Published in final edited form as:
Nat Rev Immunol. 2010 June ; 10(6): 387–402. doi:10.1038/nri2765.

The SYK tyrosine kinase: a crucial player in diverse biological functions

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Abstract

Spleen tyrosine kinase (SYK) has been known to relay adaptive immune receptor signalling. However, recent reports indicate that SYK also mediates other, unexpectedly diverse biological functions including cellular adhesion, innate immune recognition, osteoclast maturation, platelet activation and vascular development. SYK is activated by C-type lectins and integrins, and activates novel targets including the CARD9/CARMA1–BCL10–MALT1 pathway and the NLRP3 inflammasome. Drosophila studies indicate evolutionary ancient origin of SYK-mediated signalling. Moreover, SYK has a crucial role in autoimmune diseases and haematological malignancies. This Review summarizes our current understanding of SYK functions and the translation of this knowledge for therapeutic purposes.

Introduction

Our understanding of signal transduction in the immune system was dramatically increased during the 1990s when it was shown that B cell receptors (BCRs), T cell receptors (TCRs) and Fc receptors (FcRs) (collectively termed classical immunoreceptors) signal by a conceptually similar mechanism. All these receptors associate with transmembrane proteins with cytoplasmic domains containing immunoreceptor tyrosine-based activation motifs (ITAMs)[1], short peptide sequences with two tyrosine residues 6-12 amino acids apart. ITAMs are rapidly phosphorylated following receptor engagement, leading to the

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Further information
Attila Mocsai's page on the Phagocytes server: www.phagocytes.net/groups/mocsai.html
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Rigel, Inc., developing SYK inhibitors: www.rigel.com
SYK in the UCSD-Nature Molecule Pages: www.signaling-gateway.org/molecule/query;jsessionid=84f9144b229c8a6520e5196a4aa29a7884b67d528a32?afcsid=A000040
SYK in the Mouse Genome Informatics Database: http://www.informatics.jax.org//searches/accession_report.cgi?id=MGI:99515

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recruitment and activation of SYK or the related ζ-chain-associated protein kinase of 70 kDa (ZAP70). This signalling model soon entered immunology textbooks and became the central paradigm of immune cell signalling.

SYK is a 72 kDa non-receptor tyrosine kinase, which contains two SRC homology 2 (SH2)-domains and a kinase domain (BOX 1), and is most highly expressed by haematopoietic cells. Mammals also express a SYK homologue, ZAP70, which is mostly restricted to T- and NK-lineage cells. SYK-related kinases are also found in invertebrates. Further facts about SYK and SYK-related molecules can be found in BOX 1.

The SYK signalling pathway was initially thought to be restricted to classical immunoreceptors of the adaptive immune response. However, later studies showing that glycoprotein VI (GpVI), a collagen-receptor expressed by platelets, also signals by a similar mechanism[2], and that thepetechiated appearance of SYK-deficient embryos was due to a defect in lymphatic vascular development[3] provided evidence for the role of SYK outside the adaptive immune response. The discovery of ITAM-based signalling in Drosophila[4] indicated the role of such pathways beyond adaptive immunity (insects do not have an adaptive immune system). Additional studies have revealed that SYK is required for several functions of innate immune cells and for certain non-immune functions such as bone resorption by osteoclasts[5]. SYK has also been shown to mediate signaling by novel classes of receptors, including integrins[6] and C-type lectins[7], that do not contain conventional ITAM sequences. Collectively, these studies have dramatically changed our view of the SYK tyrosine kinase. This Review summarizes our current understanding of the diverse roles of SYK, in both immune and non-immune biological responses, and describes how this knowledge is being translated for therapy of human diseases.

ITAM-based signaling by classical immunoreceptors and C-type lectins

Classical immunoreceptors include BCRs, TCRs and various FcRs which mediate, either directly or indirectly, the adaptive recognition of self and foreign antigens. Immunoreceptor-associated transmembrane adaptors or, in the case of FcγRIIA, the ligand binding receptor chain itself contain one or more ITAMs (FIG. 1A), and receptor ligation leads to the phosphorylation of these ITAMs, primarily by members of the SRC tyrosine kinase family. Through interaction with their tandem SH2 domains, dual-phosphorylated ITAMs recruit SYK, in the case of the BCR or FcR, or ZAP70, in the case of the TCR, triggering kinase activation and downstream signalling. It should be mentioned that ZAP70 is significantly more dependent on SRC-family kinases than SYK, likely because SYK can phosphorylate the ITAM tyrosines itself[8,9].

Signalling downstream of SYK and ZAP70

Of the several intermediates implicated in relaying SYK- or ZAP70-mediated downstream signalling, VAV family members, phospholipase Cγ (PLCγ) isoforms, the regulatory subunits of phosphoinositide 3-kinases (PI3Ks) and the SH2 domain-containing leukocyte protein (SLP) family members SLP76 and SLP65 are directly associated with SYK (FIG. 1B) and/or ZAP70. These molecules probably participate in forming receptor-proximal signalling complexes and trigger downstream processes including Ca^{2+} and protein kinase C

(PKC) signalling, RAS homologue (RHO)-family and Pyk2-mediated cytoskeletal rearrangement, reactive oxygen species (ROS) production and phagocytosis, PI3K-mediated TEC-family and AKT signaling pathways, as well as transcriptional regulation through the RAS–extracellular signal-regulated kinase (ERK) and Ca^{2+}-NFAT pathways (FIG. 1B). While the transmembrane adaptor linker for activation of T cells (LAT) is an important component of TCR and FcεRI signalling, it is at present unclear whether any LAT-related molecules play similar roles in other lineages[10].

Ligation of classical immunoreceptors also triggers pro-inflammatory transcriptional programmes, through activation of the nuclear factor-κB (NFκB) transcription factor. Recent studies have revealed that ITAM-based signalling events lead to caspase-recruitment domain (CARD)-mediated heterotypic aggregation of CARD9 or CARMA1 (also known as CARD11) with the adaptor protein B cell lymphoma 10 (BCL10) and the associated paracaspase MALT1[11-16], triggering activation of the NF-κB pathway. Whereas CARMA1 relays antigen receptor-mediated activation of the BCL10–MALT1 complex in B cells, T cells, NK cells and mast cells, CARD9 is responsible for BCL10–MALT1 activation in macrophages and dendritic cells (DCs)[11-17]. The role of this pathway in fungal recognition will be discussed below whereas further details of CARMA1/CARD9 signaling can be found in an excellent recent review[18].

Negative regulation of SYK function

The action of SYK is often counteracted by phosphatases such as PTPN6 (SHP-1), and it is the balance of SYK and PTPN6 activities that determines the ultimate signalling output[19]. The expression of SYK is also negatively regulated by the E3 ubiquitin ligase Casitas B-lineage lymphoma (CBL), leading to ubiquitination and degradation of SYK[20-22].

Role of SYK and ZAP70 in leukocyte function

As B cell and T cell development requires signal transduction by properly rearranged antigen receptors, SYK deficiency leads to complete absence of mature B cells[23,24] and ZAP70-deficient humans and mice have severe defects in T cell development[25-27]. More detailed studies revealed that SYK plays a major though not indispensable role in the transition from the pro-B to pre-B cell stage, whereas it is completely required to pass a novel Rac-dependent checkpoint controlling the entry of immature B-cells into the splenic white pulp (termed transitional type 0 or T0 stage)[23,28]. Initially, signalling by the BCR and TCR was thought to be specifically mediated by SYK and ZAP70, respectively. Unexpectedly, ZAP70 was recently found to be expressed throughout B cell development and to participate in pre-BCR signalling, and therefore also in allelic exclusion and transition from the pro-B to the pre-B cell stage[29]. On the other hand, SYK was shown to have a considerable role inpre-TCR signalling, which occurs during the transition from the double-negative 3 (DN3) to the DN4 stage of early thymocyte development[30]. Hence, SYK and ZAP70 have overlapping functions in early lymphocyte development.

SYK is also required for FcεRI signalling in mast cells[31] and FcγR signalling in neutrophils and macrophages[32,33], whereas FcγR-mediated antibody-dependent cell-mediated cytotoxicity (ADCC) reactions are defective in natural killer (NK) cells that lack both SYK

and ZAP70[34]. The reader is referred to excellent recent and past reviews on further aspects of those signal transduction processes[9,35-38].

ITAM-mediated tonic signalling

ITAM-mediated signalling was initially thought to only occur following ligand-induced receptor crosslinking. However, recent studies revealed that the disruption of BCR signalling by inactivation of the ITAM-bearing Igα chain leads to a rapid loss of resting mature B cells[39]. These studies indicated that the maintenance of mature B cells requires ITAM-dependent constitutive ("tonic") signalling, apparently in the absence of a BCR ligand. It has yet to be tested whether this tonic signaling requires SYK, though the effect of SYK inhibitors on BCR-expressing B-cell lymphomas suggest that this is the case (see below).

Recent studies have also revealed antigen-independent signal transduction by FcεRI on mast cells[40,41]. Since these responses require receptor aggregation and SYK, they appear to conform to conventional ITAM–SYK-mediated signaling.

Signaling by hemITAM-containing C-type lectins

It has long been thought that the two phosphorylated tyrosine residues have to reside within a single peptide chain (as in a classical ITAM) to be able to recruit and activate SYK. However, a novel family of C-type lectins, including the fungal recognition receptor CLEC7 (Dectin-1) or the platelet receptor CLEC2 are able to activate SYK even though these receptors only have a single Tyr-X-X-Leu motif (termed "hemITAM") in their cytoplasmic tail[42,43]. Further studies have indicated the role of the tyrosine residue in the Tyr-X-X-Leu motif, as well as both SYK SH2 domains in signal transduction[43,44], suggesting that hemITAM-containing C-type lectins activate SYK by dimerisation followed by engagement of the two SH2-domains of SYK by phoshorylated hemITAMs on two separate receptor chains (FIG. 1A). Evidence for this stoichiometry has recently been provided for the CLEC2-SYK interaction[45]. The functional role of SYK in signaling by C-type lectins will be discussed below; other aspects of these receptors can be found in excellent recent reviews[7,46].

Structural basis of SYK function

Three different SYK activity states

Immunoreceptor signalling through SYK requires the kinase activity of SYK, as well as both SH2 domains. The kinase domain of SYK is inactive in the resting state of the protein but can be activated by binding of both SH2 domains to dually phosphorylated ITAMs (FIG 2A). Phosphorylation of tyrosine residues within the linker regions (interdomain A or B) also results in kinase activation even in the absence of phospholytaled ITAM binding (see below). This observation led to the proposal that SYK functions as an "OR" logic gate switch[47] (FIG. 2A). The physiological relevance of this activation will be discussed below.

Autoinhibition of the kinase domain

Although no high-resolution 3D structure of the entire SYK protein is available, the structure for the related ZAP70 has shown that the kinase adopts an autoinhibited conformation in the absence of phosphorylated ITAM binding[48,49]. In this structure, binding between interdomain A, interdomain B and parts of the kinase domain hold the catalytic centre in an inactive conformation. Engagement of the SH2 domains by a phosphorylated ITAM disassembles this tripartite binding, allowing the kinase domain to adopt an active conformation.

Several studies indicate that, analogous to ZAP70, SYK is also regulated by similar autoinhibition (FIG. 2A). A low-resolution 3D structure of SYK shows a compact structure, similar to the autoinhibited ZAP70[50]. Deletion of both SH2 domains, or of interdomain A alone[51,52], as well as binding of a phosphorylated ITAM to the SH2 domains[52] results in SYK activation, consistent with being relieved from an autoinhibited conformation.

Interaction of SYK with phosphorylated ITAMs

Binding of the SH2 domains of SYK to phosphorylated ITAMs is a critical step in SYK activation and downstream signalling. A high-resolution three-dimensional structure of the SH2 domains of SYK in complex with the doubly phosphorylated ITAM of CD3ε showed that the N-terminal SH2 domain binds the C-terminal phosphorylated tyrosine of the ITAM and vice versa[53] (FIG. 1A). The structure shows significant flexibility[53,54], which may account for the ability of SYK, in contrast to ZAP70, to bind to phosphorylated ITAMs with a broad range of spacing between the phosphorylated tyrosine residues (including the long-spaced FcγRIIA ITAM[55]), as well as to bridge two hemITAMs in C-type lectins[43-45] (FIG. 1A).

Role of phosphorylated tyrosines in SYK

There are ten tyrosines in SYK that are sites of autophosphorylation (FIG. 2B)[56], most of which have been shown to participate in SYK-mediated signal transduction. Unless otherwise stated, amino acids are numbered as in mouse Syk.

BCR activation triggers phosphorylation of Tyr130 in interdomain A[56], leading to dissociation of SYK from the phosphorylated ITAMs of the BCR complex[57,58] (FIG. 2C). Mutation of Tyr130 to the phosphomimetic glutamate also decreases ITAM binding and results in increased kinase activity, once again pointing to an inhibitory role for interdomain A[57].

Phosphorylated Tyr317 is a binding site for the E3 ubiquitin ligase CBL (FIG. 2B), which is involved in the ubiquitination and degradation of SYK[20,21]. Accordingly, mutation of this residue results in augmented FcεRI- and BCR-mediated signalling[59,60]. Phosphorylated Tyr317 also binds the C-terminal SH2 domain of the PI3K regulatory subunit p85α, whereas the N-terminal SH2 domain of p85α binds phosphorylated Tyr342 and phosphorylated Tyr346[61] (FIGS 2B-C). Mutation of all three tyrosines abrogates FcεRI-induced AKT activation, consistent with an inability to activate PI3K[62].

In the ZAP70 structure, Tyr315 and Tyr319 in interdomain B and Tyr597 and Tyr598 in the kinase domain stabilize the autoinhibited conformation[48]. The mutation of these residues in ZAP70 or the mutations of the analogous residues in SYK (Tyr342, Tyr346, Tyr624 and Tyr625) results in kinase activation, further indicating that SYK adopts an autoinhibited conformation[63-65]. All four residues are phosphorylated following BCR stimulation[56], likely contributing to kinase activation by destabilizing the autoinhibited conformation of SYK (FIG. 2A), as well as providing docking sites for other signalling molecules which stabilise the active conformation and initiate downstream signaling (FIG. 2C).

Phosphorylated Tyr342 binds to the SH2 domain of VAV1 and both phosphorylated Tyr342 and phosphorylated Tyr346 bind to the C-terminal SH2 domain of PLCγ1[66,67] (FIGS 2B-C). Interestingly, this latter binding is mediated by two phosphorylated tyrosines binding to one SH2 domain[68]. Mutation of these residues inhibits FcεRI signalling in mast cells[62,69] and phenocopies the cytoskeletal changes observed in SYK-deficient neutrophils[70].

When phosphorylated, Tyr624 binds the SH2 domain of SLP65[71] (FIGS 2B-C). This binding activates the kinase, which is consistent with the putative role of Tyr624 in stabilizing the autoinhibitory conformation. This SLP65–SYK association is also important for BCR signalling and B cell development[71]. Although no direct interaction between Tyr624 and the SLP65-related SLP76 adaptor has been reported, such a direct functional association would not be surprising given the strikingly similar phenotypes of SYK-deficient[3,23,24,33,72,73] and SLP76-deficient mice[3,74,75].

Integration of SYK activation

The most likely physiological relevance of the dual activation of SYK either by ITAM binding or by phosphorylation of linker tyrosines is that initial ITAM binding triggers rapid activation of Syk whereas subsequent phosphorylation leads to prolonged activation and downstream signaling even in the absence of binding to phosphorylated ITAMs (FIG. 2C). Importantly, SYK itself can catalyze the autophoshorylation of its linker tyrosines, leading to sustained SYK activation after a transient ITAM phosphorylation. Inaddition, SYK itself can phosphorylate ITAMs, suggesting the existence of a positive-feedback loop during initial ITAM-mediated SYK activation[52] (FIG. 2C).

The role of SYK in cellular adhesion

Integrin signal transduction

Integrins are a family of heterodimeric transmembrane receptors that participate in leukocyte adhesion and migration (through the β2 integrins lymphocyte function-associated antigen 1 (LFA1) and macrophage receptor 1 (MAC1)), platelet activation (αIIbβ3 integrin) and osteoclast development and function (αVβ3 integrin). Given their structural and functional differences, integrins and classical immunoreceptors were long thought to signal by conceptually different mechanisms.

Evidence for the use of similar signalling mechanisms by integrins and immunoreceptors came from studies showing defective integrin-mediated signalling in SYK-deficient neutrophils[72], monocytes/macrophages[76,77], platelets[73] and osteoclasts[78]. Further in vivo

studies showed that SYK is necessary for firm leukocyte adhesion to the inflamed endothelium[79] and the development of a MAC1-dependent vasculopathy reaction[80].

The mechanism of SYK activation by integrins has long been debated, primarily since initial studies in heterologous expression systems indicated that the αIIbβ3 integrin activates SYK by an ITAM-independent mechanism through direct association between the cytoplasmic tail of the integrin β-chain and the non-ITAM-binding surfaces of the N-terminal SH2 domain of SYK[81-83] (FIG. 3A).

More recent analyses of primary cells (rather than heterologous expression systems) came to a very different conclusion. Structure-function studies indicated a crucial role of phosphorylated tyrosine binding by the SH2 domains of SYK for integrin-mediated functions in neutrophils, macrophages and platelets[77,84]. The ITAM-containing proteins linking β2 integrin signalling to SYK activation in neutrophils and macrophages have been identified as the DAP12 and the FcR γ-chain (FcRγ) transmembrane adaptor molecules[77] (FIG. 3B). Later studies indicated that integrin signalling in osteoclasts[85], DCs[86], microglia[87] and platelets[88] also require ITAM-containing transmembrane adaptors. However, the coupling between integrins and the ITAM-containing adaptor molecules is poorly understood, even though preliminary studies[77] suggested the involvement of as yet unidentified DAP12--associated molecules (FIG. 3B).

Importantly, the above ITAM-independent[81,84] and ITAM-mediated[77,84-88] mechanisms of integrin–SYK coupling are not mutually exclusive. Indeed, the most likely scenario is that the two mechanisms jointly regulate the activity of SYK[6,85] (FIG. 3C), allowing the fine control of adhesive interactions of haematopoietic lineage cells.

It should also be noted that whereas SYK is crucial for several integrin-mediated functions, it makes limited contribution to β2 integrin-mediated migration of neutrophils[70,72].

Leukocyte selectin functions

Selectins are transmembrane glycoproteins that are involved in leukocyte rolling on the endothelium. Endothelial P-selectins mediate steady-state leukocyte rolling, whereas slow rolling in an inflammatory environment is triggered by endothelial E-selectins. Recent evidence indicates a role for SYK in signal transduction by P-selectin glycoprotein ligand 1 (PSGL1), the major selectin receptor on the leukocyte surface. SYK is activated by, and associated with, PSGL1 in leukocytes[89,90]. Abrogation of SYK activity inhibits PSGL1-mediated transcriptional regulation[89], whereas SYK-deficient neutrophils show a defect in slow rolling, suggesting that SYK is involved in PSGL1-induced inside-out signalling that activates LFA1[90] (FIG. 3D). Both groups concluded that PSGL1-induced SYK activation proceeds through an ITAM-mediated pathway[89,91]. However, one report concluded that SYK is activated through the ITAM-like motif-containing ezrin, radixin and moesin (ERM) family proteins[89], whereas the other study suggested the role of the ITAM-bearing adaptors DAP12 and FcRγ phosphorylated by the SRC-family kinase FGR[91] (FIG. 3D).

Role of SYK in innate pathogen recognition

The innate immune system uses germline-encoded pattern recognition receptors (PRRs) to detect pathogen-associated molecular patterns (PAMPs) and activate immune responses. Recently, SYK has emerged as a key component of these pathways (FIG. 4A).

Fungal recognition

The first insights into SYK-dependent PRR signalling came from studies of the C-type lectin CLEC7A (Dectin-1), a major mammalian PRR for fungal β-glucans[43] with a hemITAM in its intracellular tail (FIG. 4A). Agonist binding triggers hemITAM phosphorylation resulting in direct recruitment of SYK to CLEC7A. As with other hemITAM-containing C-type lectins, CLEC7A-mediated SYK activation requires a single tyrosine residue in a Tyr-X-X-Leu motif. It is reasonable to assume that two hemITAM-phosphorylated CLEC7A molecules engage the tandem SH2 domains of a single SYK molecule in a 2:1 stoichiometry[7], as has recently been shown for the CLEC7A-related CLEC2 receptor in platelets[45].

SYK-mediated signalling following CLEC7A ligation triggers ROS production[92,93] and phagocytosis of yeast particles by DCs[43]. Moreover, SYK couples PAMP recognition to *de novo* gene transcription and induces chemokine and cytokine production through several downstream pathways including the ERK signalling cascade and activation of transcription factors such as NFAT[94] and NF-κB[11,43,93,95-97]. Although the precise mechanisms of SYK-mediated phagocytosis and ROS production are poorly understood, the CLEC7A–SYK-induced NF-κB response is transduced through CARD9 which engages the BCL10 adaptor and the MALT1 paracaspase[11,98,99] (FIG. 4B). Consistent with an essential role of the CLEC7A–SYK–CARD9 axis for antifungal host defence, CARD9-deficient mice and humans are highly susceptible to fungal infections[11,100]. It is at present unclear how SYK activates CARD9 but this mechanism likely differs from the PKC-mediated activation of CARMA1 in lymphocytes since the critical PKC phosphorylation sites of CARMA1 are not conserved in CARD9[18]. Moreover, the specific role of SYK-triggered ERK or NFAT activation in anti-fungal immunity has not yet been defined either.

Although the CLEC7A–SYK–CARD9 pathway is a *bona fide* PRR pathway that can couple innate to adaptive immunity, it induces a distinct pattern of DC cytokines with particularly robust IL-2, IL-10 and IL-23 production[96], leading to the initiation of potent T helper 17 (T_H17) cell responses[96,101].

SYK-deficient DCs have a more profound block in IL-2 or IL-10 synthesis upon yeast stimulation than those lacking CLEC7A, suggesting redundancy at the receptor level[96]. Indeed, two additional SYK-coupled PRRs for fungi, CLEC6A (Dectin-2) and CLEC4E (Mincle), were recently identified[102-105]. Instead of containing a cytoplasmic hemITAM, CLEC6A and CLEC4E associate with and signal through the ITAM-containing FcRγ adaptor[103,105] (FIGS 4A-B). Both CLEC6A–SYK and CLEC4E–SYK signalling induce CARD9- and NF-κB-mediated pro-inflammatory responses which promote adaptive immunity and robust T_H17-type responses[102,106,107].

Inflammasome activation

IL-1β is a SYK-dependent cytokine with essential role in antifungal immunity[93]. Production of mature IL-1β requires NF-κB-mediated transcription of pro-IL-1β and proteolytic processing of pro-IL-1β by caspase-1-containing multiprotein complexes termedinflammasomes[108]. Live yeast cells specifically activate the NLRP3 inflammasome[93]. SYK controls both pro-IL-1β synthesis and NLRP3 activation in response to fungal infection[93]. Whereas pro-IL-1β synthesis is regulated through the SYK–CARD9 pathway, NLRP3 activation occurs through a SYK-dependent but largely CARD9-independent mechanism[93] (FIG. 4B). Since NLRP3 activation by malarial haemozoin also requires SYK[109], the role of SYK in NLRP3 activation is not restricted to antifungal immunity.

The NLPR3 inflammasome is activated in response to various distinct danger signals[108]. Upstream mechanisms that trigger NLRP3 activation in multiple scenarios are ROS production, potassium efflux and sometimes release of lysosomal proteases into the cytoplasm[108]. NLRP3 activation by fungi requires SYK-triggered ROS generation and potassium efflux[93] (FIG. 4B). However, since SYK activation by zymosan or non-viable yeast is not sufficient for inflammasome activation[93,110], yet unidentified other components are likely also required for the activation of the NLRP3 inflammasome following yeast infection (FIG. 4B).

Bacterial and viral recognition

There is increasing evidence that SYK-coupled PRRs also have important roles in the innate recognition of bacteria, with CLEC7A, CLEC6A and CLEC4E all implicated in mycobacterial PAMP sensing[46] (FIG. 4A). Although the mycobacterial structures recognized by CLEC7A and CLEC6A remain to be identified, CLEC4E has been reported to be the long-sought receptor for the mycobacterial cord factor trehalose-6,6′-dimycolate (TDM), the most thoroughly studied immunostimulant of *Mycobacterium tuberculosis*[107,111]. Myeloid cell activation by TDM, or its less toxic analogue trehalose-6,6-dibehenate, uses ITAM–SYK signalling through FcRγ, SYK and the CARD9–BCL10–MALT1 complex[106,107].

In addition to CLEC7A, CLEC6A and CLEC4E, there are further myeloid C-type lectin receptors with still unidentified functions[46], and other innate immune receptors have also been proposed to signal through SYK[112]. Furthermore, genetic deletion of SYK has been shown to attenuate antibacterial host defence in mice[113]. The dengue virus is detected by the C-type lectin CLEC5A (myeloid DAP12-associating lectin 1(MDL1)), which induces pro-inflammatory cytokine production through the ITAM-containing DAP12 adaptor[114] (FIG. 4A). Therefore, SYK is presumably also involved in signaling PRR-mediated recognition of certain viruses.

Signalling tissue damage in mammals and invertebrates

The innate immune system is also activated by non-infectious stimuli, such as cell death caused by sterile injury or developmental processes, or sterile inflammation during autoimmune diseases.

There is accumulating evidence that SYK-coupled C-type lectins can detect host-derived molecules from damaged cells. CLEC4E, an FcRγ-associated fungal and mycobacterial PRR (see above), can sense necrotic cells after prolonged culture *in vitro*[115] and it is reasonable to assume that this response also requires the ITAM-mediated activation of SYK (FIG. 4A). The CLEC4E ligand expressed by necrotic cells has been identified as the spliceosome-associated protein 130 (SAP130)[115]. A second SYK-coupled receptor for dead cell recognition is CLEC9A (dendritic cell NK lectin-group receptor 1 (DNGR1)). Similar to CLEC7A, CLEC9A has a cytoplasmic hemITAM with a single Tyr-X-X-Leu motif (FIG. 4A). CLEC9A is highly expressed by subsets of DCs and monocytes and recognizes as yet unidentified ligands that are exposed during cell necrosis[116]. CLEC9A promotes DC-mediated cross-presentation of dead cell-derived antigens through a hemITAM-SYK interaction[117].

The (hem)ITAM–SYK-mediated damage-recognition pathway likely represents an ancient mechanism as it is also found in invertebrates. The clearance of neuronal cell corpses by phagocytic glial cells in the Drosophila brain requires Draper, an ITAM-containing phagocytic receptor[118] (FIG. 4A). Draper ligation by phagocytic targets triggers ITAM phosphorylation by the SRC-related kinase SRC42A, leading to subsequent association with the SYK-related kinase SH2 domain ankyrin repeat kinase (SHARK) (FIG. 4A) which is essential for Draper function in glial cells *in vivo*[4].

Taken together, the above pathways indicate that the (hem)ITAM–SYK-mediated damage recognition pathway is an ancient mechanism that it shared between insects and mammals, and that this pathway overlaps with innate recognition of pathogens.

Other immune functions of SYK

SYK has been implicated in several other signalling pathways within immune cells. The DAP12-associated triggering receptor expressed on myeloid cells (TREM) molecules participate in a number of inflammation-related biological activities, including modulation of Toll-like receptor function, through activation of SYK[119]. CLEC4C (blood dendritic cell antigen 2 (BDCA2)), an FcRγ-associated receptor expressed on DCs, also signals through SYK but, in contrast to C-type lectins involved in pathogen recognition, does not activate the NFκB[46]. A number of other well-known immune cell receptors do not contain ITAMs, yet appear to also utilize ITAM–SYK-mediated accessory signals. Interferon-γ (IFNγ)-mediated enhancement of IFNα-induced STAT1 activation requires DAP12, FcRγ and SYK, possibly through interaction of DAP12- and FcRγ-containing receptors with the IFNα receptor[120]. Basophils lacking either FcRγ or SYK show reduced IL-3-induced IL-4 secretion (but not proliferation), supposedly through direct association between FcRγ and the IL-3 receptor β_c subunit[121] and DAP12 have been implicated in signaling from the macrophage colony-stimulating factor (M-CSF) receptor cFMS in an ITAM-dependent manner also requiring PYK2[78,122]. MHC class II molecules on B cells associate with, and trigger the phosphorylation of, the ITAM-bearing Igα and Igβ adaptors[123].

NK cells express SYK and ZAP70, both of which are involved in FcγR-mediated ADCC responses[34]. NK cells also express the ITAM-bearing transmembrane adaptor DAP12,

which is coupled to several activating NK cell receptors. However, although SYK and/or ZAP70 are required for calcium signalling and cytokine release following activation of DAP12-associated receptors[124,125], NK cells lacking both SYK and ZAP70 are able to kill target cells through a DAP10- and PI3K-mediated parallel pathway[125,126].

Crystals of monosodium urate activate DCs by a receptor-independent pathway[127], which involves aggregation of cholesterol and activation of a SYK-dependent signalling pathway. The mechanism of this activation is incompletely understood, but might involve the recruitment of ITAM-associated receptors. Importantly, this mechanism may contribute to the development of crystal-induced diseases such as gout.

SYK had also been proposed to participate in G-protein-coupled receptor signalling[128]. However, genetic studies in neutrophils and mast cells[128] argue against that possibility.

Non-immune functions of SYK

Although ITAM–SYK signalling has long been regarded to be specific for the immune system, recent studies indicate several non-immune functions of SYK.

Bone metabolism

Osteoclasts are macrophage-related bone-resorbing cells that develop from early myeloid precursors under the control of the TNF-related protein receptor activator of NF-κB (RANK) ligand expressed by osteoblasts. Differentiated preosteoclasts fuse to form large multi-nucleated cells, which then bind to and resorb the bone surface (FIG. 5A, inset). Importantly , the αVβ3 integrin participates in diverse functions of osteoclasts, including the sealing the resorption area from the surrounding environment (FIG. 5A, inset). Loss-of-function mutations of DAP12 (Nasu-Hakola disease) cause bone abnormalities in humans[129], indicating a possible role of ITAM-based signalling in bone metabolism. Indeed, DAP12-deficient mice show moderate osteopetrosis[130,131] while mice lacking both DAP12 and FcRγ are severely osteopetrotic[5,132]. While FcRγ is involved in osteoblast–osteoclast interactions, DAP12 relays an osteoblast-independent signal[5,132] (FIG. 5A). Importantly, SYK, which is activated downstream of DAP12 and FcRγ in an ITAM-dependent manner, is also required for osteoclast development and function[5,85,133] (FIG. 5A). Although the precise mechanism of DAP12 and FcRγ activation is poorly understood, it likely involves the FcRγ-associated receptors osteoclast-associated receptor (OSCAR) and paired immunoglobulin-like receptor A (PIR-A), and the DAP12-associated TREM2 molecule[132] (FIG. 5A). This ITAM-mediated pathway has been proposed to be a costimulatory pathway to RANK ligand signaling[132], as well as a mediator of αVβ3 integrin signal transduction[5,85] (FIG. 5A).

Platelet functions

Several platelet functions rely on SYK signalling. GpVI, the major collagen receptor of platelets, is an FcRγ-associated receptor closely related to FcαRs, which may explain that GpVI, like other FcR family members, signals through SYK[2] (FIG. 5B). The C-type lectin CLEC2 has also been shown to signal through SYK in platelets[42]. Similar to other hemITAM-cotaining receptors, CLEC2 has a single cytoplasmic Tyr-X-X-Leu motif which

engages both SH2-domains of Syk by dimerisation of the receptor[45] (FIG. 5B). SYK also mediates outside-in signalling by the αIIbβ3 integrin of platelets[73]. The mechanism of SYK activation by αIIbβ3 was shown to be ITAM-dependent[84] (see above) and to require, at least in human cells, the ITAM-containing FcγRIIA[88] molecule (FIG. 5B). Since CLEC2[134] and the SYK substrate SLP-76[135] are required for arterial thrombus formation, it is likely that SYK also participates in *in vivo* haemostatic functions of platelets.

Vascular development

One of the most striking phenotypes of *Syk*-deficient fetuses is the appearance of blood-filled subcutaneous structures originally described as petechial haemorrhages[23,24] but later identified as blood-filled lymphatic vessels[3], indicating that SYK is required for the separation of lymphatic vessels from the general circulation. Surprisingly, this vascular defect could be transferred to lethally-irradiated wild-type recipients by bone marrow transplantation[33], indicating the role of SYK expression within the haematopoietic compartment. Two subsequent studies proposed very different explanations, suggesting either that the defect lies in circulating endothelial progenitor cells (which share their origin with true blood cells)[136] or that the vascular phenotype is due to a gain-of function effect whereby tissue accumulation of $Syk^{-/-}$ myeloid cells leads to excessive release of pro-angiogenic factors triggering hyperproliferation of the lymphatic vasculature[137]. However, the former mechanism contradicts the lack of SYK expession in the endothelial lineage[137] whereas the latter mechanism does not appear to explain the correction of the vascular phenotype by the presence of as little as 5-10% wild type cells in the hematopoietic compartment in mixed bone marrow chimeras[3].

A series of very recent studies propose an exciting third explanation (FIG. 5C). Blood and lymph vessels fail to separate in platelet-deficient fetuses[138], as well as in mice lacking either the platelet receptor CLEC2[139], or the CLEC2 ligand podoplanin which is expressed on lymphatic (but not blood) endothelial cells[139-141]. A similar vascular phenotype is also induced by platelet-specific deletion of SLP76[139] (the deficiency of which also leads to blood-lymphatic vascular separation defect[3]) or of SYK itself (E. Schweighoffer and V. T., unpublished observation; and C. Bertozzi and M. Kahn, personal communication). Lymphatic endothelial cells also induce aggregation of platelets in a SLP76-dependent manner[139]. Together with the ability of podoplanin to activate platelets via CLEC2[142] and the fact that CLEC2 signals through SYK and PLCγ2[42], these results suggest that podoplanin on lymphatic endothelial cells triggers platelet activation and aggregation through CLEC2, SYK, SLP76 and PLCγ2 (FIG 5C), thereby closing off any blood-lymphatic shunts by a yet unidentified mechanism that possibly involves platelet aggregation. As a consequence, newly formed lymphatic sacks are separated from the blood vasculature, and a long-term incompatibility between the two vascular systems is establishes.

Other non-haematopoietic functions

SYK is also expressed by cells of non-haematopoietic origin including normal mammary glands and mammary gland epithelial cell lines[143], as well as various other tissues (reviewed in REFS [144,145]). While sporadic studies have proposed that SYK plays a role in processes

including signaling by integrins and TNF-receptors, transcriptional regulation and mitotic progression in these cells (reviewed in REF.[144]), this area is still poorly understood with very little mechanistic information available.

A SYK-related kinase has also been proposed to participate in gonadal development in the parasitic helminth Schistosoma mansoni[146], suggesting that the role of SYK-related kinases in non-hematopoietic tissues may also have an ancient evolutionary origin.

SYK in disease pathogenesis and therapy

Allergy and autoimmunity

The diverse roles of SYK in the immune system suggest that it may participate in allergic and/or autoimmune diseases. Indeed, SYK-deficient bone marrow chimaeras are protected from reverse passive Arthus reaction[70] and autoantibody-mediated experimental arthritis[147]. A supposedly SYK-selective inhibitor, R112, alleviates the symptoms of allergic rhinitis in human patients[148]. An R112-related inhibitor, R406, as well as its orally bioavailable prodrug, R788 (also known as fostamatinib), delayed the onset and reduced the severity of autoimmune arthritis in animal models[149,150] and provided clinical benefit in rheumatoid arthritis patients[151]. More recently, R788/fostamatinib has shown beneficial effects in autoantibody-induced thrombocytopenia in mice, and autoimmune thrombocytopenic purpura in humans[152]. Various aspects of the mechanism of action of SYK-inhibitors in clinical use are discussed in BOX 2.

Haematological malignancies

SYK has been linked to the development or maintenance of hematological malignancies including B-lineage leukemias and lymphomas (FIG. 6).

The first study linking SYK to a haematological malignancy described marked reduction of SYK expression in highly aggressive childhood pro-B cell acute lymphoblastic leukaemia (ALL) but not in more differentiated pre-B cell ALL[153]. Since SYK is required for the transition of pro-B to pre-B cells[23], it is tempting to speculate that the loss of SYK contributes to transformation of B cell precursors by preventing further differentiation (FIG. 6), as has been proposed for SLP65, the lack of which contributes to pre-B cell leukemia in both humans and mice[154,155]. However, the lack of an independent confirmation of SYK deficiency in pro-B ALL, and the uncertainties related to extrapolating prior findings on SLP65 to SYK point to the need for further studies on the role of SYK in pro-B cell leukemia.

SYK has very different and much better characterised roles in lymphomas derived from B-lineage cells beyond the pro-B cell stage. Likely related to the role of SYK in promoting proliferation and blocking apoptosis in pre-B cells, exogenous expression of SYK was able to transform normal pre-B cells, and endogenous SYK was required for transformation of pre-B cells by exogenous c-MYC[156] (FIG. 6). Furthermore, SYK inhibition promoted apoptosis of primary human B-cell precursor ALL (most likely pre-B cell ALL) cells both in vitro and in vivo, suggesting a potential therapeutic application of SYK inhibitors in certain B-cell ALL patients[157].

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Transformation of mature B-cells mostly leads to various forms of B-cell lymphoma or B-lineage chronic lymphocytic leukemia (CLL). ITAM-mediated tonic BCR signaling is required for the survival of resting mature B cells and certain B cell lymphomas[39,158,159]. This signal likely acts through SYK which could explain the expression of constitutively active SYK in B-cell lymphomas and B-lineage CLL cells[160-162]. SYK is also required for processing stroma cell-derived pro-survival signals, such as adhesion and homing, by CLL cells independently of BCR signaling[163]. These results suggest that SYK contributes to the survival and maintenance of B cell malignancies (FIG. 6). Accordingly, abrogation of SYK activity inhibited the growth of various B cell lymphomas[160,164,165], and R788/fostamatinib therapy prolonged the survival of patients with diverse types of B cell lymphoma or B-lineage CLL[166].

SYK may also participate in haematological malignancies of non-B-cell origin. T-cell lymphomas often overexpress enzymatically active SYK[167], may contain chromosomal translocations leading to the expression of an IL-2-inducible T cell kinase (ITK)–SYK fusion protein[168], and are sensitive to silencing of SYK expression[169]. A chromosomal translocation generating a TEL–SYK fusion protein causes myelodysplastic syndrome, a preleukemic state predisposing for the development of acute myeloid leukaemia[170], while SYK appears to be an important component of disease pathogenesis in acute myeloid leukaemia itself[171]. These results may further extend the possible use of SYK inhibitors in human therapy.

SYK signalling by viral oncogenes

SYK is also required for the oncogenic activity of several ITAM-containing viruses[172]. The ITAM of the viral LMP2A protein and host cell SYK are both required for Epstein-Barr virus-induced oncogenic transformation[173,174]. The ITAM-containing envelope surface protein contributes to mouse mammary tumour virus-induced transformation in an ITAM- and SYK-dependent manner[175,176]. Furthermore, the ITAM-containing K1 protein of Kaposi's sarcoma-associated herpesvirus initiates ITAM- and, presumably, SYK-dependent signalling pathways to promote lytic viral replication in infected B cells[177,178]. The possible role of these and other viruses in breast cancer[179] raise the possibility of a tumor-promoting role for SYK in certain tumours.

Other non-haematopoietic tumours

In contrast, SYK has been proposed to suppress the growth of other (supposedly non-viral-induced) tumours of non-haematopoietic origin. SYK expression inversely correlated with invasiveness in breast cancer cell lines[143] and exogenous expression of SYK inhibited tumour growth, while expression of a dominant-negative SYK was found to promote tumorigenesis[143]. SYK expression also inversely correlated with the severity of breast cancer and other epithelial cell-derived tumours in human patients[143,144,180-182], with particularly poor prognosis when low nuclear levels of SYK was present[183,184].

The decreased level of SYK expression in cancer cells likely results from hyper-methylation of the *SYK* promoter, rather than mutations or genetic deletion of *SYK* itself[144,180,182], while the loss of nuclear localization of SYK is likely due to alternative splicing leading to the

expression of the SYK-B isoform, which lacks a nuclear localization signal[185]. However, it is presently unclear how loss of SYK expression results in poor prognosis in epithelial cell-derived tumours, though it may be related to any of the previously mentioned possible physiological functions of SYK in these lineages (reviewed in ref.[144]).

Conclusions and perspective

The SYK tyrosine kinase was originally thought to only contribute to signalling responses of immunoreceptors of the adaptive immune response. However, more recent studies have identified a large number of novel functions of SYK both in the immune system and beyond. Moreover, the identification of a similar pathway in Drosophila indicates that this signalling emerged significantly earlier than the adaptive immune response. Collectively, these studies indicate that SYK plays a much more diverse role in vertebrate and invertebrate biology than previously anticipated.

In addition to the diverse roles of SYK in basic biological processes, it is also involved in the pathogenesis of several human diseases, including allergy, autoimmunity and various haematological malignancies. The promising results of SYK-inhibitors in recently completed human clinical trials suggest that SYK may become a major therapeutic target in those diseases in the future

The new observations of diverse biological functions for SYK also raise a number of questions. How do the initial steps of ITAM signalling occur? How do integrins and selectins “hook up” to an ITAM-based signalling pathway? How are DAP12 and FcRγ coupled to osteoclast development? How does SYK in platelets contribute to vascular development? What downstream pathways mediate the diverse functions of SYK? What are the roles of SYK in epithelial cells and are ITAMs involved in these functions? Is SYK a suitable target in thrombocytic diseases or pathological bone loss? Would targeting SYK be effective in treating metabolic disorders of inflammatory origin? Will long-term treatment with SYK inhibitors result in unexpected side effects, e. g. related to the role of SYK in innate immunity and its tumor suppressor function in epithelial cells? Answering these and other questions will keep scientists interested in the biological functions of SYK busy for many years to come.

Acknowledgement

We apologize to the many colleagues whose work could not be discussed due to space restriction; as well as for the overt focus of this Review on mouse knockout studies. We thank the members of our laboratories for stimulating discussions and Mark Kahn for sharing unpublished results. The authors' laboratories are supported by the European Research Council, the Wellcome Trust and the Hungarian Office for Research and Technology (A. M.), the German Research Foundation and the German Cancer Aid (J. R.), as well as by the UK Medical Research Council (V. T.).

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Glossary terms

immunoreceptor tyrosine-based activation motifs (ITAMs)	An ITAM is a short peptide motif containing two tyrosine residues that is found in the cytoplasmic tail of several signalling adaptor proteins and is necessary to recruit proteins that are involved in triggering activating signalling proteins. The consensus sequence is Tyr-X-X-(Leu/Ile)-$X_{6–12}$-Tyr-X-X-(Leu/Ile), where X denotes any amino acid.
petechia	A small red spot on the skin that results from a small bleeding due to a broken blood vessels. A large number of petechiae usually indicates a platelet defect. Note that, in contrast to the original assumptions, the red spots of Syk-deficient embryos are not *bona fide* petechiae since they are caused by a vascular developmental defect.
Glycoprotein VI (GpVI)	One of several collagen receptors expressed on platelets. GpVI is a transmembrane glycoprotein closely related to Fcα-receptors. It associates with, and signals through, the ITAM-containing FcRγ adaptor.
haemostasis	The combination of events that result in cessation of bleeding. Heamostatic processes include constriction of blood vessels, platelet aggregation and coagulation (clotting) of the blood.
osteoclasts	Large multinucleated cells that arise from macrophage precursors and are specialized for bone resorption. Once firmly adhered to the

	bone surface, osteoclasts release hypochlorous acid (HCl) and hydrolytic enzymes to digest the bone tissue.
antibody-dependent cell-mediated cytotoxicity (ADCC)	A cytotoxic mechanism by which an antibody-coated target cell is directly killed by a leukocyte that expresses Fc receptors, such as a natural killer cell, macrophage or neutrophil.
pre-B cell receptor (pre-BCR)	A receptor that is formed at the surface of pre-B cells by the pairing of rearranged immunoglobulin heavy chains with surrogate heterodimer of Igα and Igβ. Signalling by the pre-BCR occurs in the absence of known ligands and is a crucial event in B cell development.
pro-B cells	Cells in the earliest stage of B cell development in the bone marrow. They are characterized by incomplete immunoglobulin heavy-chain rearrangements and are defined as $CD19^+$cytoplasmic IgM^- or, sometimes, as $B220^+CD43^+$ (by the Hardy classification scheme).
pre-B cell	Cells in a stage of B cell development in the bone marrow that are characterized by complete immunoglobulin heavy-chain rearrangement in the absence of immunoglobulin light-chain rearrangement. They express the pre-B cell receptor, which comprises a pseudo light chain and a heavy chain.
transitional type 0 (T0) stage	Transitional B cells are recent emigrants from the bone marrow that are in the process of maturing into follicular and marginal zone B cells. They can be found in the spleen and typically have a half-life of a few days. Transitional B cells have been subdivided into subsets type 0 – 3 (T0 – T3) based on expression of IgM, IgD, CD93 and CD23. T0 B cells are the earliest immigrants from the marrow and are located in the red pulp of the spleen.
pre-T cell receptor (pre-TCR)	A cell surface receptor complex consisting of TCRβ, pre-TCRα and CD3 that is expressed by immature $CD4^-CD8^-$ thymocytes. Signalling through this complex is essential for maturation to the $CD4^+CD8^+$ stage
paracaspase	A group of proteolytic enzymes distinct from, but closely related to, the caspases. The most important mammalian paracaspase is the mucosa-associated lymphoid tissue 1 (MALT1) paracaspase.
E3 ubiquitin ligase	Ubiquitination of proteins target them for proteasomal degradation, hence it determines the half-life of proteins. E3 ubiquitin ligases are proteins responsible for specifically targeting ubiquitin to the proteins to be degraded. The other two ubiquitin ligase families

	(E1 and E2) are required to supply ubiquitin for E3 ubiquitin ligases.
hemITAM	A peptide motif in the cytoplasmic tail of various C-type lectins, characterized by the presence of a single Tyr-X-X-Leu sequence (in contrast to the two such sequences in full ITAMs). Phosphorylation of the Tyr residue in the hemITAMs leads to binding to the SYK SH2-domains in a 2:1 stoichiometry.
phosphomimetic	An amino acid whose conformation resembles that of a phosphate residue, or that of the phosphorylated form of another amino acid. Due to their negative charge, the most important phosphomimetic amino acids are glutamate and aspartate.
vasculopathy	A disorder of blood vessels, such as the inflammation of the vessel wall.
heterologous expression systems	An analytical tool relying on the forced expression of a protein in a cell type (usually a long-prpagated cell line) where it is not expressed normally. Heterologous expression systems are highly powerful research tools but may lead to artefactual results due to the expression of proteins in a foreign environment.
inside-out signalling	The process by which intracellular signalling mechanisms result in the activation of a cell surface receptor, such as integrins. By contrast, outside-in signalling is the process by which ligation of a cell surface receptor activates signalling pathways inside the cell.
T helper 17 (T_H17) cell	A subset of $CD4^+$ T helper cells that produce interleukin-17 (IL-17) and that are thought to be important in inflammatory and autoimmune diseases. Their generation involves IL-23 and IL-21, as well as the transcription factors RORγt (retinoic-acid-receptor-related orphan receptor-γt) and STAT3 (signal transducer and activator of transcription 3).
inflammasome	An inflammasome is a molecular complex of several proteins that upon assembly cleaves pro-IL-1, thereby producing active IL-1.
zymosan	A protein-carbohydrate complex prepared from yeast wall extract. Its polysacharide components (β-glucans) contains D-glucose monomers linked by β-type glycosidic bonds. β-glucans are particularly strong activators on anti-fungal host defence.
monosodium urate	Monosodium salt of uric acid. It has a tendency to precipitate in needle-shaped monosodium urate crystals in the joints, leading to gout, a painful joint inflammatory disease.
osteoblasts	Bone-lining fibroblast-related cells of non-haematopoietic origin that specialize themselves for the generation of bone matrix and the regulation of bone metabolism. Through expression of receptor-

	activator of NF-κB (RANK) ligand, they also induce development and activation of osteoclasts.
receptor activator of NF-κB (RANK)	A cytokine receptor closely related to tumor necrosis factor (TNF) receptors that is primarily expressed in the osteoclast lineage. Ligation of RANK on early marophage precursors by RANK ligand expressed on osteoblasts triggers re-programming of the cell leading to osteoclast development.
Nasu-Hakola disease	An inherited human disease characterised by presenile dementia and bone cysts. It is also known as polycystic lipomembranous osteodysplasia with sclerosing leucoencephalopathy (PLOSL). Nasu-Hakola disease is caused by loss-of-function mutations in the ITAM-cotaining DAP12 adapter or the DAP12-associated TREM2 receptor. The clinical manifestations of Nasu-Hakola disease are likely due to the loss of DAP12 or TREM2 in osteoclasts and microglia.
osteopetrosis	A rare inherited disease characterized by pathological accumulation of mineralised bone matrix in the bones. Despite the increased bone mass, osteopetrotic bones are very fragile due to abnormal bone composition and structure. Osteopetrosis is caused by the lack or disfunction of osteoclasts. Therefore, it can be cured by bone marrow transplantation.
podoplanin	A 43 kDa syaloglycoprotein originally identified from the podocytes of glomerular membrane but later shown to be expressed by several other cell types incuding lymphatic endothelial cells and tumor cells. Its name comes for extensive flattening of podoplanin-deficient podocytes. Podoplanin is a natural ligand of CLEC2 and induces CLEC2-mediated platelet aggregation.
rhodocytin	A snake venom toxin that triggers platelet activation by binding to the CLEC2 receptor on platelets. Rhodocytin is a disulphide-linked heterodimer reminiscent of certain C-type lectins.
reverse passive Arthus reaction	An antibody-mediated local hypersensitivity reaction triggered by systemic injection of a antigen, followed by local (e. g. subcutaneous) injection of antibody against the given antigen. The local formation of immune complexes results in local edema and inflammation, which is mediated by cell surface Fc-receptors and activation of the complement cascade.
C-type lectins	Carbohydrate-recognizing proteins (lectins) that require Ca^{2+} ions for their proper function. A characteristic feature of their structure is a carbohydrate recognition domain (CRD). C-type lectins include soluble molecules such as the complement-activating

mannose binding lectin, as well as cell surface receptors such as various CLEC proteins (e.g. CLEC7A or CLEC4E).

slow rolling The reduction of leukocyte rolling velocity in an inflammatory environment. Under normal conditions, leukocytes roll on the surface of endothelial cells at a speed of approx. 40 μm/s, primarily determined by the low-level expression of endothelial P-selectins. At the site of inflammation, E-selectins appear at high density on the endothelial surface, leading to slowing down of the rolling cells to approx. 5 μm/s.

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Box 1

Facts and figures on SYK and SYK-related molecules

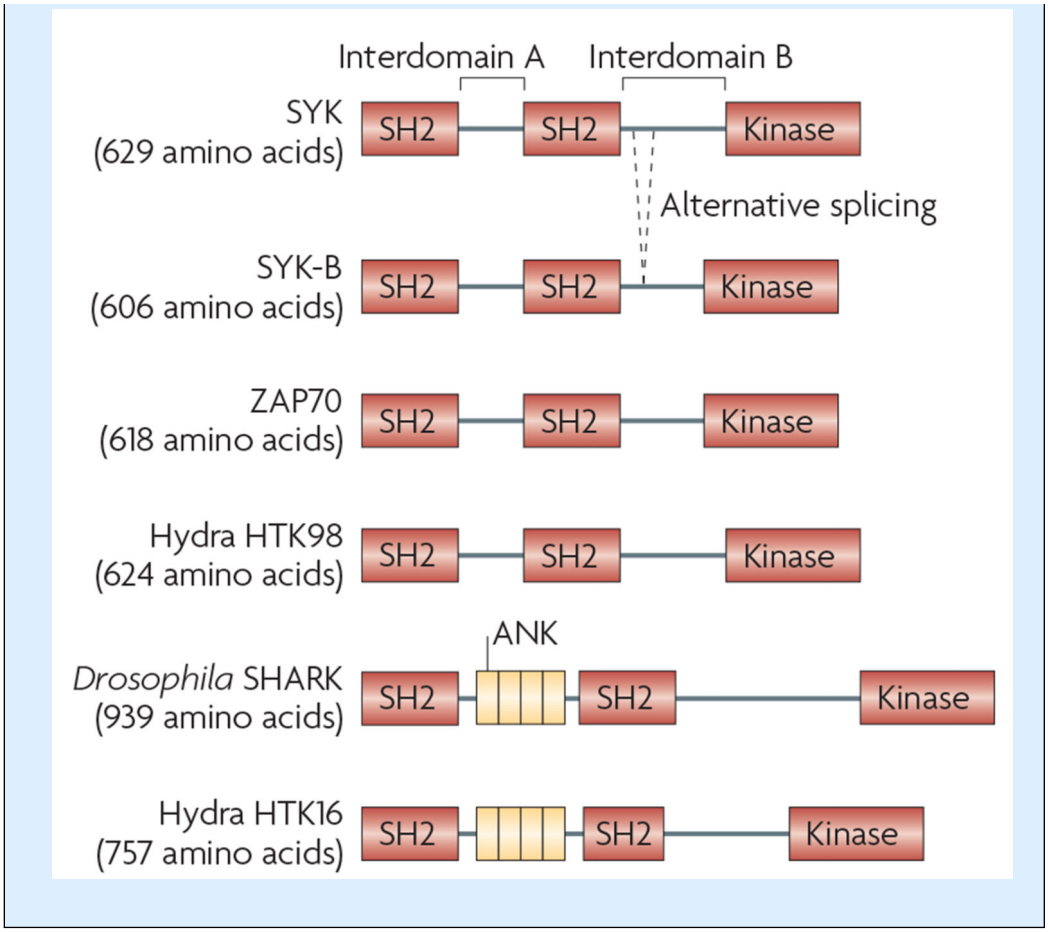
SYK contains two tandem SH2 domains and a C-terminal tyrosine kinase domain. These domains are linked by two linker regions: interdomain A between the two SH2 domains and interdomain B between the C-terminal SH2 domain and the kinase domain. An alternatively spliced form of SYK (known as SYK-B) lacks 23 amino acids of interdomain B, including a nuclear localization signal[9,185].

SYK is highly expressed by all haematopoietic lineage cells. While it was initially thought to be a haematopoietic cell-specific kinase, later studies showed its expression in other tissues, as well[145]. Though the expression of SYK is tightly regulated (see REF. [30]) the mechanism of this regulation, or that of the generation of the SYK-B isoform, is poorly understood.

Mammals also express the SYK-related molecule ZAP70, the expression of which is mostly confined to the T and NK cell lineages. Readers interested in further details of ZAP70 biology are referred to an excellent recent review[36].

SYK-family kinases have an evolutionary ancient origin but they have appeared and disappeared during the evolution of animals[186]. Drosophila express a single SYK-related molecule, SHARK[4], which contains ankyrin-like (ANK) repeats between its two SH2 domains. Interestingly, Hydrae and sponges express two SYK-related kinases, one (HTK98) similar to the vertebrate SYK, and the other (known as HTK16) more closely related to Drosophila SHARK[186,187]. By contrast, the *Caenorhabditis elegans* genome does not contain any SYK-related tyrosine kinases[188].

The *Syk* gene has been disrupted in mice by two independent groups[23,24]. The major phenotypes displayed by SYK-deficient mice are perinatal lethality, a petechiated *in utero* appearance and the lack of mature B cells. Two groups have also reported mutations allowing the Cre-mediated conditional deletion of *Syk*[189,190]. To the best of our knowledge, no human patients or animal strains with a spontaneous germline mutation in *SYK* have been described.



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Box 2

What is the mechanism of action of SYK inhibitors in clinical use?

While SYK inhibitors have shown positive effects in allergy[148], autoimmune diseases[151,152] and B-lineage malignancies[166], the mechanism of their action is incompletely understood. This is in part due to the diverse roles of SYK in immunological functions. As an example, B-cell-mediated antigen presentation and autoantibody formation, Fc-receptor-mediated myeloid cell functions and β2 integrin-mediated leukocyte activation have all been implicated in the pathogenesis of rheumatoid arthritis and they all have been shown to require SYK (see ref. [147] for further details). The rapid effects of R112 treatment in allergic rhinitis[148] and the effectiveness of SYK inhibitors in autoantibody-induced passive models of arthritis[149] and autoimmune thrombocytopenia[152] also suggest that SYK inhibitors target disease components other than BCR-mediated B-cell functions. Likewise, though initial studies suggested that the only role of SYK in B-cell lymphomas is to relay ITAM-mediated tonic BCR signaling, a recent report suggested that SYK also contributes to the survival of B-cell lymphomas through processing BCR-independent adhesive and homing signals[163].

A further complicating issue is that R406, the active metabolite of the R788/fostamatinib prodrug, is an ATP-competitive kinase inhibitor and, similar to most such inhibitors, has rather limited specificity towards SYK. Indeed, R406 has been shown to inhibit a number of other kinases and non-kinase targets at concentrations comparable to those inhibiting SYK[149]. Of those targets, the Flt3, c-Kit, Lck and Jak1/3 tyrosine kinases and the adenosine A3 receptor have been implicated in autoimmune diseases, providing additional or alternative explanations for the clinical effect of R788/fostamatinib in human patients (see ref. [147] for further details).

Taken together, it is reasonable to assume that the clinical effects of R788/fostamatinib are likely mediated by inhibition of a number of SYK-dependent and SYK-independent immune signaling pathways.

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Online summary

- SYK is a nonreceptor tyrosine kinase that has long been thought to exclusively mediate signalling by receptors of the adaptive immune response (BCR and FcR). However, recent studies indicate that it also participates in innate immunity and non-immune functions.
- SYK activation proceeds through binding of its two SH2 domains to ITAM tyrosines phosphorylated by SRC family kinases. Binding to phosphorylated ITAMs relieves SYK from an intramolecular autoinhibition and triggers its association with receptor-proximal signalling molecules.
- SYK mediates integrin signalling in neutrophils, macrophages and platelets, signalling by the PSGL1 selectin ligand, as well as the development of osteoclasts. These responses are mediated by ITAM-containing adaptor molecules (mostly DAP12 and FcRγ).
- SYK participates in innate recognition of fungal and other microbial pathogens, as well as of tissue damage, by C-type lectins. SYK activation by C-type lectins activates the CARD9-BCL10-MALT1 pathway and it is also required for NLRP3 inflammasome activation following fungal infection.
- The phosphorylated ITAM-mediated activation of the SYK homologue Shark is required to remove cellular debris in Drosophila. This finding indicates an ancient origin of the ITAMSYK signalling pathway.
- SYK is required for the separation of newly formed lymphatic vessels from the general vasculature. This function is likely mediated by the SYK-coupled CLEC2 receptor in platelets.
- SYK has a central role in the development of allergic and autoimmune diseases, as well as in haematological malignancies such as B cell lymphomas. A novel SYK inhibitor has shown beneficial effects in human rheumatoid arthritis and B cell lymphomas.

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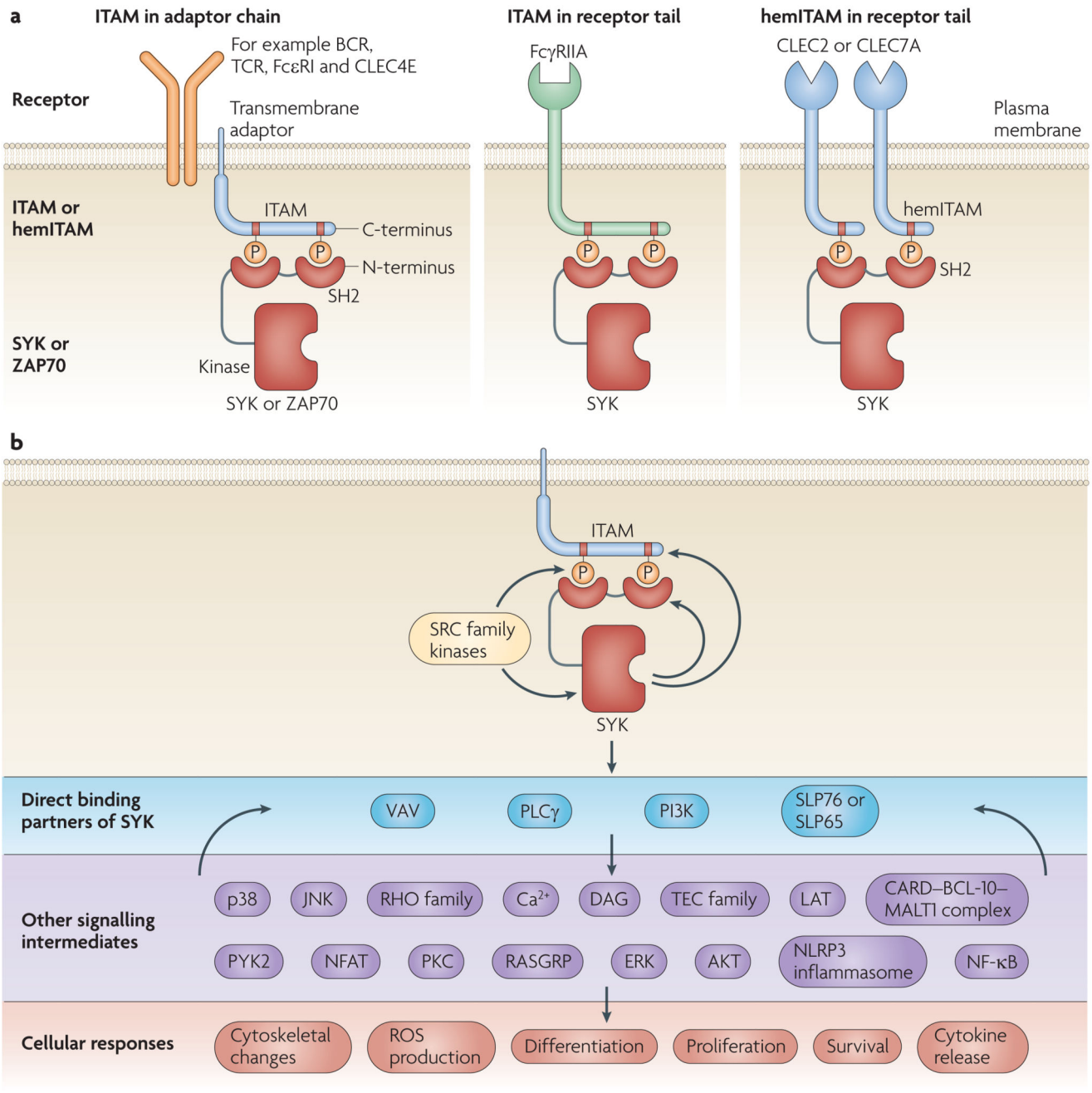

Figure 1. General mechanism of SYK-mediated signalling

a | Recruitment of SYK or ZAP-70 to plasma membrane receptors through binding of the tandem SH2 domains of SYK/ZAP-70 to two phosphotyrosine residues in the receptor complex. The two phosphorylated tyrosines are either within a single ITAM motif or in two hemITAMs on two separate receptor peptide chains. The ITAM motifs are either present in receptor-associated transmembrane adaptors or in the cytoplasmic tail of the receptor chain itself. **b** | General scheme of signal transduction through SYK. The signal transduction is mostly initiated by phosphorylation of ITAM tyrosines by SRC-family kinases. Recruitment

of SYK to dually phosphorylated ITAMs triggers activation of SYK and its direct binding to members of the VAV and PLCγ families, the p85α subunit of PI3-kinases (PI3K), as well as the SLP76/SLP65 adaptors. These direct binding partners activate downstream signaling components which eventually trigger various cellular responses. The SYK-mediated signaling pathways are also regulated by several feedback-mechanisms such as the phosphorylation of ITAM tyrosines by SYK or the regulation of direct SYK binding partners by further downstream molecules. SYK activation by hemITAM-containing receptors likely proceeds through similar mechanisms.

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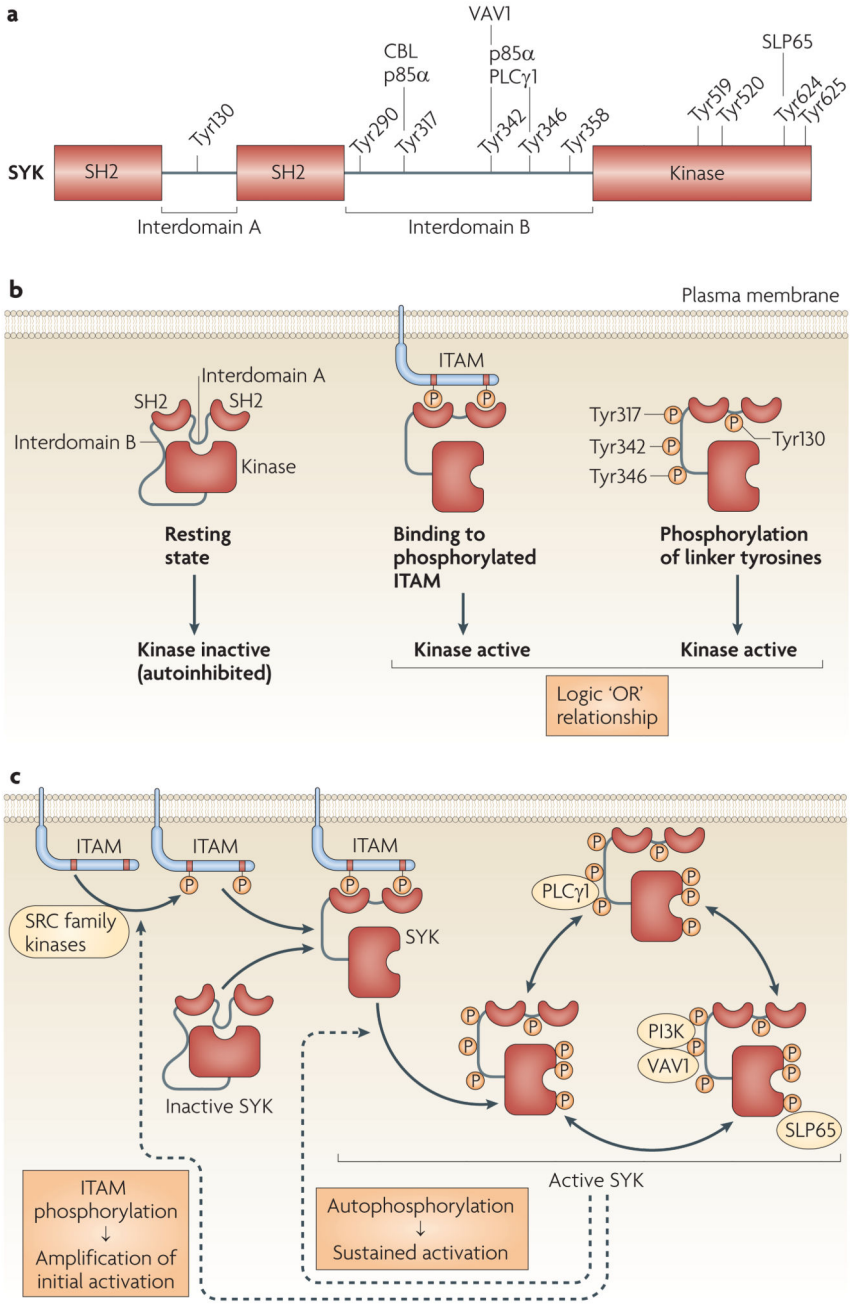

Figure 2. Structural basis of SYK activation

a | Three different states of SYK activation. SYK is autoinhibited in its resting state, due to the binding of interdomain A ("A") and interdomain B ("B") to the kinase domain, in particular, the C-terminal end. This autoinhibited conformation can be suspended by binding of the two SH2 domains to dually phosphorylated ITAMs *or* by phosphorylation of linker tyrosines in interdomain A or B. There is a logic "OR" relationship between those two activation mechanisms. **b** | The domain structure of SYK with tyrosine residues shown to be sites of autophosphorylation. Proteins shown to bind to phosphorylated tyrosines are

indicated above the tyrosine residues. p85α, the 85 kDa PI3K regulatory subunit. **c** | Combined mechanism of SYK activation. Prior to activation, SYK is held in an autoinhibited conformation by interactions between interdomains A and B and the kinase domain. Initial phosphorylation of ITAM tyrosines by SRC-family kinases provides docking sites for the SYK SH2 domains inducing conformational changes and kinase activation. SYK activation results in autophoshorylation on multiple residues, leading to release from the ITAM and sustained SYK activation. SYK then recruits direct binding partners to its phosphorylated tyrosine residues, triggering downstream signaling. Phosphorylation of ITAM tyrosine by SYK provides a positive feedback loop. Amino acid numbers correspond to the sequence of mouse SYK. Phosphorylation of the linker tyrosines are highlighted in orange colour.

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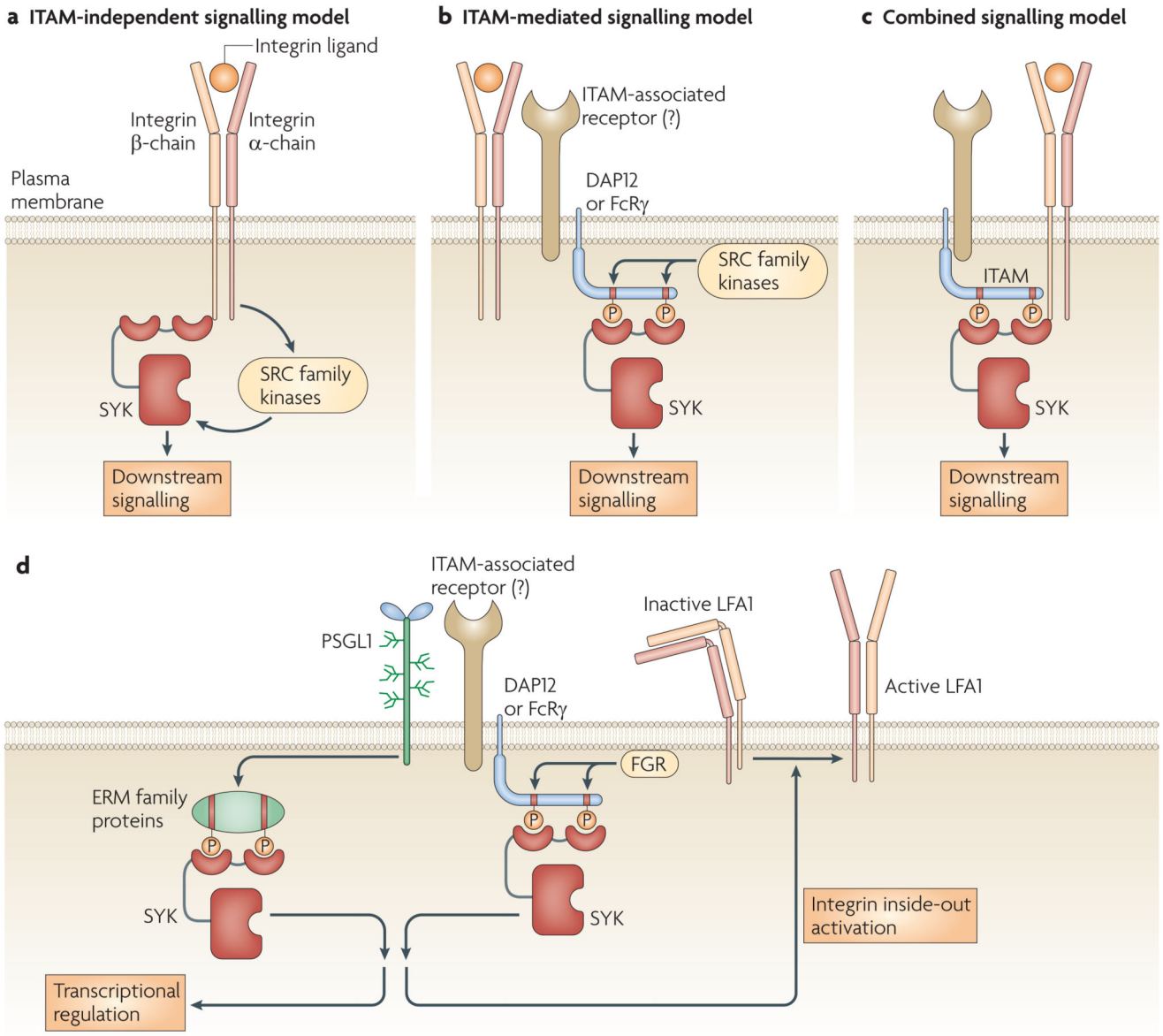

Figure 3. The role of SYK in integrin and PSGL1 signalling

a-c | Mechanism of integrin-mediated SYK activation. **a** | In the original, ITAM-independent model, integrins activate SYK without phosphorylated tyrosine binding to the SYK SH2 domains. The interaction is mediated by the intracellular domain of the integrin β-chain and the non-phosphorylated tyrosine-binding surface of the N-terminal SH2-domain of SYK. **b** | In the ITAM-mediated model, integrin ligation triggers SRC-family-mediated phosphorylation of ITAM-bearing transmembrane adaptor molecules (DAP12 and FcRγ), leading to recruitment of SYK through its tandem SH2 domains. This signalling likely involves DAP12- and/or FcRγ-associated transmembrane receptors. **c** | In the combined model, the non-phosphorylated tyrosine-binding surface of the SYK SH2 domains bind to the C-terminal end of the integrin β-chain while the two SH2 domains bind to the phosphorylated ITAM tyrosines. **d** | SYK-mediated signalling by PSGL1. Ligation of

leukocyte PSGL1 triggers phosphorylation of the ITAM tyrosines of ERM family proteins which recruit SYK via its tandem SH2 domains. A supposedly parallel mechanism under flow conditions leads to phosphorylation of the ITAM tyrosines of the DAP12 and FcRγ transmembrane adaptors by the FGR tyrosine kinase, leading to recruitment of SYK through its tandem SH2 domains. SYK activation triggers transcriptional changes and inside-out activation of LFA1.

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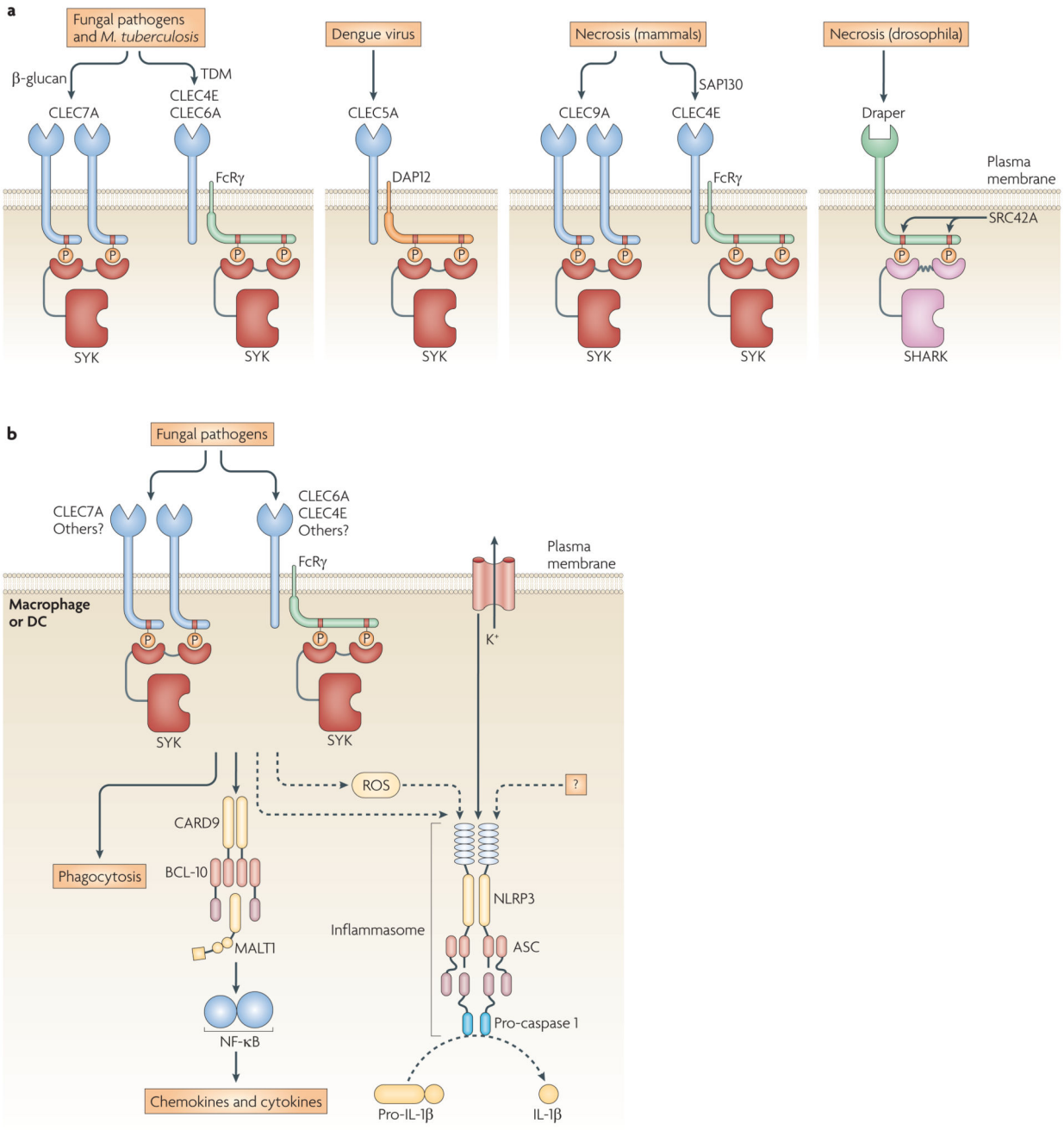

Figure 4. SYK-dependent innate pathogen- and damage-recognition pathways

a | Innate immune receptors coupled to SYK or SYK-related molecules sense foreign pathogens and tissue damage. Fungal pathogens and *M. tuberculosis* engage CLEC7A (Dectin-1), CLEC6A (Dectin-2) and CLEC4E (Mincle); Dengue virus activates CLEC5A; necrotic cells activate the mammalian CLEC9A and CLEC4E, and the Drosophila Draper receptors. The ligand-binding chains of those receptors either have a hemITAM or a complete ITAM, or associate with the ITAM-bearing transmembrane adaptors FcRγ or DAP12. All of these receptors activate SYK-family kinases (SYK or SHARK) by SH2-

mediated recruitment to phosphorylated tyrosines. The kinase responsible for phosphorylation of the Draper ITAM is the Drosophila SRC-family kinase SRC42A. **b** | Mechanism of pro-inflammatory responses triggered by recognition of fungal pathogens by C-type lectins. CLEC7A binds SYK directly through an intracellular hemITAM, whereas CLEC6A and CLEC4E engage SYK through the ITAM-containing FcRγ adaptor. Pathogens trigger SYK-mediated phagocytosis, cytokine and chemokine production, and ROS generation. SYK-mediated NF-κB signalling is controlled by the CARD9–BCL10–MALT1 complex. Additionally, SYK is required for IL-1β processing by the NLRP3 inflammasome, which is activated by SYK-dependent ROS production, potassium ion (K^+) efflux and presumably additional unknown danger signals (“?”).

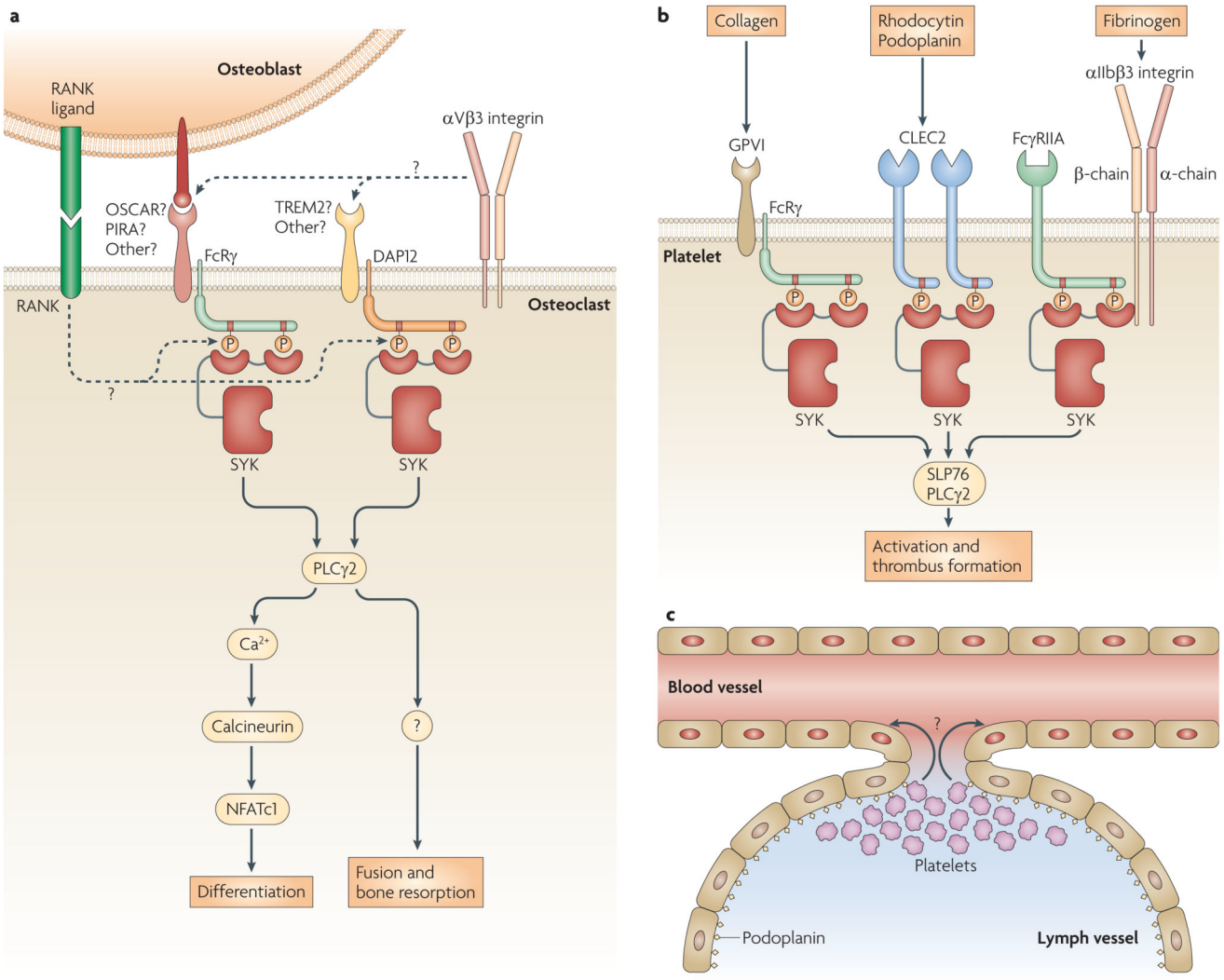

Figure 5. Non-immune functions of SYK

a | The role of DAP12 and FcRγ in osteoclasts. In addition to a RANK–RANK ligand interaction, osteoblasts also express yet unidentified ligands that engage a putative FcR-associated receptor (most likely OSCAR or PIR-A) on the osteoclast surface. Osteoclasts also express putative DAP12-associated receptors (most likely TREM2) which bind to yet unidentified ligands that are not expressed by osteoblasts. Both FcRγ and DAP12 promote osteoclast development and function through ITAM-mediated SYK activation and the PLCγ2-Calcineurin-NFATc1 pathway. Inset: Mechanism of osteoclast development. Osteoclasts develop from myeloid progenitors re-programmed by osteoblast-derived RANK ligand, leading to biochemical differentiation. Pre-osteoclasts then fuse to generate mature multi-nucleated osteoclasts and resorb the bone surface. αVβ3 integrins are required for sealing the resorption area. **b** | SYK-mediated signaling in platelets. Collagen activates the FcRγ-associated GpVI receptor on platelets and triggers Syk activation by an ITAM-mediated manner. The snake venom rhodocytin, the endogenous podoplanin and possible other platelet agonists activate the CLEC2 receptor which recruits SYK to phosphorylated

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tyrosine in the CLEC2 hemITAMs at a 2:1 stoichiometry. Fibrinogen engages the αIIbβ3 integrin which associates with the ITAM-containing FcγRIIA receptor. This complex likely activates SYK through both conventional ITAM-mediated SYK activation pathways and by binding of the integrin β-chain to the N-terminal SH2-domain of SYK in a phosphotyrosine-independent manner. All three cases of SYK activation lead to platelet activation through PLCγ2. **c** | The role of SYK in separation of blood and lymphatic vessels. Lymphatic vessels express podoplanin which trigger platelet activation and aggregation. This and possible other mechanisms ("?") lead to closing off any blood-lymphatic shunts. The mechanism of podoplanin-mediated platelet activation involves the CLEC2 receptor which activates SYK through hemITAM phosphorylation, as well as SLP76 and PLCγ2 acting downstream of SYK.

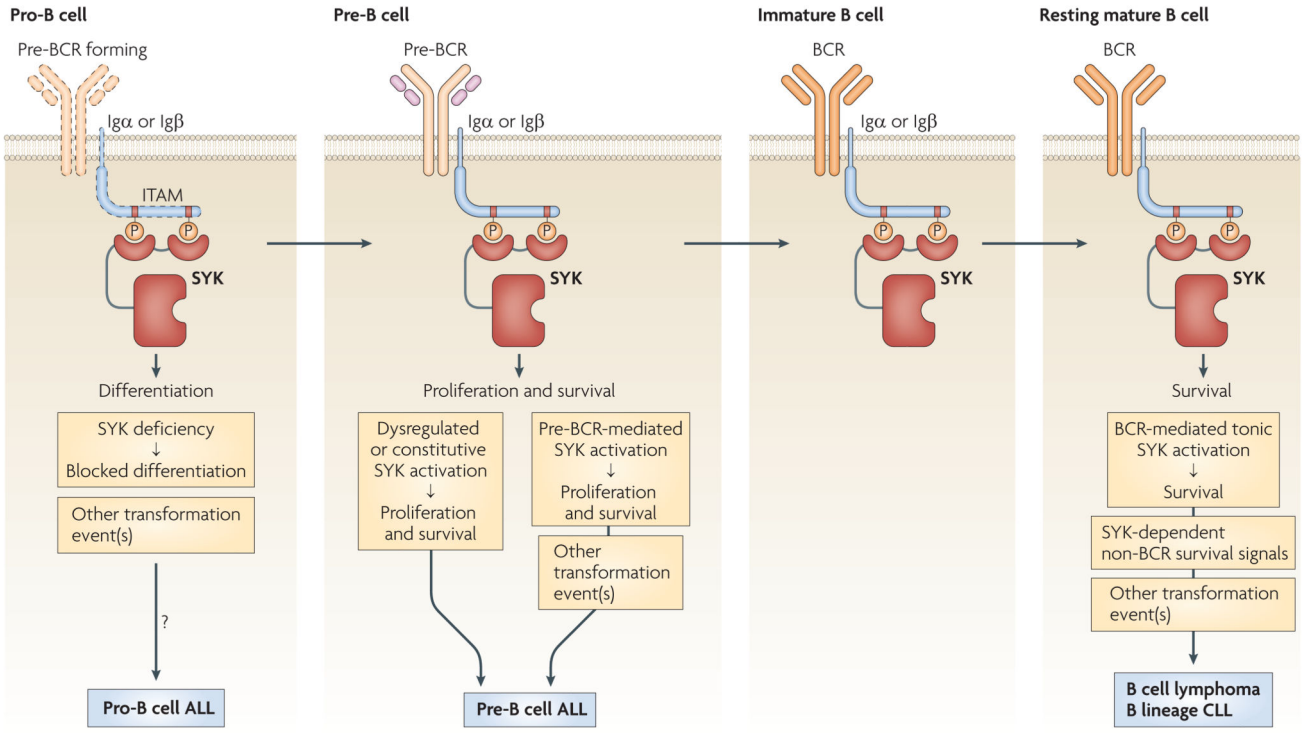

Figure 6. The Role of SYK in malignancies of the B-cell lineage

SYK is required for various processes of B-cell development, including transition from pro-B to pre-B cells, proliferation and survival of pre-B cells, as well as the survival of mature B-cells. When other transforming factors are present, loss of SYK in pro-B cells may contribute to development of pro-B cell ALL, possibly by blocking further differentiation. Deregulated or constitutive activation of SYK is able to transform pre-B cells to pre-B cell ALL. In addition, pre-BCR-induced SYK activation is required for transformation of pre-B cells to pre-B cell ALL by other transforming factors (e. g. c-MYC). BCR-mediated tonic activation of SYK, as well as other SYK-dependent but BCR-independent signals are required for survival of mature B-cell lymphomas and B-lineage CLLs.

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