GPVI-mediated activation cascade

Akkerman, JW., Annibali, D., Greene, LA., Harper, MT., Jones, ML., Jupe, S., Kunapuli, SP., Matthews, L., Nasi, S., Orlic-Milacic, M., Poole, AW., Thorpe, L., Yuzugullu, H., Zhao, JJ.

European Bioinformatics Institute, New York University Langone Medical Center, Ontario Institute for Cancer Research, Oregon Health and Science University.

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This is just an excerpt of a full-length report for this pathway. To access the complete report, please download it at the Reactome Textbook.

16/11/2022
Introduction

Reactome is open-source, open access, manually curated and peer-reviewed pathway database. Pathway annotations are authored by expert biologists, in collaboration with Reactome editorial staff and cross-referenced to many bioinformatics databases. A system of evidence tracking ensures that all assertions are backed up by the primary literature. Reactome is used by clinicians, geneticists, genomics researchers, and molecular biologists to interpret the results of high-throughput experimental studies, by bioinformaticians seeking to develop novel algorithms for mining knowledge from genomic studies, and by systems biologists building predictive models of normal and disease variant pathways.

The development of Reactome is supported by grants from the US National Institutes of Health (P41 HG003751), University of Toronto (CFREF Medicine by Design), European Union (EU STRP, EMI-CD), and the European Molecular Biology Laboratory (EBI Industry program).

Literature references


Reactome database release: 82

This document contains 1 pathway and 25 reactions (see Table of Contents)
The GPVI receptor is a complex of the GPVI protein with Fc epsilon R1 gamma (FcR). The Src family kinases Fyn and Lyn constitutively associate with the GPVI-FcR complex in platelets and initiate platelet activation through phosphorylation of the immunoreceptor tyrosine-based activation motif (ITAM) in the FcR gamma chain, leading to binding and activation of the tyrosine kinase Syk. Downstream of Syk, a series of adapter molecules and effectors lead to platelet activation.

The GPVI receptor signaling cascade is similar to that of T- and B-cell immune receptors, involving the formation of a signalosome composed of adapter and effector proteins. At the core of the T-cell receptor signalosome is the transmembrane adapter LAT and two cytosolic adapters SLP-76 and Gads. While LAT is essential for signalling to PLCgamma1 downstream of the T-cell receptor, the absence of LAT in platelets only impairs the activation of PLCgamma2, the response to collagen and GPVI receptor ligands remains sufficient to elicit a full aggregation response. In contrast, GPVI signalling is almost entirely abolished in the absence of SLP-76.

**Literature references**


**Editions**

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**Fyn/Lyn-mediated phosphorylation of FcR1 gamma**

**Location:** GPVI-mediated activation cascade

**Stable identifier:** R-HSA-114600

**Type:** transition

**Compartments:** plasma membrane, cytosol

At the beginning of this reaction, 1 molecule of 'GP VI:Fc Epsilon R1 gamma:Collagen IV complex', and 1 molecule of 'ATP' are present. At the end of this reaction, 1 molecule of 'ADP', and 1 molecule of 'GP VI:phosphorylated Fc Epsilon R1 gamma:Collagen IV complex' are present.

This reaction is mediated by the 'protein-tyrosine kinase activity' of 'GP VI: Fc Epsilon R1 gamma: Collagen IV: SRC'.

**Followed by:** Binding of Syk tyrosine kinase

**Literature references**

Binding of Syk tyrosine kinase

Location: GPVI-mediated activation cascade

Stable identifier: R-HSA-139842

Type: binding

Compartments: plasma membrane, cytosol

Syk binds to the phosphorylated ITAM motif of Fc epsilon R1 gamma chain, each SH2 domain binding a phosphorylated tyrosine. Unlike Zap70, Syk appears to autophosphorylate, so does not require Src family kinases for activation.

Preceded by: Fyn/Lyn-mediated phosphorylation of FcR1 gamma

Followed by: GPVI stimulates PI3K beta, gamma, Syk autophosphorylates
**SYK autophosphorylates ➔**

**Location:** GPVI-mediated activation cascade

**Stable identifier:** R-HSA-453200

**Type:** transition

**Compartments:** plasma membrane, cytosol

Binding of Syk causes conformational changes that lead to Syk activation by autophosphorylation. Syk can be activated by a number of phosphorylation events, and it has been proposed that Syk may function as a switch whereby any of several possible stimuli trigger the acquisition of similar activated conformations. (Tsang et al. 2008). These phosphorylations both modulate Syk's catalytic activity (Keshvara et al. 1997) and generate docking sites for SH2 domain-containing proteins, such as c-Cbl, PLC, and Vav1.

Syk tyrosine phosphorylation is reduced in the presence of the ITIM-containing immunoglobulin superfamily transmembrane protein G6B (Mori et al. 2008).

**Preceded by:** Binding of Syk tyrosine kinase

**Followed by:** p-Y348-SYK dissociates

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https://reactome.org
p-Y348-SYK dissociates

**Location:** GPVI-mediated activation cascade

**Stable identifier:** R-HSA-453183

**Type:** dissociation

**Compartments:** plasma membrane, cytosol

Structural and biophysical studies indicate that the adaptability of the Syk tandem SH2 domains is made possible by relatively weak interactions between the two SH2 domains and the flexibility of interdomain A (Zhang et al. 2008).

A large proportion of phosphorylated Syk is released into the cytosol. One factor that has been proposed for modulating the interactions of Syk with the receptor ITAM is the phosphorylation of Syk on Y130 (Keshvara et al. 1997).

**Preceded by:** SYK autophosphorylates

**Followed by:** Syk/Lck phosphorylate LAT, p-Y348-SYK binds VAV family, Syk activation leads to SLP-76 activation

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p-Y348-SYK binds VAV family

Location: GPVI-mediated activation cascade

Stable identifier: R-HSA-437932

Type: binding

Compartments: cytosol

Inferred from: Pig Syk binds human Vav1 (Sus scrofa), Syk binds Vav2 (Mus musculus)

The SH2 region of Vav1 binds to Syk at a site including phosphorylated tyrosine Y348. Mutation of this residue to F abolishes binding and subsequent Vav1 phosphorylation. Vav2 has also been shown to bind Syk.

Preceded by: p-Y348-SYK dissociates

Followed by: p-Y348-SYK phosphorylates VAV family

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https://reactome.org
Tyrosine phosphorylation is believed to be a general activation mechanism for the Vav family. VAV1 Tyr-174 binds to the Dbl homology region, inhibiting GEF activity. Phosphorylation of this residue by Syk relieves inhibition, activating Vav1. In Jurkat cells T-cell receptor activation leads to increased Vav2 tyrosine phosphorylation; the expression of Lck, Fyn, Zap70, or Syk stimulated this phosphorylation.

Vav is regulated downstream of the thrombin and thrombopoietin receptors (Miyakawa et al. 1997) and integrins, including the major platelet integrin alphaIIbbeta3. Vav family proteins are involved in filopodia and lamellipodia formation; mouse platelets deficient in Vav1 and Vav3 exhibit reduced filopodia and lamellipodia formation during spreading on fibrinogen. This is accompanied by reduced alphaIIbbeta3-mediated PLCgamma2 tyrosine phosphorylation and reduced Ca(2+) mobilization (Pearce et al. 2007).

Preceded by: p-Y348-SYK binds VAV family

Followed by: VAV1 is a GEF for Rho/Rac family GTPases, PIP2 binds inhibiting VAV, PI(3,4,5)P3 binds VAV1,2,3, VAV3 is a GEF for Rho/Rac family kinases, VAV2 is a GEF for Rho/Rac family kinases

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VAV1 is a GEF for Rho/Rac family GTPases

Location: GPVI-mediated activation cascade

Stable identifier: R-HSA-442273

Type: transition

Compartments: cytosol

Vav family members are guanine nucleotide exchange factors (GEFs) for Rho-family GTPases. Vav1 is a GEF for Rac1, Rac2 and RhoG, and possibly RhoA and Cdc42

Preceded by: PI(3,4,5)P3 binds VAV1,2,3, p-Y348-SYK phosphorylates VAV family

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VAV2 is a GEF for Rho/Rac family kinases

Location: GPVI-mediated activation cascade

Stable identifier: R-HSA-442291

Type: transition

Compartments: cytosol

Members of the Vav family are guanine nucleotide exchange factors (GEFs) for Rho-family GTPases. Vav2 is a GEF for RhoA, RhoB and RhoG, and possibly Rac1 and Cdc42

Preceded by: p-Y348-SYK phosphorylates VAV family

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VAV3 is a GEF for Rho/Rac family kinases

Location: GPVI-mediated activation cascade

Stable identifier: R-HSA-442314

Type: transition

Compartments: cytosol

Vav3 is a guanine nucleotide exchange factors (GEF) for RhoA, RhoB and to a lesser extent Rac1.

Preceded by: p-Y348-SYK phosphorylates VAV family

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Syk/Lck phosphorylate LAT

**Location:** GPVI-mediated activation cascade

**Stable identifier:** R-HSA-434836

**Type:** transition

**Compartments:** plasma membrane, cytosol

Activated Syk (or possibly the related kinase Lck) phosphorylates two key tyrosine residues of LAT.

**Preceded by:** p-Y348-SYK dissociates

**Literature references**


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Stimulation of platelets with collagen-related peptide leads to tyrosine phosphorylation of SLP-76, an adaptor protein with multiple binding domains (Gross et al. 1999). Phosphorylation of SLP-76 is mediated by Syk, analogous to the role of ZAP-70 in phosphorylating T-cell SLP-76 (Bubeck-Wardenberg et al. 1996, Hussain et al. 1999, Fasbender et al. 2017). SLP-76 was shown to bind to tyrosine-phosphorylated C-terminal tail of SYK (de Castro et al. 2012). The phosphorylated tyrosine residues provide a binding site for the SH2 domains of downstream signalling proteins like Vav, Itk and ADAP (Jordan et al. 2003). Platelets from mice defective in SLP76 do not connect GPVI engagement with downstream signaling (Clements et al. 1999, Judd et al. 2000). GPVI signaling via SLP-76 does not appear to require LAT or GADS (Judd et al. 2002) suggesting that the mechanism is not identical to that of T-cells. LAT and SLP-76 are both required for P-selectin expression and degranulation but may function independently, or rely on proteins not required by T-cells (Jordan et al. 2003).

**Preceded by:** p-Y348-SYK dissociates

**Followed by:** p-SLP-76 binds VAV, SLP-76 stimulates PLC gamma 2

**Literature references**


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SLP-76 stimulates PLC gamma 2

Location: GPVI-mediated activation cascade

Stable identifier: R-HSA-429497

Type: omitted

Compartments: plasma membrane

SLP-76 has a well-established role in recruitment of PLC gamma 1 in immunoreceptor signalling; its role in the recruitment of PLC gamma 2 in integrin signalling is less clear. Results from SLP-76 null mice imply a functional role in GPVI signalling. Platelets from SLP-76 null mice exhibit a marked reduction in spreading and a decrease in whole cell phosphotyrosine levels when adhered to a fibrinogen-coated surface. In vivo reconstitution of SLP-76 by retroviral gene transfer corrects bleeding diathesis and restores normal responses to both collagen and fibrinogen (Judd et al., 2000).

Preceded by: Syk activation leads to SLP-76 activation

Followed by: PLC gamma 2-mediated PIP2 hydrolysis

Literature references


Editions

2009-06-03 Authored Akkerman, JW.
2009-11-02 Reviewed Poole, AW., Jones, ML., Harper, MT.
2009-11-03 Edited Jupe, S.
p-SLP-76 binds VAV

Location: GPVI-mediated activation cascade

Stable identifier: R-HSA-430158

Type: binding

Compartments: cytosol

SLP-76 is a hematopoietic cell-specific adapter protein. Studies indicate that three phosphotyrosines in SLP-76 (Y113, Y128, and Y145) are required for interactions with the SH2 domains of Vav1 (and Nck and Itk). This interaction is essential for membrane recruitment of Vav1. Similarly, association of Vav3 with SLP-76 was found to be essential for membrane recruitment. Vav2 has been shown to interact with SLP-76 in resting Jurkat cells.

Preceded by: Syk activation leads to SLP-76 activation

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**PLC gamma 2-mediated PIP2 hydrolysis**

**Location:** GPVI-mediated activation cascade

**Stable identifier:** R-HSA-114689

**Type:** transition

**Compartments:** plasma membrane, cytosol

At the beginning of this reaction, 1 molecule of '1-Phosphatidyl-D-myo-inositol 4,5-bisphosphate' is present. At the end of this reaction, 1 molecule of '1D-myo-Inositol 1,4,5-trisphosphate', and 1 molecule of '1,2-Diacylglycerol' are present.

This reaction is mediated by the 'phospholipase C activity' of 'Phosphorylated phospholipase C gamma 2'.

**Preceded by:** SLP-76 stimulates PLC gamma 2

**Literature references**


**Editions**

2009-09-09 Edited Jupe, S.
PI(3,4,5)P3 binds VAV1,2,3

Location: GPVI-mediated activation cascade

Stable identifier: R-HSA-434637

Type: binding

Compartments: plasma membrane, cytosol

Inferred from: PIP3 stimulates Vav1 (Mus musculus)

Vav interacts directly with PIP2 and PIP3, with a fivefold selectivity for PIP3 over PIP2. PIP3 gives a two-fold stimulation of Vav1 GEF activity while PIP2 leads to 90% inhibition. Binding probably occurs through the PH domain, known to bind phosphoinositides.

Preceded by: PI3K alpha, beta, gamma convert PIP2 to PIP3, p-Y348-SYK phosphorylates VAV family

Followed by: VAV1 is a GEF for Rho/Rac family GTPases

Literature references

**PIP2 binds inhibiting VAV**

**Location:** GPVI-mediated activation cascade

**Stable identifier:** R-HSA-434633

**Type:** binding

**Compartments:** plasma membrane, cytosol

**Inferred from:** PIP2 inhibits Vav1 (Mus musculus)

Vav interacts directly with PIP2 and PIP3, with a fivefold selectivity for PIP3 over PIP2. PIP3 gives a two-fold stimulation of Vav1 GEF activity while PIP2 leads to 90% inhibition. Binding probably occurs through the PH domain, known to bind phosphoinositides.

**Preceded by:** PI3K alpha, beta, gamma convert PIP2 to PIP3, p-Y348-SYK phosphorylates VAV family

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GPVI downstream signaling involves PI3K. Mouse knockouts of PI3Kbeta/PI3Kgamma suggest that though both isoforms are required for a full platelet response, only beta is absolutely required for Akt phosphorylation, Rap1 activation, and platelet aggregation downstream. The pathway connecting GPVI to PI3K is unclear. Two possible routes are suggested by interactions of the PI3K p85 regulatory subunit with LAT and with peptides representing the ITAM motif of Fc Epsilon R1 gamma.

**Preceded by:** Binding of Syk tyrosine kinase

**Followed by:** PI3K alpha, beta, gamma convert PIP2 to PIP3

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PI3K alpha, beta, gamma convert PIP2 to PIP3

Location: GPVI-mediated activation cascade

Stable identifier: R-HSA-437162

Type: transition

Compartments: plasma membrane, cytosol

Class I Phosphoinositide 3-kinases (PI3Ks) are heterodimeric proteins, each having a catalytic subunit of 110-120 kDa and an associated regulatory subunit. PI3Ks alpha, beta and delta share a common regulatory p85 subunit, PI3K gamma has a p101 regulatory subunit. All the class I PI3Ks are able to phosphorylate PtdIns, PtdIns-4-P, or PtdIns-4,5-P2 (PIP2) on the free 3-position, and have a strong preference for PIP2. They are activated by receptor tyrosine kinases and by Ras and Rho family GTPases.

Preceded by: GPVI stimulates PI3K beta, gamma

Followed by: PIP2 binds inhibiting VAV, PI(3,4,5)P3 binds VAV1,2,3, PIP3 recruits PDPK1 to the membrane

Literature references


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PIP3 recruits PDPK1 to the membrane

**Location:** GPVI-mediated activation cascade

**Stable identifier:** R-HSA-2316429

**Type:** binding

**Compartments:** plasma membrane, cytosol

PIP3 generated by PI3K recruits phosphatidylinositide-dependent protein kinase 1 (PDPK1 i.e. PDK1) to the membrane, through its PH (pleckstrin-homology) domain. PDPK1 binds PIP3 with high affinity, and also shows low affinity for PIP2 (Currie et al. 1999).

**Preceded by:** PI3K alpha, beta, gamma convert PIP2 to PIP3

**Followed by:** PDPK1 binds PRKCZ

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PDPK1 binds PRKCZ

**Location:** GPVI-mediated activation cascade

**Stable identifier:** R-HSA-437192

**Type:** binding

**Compartments:** plasma membrane, cytosol

**Inferred from:** PDK1 binds PKC zeta (Rattus norvegicus)

3-phosphoinositide dependent protein kinase-1 (PDPK1, also known as PDK1) and Protein kinase C zeta type (PRKCZ, also known as PKC zeta) are associated in fibroblasts.

**Preceded by:** PIP3 recruits PDPK1 to the membrane

**Followed by:** PDPK1 activates PRKCZ

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PDPK1 activates PRKCZ

**Location:** GPVI-mediated activation cascade

**Stable identifier:** R-HSA-437195

**Type:** transition

**Compartments:** plasma membrane, cytosol

**Inferred from:** PDK1 activates PKC zeta (Rattus norvegicus)

3-phosphoinositide dependent protein kinase-1 (Pdpk1, also known as Pdk1 and PKB kinase because of its activity at Protein kinase B) phosphorylates T410 of protein kinase C zeta type (Prkcz, also known as PKC zeta), leading to activation. The motif surrounding T410 is highly conserved in other PKC family members suggesting that Pdpk1 might activate other PKCs.

**Preceded by:** PDPK1 binds PRKCZ

**Literature references**


**Editions**

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<td>Poole, AW., Jones, ML., Harper, MT.</td>
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G6B binds PTPN6, PTPN11

**Location:** GPVI-mediated activation cascade

**Stable identifier:** R-HSA-5684169

**Type:** binding

**Compartments:** plasma membrane, cytosol

G6B is a member of the immunoglobulin superfamily. The G6B-B variant is the only variant to contain both a transmembrane region and two immunoreceptor tyrosine-based inhibitory motifs (ITIMs) that support binding to the SH2 domain-containing protein tyrosine phosphatases PTPN6 (SHP1) and PTPN11 (SHP2) (de Vet et al. 2001, Senis et al. 2007). ITIMs are defined by the consensus sequence (L/I/V/S)-X-Y-X-X-(L/V) and are commonly present in pairs separated by 15 to 30 amino acid residues. ITIM-containing receptors were originally identified by their ability to inhibit signaling by ITAM receptors (Bijsterbosch & Klaus 1985). Expression of the GPVI-FcR gamma-chain complex or C-type lectin domain family 1 member B (CLEC1B, CLEC2) in DT40 (chicken) B cells leads to the generation of both constitutive and agonist-induced signals that are inhibited by G6B. This effect is dependent on the two ITIMs in the cytosolic tail of G6B, but is reported to be independent of the two SH2 domain-containing tyrosine phosphatases PTPN6 and PTPN11, and the inositol lipid 5'-phosphatase SHIP1 (Mori et al. 2008). A more recent study (Coxon et al. 2011) found that other SH2 domain-containing proteins including SYK and PLCgamma2 also recognize G6B phosphomotifs, which may explain why G6B remains inhibitory in the absence of both PTPN6 and PTPN11.

The tandem SH2 domains of PTPN11 have a 100-fold higher binding affinity for G6B than that of PTPN6. PTPN6 has an absolute binding requirement for phosphorylation at both ITAM motifs, while PTPN11 can associate with G6B when only one motif is phosphorylated. The presence of dual phosphorylated G6B in washed human platelets reduced the EC(50) for both CRP and collagen-induced aggregation (Coxon et al. 2011). G6B is proposed to inhibit sustained constitutive signaling from GPVI-FcRgamma and CLEC1B (Mori et al. 2008).

**Literature references**


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C-type lectin domain family 1 member B (CLEC1B, CLEC2) is a 32-kDa C-type lectin-like receptor that dimerizes to form the platelet receptor for the snake venom toxin rhodocytin and the endogenous lymphatic endothelial marker, podoplanin (PDPN) (Suzuki-Inoue et al. 2006, 2007, Christou et al. 2008, Watson et al. 2009). PDPN is a sialomucin-like glycoprotein with a wide tissue distribution. It is found at a high level in lung type I alveolar cells, kidney podocytes, choroid plexus epithelium, lymphatic endothelial cells and fibroblastic reticular cells within secondary lymphoid organs. PDPN is not found on vascular endothelial cells. It is up-regulated in a variety of tumors and on macrophages following lipopolysaccharide stimulation. Cells expressing PDPN or recombinant forms of its extracellular domain have been shown to induce platelet activation (Pollitt et al. 2014).

Followed by: Unknown kinase phosphorylates CLEC1B dimer:PDPN

Literature references


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Following stimulation by rhodocytin CLEC1B is phosphorylated on the YxxL or hemi-ITAM motif. The kinase responsible for this is not clear. Phosphorylation is suggested to allow the tandem SH2 domains of SYK to bind phosphorylated CLEC1B hemi-ITAM sites (Suzuki-Inoue et al. 2006). GPVI ITAMs are phosphorylated by the Src family kinases FYN and LYN, which results in SYK binding, but CLEC1B appears to be phosphorylated mainly by SYK. The SYK-specific inhibitor R406 inhibits CLEC1B phosphorylation in response to rhodocytin, suggesting SYK is responsible for hemi-ITAM phosphorylation in human platelets (Spalton et al. 2009). However the Src family-specific kinase inhibitor PP2 also inhibits CLEC1B tyrosine phosphorylation (Suzuki-Inoue et al. 2006), suggesting that CLEC1B is phosphorylated by Syk and Src family kinases in human platelets (Suzuki-Inoue et al. 2006, Suzuki-Inoue 2011). Severin et al. (2011) reported that phosphorylation of CLEC1B by rhodocytin is abolished in Syk-deficient mice, while phosphorylation is not altered in mice deficient in the major platelet Src family kinases Fyn, Lyn, Src, or the tyrosine phosphatase CD148, which regulates the basal activity of Src family kinases. The same group also reported that PP2 does not inhibit phosphorylation of mouse Clec1b by rhodocytin, in contrast to the reported effect in human platelets (Suzuki-Inoue et al. 2006), suggesting that Syk phosphorylates Clec1b independently of the Src family kinases in mice.

**Preceded by:** CLEC1B dimer binds PDPN

**Followed by:** p-Y7-CLEC1B dimer:PDPN binds SYK

**Literature references**

Following the phosphorylation of CLEC1B on its hemi-ITAM motif it can bind the kinase SYK (Suzuki-Inoue et al. 2006, 2007, Spalton et al. 2009, Severin et al. 2011). Beyond SYK, CLEC1B signalling is similar to that of GPVI:Fcr1 gamma. Murine platelets deficient in Syk or PLC gamma 2 fail to respond to rhodocytin, suggesting they are crucial for Clec1b signal transduction. Mice deficient in the adaptor proteins Linker for activation of T-cells family member 1 (LAT), LCP2 (SLP-76) or the guanine nucleotide exchange factors Vav1-3 are able to respond to high concentrations of rhodocytin, suggesting that these molecules participate in Clec1b signaling but do not prevent signaling when absent (Suzuki-Inoue et al. 2006, Finney et al. 2011).

Clec1b signaling is reduced in the presence of the ITIM-containing immunoglobulin superfamily transmembrane protein G6B (Mori et al. 2008). G6B is thought to act by reducing Syk tyrosine phosphorylation (Mori et al. 2008) but it is possible that the target of inhibition is elsewhere in the CLEC1B signaling cascade.

**Preceded by:** Unknown kinase phosphorylates CLEC1B dimer:PDPN

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