Received: 25 June 2020 | Revised: 17 January 2021 | Accepted: 19 January 2021

DOI: 10.1002/rth2.12495

rpth research & practice in thrombosis & haemostasis

ILLUSTRATED REVIEW

Platelet Src family kinases: A tale of reversible phosphorylation

Yotis A. Senis PhD, MSc[1] | Zoltan Nagy PhD, MSc[2] | Jun Mori PhD, DDS[3] | Sophia Lane[4] | Patrick Lane[4]

[1]Unité Mixte de Recherche-S 1255, Fédération de Médecine Translationnelle de Strasbourg, Université de Strasbourg, Institut National de la Santé et de la Recherche Médicale, Etablissement Français du Sang Grand Est, Strasbourg, France

[2]Institute of Experimental Biomedicine, University Hospital and Rudolf Virchow Center, University of Würzburg, Würzburg, Germany

[3]Research and Development, Align Technology Inc., Yokohama, Japan

[4]Illustration and Design, ScEYEnce Studios, Elkins Park, PA, USA

Correspondence
Yotis A. Senis, Etablissement Français du Sang Grand Est, Inserm UMR-S1255, 10 Rue Spielmann, 67065 Strasbourg, France.
Email: yotis.senis@efs.sante.fr

Funding information
Funding was provided by BHF, Inserm and EFS (YAS). Additional sources of funding can be found in Acknowledgements.

Handling Editor: Dr Alisa Wolberg.

Abstract

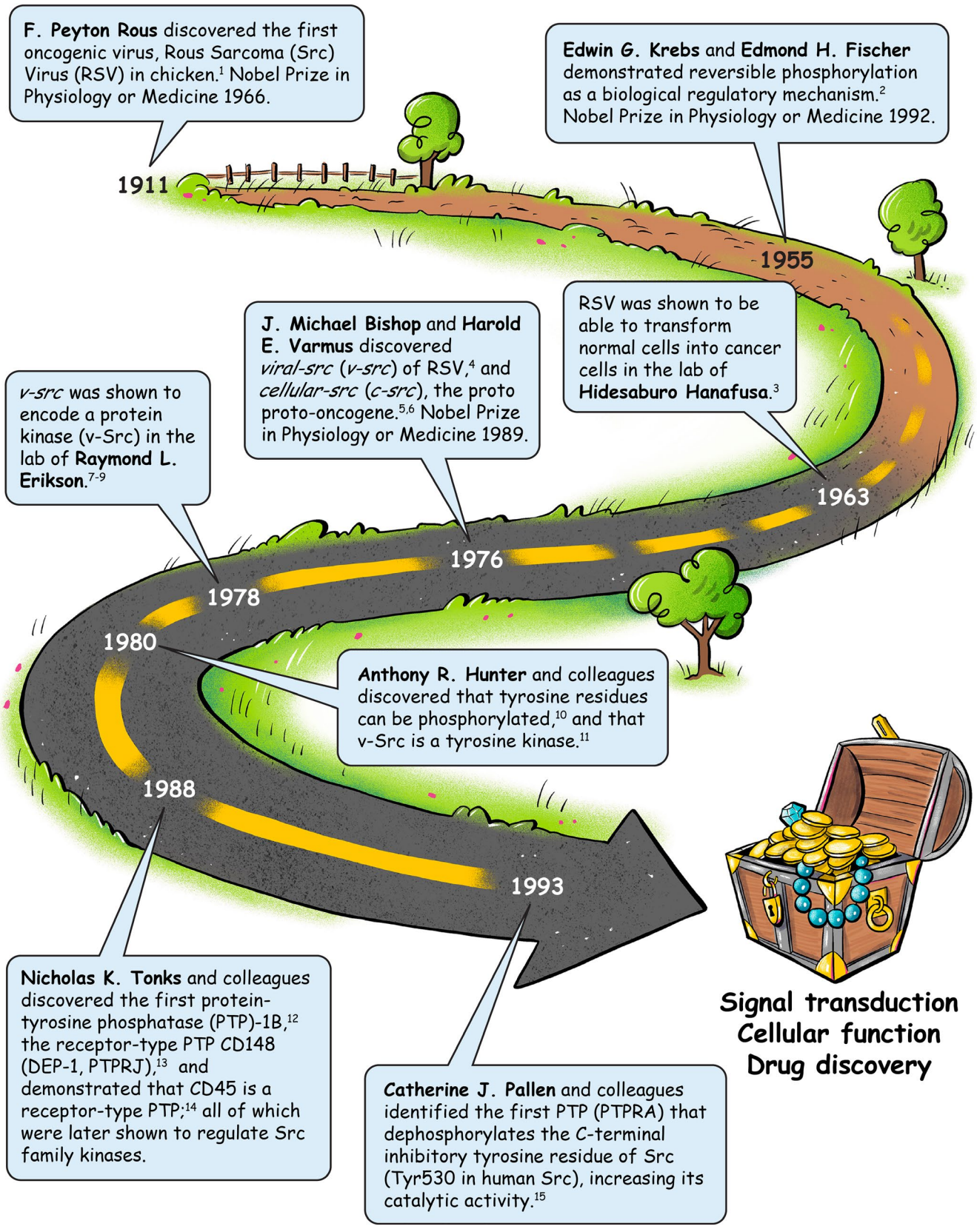
Sarcoma (Src) family kinases (SFKs) have occupied a central place in platelet research for over 40 years. Discovered by virologists and oncologists as the proto *proto-oncogene*, Src tyrosine kinase spurred a phenomenal burst of research on reversible tyrosine phosphorylation and signal transduction. For a time, platelets were adopted as the model of choice for studying the biological functions of Src, owing to their ease of isolation, high Src expression, and lack of a nucleus, only to be abandoned due to challenges of culturing and manipulating using common molecular biology-based techniques. For platelet biologists, SFKs have remained an important area of investigation, initiating and amplifying signals from all major adhesion, activation, and inhibitory receptors, including the integrin αIIbβ3, the collagen receptor complex glycoprotein VI–Fc receptor γ-chain, the G protein–coupled ADP receptor $P2Y_{12}$ and the inhibitory receptors platelet endothelial cell adhesion molecule-1 and G6b-B. The vital roles of SFKs in platelets is highlighted by the severe phenotypes of *null* and *gain-of-function* mutations in SFKs in mice and humans, and effects of pharmacologic inhibitors on platelet activation, thrombosis, and hemostasis. The recent description of critical regulators of SFKs in platelets, namely, C-terminal Src kinase (Csk), Csk homologous kinase (Chk), the receptor-type protein-tyrosine phosphatase receptor type J (PTPRJ) helps explain some of the bleeding side effects of tyrosine kinase inhibitors and are novel therapeutic targets for regulating the thrombotic and hemostatic capacity of platelets. Recent findings from Chk, Csk, and PTPRJ knockout mouse models highlighted that SFKs are able to autoinhibit by phosphorylating their C-terminal tyrosine residues, providing fundamental insights into SFK autoregulation.

KEYWORDS
kinase, phosphatatase, platelets, Src, tyrosine phosphorylation

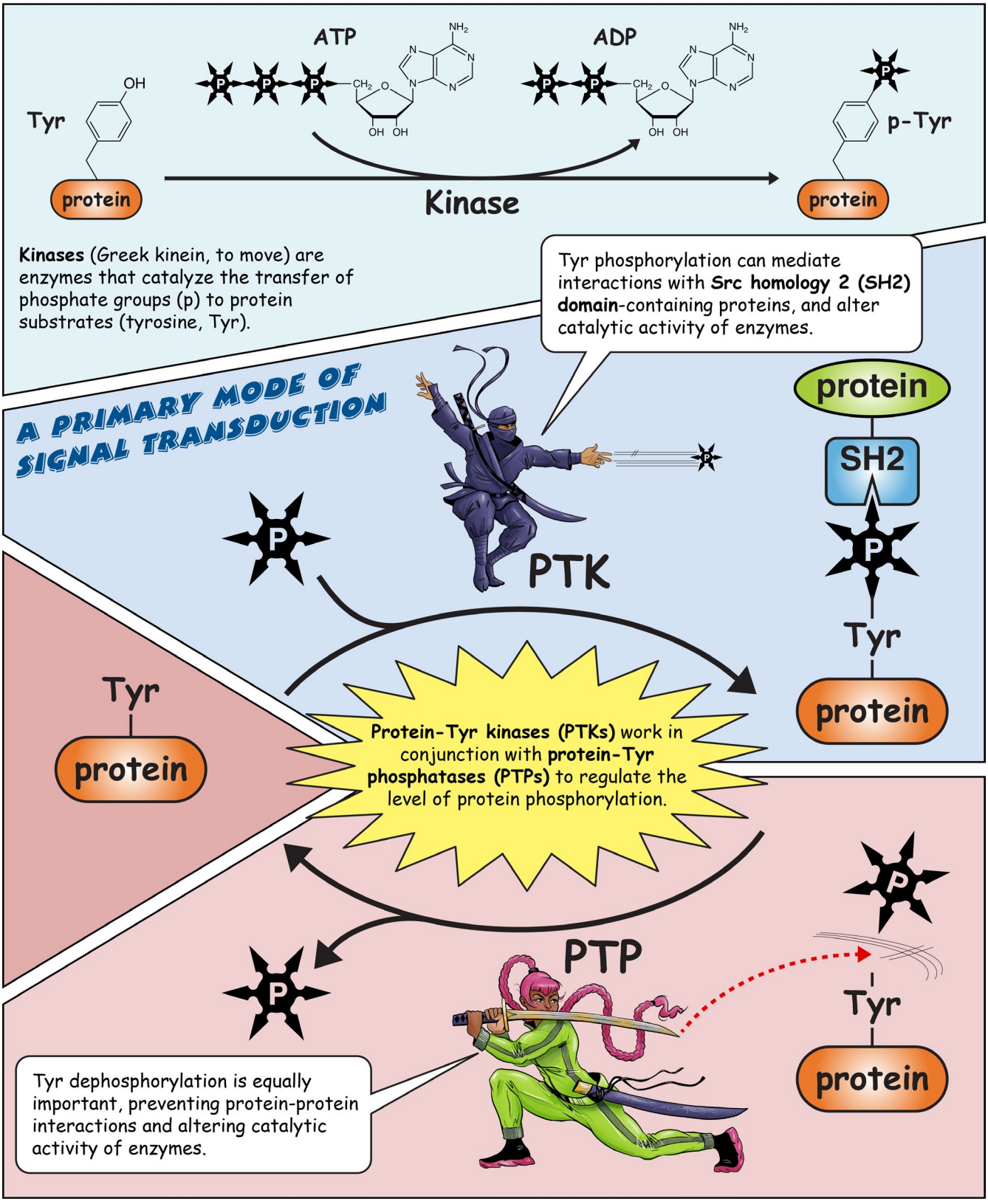
Essentials

- Sarcoma (Src) family kinases (SFKs) are essential for initiating and amplifying platelet activation.
- Reversible phosphorylation is a primary mode of regulation of SFK activity.
- The tyrosine kinases C-terminal Src kinase (Csk) and Csk homologous kinase and phosphatase protein-tyrosine phosphatase receptor type J are critical regulators of SFKs.
- Autophosphorylation provides an additional level of SFK regulation.

THE WINDING PATH OF DISCOVERY

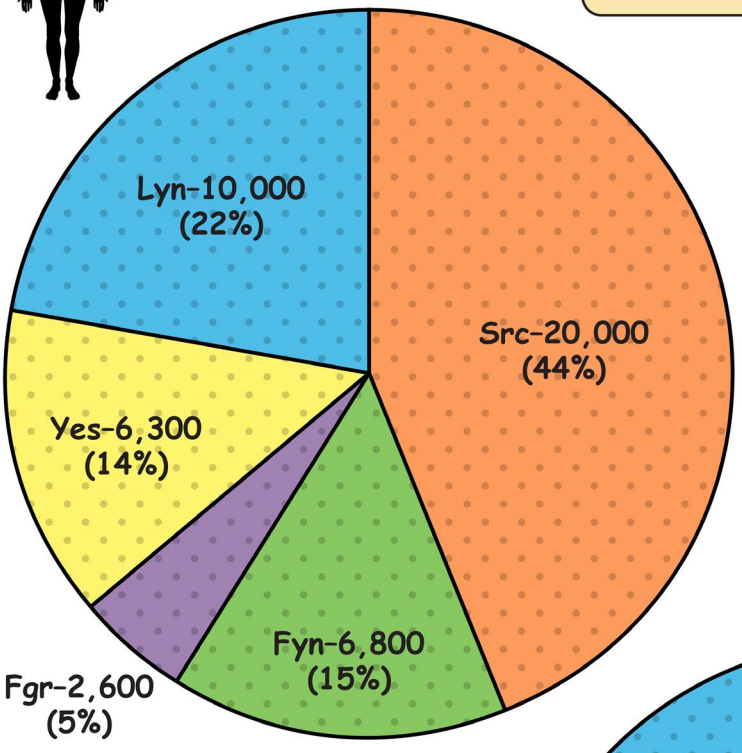


REVERSIBLE TYROSINE PHOSPHORYLATION



PLATELET SRC FAMILY KINASES

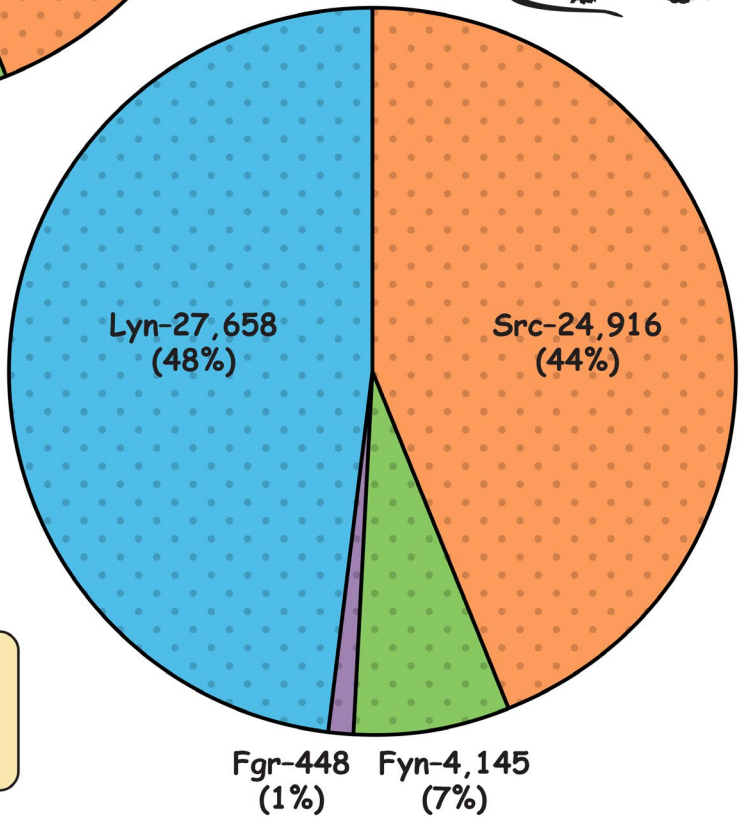
Human



Joan S. Brugge identified v-Src while in the lab of **Raymond L. Erikson**,[7] and her lab later showed that platelets express one of the highest levels of c-Src (Src) of any cell type.[16]

Src family kinases (SFKs) include Src, Lyn, Fyn, Fgr, Lck, Hck, Blk, Yes, of which Src, Lyn and Fyn are highly expressed in platelets.

Mouse



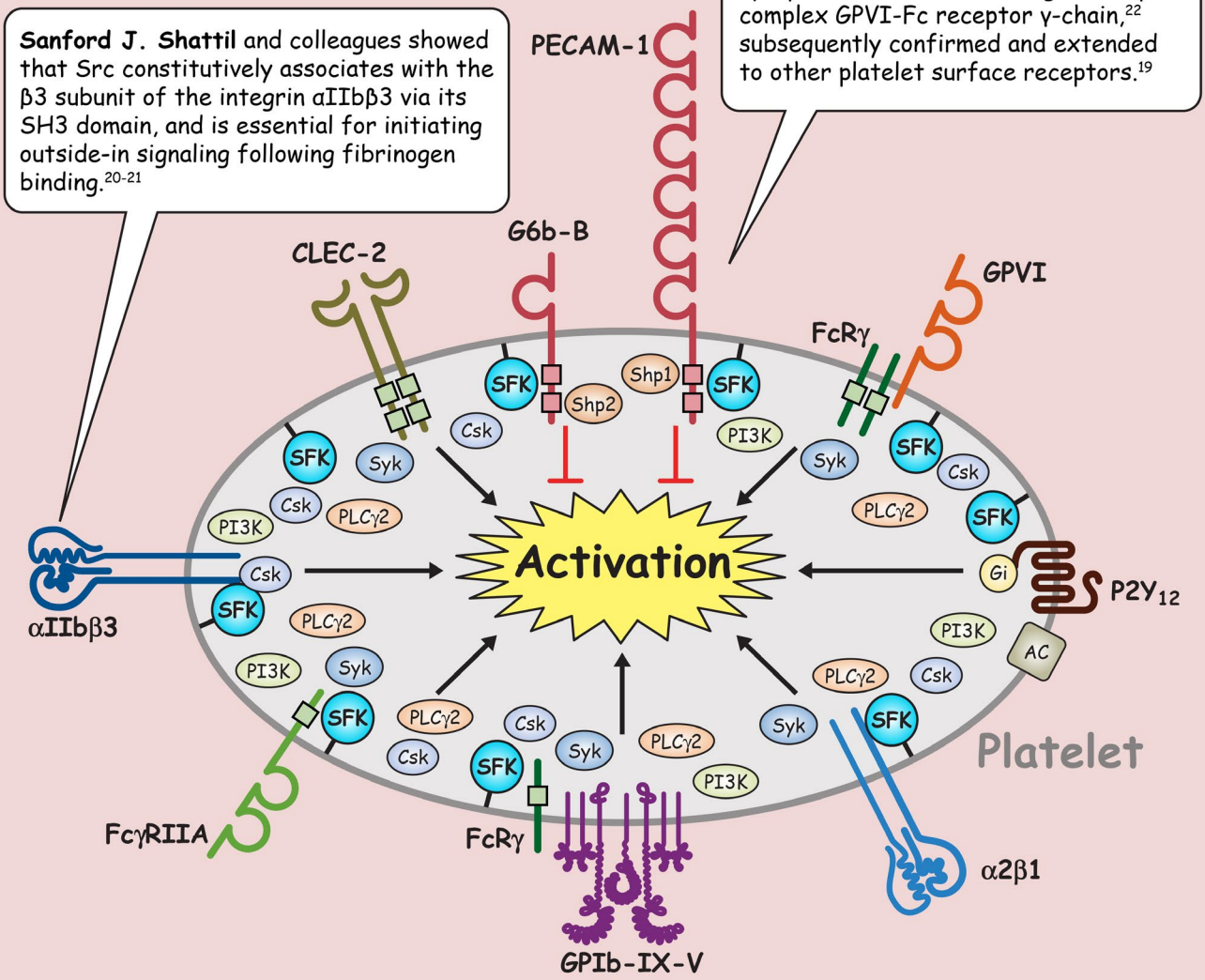
Copy numbers and proportions of most abundant SFKs expressed in human and mouse platelets, determined by proteomic-based approaches.[17,18]

SFKs are essential for platelet activation

SFKs are critical for initiating and amplifying signals from platelet adhesion (αIIbβ3, α2β1, GPIb-XI-V), activation (GPVI, CLEC-2, FcγRIIA, $P2Y_{12}$) and inhibitory (G6b-B, PECAM-1) receptors.[19]

Sanford J. Shattil and colleagues showed that Src constitutively associates with the β3 subunit of the integrin αIIbβ3 via its SH3 domain, and is essential for initiating outside-in signaling following fibrinogen binding.[20-21]

Hiroshi Takayama and colleagues showed physical and functional associations of Lyn and Fyn with the cytoplasmic tail of the collagen receptor complex GPVI-Fc receptor γ-chain,[22] subsequently confirmed and extended to other platelet surface receptors.[19]



Src family kinase (SFK), C-terminal Src kinase (Csk), spleen tyrosine kinase (Syk), phospholipase Cγ2 (PLCγ2), phosphoinositide 3′-kinase (PI3K), adenylate cyclase (AC), Src homology 2 domain-containing tyrosine phosphatase 1 and 2 (Shp1, Shp2), immunoreceptor tyrosine-based activation motif ▢, immunoreceptor tyrosine-based inhibition motif ▢

STRUCTURE AND PHOSPHO-REGULATION OF SRC

All SFKs share the same structural features. In addition, Lyn, Fyn, Lck and Yes are palmitoylated, affecting membrane-localization.[23]

Human c-Src

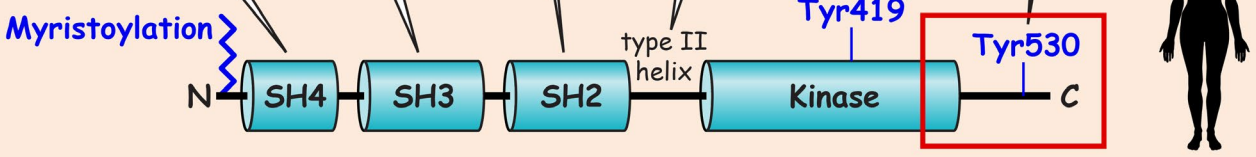
SH2 domain identified by **Anthony J. Pawson** and colleagues binds phospho-tyrosine residues and mediates protein-protein interactions.[24]

Two highly important regulatory phosphorylation sites are Tyr419 (activation loop) and Tyr530 (C-terminal inhibitory tail).[25-27]

SH4 membrane-localization domain

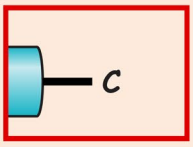
SH3 PxxP type II helix recognition domain

Type II helix linker region mediates interaction with SH3 domain



Chicken v-Src

v-Src lacks the C-terminal inhibitory tyrosine residue (Tyr527 in chicken Src), resulting in higher activity and transforming ability.



REVERSIBLE PHOSPHORYLATION OF TYR530 AND TYR419

The structure of Tyr530 phosphorylated Src was solved by the group of **Michael J. Eck**.[28]

In humans, the E527K Src variant affects the Tyr530 phosphorylation site leading to constitutively active kinase, bleeding, thrombocytopenia, myelofibrosis and bone pathologies.[29]

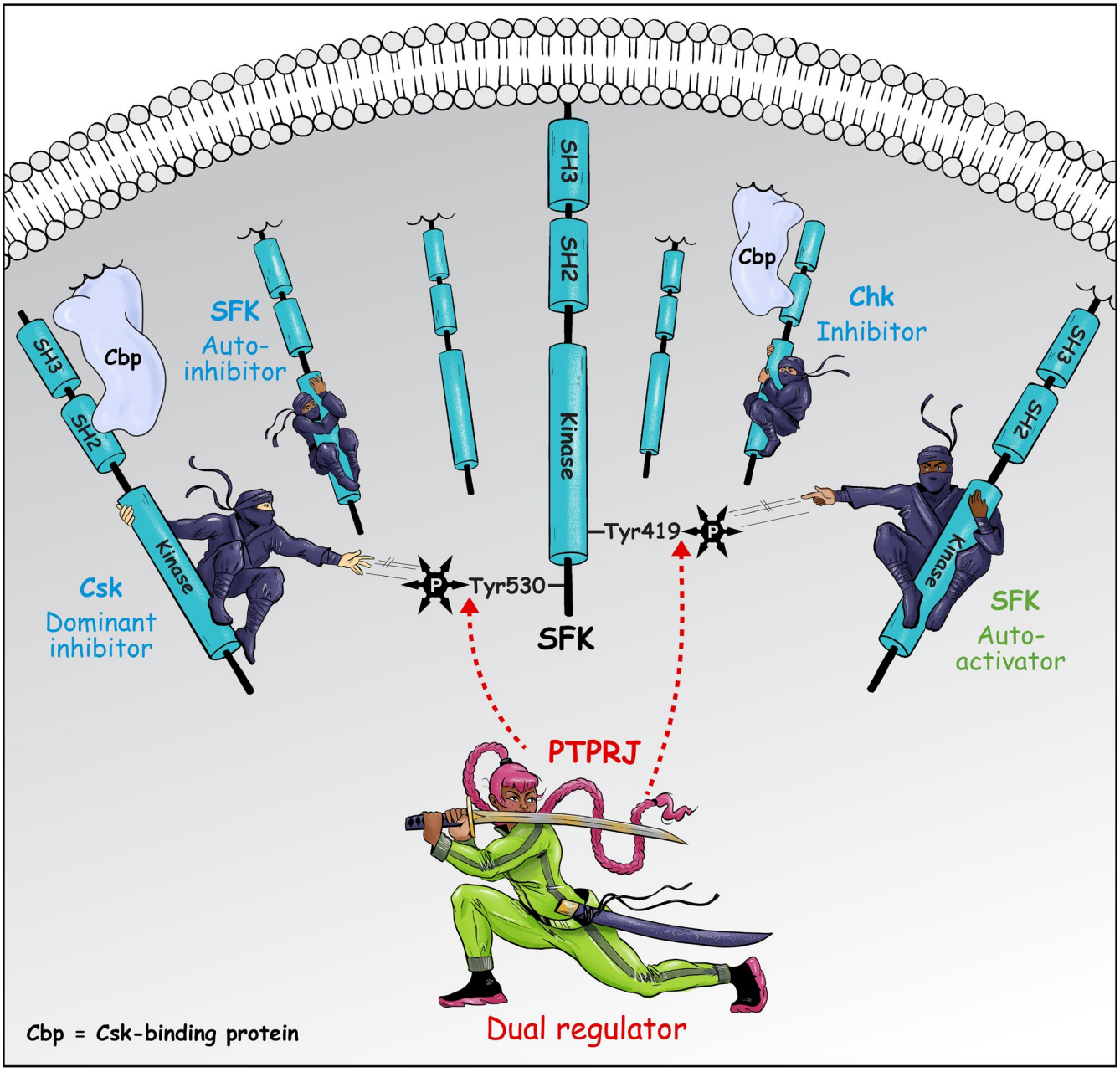
Phosphorylation

- Tyr530 by C-terminal Src kinase (Csk), Csk homologous kinase (Chk) and SFKs inhibits SFK activity.[30-32]
- *trans*-autophosphorylation of Tyr419 by SFKs increases SFK activity.

Dephosphorylation

- p-Tyr530 by the receptor-type PTPs PTPRJ, CD45, PTPRA, PTPRE and non-receptor PTPs PTP-1B, SHP1, SHP2 increases SFK activity.[27, 33-35]
- p-Tyr419 by PTPRJ, CD45 decreases SFK activity.[27, 33, 36]

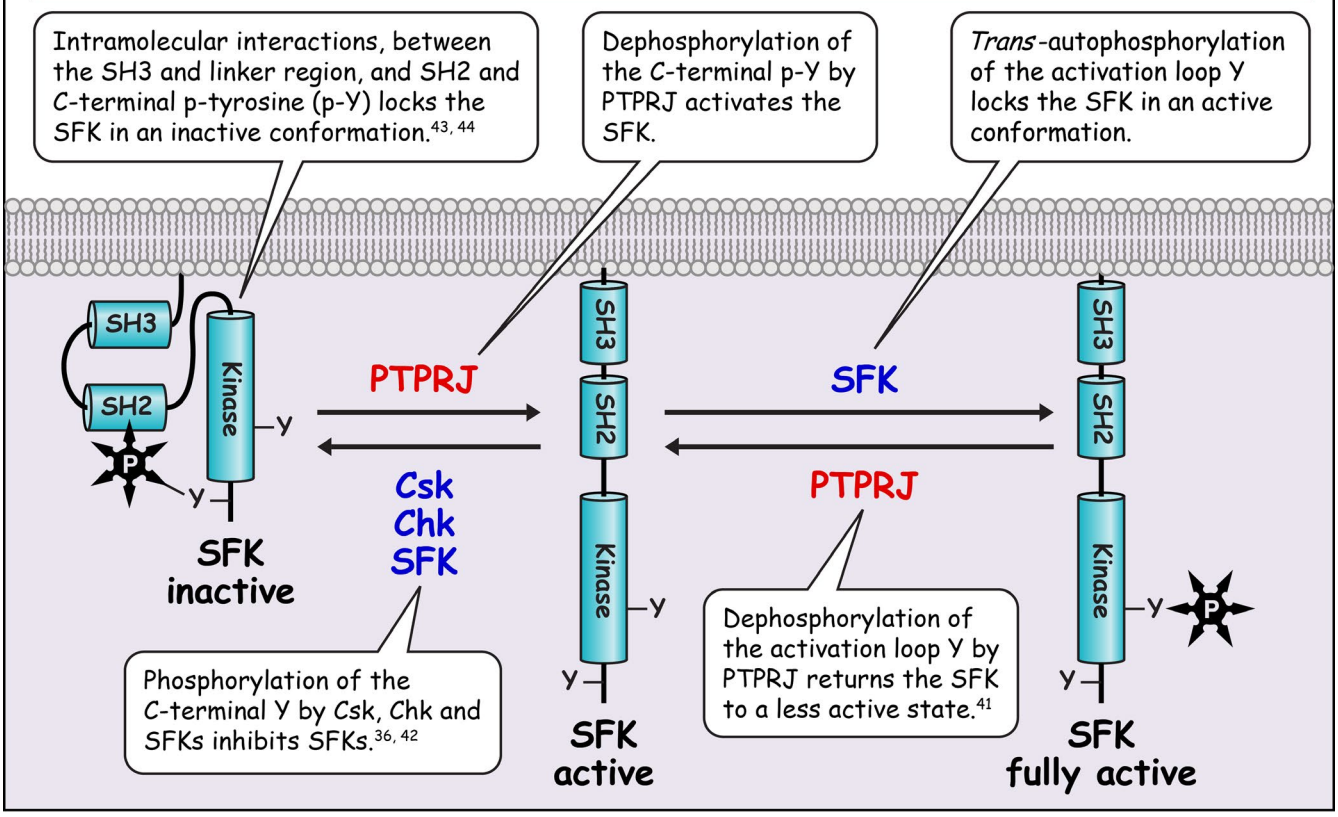
KINASE/PHOSPHATASE REGULATORS OF PLATELET SFKs



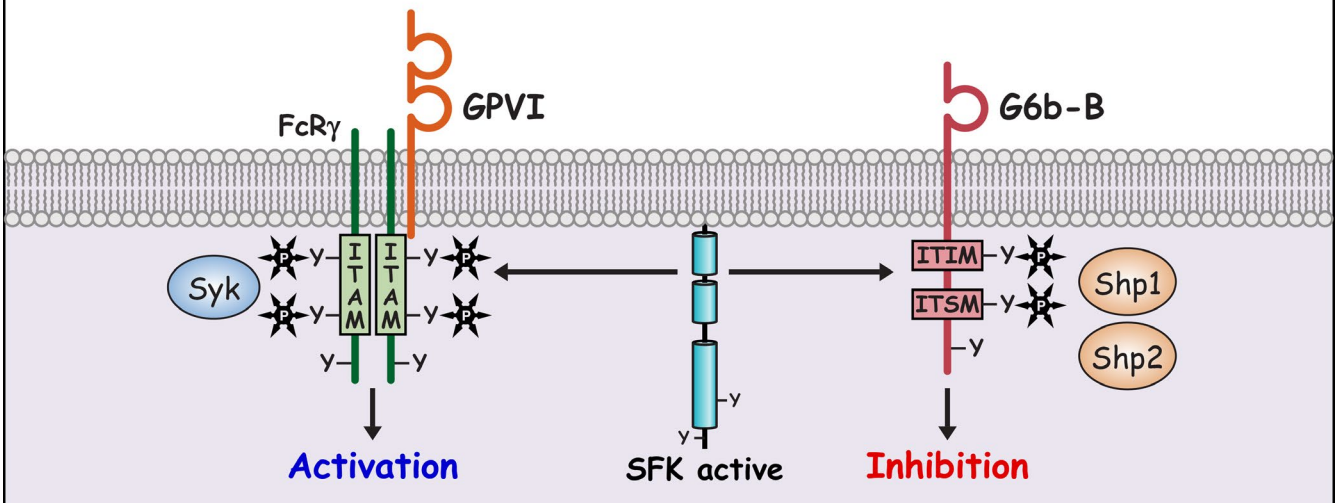
AUXILIARY PTP REGULATORS

THE SFK EQUILIBRIUM IN PLATELETS

SFKs are tightly regulated in platelets by the interplay of Csk, Chk, SFKs and PTPRJ.[36, 41, 42] Resting platelets contain basal SFK activity, allowing them to rapidly respond to vascular injury. Why this does not lead to unwanted signalling is partially explained by accessibility of downstream substrates.



CONCOMITANT ACTIVATION AND INHIBITION

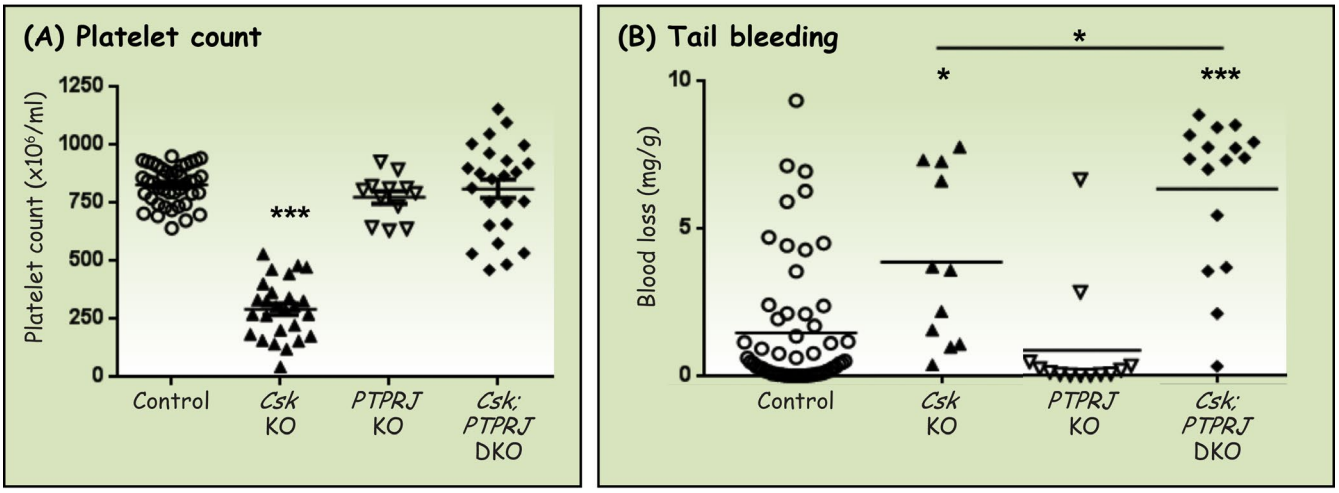


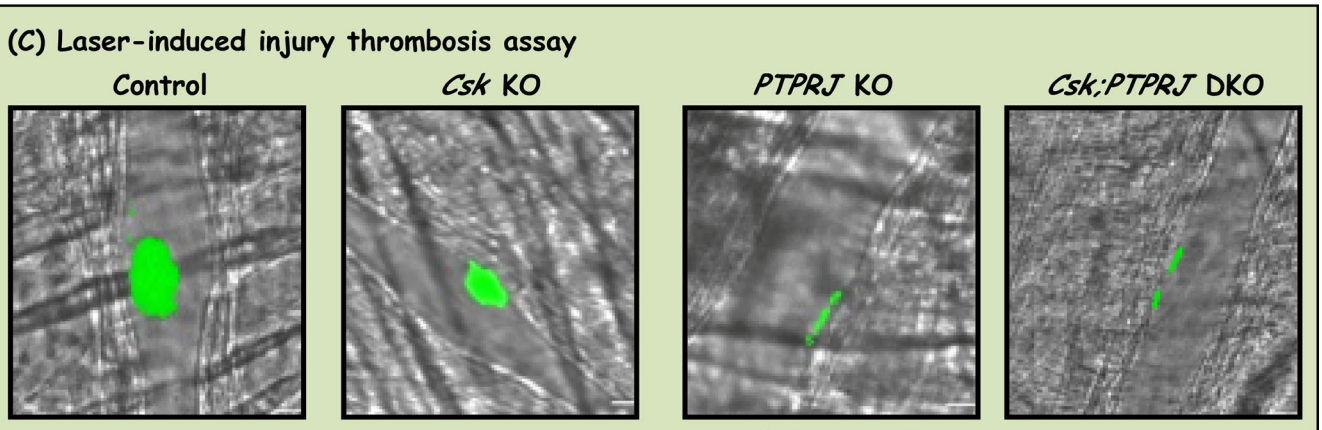
ITAM
Immunoreceptor Tyrosine-based Activation Motif
(YxxL/Ix[6-8]YxxL/I)

ITIM
Immunoreceptor Tyrosine-based Inhibition Motif
(I/V/LxYxxL/V)

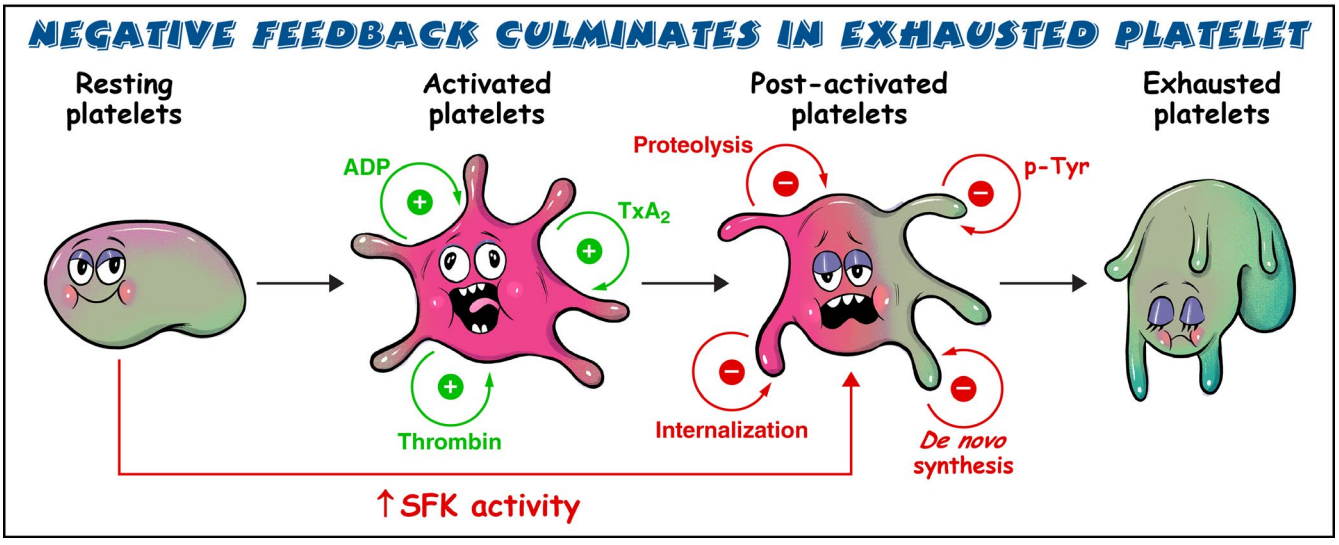
ITSM
Immunoreceptor Tyrosine-based Switch Motif
(TxYxxV/I)

PATHOLOGICAL CONSEQUENCES OF SFK DISEQUILIBRIUM





Bleeding and thrombotic complications in *Pf4-Cre*$^{+}$;*Csk*$^{fl/fl}$;*PTPRJ*$^{fl/fl}$ conditional double knockout (DKO) mice, despite normal platelet counts. (A-C) Platelet count in control, Csk, PTPRJ and Csk;PTPRJ conditional KO and DKO mouse models (platelets green; scale bar: 10 mm).[36] This research was originally published in Blood.[36] ©American Society of Hematology.

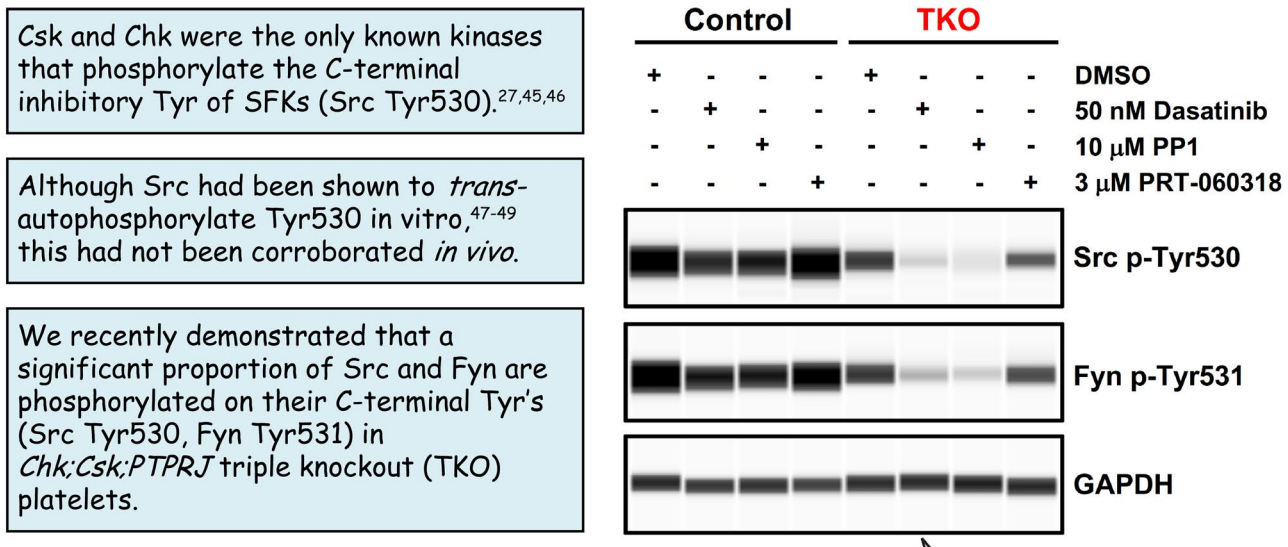


AUTOPHOSPHORYLATION OF SFK INHIBITORY TYR

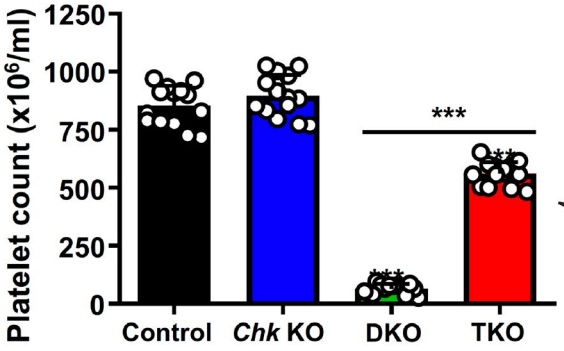
Csk and Chk were the only known kinases that phosphorylate the C-terminal inhibitory Tyr of SFKs (Src Tyr530).[27,45,46]

Although Src had been shown to *trans*-autophosphorylate Tyr530 in vitro,[47-49] this had not been corroborated *in vivo*.

We recently demonstrated that a significant proportion of Src and Fyn are phosphorylated on their C-terminal Tyr's (Src Tyr530, Fyn Tyr531) in *Chk;Csk;PTPRJ* triple knockout (TKO) platelets.



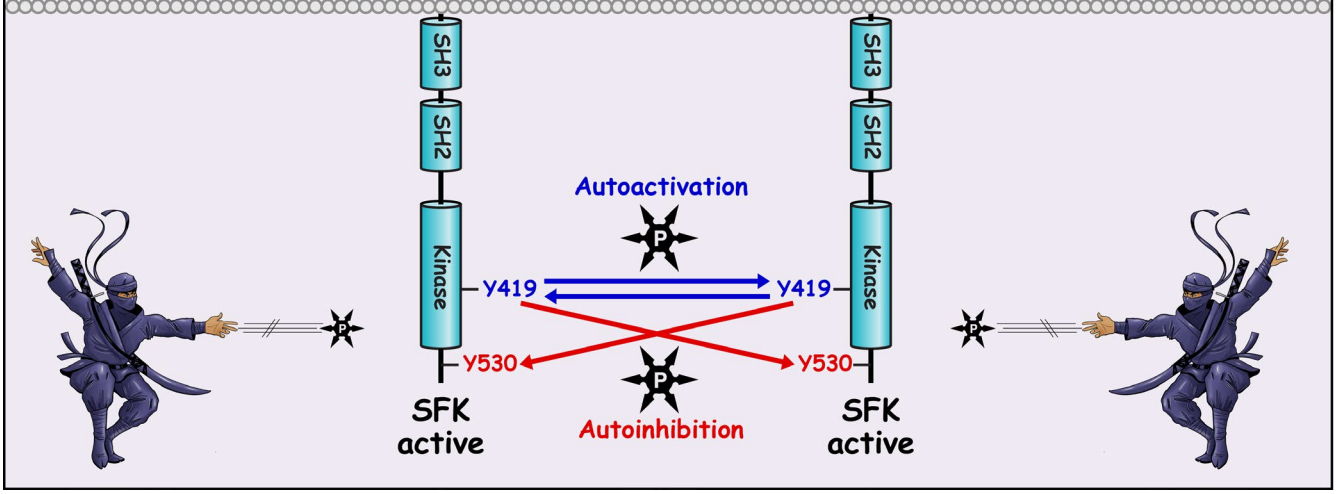
The SFK inhibitors dasatinib and PP1 reduced C-terminal Tyr phosphorylation in control and TKO platelets, whereas the Syk inhibitor PRT-060318 had no effect.[42]



Severe thrombocytopenia in *Chk;Csk* double KO (DKO) mice was partially rescued in TKO mice.[42]

This research was originally published in Blood.[42]

SFK AUTO-REGULATION

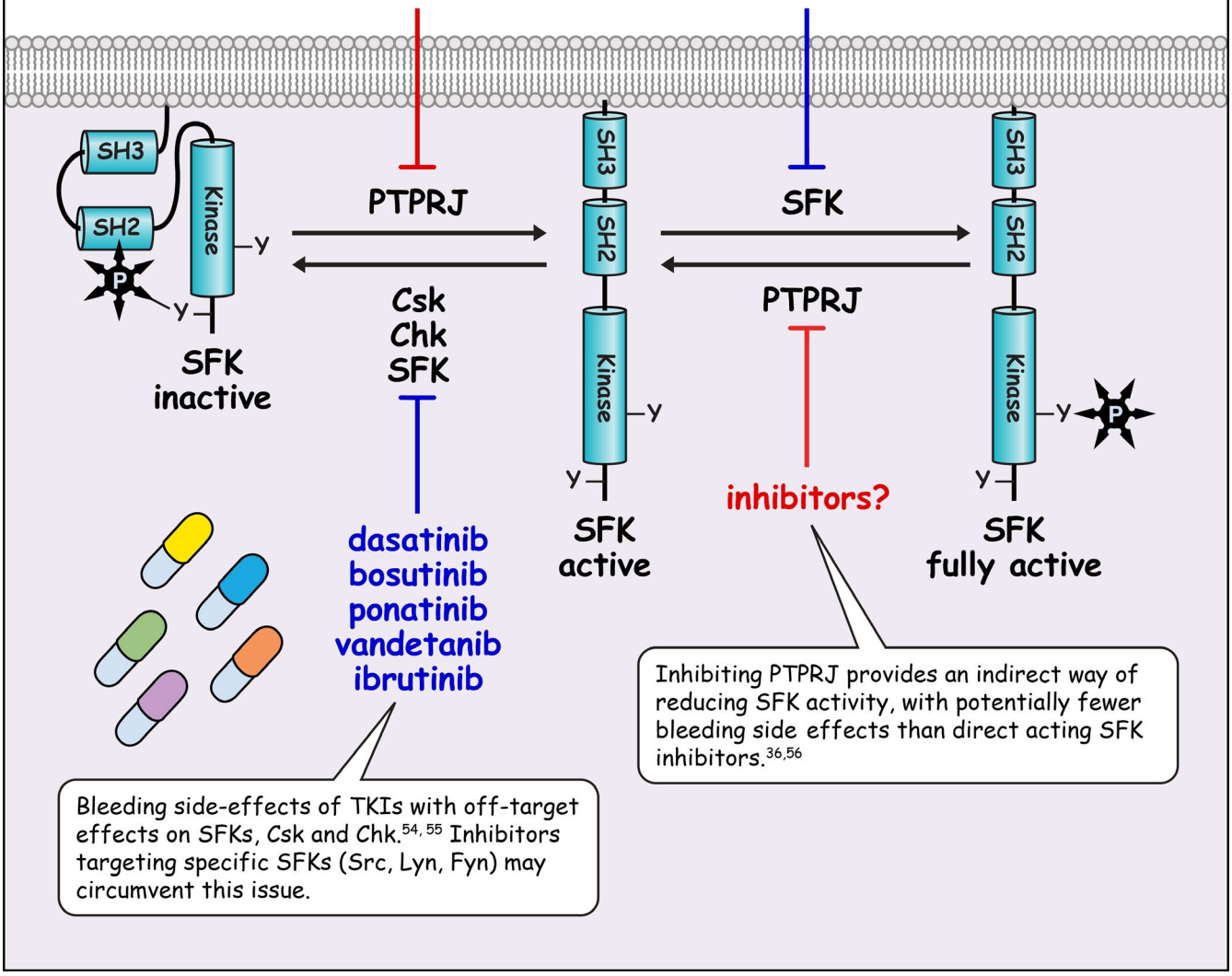


TARGETING SFKS AND THEIR REGULATORS

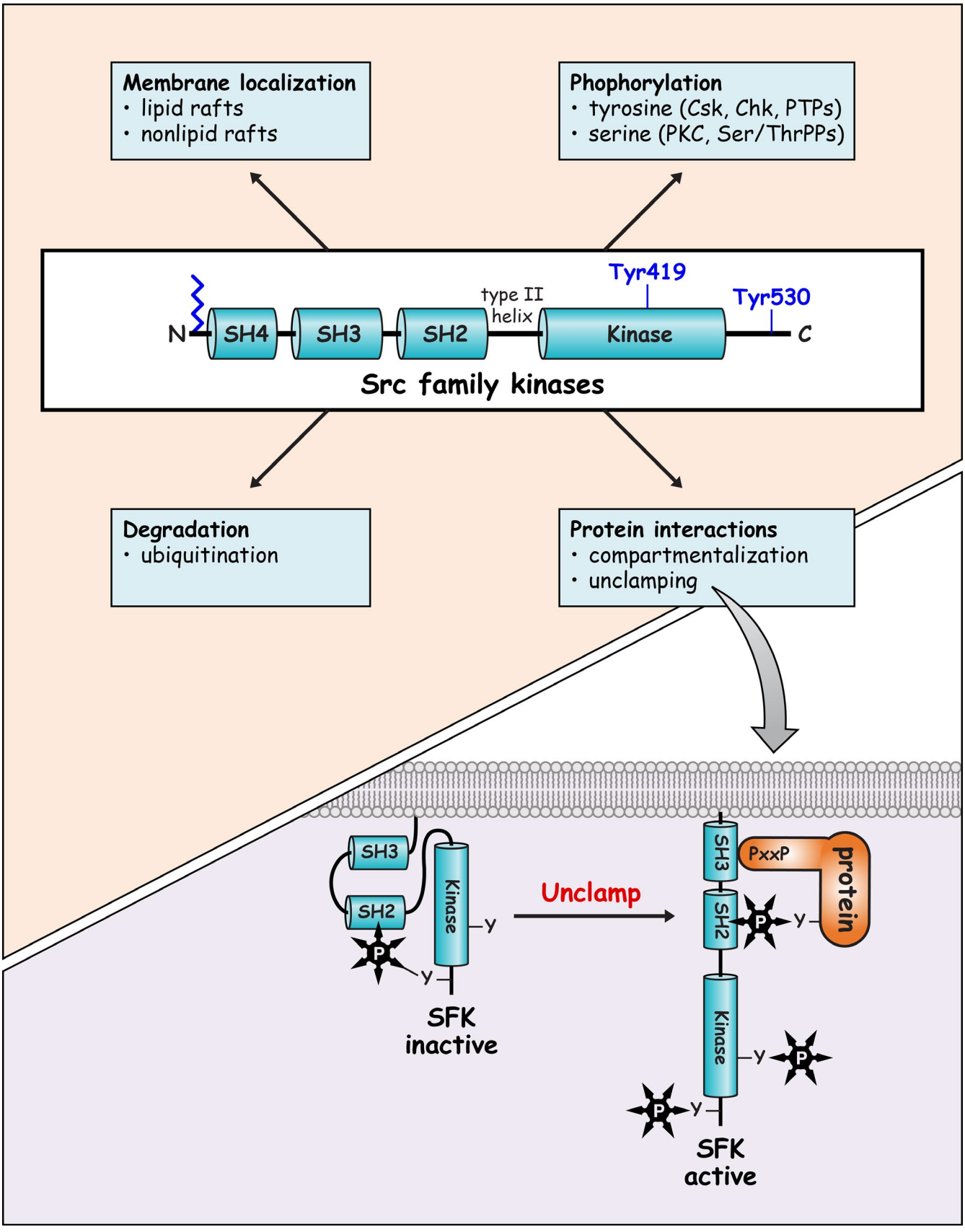
SFKs are essential for cell proliferation, adhesion, migration, survival, angiogenesis and invasion, and are targeted in a variety of pathologies, including cancer, autoimmunity and cardiovascular disease.[50, 51]

Dasatinib, bosutinib, ponatinib, vandetanib are orally active tyrosine kinase inhibitors (TKIs) with off-target effects on SFKs, used in the treatment of various cancers.[50, 52] Ibrutinib is a Btk inhibitor with off-target effects on Csk, also used in the treatment of cancer.[53] All have bleeding side effects.

CIRCUMVENTING BLEEDING SIDE EFFECTS



OTHER MODES OF SFK REGULATION



ACKNOWLEDGEMENTS

We thank Dr Alexandra Mazharian for critical reading, discussion, and suggestions. ZN was funded by the British Heart Foundation (BHF) and University Hospital Würzburg, and acknowledges support from Prof Bernhard Nieswandt (University of Würzburg); JM was funded by the BHF; and YAS was funded by the BHF, Etablissement Français du Sang, and Inserm.

AUTHOR CONTRIBUTIONS

YAS developed theme, prepared images, and wrote and revised the manuscript. ZN prepared images and revised the manuscript. JM developed theme and prepared images. SL developed theme, illustration, and design. PL developed theme, illustration, and design.

RELATIONSHIP DISCLOSURE

The authors report no conflicts of interest to disclose.

ORCID

Yotis A. Senis https://orcid.org/0000-0002-0947-9957
Zoltan Nagy https://orcid.org/0000-0001-6517-2071
Jun Mori https://orcid.org/0000-0002-6212-1604

TWITTER

Yotis A. Senis @YotisSenis
Zoltan Nagy @ZoltanNagyPhd

REFERENCES

1. Rous P. A Sarcoma of the fowl transmissible by an agent separable from the tumor cells. *J Exp Med*. 1911;13(4):397-411.
2. Fischer EH, Krebs EG. Conversion of phosphorylase b to phosphorylase a in muscle extracts. *J Biol Chem*. 1955;216(1):121-132.
3. Hanafusa H, Hanafusa T, Rubin H. The defectiveness of Rous sarcoma virus. *Proc Natl Acad Sci U S A*. 1963;49:572-580.
4. Stehelin D, Varmus HE, Bishop JM, Vogt PK. DNA related to the transforming gene(s) of avian sarcoma viruses is present in normal avian DNA. *Nature*. 1976;260(5547):170-173.
5. Stehelin D, Fujita DJ, Padgett T, Varmus HE, Bishop JM. Detection and enumeration of transformation-defective strains of avian sarcoma virus with molecular hybridization. *Virology*. 1977;76(2):675-684.
6. Oppermann H, Levinson AD, Varmus HE, Levintow L, Bishop JM. Uninfected vertebrate cells contain a protein that is closely related to the product of the avian sarcoma virus transforming gene (src). *Proc Natl Acad Sci U S A*. 1979;76(4):1804-1808.
7. Brugge JS, Erikson RL. Identification of a transformation-specific antigen induced by an avian sarcoma virus. *Nature*. 1977;269(5626):346-348.
8. Collett MS, Erikson RL. Protein kinase activity associated with the avian sarcoma virus Src gene product. *Proc Natl Acad Sci U S A*. 1978;75(4):2021-2024.
9. Erikson E, Collett MS, Erikson RL. In vitro synthesis of a functional avian sarcoma virus transforming-gene product. *Nature*. 1978;274(5674):919-921.
10. Eckhart W, Hutchinson MA, Hunter T. An activity phosphorylating tyrosine in polyoma T antigen immunoprecipitates. *Cell*. 1979;18(4):925-933.
11. Hunter T, Sefton BM. Transforming gene product of Rous sarcoma virus phosphorylates tyrosine. *Proc Natl Acad Sci U S A*. 1980;77(3):1311-1315.
12. Tonks NK, Diltz CD, Fischer EH. Characterization of the major protein-tyrosine-phosphatases of human placenta. *J Biol Chem*. 1988;263(14):6731-6737.
13. Ostman A, Yang Q, Tonks NK. Expression of DEP-1, a receptor-like protein-tyrosine-phosphatase, is enhanced with increasing cell density. *Proc Natl Acad Sci U S A*. 1994;91(21):9680-9684.
14. Tonks NK, Charbonneau H, Diltz CD, Fischer EH, Walsh KA. Demonstration that the leukocyte common antigen CD45 is a protein tyrosine phosphatase. *Biochemistry*. 1988;27(24):8695-8701.
15. Zheng XM, Wang Y, Pallen CJ. Cell transformation and activation of pp60c-src by overexpression of a protein tyrosine phosphatase. *Nature*. 1992;359(6393):336-339.
16. Golden A, Nemeth SP, Brugge JS. Blood platelets express high levels of the pp60c-src-specific tyrosine kinase activity. *Proc Natl Acad Sci U S A*. 1986;83(4):852-856.
17. Burkhart JM, Vaudel M, Gambaryan S, et al. The first comprehensive and quantitative analysis of human platelet protein composition allows the comparative analysis of structural and functional pathways. *Blood*. 2012;120(15):e73-82.
18. Zeiler M, Moser M, Mann M. Copy number analysis of the murine platelet proteome spanning the complete abundance range. *Mol Cell Proteomics*. 2014;13(12):3435-3445.
19. Senis YA, Mazharian A, Mori J. Src family kinases: at the forefront of platelet activation. *Blood*. 2014;124(13):2013-2024.
20. Arias-Salgado EG, Lizano S, Sarkar S, Brugge JS, Ginsberg MH, Shattil SJ. Src kinase activation by direct interaction with the integrin beta cytoplasmic domain. *Proc Natl Acad Sci U S A*. 2003;100(23):13298-13302.
21. Obergfell A, Eto K, Mocsai A, et al. Coordinate interactions of Csk, Src, and Syk kinases with [alpha]IIb[beta]3 initiate integrin signaling to the cytoskeleton. *J Cell Biol*. 2002;157(2):265-275.
22. Ezumi Y, Shindoh K, Tsuji M, Takayama H. Physical and functional association of the Src family kinases Fyn and Lyn with the collagen receptor glycoprotein VI-Fc receptor gamma chain complex on human platelets. *J Exp Med*. 1998;188(2):267-276.
23. Resh MD. Myristylation and palmitylation of Src family members: the fats of the matter. *Cell*. 1994;76(3):411-413.
24. Moran MF, Koch CA, Anderson D, et al. Src homology region 2 domains direct protein-protein interactions in signal transduction. *Proc Natl Acad Sci U S A*. 1990;87(21):8622-8626.
25. Iba H, Cross FR, Garber EA, Hanafusa H. Low level of cellular protein phosphorylation by nontransforming overproduced p60c-src. *Mol Cell Biol*. 1985;5(5):1058-1066.
26. Cooper JA, Gould KL, Cartwright CA, Hunter T. Tyr527 is phosphorylated in pp60c-src: implications for regulation. *Science*. 1986;231(4744):1431-1434.
27. Roskoski R Jr. Src kinase regulation by phosphorylation and dephosphorylation. *Biochem Biophys Res Commun*. 2005;331(1):1-14.
28. Xu W, Harrison SC, Eck MJ. Three-dimensional structure of the tyrosine kinase c-Src. *Nature*. 1997;385(6617):595-602.
29. Turro E, Greene D, Wijgaerts A, et al. A dominant gain-of-function mutation in universal tyrosine kinase SRC causes thrombocytopenia, myelofibrosis, bleeding, and bone pathologies. *Sci Transl Med*. 2016;8(328):328ra330.
30. Okada M, Nakagawa H. A protein tyrosine kinase involved in regulation of pp60c-src function. *J Biol Chem*. 1989;264(35):20886-20893.
31. Nada S, Okada M, MacAuley A, Cooper JA, Nakagawa H. Cloning of a complementary DNA for a protein-tyrosine kinase that specifically phosphorylates a negative regulatory site of p60c-src. *Nature*. 1991;351(6321):69-72.
32. Nada S, Yagi T, Takeda H, et al. Constitutive activation of Src family kinases in mouse embryos that lack Csk. *Cell*. 1993;73(6):1125-1135.
33. Ellison S, Mori J, Barr AJ, Senis YA. CD148 enhances platelet responsiveness to collagen by maintaining a pool of active Src family kinases. *J Thromb Haemost*. 2010;8(7):1575-1583.

34. Mori J, Wang YJ, Ellison S, et al. Dominant role of the protein-tyrosine phosphatase CD148 in regulating platelet activation relative to protein-tyrosine phosphatase-1B. *Arterioscler Thromb Vasc Biol*. 2012;32(12):2956-2965.
35. Senis YA. Protein-tyrosine phosphatases: a new frontier in platelet signal transduction. *J Thromb Haemost*. 2013;11(10):1800-1813.
36. Mori J, Nagy Z, Di Nunzio G, et al. Maintenance of murine platelet homeostasis by the kinase Csk and phosphatase CD148. *Blood*. 2018;131(10):1122-1144.
37. Bjorge JD, Pang A, Fujita DJ. Identification of protein-tyrosine phosphatase 1B as the major tyrosine phosphatase activity capable of dephosphorylating and activating c-Src in several human breast cancer cell lines. *J Biol Chem*. 2000;275(52):41439-41446.
38. Somani AK, Bignon JS, Mills GB, Siminovitch KA, Branch DR. Src kinase activity is regulated by the SHP-1 protein-tyrosine phosphatase. *J Biol Chem*. 1997;272(34):21113-21119.
39. Zhang SQ, Yang W, Kontaridis MI, et al. Shp2 regulates SRC family kinase activity and Ras/Erk activation by controlling Csk recruitment. *Mol Cell*. 2004;13(3):341-355.
40. Gil-Henn H, Elson A. Tyrosine phosphatase-epsilon activates Src and supports the transformed phenotype of Neu-induced mammary tumor cells. *J Biol Chem*. 2003;278(18):15579-15586.
41. Senis YA, Tomlinson MG, Ellison S, et al. The tyrosine phosphatase CD148 is an essential positive regulator of platelet activation and thrombosis. *Blood*. 2009;113(20):4942-4954.
42. Nagy Z, Mori J, Ivanova VS, Mazharian A, Senis YA. Interplay between the tyrosine kinases Chk and Csk and phosphatase PTPRJ is critical for regulating platelets in mice. *Blood*. 2020;135(18):1574-1587.
43. Xu C, Gagnon E, Call ME, et al. Regulation of T cell receptor activation by dynamic membrane binding of the CD3epsilon cytoplasmic tyrosine-based motif. *Cell*. 2008;135(4):702-713.
44. Nika K, Soldani C, Salek M, et al. Constitutively active Lck kinase in T cells drives antigen receptor signal transduction. *Immunity*. 2010;32(6):766-777.
45. Thomas JE, Soriano P, Brugge JS. Phosphorylation of c-Src on tyrosine 527 by another protein tyrosine kinase. *Science*. 1991;254(5031):568-571.
46. Jove R, Kornbluth S, Hanafusa H. Enzymatically inactive p60c-src mutant with altered ATP-binding site is fully phosphorylated in its carboxy-terminal regulatory region. *Cell*. 1987;50(6):937-943.
47. Cooper JA, MacAuley A. Potential positive and negative autoregulation of p60c-src by intermolecular autophosphorylation. *Proc Natl Acad Sci U S A*. 1988;85(12):4232-4236.
48. MacAuley A, Okada M, Nada S, Nakagawa H, Cooper JA. Phosphorylation of Src mutants at Tyr 527 in fibroblasts does not correlate with in vitro phosphorylation by CSK. *Oncogene*. 1993;8(1):117-124.
49. Osusky M, Taylor SJ, Shalloway D. Autophosphorylation of purified c-Src at its primary negative regulation site. *J Biol Chem*. 1995;270(43):25729-25732.
50. Kim LC, Song L, Haura EB. Src kinases as therapeutic targets for cancer. *Nat Rev Clin Oncol*. 2009;6(10):587-595.
51. Zhang S, Yu D. Targeting Src family kinases in anti-cancer therapies: turning promise into triumph. *Trends Pharmacol Sci*. 2012;33(3):122-128.
52. Roskoski R Jr. Src protein-tyrosine kinase structure, mechanism, and small molecule inhibitors. *Pharmacol Res*. 2015;94:9-25.
53. Honigberg LA, Smith AM, Sirisawad M, et al. The Bruton tyrosine kinase inhibitor PCI-32765 blocks B-cell activation and is efficacious in models of autoimmune disease and B-cell malignancy. *Proc Natl Acad Sci U S A*. 2010;107(29):13075-13080.
54. Gratacap MP, Martin V, Valera MC, et al. The new tyrosine-kinase inhibitor and anticancer drug dasatinib reversibly affects platelet activation in vitro and in vivo. *Blood*. 2009;114(9):1884-1892.
55. Levade M, David E, Garcia C, et al. Ibrutinib treatment affects collagen and von Willebrand factor-dependent platelet functions. *Blood*. 2014;124(26):3991-3995.
56. Senis YA, Barr AJ. Targeting receptor-type protein tyrosine phosphatases with biotherapeutics: is outside-in better than inside-out? *Molecules*. 2018;23(3):569.

How to cite this article: Senis YA, Nagy Z, Mori J, Lane S, Lane P. Platelet Src family kinases: A tale of reversible phosphorylation. *Res Pract Thromb Haemost*. 2021;5:376–389. https://doi.org/10.1002/rth2.12495