Signaling and regulation of the platelet glycoprotein Ib–IX–V complex

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Purpose of review

The platelet adhesion receptor, the glycoprotein Ib–IX–V complex, not only mediates platelet adhesion but also transmits signals leading to platelet activation, aggregation and secretion. Significant progress has been made recently on the signaling pathways and regulatory mechanisms involving glycoprotein Ib–IX–V function.

Recent findings

The interaction of glycoprotein Ib–IX–V with its ligand, von Willebrand factor, is dually controlled by von Willebrand factor conformation and intracellular signal-mediated regulation of glycoprotein Ib–IX–V receptor function that requires the ζ isoform of the 14-3-3 protein family (14-3-3ζ). Glycoprotein Ib–IX–V signaling is mediated by the Src family of protein kinases, phospholipase C, calcium elevation, phosphoinositol 3-kinase, and multiple amplification mechanisms including the nitric oxide–cGMP pathway, the mitogen-activated protein kinase pathway, the immunoreceptor tyrosine-based activation motif pathway, and ADP and thromboxane A_2 pathways.

Summary

Progress in understanding the mechanism(s) regulating glycoprotein Ib–IX–V should help develop inhibitors and modifiers that interfere or augment its von Willebrand factor binding function and thus be useful for treating thrombosis and bleeding disorders. Characterization of intracellular molecules and pathways in glycoprotein Ib–IX–V signaling has implications in the development of new agents and for the use of existing drugs that affect glycoprotein Ib–IX–V signaling.

Keywords

glycoprotein Ib–IX, platelet, platelet adhesion, platelet aggregation, von Willebrand factor

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Abbreviations

CHO	Chinese hamster ovary
ERK	extracellular stimuli-responsive kinase
GP	glycoprotein
ITAM	immunoreceptor tyrosine-based activation motif
PI3K	phosphoinositide 3-kinase
SFK	Src family of protein kinase
TXA_2	thromboxane A_2
VWF	von Willebrand factor

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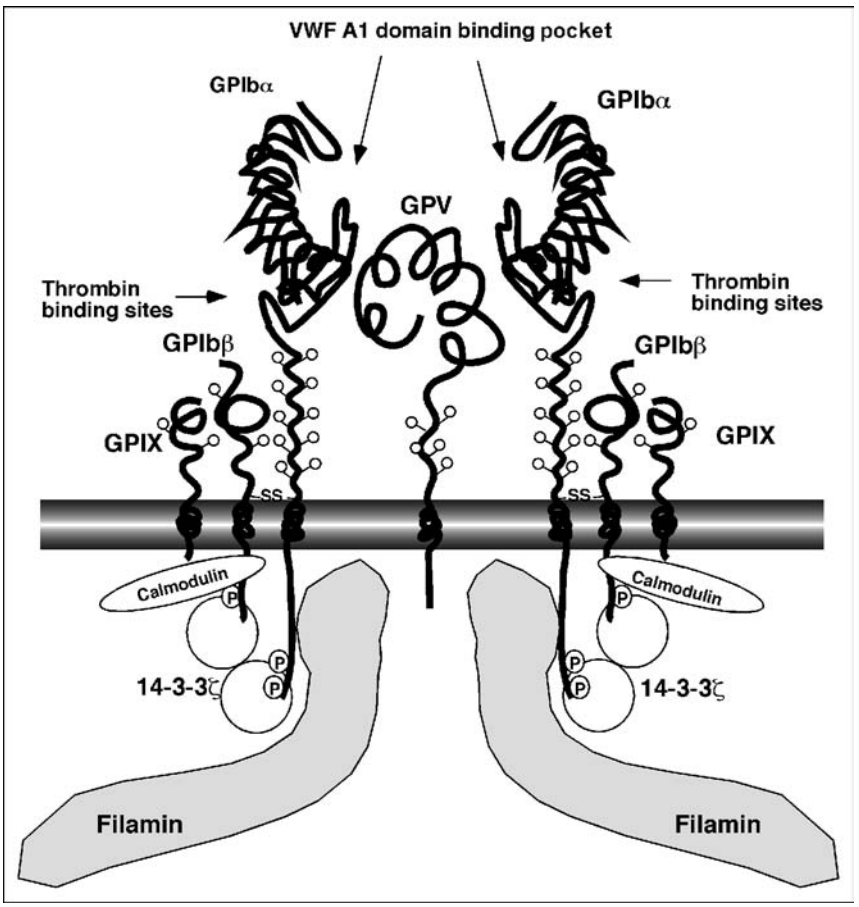
Introduction

Under high shear rate flow conditions often present in arteries and capillaries, platelet adhesion to the site of vascular injury requires interaction between subendothelial matrix-bound von Willebrand factor (VWF) and its platelet receptor, glycoprotein Ib–IX–V (GPIb–IX–V). GPIb–IX–V consists of four transmembrane proteins (Fig. 1). GPIb is composed of a disulfide-linked α chain (GPIbα) and β chain (GPIbβ) (recently reported to be in a 1 : 2 ratio [1•]). GPIb forms a 1 : 1 complex with the GPIX. The GPIb–IX complex is sufficient for both ligand-binding and signaling functions. Another subunit, GPV is expressed in a loosely associated 1 : 2 complex with GPIb–IX. The structures of the GPIbα N-terminal ligand binding domain, alone and complexed with either the VWF A1 domain [2,3] or thrombin [4,5], and a filamin complex with a GPIbα cytoplasmic domain peptide [6] have been reported. In addition to serving as a receptor for VWF and thrombin, GPIb–IX interacts with the leukocyte integrin $\alpha_m\beta_2$ [7], P-selectin [8], coagulation factors XI and XII [9,10], high molecular weight kininogen [11], and thrombospondin-1 [12]. GPIb–IX–V is thus also important in platelet adhesion to activated endothelial cells, in leukocyte recruitment to sites of vascular injury [13], and in thrombin-induced platelet activation and coagulation. Interestingly, the lack of the extracellular domain of GPIbα not only diminishes initial platelet adhesion to the injured vascular wall, but also impairs the recruitment of platelets to already formed thrombi [14••]. Furthermore, the interaction of GPIb–IX with VWF transmits signals leading to activation of the integrin $\alpha_{IIb}\beta_3$, which mediates stable platelet adhesion, spreading and aggregation, and is involved in the formation of procoagulant microparticles [15•,16]. The signaling function of GPIb–IX–V is therefore as important as its function in mediating transient platelet attachment to VWF. The VWF binding function of

Figure 1 A schematic illustration of the glycoprotein Ib–IX–V (GPIb-IX-V) complex and associated proteins



The N-terminal domain of GPIbα contains binding sites for von Willebrand factor (VWF) and thrombin. The cytoplasmic domain of GPIbα contains the phosphorylation-dependent 14–3–3 binding sites, and also the binding site for filamin that links GPIb–IX to actin cytoskeleton underlining the membrane. The cytoplasmic domain of GPIbβ is phosphorylated by protein kinase A (PKA) and this phosphorylation is required for 14–3–3 binding. The membrane proximal region in the cytoplasmic domain of GPIbβ and GPV contain calmodulin-binding sites.

GPIb–IX–V has to be stringently regulated, since inadvertent binding of VWF to GPIb–IX–V causes both thrombosis and bleeding due to VWF-mediated platelet aggregation and the subsequent clearance of platelets and VWF multimers from the circulation, as exemplified in thrombotic thrombocytopenic purpura. Thus, an important aspect of GPIb–IX–V signaling are those signals that regulate its receptor function. This review will discuss recent progress in understanding GPIb–IX–V-dependent signaling pathways and the mechanisms that regulate the function of GPIb–IX–V.

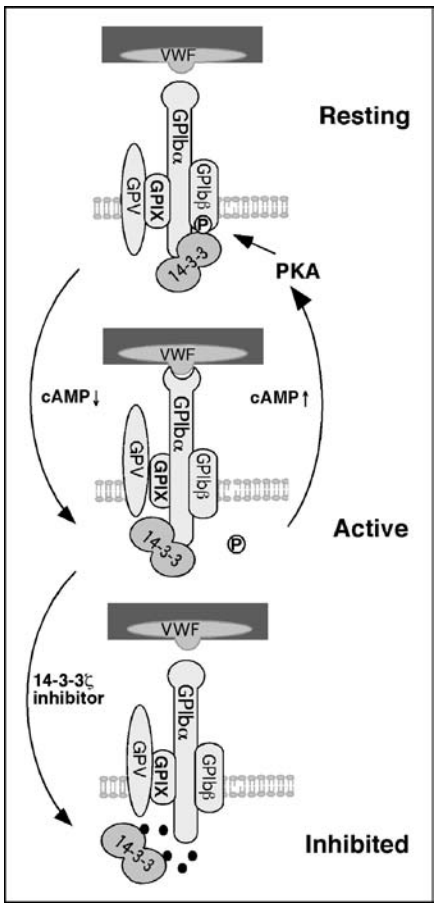
Regulation of the von Willebrand factor binding function of glycoprotein Ib–IX

The regulation of the interaction between VWF and GPIb–IX is two-fold. On the one hand, this interaction is regulated by 'conformational changes' in the A1 domain of VWF. The active 'conformation' of VWF is induced when VWF is immobilized onto surfaces, when bound to collagen in the subendothelial matrix or under high shear stress. Under experimental conditions, ristocetin and botrocetin also induce the binding of soluble VWF to GPIb–IX, and thus GPIb–IX-mediated platelet agglutination and aggregation. On the other hand, the receptor function of GPIb–IX is also regulated by intracellular signaling. Ristocetin-induced platelet aggregation and VWF binding is inhibited by the cAMP-elevating platelet antagonists, prostaglandin I_2 and E_1 [17,18]. cAMP-dependent protein kinase (PKA) phosphorylates the GPIbβ cytoplasmic domain at Ser^{166} [19]. A conservative mutation of Ser^{166} to Ala prevents phosphorylation at Ser^{166}, and results in enhanced VWF binding to GPIb–IX expressed in Chinese hamster ovary (CHO) cells [18,20], suggesting that phosphorylation of GPIbβ negatively regulates the VWF binding function of GPIb–IX.

The role of 14-3-3ζ in regulating the von Willebrand factor binding function of glycoprotein Ib–IX

The intracellular adaptor/signaling molecule, 14–3–3ζ, is expressed as a homodimer, containing two ligand binding sites that recognize phosphoserine-dependent ligands

Figure 2 A 'toggle switch' model of glycoprotein Ib–IX–V (GPIb–IX–V) regulation



Increases in intracellular cAMP induce GPIbβ phosphorylation, allowing 14–3–3ζ interaction with both GPIbα and GPIbβ, locking GPIb–IX in an 'off' or 'resting' state. A decrease in cAMP dissociates 14–3–3ζ from GPIbβ and thus 'switches on' the von Willebrand factor (VWF) binding function. Inhibition of 14–3–3ζ binding to GPIbα disrupts the 'toggle switch', inhibiting the VWF binding function of GPIb–IX–V. Adapted with permission from Dai *et al.* [20]. PKA, protein kinase A.

including GPIb–IX. The RYSGHpSL^{610} sequence at the C-terminus of GPIbα with constitutively phosphorylated Ser^{609} is the primary site required for high-affinity 14–3–3ζ binding to GPIb–IX [21,22]. Additional 14–3–3ζ interaction sites have also been reported in GPIbα Arg^{557}–Gly^{575} and Leu^{580}–Ser^{590} [23,24] and in GPIbβ. The site in GPIbβ requires phosphorylation of GPIbβ at Ser^{166} [23]. Thus, a 14–3–3ζ dimer may potentially interact with GPIbα alone or with GPIbα and GPIbβ, depending on the phosphorylation state of GPIbβ (Fig. 2). Dephosphorylation of GPIbβ induces 'activation' of VWF binding to GPIb–IX, which, however, is diminished by inhibition of 14–3–3ζ binding to the C-terminus of GPIbα, either by mutagenesis or by a myristoylated inhibitor peptide of GPIbα–14–3–3 interaction [20]. This 14–3–3 inhibitor also inhibits VWF binding to GPIb–IX in human platelets [20]. Based on these findings, a toggle switch model of GPIb–IX regulation by 14–3–3ζ has been proposed (Fig. 2). In this model, PKA phosphorylation of GPIbβ at Ser^{166} allows binding of 14–3–3ζ to both GPIbα and GPIbβ, locking GPIb–IX in a low-affinity state. Dephosphorylation of GPIbβ dissociates 14–3–3ζ from GPIbβ, allowing the 14–3–3ζ dimer to interact with two sites in GPIbα or with GPIbα and another protein, switching GPIb–IX to a 14–3–3ζ-dependent 'active' state. Controversially, deletion of the C-terminal six residues of human GPIbα inhibited VWF binding when expressed with human GPIbβ–IX in CHO cells [25], but not when expressed with mouse GPIbβ–IX–V in mouse platelets [26]. Nevertheless, the data that the inhibitor of 14–3–3ζ–GPIbα interaction diminishes VWF binding with human platelets suggests a potential target for antithrombotic drug development.

The relationship between the von Willebrand factor binding function of glycoprotein Ib–IX and the cytoskeleton

Phosphorylation of GPIbβ (Ser^{166}) inhibits actin polymerization during platelet activation, suggesting a role in regulating actin cytoskeletal organization [27]. The inhibitory effect of prostaglandin I_2 on ristocetin-induced platelet agglutination is abolished by low concentrations of cytochalasin D [17]. Shear-induced, VWF-dependent platelet aggregation is also enhanced by actin depolymerizing agents, cytochalasin D and latrunculin B, even in Glanzmann's thrombasthenic platelets (which lack $\alpha_{IIb}\beta_3$), excluding an effect on integrin activation [28]. Cytochalasin D also diminishes the inhibitory effect of prostaglandin E_1 on VWF binding to platelets [18], suggesting that the cAMP-dependent inhibition of VWF binding to GPIb–IX is associated with its effect on the actin cytoskeleton. This view is supported by the observation that a GPIbα mutation that abolishes filamin binding enhances VWF binding function under the experimental conditions in which GPIbβ-phosphorylated wild type GPIb–IX shows diminished VWF binding [29]. Recent reports suggest that the peptides corresponding to the filamin binding site in GPIbα, when allowed to enter cells, inhibit VWF-induced platelet activation [30,31]. The effects of these peptides, however, do not appear to be on the VWF binding function of GPIb–IX (K. Dai, X. Du, unpublished data), but are associated with secretion of platelet ADP, and thus possibly ADP-mediated platelet desensitization [31]. The precise role of the cytoskeleton in regulating GPIb–IX function thus requires further clarification.

Proteolytic regulation of glycoprotein Ib–IX

Cleavage of the extracellular domain of GPIbα by many proteases, including calpain, trypsin, and elastase, effectively abolishes its ligand binding function. Cleavage of

GPV by thrombin has been suggested to promote GPIb–IX-mediated platelet activation [32]. Recent studies show that GPIbα and GPV are cleaved by the metalloprotease, ADAM17 [33,34]. Interestingly, cleavage is induced by aspirin as well as platelet agonists [33,34].

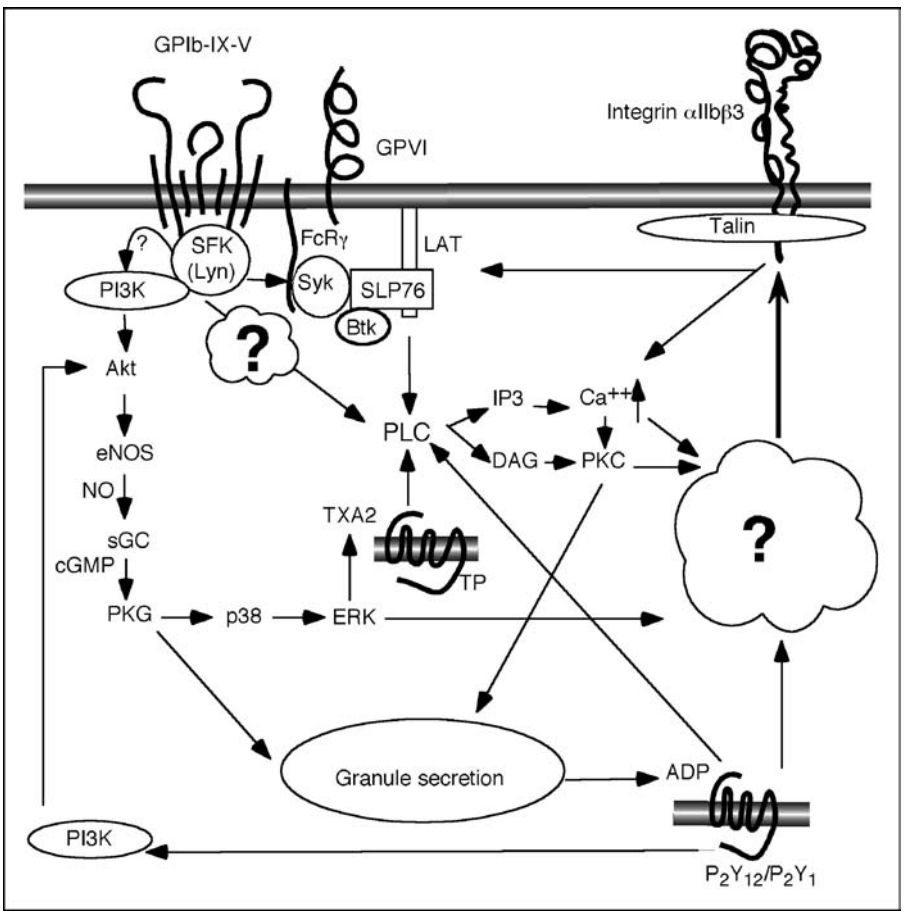
Glycoprotein Ib–IX-dependent signaling

VWF binding to GPIb–IX is known to induce activation of integrin $\alpha_{IIb}\beta_3$ and thus integrin-dependent platelet aggregation, spreading and secretion. VWF binding to GPIb–IX induces intracellular signaling events including calcium elevation, protein phosphorylation, phospholipase activation, thromboxane A_2 (TXA_2) production, phosphatidyl inositol metabolism and phosphoinositide 3-kinase (PI3K) activation [35–38]. GPIb–IX is also important in low-dose thrombin-induced signaling [39,40•] and mediates platelet activation by the antiβ2 glycoprotein I (β2GPI)–β2GPI complex in antiphospholipid syndrome [41]. GPIb–IX–V is a 'weak' agonist receptor. Thus, the full-scale platelet activation induced via GPIb–IX–V *in vitro* can be optimally shown only with platelets that are not desensitized during their isolation, which explains occasional reports that have queried the significance of GPIb–IX signaling [42]. Significant progress has been made in identifying molecules and pathways that are important in GPIb–IX signaling (Fig. 3), including molecules important in early GPIb–IX-specific signaling, in addition to those involved in common platelet activation or integrin signaling pathways and thus also important in GPIb–IX-mediated platelet activation (or its amplification), and those important in both the early GPIb–IX-specific signaling and common platelet activation pathways.

Early glycoprotein Ib–IX signaling mechanisms and the role of the Src family of protein kinases

The GPIb–IX-associated molecules, such as 14–3–3ζ, calmodulin, filamin and PI3K, are obvious candidates in the search for molecules important in early GPIb–IX signaling. Deletion of the entire C-terminal 14–3–3ζ binding domain in GPIbα diminishes GPIb–IX-mediated integrin activation in transfected CHO cells [43]. This effect can be explained not only by the inhibition of GPIb–IX signaling but also by the important role of 14–3–3ζ in the VWF binding function of

Figure 3 Glycoprotein Ib–IX (GPIb–IX) signaling pathways



For clear illustration, the pathways are simplified, omitting many established details. Btk, Bruton tyrosine kinase; DAG, diacylglycerol; eNOS, endothelial nitric oxide synthase; ERK, extracellular stimuli-responsive kinase; FcRγ, Fc receptor γ chain; IP3, inositol 1,4,5 triphosphate; LAT, linker for activation of T cells; NO, nitric oxide; PI3K, phosphoinositide 3-kinase; PKC, protein kinase C; PKG, protein kinase G; PLC, phospholipase C; SFK, Src family of protein kinase; sGC, soluble guanylyl cyclase; TP, the thromboxane-prostanoid receptor; TXA_2, thromboxane A_2. P2Y12 (Gi-coupled) and P2Y1 (Gq-coupled) are two different ADP receptors.

GPIb–IX [20]. Deletion of the C-terminal five amino acid residues of GPIbα reportedly also inhibits thrombin-induced, GPIb–IX-dependent activation of fibrin binding to $\alpha_{IIb}\beta_3$ [40•]. The complexity in the role of the 14–3–3 binding site in GPIbα is reflected by the report that a similar deletion of the GPIbα C-terminus increases cell spreading on VWF and fibrinogen. This was interpreted as the 14–3–3ζ–GPIb–IX interaction sequestering 14–3–3ζ and thus inhibiting the role of 14–3–3ζ in promoting integrin-induced activation of CDC42 and Rac [44]. This interpretation, however, does not explain why 14–3–3ζ inhibitors do not affect platelet activation induced by GPIb–IX-independent agonists [20].

14–3–3ζ has been reported to potentially mediate an association between GPIb–IX and PI3K [45]. A role of PI3K in GPIb–IX signaling has been suggested by the inhibitory effect of PI3K inhibitors [38,43]. Importantly, the Src family of protein kinase (SFK) members, c-Src and Lyn, have been reported to become associated with GPIb–IX following VWF stimulation [46]. This association appears to require the PI3K p85 subunit, suggesting the complex formation between SFK and PI3K. Functionally, VWF-induced platelet activation is abolished by inhibitors of SFK [47–49]. Furthermore, Lyn knockout mouse platelets showed diminished integrin-dependent platelet aggregation induced by botrocetin [50]. Thus, Lyn is important in GPIb–IX signaling.

Platelet adhesion to VWF initiates distinct waves of calcium elevation [51,52]. The early waves of calcium elevation are associated with GPIb–IX signaling and independent of PI3K. The later calcium elevation is integrin dependent [51,52]. Phospholipase Cγ2 appears to play an important role in GPIb–IX-mediated calcium elevation [47] and platelet aggregation [50]. SFK inhibitors abolish phospholipase Cγ2 activation [47], both waves of calcium elevation and PI3K activation [48], suggesting that SFK may be important early in GPIb–IX signaling.

The nitric oxide–cGMP–protein kinase G signaling pathway

Recent studies indicate that cGMP-dependent protein kinase (PKG) I plays a stimulatory role in GPIb–IX-mediated integrin activation and platelet aggregation [53]. PKG appears to be involved both in the GPIb–IX-specific signaling pathway leading to integrin activation and in the common aggregation-dependent granule secretion pathway that is important in amplification and stabilization of platelet aggregation [54,55,56•]. PKG is activated by intracellular cGMP. VWF binding to GPIb–IX activates the PI3K–Akt pathway (H. Yin, X. Du, unpublished data), which induces nitric oxide synthesis in platelets [56•,57•]. Nitric oxide stimulates cGMP synthesis. VWF-induced platelet cGMP elevation has been reported by at least two independent studies [53,57•]. A contradictory report may be explained in that the study was performed under the experimental conditions that apparently desensitize platelets [42]. The finding that the nitric oxide–cGMP–PKG pathway stimulates VWF-induced platelet activation is counter-intuitive to the concept that nitric oxide and cGMP inhibit platelet activation. This contradiction, however, is explained by the finding that nitric oxide and cGMP play timing and concentration-dependent biphasic roles in platelets [53,55,58]. Nitric oxide and cGMP promote platelet activation at low concentrations induced during platelet activation, but become inhibitory at higher concentrations.

The roles of ADP and thromboxane A_2

Botrocetin or ristocetin-induced, GPIb–IX-dependent platelet aggregation occurs in two waves. The first wave represents both the VWF-mediated crosslinking of platelets (agglutination) and integrin-dependent platelet aggregation. The second wave is the secretion and integrin-dependent irreversible platelet aggregation. The second wave of platelet aggregation is inhibited by antagonists of ADP and TXA_2, and thus requires both ADP and TXA_2 signaling pathways [49,59]. Under high shear flow conditions *in vitro* and *in vivo*, a two-stage platelet aggregation process has also been described. The early unstable platelet aggregation is independent of ADP but the second stage stable aggregation requires ADP [60•]. GPIb–IX signaling induces TXA_2 synthesis. TXA_2 synthesis, however, is significantly inhibited by integrin $\alpha_{IIb}\beta_3$ blockers, suggesting that a major part of TXA_2 synthesis requires integrin signaling that follows the early phase of platelet aggregation [50]. In the absence of fibrinogen, the integrin-dependent platelet aggregation induced by ristocetin requires TXA_2-dependent granule secretion [49]. The first wave integrin-dependent platelet aggregation in plasma, however, is not abolished by aspirin, indicating that VWF induces initial integrin activation via a TXA_2-independent pathway [61]. This notion is consistent with the findings that platelet activation induced by immobilized recombinant VWF functional domain can be independent of both ADP and TXA_2 [48]. Thus, both ADP and TXA_2 pathways are important amplification mechanisms that greatly enhance GPIb–IX-mediated platelet activation.

Mitogen-activated protein kinases

Several studies have now shown that extracellular stimuli-responsive kinase (ERK) is activated following VWF binding to GPIb–IX and is important in GPIb–IX-mediated platelet activation *in vitro* and *in vivo* [49,61,62,63••,64]. Inhibitors of the ERK pathway inhibit the GPIb–IX-mediated second wave of platelet aggregation and TXA_2 production [49,61]. These inhibitors, however, also inhibited integrin-dependent first-wave

platelet aggregation even in the presence of aspirin in platelet-rich plasma [61]. Furthermore, dominant negative mutants of the upstream enzymes of the ERK pathway (c-Raf and MEK) inhibited ristocetin-induced integrin activation in transfected CHO cells [61]. Thus ERK is important not only in stimulating the TXA_2 pathway, but also with respect to the TXA_2-independent integrin activation pathway. More recently, another mitogen-activated protein kinase (MAPK), p38, has been shown to be important in GPIb–IX signaling, and in activation of the ERK pathway [63••]. GPIb–IX-mediated p38 and ERK activation is downstream of SFK [49], and the cGMP–PKG pathway [61,63••]. These studies delineate a new signaling pathway in which GPIb–IX sequentially activates SFK, nitric oxide–cGMP–PKG, p38 and ERK pathways, leading to integrin activation.

The immunoreceptor tyrosine-based activation motif signaling pathway

Human platelets express two immunoreceptor tyrosine-based activation motif (ITAM) receptors, the Fc receptor γ chain (FcRγ) and Fcγ receptor IIA (FcγRIIA). The ITAM domain of these receptors, when dual tyrosine phosphorylated, interacts with syk, which signals through the adapter proteins, SLP76 and LAT, leading to activation of phospholipase C γ2, thus calcium mobilization and protein kinase C activation. There have been data suggesting an association of GPIb–IX with FcγRIIA [65] and FcRγ [66]. VWF binding to GPIb–IX induces tyrosine phosphorylation of ITAMs [48]. Wild type mouse platelets that do not express FcγRIIA, and FcγR-deficient mouse platelets (which express neither), however, still respond to VWF, indicating that the ITAM pathway is not essential for the early GPIb–IX signaling leading to integrin activation [48,50]. Thus, the ITAM pathway may be an amplification mechanism that promotes GPIb–IX-induced platelet secretion [50]. This pathway is negatively regulated by platelet–endothelial cell adhesion molecule-1 [67]. Interestingly, FcRγ plays a role in amplifying integrin outside-in signaling [68], and is the major signaling molecule for the collagen receptor, GPVI. In this regard, it has been reported that GPIb–IX is associated with GPVI in platelets [69], and an anti-GPIbα monoclonal antibody inhibits collagen and collagen-related peptide-induced platelet aggregation [69,70]. Thus, it is also possible that the GPIb–IX signaling pathway and the GPVI-mediated ITAM pathway act synergistically to induce platelet activation.

Other signaling molecules involved in glycoprotein Ib–IX signaling

Liu *et al.* [71•] reported that the botrocetin-induced second wave of platelet aggregation is abolished in mouse platelets expressing functionally defective Bruton tyrosine kinase (Btk), suggesting a role of Btk in GPIb–IX signaling. Kasirer-Friede *et al.* [72•] reported that the adhesion and degranulation promoting adapter protein (ADAP) is important in integrin activation induced by GPIb–IX and other agonists. Finally, the platelet purinergic receptor, P2X1, has been shown to augment platelet activation induced by GPIb–IX and other agonists [73].

Conclusion

Significant progress has been made in identifying molecules and pathways important in GPIb–IX regulation and signaling (Fig. 3). The identification of 14–3–3ζ-dependent regulation of VWF binding to GPIb–IX and the development of 14–3–3ζ inhibitors provides a potential target for antithrombotic drug development. The molecular mechanisms of GPIb–IX regulation, the 'specific' molecular pathway for early GPIb–IX signaling, as well as the interplays between the multiple 'amplification' pathways, however, remain major challenges to investigators in this field.

Acknowledgement

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 298–299).

1 Luo SZ, Mo X, Afshar-Kharghan V, *et al.* Glycoprotein Ib{alpha} forms • disulfides with two glycoprotein Ib{beta} subunits in the resting platelet. Blood 2007; 109:603–609.

Challenging the current concept that GPIbα and GPIbβ form a 1 : 1 complex, this paper presents data suggesting that 2 GPIbβ chains are complexed with one GPIbα chain.

2 Huizinga EG, Tsuji S, Romijn RA, *et al.* Structures of glycoprotein Ibalpha and its complex with von Willebrand factor A1 domain. Science 2002; 297: 1176–1179.

3 Uff S, Clemetson JM, Harrison T, *et al.* Crystal structure of the platelet glycoprotein Ib(alpha) N-terminal domain reveals an unmasking mechanism for receptor activation. J Biol Chem 2002; 277:35657–35663.

4 Celikel R, McClintock RA, Roberts JR, *et al.* Modulation of alpha-thrombin function by distinct interactions with platelet glycoprotein Ibalpha. Science 2003; 301:218–221.

5 Dumas JJ, Kumar R, Seehra J, *et al.* Crystal structure of the GpIbalpha–thrombin complex essential for platelet aggregation. Science 2003; 301: 222–226.

6 Nakamura F, Pudas R, Heikkinen O, *et al.* The structure of the GPIb–filamin A complex. Blood 2006; 107:1925–1932.

7 Simon DI, Chen Z, Xu H, *et al.* Platelet glycoprotein ibalpha is a counterreceptor for the leukocyte integrin Mac-1 (CD11b/CD18). J Exp Med 2000; 192:193–204.

8 Romo GM, Dong JF, Schade AJ, *et al.* The glycoprotein Ib–IX–V complex is a platelet counterreceptor for P-selectin. J Exp Med 1999; 190:803–814.

9 Baglia FA, Shrimpton CN, Emsley J, *et al.* Factor XI interacts with the leucine-rich repeats of glycoprotein Ibalpha on the activated platelet. J Biol Chem 2004; 279:49323–49329.

10 Bradford HN, Pixley RA, Colman RW. Human factor XII binding to the glycoprotein Ib–IX–V complex inhibits thrombin-induced platelet aggregation. J Biol Chem 2000; 275:22756–22763.

11 Bradford HN, Dela Cadena RA, Kunapuli SP, *et al.* Human kininogens regulate thrombin binding to platelets through the glycoprotein Ib–IX–V complex. Blood 1997; 90:1508–1515.

12 Jurk K, Clemetson KJ, de Groot PG, *et al.* Thrombospondin-1 mediates platelet adhesion at high shear via glycoprotein Ib (GPIb): an alternative/backup mechanism to von Willebrand factor. FASEB J 2003; 17:1490–1492.

13 Wang Y, Sakuma M, Chen Z, *et al.* Leukocyte engagement of platelet glycoprotein Ibalpha via the integrin Mac-1 is critical for the biological response to vascular injury. Circulation 2005; 112:2993–3000.

14 •• Bergmeier W, Piffath CL, Goerge T, *et al.* The role of platelet adhesion receptor GPIbalpha far exceeds that of its main ligand, von Willebrand factor, in arterial thrombosis. Proc Natl Acad Sci U S A 2006; 103:16900–16905.
This report provides convincing data that the role of GPIb–IX is not limited to initial platelet adhesion to the site of vascular injury, but is also important in growth and stability of thrombi.

15 • Reininger AJ, Heijnen HF, Schumann H, *et al.* Mechanism of platelet adhesion to von Willebrand factor and microparticle formation under high shear stress. Blood 2006; 107:3537–3545.
This paper shows a mechanism by which platelet adhesion to VWF induces formation of platelet-derived microparticles important in facilitating the coagulation process.

16 Pontiggia L, Steiner B, Ulrichts H, *et al.* Platelet microparticle formation and thrombin generation under high shear are effectively suppressed by a monoclonal antibody against GPIba. Thromb Haemost 2006; 96:774–780.

17 Coller BS. Inhibition of von Willebrand factor-dependent platelet function by increased platelet cyclic AMP and its prevention by cytoskeleton-disrupting agents. Blood 1981; 57:846–855.

18 Bodnar RJ, Xi X, Li Z, *et al.* Regulation of glycoprotein Ib–IX–von Willebrand factor interaction by cAMP-dependent protein kinase-mediated phosphorylation at Ser 166 of glycoprotein Ibbeta. J Biol Chem 2002; 277:47080–47087.

19 Wardell MR, Reynolds CC, Berndt MC, *et al.* Platelet glycoprotein Ib beta is phosphorylated on serine 166 by cyclic AMP-dependent protein kinase. J Biol Chem 1989; 264:15656–15661.

20 Dai K, Bodnar R, Berndt MC, Du X. A critical role for 14–3–3zeta protein in regulating the VWF binding function of platelet glycoprotein Ib–IX and its therapeutic implications. Blood 2005; 106:1975–1981.

21 Du X, Fox JE, Pei S. Identification of a binding sequence for the 14–3–3 protein within the cytoplasmic domain of the adhesion receptor, platelet glycoprotein Ibα. J Biol Chem 1996; 271:7362–7367.

22 Bodnar RJ, Gu M, Li Z, *et al.* The cytoplasmic domain of the platelet glycoprotein Ibα is phosphorylated at serine^{609}. J Biol Chem 1999; 274: 33474–33479.

23 Andrews RK, Harris SJ, McNally T, Berndt MC. Binding of purified 14–3–3 zeta signaling protein to discrete amino acid sequences within the cytoplasmic domain of the platelet membrane glycoprotein Ib–IX–V complex. Biochemistry 1998; 37:638–647.

24 Mangin P, David T, Lavaud V, *et al.* Identification of a novel 14–3–3{zeta} binding site within the cytoplasmic tail of platelet glycoprotein Ib{alpha}. Blood 2004; 104:420–427.

25 Dong JF, Li CQ, Sae TG, *et al.* The cytoplasmic domain of glycoprotein (GP) Ibalpha constrains the lateral diffusion of the GP Ib–IX complex and modulates von Willebrand factor binding. Biochemistry 1997; 36:12421–12427.

26 Kanaji T, Russell S, Cunningham J, *et al.* Megakaryocyte proliferation and ploidy regulated by the cytoplasmic tail of glycoprotein Ibalpha. Blood 2004; 104:3161–3168.

27 Fox JE, Berndt MC. Cyclic AMP-dependent phosphorylation of glycoprotein Ib inhibits collagen-induced polymerization of actin in platelets. J Biol Chem 1989; 264:9520–9526.

28 Dopheide SM, Maxwell MJ, Jackson SP. Shear-dependent tether formation during platelet translocation on von Willebrand factor. Blood 2002; 99:159–167.

29 Englund GD, Bodnar RJ, Li Z, *et al.* Regulation of von Willebrand factor binding to the platelet glycoprotein Ib–IX by a membrane skeleton-dependent inside-out signal. J Biol Chem 2001; 276:16952–16959.

30 Feng S, Resendiz JC, Lu X, Kroll MH. Filamin A binding to the cytoplasmic tail of glycoprotein Ibalpha regulates von Willebrand factor-induced platelet activation. Blood 2003; 102:2122–2129.

31 David T, Ohlmann P, Eckly A, *et al.* Inhibition of adhesive and signaling functions of the platelet GPIb–V–IX complex by a cell penetrating GPIbalpha peptide. J Thromb Haemost 2006; 4:2645–2655.

32 Ramakrishnan V, DeGuzman F, Bao M, *et al.* A thrombin receptor function for platelet glycoprotein Ib–IX unmasked by cleavage of glycoprotein V. Proc Natl Acad Sci U S A 2001; 98:1823–1828.

33 Aktas B, Pozgajova M, Bergmeier W, *et al.* Aspirin induces platelet receptor shedding via ADAM17 (TACE). J Biol Chem 2005; 280:39716–39722.

34 Rabie T, Strehl A, Ludwig A, Nieswandt B. Evidence for a role of ADAM17 (TACE) in the regulation of platelet glycoprotein V. J Biol Chem 2005; 280:14462–14468.

35 De Marco L, Girolami A, Russell S, Ruggeri ZM. Interaction of asialo von Willebrand factor with glycoprotein Ib induces fibrinogen binding to the glycoprotein IIb/IIIa complex and mediates platelet aggregation. J Clin Invest 1985; 75:1198–1203.

36 Gralnick HR, Williams SB, Coller BS. Asialo von Willebrand factor interactions with platelets: interdependence of glycoproteins Ib and IIb/IIIa for binding and aggregation. J Clin Invest 1985; 75:19–25.

37 Kroll MH, Harris TS, Moake JL, *et al.* von Willebrand factor binding to platelet GpIb initiates signals for platelet activation. J Clin Invest 1991; 88:1568–1573.

38 Yap CL, Anderson KE, Hughan SC, *et al.* Essential role for phosphoinositide 3-kinase in shear-dependent signaling between platelet glycoprotein Ib/V/IX and integrin alpha(IIb)beta(3). Blood 2002; 99:151–158.

39 Greco NJ, Tandon NN, Jones GD, *et al.* Contributions of glycoprotein Ib and the seven transmembrane domain receptor to increases in platelet cytoplasmic [Ca2+] induced by alpha-thrombin. Biochemistry 1996; 35:906–914.

40 • Pabon D, Jayo A, Xie J, *et al.* Thrombin induces GPIb–IX-mediated fibrin binding to alphabeta in a reconstituted Chinese hamster ovary cell model. J Thromb Haemost 2006; 4:2238–2247.
This paper suggests that thrombin induces activation of fibrin-binding to integrin $\alpha_{IIb}\beta_3$ via a signaling pathway that requires GPIb–IX. Deletion of the 14–3–3ζ binding region in the cytoplasmic domain of GPIbα inhibits GPIb–IX-mediated fibrin binding to $\alpha_{IIb}\beta_3$, suggesting that 14–3–3ζ–GPIba interaction is important in this signaling process.

41 Shi T, Giannakopoulos B, Yan X, *et al.* Antibeta2-glycoprotein I antibodies in complex with beta2-glycoprotein I can activate platelets in a dysregulated manner via glycoprotein Ib–IX–V. Arthritis Rheum 2006; 54:2558–2567.

42 Du X, Marjanovic JA, Li Z. On the roles of cGMP and glycoprotein Ib in platelet activation. Blood 2004; 103:4371–4372.

43 Gu M, Xi X, Englund GD, *et al.* Analysis of the roles of 14–3–3 in the platelet glycoprotein Ib–IX-mediated activation of integrin αIIbβ3 using a reconstituted mammalian cell expression model. J Cell Biol 1999; 147:1085–1096.

44 Bialkowska K, Zaffran Y, Meyer SC, Fox JE. 14–3–3 zeta mediates integrin-induced activation of Cdc42 and Rac: platelet glycoprotein Ib–IX regulates integrin-induced signaling by sequestering 14–3–3 zeta. J Biol Chem 2003; 278:33342–33350.

45 Munday AD, Berndt MC, Mitchell CA. Phosphoinositide 3-kinase forms a complex with platelet membrane glycoprotein Ib–IX–V complex and 14–3–3zeta. Blood 2000; 96:577–584.

46 Wu Y, Asazuma N, Satoh K, *et al.* Interaction between von Willebrand factor and glycoprotein Ib activates Src kinase in human platelets: role of phosphoinositide 3-kinase. Blood 2003; 101:3469–3476.

47 Mangin P, Yuan Y, Goncalves I, *et al.* Signaling role for phospholipase C gamma 2 in platelet glycoprotein Ib alpha calcium flux and cytoskeletal reorganization: involvement of a pathway distinct from FcR gamma chain and Fc gamma RIIA. J Biol Chem 2003; 278:32880–32891.

48 Kasirer-Friede A, Cozzi MR, Mazzucato M, *et al.* Signaling through GP Ib–IX–V activates alpha IIb beta 3 independently of other receptors. Blood 2004; 103:3403–3411.

49 Garcia A, Quinton TM, Dorsam RT, Kunapuli SP. Src family kinase-mediated and Erk-mediated thromboxane A2 generation are essential for VWF/GPIb-induced fibrinogen receptor activation in human platelets. Blood 2005; 106:3410–3414.

50 Liu J, Pestina TI, Berndt MC, *et al.* Botrocetin/VWF-induced signaling through GPIb–IX–V produces TxA2 in an alphaIIbbeta3- and aggregation-independent manner. Blood 2005; 106:2750–2756.

51 Mazzucato M, Pradella P, Cozzi MR, *et al.* Sequential cytoplasmic calcium signals in a 2-stage platelet activation process induced by the glycoprotein Ibalpha mechanoreceptor. Blood 2002; 100:2793–2800.

52 Nesbitt WS, Kulkarni S, Giuliano S, *et al.* Distinct glycoprotein Ib/V/IX and integrin alpha IIbbeta 3-dependent calcium signals cooperatively regulate platelet adhesion under flow. J Biol Chem 2002; 277:2965–2972.

53 Li Z, Xi X, Gu M, *et al.* A stimulatory role for cGMP-dependent protein kinase in platelet activation. Cell 2003; 112:77–86.

54 Li Z, Zhang G, Marjanovic JA, *et al.* A platelet secretion pathway mediated by cGMP-dependent protein kinase. J Biol Chem 2004; 279: 42469–42475.

55 Marjanovic JA, Li Z, Stojanovic A, Du X. Stimulatory roles of nitric oxide synthase 3 and guanylyl cyclase in platelet activation. J Biol Chem 2005; 280:37430–37438.

56 Stojanovic A, Marjanovic JA, Brovkovych VM, *et al.* A phosphoinositide
• 3-kinase–AKT–nitric oxide–cGMP signaling pathway in stimulating platelet secretion and aggregation. J Biol Chem 2006; 281:16333–16339.
This paper provides evidence that PI3K can signal by activating Akt, which activates the nitric oxide–cGMP pathway, leading to platelet secretion and amplification of platelet aggregation. The data also suggest that nitric oxide plays biphasic roles in platelet activation. This paper does not provide, however, direct evidence whether PI3K signals via this pathway during GPIb–IX-mediated platelet activation.

57 Riba R, Oberprieler NG, Roberts W, Naseem KM. von Willebrand factor
• activates endothelial nitric oxide synthase in blood platelets by a glycoprotein Ib-dependent mechanism. J Thromb Haemost 2006; 4:2636–2644.
This paper provides data that VWF binding to GPIb–IX induces nitric oxide synthesis and elevation of cGMP, suggesting that GPIb–IX mediates activation of endothelial nitric oxide synthase which is responsible for cGMP elevation.

58 Li Z, Ajdic J, Eigenthaler M, Du X. A predominant role for cAMP-dependent protein kinase in the cGMP-induced phosphorylation of vasodilator-stimulated phosphoprotein and platelet inhibition in humans. Blood 2003; 101:4423–4429.

59 Liu J, Pestina TI, Berndt MC, *et al.* The roles of ADP and TXA in botrocetin/VWF-induced aggregation of washed platelets. J Thromb Haemost 2004; 2:2213–2222.

60 Maxwell MJ, Westein E, Nesbitt WS, *et al.* Identification of a two-stage platelet
• aggregation process mediating shear-dependent thrombus formation. Blood 2007; 109:566–576.
GPIb–IX-mediated two-wave platelet aggregation induced by botrocetin and ristocetin is known. This study provides data showing that the two-wave aggregation also occurs during platelet adhesion under high shear flow conditions *in vitro* and *in vivo*.

61 Li Z, Xi X, Du X. A mitogen-activated protein kinase-dependent signaling pathway in the activation of platelet integrin αIIbβ3. J Biol Chem 2001; 276:42226–42232.

62 Mazharian A, Roger S, Maurice P, *et al.* Differential involvement of ERK2 and p38 in platelet adhesion to collagen. J Biol Chem 2005; 280:26002–26010.

63 Li Z, Zhang G, Feil R, *et al.* Sequential activation of p38 and ERK pathways by
•• cGMP-dependent protein kinase leading to activation of the platelet integrin {alpha}IIb{beta}3. Blood 2006; 107:965–972.
This study shows that VWF binding to GPIb–IX sequentially activates the cGMP–PKG pathway, the p38 MAPK pathway, and the ERK pathway, and thus reveals a new signaling pathway leading to integrin activation. The roles of cGMP, p38 and ERK are not only shown in platelets, but also in transfected CHO cells that do not have platelet granules, suggesting that the roles of these molecules in GPIb–IX-mediated integrin activation are independent of granule secretion.

64 Oury C, Daenens K, Hu H, *et al.* ERK2 activation in arteriolar and venular murine thrombosis: platelet receptor GPIb vs. P2X. J Thromb Haemost 2006; 4:443–452.

65 Sullam PM, Hyun WC, Szollosi J, *et al.* Physical proximity and functional interplay of the glycoprotein Ib–IX–V complex and the Fc receptor FcgammaRIIA on the platelet plasma membrane. J Biol Chem 1998; 273:5331–5336.

66 Wu Y, Suzuki-Inoue K, Satoh K, *et al.* Role of Fc receptor gamma-chain in platelet glycoprotein Ib-mediated signaling. Blood 2001; 97:3836–3845.

67 Rathore V, Stapleton MA, Hillery CA, *et al.* PECAM-1 negatively regulates GPIb/V/IX signaling in murine platelets. Blood 2003; 102:3658–3664.

68 Liu J, Ware J, Jackson CW, Gartner TK. FcRgamma-chain dependent alphallbbeta3 elicited outside-in signaling. J Thromb Haemost 2007; 5:426–428.

69 Arthur JF, Gardiner EE, Matzaris M, *et al.* Glycoprotein VI is associated with GPIb–IX–V on the membrane of resting and activated platelets. Thromb Haemost 2005; 93:716–723.

70 Ruan CG, Du XP, Xi XD, *et al.* A murine antiglycoprotein Ib complex monoclonal antibody, SZ 2, inhibits platelet aggregation induced by both ristocetin and collagen. Blood 1987; 69:570–577.

71 Liu J, Fitzgerald ME, Berndt MC, *et al.* Bruton tyrosine kinase is essential for
• botrocetin/VWF-induced signaling and GPIb-dependent thrombus formation in vivo. Blood 2006; 108:2596–2603.
This study identifies that Btk is important in GPIb–IX-dependent platelet activation. Btk activation appears to be downstream of SFK and PI3K, and also requires SLP76, a molecule involved in the ITAM pathway. It remains to be established whether Btk is important in the early GPIb–IX signaling pathway or in the ITAM pathway or other amplification pathways.

72 Kasirer-Friede A, Moran PB, Nagrampa-Orje J, *et al.* ADAP is required for
• normal {alpha}IIb{beta}3 activation by VWF/GP Ib-IX-V and other agonists. Blood 2007; 109:1018–1025.
This study suggests an important role for ADAP in the integrin activation pathways induced by several platelet agonists including GPIb–IX.

73 Oury C, Sticker E, Cornelissen H, *et al.* ATP augments von Willebrand factor-dependent shear-induced platelet aggregation through Ca2+-calmodulin and myosin light chain kinase activation. J Biol Chem 2004; 279: 26266–26273.