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The Organizing Principle of Platelet Glycoprotein Ib-IX-V Complex

Renhao Li[*] and **Jonas Emsley**[†]

[*]Department of Pediatrics, Aflac Cancer and Blood Disorders Center, Emory University School of Medicine, Atlanta, GA, USA

[†]Center for Biomolecular Sciences, School of Pharmacy, University of Nottingham, Nottingham, UK

Summary

The glycoprotein (GP)Ib-IX-V complex is the platelet receptor for von Willebrand factor and many other molecules critically involved in he most as is and thrombosis. The lack of functional GPIb-IX-V complexes on the platelet surface is the cause of Bernard-Soulier syndrome, a rare hereditary bleeding disorder also associated with macro thrombocytopenia. The GPIb-IX-V complex contains GPIbα, GPIbβ, GPIX and GPV subunits, all of which are type I trans membrane proteins containing leucine-rich repeat domains. Although all the subunits were identified decades ago, not until recently did the mechanism of complex assembly begin to emerge from a systematic characterization of inter-subunit interactions. This review summarizes forces driving the assembly of the GPIb-IX-V complex, discusses their implication on the pathogenesis of Bernard-Soulier syndrome, and identifies questions that remain about the structure and organization of GPIb-IX-V.

Keywords

GPIb-IX-V complex; platelet glycoprotein; Bernard-Soulier syndrome; leucine-rich repeat domain; transmembrane domain

Introduction

The glycoprotein (GP)Ib-IX-V complex is expressed exclusively on platelets and mega karyocytes. It is the second most abundant receptor complex on human platelets. The importance of this receptor complex to he most as is was demonstrated when GPIb was discovered as the missing protein in patients with the Bernard-Soulier syndrome (BSS), a hereditary bleeding disorder [1]. Although GPIb-IX-V primarily functions as a platelet receptor for von Willebrand factor (VWF), it can also bind to other ligands present in circulation such as thrombin, P-select in, integr in $\alpha_M\beta_2$, factor XI, factor XII, high-molecular-weight kininogen[2–8], as well as a number of snake venom proteins[recently reviewed in 9]. Eliminating these interactions either by employing specific inhibitors or genetically removing target proteins in animal models has established critical roles of GPIb-IX-V in thrombosis, inflammation, metastasis, and the life cycle of platelets [10–17].

[*]Corresponding author: Renhao Li, Department of Pediatrics, Emory University School of Medicine, 2015 Upper gate Drive NE, Atlanta, GA 30322. Phone: 404-727-8217; Fax: 404-727-4859; renhao.li@emory.edu.

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GPIb-IX-V contains 4 different subunits: GPIbα, GPIbβ, GPIX and GPV. Although the association of these subunits was demonstrated more than 20 years ago [18, 19], not until recently was the mechanism of complex assembly elucidated. Recent reports showed that GPIb-IX is a highly integrated membrane protein complex that is weakly associated with GPV. While the full-length GPIb-IX complex is stable and can be purified directly from platelet lysates [20], many segments of GPIb-IX are not when individually produced. In fact, there have been only two reports of direct observation of interactions between GPIb-IX-derived protein domains, both of which involved highly specialized conditions and methods[21, 22]. This review summarizes recent progress in understanding the structure and organization of human GPIb-IX-V, discusses the implication of these studies on the pathogenesis of BSS, and lists remaining questions about the organization of the complex.

Structure of each GPIb-IX-V subunit

To understand the organization of GPIb-IX-V, it is necessary to discuss the primary domain structure of each subunit. Each subunit of the complex is a type I transmembrane protein, consisting of a large N-terminal extra cellular domain, a single-pass transmembrane (TM) helix and a relatively short cytoplasmic tail de void of enzymatic activity. The extra cellular domain of each subunit contains a leucine-rich repeat (LRR) structural domain of variable size.

GPIbα is the major subunit and possesses all of the known extra cellular ligand-binding sites in the complex (Fig. 1). The crystal structure of its N-terminal LRR domain reveals a single disulfide bond (Cys4-Cys17) in the N-capping region, 7 LRR sequences in the central parallel β-coil region, and two disulfide bonds (Cys209-Cys248, Cys211-Cys264) in the C-capping region[23].The parallel β-coil region is composed of stacking layers of 3-sided coils (Fig. 1B). Each side of the coil is named the linker, convex loop and concave β-strand. Two N-glycosylation sites (Asn 21, Asn 159) are located in the β-coil region. Following the LRR domain are an acidic residue-rich sequence containing sulfated tyrosines and the thrombin-binding site, a heavily O-glycosylated region known as macroglyco peptide, and a stalk region of approximately 40–50 residues (Fig. 1A). Two vicinal cysteine residues (Cys484, Cys485)at the junction of extra cellular and TM domains form disulfide bonds with GPIbβ[24].The GPIbα cytoplasmic tail can bind to filamin A, 14-3-3ς and phosphoinositide 3-kinase[25–28]. Several residues in the cytoplasmic domain, including Ser587, Ser590 and Ser609, can be phosphorylated [29–31].

In the GPIbβ extra cellular domain, both N- and C-capping regions flanking the LRR sequence contain 2 interlocking disulfide bonds [22]. Containing only a single LRR, the parallel β-coil region is much less curved than that in GPIbα(Fig. 1B).There is one N-glycosylation site (Asn41). Residue Cys122 located at the junction of extra cellular and TM domains forms a disulfide bond with GPIbα (Fig. 1C), whereas residue Cys148 located at the junction of TM and cytoplasmic domains is palmitoy lated [32]. The GPIbβ cytoplasmic domain contains approximately 30 residues. Its juxtamembrane region is enriched in basic residues and can associate with calmodulin and TNF receptor-associated factor 4[33, 34].Residue Ser166 located in the distal region is phosphorylated and is involved in association with 14-3-3ς[35, 36].

The GPIX extra cellular domain, also containing a single LRR sequence, shares over 45% sequence identity with its GPIbβ counterpart. It is therefore expected to take on a similar structure. However, its TM and cytoplasmic sequences differ significantly from their GPIbβ counterparts. The GPIX cytoplasmic tail contains only 8 residues and is not known to associate with any intracellular proteins. Like GPIbβ, GPIX contains amyristoylated residue

Cys154 at the junction of TM and cytoplasmic domains [32]. It should be noted that mouse GPIbβ and GPIX do not have acylated cysteines.

The extra cellular domain of GPV contains 13 LRR sequences that are flanked by N- and C-capping regions, both of which contain 2 interlocking disulfide bonds. Following the LRR domain area stalk region, the TM helix, and a short cytoplasmic tail that contains several basic residues.

TM and extra cellular domain interactions in the assembly of GPIb-IX

GPIb-IX is a highly stable complex containing GPIbα, GPIbβ and GPIX at a ratio of 1:2:1[18, 20, 24]. Mutations from BSS patients map to GPIbα, GPIbβ or GPIX genes, indicating that a mutation in any of the three subunits can abolish GPIb-IX expression in platelets [37]. A requirement for the concurrent expression of all three subunits to enable efficient expression of GPIb-IX on the cell surface has also been reproduced in transfected mammalian cells [38].Further dissection of GPIb-IX expression in transfected cells suggests that GPIbα, GPIbβ and GPIX interact with and stabilize one another[39–42].Thus, the expression level of GPIb-IX on the cell surface can be used as an indicator for complex assembly. Coupling the mutational analysis with structural and biophysical characterization, recent studies have identified three inter-subunit interactions in GPIb-IX— disulfide bonds between GPIbα and GPIbβ, interactions between TM domains, and interactions between GPIbβ and GPIX extra cellular domains(Fig. 1C).

It was demonstrated recently that GPIbα is connected via disulfide bonds with two GPIbβ subunits [24]. Residues Cys484 and Cys485 in GPIbβ and Cys122 in GPIbα are involved since mutating anyone of these residues to serine abolish esdisulfide formation between the subunits[24, 43]. However, these mutations did not significantly impact the expression level of GPIb-IX in transfected cells, suggesting that these disulfide bonds are not necessary for proper assembly of the complex[24, 43].

Interaction between the TM domains of GPIb-IX was first suggested when it was observed that replacing the TM domain of any subunit with a poly-leucine-alanine sequence resulted in a significant decrease in GPIb-IX expression on the surface of transfected cells [44, 45]. Consistent with these observations, the extra cellular domain of GPIbα, but not the combined extra cellular and TM domains, can be replaced with their counterparts from the α-subunit of interleukin-4 receptor without affecting GPIb-IX expression in transgenic mice[11]. In addition, a frame-shift mutation in the GPIbβ gene that produced new TM and cytoplasmic domains was identified in a patient with BSS [46], as was a nonsense mutation in the GPIX gene that eliminated its TM and cytoplasmic domains in another patient with BSS [47]. Besides decreasing expression of the GPIb-IX complex, formation of native juxtamembrane disulfide bonds between GPIbα and GPIbβ is also perturbed or disrupted when the TM domains of GPIb-IX are replaced [44, 45].

Direct interaction between the TM domains of GPIb-IX has been demonstrated using recombinant peptides containing individual GPIb-IX TM sequences. Mixing GPIbα and GPIbβ TM peptides in membrane-mimicking detergent micelles under reversible redox conditions can produce a GPIbβ-GPIbα-GPIbβ ($\alpha\beta_2$) tri peptide containing native disulfide bonds[24]. Addition of the GPIX TM peptide to the mixture facilitates $\alpha\beta_2$ complex formation, mimicking the effect of GPIX on GPIbα-GPIbβ disulfides formation in transfected cells[21]. Further, fluorescence resonance energy transfer was observed between Cy 3-labeled $\alpha\beta_2$ and a Cy 5-labeled GPIX TM peptide, while no energy transfer was observed between $\alpha\beta_2$ and a mutant D135K GPIX TM peptide[21]. These results showed that GPIb-IX TM domains interact with one another to form a parallel 4-helical bundle in the membrane and that formation of GPIbα-GPIbβ disulfide bonds depends on interactions

between the helices. In this 4-helical bundle, two GPIbβ TM helices should be located in diagonal to accommodate the distance constraints imposed by the two adjacent GPIbα-GPIbβ disulfide bonds (Fig. 1C).

There are conserved polar residues in GPIbα (Ser503), GPIbβ(Gln132, His139) and GPIX (Asp135) TM domains. Mutating these residues to a polar residues (*e.g.* H139L) or to residues of opposite charge (*e.g.* D135K) significantly decreases surface expression of GPIb-IX and hampers formation of native GPIbα-GPIbβ disulfide bonds[44, 45]. In the hydrophobic membrane, polar residues in a TM helix prefer to interact with other polar residues in other helices [48]. The polar residues in the GPIb-IX TM domains are clustered at two distinct depths, and likely interact with other polar residues at the same depth (*e.g.* Gln129 in GPIbβ/Asp135 in GPIX, Ser503 in GPIbα/His139 in GPIbβ).Further, scanning mutagenesis has identified several a polar residues, particularly leucine residues in the GPIX TM domain, as critical to proper assembly of GPIb-IX, suggesting that these residues are at the interfacial region within the helical bundle [44, 45]. Overall, it appears that a combination of polar interactions and leucine zipper interactions mediates association of the GPIb-IX TM helices. It is noteworthy that both acylated residues (Cys148 in GPIbβ and Cys154 in GPIX) are located on the opposite side in each TM helix from aforementioned polar residues, which is consistent with the expectation that the attached hydrocarbon acyl chain would interfere with association of GPIb-IX TM helices and thus should not be at the interface.

The interaction between extra cellular domains of GPIbβ and GPIX was first postulated when mutations in the GPIbβ extra cellular domain were found to abolish its ability to support surface expression of GPIX in transfected cells[40]. However, this interaction has resisted direct detection by conventional means for two reasons. First, while the isolated GPIbβ extra cellular domain can be secreted from transfected cells as a stable protein, the highly homologous GPIX extra cellular domain does not express as a well-folded protein and is presumably unstable [49]. This is consistent with a recent report that GPIX is a preferred substrate of molecular chaperone gp96/grp94[50]. Second, the effect of the extra cellular domain interaction is only manifest in the presence of neighboring TM domains. In contrast to full-length GPIbβ that can facilitate surface expression of GPIX in transfected cells, the isolated GPIbβ extra cellular domain is not able to support secretion of the GPIX extra cellular domain nor surface expression of full-length GPIX[49].

However, the interaction between extra cellular domains of GPIbβ and GPIX has been demonstrated using a GPIbβ/GPIX chimeric extra cellular domain called GPIbβ_{Eabc}49]. In this chimera protein, three convex loops from the GPIX extra cellular domain (Ala29-Arg36, Ser49-Gln60 and Ser76-Asp86) were grafted onto the GPIbβ scaffold. Like the isolated GPIbβ extra cellular domain, GPIbβ_{Eabc} is monomeric in aqueous solutions [22], but unlike the GPIbβ extra cellular domain, GPIbβ_{Eabc} is a homo tetramer in a crystal where the protein concentration is typically in millimolar range, much higher than in aqueous solutions. This suggests that the tetramerization constant for GPIbβ_{Eabc} is relatively weak. The crystal structure of the GPIbβ_{Eabc} homo tetramer shows that each interface between two GPIbβ_{Eabc} monomers consists of mostly residues from the C-capping region of GPIbβ on one side and residues from convex loops of GPIX on the other (Fig. 1C). In particular, the side chain of Tyr106 in GPIbβ interacts with hydrophobic side chains of several GPIX residues that form a shallow pocket at the interface [22]. Mutagenesis of crucial residues at the interface such as Tyr106 confirmed that the GPIbβ/GPIX interface shown in the crystal structure is a valid representation of that in the full-length complex.

There are one GPIX and two GPIbβ subunits in GPIb-IX. However, it was unclear how a single GPIX extra cellular domain, which does not appear to have an internal two-fold

symmetry, supports distinct binding sites for two GPIbβ extra cellular domains. The crystal structure of $GPIb\beta_{Eabc}$ tetramer not only reveals structural details of one GPIbβ/GPIX interface, but also provides clues about the other. In the $GPIb\beta_{Eabc}$ tetramer, the C-termini of all 4 monomers are located on the same side[22]. The distances between them match those between the N-termini of a 4-helical bundle. Therefore, if GPIbβ and GPIX extra cellular domains assemble as in the $GPIb\beta_{Eabc}$ tetramer, they can be accommodated on top of the adjacent TM helical bundle (Fig. 1C). Thus, the crystal structure of the $GPIb\beta_{Eabc}$ tetramer predicts that the second GPIbβ/GPIX interface, mimicking the first one, is between the C-capping region of GPIX and convex loops of second GPIbβ (Fig. 1C). Evidence supporting this suggestion came from a recent analysis that placed residue Pro74, located in the third convex loop of GPIbβ extra cellular domain, at or near the GPIbβ/GPIX interface [22]. Structural details of the second GPIbβ/GPIX interface, however, await further elucidation.

Lack of contribution of other regions to GPIb-IX assembly

Although the stalk region of GPIbα is located in proximity to the GPIbβ extra cellular domain in GPIb-IX, there is no evidence supporting a direct interaction between them. Replacing the stalk region of GPIbα, along with the rest of the extra cellular domain, with the extra cellular domain of the α-subunit of human interleukin-4 receptor, preserved surface expression levels of GPIbβ and GPIX in platelets[11]. Furthermore, co-immuno precipitation of a mutant GPIb-IX in which both GPIbα-GPIbβ disulfide bonds are removed due to C484S/C485S mutations in GPIbα showed that the noncovalent association of GPIbα with GPIbβ is mostly through the TM helices, in contrast to that of GPIbβ with GPIX in which both TM and extra cellular domains are involved [51].

Because truncation and single-site mutations in the GPIbα cytoplasmic domain designed to perturb the interaction of GPIbα with intracellular binding proteins such as filamin A did not reveal significant decreases in the level of GPIb-IX expression on the surface of transfected cells, it is likely that the GPIbα cytoplasmic domain does not actively participate in complex assembly[52–54]. Nonetheless, the expression level of GPIb-IX is decreased in mouse platelets lacking filamin A, but its expression is not altered in mega karyocytes. The decrease is likely due to an increase in metallo protease levels as a result of filamin A deletion rather than due to a defect in GPIb-IX assembly [55]. Similar to GPIbα, neither deleting the GPIX cytoplasmic domain nor replacing it with a poly-alanine sequence affected GPIb-IX assembly in transfected cells[45]. Fatty acids attached in human GPIbβ and GPIX cytoplasmic domains are not required for GPIb-IX assembly, since mutating both Cys148 in GPIbβ and Cys154 in GPIX did not alter GPIb-IX structure nor impact its expression [56].

Although deleting the membrane-distal portion of the GPIbβ cytoplasmic domain also did not affect GPIb-IX assembly, deleting the juxtamembrane region (residues 149–154) or replacing it with a poly-alanine sequence significantly decreased surface expression of GPIb-IX and interfered with formation of GPIbα-GPIbβ disulfide bonds as well[57, 58]. In contrast, replacing the juxtamembrane region of the GPIbα cytoplasmic domain with a poly-alanine sequence did not produce similar disruptive effects on GPIb-IX assembly (X. Mo and R. Li, unpublished results). This suggests that the juxtamembrane region of GPIbα and GPIbβ cytoplasmic domains do not interact. The juxtamembrane region of the GPIbβ cytoplasmic domain is the only region of the three GPIb-IX subunits that is enriched in basic residues. Basic residues in the juxtamembrane region of cytoplasmic domains often interact with negatively charged lipids in cell membranes, helping to mediate protein topology and vesicle trafficking in the cell [59, 60]. Therefore, it is conceivable that removing the basic

residues from the GPIbβ cytoplasmic domain could result in alteration of the GPIb-IX topology and decrease of its expression on the cell surface.

Weak association of GPV with GPIb-IX through TM domains

In contrast to the tight association between GPIb and GPIX [18], GPV is weakly associated with GPIb since immuno precipitation of GPV from platelet lysates using anti-GPIbα antibodies was only observed in the presence of digit on in but not NP-40 as the lys is detergent [19]. The weak association can potentially explain why in platelets GPV is shed faster than GPIbα[61]. Although deletion of GPV has little impact on the surface expression level of GPIb-IX in mice [62, 63], GPIb-IX is essential to efficient expression of GPV in the plasma membrane of transfected cells [64, 65]. Consistent with this observation, decreased GPV expression is often reported in platelets of BSS patients [37, 66].

Assessment of GPV expression in transfected Chinese hamster ovary (CHO) cells that also express various combinations of GPIb-IX subunits identified GPIbα as the critical subunit in GPIb-IX supporting efficient surface expression of GPV [64]. A recent study identified the TM helices of GPV and GPIbα as essential to the GPIb-IX enhancement of GPV expression in transfected CHO cells[67]. Scanning mutagenesis identified key residues in GPV and GPIbα TM helices that presumably form an interface between the two proteins (Fig. 2). Whether other domains of GPV are involved in the interaction with GPIb-IX is not known, although the sensitivity of GPIb-IX-V integrity to nonionic detergents such as NP-40 but not to digit on in suggests that the association is driven largely by polar interactions in the TM domains [68–70]. These results are consistent with the current structural model of GPIb-IX, as GPIbα TM residues important for GPV association do not overlap with those important for association with GPIbβ and GPIX. Moreover, the interface identified on the GPIbα TM helix is the only accessible region in the GPIb-IX TM helical bundle because direct contact with GPIbβ or GPIX TM helices would cause a severe steric clash with their extra cellular domains (Fig. 2). Unlike GPIbβ and GPIX, both GPIbα and GPV have a long stalk region preceding their TM helices, which should be flexible to accommodate the close contact between their TM helices.

Based on the expression levels of GPIb-IX and GPV on the platelet surface measured by antibody binding, it has been suggested that they form a complex with a stoichiometry of 2:1[19]. It was further suggested that GPV is sandwiched between two GPIbα subunits in the GPIb-IX-V complex [64]. However, there is no direct evidence supporting the 2:1 stoichiometry. Rather, a recent quantitative survey of proteins in platelets put the number of GPV molecules on par with that of GPIX [71]. Moreover, it is noteworthy that the putative GPIbα-binding interface in the GPV TM domain is restricted to one side of the α-helix, which is too small to support simultaneous association with two GPIbα TM helices[67]. The structural detail of the association of GPIb-IX with GPV awaits further elucidation, but at the present time one should not rule out the possibility that a population of GPIb-IX on the platelet surface is free of GPV association and may instead associate with other membrane receptors such as GPVI, Fcγ RIIA and apolipoprotein E receptor 2[72–74].

Differential pathogenesis of BSS mutations

BSS is characterized by the abnormally low expression of functional GPIb-IX-V complex on the platelet surface[37, 66]. Investigation of the structure and organization of the GPIb-IX-V complex has helped to identify mechanisms by which BSS mutations disrupt GPIb-IX assembly and expression. BSS mutations have been mapped to GPIbα, GPIbβ or GPIX genes, with new ones still being discovered[e.g. 75, 76]. For brevity reasons, references for many of the BSS mutations discussed below are not provided here but they have been compiled in recent reviews [37, 66, 77]. Since it is unlikely that each newly discovered BSS

mutation, or any mutation in GPIb-IX-V genes identified from a patient with bleeding diathesis, will be characterized extensively for its pathogenic origin, a brief review of the mechanisms of known BSS mutations will be helpful in future diagnosis of BSS and in identification of GPIb-IX mutations with novel mechanistic implications. For brevity reasons, only mutations that cause a change in GPIb-IX protein sequences will be discussed here. Those likely affecting transcription, translation or translocation process of GPIb-IX have been reviewed previously [37, 66].

Nonsense or frame-shift mutations in the GPIb-IX genes result in the production of a GPIb-IX subunit that is significantly shortened or altered from the native sequence and is missing large segments important for structural integrity of the host subunit and/or assembly of the entire complex. Since the extra cellular and TM domains participate in GPIb-IX assembly, it is not coincidental that all nonsense or frame-shifting mutations reported to date have been restricted to these two domains and not the cytoplasmic domains.

A missense BSS mutation can disrupt GPIb-IX assembly via two distinct but related mechanisms. First, a mutation can disrupt folding of the host domain or significantly destabilize it. Extensive characterization of the LRR domain has identified residues or positions that are critical to its structural integrity [78]. Therefore, it is understandable that mutations alter in gendogenous Cys residues in the disulfide bonds (*e.g.*. C209S in GPIbα, C5Y in GPIbβ, and C8R, C8W, C73Y, C97Y in GPIX) or adding additional Cys residues in N- and C-capping regions (*e.g.* R17C, Y88C in GPIbβ, and F55C, Y79C in GPIX) interfere with formation of interlocking disulfide bonds in both capping regions. Mutations that change key residues in the LRR sequence motif including the conserved Asn residue (*e.g.* N110D, L129P, W207G in GPIbα, N64T, P65R in GPIbβ, and L40P, N45S, L47P in GPIX) likely disrupt internal side chain packing of the central parallel β-coil. Mutations that remove large hydrophobic side chains from or add polar side chains to the interior of the LRR domain (*e.g.* Y54D, C65 Rin GPIbα) should have a similarly disruptive result. Effects of other mutations are more difficult to predict because they are located in a more flexible region or they affect residues with side chains exposed to the solvent (*e.g.* P29L, P96S in GPIbβ, and D21G, F55S in GPIX). To experimentally determine the effect of a mutation on LRR domain folding, one needs to express the mutant LRR domain in transfected cells and to analyze its expression, secretion as well as formation of intra molecular disulfide bonds [22, 49]. This is only applicable for mutations in GPIbα and GPIbβ LRR domains, since the GPIX extra cellular domain cannot be expressed in isolation [49].

Compared to the extra cellular domain, it is easier to predict the effect of a single-site mutation in the TM domain because the α-helical structure of the TM domain depends mostly on backbone, not side chain, atoms. Typically a missense mutation, even when it involves Gly or Pro, is not expected to significantly alter the α-helical structure of a TM domain[48]. However, it is possible for a mutation (*e.g.* a polar-to-polar mutation) to significantly lower the hydrophobicity of the TM domain, thereby impairing proper translocation of the host subunit to the membrane or leading to its rapid degradation[79, 80]. Such a mutation would therefore be expected to abolish proper expression of GPIb-IX in the membrane. The A140T mutation in GPIX [81] appears to fit into this mechanism, but its effect is mild or even negligible in many cases [45, 82, 83].

Second, a mutation causing BSS might not disrupt folding of the host domain but instead disrupt its interaction with other GPIb-IX subunits. Two mutations in the GPIbβ extra cellular domain, P74R and A108P, were recently shown to use this mechanism[22]. It is also possible for a mutation in the GPIX extra cellular domain to disrupt GPIb-IX assembly by the same mechanism, although the effect of such a mutation on the folding of GPIX extra cellular domain is difficult to assess[22]. In addition, a missense BSS mutation in the TM

domain can also utilize this mechanism if it does not alter the α-helical structure of the host TM domain. Recentlya BSS patient was reported to have a homozygous Q129H/L132P double mutation in the GPIbβ TM domain and concurrently a heterozygous Y492H mutation in the GPIbα TM domain [76]. Gln129 in the GPIbβ TM domain is an important residue to GPIb-IX assembly because of its interaction with GPIbα and GPIX counter parts [44, 45]. Tyr492 in GPIbα is located at the same depth in the membrane as Gln129 in GPIbβ. Changing it to histidine is likely to alter the nearby interactions involving Gln129.

Summary and remaining questions

To summarize, recent studies have demonstrated that the interaction among GPIbα, GPIbβ and GPIX TM helices, in cooperation with those among GPIbβ and GPIX extra cellular domains, provide the driving force for GPIb-IX assembly (Fig. 1). These interactions stabilize individual domains in the complex and prevent their premature degradation in the cell. Juxta membrane disulfide bonds between GPIbα and GPIbβ further stabilize the complex. GPV is weakly associated with the tightly integrated GPIb-IX complex, mostly through the interaction between GPV and GPIbα TM helices (Fig. 2). This model provides an explanation for the requirement of all subunits for efficient GPIb-IX expression in the plasma membrane, as well as for most of the mutations identified in BSS patients. At the same time, it is important to note that this model needs further refinement and revision, with the following observations about GPIb-IX, for instance, still a waiting explanation.

First, most BSS patients exhibit an autosomal recessive mode of inheritance, but in a few cases (N41H, Y54D, L57F, A156V in GPIbα), BSS appears to be autosomal dominant. BSS patients with heterozygous mutations in GPIbβ have also been reported, although detailed family pedigrees are not available [84, 85]. The affected patients generally have a mild bleeding tendency and mild macro thrombocytopenia. Considering the role of GPIb-IX in platelet adhesion, these clinical symptoms may be explained by the decreased but not totally abolished expression level of GPIbα. However, people with other heterozygous mutations, including nonsense mutations that should result in a 50% decrease of the GPIbα expression level, display neither a bleeding diathesis nor a clear difference in platelet size. Why the autosomal dominant inheritance is limited to the aforementioned mutations in GPIbα remains to be determined.

Second, a BSS patient was found to have a frame-shift mutation in one GPIbβ allele and a C122S mutation in the other [43]. The frame-shift mutation caused replacement of the GPIbβ TM and cytoplasmic domains with a42-residue sequence that does not participate in the GPIb-IX assembly. Although C122S abolishes formation of disulfide bonds between GPIbα and GPIbβ, it does not significantly decrease the complex expression in transfected cells [24, 43]. However, the expression levels of GPIbα, GPIbβ and GPIX in the patient's platelets were significantly lower than 50% of normal[43]. Lack of GPIbα-GPIbβ disulfide bonds may reduce the GPIb-IX stability, but the molecular mechanism by which the C122S mutation reduces GPIb-IX expression on the platelet surface remains to be defined.

Third, there are intriguing reports that dimerization or clustering of GPIb-IX plays a role in platelet activation and clearance. Because VWF is a multimeric protein, it is expected to bring together multiple copies of GPIbα in the plasma membrane, which in turn may partition into detergent-resistant membrane rafts where signaling molecules including kinases are enriched [86–88]. Consistent with this clustering hypothesis, the dimeric VWF A1 domains or some bivalent monoclonal antibodies targeting GPIbα, but not monomeric A1 or Fab fragments of the antibodies, can induce platelet aggregation [89–91]. Similarly, infusion of monoclonal antibodies targeting the extra cellular domain of murine GPIbα causes acute thrombocytopenia in mice via a mechanism that is independent of Fc receptors

[92]. The bivalent $F(ab')_2$ antibody fragment, but not its monomeric Fab fragment, can achieve the same effect [15, 92]. These results suggest that dimerization of GPIbα has an important biological function. However, not all antibodies targeting GPIbα activate platelets; some even show inhibitory capabilities[93]. Moreover, antibodies targeting GPIbβ and GPIX, which presumably achieve the same dimerizing effect of GPIb-IX as those targeting GPIbα, do not activate platelets or cause acute thrombocytopenia in mice [92, 94]. Consistently, artificially induced dimerization of the GPIX cytoplasmic domain in the GPIb-IX complex expressed in CHO cells is not sufficient to induce down stream activation of integrin α IIbβ 3, although it increases the avidity of association of GPIbα with VWF [95, 96]. Overall, these observations argue for an ordered dimerization of GPIbα rather than a non-specific cluster as a true signal generator. The presence of such a signaling GPIbβ dimer remains to be confirmed and its defining features elucidated.

Finally, a fundamental question about GPIb-IX assembly remains: what is the purpose of having such an elaborate interaction network in human GPIb-IX to ensure coexpression of GPIbα with GPIbβ and GPIX? It would seem wasteful if the sole purpose of having three subunits was to aid expression of the fourth one. Even when GPIbβ is partly justified because the GPIbβ cytoplasmic domain plays a role in modulating GPIbα function [97–99], why does GPIbα coexpress with GPIX? There are no obvious GPIb-IX para logs in other types of cells. With the implicit assumption that human GPIb-IX evolves from a simpler complex, comparing GPIb-IX assembly across species may lead to further understanding of its origin and function.

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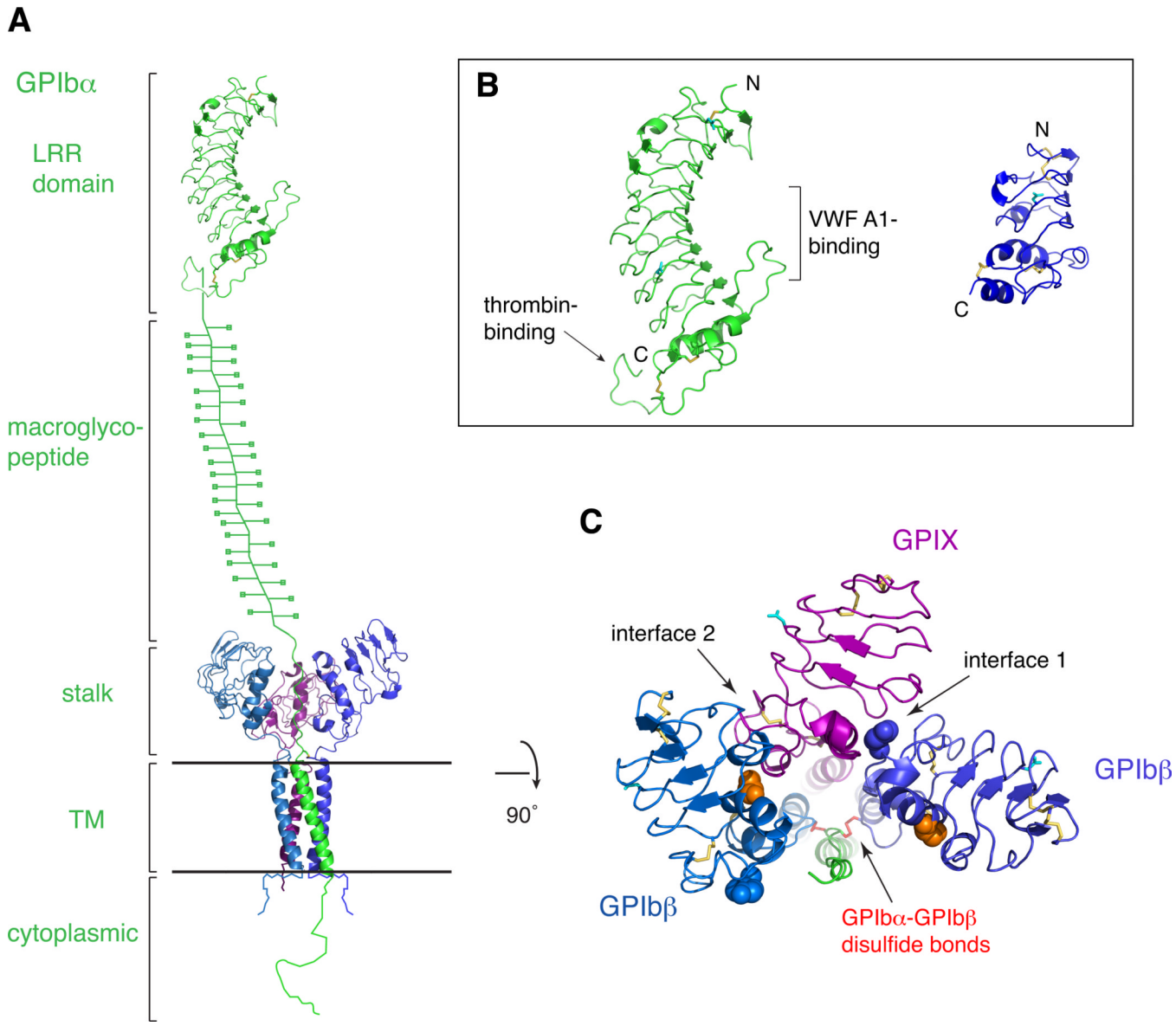

Figure 1.

The organization of GPIb-IX complex. GPIbα (green), GPIbβ (blue) and GPIX (purple) subunits are colored differently. *A*, A cartoon illustration of the GPIb-IX complex largely drawn in ribbon diagrams. Various parts of GPIbα are labeled on the left. *B*, Ribbon diagrams showing the crystal structures of the GPIbα N-terminal domain (green) and GPIbβ extra cellular domain (blue). The PDB IDs are 1 GWB[23] and 3 RFE[22], respectively. Both structures are positioned with the N-terminus on top, the concave β-strands on the right and convex loops left. Disulfide bonds in each LRR domain are shown in yellow, and side chains of Asn for N-glycosylation in cyan. The binding region for the A1 domain of VWF is marked in the GPIbα N-terminal domain. It should be noted that the conformation of the thrombin-binding sequence that contains several sulfated tyrosines is very flexible. *C*, The top view of the membrane-proximal portion of GPIb-IX that contains the stalk region of GPIbα, the extra cellular domains of GPIbβ and GPIX, and a portion of the TM helical bundle. This illustration is adapted from a previous publication [22]. The disulfide bonds between GPIbα and GPIbβ are highlighted in red. Side chains of Tyr106 in GPIbβ are

shown in blue spheres, one of which are located at the interface 1 between GPIbβ and GPIX. Residue Pro74 in GPIbβ are shown in orange spheres, one of which are located at or close to the interface 2.

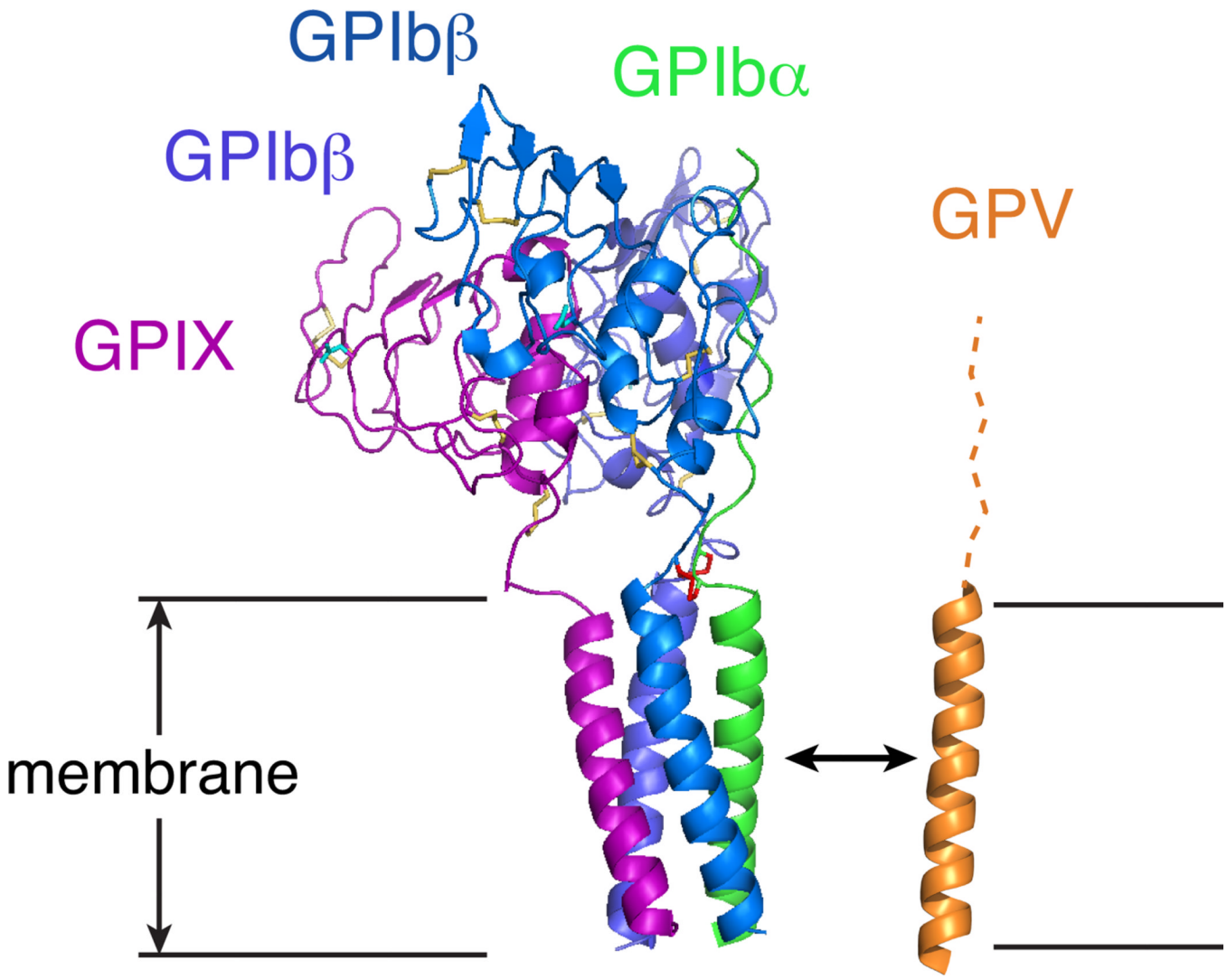

Figure 2.
The interaction of GPV with GPIb-IX through TM domains. The membrane-proximal portion of GPIb-IX, in the same color scheme as in Figure 1, is shown at an angle to demonstrate the accessibility of the GPIbα TM helix to direct association with the GPV TM helix (in orange color) as well as the inaccessibility of the GPIX TM helix. This is adapted from a recent publication[67].