Developing Relationships: Arterial Platelet Adhesion, Glycoprotein Ib, and Leucine-Rich Glycoproteins

By Gerald J. Roth

The basic fact of platelet function is simple: “Platelets plug holes in blood vessels.” Circulating platelets monitor the vascular lining, sticking to damaged areas with exposed subendothelium. Additional platelets then join those already attached to form a plug that closes the rent in the vessel wall. Platelet-to-vessel adhesion is a discrete functional event as defined by two key concepts. First, distinct aspects of platelet function govern plug formation: adhesion (platelet-to-nonplatelet attachment), aggregation (platelet-to-platelet attachment), and secretion (release of granular material). Second, distinct forms of platelet adhesion are used in plug formation depending on the rate of blood flow, high (arterial) or low (venous). Adhesion precedes aggregation and secretion and initiates hemostatic and thrombotic events.

In arterial blood, adhesion requires stopping and anchoring the moving platelet, a cellular process that involves specific molecules. Of the many surface molecules present, one receptor-ligand pair stands out as particularly well adapted to effect “stopping and anchoring.” In this pair, the platelet receptor (glycoprotein Ib [GP Ib]) of an unactivated platelet binds with the vascular ligand (von Willebrand factor [vWF]) of subendothelium only in the presence of shearing forces generated by blood flow. Hence, the binding of GP Ib to vWF needs movement to stop movement. This unique quality is termed “shear rate-dependence,” and the process is well described at a cellular level but is not yet understood at a molecular level. The GP Ib-vWF interaction plays a critical role in platelet function and hemostasis because abnormalities in either molecule give rise to clinical bleeding. Recently, progress has been made in understanding the structure and function of GP Ib/vWF. For example, GP Ib is a “leucine-rich” GP (LRG) with specific binding sites for vWF. This review emphasizes the structure and function of platelet GP Ib and evaluates the GP Ib-vWF interaction as an aspect of arterial platelet adhesion. Potential mechanisms that correlate flow/shear forces with GP Ib/vWF function are proposed in the light of current knowledge.

GP Ib: A member of a surface membrane complex

Nomenclature and structure. The name GP Ib derives from the original separation of platelet surface GP into three broad groups (I, II, III) and from the subsequent identification of individual group members by the use of improved electrophoretic techniques (e.g., IA, Ib, Ic). Platelet GP Ib is a surface membrane heterodimer, Mr 165,000, consisting of a larger α chain (GP Ibα; Mr 143,000) and a smaller disulfide-linked β chain (GP Ibβ; Mr 22,000). Genetic heterogeneity in GP Ib size has been described in several populations. In the platelet membrane, GP Ib forms a noncovalent complex with a second, smaller protein, GP IX (Mr 20,000). Approximately 25,000 copies of the GP Ib-IX complex are found in a normal platelet, and little or no free, uncomplexed GP Ib or IX can be identified. Morphologically, the GP Ib-IX complex is an elongated rod, 60 nm in length, with a longer extracellular amino-terminus extending approximately 50 nm from the plasma membrane and a shorter carboxy-terminus made up of the transmembrane and intracellular portions of the involved proteins. Functionally, the binding of the GP Ib-IX complex to vWF is governed by the GP Ibα chain without any appreciable contribution from either GP Ibβ or GP IX.

The largest member of the GP Ib-IX complex, the GP Ibα chain, is heavily glycosylated, consisting of approximately equal mass of carbohydrate and protein. In addition, the α chain has distinct structural domains as defined by proteolytic cleavage of the parent molecule. Proteolysis by calpain near the transmembrane region releases the soluble extracellular domain, glycocalcin (Mr 120,000), named for its presence in the glycoalyx, the carbohydrate-rich coating of platelet plasma membranes. Glycocalcin can be split into two parts by trypsin, giving rise to an NH2-terminal fragment (Mr 45,000) that contains binding activity for both vWF and thrombin and a glycosylated COOH-terminal portion, the macroglycopeptide domain. Most of the carbohydrate in GP Ibα is present in the macroglycopeptide region in the form of hexasaccharide chains of the O-linked, mucin type. These chains are capped by sialic acid that contains much of the negative charge on the platelet surface. Although the functional significance of the macroglycopeptide domain is unknown, its length (extending about 15 nm from the plasma membrane), number (25,000 copies/platelet), location (surface), and composition (carbohydrate) are all consistent with the idea that the structure is a prominent part of the surface glycoalyx. The other members of the GP Ib-IX complex, GP Ibβ and GP IX, are distinguished primarily by their linkage to GP Ibα. The primary structure of the proteins is discussed in a later section.

Bernard-Soulier syndrome and GP Ib function. In 1948, two French physicians, Drs Bernard and Soulier, described a young patient with a low platelet count, large platelets, and a severe bleeding disorder in the setting of an inherited bleeding diathesis. Additional families with a similar congenital disorder of platelet function and a concomitant deficiency of GP Ib have been described, leading to the development of the Bernard-Soulier syndrome and GP Ib function.

From the Hematology Section, Medical and Research Services, Seattle Veterans Hospital; and the University of Washington, Seattle. Submitted April 25, 1990; accepted August 30, 1990. Supported by a Merit Review Grant from the Veterans Administration, by Grant HL 39947 from the National Institutes of Health, and by Grant 90-904 from the American Heart Association. Address reprint requests to Gerald J. Roth, MD, Seattle VAMC (111), 1660 S Columbian Way, Seattle, WA 98108. © 1991 by The American Society of Hematology. 0006-4971/91/7701-0025$3.00/0
current definition of Bernard-Soulier syndrome (BSs) as thrombocytopenia, large circulating platelets, and a deficiency of GP Ib. BSs circulating platelets lack two other surface GPs: GP IX, presumably due to the absence of its GP Ib partner; and GP V (Mr 82,000). GP V, a thrombin substrate, is not physically associated with the surface GP Ib-IX complex. However, in view of the shared defect of all three proteins in Bernard-Soulier syndrome platelets, GP V is related to the GP Ib-IX complex through an undefined common requirement for synthesis, processing, or expression.

Information gained from the study of Bernard-Soulier platelets underpins our current knowledge of GP Ib function (Table 1). These platelets have a selective defect in vWF-dependent adhesion and lack the ability to bind vWF in the presence of ristocetin. Plasma vWF is an elongated multimeric protein (length up to 2 μm) that binds to vascular components and serves as an intermediary between the vessel wall and GP Ib on the surface of unactivated platelets. The specific biochemical abnormality in BSs platelets was identified as deficient GP Ib, and by selective proteolytic removal of all resultant defective vWF binding and adhesion. Similar defects in vWF caused by congenital abnormalities (von Willebrand disease [vWD]) or by anti-vWF antibodies cause abnormal GP Ib binding and decreased adhesion. Therefore, the functional role of this receptor-ligand interaction is amply documented as mediating the shear rate-dependent, activation-independent attachment of platelets to vessels in the arterial circulation.

The antibiotic ristocetin induces platelet agglutination through the specific binding of vWF to platelet GP Ib. Because soluble vWF has no affinity for GP Ib in the absence of ristocetin-like agents, these compounds provide very useful reagents for laboratory study of the GP Ib-vWF interaction. Earlier observations of deficient ristocetin-induced agglutination in BSs and vWD provided critical insight into the molecular defects in the disorders; currently, ristocetin is used to define specific binding domains within the molecules. However, ristocetin is not a normal constituent of the human body, and the agent cannot be construed as a “physiologic” inducer of the GP Ib-vWF interaction. An approximation of the in vivo GP Ib-vWF interaction can be observed using perfusion systems that pump blood over vascular surfaces and provide conditions that simulate those of the arterial circulation (shear, vascular surfaces). However, perfusion systems are not easily adapted to the study of individual molecules. Herein lies a major problem in understanding GP Ib-vWF function at a molecular level. “Unphysiologic” agents such as ristocetin are conveniently applied to the study of molecular function while “physiologic” flow systems are not.

Additional features of the GP Ib-related proteins. As a transmembrane complex, GP Ib-IX links the extracellular events of platelet adhesion to structures and functions within the platelet. The linkage involves a direct attachment of the Ib-IX complex to actin-binding protein, a connection that functions to maintain the shape of the platelet. For example, the giant platelets and increased membrane fluidity seen in BSs may reflect the lack of this critical link between the plasma membrane and cytoskeleton. Transmembrane signalling through the GP Ib-IX complex may be regulated by cleavage of actin-binding protein by calpain during platelet activation and perhaps by phosphorylation of a carboxy-terminal site in GP Ib. Both GP Ib and GP V interact with exogenous thrombin, the former as a binding site and the latter as a substrate for proteolysis. However, neither protein appears to function as the receptor for thrombin because the absence of these proteins does not eliminate the platelet response to the agonist. Finally, both the GP Ib-IX complex and GP V provide antigenic sites for both drug-dependent and autoimmune antibodies directed against the platelet surface.

Table 1. Timeline of Earlier Observations Concerning Platelet GP Ib

<table>
<thead>
<tr>
<th>Date</th>
<th>Observation</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1948 BSs</td>
<td>Bleeding, giant platelets, thrombocytopenia</td>
<td>38</td>
</tr>
<tr>
<td>1969 BSs</td>
<td>platelets lack surface sialic acid</td>
<td>37</td>
</tr>
<tr>
<td>1973-1974</td>
<td>BSs platelets defective in adhesion and defective in vWF binding</td>
<td>8, 14, 46</td>
</tr>
<tr>
<td>1972-1977</td>
<td>GP Ib defined as distinct α/β heterodimer</td>
<td>16-18</td>
</tr>
<tr>
<td>1977-1978</td>
<td>Thrombin binds GP Ib and cleaves GP V</td>
<td>31, 32, 43</td>
</tr>
<tr>
<td>1975-1983</td>
<td>BSs platelets lack GPs Ib, V and IX; GP Ib present in the Ib-IX complex</td>
<td>22, 23, 40, 41</td>
</tr>
</tbody>
</table>

QUALITIES OF GP Ib/vWF-DEPENDENT PLATELET ADHESION

Contact between a circulating, unactivated platelet and arterial subendothelium constitutes a fundamental initial event in GP Ib/vWF-dependent platelet adhesion. Starting with morphologic observations of the contact process in perfusion systems, the physiology of GP Ib/vWF-dependent adhesion has been defined in terms of its shear rate-dependence/activation-independence and the additional parameters discussed below.

Shear rate- and transport-dependence. Shear arises as parallel layers of material move in different directions or move in the same direction at differing speeds. In the case of arterial blood flow, shear develops between concentric layers of blood moving at differing speeds through the vessel. Within the central region of rapid axial flow, minimal differences are present in “layers” of blood, but maximal differences arise at the blood-to-vessel interface. Platelet adhesion through GP Ib-vWF occurs at blood-to-vessel interfaces in the presence of “sufficient” shear forces, expressed as wall shear rates of greater than 650 s⁻¹. Flow conditions that produce “sufficient” wall shear rates (650 to 3,000 s⁻¹) are present in arteries and arterioles.
referred to as “arterial/rapid/high” flow and calculated on the basis of maximal flow in pulsatile systems. Shear rates that exceed those found in normal arteries, greater than 3,000 s⁻¹, can activate platelets directly although the physiologic role of such “shear-induced platelet aggregation” is unclear. In contrast, the slower rate of blood flow in veins generates wall shear rates of 650 s⁻¹ or less, and these conditions generally do not evoke the GP Ib/vWF-dependent adhesion mechanism.

Arterial blood flow also “transports” platelets to the vessel interface. Transport depends on the rheologic properties of red blood cells (RBCs), leading to a concentration of RBCs in the axial stream and displacement of platelets laterally to the periphery of the moving blood. In the absence of RBCs, platelet adhesion does not occur under arterial flow conditions because platelets are not “transported” to the vessel wall. However, blood flow contributes more to arterial platelet adhesion than simple transport alone because, in the absence of flow or an exogenous stimulant such as ristocetin, the static apposition of platelet GP Ib to vWF leads to little or no appreciable interaction (discussed below).

**Activation-independence.** Platelet-to-vessel wall contact is the first observable event in GP Ib/vWF-dependent platelet adhesion to subendothelium. According to their morphologic appearance, contact platelets are not degranulated and have not undergone activation. Because initial platelet contact through GP Ib-vWF involves unactivated platelets, the entire adhesion process can be termed “activation-independently,” despite the fact that contact itself activates platelets through the GP Ib receptor and leads to platelet “spreading” on the vascular surface.

**Sequential nature starting at the subendothelial surface.** Intact, normal endothelium does not react with vWF or GP Ib, and the initiating event in arterial platelet adhesion appears to be the disruption or loss of the vascular endothelial lining due to injury or disease. With a breach in the endothelial lining, subendothelial components come in contact with the blood and provide a surface for vWF binding. Subendothelium contains a variety of extracellular matrix (ECM) components such as fibrillar/nonfibrillar collagens, microfibrils, elastin, and proteoglycans; all of which may provide binding sites for vWF. The exact vascular component for in vivo vWF binding remains undefined. The possibility exists that no single vascular component subserves vWF binding and subsequent platelet adhesion, and that multiple elements can provide the vascular substrate for vWF function. For example, in vitro perfusion studies show that multiple surfaces (collagens, subcellular matrices, intact subendothelium) can support GP Ib-vWF–dependent platelet adhesion. Also, the binding site for vWF within vWF itself appears to be somewhat nonspecific, capable of interacting with a variety of ECM elements.

Two forms of vWF, one circulating in plasma as a soluble protein and the other bound to the vascular subendothelium, are potentially available as the intermediary between the vessel wall and GP Ib on the circulating platelet. The bound vWF can originate within previously overlying endothelium that synthesizes vWF and secretes it directly through its basal surface into the subendothelium. A third form of vWF exists within platelet granules and contributes to the adhesion of activated platelets.

**Potential mechanisms.** Two possible mechanisms for GP Ib/vWF-dependent adhesion are shown in Fig 1. The mechanisms are not mutually exclusive nor do they include all possibilities. The model shown in Fig 1A (top, soluble vWF) outlines an effect of shear on platelet GP Ib, leading to the binding of circulating or soluble vWF to the platelet and subsequent adhesion of the vWF-platelet complex to the vascular site. The model shown in Fig 1B (bottom, bound vWF) indicates that vWF bound to the subendothelium may change its conformation with shear and become reactive with GP Ib to mediate adhesion.

Perfusion studies demonstrate that vWF bound to vascular subendothelium can mediate GP Ib-dependent adhesion in the absence of additional circulating ligand. These observations can be interpreted as suggesting that vWF, regardless of flow, undergoes a conformational change on binding to the vessel wall and becomes reactive with platelet GP Ib. If such a change actually occurs, then vascular elements can be expected to confer GP Ib-reactivity to bound vWF under static conditions. Direct experiments to test this hypothesis have not given clear-cut results, and the hypothesis remains unproven. For example, unique vascular components for vWF binding have not
been identified. VWF itself binds to a variety of ECM components and vWF bound to collagen does not display a significantly increased affinity for GP Ib. These negative or equivocal findings indicate that the molecular mechanism for shear rate-dependent platelet adhesion through GPIb/vWF is still unknown. The key element that is missing in current theories is the effect of shear/flow. Apparently, mechanical force itself, in the form of shear, can exert a critical effect on GP Ib and/or vWF and lead to a specific interaction between the two molecules.

Other receptors/ligands related to GP Ib/vWF. Although attachment of contact platelets to subendothelium depends primarily on GP Ib-vWF, such contact is only one step in a continuum of events that begins with a circulating platelet and ends with a platelet plug. Once contact occurs between GP Ib and vWF, intraplatelet activation ensues and other receptor-ligand pairs come into play. For example, the activation-dependent fibrinogen receptor, GP IIB/IIIa (α2β3), appears to mediate platelet spreading by binding to subendothelial vWF and similar function may be provided by related receptors such as the vitronectin receptor (α5β3) and the activation-independent fibronectin receptor, GP Ic/IIa (α5β1-VLASS) through interactions with their respective ligands. Additional surface receptors may contribute to the contact to spread process by binding directly to ECM components without intervening ligands. Examples are the cation-dependent collagen receptor, GP Ia/IIa (α1β1-VLASS2) and GP IV, an additional collagen receptor. Finally, other, less well-characterized receptors such as a second form of GP Ic/IIa (α5β1-VLASS6) are present as detected by structural or functional studies. The nomenclature for these receptors is complex because it includes a "GP" designation as a platelet surface GP, an αβ heterodimer chain designation for subfamilies of the integrin superfamily, and names such as VLA, referring to the very late activation antigens. Other than GP Ib/vWF, the receptor-ligand pairs noted above have not been characterized as both shear rate-dependent and activation-independent, and they do not appear to substitute for GP Ib-vWF in providing for the attachment of contact platelets.

The ability of GP Ib to mediate platelet activation following contact indicates that the receptor has function in addition to vWF binding and is subject to regulation. For example, vWF binding to surface GP Ib is affected by intraplatelet events such as variations in cAMP levels, thrombin stimulation, and perhaps by phosphorylation of GP Ibα. After the contact and spreading steps of adhesion, platelet aggregates (platelet-platelet interactions) appear as part of thrombus formation at sites of initial platelet attachment. The activation-dependent GP IIB/IIIa receptor plays a major role in aggregation, using fibrinogen as its adhesive GP ligand.

GP Ib/vWF as one aspect of arterial platelet adhesion. Because patients who lack GP Ib/vWF suffer from clinically significant bleeding, the GP Ib-vWF interaction plays a major role in providing the normal arterial circulation and makes a critical contribution to normal hemostasis. The correlation between the in vitro perfusion findings and the clinical observations suggests that the perfusion systems approximate the flow conditions in normal arteries. However, the clinician confronts arterial platelet adhesion in conditions that may differ markedly from the idealized conditions of perfusion systems. A relevant example would be platelet adhesion to an atherosclerotic arterial wall, initiating thrombus formation. In general, available perfusion studies do not address the problems presented by abnormal vessels and circulating activated platelets in the presence of nonlaminar flow with regions of turbulence and stasis. Fuster et al. and Nichols et al. have studied thrombus formation in vWF-deficient swine and demonstrated a role for vWF in thrombotic events on both injured and atherosclerotic vessels. Current information is insufficient to judge the clinical significance of GP Ib/vWF in arterial thrombosis, and further studies are needed.

GP Ib/vWF abnormalities associated with increased function. Excessive reactivity between GP Ib and vWF has been described as a consequence of both ristocetin administration and pseudo-vWD. As noted above, ristocetin induces vWF binding to platelet GP Ib in the absence of blood flow and blood vessels. In addition, ristocetin invariably caused thrombocytopenia when used clinically as a systemic antibiotic, presumably due to generalized binding of vWF to platelet GP Ib and the subsequent destruction of the vWF-coated platelets by the monocyte-phagocyte system. Pseudo-vWD is a congenital disorder of platelet GP Ib marked by excessive reactivity between vWF and GP Ib, thrombocytopenia, and autosomal dominant inheritance. The genetic defect appears to result in a qualitative change in the GP Ib chain that permits the receptor to react with the ligand in the absence of both flow and blood vessels.

Implications of hyperfunction/shear-independence: Localization/reversibility. As examples of hyperfunction, pseudo-vWD and ristocetin administration produce the same pathophysiologic result: enhanced reactivity between GP Ib-vWF and thrombocytopenia. In both cases, the abnormal GP Ib-vWF interaction is not shear rate-dependent and occurs in all regions of circulation with resultant platelet destruction. Conversely, the normal shear rate-dependent interaction between GP Ib and vWF is not generalized throughout the circulation but localized at the blood-to-vessel interface. Shear rate-dependence implies that maximal reactivity between receptor and ligand will arise at vascular surfaces where shearing forces are maximal. In regions of the circulation with reduced shear or slow blood flow, the affinity between normal vWF and normal GP Ib appears to be minimal or nonexistent. The on-again/off-again aspect suggests that the normal Ib-vWF interaction is reversible, depending on flow conditions and the location of the reactants in regard to the vessel wall. Therefore, the quality of shear rate-dependence implies that the GP Ib-vWF reaction is both localized and reversible. As a hypothetical example, a moving platelet at the vascular interface in the presence of shearing forces (localization) may bind vWF to its surface through GP Ib, but the platelet-bound vWF may later dissociate (reversibility) when flow is decreased or...
when the platelet moves away from the vessel wall into a region of decreased shear (Fig 1A, soluble model).

Ristocetin and an analogy. Use of ristocetin permits quantitative study of the GP Ib-\(\nu\)-VF reaction and identification of binding domains.\(^{15,56,127-129}\) Antibodies that block ristocetin-induced binding also block GP Ib-\(\nu\)-VF dependent adhesion in perfusion systems, indicating that ristocetin-induced binding is a close facsimile of shear-dependent adhesion.\(^{19}\) The mechanism of the ristocetin effect appears to involve positive and negative charges because polycations can induce GP Ib-\(\nu\)-VF binding.\(^{13}\) Furthermore, neuraminidase treatment of \(\nu\)VF to remove negatively charged sialic acid leads to specific binding of asialo-\(\nu\)VF to GP Ib.\(^{132}\) However, as noted earlier, ristocetin cannot substitute for flow in producing shear-dependence adhesive interactions; and physiologic, shear-dependent adhesion remains an elusive subject for molecular analysis. The situation is analogous to that described in the story of an inebriated citizen who loses his wallet in a dark alley but searches for it in the glow of a nearby street light. The "wallet" of the mechanism for shear-dependent adhesion remains in an unilluminated recess reserved for problems that combine mechanical force with protein-protein interactions. The "glow" of valuable information from perfusion- and ristocetin-based studies has not yet penetrated the recess.

A current challenge. A major current challenge in the GP Ib/\(\nu\)-VF field is to understand the nature of shear rate-dependence. Available knowledge does not identify the molecule (Ib and/or \(\nu\)VF) affected by shear (Fig 1). As described in the next section, information from cDNA cloning work provides clues to the potential relationship between shear and the structure/function of GP Ib.

**PRIMARY STRUCTURES OF THE GP Ib-IX COMPLEX**

cDNA cloning of the GP Ib-IX complex. The cDNAs encoding GP Ib\(_\alpha\), GP Ib\(_\beta\), and GP IX have been cloned and sequenced to provide the primary structure of each of the three polypeptides.\(^{13,133-137}\) Clones were obtained from a cDNA library constructed with poly (A)\(^+\) RNA obtained from phorbol-treated human erythroleukemia (HEL) cells.\(^{138,139}\) As shown in Fig 2, full-length cDNA sequences were obtained with both 5' and 3' noncoding regions, poly(A) tails, and lengths consistent with the size of the individual transcripts detected by Northern blot analysis of platelet poly (A)\(^+\) RNA.\(^{140}\) The transcript and cDNA sizes are: 2.4 kb to 2,420 b, 1.1 kb to 968 b, 1.0 kb to 896 b for GP Ib\(_\alpha\), GP Ib\(_\beta\), and GP IX, respectively. Start codons were identified according to methionine residues located at the beginning of putative signal sequences, approximately 25 residues in length, that preceded the determined NH\(_2\) terminus of each mature protein.\(^{136,137}\)

Domain structure and structural features of GP Ib-IX and GP V. As shown by hydropathy plots of predicted primary structures (Fig 2),\(^{141}\) two hydrophobic domains are present at the beginning and near the end of each protein, the first representing a putative signal sequence and the second a transmembrane domain. A noteworthy aspect of the hydropathy plots is the long stretch of hydrophilic sequence in the central portion of GP Ib\(_\alpha\) (residues 225 to 420, Fig 2A) that includes a region of O-linked carbohydrate and a hinge region with binding sites for \(\nu\)VF and thrombin as discussed below.\(^{142,144}\)

The mature GPs Ib\(_\alpha\), Ib\(_\beta\), and IX consist of 610, 181, and 160 amino acids, respectively, with larger extracellular and smaller intracellular domains (Fig 3). The most striking feature of the primary structure of these proteins is the presence of unique 24-amino acid sequences that distinguish the LRGs (Fig 4).\(^{11,145,146}\) LRG sequences are usually found in a series of tandem repeats as in GP Ib\(_\alpha\) with its seven tandem LRG repeats located toward the amino terminus of the protein (residues 36 to 200; Figs 2A, 3, and 4). Both GP Ib\(_\alpha\) and GP IX are unusual LRG proteins, having only one LRG segment. Each of the three members of the Ib-IX complex have conserved flanking sequences of approximately 22 residues that border the central LRG structure on both the amino- and carboxy-terminal sides (Figs 3 and 4). The potential significance of the "flank-LRG center-flank" structure in these proteins is discussed along with the LRG family in the next section.

The \(\alpha\) chain of GP Ib contains a second set of tandem repeats between residues 363 and 414 made up of five segments, each of approximately nine amino acids, that resemble similar carbohydrate-linked (O-CHO) sequences found in a number of unrelated GPs.\(^{13}\) The sequence from residue 310 to 420 that includes the five repeats is enriched in serine/threonine and appears to contain the bulk of the O-linked carbohydrate of GP Ib\(_\alpha\) that constitutes the main feature of the "macroglycopeptide" domain.\(^{34}\) The functional role of the multiple oligosaccharide chains concentrated into one limited domain is unknown, but as noted earlier this structure may constitute a major part of the platelet glyocalyx.\(^{26}\) Furthermore, the structure is implicated in GP Ib\(_\alpha\) function by its proximity to the binding site for \(\nu\)VF.\(^{142,143}\) In addition to O-linked oligosaccharides, GP Ib\(_\alpha\) contains four N-linked glycosylation sites while the \(\beta\) chain and GP IX each have a single N-linked site.\(^{13,133,134}\)

Between the flank-LRG center-flank region on the aminoterminal side and the O-carbohydrate region on the carboxyterminal side lies an interconnecting hinge region of the \(\alpha\) chain, residues 220 to 310. A portion of the hinge region, residues 269 to 301, is distinguished by a concentration of charged residues, beginning with a series of negative charges and switching abruptly at residue 288 to a series of positive charges. Residues 269 to 287 include 10 negatively charged amino acids while residues 288 to 301 contain five positive charges. Work with peptide inhibitors and with expression systems implicate this charged "hinge" domain as a major binding site for \(\nu\)VF within the \(\alpha\) chain of GP Ib.\(^{142,146}\) The presence of charged residues in a \(\nu\)VF-binding site of Ib\(_\alpha\) fits with the concept that charge plays a role in ristocetin, polycation, and neuraminidase-induced interactions between GP Ib and \(\nu\)VF.\(^{133,132}\) However, the precise manner in which charge might relate to \(\nu\)VF binding remains unexplained, and any effect of flow or shear on the hinge region is undefined.

The \(\alpha\) chain of GP Ib is covalently attached to the \(\beta\) chain through disulfide bond(s), and the GP Ib heterodimer is
noncovalently bound to GP IX (Fig 3). In the α and the β chains, cysteines are located adjacent to the transmembrane domain and are likely to form the disulfide link. The β chain is subject to cAMP-dependent phosphorylation at both a major site (residue 166) and a nearby minor site (residue 172). The phosphorylation event inhibits actin polymerization and may influence the link between the GP Ib-IX complex and the platelet interior. An unpaired
cysteine in the intracellular portion of the \( \beta \) chain could provide a mixed disulfide link to intraplatelet components.\(^{146} \)

The intracellular domains of the GP Ib-IX complex are not extensive, consisting of only six amino acids in the case of vascular surfaces and to platelet GP Ib. Collagen binding repeats and associated O-linked carbohydrate (\(|\beta|\)) or as seven tandem repeats (\(|\beta|\), Ib, and IX) Calpain releases the extracellular portion of GP Ib\(_a\), glycolycin, with its macroglycopeptide domain marked by five, 9-amino acid tandem repeats and associated O-linked carbohydrate. The hinge region between the LRG structure and the O-carbohydrate region of Ib\(_a\) mediates binding to vWF and thrombin. GP Ib\(_a\) contains a cAMP-dependent phosphorylation (PO) site.

**Fig 3.** Sketch of the primary structure of the GP Ib-IX complex. Proteins are depicted as open bars (\(|\beta|\)) with superscripts referring to amino acid number and subscripts to NH\(_2\) and COOH-termini. Transmembrane domains demarcate larger extracellular from smaller intracellular regions extending on either side of the plasma membrane (\(|\beta|\)). Disulfide bonds, S-S, link the GP Ib \( \alpha \) and \( \beta \) chains while GP IX is noncovalently associated with Ib. Flank-LRG-center-flank structures include conserved, 22-amino acid flanking sequences (\(|\beta|\) on either side of central, 24-amino acid LRG sequences present as one segment (\(|\beta|\), Ib, and IX) or as seven tandem repeats (\(|\beta|\), Ib, and IX). Calpain releases the extracellular portion of GP Ib\(_a\), glycolycin, with its macroglycopeptide domain marked by five, 9-amino acid tandem repeats and associated O-linked carbohydrate (\(|\beta|\)). The hinge region between the LRG structure and the O-carbohydrate region of Ib\(_a\) mediates binding to vWF and thrombin. GP Ib\(_a\) contains a cAMP-dependent phosphorylation (PO) site.

The primary structure of the associated protein, GP V, is not known in its entirety, but recent evidence indicates that it contains LRG segments.\(^{150,151} \)

The intracellular domains of the GP Ib-IX complex are not extensive, consisting of only six amino acids in the case of GP IX. GP IX has few structural features other than LRG-related sequences, but it contains an unpaired cysteine in its transmembrane domain that is a likely site for fatty acid acylation by palmitate and may contribute to the membrane binding capacity of the complex.\(^{159} \)

**Fig 4.** Synopsis of LRG sequences currently reported. (A) Single and consensus LRG sequences. The single LRG sequences of GPs Ib,\(^{129} \) and IX\(^{129} \) are shown above consensus sequences for the LRG repeats of: the \( \alpha \) chain of GP Ib (GP Ib\(_a\), seven repeats),\(^{129} \) the leucine-rich \( \alpha \) core protein of human serum (LRG\(_a\), nine repeats),\(^{129} \) human proteoglycan core protein (PG40, 10 repeats similar to bovine PG1, references 160 and 161),\(^{129} \) Drosophila photoreceptor cell chaoptin (chaoptin, 41 repeats),\(^{129} \) porcine RNAase inhibitor-type A repeats (P-RNA-IN-A, 8 repeats),\(^{129} \) and overall consensus sequence (consensus) for all of the sequences listed. X, absence of conserved residue; \(-\), gap inserted in the sequence; h, hydrophobic residue (I, L, V, F). Conserved residues are enclosed in boxes. The same abbreviations are used in (B) and (C). (B) Amino acid sequences flanking LRG regions on the NH\(_2\)-terminal side of the LRG segment of GP IX is shown along with similar NH\(_2\)-terminal flanking sequences of approximately 22 amino acids present in GP Ib\(_a\), GP Ib\(_b\), human proteoglycan core protein, human serum leucine-rich \( \alpha \) core protein, Drosophila photoreceptor cell chaoptin, and a consensus sequence for the NH\(_2\)-terminal flanking sequences. (C) Amino acid sequences flanking LRG regions on the COOH-terminal side of the LRG segment of GP IX is shown along with similar COOH-terminal flanking sequences of approximately 22 amino acids present in GP Ib\(_a\), GP Ib\(_b\), human serum leucine-rich \( \alpha \) core protein, Drosophila photoreceptor cell chaoptin, and a consensus sequence for the COOH-terminal flanking sequences. Fig 4 is reprinted with permission (Proc Natl Acad Sci USA 86:6773, 1989).\(^{129} \)
leucine-rich α2 GP (LRG protein), a trace component of normal human serum.11 This protein lacks a known source or function but contains nine tandem (one after the other) segments, each of 24 amino acids, with sequence homology. These repeats are distinguished by conserved leucines at positions 1, 3, 8, 11, 15, 19, and 22 as shown in Fig 4. The consensus LRG sequence for the nine repeats in the leucine-rich α2 GP (LRG in Fig 4A) includes a variety of other amino acids (hydrophilic/hydrophobic, conserved/nonconserved) interspersed between the leucines that give the structure its name.

A second LRG protein, yeast adenylate cyclase, was reported in 1985 and was shown to contain 26 tandem LRG repeats.145 The consensus sequence for the LRG segments of yeast adenylate cyclase differs somewhat from that for LRG protein itself (line 8, Fig 4A). However, the homology between the two was clear-cut and indicated that the LRG sequence would be present in additional, otherwise unrelated proteins.13,131,134,146-148

The finding of LRG segments in different proteins led to the recognition of conserved flanking sequences on either side of a central LRG region in some LRG proteins (Fig 4, B and C). Flanking sequences exist as single, nonrepeated segments of approximately 22 amino acids, and those on the amino-terminal side differ from those on the carboxy-terminal side. The proteins of the platelet GP Ib-IX complex provide the best example of the flank-LRG center-flank structure. The simple, and perhaps ancestral, flanked single LRG center-flank found in GP Ibα and GP IX contrasts with the flank-seven LRG center-flank of GP Ibβ, suggesting that flanking sequences may be associated in an undefined manner to the evolution of LRG proteins by facilitating the tandem duplication of the central LRG repeats.134

The LRG family of proteins. As noted in Table 2, the LRG motif appears in diverse proteins with differing functions. The entries in Table 2 are given in roughly chronological order according to the year of the report with the proband, the leucine-rich α2 GP, listed first. One can predict that the LRG family will prove to include many additional members in multiple tissues and species.

Functions of LRG segments. In view of its wide distribution, the LRG segment has functional significance, particularly in the form of multiple tandem repeats. Apparently the interposition of different amino acids between the conserved (usually leucine) residues in the 24-amino acid structure provides for specific functions in individual LRG proteins. "Variations" on the basic LRG "theme" may give additional functional properties as indicated by the five-amino acid insert within the LRG segments of the RNase inhibitors138,139 and the truncated LRG segments in monocyte CD14 and Yersinia yopM.137,143

The LRG segments of the RNase inhibitors and PGII possess highly specific function, binding to pancreatic RNase and transforming growth factor β, respectively.146,147 Although no direct data are available, LRG segments may also interact with lipid structures in view of their amphipathic, hydrophilic/hydrophobic character. The general property of protein:protein interaction and cell:cell or cell:matrix adhesion appears to be a common feature related to the function of the LRG segment. For example, the proteoglycan core proteins and the oligodendrocyte-myelin GP appear to mediate interactions with extracellular matrix, while the Drosophila LRG proteins (chaoptin, toll) appear to orient specific cells (photoreceptor cells, embryos) to adjacent structures. However, an adhesive quality is not obvious in other members of the LRG family such as the transmembrane, G-protein linked lutropin receptor.

Correlates between "leucine-zippers" and LRG segments. "Leucine zipper" proteins such as the c-fos and c-jun proto-oncogene products regulate transcription by binding

---

Table 2. Current Members of the Leucine-Rich GP Family

<table>
<thead>
<tr>
<th>Protein</th>
<th>Function</th>
<th>Species/Tissue</th>
<th>No. LRG Repeats</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leucine-rich α2 GP</td>
<td>?</td>
<td>Human plasma</td>
<td>9</td>
<td>11 (1985)</td>
</tr>
<tr>
<td>Adenylate cyclase</td>
<td>cAMP</td>
<td>Yeast</td>
<td>26</td>
<td>145 (1985)</td>
</tr>
<tr>
<td>Proteoglycan core</td>
<td>? Adhesion</td>
<td>Human fibroblast</td>
<td>10</td>
<td>154 (1986)</td>
</tr>
<tr>
<td>Platelet GP Ibα</td>
<td>Adhesion</td>
<td>Human platelet</td>
<td>7</td>
<td>13 (1987)</td>
</tr>
<tr>
<td>Platelet GP Ibβ</td>
<td>Adhesion</td>
<td>Human platelet</td>
<td>1</td>
<td>133 (1988)</td>
</tr>
<tr>
<td>Platelet GP IX</td>
<td>Adhesion</td>
<td>Human platelet</td>
<td>1</td>
<td>134 (1989)</td>
</tr>
<tr>
<td>toll Protein</td>
<td>Morphogenesis</td>
<td>Drosophila embryo</td>
<td>15</td>
<td>155 (1987)</td>
</tr>
<tr>
<td>Chaoptin</td>
<td>Photo-reception</td>
<td>Drosophila eye</td>
<td>41</td>
<td>156 (1987)</td>
</tr>
<tr>
<td>Monocyte CD14</td>
<td>Differentiation</td>
<td>Human monocyte</td>
<td>3-trunc,*</td>
<td>157 (1988)</td>
</tr>
<tr>
<td>RNase inhibitor</td>
<td>RNase inhibitor</td>
<td>Porcine liver</td>
<td>15 + 5*</td>
<td>158 (1989)</td>
</tr>
<tr>
<td>RNase inhibitor</td>
<td>RNase inhibitor</td>
<td>Human placenta</td>
<td>14 + 5</td>
<td>159 (1989)</td>
</tr>
<tr>
<td>Lutropin-chorio gonadotropin recept.</td>
<td>Lutropin receptor</td>
<td>Rat luteum</td>
<td>15</td>
<td>162 (1989)</td>
</tr>
<tr>
<td>Oligodendrocyte-Myelin GP</td>
<td>Neural function</td>
<td>Human nerve</td>
<td>8</td>
<td>165 (1990)</td>
</tr>
</tbody>
</table>

*Monocyte CD14 contains truncated (trunc.) LRG sequences, approximately half the length of the usual segment.
†The RNase inhibitor contains two forms (A and B) of LRG repeats. The A form has a five-amino acid insert (+ 5) while the B has a four-residue insert within the usual 24 amino acids.
‡The yopM product contains approximately six dispersed sequences that resemble portions of LRG segments.
to AP-1 sites in genomic DNA. Positioned at intervals within these proteins, leucines form a hydrophobic ridge on one side of an α helix and "zip" together with a complementary α helix to produce active regulatory factors. Both the innate structure of the leucine molecule and the spacing of the leucines appear to contribute to the formation of functional dimers. For example, other related hydrophobic amino acids such as isoleucine/valine cannot substitute for functional dimers. For example, other related hydrophobic amino acids such as isoleucine/valine cannot substitute for functional dimers. Moreover, leucines are present at positions 1, 8, 15, and 22 in many LRG segments (every seventh position), although such spacing would be interrupted after each 24-amino acid cycle. Both the leucine-predominance and the leucine-spacing within LRG sequences suggest that some parallels may exist between the functional properties of this structure and "leucine zippers." Such parallels might include the association of the LRG segments within a single LRG protein or between two different LRG proteins. However, the parallels are by no means complete because the high proline content in LRGs would impair α helix formation and another repetitive spacing of leucines in LRGs (positions 3, 11, 19: every eighth position, continuous between segments) differs from that of "zippers."

A POTENTIAL FUNCTION FOR THE LRG STRUCTURE OF Ibα

The binding site for vWF within GP Ibα is located between residues 225 and 310, based on studies of ristocetin-induced binding. The site lies outside the LRG structure of the α chain, residues 15 to 221, and therefore the LRG segments of GP Ibα do not appear to bind directly to vWF. However, the conservation of the LRG segments in the GP Ib-IX complex suggests that they possess function. The LRG segments of Ibα could contribute indirectly to function by participating in a shear-dependent conformational change within the molecule that exposes the binding site for vWF. The conformational change could lead to an interaction of the LRG segments with components of the platelet surface. The unique property of the change would be its shear-dependence, perhaps involving movement within the "hinge" binding domain. The hypothesis combines the striking structural feature of the Ib-IX complex (LRGs) with the major functional feature of the Ib-vWF interaction (shear-dependence). No direct evidence is available to support this model (Fig 1A), but the hypothesis is consistent with available structural and functional information on GP Ib and the GP Ib-vWF interaction.

CURRENT AND PROSPECTIVE STUDIES OF GP Ib-IX

Structure and chromosomal localization of the GP Ib-related genes. Genomic sequences coding for the α chain of human GP Ib have been cloned and characterized, showing a simple intron-depleted structure and several potential promoter sequences in the 5' upstream region. A single 233-bp intron is located six bases upstream from the start codon in the 5' noncoding portion of the cDNA sequence. The GP Ibα gene is located on the distal short arm of chromosome 17; specifically, 17p12-ter. Genomic cloning studies of GP Ibα and GP IX have not been reported, and the cDNA for GP V has not been characterized. However, Southern blot analysis and chromosomal hybridization studies with the GP IX cDNA probe are consistent with the presence of a small, single-copy gene for human GP IX, located on chromosome 3. Therefore, the genes for the GP Ib-related proteins (GP Ibα, V, IX) may prove to share a simple, intron-depleted structure. However, they are dispersed on at least two chromosomes, and the transcripts for the proteins are distinct entities because the genes are not linked in any physical way.

Tissue-specific and developmental regulation of the GP Ib-related genes. Surface GP Ib appears to be restricted to megakaryocytes and to cell lines with megakaryocytic features. The studies have used both Northern blot analysis of transcripts from various hematopoietic cells and monospecific antibodies for detection of cell surface antigens. GP Ib-like proteins that resemble GP Ibα either functionally or antigenically, have been described on the surface of endothelial cells and platelets. However, endothelial cells may lack transcripts for GP Ibα and transcripts for possible GP Ib-like proteins in platelets have not been characterized.

Regulation of the GP Ib-related genes during megakaryocyte development has not been explored in detail. Megakaryocyte-like cell lines increase their synthesis of GP Ibα in response to phorbol esters, and GP Ibα accumulates in normal megakaryocytes during maturation. However, no detailed information defines the megakaryocyte progenitors/precursors that synthesize GP Ibα or indicates the mode of developmental gene regulation (transcriptional or nontranscriptional). The synthesis of megakaryocyte proteins such as GP Ibα should be regulated, at least in part, by thrombopoietin as the "late" regulator of megakaryocyte differentiation and maturation. In addition, the transcription factor, GF-1, may play a role in regulation because it is involved in megakaryocyte differentiation. However, no direct studies on GP Ibα gene regulation are available, and the nature of thrombopoietin itself is unclear.

The molecular basis of BSs and pseudo-vWD. Bernard-Soulier platelets lack three surface GPs (GP Ibα, V, and IX), and therefore, BSs resembles congenital abnormalities of integrins because both disorders result in a deficiency of multiple surface proteins. Integrins are surface αβ heterodimers that subserve adhesion and assemble through a pattern of a common β subunit linked to one of several α subunits. Normal surface expression of an αβ integrin heterodimer requires normal synthesis, processing, and assembly of both the α and β chains. Congenital disorders of integrins such as Glanzmann’s thrombasthenia and leukocyte adhesion deficiency appear to arise from genetic defects limited to one chain that result in deficient surface expression of both chains. Applying the information from the integrins to BSs, one may begin with the premise that all four polypeptides (Ibα, V, and IX) are
required for normal expression of the mature surface GP$s (the GP Ib-IX complex and GP V). A defect in any aspect (synthesis, processing, assembly) of any one of the four polypeptides could result in abnormal expression of the other three. Some phenotypic variation could result from specific single defects, and phenotypic variants of BSs have been described.12,24,189 However, descriptions of molecular genetic defect(s) in BSs are few. A heterozygous point mutation in the GP Ib, gene has been reported in a patient with atypical pseudo-vWD. Current information suggests that specific single defects, and phenotypic variants of BSs appear to possess normal genes for GP Ib, and Southern blot analyses of the GP Ib, gene in BSs patients show a normal pattern.192 Continuing work may identify mutations within GP Ib-related genes in BSs that will indicate which gene product(s) is required for expression of the surface proteins. 

To date, no molecular genetic defects have been described in pseudo-vWD. Current information suggests that the defect lies in the coding region of the GP Ib, gene.134,135

**CONCLUDING REMARKS**

Knowledge of platelet GP Ib has progressed since the description of the BSs in 1948. The protein is associated with other elements both on the outside (GP IX) and on the inside (actin-binding protein) of the platelet plasma membrane, and it shares the LRG structure with its related proteins (GP V, GP IX) and with the other members of the LRG family. The essential feature of platelet GP Ib is linkage; specifically, linkage of three events across the platelet surface membrane. The first event is extracellular, a shear-dependent interaction with vWF. The second is cellular, the adherence of the platelet to an arterial vascular site. The third is intracellular, activation of the previously resting platelet. The molecular mechanisms for these events are yet to be defined. The field is ripe for conjecture and for further insight into the relationships among mechanical force, a critical protein–protein interaction, and subsequent hemostatic and thrombotic events.

**ACKNOWLEDGMENT**

I thank Bea Yancy for secretarial assistance; the Pheresis Unit of the Puget Sound Blood Center for platelet pheresis specimens; and my colleagues Mark J. Hickey, Stuart A. Williams, Charles R. Tweedy, and Todd A. Church for helpful discussions. James G. White, MD, generously provided the photomicrograph for the cover illustration.

**REFERENCES**

22. Clementson KJ, McGregor JL, James E, Dechavanne M,


71. Moske JL, Turner NA, Stathopoulos NA, Nolasso L, Hellem J: Shear-induced platelet aggregation can be mediated by vWF released from platelets, as well as by exogenous large or unusually large vWF multimers, requires adenosine diphosphate, and is resistant to aspirin. Blood 71:1366, 1988.


99. Weiss HJ, Turitto VT, Baumgartner HR: Platelet adhesion and thrombus formation on subendothelium in platelets deficient
in glycoprotein IIb/IIa, Ib and storage granules. Blood 67:322, 1986


112. Nievlestein PFEM, Sivasa JJ: Glycoprotein IIb-IIa and RGD(S) are not important for fibronectin-dependent platelet adhesion under flow conditions. Blood 72:82, 1988


133. Lopez JA, Chung DW, Fujikawa K, Hagen FS, Davie EW, Roth GJ: The alpha and beta chains of human platelet glycopro- tein Ib are both transmembrane proteins containing a leucine-rich amino acid sequence. Proc Natl Acad Sci USA 85:2135, 1988


140. Roth GJ, Hickey MJ, Chung DW, Hickstein DD: Circulating human blood platelets retain appreciable amounts of poly (A)+ RNA. Biochem Biophys Res Commun 166:705, 1989
144. Handin RJ, Petersen E: Production of recombinant glycoprotein Ibalpha fragments and delineation of the von Willebrand factor binding site. Blood 74:477a, 1989 (abstr, suppl)
148. Kalomiris EL, Coller BS: Thiol-specific probes indicate that the beta-chain of platelet glycoprotein Ib is a transmembrane protein with a reactive endofacial sulfhydryl group. Biochemistry 24:5430, 1985
Developing relationships: arterial platelet adhesion, glycoprotein Ib, and leucine-rich glycoproteins

GJ Roth