

Platelets: Physiology and Biochemistry

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ABSTRACT

Platelets are specialized blood cells that play central roles in physiologic and pathologic processes of hemostasis, inflammation, tumor metastasis, wound healing, and host defense. Activation of platelets is crucial for platelet function that includes a complex interplay of adhesion and signaling molecules. This article gives an overview of the activation processes involved in primary and secondary hemostasis, for example, platelet adhesion, platelet secretion, platelet aggregation, microvesicle formation, and clot retraction/stabilization. In addition, activated platelets are predominantly involved in cross talk to other blood and vascular cells. Stimulated “sticky” platelets enable recruitment of leukocytes at sites of vascular injury under high shear conditions. Platelet-derived microparticles as well as soluble adhesion molecules, sP-selectin and sCD40L, shed from the surface of activated platelets, are capable of activating, in turn, leukocytes and endothelial cells. This article focuses further on the new view of receptor-mediated thrombin generation of human platelets, necessary for the formation of a stable platelet-fibrin clot during secondary hemostasis. Finally, special emphasis is placed on important stimulatory and inhibitory signaling pathways that modulate platelet function.

KEYWORDS: Platelet activation, platelet adhesion, platelet aggregation, procoagulant activity, thrombin generation

Objectives: On completion of the article, the reader should be able to (1) recite some physiological functions of platelets in hemostasis and (2) describe the principle of the receptor-mediated model of thrombin generation on the platelet surface.

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Although the platelet was initially viewed only as a bystander in haemostasis, it is now evident that the platelet is in fact a key mediator of thrombosis as well as inflammation.

D.L. Bhatt and E.J. Topol¹

Platelets, the smallest of the human blood cells ($3.6 \times 0.7 \mu\text{m}$), are central players in processes of hemo-

stasis and thrombosis. In addition, platelets are specialized cells of the innate immune defense, modulators of the inflammatory response, and involved in wound healing as well as in hematogenic metastasis. They are released from megakaryocytes in the bone marrow as anucleated fragments into the circulation. When a vessel wall is damaged, platelets are recruited from the circulation to the unveiled subendothelial matrix

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Table 1 Agonists, Ligands, and Receptors Important for Platelet Function

Platelet Function	Agonists, Ligands	Receptors
Initial and firm adhesion	vWF	GPIb/V/IX
	TSP1	GPIb/V/IX, CD36
	Collagen	$\alpha_2\beta_1$, GPVI, CD36
	Fibrinogen	$\alpha_{IIb}\beta_3$
	Fibronectin	$\alpha_5\beta_1$ ⁷³
	Vitronectin	$\alpha_v\beta_3$ ⁷⁷
	Laminin	$\alpha_6\beta_1$ ⁷⁴
	High shear stress	GPIb/V/IX
Activation and amplification	Thrombin	PAR1, PAR4, GPIb/V/IX
	ADP	P2Y ₁ , P2Y ₁₂
	TxA ₂	TP α , TP β
	Epinephrine	α_2A
	Serotonin	5-HT2A
	MMP-2, MMP-1 ^{75,76}	?
	Immune complexes	Fc γ IIa
	Complement factors	C1q, C3a, C5a receptors
	Plasmin	?
Aggregation/amplification and stabilization	Streptokinase	?
	Fibrin	Activated $\alpha_{IIb}\beta_3$
	vWF	Activated $\alpha_{IIb}\beta_3$, GPIb/V/IX
	TSP-1 ⁷⁷	Activated $\alpha_{IIb}\beta_3$, CD36,IAP
	Fibronectin	Activated $\alpha_{IIb}\beta_3$
	sCD40L	Activated $\alpha_{IIb}\beta_3$
	Gas6	AxI ^{78,79}
	SDF-1, TARC, MDC	CXCR4, CCR4 ⁸⁰⁻⁸²

vWF, von Willebrand factor; TSP1, thrombospondin-1; ADP, adenosine diphosphate; TxA₂, thromboxane A₂; MMP, matrix metalloproteinase; IAP, integrin associated protein; SDF, stromal cell-derived factor; TARC, thymus and activation-regulated chemokine; MDC, macrophage-derived chemokine.

forming a hemostatic plug to close the leak in the vessel wall. On the other hand, platelets form a thrombus at sites of ruptured atherosclerotic plaques, and in this way trigger heart attacks and strokes. The immobilization of platelets at sites of vascular injury requires specific platelet-vessel wall- (adhesion) and platelet-platelet-interactions (aggregation). The major agonists and adhesion molecules/receptors that mediate these interactions are summarized in Table 1.

MECHANISMS OF PLATELET ACTIVATION

The *adhesion* of platelets to the subendothelial matrix is the initial step in primary hemostasis. Platelets interact with extracellular matrix proteins via specific adhesive glycoproteins (GP). Binding of biochemical agonists to their receptors, receptor cross-linking, or changes in the plasma membrane induce a complex cascade of signals, transduced from the membrane into the cytoplasm, which results in platelet activation (outside-in signaling).² In contrast to resting platelets, which are discoid with homogeneously distributed granules (Fig. 1A),

activated platelets show a change in the assembly of cytoskeleton proteins resulting in a shape change with extensive formation of pseudopodia originating from the plasma membrane.³ Further, the granules centralize and fuse with the plasma membrane via exocytosis with *secretion* of the granule content (Fig. 1B). Some secretion products, such as adenosine diphosphate (ADP) and serotonin, potentiate the stimulation of more platelets, which are attracted to the damaged vessel wall. The activation of platelets is associated with the binding of fibrinogen to its major receptor GPIIb/IIIa ($\alpha_{IIb}\beta_3$ -integrin), which is essential for platelet bridging and subsequent *aggregation*. During secondary hemostasis, the amplification of platelet stimulation leads to *procoagulant activity*, *thrombin generation*, and formation of a stable platelet-fibrin plug with subsequent clot retraction.

Platelet Adhesion

In certain conditions of flow, platelets have to slow down to stop at sites of vascular damage. The high molecular weight (1–10 MDa) multimeric plasma protein von

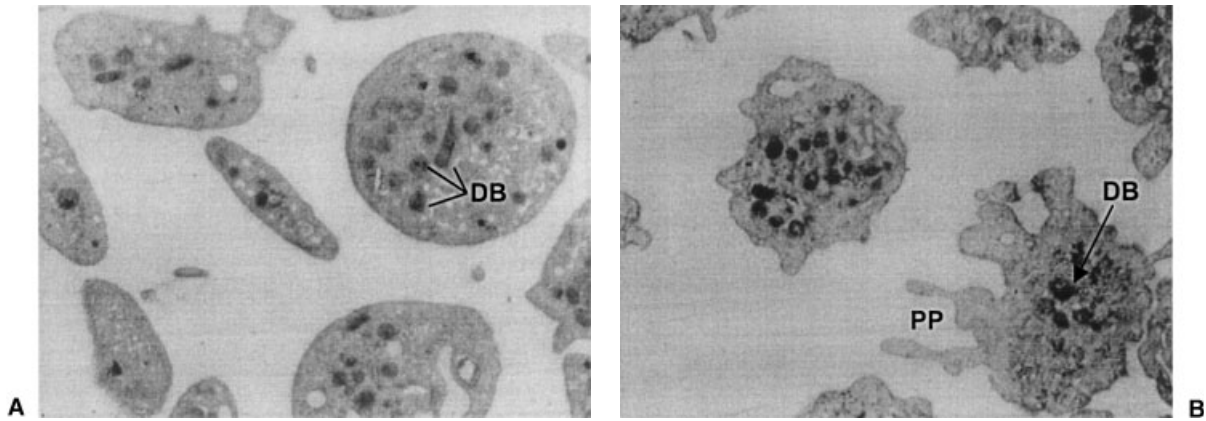


Figure 1 Morphology of human platelets. (A) Thin section of discoid resting platelets with evenly distributed granules. (B) Thin section of stimulated platelets, showing formation of pseudopodia and centralization of granules. DB, dense body; PP, pseudopodium. Magnification $\times 21,000$.

Willebrand factor (vWF) associates with the major matrix protein collagen on the surface of the subendothelium and serves as a substrate for platelet adhesion, predominantly under high shear. The multiple binding sites of vWF multimers enables first contacts to the GPIb/V/IX complex on platelets leading to formation of firm bonds and platelet capture. In contrast to vWF monomers, only dimers and multimers are able to cross-link and to activate the GPIb/V/IX complex. Conformational changes in the GPIb/V/IX or vWF molecule are thought to modulate these interactions. Under physiological conditions it is supposed that binding of vWF to collagen enables binding to GPIb/V/IX.⁴ Even point mutations in the vWF or GPIb induced spontaneous ligand binding. The antibiotic ristocetin or the snake venom ingredient botrocetin are used to induce vWF-GPIb/V/IX interaction in vitro. The modular glycoprotein thrombospondin-1 (TSP1), which is also integrated in the subendothelial matrix, has been identified to serve as an alternative adhesion substrate to vWF via GPIb under high shear conditions.⁵ In addition to PSGL1, the GPIb/V/XI complex enables rolling of activated platelets on the endothelium through endothelial P-selectin.^{6,7}

Under static or low shear conditions, platelets adhere predominantly to collagen of the subendothelium. Collagen binds initially to GPIa/IIa, cross-links many of these integrin molecules, and in this way activates platelets.⁸ Patients who lack platelet GPIa/IIa have bleeding problems.⁹ Other collagen receptors, such as CD36 and GPVI, play important roles in collagen-induced signaling.^{10,11} GPVI, the major signaling receptor, is a member of the immunoglobulin superfamily and is linked to the Fc receptor γ chain. Its signaling pathway is similar to lymphocyte signaling.¹² Stimulation of platelets as a result of adhesion leads to spreading, activation of GPIIb/IIIa, enabling binding of soluble fibrinogen, and granule secretion (Fig. 2).

Platelet Secretion

Activated platelets release several granule components which modulate functions of interacting platelets and blood and vascular cells. Several secretion products of immobilized platelets stimulate additional circulating platelets which are recruited to form aggregates. The dense bodies of platelets contain important secondary agonists like ADP or serotonin. About 50% of platelet ADP is stored in the dense bodies (storage pool), which is released after platelet activation but cannot be refilled. In contrast, the metabolic pool of adenine nucleotides, localized in the cytoplasm but not connected to the dense bodies, is able to synthesize new ADP but cannot be released.¹³ ADP is predicted to be the prominent amplifier of initial platelet activation.¹⁴ There are two important ADP receptors on the platelet surface. The P2Y₁-receptor mediates mobilization of Ca^{2+} and shape change and transient aggregation.¹⁵ The P2Y₁₂-receptor is believed to potentiate platelet secretion and to be involved in sustained irreversible aggregation.¹⁶ Enzymatic conversion of released ADP to inactive adenosine monophosphate (AMP) by endothelial ecto-ADPase/CD39 limits platelet activation by ADP.¹⁷ A lack of the second aggregation wave after collagen stimulation characterizes disorders in ADP-mediated platelet activation.

Serotonin (5-hydroxytryptamine, 5-HT), a well-known strong vasoconstrictor, binds to the Gq-coupled 5HT_{2A}-receptor and amplifies together with ADP the platelet response. In addition, serotonin may play a procoagulant role in augmenting the retention of procoagulant proteins like fibrinogen and thrombospondin (TSP) on the platelet surface.¹⁸ The dense tubular system contains a Ca^{2+} pool which is mobilized during platelet activation. Ca^{2+} fluxes are central triggers in platelet activation, platelet attraction, and platelet aggregation.¹⁹ The α -granules contain large adhesive proteins (vWF, TSP1, vitronectin, fibronectin), mitogenic factors (PDGF, VEGF, TGF β), coagulation

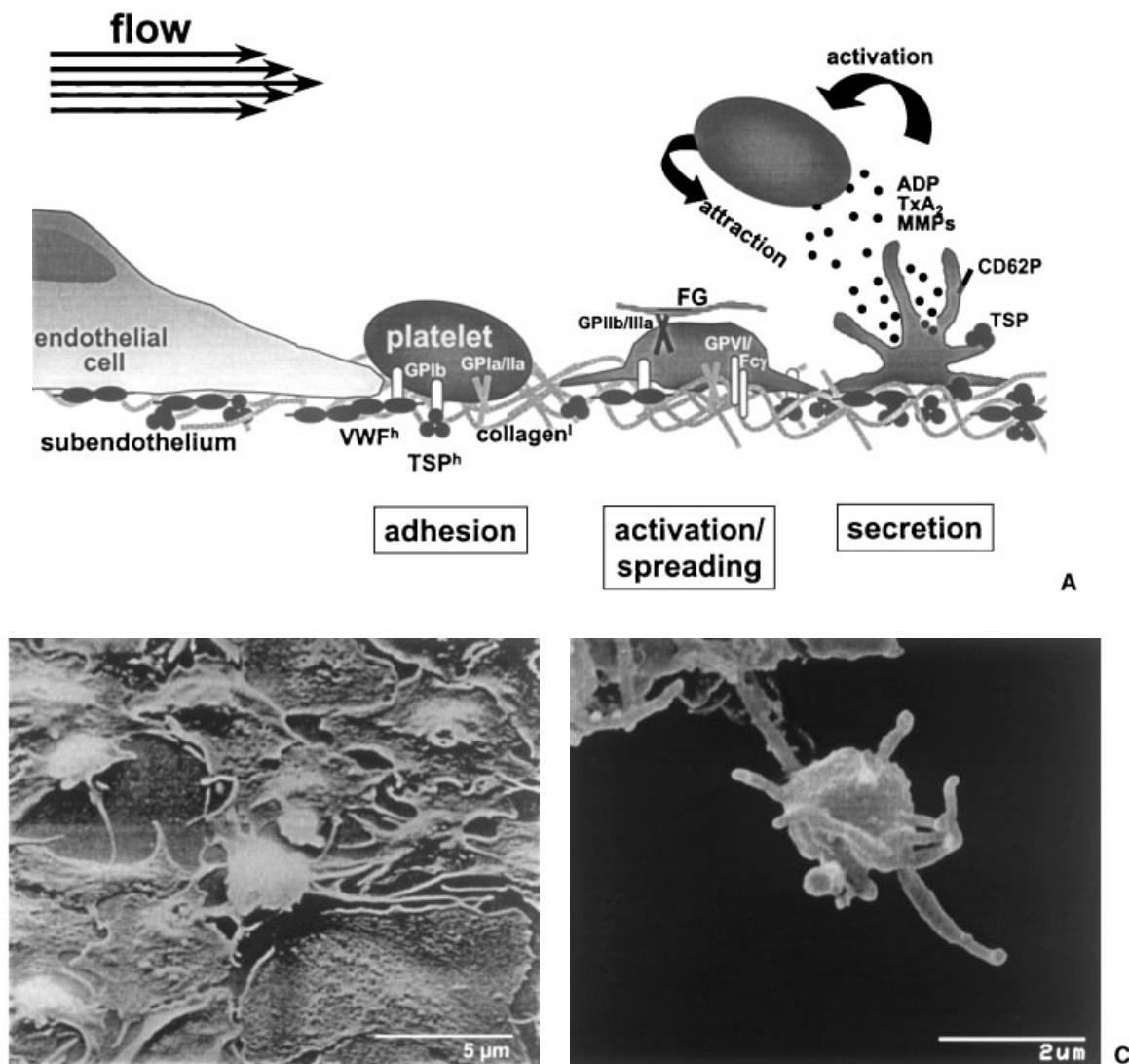


Figure 2 Primary hemostasis. (A) Platelets recruited from the circulation to the subendothelium of a damaged vessel wall adhere to vWF and TSP via GPIb/V/IX under high shear conditions (h). Collagen serves as adhesive substrate for platelets (via GPIa/IIa and GPVI/Fcγ) under low shear conditions (l). Receptor clustering through multiple binding sites of matrix proteins induce activation of GPIIb/IIIa with subsequent binding of fibrinogen (FG) and platelet spreading. Activated platelets secrete several adhesive proteins, including TSP, which binds back to the platelet surface. Secreted secondary agonists (ADP, TxA₂ and MMPs) amplify activation and attraction of additional circulating platelets. (B) REM-preparation of human platelets adhered and spread on immobilized collagen. (C) Scanning electron microscope preparation of a thrombin-stimulated human platelet with marked pseudopodia. vWF, von Willebrand factor; TSP, thrombospondin, ADP, adenosine diphosphate; TxA₂, thromboxane A₂; MMPs, matrix metalloproteinases.

factors (factors V, VII, XI, XIII), and protease inhibitors (protein C, PAI-1, TFPI), which are released immediately after platelet activation. Some of the α -granule proteins are synthesized by megakaryocytes (TSP1,²⁰ β -thromboglobulin, platelet factor 4); others are endocytosed from the plasma (immunoglobulins, fibrinogen, vitronectin). Various glycoproteins, for example, P-selectin (CD62P), are exclusively localized on the α -granule membrane of resting platelets. Upon secretion the membrane of the α -granule membrane fuses with the plasma membrane and exposes CD62P on the platelet surface. P-selectin and other activation-dependent glycoproteins, including CD40L, mediate platelet binding

to neutrophils and monocytes.²¹ Leukocytes are able to roll on platelets, which are immobilized on the subendothelium, in a P-selectin-dependent manner (Fig. 2).²²

Platelet Aggregation

The aggregation of platelets is characterized by the accumulation of platelets into a hemostatic plug (Fig. 3). The central platelet receptor in this process is the GPIIb/IIIa ($\alpha_{IIb}\beta_3$ -integrin) linking activated platelets through fibrinogen bridges. A resting platelet presents ~40,000 to 50,000 GPIIb/IIIa complexes on its surface. In its nonactive state this integrin cannot

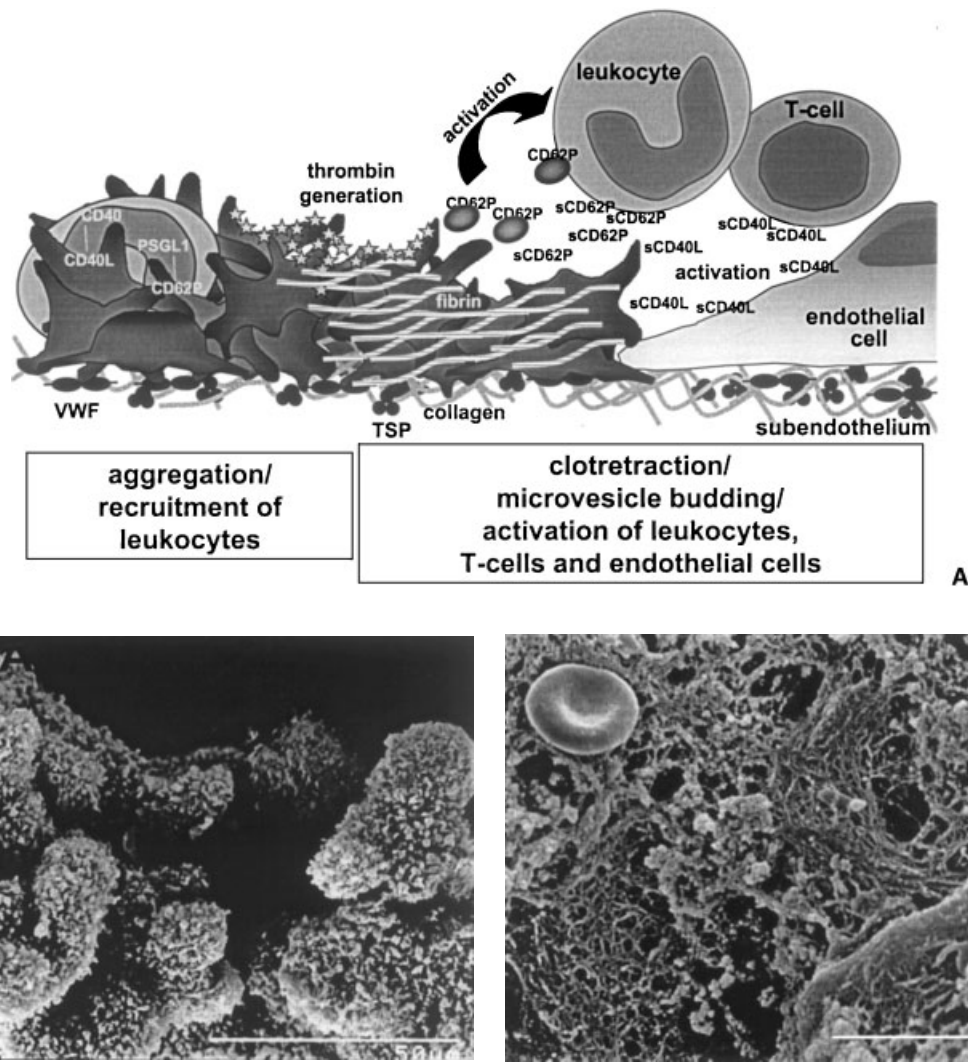


Figure 3 Aggregation and secondary hemostasis. (A) Fibrinogen or in high shear environments vWF bridges platelets through activated GPIIb/IIIa, leading to formation of an unstable platelet plug. Leukocytes are recruited to aggregated platelets via CD40/CD40L and PSGL1/CD62P interactions. Increased amounts of thrombin are generated on the platelet plug, which converts bound fibrinogen to fibrin, leading to plug stabilization and clot retraction. Microparticles as well as adhesion molecules (sCD62P, sCD40L) are shed from the platelet surface into the circulation as stimuli for leukocytes, T cells, and endothelial cells. (B) Scanning electron microscope preparation of a nonretracted platelet plug, induced by collagen. (C) REM-preparation of a platelet-fibrin clot with recruited red cell(s). vWF, von Willebrand factor; TSP, thrombospondin.

bind soluble ligands like plasma fibrinogen, vWF, TSP, fibronectin, or vitronectin. Only stimulation of a platelet leads to an increase in GPIIb/IIIa molecules, via α -granule exocytosis, and to activation of surface-exposed GPIIb/IIIa, enabling binding of soluble ligands. On the other hand, immobilized fibrinogen on stimulated platelets serves as an adhesive substrate for resting platelets through GPIIb/IIIa that leads to amplification of primary aggregation.²³ Interaction between GPIIb/IIIa and its ligand is associated with molecular conformational changes, resulting in a firm connection. Activation-dependent changes in the conformation of GPIIb/IIIa can be detected biophysically or by specific antibodies. Ligand binding to GPIIb/IIIa induces additional conformational changes which lead to phosphor-

ylation of tyrosines of the cytoplasmatic GPIIIa-chain.^{24,25} Disulfide changes in the GPIIb/IIIa complex by the surface-associated protein disulfide isomerase induce high affinity binding sites for fibrinogen.²⁶ Fibrinogen links discoid platelets during the initial platelet-platelet contact.²⁷ In high shear environments, as found in arterioles and stenosed arteries, platelet activation/aggregation can be induced by shear itself. In this case, platelets are first linked by vWF bridges via the GPIb/V/IX complex. This interaction leads to activation of GPIIb/IIIa (inside-out signaling) and in turn to stable vWF-mediated platelet aggregates.²⁸

Low-density lipoproteins (LDL) are supposed to be also a ligand of GPIIb/IIIa and a modulator of

platelet function. Increased levels of LDL are found in the plasma from patients who discontinued statin treatment and these elevated LDL levels are associated with platelet hyperactivity. The possible link between LDL and a corresponding prothrombotic state may explain the increased cardiovascular event rate after statin discontinuation.²⁹ In addition, platelets recruit leukocytes and T-cells into the growing plug. Interactions via P-selectin/PSGL1 or CD40L/CD40 mediate activation of leukocytes which trigger or limit thrombus growth.³⁰ Circulating platelet-monocyte aggregates are supposed to play a role in enhancing formation of atherosclerotic plaques as well as in graft occlusion after peripheral vascular surgery.^{31,32} Formation of platelet-leukocyte associates via GPIb on platelets and Mac-1 on leukocytes may be a cause of the phenomenon of rapid clearance of transferred cooled platelets with activated clustered GPIb/V/IX complexes.³³

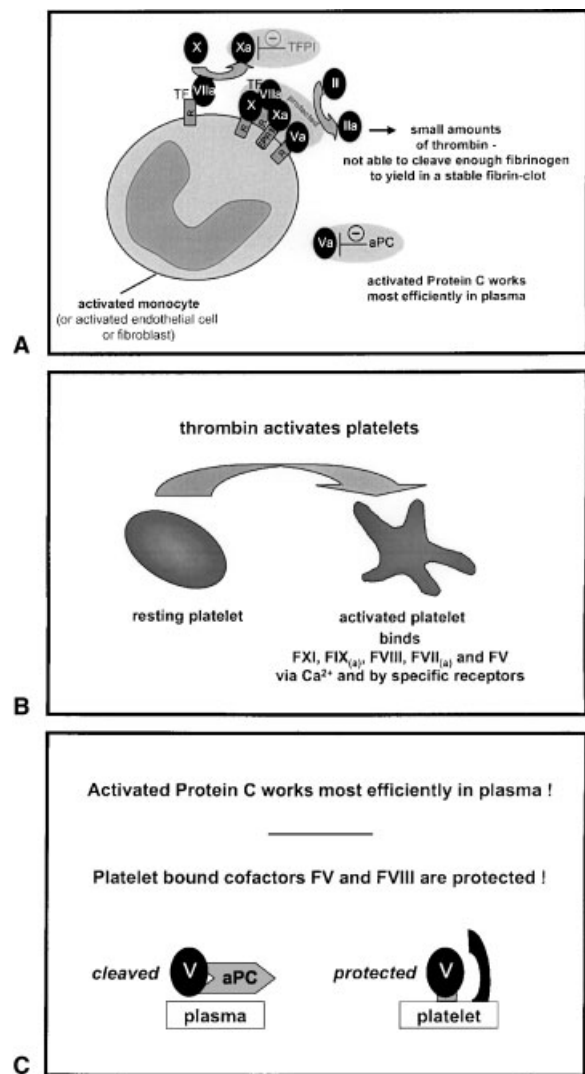
PROCOAGULANT ACTIVITY – MODEL OF RECEPTOR-MEDIATED THROMBIN GENERATION ON PLATELETS

The formation of a stable platelet plug during secondary hemostasis is characterized by thrombin-mediated conversion of fibrinogen to fibrin. Thrombin is generated on surfaces of blood and vascular cells. However, the platelet membrane contains a specific lipid assembly and receptors with high-affinity binding sites for clotting factors, a favored preferential and specialized locus to induce and modulate secondary hemostatic processes.^{34,35}

The plasma membrane of platelets consists predominantly of phospholipids (~70%), cholesterol, and glycolipids. The major phospholipids are phosphatidylcholine (PC), sphingomyelin (SphM), phosphatidylethanolamine (PE), phosphatidylserine (PS) and phosphatidylinositol (PI). They are localized asymmetrically in the plasma membrane, with a concentration of SphM and PC in the outer leaflet and of PE and acetylated arachidonic acid in the inner leaflet. Enzymes are distributed in a specific manner in different platelet membranes. It is postulated that only the plasma membrane contains adenylate cyclase. In contrast, enzymes responsible for thromboxane A₂-synthesis, such as phospholipase A₂, diglycerol lipase, cyclooxygenase (CO), and thromboxane synthase, are restricted to the intracellular dense tubular membranes.³⁶ Platelet activation is associated with a flip-flop move of anionic phospholipids, for example, PS, from the inner to the outer leaflet, leading to an increase of PS from 2 to 12% of the phospholipid content. The exposure of anionic phospholipids on the platelet surface can be monitored by labeling of platelets with annexin V, a specific ligand for amino phospholipids.³⁷ The exposure of PS on aggregated platelets provides a catalytic surface for procoagulant

processes, enabling thrombin generation at the site of injury.³⁸ A recently identified alternatively spliced form of human tissue factor (TF) exhibits procoagulant activity when exposed as soluble molecule to phospholipids, suggesting that soluble TF contributes to thrombus growth.³⁹

In contrast to the classic view of secondary hemostasis, a model of controlled thrombin generation on the platelet surface may explain many unresolved questions regarding hemophilia or the molecular mechanism of FVIIa in the clotting process. Small amounts of thrombin are formed on the surface of a TF presenting cell (fibroblast or activated monocyte or activated endothelial cell, respectively). These amounts of thrombin are not able to produce a stable fibrin clot, but are enough to activate platelets. Activated platelets can then bind coagulation factors and cofactors via Ca²⁺ and by specific receptors.^{40,41} Platelet-bound cofactors FV and FVIII are protected against cleavage by activated protein



C.⁴² On the surface of the platelet, FXIa binds to its receptor GPIb and activates FIX. In contrast to FXa, which is readily inhibited by TF pathway inhibitor (TFPI) as soon as it enters the plasma, FIXa, built on

TF/FVIIa presenting cells can in addition diffuse to the activated platelets. On the platelet surface, the Xase complex and the prothrombinase complex have optimal conditions.^{43,44} The concerted actions of coagulation

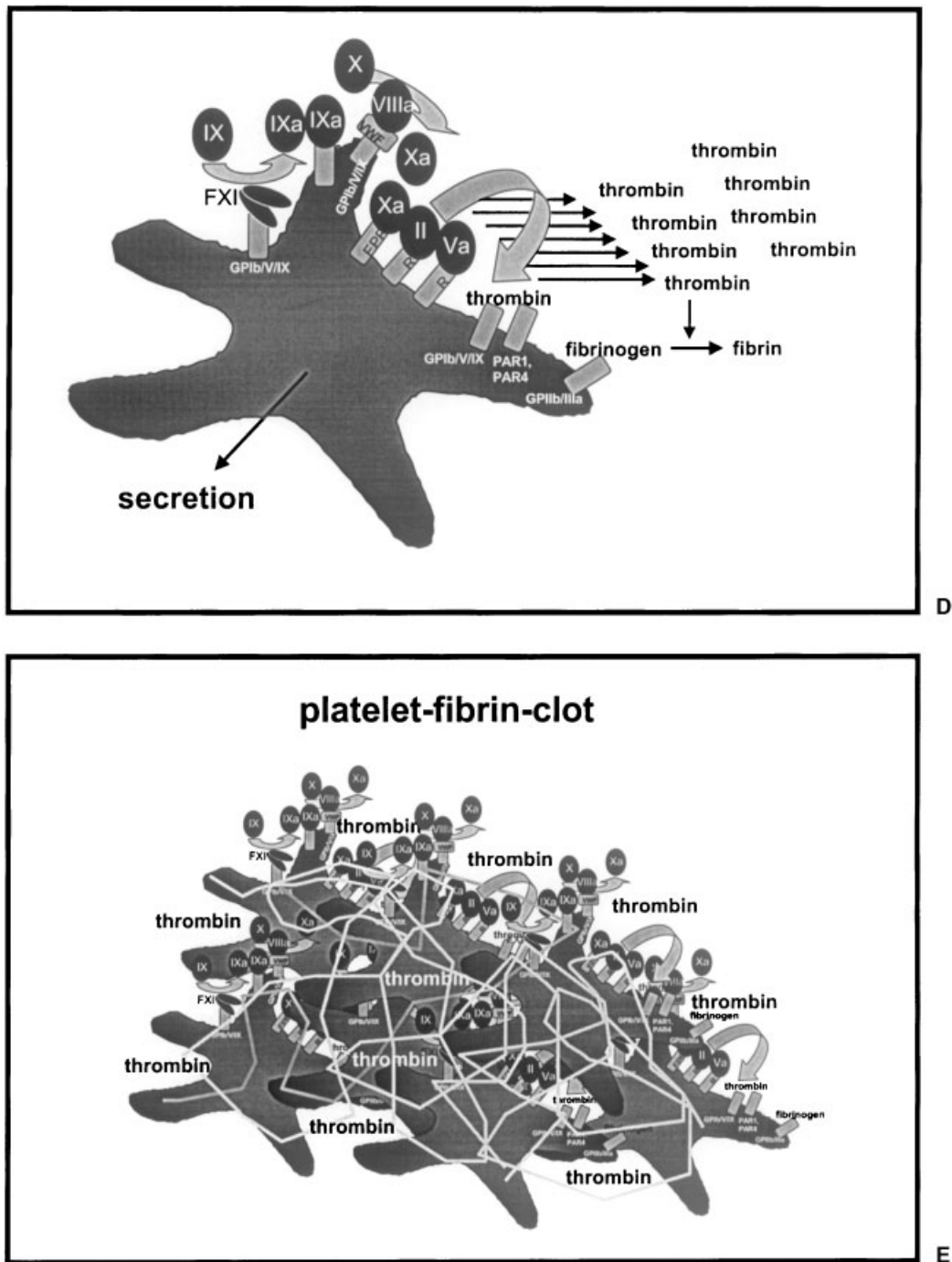


Figure 4 Model of receptor-mediated thrombin generation. (A) Small amounts of thrombin formed on the surface of a tissue factor (TF) presenting cell (fibroblast or activated monocyte or activated endothelial cell, respectively). These amounts of thrombin are not able to produce a stable fibrin clot, but are enough to activate platelets. (B) Activated platelets can then bind coagulation factors and cofactors via Ca^{2+} and by specific receptors. (C) Platelet-bound cofactors FV and FVIII are protected against cleavage by activated protein C. (D) On the surface of the platelet FXIa binds to its receptor GPIb and activates FIX. In contrast to FXa that is readily inhibited by tissue factor pathway inhibitor (TFPI) as soon as it enters the plasma, FIXa, built on TF/FVIIa presenting cells can in addition diffuse to the activated platelets. On the platelet surface the Xase-complex and the prothrombinase-complex have optimal conditions. (E) The concerted actions of coagulation factors on the platelet surface lead to a burst of thrombin formation, so that a stable fibrin clot can be formed. aPC, activated protein C; R, receptor; EPR1, effector cell protease receptor 1, PAR, protease activated receptor.

factors yield on the platelet surface in a burst of thrombin formation, so that a stable fibrin clot can be formed (Fig. 4).^{45,46}

MICROVESICLE RELEASE/SHEDDING OF ADHESION MOLECULES

Strong agonists like collagen in combination with thrombin or complement (C5b-9) induce shedding of microvesicles from the platelet surface. This "budding" process is due to Ca^{2+} -mediated activation of calpain and leads to vesicles containing exclusively intracytoplasmic substances.^{47,48} These vesicles are procoagulant and show similar surface expression of activation-dependent adhesion molecules (P-selectin, CD40L) as stimulated platelets. Platelet-derived microparticles are found to be increased in the circulation of patients with sepsis or after cardiopulmonary bypass and are thought to be associated with thrombotic diseases.⁴⁹ Therefore, defects in shedding of platelet-microvesicles is associated with bleeding disorders. It has been shown that platelet-microvesicles bind to and activate leukocytes and endothelial cells, bridging leukocytes to each other or leukocytes with endothelial cells via PSGL1 L-selectin, P-selectin/PSGL1, and CD40L/CD40, respectively.⁵⁰ Upon platelet stimulation, surface-expressed CD62P as well as CD40L are further cleaved in soluble fragments.^{51,52} sCD62P is known to induce a procoagulant state of monocytes. Platelet-derived sCD40L is sup-

posed to be a potent stimulus for T-cells, endothelial cells, and platelets and seems to be necessary for stability of arterial thrombi (Fig. 3).⁵³⁻⁵⁵

SIGNAL TRANSDUCTION – STIMULATORY SIGNALING

Stimulatory platelet signaling as a result of receptor ligation and receptor cross-linking leads to production and release of several intracellular messenger molecules: Ca^{2+} , products of the phospholipase C (PLC)-mediated phosphoinositid hydrolysis, diacylglycerol, inositol-1,4,5-triphosphat (IP3), and thromboxane A_2 (TxA_2). Platelet agonists like ADP, TxA_2 , epinephrine, serotonin, and thrombin interact with seven specific transmembrane receptors that are coupled by GTP-binding heterotrimeric G-proteins, initiating several signaling pathways (Fig. 5). Signaling through receptors coupled to the Gq-family of G-proteins (PAR1, PAR4, TxA_2 -receptor, 5-HT $_{2A}$ -receptor) leads to activation of PLC. PLC catalyzes the hydrolysis of phosphatidyl inositolbisphosphate (PIP2) to IP3, which induces the mobilization of Ca^{2+} from the dense tubula system. An increase in intracellular Ca^{2+} is associated with a phosphorylation of the myosin-light-chain by myosin-light-chain kinase, a process that is necessary for shape change. In addition, receptor signaling through G $_{12/13}$ -proteins (PAR1, PAR4), contributes to shape change, too.⁵⁶ Granule secretion is one relevant process in response to Ca^{2+}

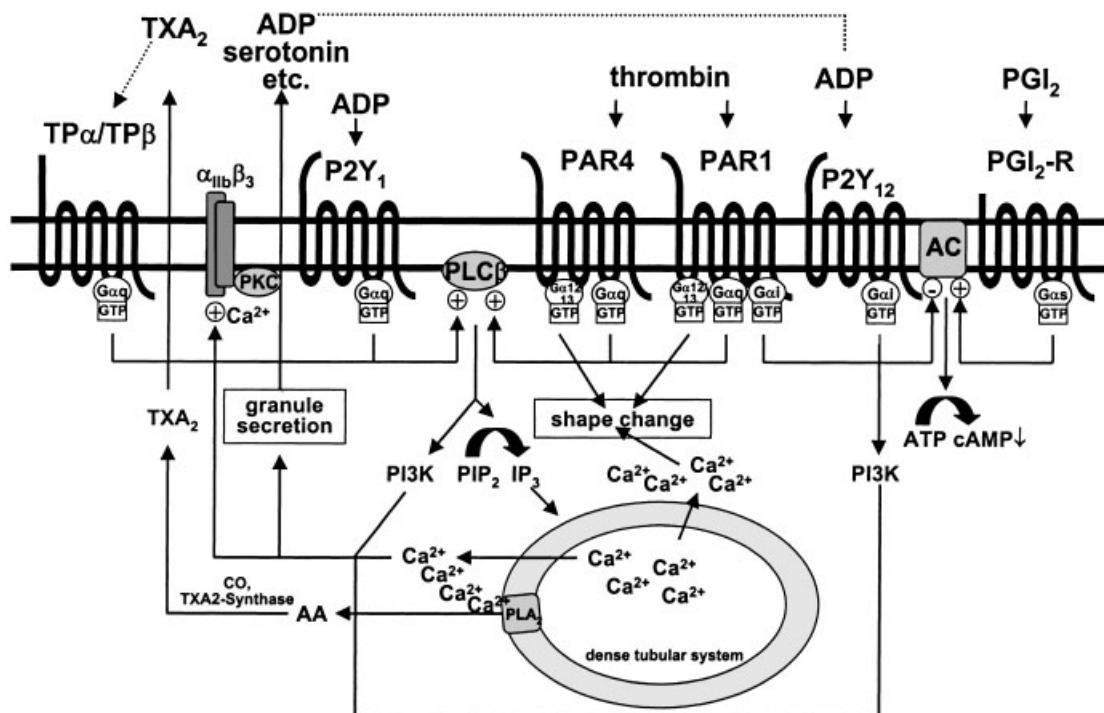


Figure 5 G-protein coupled seven-transmembrane receptor signaling in platelets. GTP, guanine triphosphate; PKC, protein kinase C; TxA_2 , thromboxane A_2 ; CO, cyclooxygenase; PI3K, phosphatidylinositol-3 kinase; PIP2, phosphatidylinositolbisphosphate; IP3, inositol-1,4,5-triphosphate; PLA, phospholipase A; AC, adenylatecyclase; cAMP, cyclic adenosine monophosphate; ADP, adenosine diphosphate; ATP, adenosine triphosphate; PGI $_2$, prostaglandin I $_2$, R, receptor; PAR, protease activated receptor.

mobilization, which leads to release of ADP from the dense bodies. ADP binds back to P2Y₁₂ and amplifies platelet activation.⁵⁷ Another activation-enhancing pathway is characterized by the synthesis and release of TxA₂ which results from Ca²⁺-dependent mobilization of arachidonic acid by phospholipase A2 and subsequent metabolism through the CO and TxA₂-synthase. Secreted TxA₂ in turn rebinds to its Gq-coupled TP-receptors and potentiates stimulatory processes.⁵⁸ This pathway is not essential for GPIIb/IIIa activation, secretion, and aggregation.⁵⁹ Therefore, blocking this pathway by acetylsalicylic acid (COX-inhibitor), TxA₂-receptor inhibitors, or TxA₂-synthase inhibitors does not inhibit platelet activation completely. Secreted ADP activates additional Gi-mediated pathways via its P2Y₁₂-receptor, leading to inhibition of adenylylase with subsequent decrease of the activation blocking messenger cAMP. Pepducins, cell-penetrating peptides, are novel intracellular inhibitors of signal transduction from receptor to G-proteins.⁶⁰ It has been shown that epinephrine signaling through Gq-coupled α_2 a receptor stimulation shares the final part of the P2Y₁₂-receptor signaling pathway.⁶¹ This “bypass effect” of epinephrine may possibly explain why thienopyridine drugs (ticlopidine, clopidogrel), affecting the P2Y₁₂-receptor, are not as successful in antithrombotic therapy of ischemic arterial vascular diseases as hoped.

Signaling via collagen, immunoglobulins and vWF depends on nonreceptor tyrosine kinases. Platelet stimulation in response to collagen involves signaling by the major collagen receptors GPIa/IIa and GPVI

(Fig. 6). The signaling via GPVI, which is associated with an Fc-receptor γ chain, is similar to antigen-receptor-mediated signaling in T and B cells.^{62,63} Cross-linking of GPVI/FcR γ chain by collagen ligation leads to phosphorylation of the immunoreceptor tyrosine-based activation motif (ITAM) of the FcR γ chain by the Src-tyrosine kinases Lyn and Fyn with subsequent binding and activation of the nonreceptor tyrosine kinase Syk. The participation of Syk and the adapters LAT and SLP76 leads to activation of phospholipase C γ 2 (PLC γ 2). The formation of lipid rafts may be essential for the integration of the key signaling complexes, leading to PLC γ 2 activation.⁶⁴ Activation of PLC γ 2 results in IP3-synthesis, and the subsequent downstream pathways are similar to those induced by Gq-coupled receptors. As a consequence GPIIb/IIIa receptors are activated, enabling binding of soluble ligands, which in turn leads to activation of Syk (outside-in signaling), contributing to actin polymerization and platelet spreading.⁶⁵ Recently it has been revealed that GPIa/IIa may couple to many of the same intracellular signaling molecules as GPVI.^{66,67} Therefore, the roles of GPVI and GPIa/IIa are not as easy to distinguish as originally thought.

SIGNAL TRANSDUCTION – INHIBITORY SIGNALING

For regulation and limitation of collagen-induced thrombus formation, platelets express platelet-endothelial cell adhesion molecule-1 (CD31), a member of

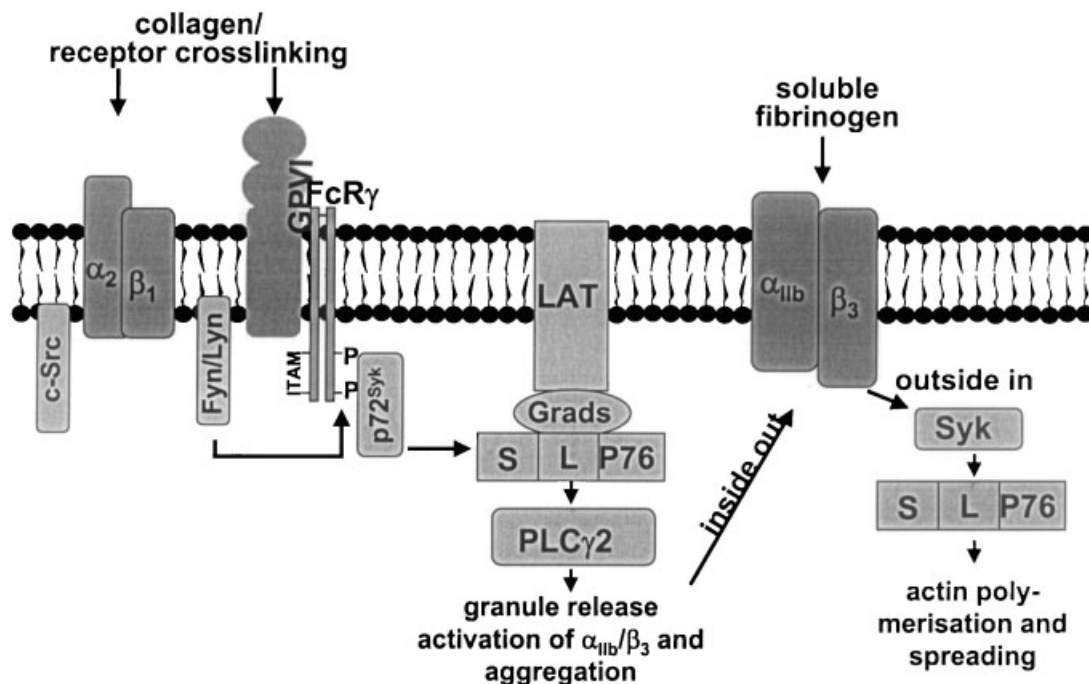


Figure 6 Nonreceptor tyrosine kinase mediated collagen signaling in platelets. Tyrosine-kinases: c-Src, Fyn/Lyn; non receptor tyrosine kinases: p72^{Syk}, Syk, adapter molecules: LAT, Grads, SLP76; PLC: phospholipase C; ITAM: immunoreceptor tyrosine based activation motif.

the inhibitory receptor family. Cross-linking of CD31 includes phosphorylation of immunoreceptor tyrosine-based inhibition motifs (ITIMS), inhibiting the actions of ITAMS.^{68,69}

In the absence of a wound, platelet activation is counteracted by signaling from prostaglandin I₂ (PGI₂) and EDRF/nitric oxide (NO), released from endothelial cells. Both platelet inhibitors induce an intracellular increase of the second messenger cAMP/cGMP by activation of adenylate cyclase (PGI₂) and guanylate cyclase (NO), respectively.⁷⁰ High concentrations of cyclic mononucleotides lead to a decrease in IP₃ synthesis and Ca²⁺ mobilization, resulting in reduction of platelet activation.^{71,72}

These fascinating little cells called platelets are much more important in hemostasis than originally thought. We are looking forward to a future that will unravel platelets' role in other physiologic and pathologic processes such as inflammation, sepsis, asthma, ischemia reperfusion injury, and host defense.

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