CHAPTER 1

Basics of Coagulation

The basic mechanics of hemostasis must be grasped in order to understand the disorders of hemostasis and the therapies designed to alter coagulation. Generally, coagulation is divided into fibrin formation, fibrinolysis, platelet function, and natural anticoagulants.

Formation of Fibrin

The coagulation cascade is a series of enzymatic steps designed to amplify the insult of initial trauma into the formation of a fibrin plug. Recent research has revealed how fibrin formation occurs in vivo rather than how it occurs in the test-tube. The in vivo pathway for the purposes of this book is called the "new pathway" of coagulation. Unfortunately, the two most common laboratory tests for coagulation and many books are still based on the test-tube models of coagulation. It is important to learn (a little bit) about the older models of coagulation to understand these two laboratory tests and much of the classic literature.

The Old Pathways (Fig. 1.1)

In the test tube tissue factor (TF)+VIIa is much more effective in activating factor X than factor XI. This pathway is initiated by adding bits of tissue ("tissue thromboplastin", usually minced animal's brains) to plasma. The brain tissue is used in these studies because it is an excellent source of both phospholipids and tissue factor. Since an extrinsic initiator, brain, was added, this pathway is known as the "*extrinsic pathway.*" The second pathway is started when blood is exposed to glass. Since nothing is added (except the glass surface) this is called the "*intrinsic pathway.*" This pathway is dependent on a different set of enzymes that result in factor XII activating factor XI. Since both pathways are the same once Factor X is formed, the path from factor X to fibrin formation is known as the "common" pathway.

To recap:

Extrinsic pathway: **TF+VIIa**→Xa+V→IIa→Clot
Intrinsic pathway: **Contact system→IXa+VIII**→Xa+V→IIa→Clot
Common pathway: **→Xa+V→IIa→Clot**

These pathways explained laboratory findings but did not match clinical observations. Patients with deficiencies of the contact system did not bleed, suggesting that the intrinsic pathway was not relevant. Hemophiliacs, on the other hand, are missing proteins VIII and IX (intrinsic pathway), which implies that the extrinsic pathway alone was not enough to support hemostasis. These contradictory observations led to the development of the "new pathway".

The Players

Most of the coagulation proteins are either enzymes (serine proteases) or cofactors (Table 1.1). A coagulation protein is a framework consisting of a serine protease

Hemostasis and Thrombosis, 2nd Edition, by Thomas G. DeLoughery.

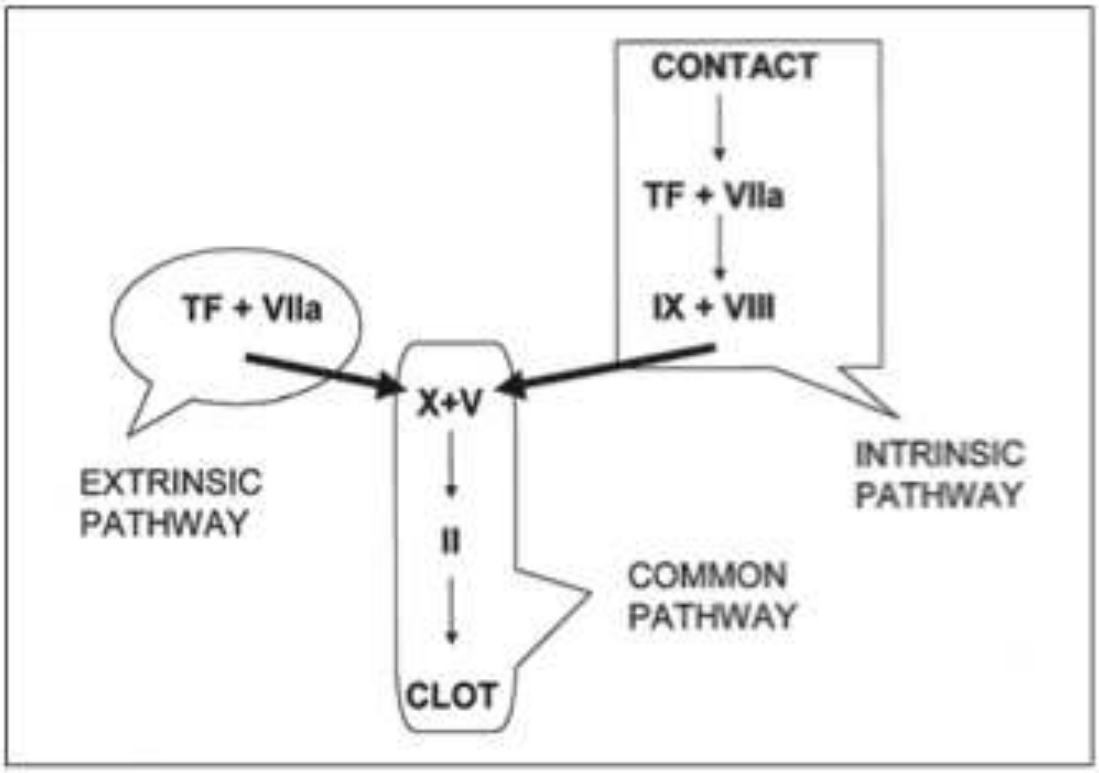


Fig. 1.1. Old Models of Coagulation.

with different protein domains added to it. The purpose of these domains is to add different capabilities to the clotting proteins.

Factors II, VII, IX, X, protein C, protein S and protein Z have vitamin K- dependent glutamic acid (GLA) domains on the amino terminus of the protein. These domains contain 9-11 glutamic acids modified to form gamma-carboxyglutamic acid (GLA). This modification allows calcium to bind to these proteins. The binding of calcium changes the conformation of the protein and serves to bind them in turn to phospholipid surfaces (Fig. 1.2). The hepatic GLA redox reaction is dependent on vitamin K ("Koagulation"). Without this vitamin, dysfunctional coagulation proteins are produced which function poorly in coagulation reactions. The drug warfarin blocks the recycling of vitamin K and leads to a reduction in functional coagulation factors.

Table 1.1 Coagulation proteins

Enzymes	Cofactors	Miscellaneous
Factor IIa	Tissue factor	Fibrinogen
Factor VIIa	Factor V	Factor XIII
Factor IXa	Factor VIII	Alpha$_2$antiplasmin
Factor Xa	Protein S	PAI-1
Factor XIa		Antithrombin
Protein C		
tPA		
Plasmin		

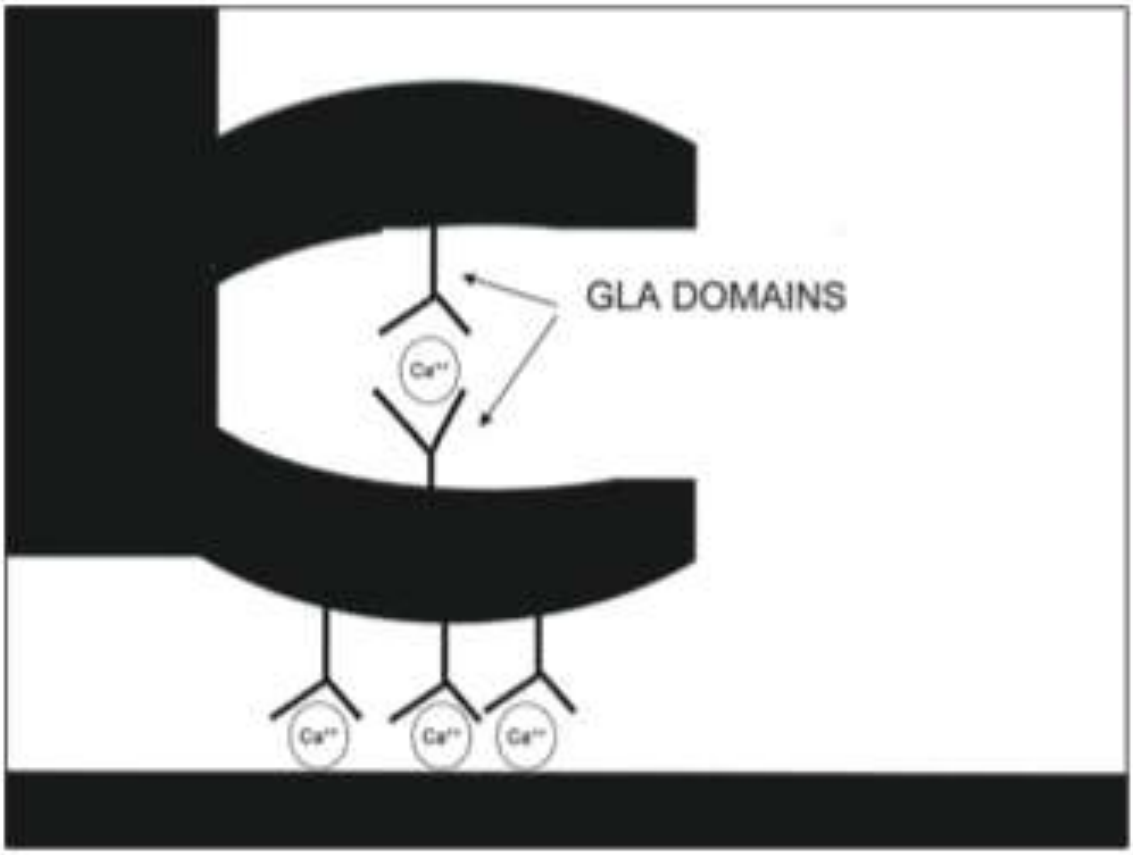


Fig. 1.2. GLA domains serves two functions. One is to bind the protein to phospholipid surfaces. The other is to fold the protein into the proper configuration.

Factor II, tissue plasminogen activator, and plasmin contain "kringle" regions (named after a Danish pastry). The kringle domains help these proteins bind to fibrinogen (Fig. 1.3).

The cofactors V and VIII are very similar molecules and require activation by thrombin. The mechanism underlying their cofactor function is unknown. The presence of these two cofactors enhances the efficiency of the coagulation factors by at least 100,000-fold.

The "Quaternary Complex"

Most coagulation reactions have four components, starting with the enzyme binding to a cofactor that is bonded by calcium to a surface (Fig. 1.4). This serves to make a little "coagulation factory" on the surface and improves the efficiency of the reaction by bringing the components together.

- **Enzyme**: (VIIa, XIa, Xa, IIa, protein C)
- **Co-factor**: (V, VIII, tissue factor, protein S)—speeds up reactions by orders of magnitude
- **Calcium**: binds protein to surfaces
- **Phospholipid surface**: Has a negative charge and speeds reactions by bringing proteins closer to each other.

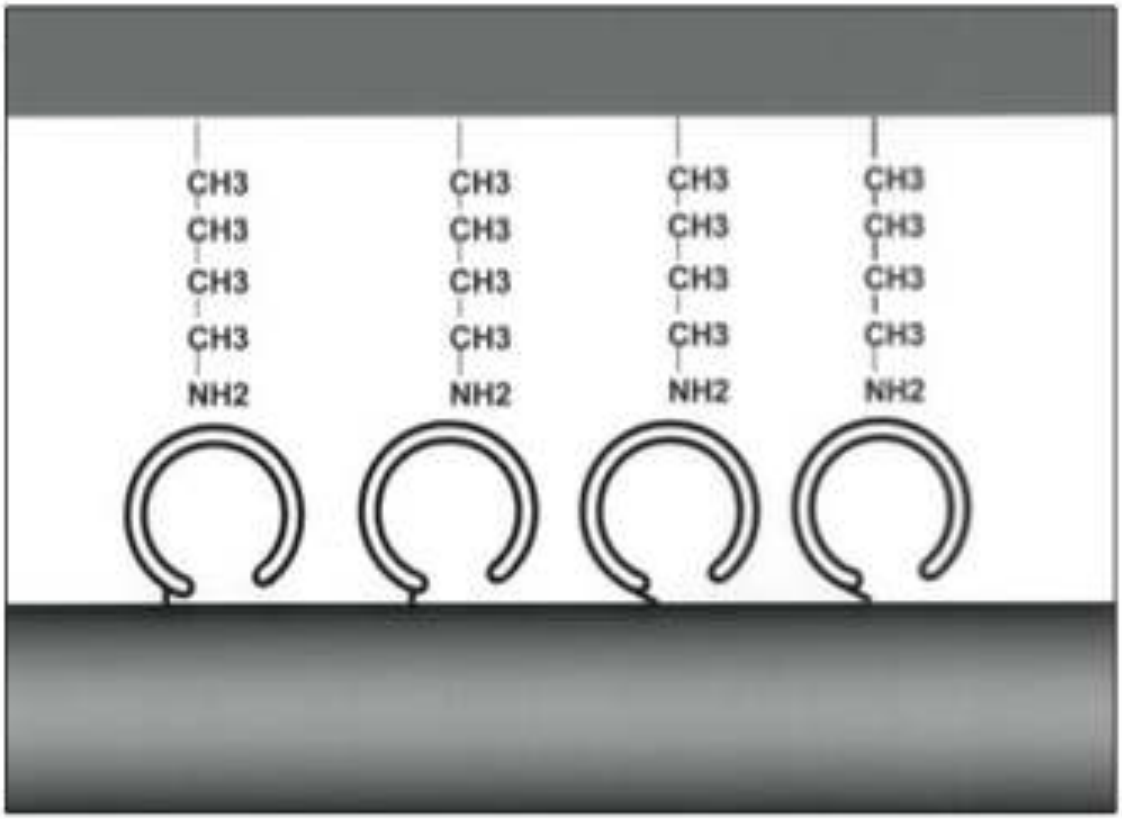


Fig. 1.3. Binding of the "Kringle" region to the lysine residue of fibrinogen.

Fig. 1.4. The quartanry complex. Active Enzyme (IXa) activates proenzyme (X) with the help of a co-factor (VIII). These enzymes are bound to the phospholipid surface by calcium.

Initiation of Coagulation

Overview: TF+VII→IX+VIII→X+V→II

The key step in the initiation of coagulation is exposure of tissue factor (TF). TF is a transmembrane surface molecule that is more or less on all cell surfaces except endothelial cells and circulating blood cells. Thus, flowing blood under normal

Table 1.2 Key coagulation reactions

Key Reactions

The New Pathway

TF+VIIa→IXa+VIII→Xa+V→IIa→Clot

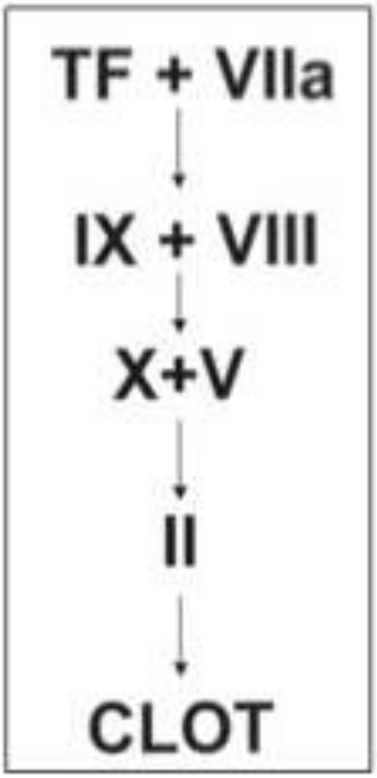
The Old Pathway

Intrinsic pathway: **Contact system →IXa+VIII→**Xa+V→IIa→Clot
Extrinsic pathway: **TF+VIIa→**Xa+V→IIa→Clot
Common pathway: **→Xa+V→IIa→Clot**

Fibrin Formation

Fibrinogen-(*THROMBIN*)→Fibrin monomer→Fibrin polymer -(*FACTOR XIII*)→Fibrin Clot

Fig. 1.5. New standard model for coagulation.



conditions is never exposed to TF. With trauma, blood spills out of the vessel and contacts TF. This is what initiates the coagulation cascade.

TF binds factor VII. This reaction would stop immediately without active factor VII (VIIa) to cleave factor IX (Fig. 1.6). However a tiny bit (0.1%) of factor VII circulates in the active form. This bit of VIIa from the blood binds TF, the TF-VIIa complex activates surrounding TF-VII complexes and these complexes start converting factor IX into IXa (Fig. 1.5).

Now factor IXa, with its cofactor VIIIa, converts X into Xa. The presence of VIIIa is crucial for the function of the Xa complex. Of note, the underlying pathology of the two most common forms of hemophilia is the absence of the two proteins in this reaction (IX and VIII) (Fig. 1.7).

Fig. 1.6. Tissue factor is exposed and binds to factor VIIa. The tissue factor-VIIa complex then activates factor IX.

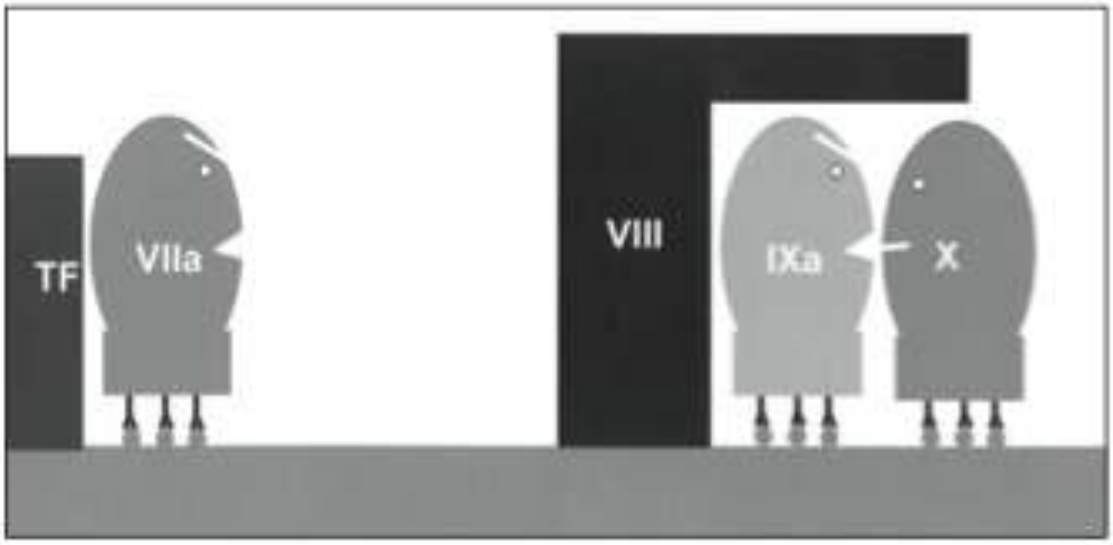


Fig. 1.7. Factor IX along with its cofactor VIII activates factor X.

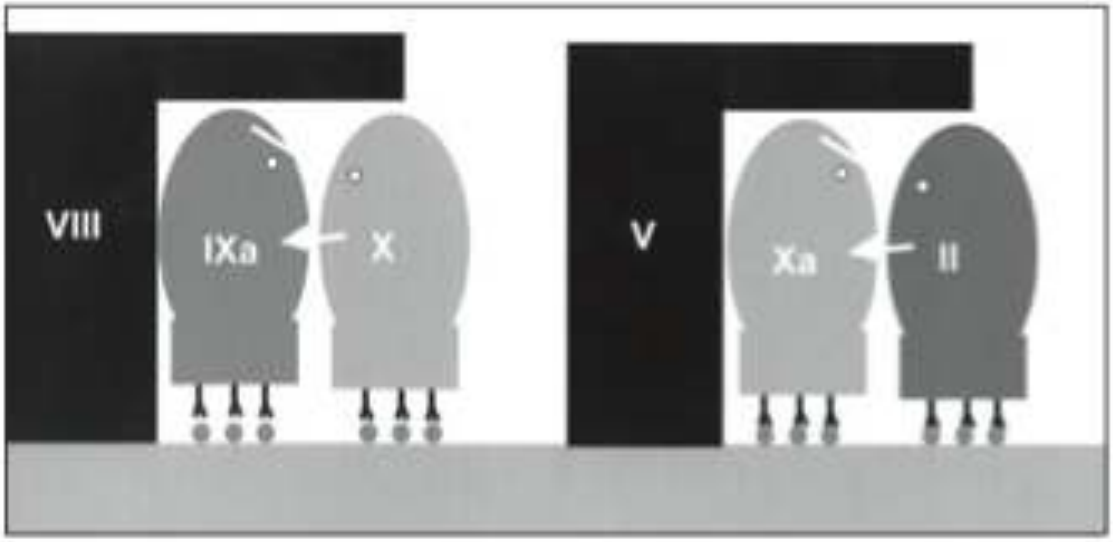


Fig. 1.8. Factor X along with its cofactor factor V activation prothrombin into thrombin.

Factor Xa binds with cofactor Va to generate thrombin (IIa) from prothrombin (II). The production of thrombin is the final step in the initiation of coagulation and is the single most crucial step in hemostasis (Fig. 1.8).

Thrombin

Thrombin is a multifunctional molecule. It functions to:

- Cleave **fibrinogen** into fibrin
- Activate **factors V and VIII**
- Activate **factor XIII**
- Activate **factor XI**
- Activate **platelets**
- Activate **thrombin activatable fibrinolysis inhibitor (TAFI)**
- Activate **fibrinolysis**
- Activate **protein C**

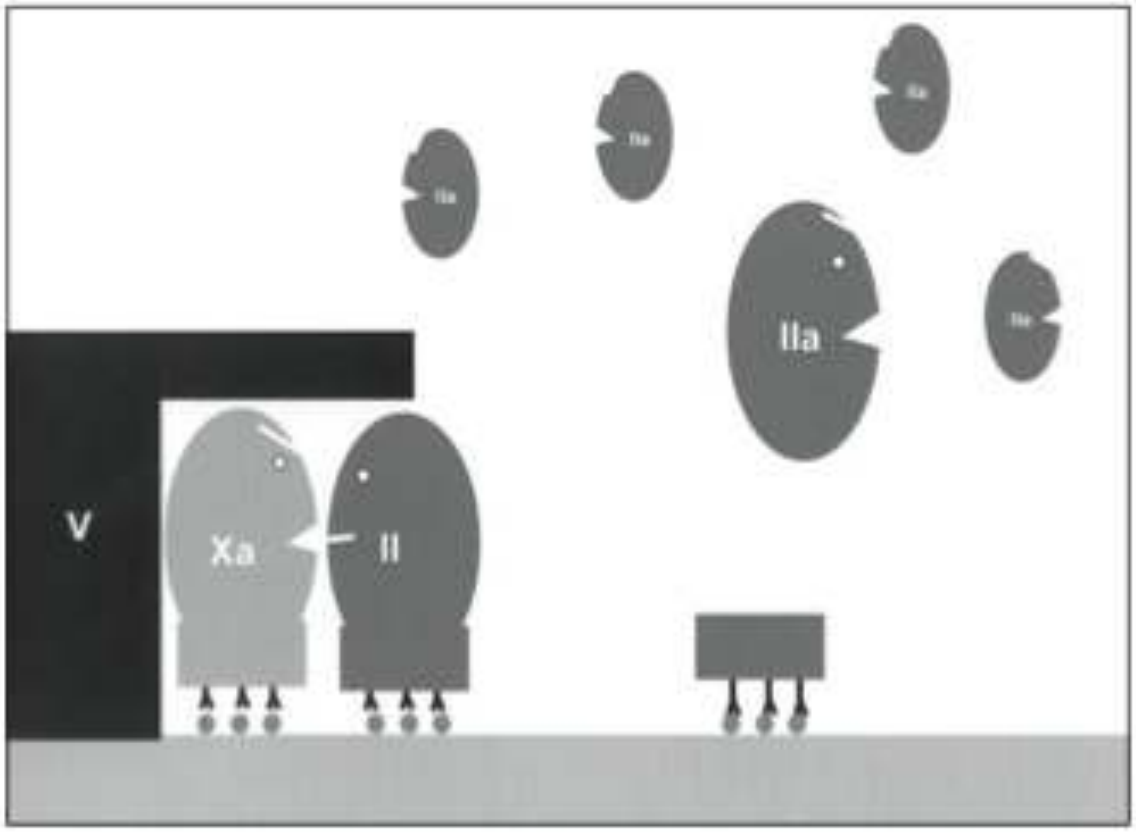


Fig. 1.9. Thrombin, freed from its GLA domains, flees to performs its many functions.

Thrombin is unique in several ways (Fig. 1.9). It does not require a co-factor for enzymatic function. When it is activated, it separates from its GLA domain so it can float around to promote clotting. Thrombin also provides both *positive feedback* by activating factors V, VIII, XI, XIII, TAFI and *negative feedback* by activating protein C and promoting fibrinolysis. Thrombin activation of factor XI provides a further positive feedback loop. Active factor XI activates IX eventually leading to more thrombin generation.

Fibrin Formation (Figs. 1.10, 1.11)

Fibrin is formed by turning soluble circulating fibrinogen into an insoluble fibrin thrombus. This is done in two steps. In the first step thrombin converts fibrinogen into fibrin monomers which spontaneously polymerize to form fibrin polymers. In the second step factor XIII stabilizes the clot by forming amide bonds between different fibrin polymers:

Fibrinogen→Fibrin monomer→Fibrin polymer→Fibrin clot

Thrombin acts on fibrinogen and clips off two peptides (fibrinopeptide A and B). This produces the *fibrin monomer*. The act of thrombin clipping off these peptides exposes polymerization sites that can bind to other fibrin monomers. The monomers polymerize together to form a loose clot. *Factor XIII* then solidifies the bond by forming glutamyl-lysine bridges between the side chains of the fibrin monomers. Note that factor XIII is the only coagulation enzyme that is *NOT* a serine protease.

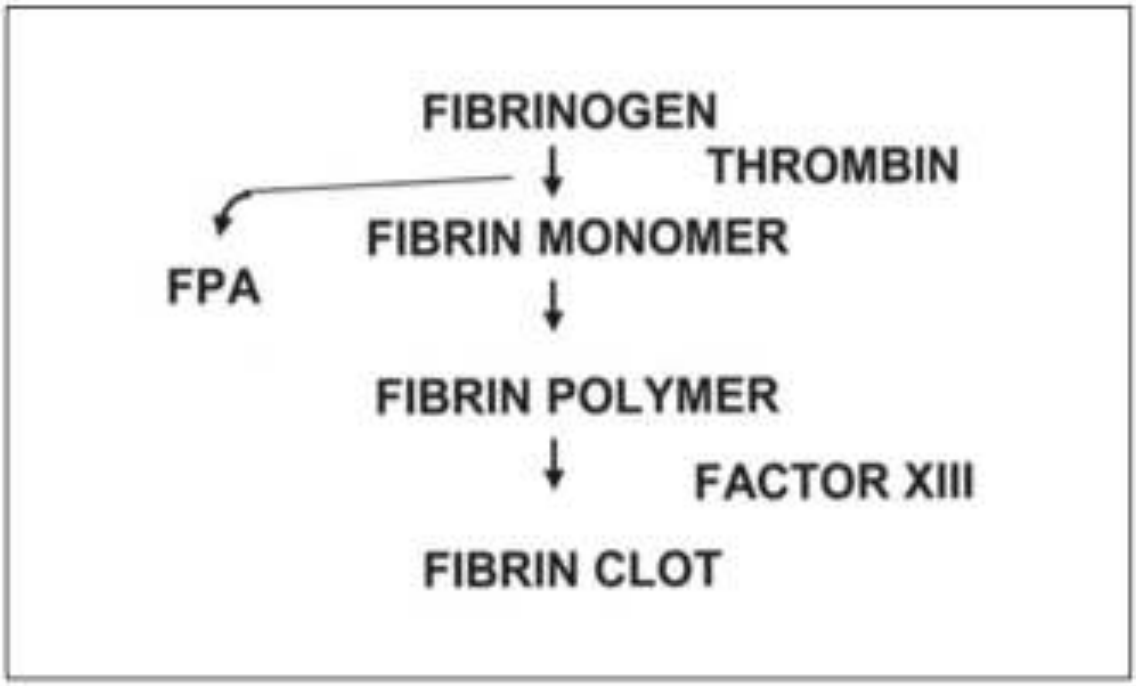


Fig. 1.10. Formation of the fibrin clot.

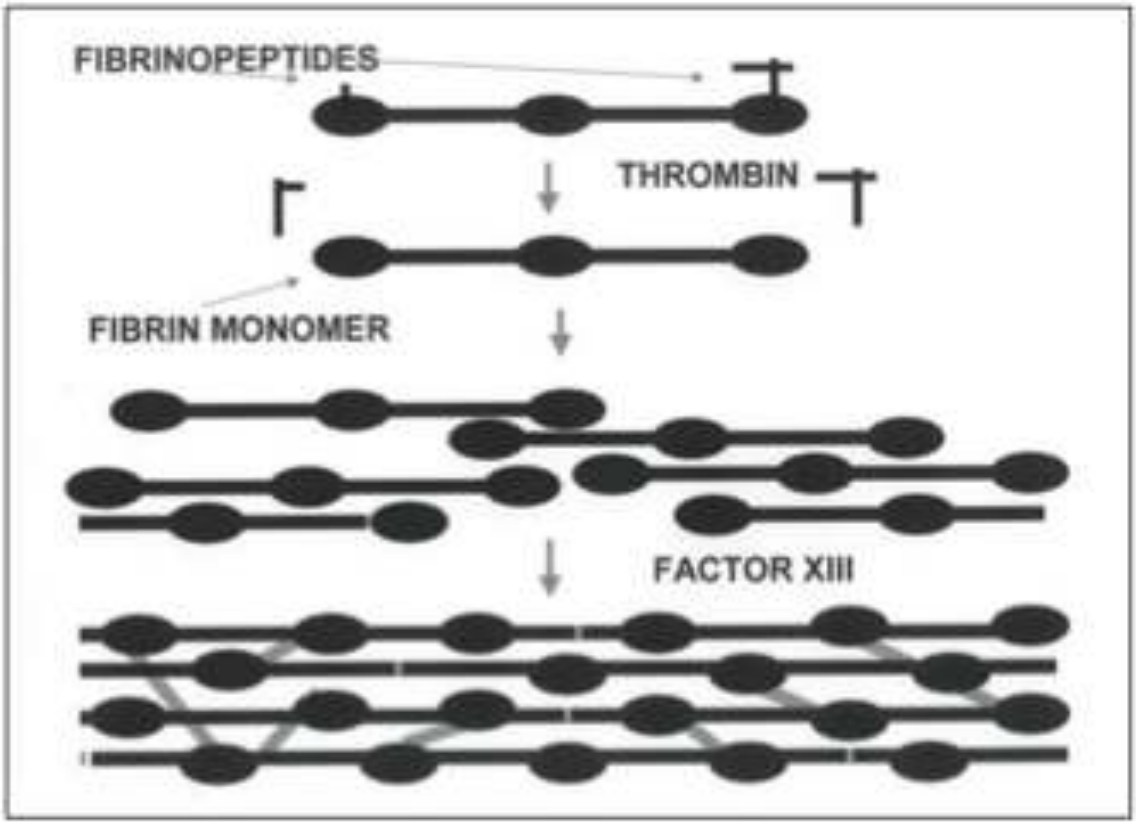


Fig. 1.11. Formation of the fibrin thrombus. Thrombin acts on fibrinogen to cleave fibrinopeptide A and B. This forms the fibrin monomer that loosely polymerizes. Factor XIII covalently bonds the fibrin monomer to form a stable thrombus.

In addition thrombin promotes coagulation by activating thrombin activatable fibrinolysis inhibitor (TAFI) (Fig. 1.12). TAFI cleaves the lysine residues to which many fibrinolytic enzymes bind, rendering the clot less likely to be dissolved.

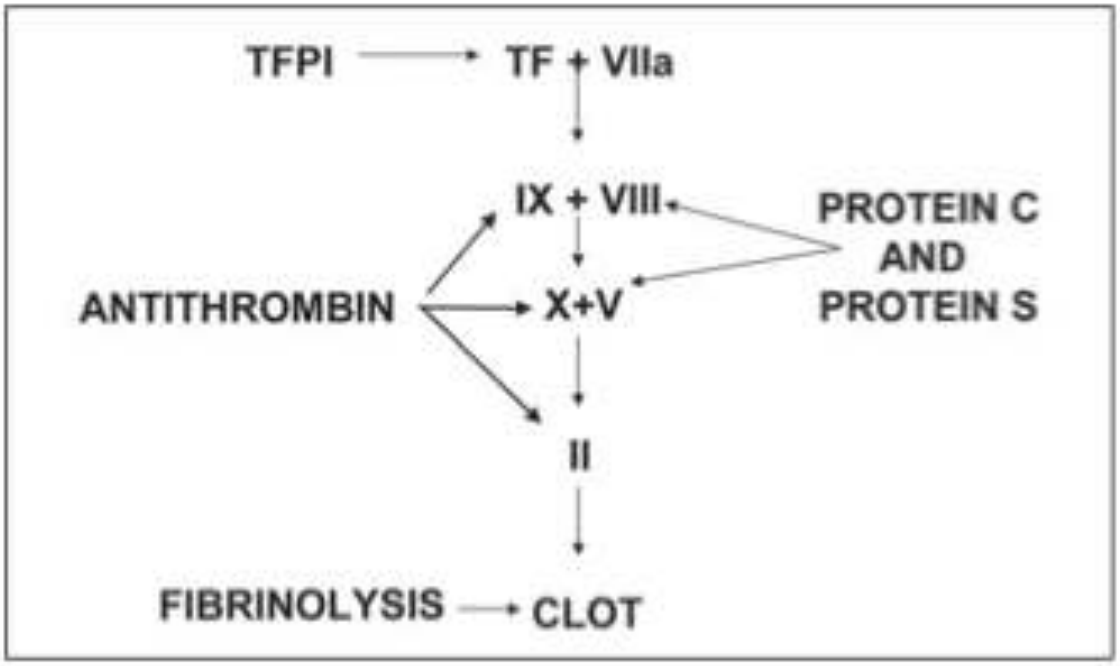


Fig. 1.12. Natural anticoagulants.

The Role of Factor XI

The "new" pathway leaves out the role of several proteins found in the older pathways. The contact system (factors XII, etc..) appears to play a role in inflammation but patients with deficiency of these proteins do not have hemostatic defects. The role of factor XI is more confusing. Although not in the new pathway, patients with deficiencies of factor XI do exhibit bleeding, especially after surgery. Thrombin can activate factor XI which then feeds back to activate factor IX. This then leads to more thrombin generation. More thrombin formation leads to activation of TAFI. This theory is consistent with the finding that patients lacking factor XI often have bleeding in sites of fibrinolytic activity such as the mouth after oral surgery.

Fibrinolysis

The fibrinolytic system is responsible for breaking down blood clots once they have formed. Obviously this is an important process to prevent thrombi from getting too large, to aid wound healing, and to prevent thrombosis in an undesirable place. Recent research has also implicated roles for proteins from the fibrinolytic system in diverse processes such as cancer metastasis and memory.

Fibrinolytic Proteins

The key proteins in the fibrinolytic system are (Fig. 1.13):

Plasmin

This is a serine protease produced by the liver which cleaves bonds in fibrin

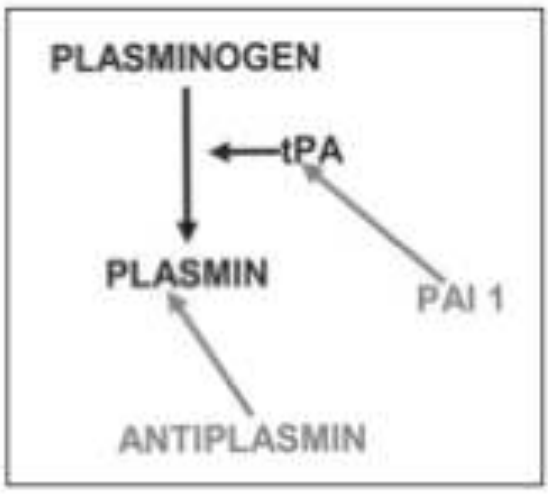


Fig. 1.13. The fibrinolytic pathway.

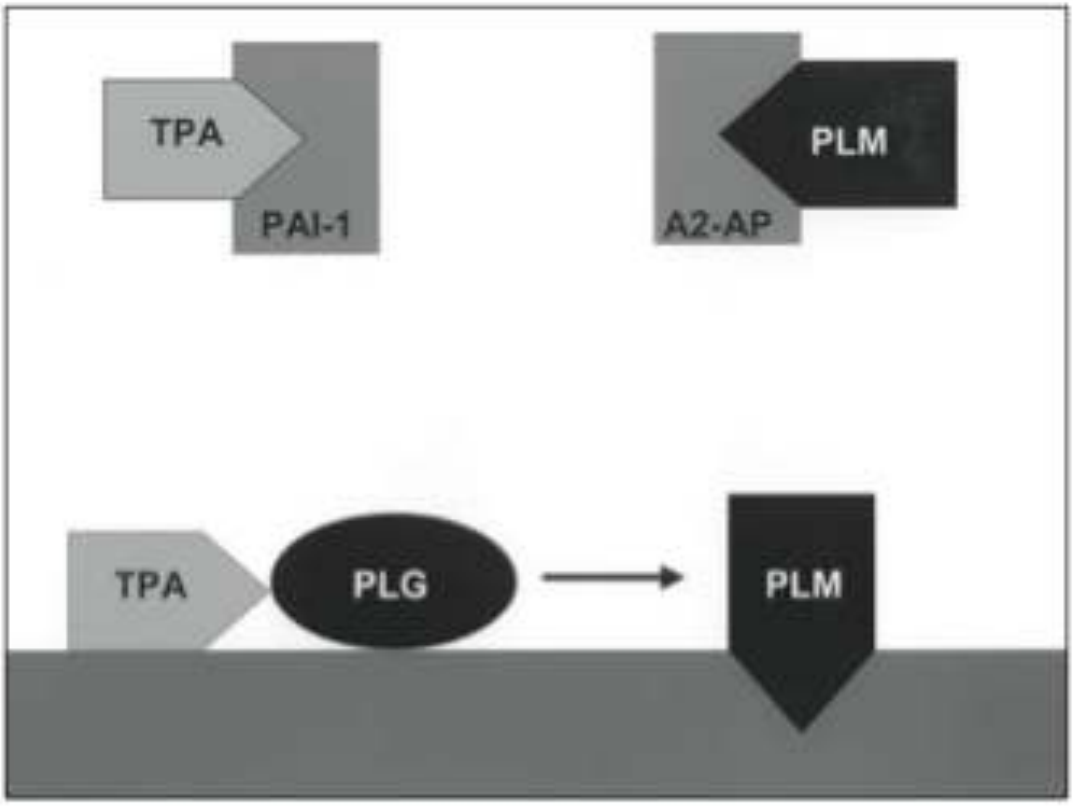


Fig. 1.14. Physiologic fibrinolysis. Free tPA can bins to fibrin and efficiently activate plasminogen to plasmin. Any free tPA or plasma is inactivated by circulating inhibitors.

and fibrinogen. Normally it circulates as an inactive precursor *plasminogen*, but it can be converted to plasmin by:

Tissue plasminogen activator (tPA): This is produced by endothelial cells. tPA is the physiologic activator of plasminogen.

Urokinase (UK): This is secreted in the urine (hence its name) and in many other cells. It is also a potent activator of plasminogen.

Several inhibitors of fibrinolysis are present to keep the fibrinolytic system in balance:

Plasminogen activator inhibitor (PAI-1): PAI-1 is made by the liver and endothelial cells. It binds and inactivates tPA.

Alpha$_2$ antiplasmin: This is made by the liver. It binds and inactivates plasmin.

Fibrinolysis—The Process

The ability of tPA to cleave plasminogen to plasmin is far greater when plasminogen and tPA are *both bound* to the fibrin clot. Moreover, when plasmin is bound to fibrin, it is protected from the action of circulating alpha$_2$-antiplasmin (Fig. 1.14).

A formed thrombus carries with it the seeds of its own destruction by incorporating plasminogen into the clot. tPA released from nearby endothelial cells percolates into the clot. The tPA binds to fibrin and then converts plasminogen to plasmin, which lyses the clot. Any excess tPA that escapes into the plasma is rapidly inactivated by PAI-1. Any plasmin that escapes in the plasma is rapidly inactivated by alpha$_2$-antiplasmin. Thus, active fibrinolysis is confined to the thrombus itself (Fig. 1.15).

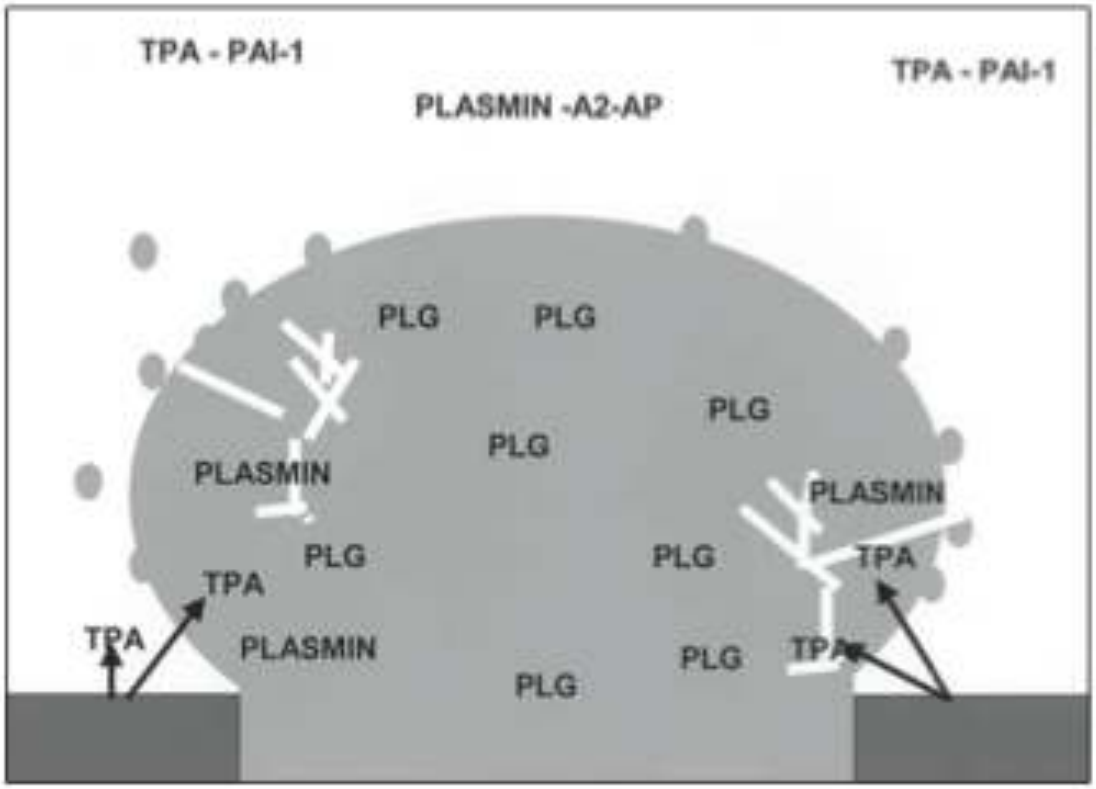


Fig. 1.15. When the thrombus reaches normal endothelial cells, the endothelial cells will secrete tPA that binds to the fibrin and cleaves plasminogen to plasmin lysis the thrombus.

Platelet Production and Life Span

Platelets are made in the bone marrow. Huge cells known as megakaryocytes (derived from hematopoietic stem cells) are the precursors to platelets; one megakaryocyte can produce 2,000 platelets. Platelets bud off the edges of the megakaryocytes and the megakaryocyte eventually perishes by evaporating. The platelet circulates in the blood for 7-10 days. Platelets either circulate freely or are sequestered in the spleen. At any given time one-third of the platelets are located in the spleen.

Thrombopoietin (TPO)

Discovered in 1994, TPO is the main growth and maturation factor for megakaryocytes. One-half of the TPO molecule is very similar to erythropoietin. TPO can make early precursor cells differentiate into megakaryocytes and can also induce generation of platelets by megakaryocytes. TPO and molecules with TPO-like activity are currently being tested in patients with severe thrombocytopenia due to chemotherapy or bone marrow transplantation.

Function of Platelets

Platelets do four things:

1. **Adhere** to damaged endothelium.
2. **Store** ADP and proteins.
3. **Aggregate** with other platelets.
4. **Provide a surface** for coagulation reactions.

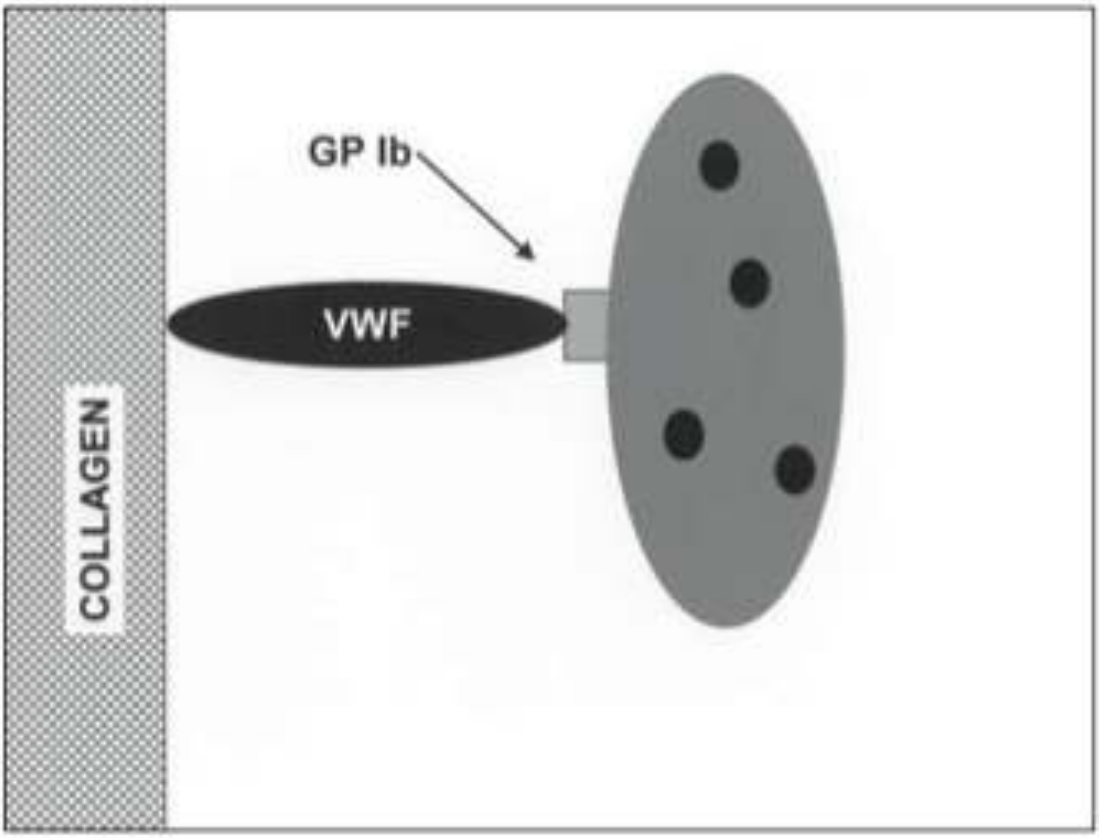


Fig. 1.16. Platelet adhesion: Platelet binding to collagen with von Willebrand factor as the glue and GP Ib as the receptor.

Platelet Adhesion (Fig. 1.16)

Damage to a blood vessel exposes *collagen* that is wrapped around the vessel. The exposed collagen reacts with and binds a large multimeric protein known as *von Willebrand factor*. Once it is bound, von Willebrand factor changes in conformation at one end so that it now can bind to platelets. The von Willebrand factor attaches to the platelet receptor *glycoprotein (Gp) Ib*. Platelet adhesion by von Willebrand factor creates a platelet monolayer over an injured surface. The binding of von Willebrand factor to Gp Ib leads to physiological changes called platelet activation.

About von Willebrand Factor

Von Willebrand Factor (vWF) is a huge molecule, up to 20 million daltons in molecular weight. It serves another role in hemostasis by carrying and protecting coagulation factor VIII. Patients who completely lack vWF also lack factor VIII, which results in a severe bleeding disorder.

Summary of Platelet Adhesion

Platelet adhesion occurs by exposing *collagen*, which leads to the binding of *von Willebrand factor* ("the glue") to the platelet receptor *GP Ib*.

Platelet Storage (Figs. 1.17, 1.18)

Platelets are filled with granules that store ADP and other proteins released when platelets are activated. *Alpha* granules store proteins and *dense* granules store chemicals. Alpha granules contain proteins such as vWF and factor V. *Dense* granules

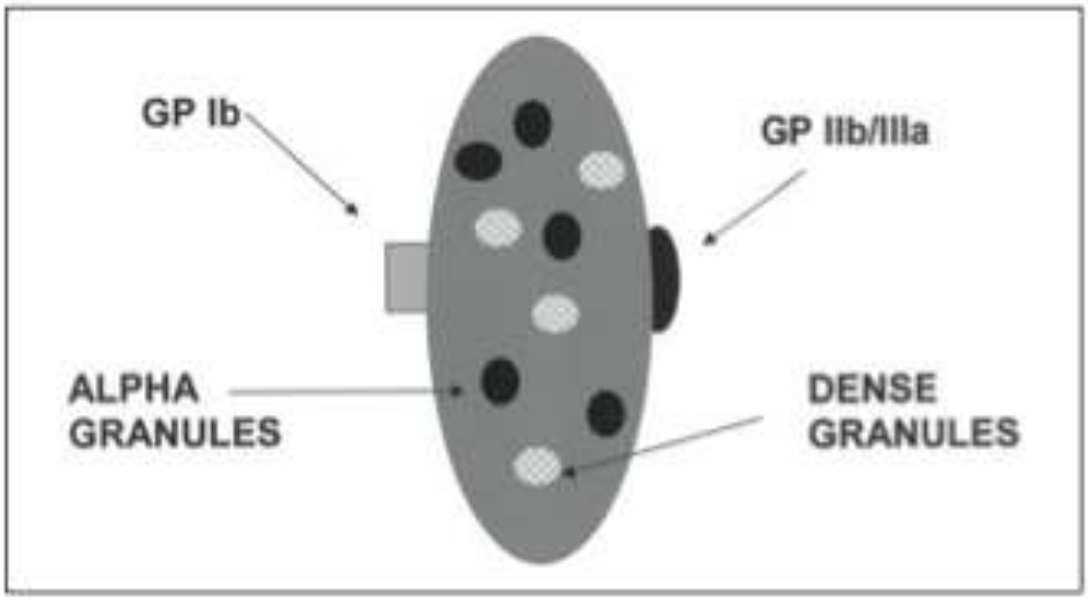


Fig. 1.17. Platelet structure demonstrating two key receptors: GP Ib and IIb/IIIa and two granules—The alpha granules and dense granules.

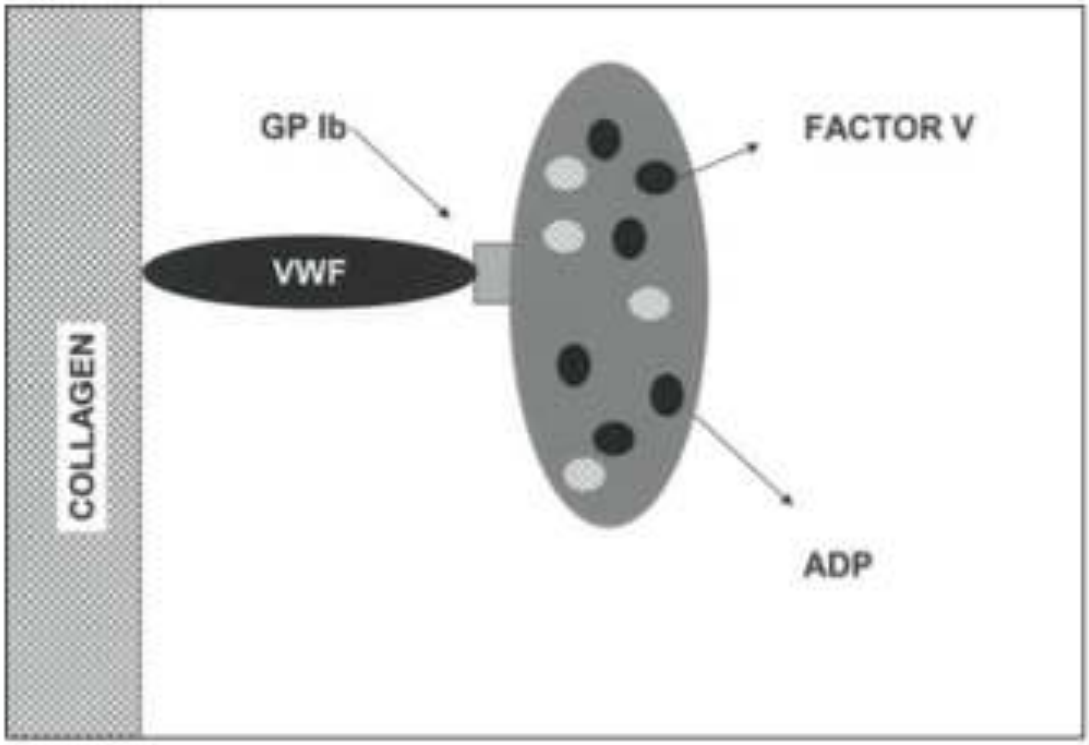


Fig. 1.18. Platelet, when activated, releases its granule contents.

contain chemicals such as serotonin and ADP which, after release, activate nearby platelets. Platelet activation also leads to production of thromboxane A_2, a key activator of platelets (recall that thromboxane A_2 synthesis is blocked by aspirin).

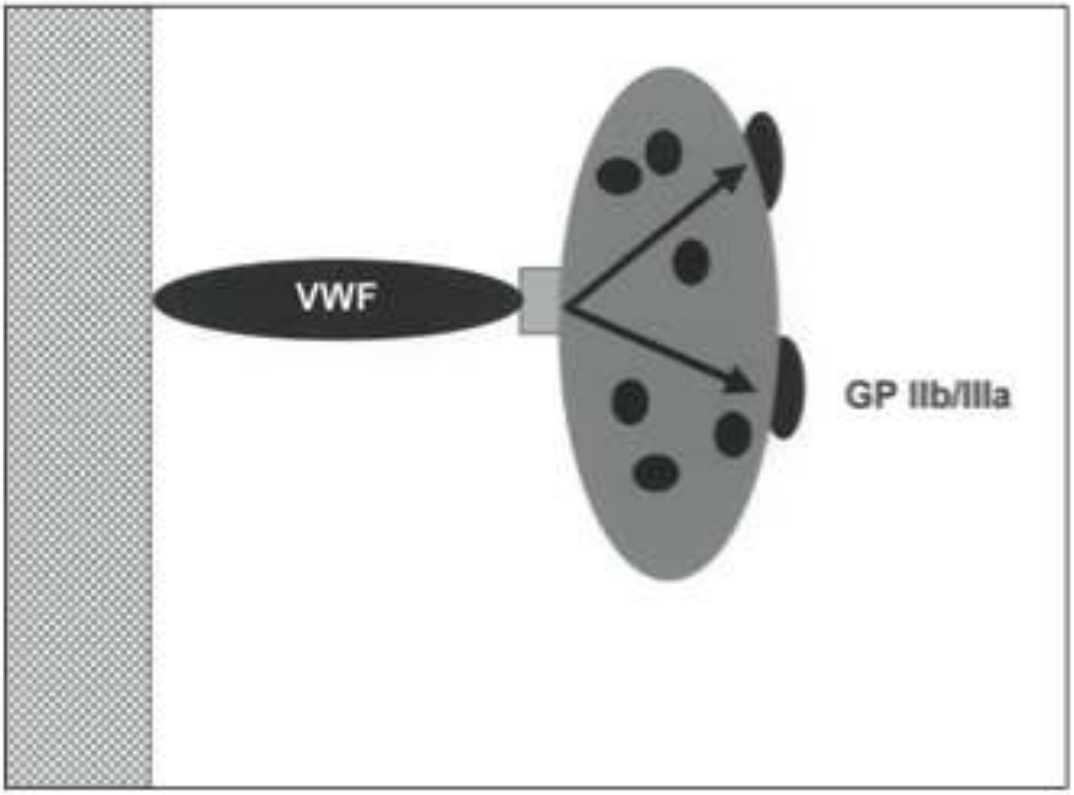


Fig. 1.19. Binding of von Willebrand factor to GP Ib initiating platelet activation and exposing GP IIb/IIIa.

Platelet Aggregation (Figs. 1.19, 1.20)

Platelet aggregation is the binding of platelets to *each other* (as opposed to adhesion where platelets *adhere* to the *vasculature*). Aggregation occurs because of the activation of a platelet receptor known as *GP IIb/IIIa*. This platelet receptor is activated by a number of processes including:

1. Binding of *vWF* to *Gp Ib*.
2. Binding of *platelet agonist* such as thromboxane A2 and ADP to platelet receptors.
3. Binding of *thrombin* to the platelet thrombin receptor. This ties the humoral phase of coagulation (tissue factor etc.) to platelet activation. Thrombin is the *MOST* potent physiologic activator of platelets known.

Activation of GP IIb/IIIa is the final common pathway for platelet aggregation. The GP IIb/IIIa receptor is a target for many powerful antiplatelet agents currently in use.

After the platelets have formed a monolayer on an injured surface, they release platelet agonists such as ADP. This activates nearby platelets causing them to activate their own GP IIb/IIIa. Fibrinogen (abundant in the plasma) then binds to all the active Gp IIb/IIIa exposed on the platelet surface. The "*glue*" for platelet aggregation is *fibrinogen*. This acts to clump platelets together into a large mass, forming a platelet plug that stops the bleeding.

Platelet Surface

Coagulation reactions take place on *surfaces*. This allows all the coagulation factors to be close to one another and increases the efficiency of the reactions. When

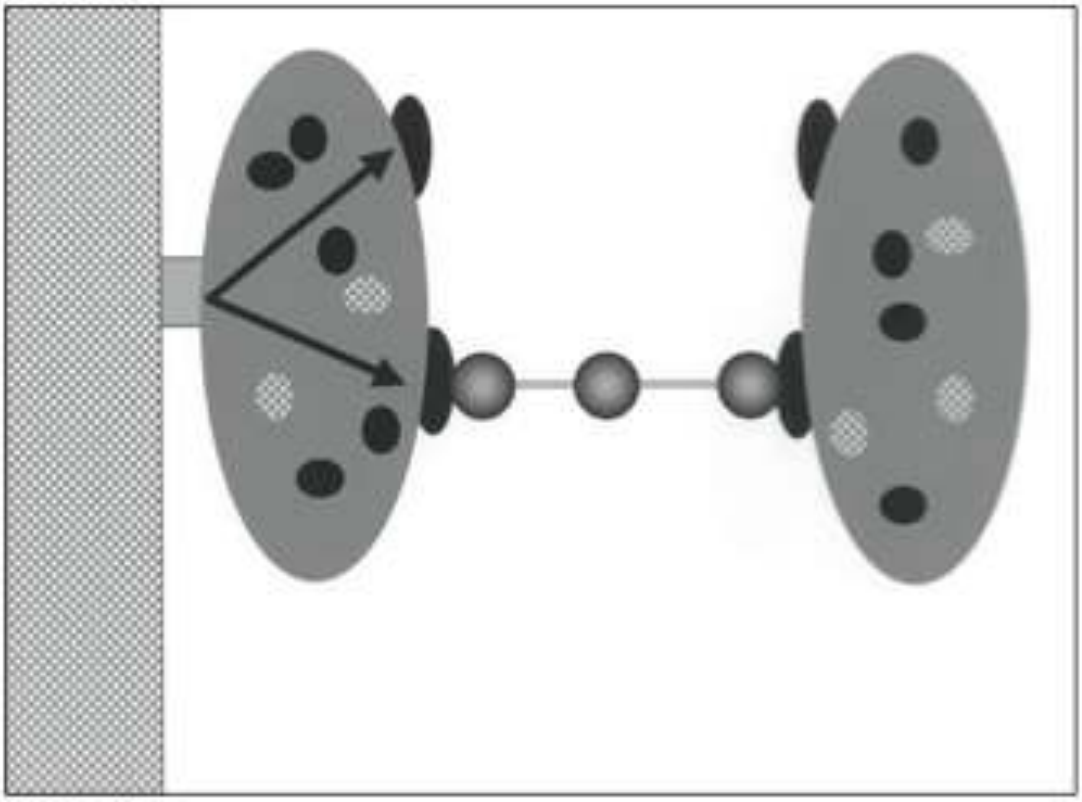


Fig. 1.20. Platelet aggregation: Binding platelet to platelet with fibrinogen as the glue and GP IIb/IIIa as the receptor.

platelets are activated, they expose a negatively charged phospholipid—*phosphatidylserine*.

Phosphatidylserine augments the binding of coagulation factors to injured surfaces. Since platelets are found at the site of injury, their surfaces provide a platform for coagulation. When platelets are activated, little blebs (called platelet *microparticles*) bubble off the surface. These microparticles increase the surface area available for coagulation reactions many times over.

Natural Anticoagulants

For every step of the coagulation cascade there exists a protein inhibitor of that step. These proteins ensure that excess thrombosis does not occur. These proteins are tissue-factor pathway inhibitor (TFPI), antithrombin (formally known as antithrombin III), protein C and protein S.

Tissue-factor pathway inhibitor is a protein that binds to factor Xa. This complex then forms a quarternary complex with tissue factor-VIIa and halts further IXa formation. It is speculated that coagulation continues via thrombin activating factor XI which in turns activates factor IX and leads to more thrombin generation.

Protein C is a serine protease that cleaves and destroys factors Va and VIIIa. Its cofactor protein S is crucial for this function. Both proteins C and S are vitamin K dependent. Protein S has several unusual features. First, it is not a serine protease. Secondly, it circulates in two forms in the plasma, a free form and a form bound to C4B-Binding protein. Only the free form can serve as a cofactor to protein C. Normally about 40% of protein S exists in the free form. Alterations in this ratio, either acquired or genetic, are responsible for many of the hypercoagulable states.

Antithrombin is a serine protease inhibitor that binds and inactivates all the serine proteases of the coagulation cascade. Its function is greatly augmented by either natural heparan or exogenous heparin. The addition of these complex polysaccharides leads to a dramatic increase in antithrombin's ability to bind and neutralize serine proteases.

Suggested Reading

Basics of Coagulation

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