Coagulation Pathway and Physiology

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Introduction

Our understanding of blood clotting is intimately tied to the history of civilization. With the advent of writing 5000 years ago, it could be argued that the first symbols used for blood, bleeding, or clotting represented the first published coagulation pathway. The ancient peoples of the world always held blood in utmost mystical esteem. Through the ages, this esteem has been transmitted to modern times in the many expressions that use "blood," such as "blood is thicker than water," "blood of our fathers," and others.

Mysticism aside, the study of blood clotting and the development of laboratory tests for blood clotting abnormalities are historically inseparable. The workhorse tests of the modern coagulation laboratory, the prothrombin time (PT) and the activated partial thromboplastin time (aPTT), are the basis for the published extrinsic and intrinsic coagulation pathways, even though it is now known that these pathways do not accurately reflect the function of blood clotting in a living organism. In this chapter, and ultimately this textbook, the many authors hope to present a clear explanation of coagulation testing and its important place in the medical armamentarium for diagnosing and treating disease.

Constituents of the Hemostatic System

With the evolution of vertebrates and their pressurized circulatory system, there had to arise some method to seal the system if injured—hence the hemostatic system. Interestingly, there is nothing quite comparable to the vertebrate hemostatic system in invertebrate species. In all vertebrates studied, the basic constituents of the hemostatic system appear to be conserved.

Figure 1-1 illustrates the three major constituents of the hemostatic pathways and how they are interrelated. Each element of the hemosta-

tic system occupies a site at the vertex of an equilateral triangle. This representation implies that each system constituent interacts with and influences all other constituents. In the normal resting state, these interactions conspire to maintain the fluidity of the blood to ensure survival of the organism. Normally, only at the site of an injury will the fluidity of the blood be altered and a blood clot form.
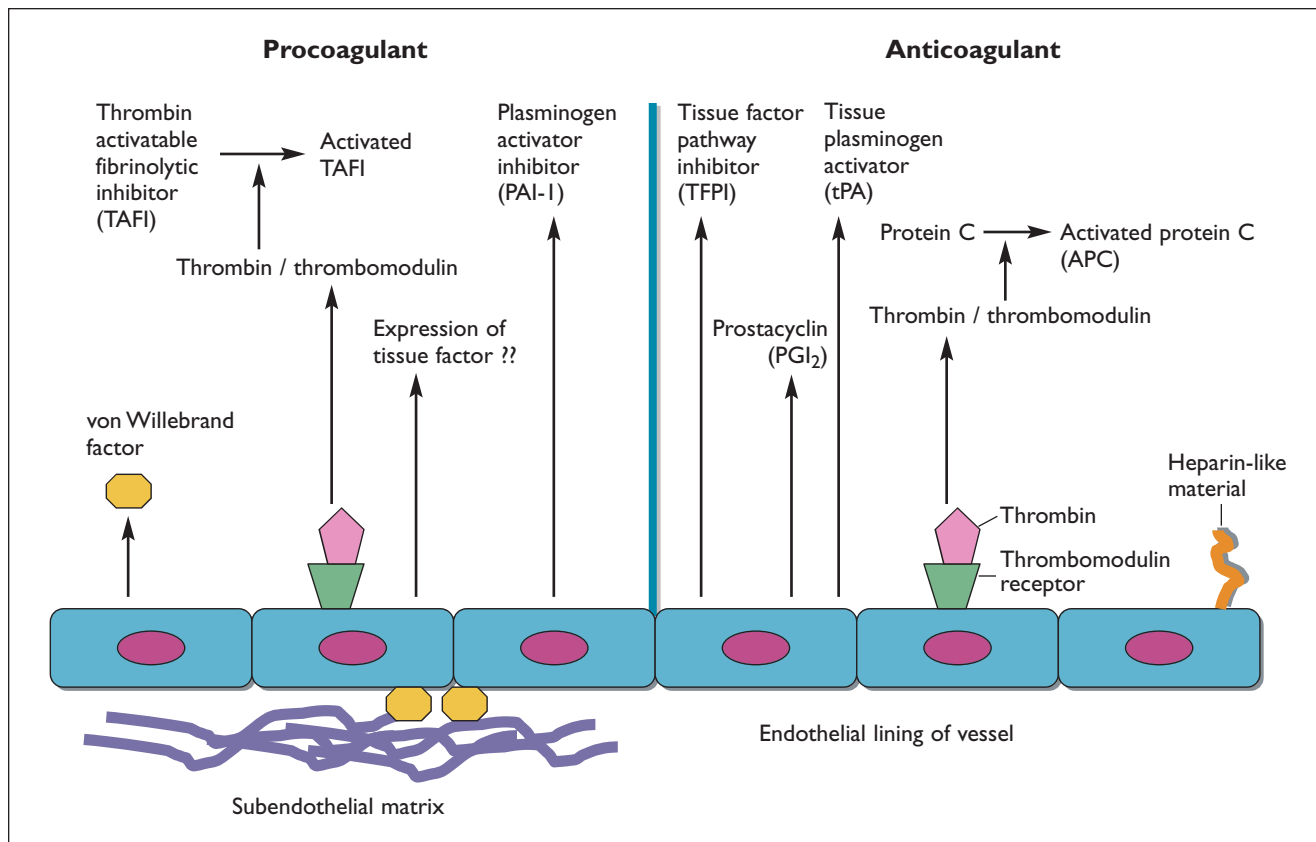
Endothelium

Figure 1-2 shows some of the basic properties of the endothelium. The endothelium normally promotes blood fluidity, unless there is an injury. With damage, the normal response is to promote coagulation at the wound site while containing the coagulation response and not allowing it to propagate beyond this site.

Until recently, the dogma of blood clotting suggested that the single, major procoagulant function of the endothelium is to make and express tissue factor with injury. Endothelial cells do not normally make tissue factor but may synthesize it following cytokine stimulation or acquire the material from activated monocytes in the circulation. Tissue factor is a glycosylated intrinsic membrane protein that is expressed on the surface of
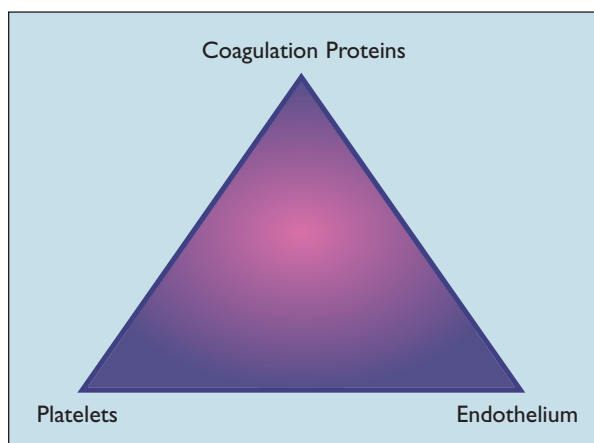
Figure 1-1. Basic representation of the elements of hemostasis.

Figure 1-2. A stylized view of endothelial functions related to procoagulation and anticoagulation. The subendothelial matrix, represented by the purple interlocking lines, is a complex of many materials. The most important constituents of the subendothelial matrix related to coagulation function are collagen and von Willebrand factor.

adventitial vascular wall cells and is exposed to flowing blood during vascular injury or endothelial denudation. Tissue factor, when bound to factor VIIa, is the major activator of the extrinsic pathway of coagulation. Classically, tissue factor is not present in the plasma but only presented on cell surfaces at a wound site. Because tissue factor is "extrinsic" to the circulation, the pathway was thusly named.

Endothelium is the major synthetic and storage site for von Willebrand factor (vWF). Von Willebrand factor is secreted from the endothelial cell both into the plasma and also abluminally into the subendothelial matrix. It is a large multimeric protein that acts as the intercellular glue binding platelets to one another and also to the subendothelial matrix at an injury site. In addition, the second major function of vWF is to act as a carrier protein for factor VIII (antihemophilic factor). Von Willebrand factor is synthesized in the endothelial cell as a dimeric protein, with a molecular weight of the monomer approximately 250,000 daltons. The propeptide of vWF, the N-terminal portion of the protein that is cleaved off prior to secretion or

storage, is important in directing the multimerization of vWF. Von Willebrand factor multimers can range in size up to 20×10^6 daltons. Von Willebrand factor binds to the platelet glycoprotein Ib/IX/V receptor and mediates platelet adhesion to the vascular wall under shear, which is discussed in more detail in chapter 2.

The remaining endothelial procoagulant functions discussed in this brief review and listed in Figure 1-2 are all parts of the fibrinolytic system, discussed in more detail in chapter 3. The fibrinolytic system is responsible for proteolysis and solubilization of the formed clot constituents to allow its removal. Plasminogen activator inhibitor acts to block the ability of tissue plasminogen activator to turn on plasmin, the primary enzyme of fibrinolysis. The thrombin activatable fibrinolytic inhibitor (TAFI), similar to the thrombin regulatory enzyme protein C, is cleaved to its activated form by the thrombin/thrombomodulin complex. The endothelial cell surface receptor thrombomodulin is a 450,000-dalton protein whose only known ligand is thrombin. Thrombin, once bound to thrombomodulin, loses some proteolytic capa-

bilities but gains the ability to either activate TAFI or protein C. Relevant to this discussion is that the activated form of TAFI catalyzes the removal of lysine residues from the fibrin clot, making it less recognizable as a substrate for plasmin and hence more persistent.

The endothelium and ultimately the entire vessel are normally geared to maintain blood fluidity in the absence of injury. To that end, the endothelium possesses a variety of functions whose ultimate goal is to promote blood fluidity. Although Figure 1-2 portrays a typical endothelium, evidence is accumulating suggesting that endothelium from different vessels and from various parts of the vascular tree is not homogeneous.

The anticoagulant functions of the endothelium inhibit all aspects of the coagulation pathways. Tissue factor pathway inhibitor (TFPI) is a 33,000-dalton low-concentration inhibitor of the extrinsic pathway that is secreted into the plasma by the endothelium. This material rapidly inhibits the tissue factor/factor VIIa complex. Prostacyclin (PGI$_2$), a prostaglandin produced by the endothelial-specific cyclooxygenase enzyme system, is a potent inhibitor of platelet aggregation. Tissue plasminogen activator (tPA) is the main enzymatic activator of the potent fibrinolytic enzyme plasmin. The fibrinolytic system is discussed in more detail in chapter 3.

Once the enzyme thrombin is bound to thrombomodulin, it also gains the ability to activate protein C to activated protein C (APC). APC, along with its cofactor protein S, phospholipid surface, and calcium ions, acts to down-regulate thrombin generation by proteolyzing the two protein cofactors in the coagulation pathways, factor Va and factor VIIIa. Finally, the last anticoagulant property of endothelium listed in Figure 1-2 is the presence of the heparin-like material on the surface. Heparan sulfate is a glycosaminoglycan that is attached to the luminal surface of the endothelium by a protein backbone. The cell-surface heparan sulfate acts as a cofactor for one of the main direct inhibitors of many of the coagulation enzymes, antithrombin. Antithrombin is a 58,000-dalton serpin (serine protease inhibitor) that has a five-stranded central β-sheet (the A-sheet), together with a heparin-binding D-helix and a mobile reactive site loop containing an Arginine393-Serine394 bond that resembles the substrate for thrombin and other serine proteases such as factor Xa, factor IXa, and factor XIa. Once thrombin cleaves the bond, the protease is inhibited by a covalent link to the antithrombin. In its native state, antithrombin inactivates the proteinases inefficiently, due to conformational inaccessibility of the arginine-serine bond. Inhibition is accelerated approximately 1000-fold by the binding of heparin to arginine residues in the D-helix of antithrombin, with a resultant conformational change and exposure of the reactive center.

Platelets

Platelets are discoid anucleate subcellular fragments that can vary in size up to 3 ∝m in diameter. They arise from the megakaryocyte in the bone marrow and circulate in blood at a platelet count that ranges from 200,000 to 400,000/∝L. The platelet has a complex ultrastructure that includes many different surface receptors, several types of storage granules, and a network of actin and myosin filaments. At the site of an injury, the platelets contact extracellular matrix components, causing a series of metabolic changes that result in the formation of a platelet plug. These metabolic changes are usually termed aggregation. Ultimately, this platelet plug is stabilized by the formation of a fibrin clot. Platelet structure and function are described in chapter 2.

Coagulation Proteins

Table 1-1 lists the proteins involved in the formation of the fibrin clot. Factors II, VII, IX, and X (as well as proteins C, S, and Z) are the zymogen forms of vitamin K-dependent serine proteases. Vitamin K is a necessary cofactor for a post-translational modification that adds a carboxyl group to the 10 to 12 glutamic acid residues in the amino terminal portion of each of these proteins. The vitamin K-dependent proteins utilize these clusters of γ-carboxyl glutamic acid (gla) residues to adhere to phospholipid surfaces and assemble multimolecular coagulation complexes. Without this important post-translational modification, the assembly of cell-based coagulation complexes is impaired, leading to ineffective fibrin formation. Another reason to understand this biochemical fact is that the most commonly used oral anticoagulant, warfarin, exerts its anticoagulant effect by inhibiting this modification and ultimately producing dysfunctional vitamin K-dependent factors. These dysfunctional factors affect coagulation tests, and the warfarin anticoagulant effect is therefore monitored by the clot-based PT assay.

Since the identification of all the factors listed in Table 1-1, accumulating epidemiologic evidence has called into question whether some of these factors truly participate in the formation of a fibrin

Table 1-1. Coagulation Factors

| Name	Description	Function
Fibrinogen (Factor I)	Molecular Weight (MW) = 340,000 daltons (Da); glycoprotein	Adhesive protein that forms the fibrin clot
Prothrombin (Factor II)	MW = 72,000 Da; vitamin K-dependent serine protease	Activated form is main enzyme of coagulation
Tissue factor (Factor III)	MW = 37,000 Da; also known as thromboplastin	Lipoprotein initiator of extrinsic pathway
Calcium ions (Factor IV)	Necessity of Ca++ ions for coagulation reactions described in 19th century	Metal cation necessary for coagulation reactions
Factor V (Labile factor)	MW = 330,000 Da	Cofactor for activation of prothrombin to thrombin
Factor VII (Proconvertin)	MW = 50,000 Da; vitamin K-dependent serine protease	With tissue factor, initiates extrinsic pathway
Factor VIII (Antihemophilic factor)	MW = 330,000 Da	Cofactor for intrinsic activation of factor X
Factor IX (Christmas factor)	MW = 55,000 Da; vitamin K-dependent serine protease	Activated form is enzyme for intrinsic activation of factor X
Factor X (Stuart-Prower factor)	MW = 58,900 Da; vitamin K-dependent serine protease	Activated form is enzyme for final common pathway activation of prothrombin
Factor XI (Plasma thromboplastin antecedent)	MW = 160,000 Da; serine protease	Activated form is intrinsic activator of factor IX
Factor XII (Hageman factor)	MW = 80,000 Da; serine protease	Factor that nominally starts aPTT-based intrinsic pathway
Factor XIII (Fibrin stabilizing factor)	MW = 320,000 Da	Transamidase that cross-links fibrin clot
High-molecular-weight kininogen (Fitzgerald, Flaujeac, or William factor)	MW = 110,000 Da; circulates in a complex with factor XI	Cofactor
Prekallikrein (Fletcher factor)	MW = 85,000 Da; serine protease	Activated form that participates at beginning of aPTT-based intrinsic pathway

clot in vivo. Although discussed more fully in the following sections, evidence suggests that factor XII and prekallikrein may not normally participate in clotting in vivo but are important in the in vitro laboratory clot assays.

The Extrinsic Pathway and the PT

The first description of the extrinsic pathway was reported by Dr. Paul Morawitz in 1905. Dr. Morawitz produced a hemostasis model incorporating all of the scientific information of his day. Figure 1-3 illustrates a version of this model.

In 1935, Dr. Armand Quick published his method for the prothrombin time—with minor variations, the same laboratory test that is still in use today. Dr. Quick, using the classic four-component extrinsic pathway model of Dr. Morawitz,

essentially made "thrombokinase" with calcium ions. This "thrombokinase" was prepared from a saline extract of rabbit brain with the addition of calcium. The more modern nomenclature for this material is thromboplastin. The basis for Dr. Quick's assay was that adding calcium ions with
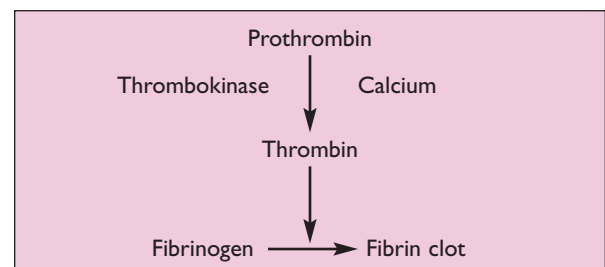
Figure 1-3. A representation of the original extrinsic pathway proposed in 1905.

an excess of thromboplastin to anticoagulated plasma was a direct measure of the prothrombin amount in the plasma—hence the name of the assay, prothrombin time. Only in the 1950s and early 1960s, with the discovery of additional coagulation factors, did the true nature of the extrinsic pathway become known. This is discussed in more detail below in the section, "The PT and aPTT Pathways."

The Intrinsic Pathway and the aPTT

Dr. Quick, in his first publication, observed that his new PT assay was not sensitive to the hemophilic defect. Patients with the symptoms of hemophilia did not usually have an abnormal PT. Evidence had been accumulating that the four-component extrinsic pathway model of blood clotting was not complete. The plasma had the potential to clot without the addition of an extrinsic material. The thromboplastin, thrombokinase, or what we now call tissue factor was not always needed to make blood clot, especially in vitro. Therefore, it appeared that plasma had within it or intrinsic to it all the factors necessary to cause blood clotting.

In 1953, Drs. Langdell, Wagner, and Brinkhous published a paper detailing a clot-based assay that was sensitive to the defect in hemophilic plasma. Instead of using a complete tissue extract, such as the prothrombin time thromboplastin reagent, their assay used only a partial extract. Hence these researchers called their assay a partial thromboplastin time (PTT). In this group's initial assay, the activator necessary to cause clotting was added separately from the PTT reagent and calcium ions. Other workers modified the assay, adding an activator to the PTT reagent, producing the modern activated thromboplastin time assay.

The PT and aPTT Pathways

The PT and aPTT assays were developed based on theories and specific testing needs, without complete knowledge of all the proteins involved in coagulation. In the period from 1935 and the inception of the PT until the early 1970s, all of the procoagulant proteins involved in forming a fibrin clot were identified. Many of these factors were identified because patients were found with deficiency states. Some of these patients had congenital bleeding disease, while others presented with an abnormal prolongation in the PT and/or the aPTT. It became clear that a fresh model of coagulation other than the classic extrinsic pathway was needed.

In the early 1960s, a new synthesis of all hemostasis knowledge was put together, and the PT or extrinsic and aPTT or intrinsic coagulation pathways were published. This provided a framework of how many of the proteins listed in Table 1-1 interact to form a blood clot. This pathway is illustrated in Figure 1-4. Although there have been some modifications since the original papers, these are the pathways with which most workers in hemostasis are familiar. From the initial publication, accumulating epidemiologic evidence suggests this formulation of the intrinsic and extrinsic hemostasis pathways might not be a correct representation of blood clotting in vivo. For example, patients deficient in factor XII, prekallikrein, or high-molecular-weight kininogen do not have a bleeding or thrombotic phenotype. All of these proteins are present at the start of the intrinsic pathway, and deficiencies of each factor can cause a significantly prolonged aPTT assay. Logically, it would make sense that a deficiency of a factor at the start of a pathway would cause bleeding pathology. However, all evidence suggests this is not so. The intrinsic and extrinsic pathways as they have existed since their inception are based on in vitro testing. The in vivo function appears to be different. **It is important to be familiar with the older pathway model because the PT and the aPTT are still useful as diagnostic tests.**

Newer Coagulation Model

Figure 1-5 illustrates a newer model of coagulation. In this figure, thrombin is depicted as the center of the coagulation universe; all aspects of hemostasis feed into the regulation and control of thrombin generation, which in turn forms the definitive clot at the site of an injury. Of note, the figure lacks several proteins normally considered part of the classic intrinsic coagulation pathway: factor XII and prekallikrein. These proteins are not currently thought to be important for in vivo coagulation activation. Although high-molecular-weight kininogen deficiency may not be associated with a bleeding diathesis, it is still part of the new coagulation pathway as it circulates in plasma bound to factor XI. Also important in this newer concept is that the majority of the steps in the coagulation cascade take place by the formation of multimolecular coagulation protein complexes on phospholipid cell surfaces.

This new coagulation model has extrinsic and intrinsic pathway limbs, but the in vivo process of hemostasis is thought only to be initiated by cell-based tissue factor expressed at an injury site.
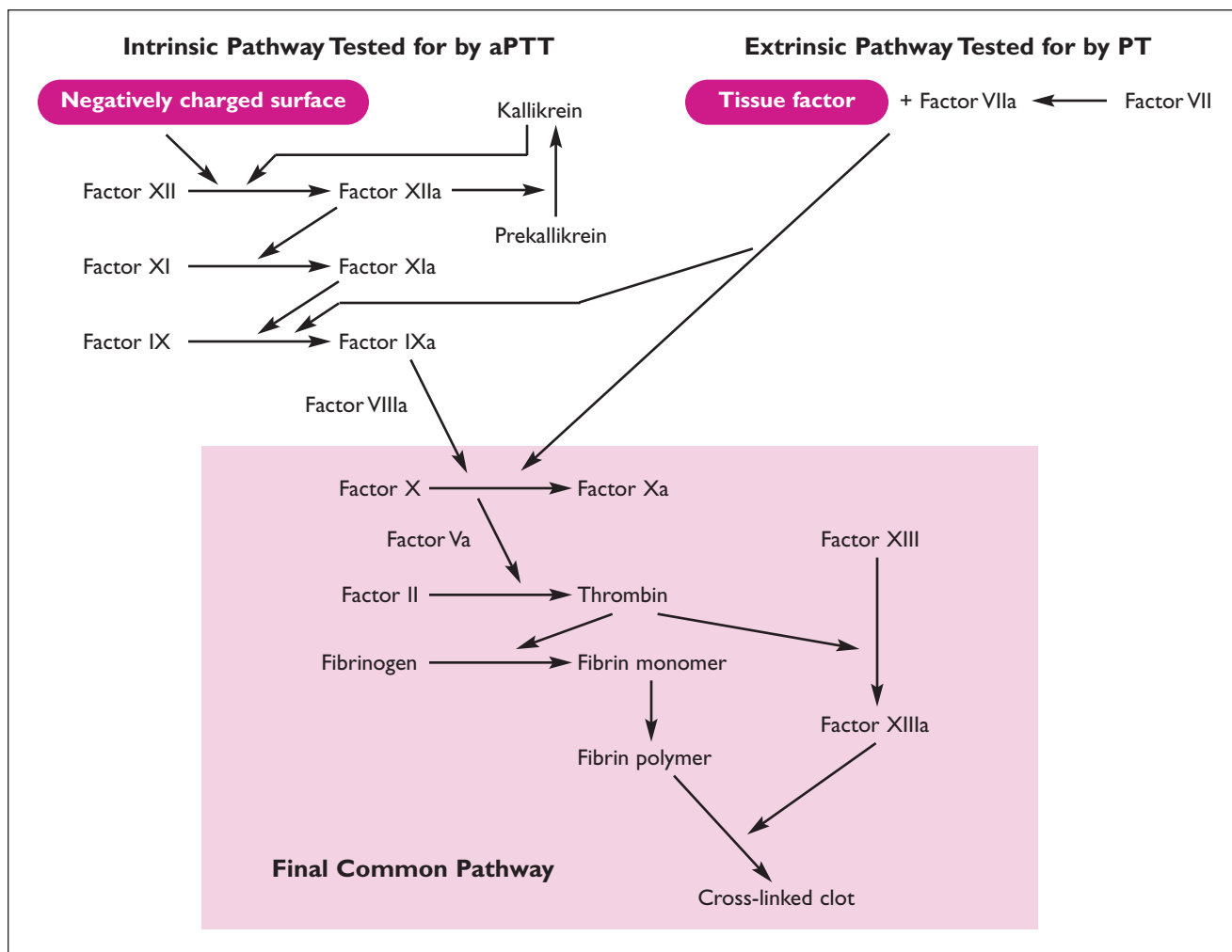
Figure 1-4. A model of the classic extrinsic and intrinsic coagulation pathways. For the sake of clarity, calcium ions and phospholipids, two important cofactors for most coagulation reactions, ha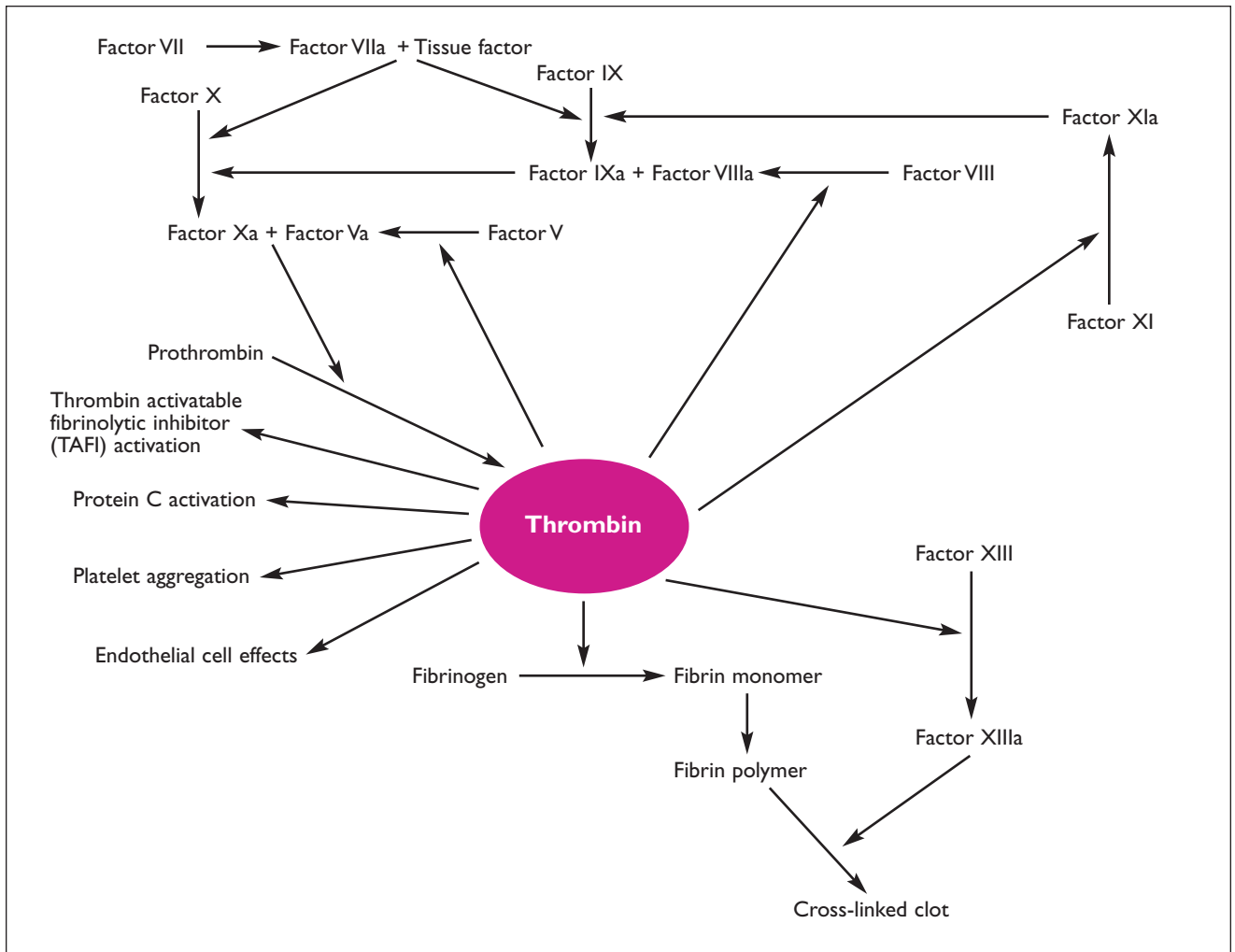ve been omitted from the figure. Ca^{++} and phospholipids are necessary for most reactions except the activation of factor XII and the activation of prekallikrein. The cofactor for the activation of prekallikrein, high-molecular-weight kininogen, has also been omitted from the figure for clarity.

Tissue factor binds to factor VII or VIIa in a 1:1 complex. Limited proteolysis leads to a tissue factor/factor VIIa complex that can activate factor X or factor IX to activated serine proteases through cleavage of an activation peptide. Once the pathway commences, the tissue factor/factor VIIa activation of factor X is rapidly shut down by an inhibitor produced by endothelial cells, tissue factor pathway inhibitor (TFPI). The newly activated factor IXa then binds to its cofactor, factor VIIIa, on a phospholipid surface to form the tenase complex that results in the activation of factor X to factor Xa.

The activation of factor X to Xa starts the final, common pathway for thrombin activation. Factor Xa combines with the cofactor, factor Va, together with calcium on phospholipid surfaces to form the prothrombinase complex. This complex then effects the conversion of prothrombin to thrombin by cleavage of an activation peptide, prothrombin F1.2.

The generation of a small amount of thrombin initiated by extrinsic means appears to be enough to start the coagulation mechanism and, if conditions are right, the expansion of thrombin generation through an intrinsic mechanism. The intrinsic limbs of the pathway include activation of factor XI to factor XIa by thrombin, with the ultimate generation of more thrombin using factor IXa and factor VIIIa to activate factor X. This pathway model also explains the mechanism of how two of the important cofactors, factor V and factor VIII, are activated by thrombin. It was known that both factor V and factor VIII had to be partially proteolyzed or activated to participate in the formation of a blood clot, but the mechanism was never heretofore part of the coagulation model.

Figure 1-5. A newer model of the coagulation pathway. For the sake of clarity, Ca^{++} and phospholipids have been omitted from the figure. These two cofactors are necessary for all of the reactions listed in the figure that result in the activation of prothrombin to thrombin. The pathway is initiated by an extrinsic mechanism that generates small amounts of factor Xa, which in turn activate small amounts of thrombin. The tissue factor/factor VIIa proteolysis of factor X is quickly inhibit- ed by tissue factor pathway inhibitor (TFPI). The small amounts of thrombin generated from the initial activation feedback to create activated cofactors, factors Va and VIIIa, which in turn help to generate more thrombin. Tissue factor/factor VIIa is also capable of indirectly activating factor X through the acti- vation of factor IX to factor IXa. Finally, as more thrombin is created, it activates factor XI to factor XIa, thereby enhancing the ability to ultimately make more thrombin.

Activation of factors V and VIII by thrombin results in a further burst of coagulation activity through increased activity of the tenase and pro-thrombinase complexes.

Fibrinogen is the ultimate substrate protein of the coagulation cascade and forms the principal structural protein of the fibrin clot. Fibrinogen, produced in the liver, is a dimer composed of three pairs of protein chains, Aα, Bβ, and γ, that are disulfide-linked at their N-terminal ends. Fibrinogen, as viewed by molecular imaging techniques, is composed of three globular domains, a central E domain flanked by two identical D domains (Figure 1-6). Thrombin cleaves small peptides, termed fibrinopeptides A and B, from the Aα and Bβ chains, respectively, to form a fibrin monomer. These monomers assemble into protofibrils in a half-staggered, side-to-side fashion that is stabilized by noncovalent interactions between fibrin molecules. The protofibrils lateral-ly associate into thicker fibrin fibers and form the fibrin clot. This clot, however, is not stable and ultimately will come apart if not covalently cross-linked. Thrombin activates factor XIII to the trans-glutaminase enzyme factor XIIIa. Factor XIIIa, act-ing upon the glutamic acid and lysine side chains
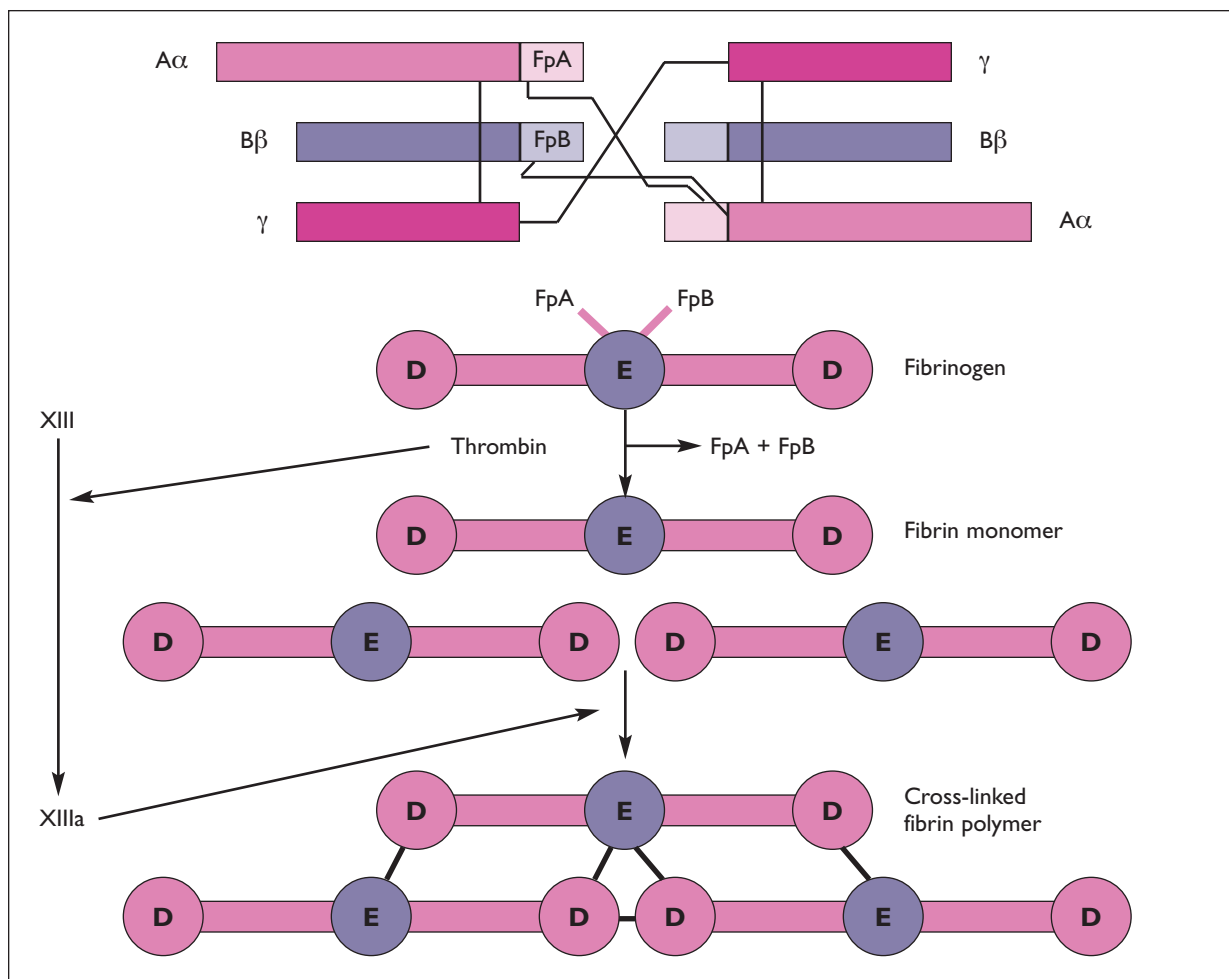
Figure 1-6. Fibrinogen is an abundant plasma protein that is a dimer of the Aα, Bβ, and γ chains connected by disulfide bonds. The fibrinogen dimer is composed of two flanking D globular domains with a central E domain. Fibrinogen forms the main structure of the fibrin clot, after cleavage of fibrinopeptides A (FpA) and B (FpB) by thrombin. The fibrin monomer assembles in a half-staggered overlap with adjoining fibrin monomers and is then covalently cross-linked into a fibrin polymer by the transamidase factor XIIIa.

in the fibrin amino acid sequence, creates covalent bonds between fibrin monomer γ chains, creating a stable clot. In addition, factor XIIIa can covalently cross-link a variety of other materials into the forming fibrin clot, including plasminogen and antiplasmin. This property of factor XIIIa is important for the penultimate purpose of the clot: wound healing and tissue repair.

Finally, Figure 1-5 alludes to the fact that there are many properties of thrombin other than the formation of the fibrin clot. Thrombin has direct effects on the other constituents of the coagulation triad: platelets and endothelial cells. Additionally, thrombin participates in its own downregulation. In the next section, some of these coagulation regulatory processes will be mentioned.

Regulatory Mechanisms

When a clot is formed, there has to be some mechanism to limit the clot to the site of an injury and ultimately to remove the clot when that injury has healed. The clot removal system, or fibrinolysis pathway, consists of the zymogen plasminogen, a variety of activators, and several inhibitors. Primary among these activators is tissue plasminogen activator (tPA), a product of endothelial cells. The fibrinolytic pathway is discussed in detail in chapter 3.

The most important coagulation proteins that are involved in regulation of thrombin generation are summarized in Table 1-2. Tissue factor pathway inhibitor has been mentioned in both the "Endothelium" and "Newer Coagulation Model"

Table 1-2. Coagulation Factor Inhibitors

Name	Description	Function
Tissue factor pathway inhibitor (TFPI)	Molecular Weight (MW) = 33,000 daltons (Da)	Inhibits the tissue factor/factor VIIa complex
Protein C	MW = 62,000 Da; vitamin K-dependent serine protease	Activated form cleaves coagulation cofactors Va and VIIIa
Protein S	MW = 75,000 Da; vitamin K-dependent protein	Cofactor for protein C
Antithrombin	MW = 58,000 Da	Serpin that directly inhibits several of the serine proteases; requires heparin as a cofactor

sections. Antithrombin also has been discussed in the "Endothelium" section of this chapter.

Once thrombin is formed, it can, as Figure 1-5 shows, be directed along an assortment of paths, which can result in a variety of important coagulation functions. If thrombin binds to the endothelial cell receptor thrombomodulin, its ability becomes limited to two paths. One of those paths is the protein C pathway. The thrombin/thrombomodulin receptor can activate protein C to its active form. Once activated, protein C and its cofactor, protein S, along with a phospholipid surface and calcium ions, can cleave factor Va and factor VIIIa to inactive forms. The presence of the cofactors factor Va and factor VIIIa is necessary for coagulation to function. Limiting the amounts of factor Va or factor VIIIa at the site of a growing clot essentially shuts down the ability to activate more thrombin and increase the amount of blood clot.

Operation of the Hemostasis and Thrombosis Pathway

The coagulation pathway is a complex interaction of many elements—the endothelium, coagulation factors, and platelets—with the ultimate goals of stemming the loss of blood at the site of an injury and laying the groundwork for injury repair and healing. Figure 1-7 illustrates a flow-chart diagram of how all the elements of the hemostatic pathways function together.
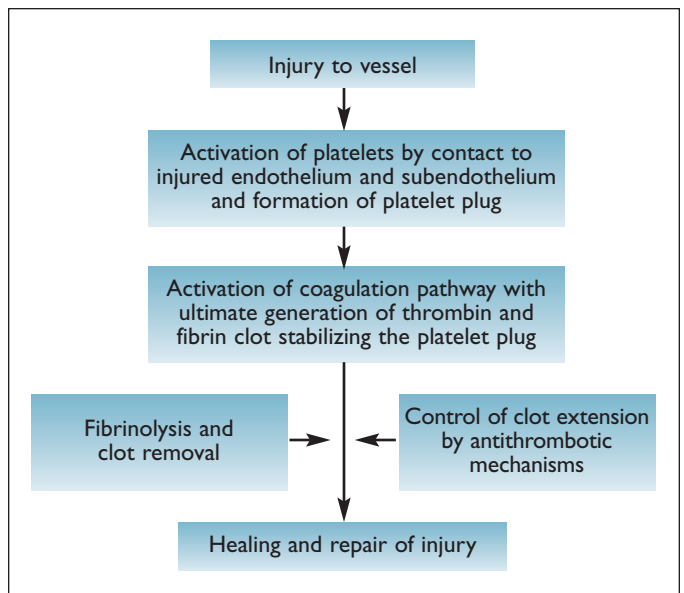
Figure 1-7. Algorithm illustrating the fundamental operation of the coagulation pathway.

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