

CHAPTER 1

Theories of Blood Coagulation: Basic Concepts and Recent Updates

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Historical background

Any mechanistic description of blood coagulation should account for a number of simple observations about the blood coagulation process. Blood that is circulating inside the body tends not to clot. However, blood that escapes from the vasculature does clot. This suggests that there is a material outside blood that is necessary for the clotting process. This point was emphasized by the study of Foa and Pellacani who showed that “tissue juice” (filtered saline extract of brain), when injected into the circulation of a rabbit, could cause intravascular thrombus formation [1]. This result was further clarified by Macfarlane and Biggs who showed that blood had all the factors needed to clot (intrinsic factors) but that this process was slow and that clotting was accelerated by the addition of tissue extracts (extrinsic factors) [2].

A clotted mass of blood was called a thrombus. When this thrombus was washed, a material, thrombin, could be eluted that would immediately clot fresh blood. It was further shown that there existed in blood an inactive agent, prothrombin, which could be converted to active thrombin. The agent responsible for this conversion was called thromboplastin (or thrombokinase). Attempts to discover the nature of thromboplastin led to much of our current mechanistic understanding of coagulation.

In 1875, Zahn made the important observation that bleeding from a blood vessel was blocked by a white (not red) thrombus [3]. Bizzozero and Hayem, working separately, studied a colorless corpuscle in blood called a thrombocyte or platelet [4,5]. This cell could be shown to be

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associated with fibrin and was postulated to be a major component of the white thrombus [4]. It was therefore suggested that there was a platelet thromboplastin that was critical for clotting (in modern usage, platelet procoagulant function is described as such and the term thromboplastin is used to mean the protein tissue factor [TF] which is the coagulation initiator in tissues).

It is known that in some families there is an inherited bleeding tendency (hemophilia). Eagle studied individuals with this disorder and showed that the platelet function in those patients was normal but that there was still a deficiency in prothrombin conversion [6]. This established that there was a plasma component required for clotting in addition to a requirement for platelets. Further studies in patients with different bleeding tendencies established that there are a number of elements that make up the plasma clotting component. Because these factors were discovered by multiple investigators in different parts of the world (and given a different name by each group), a systematic nomenclature was established using Roman numerals [7] (Table 1.1).

While studies on deficient plasmas had established what the important components were, the mechanisms of action and the interactions between these components were not immediately clear. Early coagulation schemes started from the model of prothrombin being converted to thrombin and visualized all of the circulating coagulation proteins as zymogens that were converted during coagulation into active enzymes [8,9]. Once the proteins involved in coagulation were isolated and their structure and functions were studied, it became clear that coagulation function was organized

Table 1.1 Systematic nomenclature of clotting factors.

Factor*	Comments
I	Fibrinogen
II	Prothrombin
III	Lipid, platelet surface, or Thromboplastin (not used)
IV	Calcium (not used)
V	
VI	Activated factor V (not used)
VII	
VIII	Hemophilia A factor
IX	Hemophilia B factor
X	
XI	Hemophilia C factor
XII	

*Activated forms of the factor are indicated by appending the letter "a" to the name.

Table 1.2 Composition and physiologic location of coagulation complexes.

Cofactor	Enzyme	Substrate	Cell surface
Tissue factor	Factor VIIa	Factor X / Factor IX	Many cells (but generally not circulating cells)
Factor VIIIa	Factor IXa	Factor X	Platelets
Factor Va	Factor Xa	Prothrombin	Platelets release factor Va on activation; many other cells
Thrombomodulin	Thrombin	Protein C	Endothelial cells
Protein S	Activated protein C	Factor Va / Factor VIIIa	Endothelial cells

around a mechanism of an active enzyme being paired with a cofactor [10]. In the absence of the cofactor, the enzyme has limited activity; typically a cofactor will accelerate the activity of a coagulation enzyme as much as 1000-fold [11]. Thus, each step in coagulation is regulated at two levels: 1) activation of the zymogen to an active enzyme and 2) the presence of (and sometimes activation of) the requisite cofactor. Since some cofactors, such as thromboplastin (tissue factor) and thrombomodulin, are integral membrane proteins, the functions of these complexes can be limited to cells and tissues that express the protein (Table 1.2).

The coagulation factors show only weak activity in solution, and binding to an appropriate cell surface accelerates their activity up to 1000-fold. This surface binding is dependent on calcium and, therefore, blood can be anticoagulated by the addition of chelating agents such as citrate or EDTA that bind calcium [12]. This chelation does not alter protein properties and can be readily reversed by reintroduction of calcium in excess of the chelating agents. Clinical assays use plasma prepared from blood chelated with citrate to analyze clotting factor function by addition of an appropriate activator and calcium and measuring the time to clot formation.

Localization of the coagulation reactions to a desired surface represents a powerful mechanism for limiting coagulation to surfaces at the site of injury. One component of coagulation factor binding to cells is the phospholipid composition of the outer leaflet of the cell membrane. Phospholipids with acidic head groups, phosphatidic acid (PA) and phosphatidylserine (PS), promote binding of coagulation factors [13]. In addition, phosphatidylserine acts as an allosteric regulator of function and accounts for the ability of PS-containing membranes to enhance coagulation factor activity [14]. While generic phospholipid surfaces can support coagulation reactions (and are used in clinical assays), it is clear that cells, in addition to having appropriate lipid surfaces, have regulatory elements that control the coagulation reactions [15,16].

Functional platelets are required as a surface for hemostasis, and patients with low platelet counts (thrombocytopenia) or platelet function defects (thrombocytopathia) such as Bernard–Soulier syndrome or Glanzmann's thrombasthenia have a bleeding tendency. Circulating platelets, like essentially all cells in blood as well as endothelial cells, have outer membranes with low levels of acidic phospholipids. When platelets adhere at a site of injury the composition of their membrane changes such that acidic phospholipids, including phosphatidylserine, are now expressed on the outer surface of the membranes [17]. This change in surface lipid composition, along with changes in platelet surface proteins and release of procoagulant factors from platelet granules, provides a surface that supports robust coagulation.

Cell-based model of coagulation

In a mild injury, the coagulation process starts with hemostatic platelet aggregates which can be found at the ends of transected blood vessels [18]. Early in the process, these aggregates consist of activated (degranulated) platelets packed together. In time, small amounts of fibrin are deposited between the platelets. At longer times more fibrin becomes associated with the platelet masses. This fibrin extends into the tissues and provides stability to the area of injury [18,19].

In hemophilia A or B, the process is somewhat different [20]. Early in the process, the platelets are loosely associated but are not activated. Even at longer times platelets are only poorly activated and fibrin is not seen between the platelets. The result is that the platelet mass is not stabilized. Whereas normal individuals show extensive fibrin extending into the tissues, in hemophilia a thin layer of fibrin can be seen only at the margins of the wound area and does not extend significantly into the tissues [19,20].

These observations of hemostasis suggest that coagulation can be conceived of as a series of overlapping steps: initiation; amplification; and propagation.

Initiation

Blood coagulation is initiated by an injury to a blood vessel; this injury could be a denudement of some of the endothelium or a break in the vessel. In either case, two processes begin immediately. One process is that platelets quickly adhere to the site of injury. This adherence requires von Willebrand factor which binds to both collagen in the exposed subendothelium and the abundant platelet protein glycoprotein Ib. This adherence brings platelets into contact with collagen which, through the platelet

collagen receptor glycoprotein VI, activates platelets [21]. This activation causes changes in platelet surface receptors and leads the platelets to degranulate. Degranulation releases a number of stored proteins including a partially activated form of factor V [22].

The second process that begins with a break in the vasculature is that plasma concentrations of coagulation proteins are brought into the area of injury and presented to extravascular cells. Cells surrounding the vasculature tend to be rich in the protein called tissue factor (thromboplastin); the high concentration of tissue factor around blood vessels has been described as contributing to a hemostatic envelope [23]. At least some of the tissue factor already has factor VII bound [24] and factor VII binds tightly to any free tissue factor. On cells this tissue factor-bound factor VII is rapidly converted to factor VIIa. This conversion can be via cellular proteases, by autoactivation by other factor VIIa molecules, or by factor Xa generated by factor VIIa–tissue factor complexes [25,26].

These factor VIIa–tissue factor complexes catalyze two reactions: activation of factor X and activation of factor IX [27]. The factor Xa that is formed can complex with the partially active factor V released from platelets; this factor Xa–Va complex converts at least some prothrombin to thrombin. Formation of factor Xa also starts the process of regulating coagulation. The inhibitor TFPI (tissue factor pathway inhibitor) can bind to factor Xa and factor VIIa to turn off the factor VIIa–tissue factor complex [28,29]. This inhibition requires factor Xa so that the factor VIIa–tissue factor complex is not turned off until some factor Xa has been formed. Factor Xa in a complex with factor Va is protected from the abundant plasma inhibitor antithrombin, but once released from the complex, factor Xa inhibition by antithrombin is rapid with an expected half-life of about 4 minutes.

Amplification

The initial thrombin formed during the initiation phase is probably not sufficient to provide for robust fibrin formation and hemostasis. However, the thrombin formed on the initiating cell can transfer to platelets where the initial hemostatic signal is amplified by activating platelets and cofactors. On the platelet surface, thrombin is relatively protected from inhibition by antithrombin (in plasma, thrombin has a half-life of just over 1 minute). Thrombin can bind to at least two receptors on the platelet surface: glycoprotein Ib and protease-activated receptor (PAR)-1 [30,31]. Thrombin binding to and cleavage of PAR-1 transmits signals that lead to platelet activation (outside-in signals) [32]. This activation results in changes in the surface lipid content with increased exposure on the outer leaflet of acid phospholipids [17]. Activation also leads to inside-out signals that alter the conformation and function of some surface proteins including the fibrin binding protein complex of glycoproteins IIb and IIIa [33].

Activation also results in release of internal stores of a number of components from alpha granules and dense granules. The released components include partially active factor V, fibrinogen, ADP which acts as signal for further platelet activation, and polyphosphates [34,35].

Thrombin bound to glycoprotein Ib can cleave PAR-1 and PAR-4 [36]. This thrombin can also cleave factor VIII, releasing factor VIIIa onto the platelet surface. Factor VIII circulates in a complex with von Willebrand factor [37]; since both thrombin and von Willebrand factor are bound to glycoprotein Ib, it suggests that factor VIII may be presented to thrombin in such a way as to allow for rapid activation. Thrombin on the platelet surface can also fully activate platelet surface factor V, a reaction that is enhanced by platelet-released polyphosphates [35]. Either the partially active factor V released from platelets or plasma-derived factor V can be activated by thrombin. Thrombin activation of platelets is augmented in platelets bound directly to collagen (as opposed to platelets aggregated onto other platelets or onto fibrin) [38]. These platelets, sometimes called COAT platelets, have higher levels of acidic phospholipids as well as significantly increased binding of factors X, IX, VIII, and V [17,39].

The amplification process leads to platelets which are primed to varying degrees for thrombin generation. These platelets have an appropriate lipid surface with activated receptors and activated cofactors bound to the surface.

Propagation

Factor IXa formed during the Initiation phase binds to the platelet surface. Factor IXa is available even in the presence of plasma levels of anti-thrombin since the half-life of activated factor IXa is about an hour in plasma. Factor IXa can bind either to platelet surface factor VIIIa [40] formed in the amplification phase or to a platelet receptor and be transferred to factor VIIIa [41]. The factor IXa/VIIIa complex activates factor X on the platelet surface. Factor Xa can then move quickly into a complex with factor Va. The resulting factor Xa/Va complex provides the rapid burst of thrombin critical to giving good fibrin structure and providing for a stable clot.

Formation of factor Xa on the platelet surface plays a critical role in regulating the clotting process [42]. The rate of factor X activation determines the rate and amount of thrombin generation. Deficiencies in platelet surface factor Xa generation caused by a lack of or reduction in factor VIII or IX levels (hemophilia A or B, respectively) result in reduced or absent factor Xa and thrombin generation. Therapies to treat hemophilia involve restoring a robust rate of platelet surface factor Xa generation [43]. Factor Xa generation appears to be regulated in part by release of TFPI from

platelets, and agents that block TFPI are under consideration as possible therapeutic agents in hemophilia [44].

Thrombin generation on platelets initiates a positive feedback loop through factor XI. Factor XI can be activated on platelets by thrombin [45,46]. This thrombin activation is enhanced by polyphosphates released from platelets [35]. The platelet surface factor XIa can activate factor IX, leading to enhanced factor Xa and thrombin generation. The amount of enhancement from factor XI shows wide variations on platelets from different individuals and may account for some of the variable bleeding associated with factor XI deficiency (hemophilia C) [45].

In some cases, particularly in cases of intravascular injury where there is substantial blood flow across the injured surface, there may be a contribution to the propagation phase from circulating tissue factor [47]. While healthy individuals have little or no circulating tissue factor [48], in some pathologic conditions, such as pancreatic cancer, there are measurable levels of circulating tissue factor in the form of microparticles [49]. If the microparticles also have surface molecules that can associate with platelets or other cells at the site of injury, then tissue factor on these microparticles may contribute to factor X activation and thrombin generation at a site of injury [50].

Very small amounts of thrombin (less than 1 nM or 0.1 U/mL) are required to promote fibrin formation, and much of the thrombin generation occurs after a clot has formed [51]. The thrombin formed binds to fibrin where it can remain active for many hours; the binding of thrombin to fibrin resulted in fibrin being described as antithrombin I [52]. Furthermore, platelet factor Xa/Va complexes appear to be active long after clot formation (hours) and can rapidly generate thrombin when presented with fresh plasma as a substrate [51]. It is likely that the persistence of fibrin-bound thrombin and the prolonged ability to generate thrombin are protective mechanisms to stabilize clots. Disruption of a clot would mean that thrombin is immediately available to cleave fibrinogen and refresh the fibrin clot. Also, new prothrombin present in plasma could be activated to further replenish thrombin stores and provide for clot stability.

Control and localization

Multiple mechanisms exist to prevent a clot from spreading into healthy vasculature. Flow is an important control mechanism and reduced flow is associated with venous thrombosis. Flow removes procoagulant proteins from the area of active thrombin generation, reducing their concentrations below the threshold required to maintain coagulation. Once thrombin, factor Xa, and other procoagulant proteases are removed from the relatively protected area of the clot, they are subject to inhibition by antithrombin, TFPI, and other plasma inhibitors. This inhibition is enhanced

by the carbohydrate components of proteoglycans found on endothelial cells [53]. Also, endothelial cells have surface-associated TFPI that promotes rapid inactivation of factor Xa [54].

Platelet activation also represents a control mechanism. Thrombin cleavage of PARs is important for platelet activation, but the final activated state is dependent on signaling through other platelet receptors [55]. Platelets bound to collagen (and that thus signal through glycoprotein IV) have very high levels of procoagulant factors and are associated with enhanced thrombin generation and fibrin formation. Other platelets appear to have less procoagulant activity and have a more structural role in stabilizing the fibrin clot [56]. It appears that growth of a clot may in part be constrained by structural platelets that do not strongly support thrombin generation and, therefore, do not strongly support the positive feedback loop that generates the burst of thrombin.

Platelets support a thrombin-driven positive feedback loop that activates additional platelets, promotes thrombin generation, and leads to fibrin formation. In contrast, thrombin on healthy endothelial cells leads to a negative feedback loop that shuts off further thrombin generation. Endothelial cells express the thrombin-binding protein, thrombomodulin [57]. Thrombin bound to thrombomodulin can no longer cleave fibrinogen; however, thrombin bound to thrombomodulin gains the ability to activate protein C [58]. Activation of protein C is enhanced by another endothelial cell protein called EPCR (endothelial cell protein C receptor) [59].

Activated protein C cleaves and inactivates both factor VIIIa and factor Va in a reaction that is enhanced somewhat by protein S [60]. This inactivation is more efficient on the endothelial cell surface than on platelets and suggests that the protein C pathway localizes thrombin generation rather than strictly shutting it down [61]. One of the sites on factor V that is cleaved by activated protein C is altered by the common factor V Leiden mutation [62]; this mutation is associated with venous thrombosis, suggesting that the negative feedback loop on healthy endothelium is a critical component of maintaining vascular patency.

Clinical assays

Coagulation function is generally measured in clinical assays that incorporate the elements of either the Initiation phase (prothrombin time or PT) or the propagation phase (activated partial thromboplastin time or aPTT). These assays are done on platelet-poor plasma so that the platelet contributions to clotting are not studied. The normal controls of these assays tend to be very reproducible, and the cell-free plasma can be frozen

and stored. Other assays integrate the initiation and propagation phases and may include platelet function, such as thrombin generation (thrombogram or calibrated automated thrombography (CAT) [63] or whole blood clotting (thromboelastogram) [64].

The PT assay is done by adding very high levels of thromboplastin (TF) to plasma. Factor VII binds to this TF and is activated. The factor VIIa/TF complex activates factor X and the factor Xa/Va complex generates thrombin which clots the plasma. The endpoint of the assay is the time required for clot formation. Because thromboplastin is generally external to the blood, the assay is sometimes referred to as assaying the extrinsic pathway.

The aPTT assay is done by adding, in the absence of calcium, a negatively charged activator to plasma. The negative charge assembles a complex of high-molecular-weight kininogen and factor XII that converts all of the factor XI in the sample to factor XIa [65]. When recalcified, the factor XIa activates factor IXa which forms a complex with factor VIIIa. This factor IXa/VIIIa complex activates factor Xa which, in complex with factor Va, cleaves prothrombin to thrombin and clots the plasma. The endpoint of the assay is the time required for clot formation. Since all of the protein components are found in plasma, this assay is sometimes referred to as assaying the intrinsic pathway.

Since thrombin generation in the aPTT requires factors IX and VIII, it is used to monitor factor levels during therapy in hemophilia. The assay is very sensitive to the levels of the contact factors, factors XI and XII. However, patients with factor XI deficiency have a variable bleeding diathesis that is not strictly correlated with plasma levels [66]. This may be a function of how factor XI interacts with platelets and would not be assayed by the aPTT. Factor XII deficiency is not associated with any bleeding, nor is the deficiency protective of thrombosis in humans [67]. Bacterial polyphosphates are able to promote factor XII activation and may play a role in coagulation associated with the innate immune response; platelet polyphosphates are shorter than bacterial polyphosphates and do not promote activation of factor XII [35].

Summary

This overview provides a conceptual model of hemostasis as being initiated by injury leading to exposure of collagen and thromboplastin. Platelets adhere and are activated. Coagulation factors are activated, assemble on the platelet surface, and give robust thrombin generation leading to stable clot formation and clot retraction. Subsequent chapters will detail mechanisms of the processes involved in promoting hemostasis. Many of the

same players (thromboplastin, platelets, coagulation factors), albeit with slightly different roles, are also involved in pathological coagulation leading to thrombosis or bleeding. Dysregulation resulting in thrombosis and other coagulation abnormalities will also be discussed in subsequent chapters.

References

1. Foà P, Pellacani P. Sul fermento übrinogeno e sullc azioni tossiclie esercitate da aleuni organi freschi. *Arch per le sc med* 1883;VII:113–65.
2. Macfarlane RG, Biggs R. A thrombin generation test; the application in haemophilia and thrombocytopenia. *J Clin Pathol* 1953;6:3–8.
3. Zahn F. Untersuchungen über Thrombose: Bildung der Thromben. *Virchows Arch Pathol Anat Physiol Klin Med* 1875;62:81–124.
4. Bizzozero J. Ueber einen neuen formbestandtheil des blutes und dessen rolle bei der thrombose und der blutgerinnung. *Virchows Arch Pathol Anat Physiol Klin Med* 1882;90:261–332.
5. Hayem G. Sur le mécanisme de l'arrêt des hémorrhagies. *C R Acad Sci* 1882;95:18–21.
6. Eagle H. Studies on blood coagulation, IV: the nature of the clotting deficiency in hemophilia. *J Gen Physiol* 1935;18:813–19.
7. Jackson CM. Recommended nomenclature for blood clotting-zymogens and zymogen activation products of the international committee on thrombosis and hemostasis. *Thromb Haemost* 1977;38:567–77.
8. Davie E, Ratnoff O. Waterfall sequence for intrinsic blood clotting. *Science* 1964; 145:1310–12.
9. Macfarlane R. An enzyme cascade in the blood coagulation mechanism, and its function as a biochemical amplifier. *Nature* 1964;202:498–9.
10. Jobin F, Esnouf MP. Studies on the formation of the prothrombin-converting complex. *Biochem J* 1967;102:666–74.
11. Rosing J, Tans G, Govers-Riemslog JW, et al. The role of phospholipids and factor Va in the prothrombinase complex. *J Biol Chem* 1980;255:274–83.
12. Pekelharig C. *Untersuchen über des Fibrinferment*. Amsterdam: J. Müller, 1892.
13. Bull RK, Jevons S, Barton PG. Complexes of prothrombin with calcium ions and phospholipids. *J Biol Chem* 1972;247:2747–54.
14. Majumder R, Weinreb G, Lentz BR. Efficient thrombin generation requires molecular phosphatidylserine, not a membrane surface. *Biochemistry* 2005;44:16998–7006.
15. Wood JP, Silveira JR, Maille NM, et al. Prothrombin activation on the activated platelet surface optimizes expression of procoagulant activity. *Blood* 2011;117: 1710–18.
16. Haynes LM, Bouchard BA, Tracy PB, Mann KG. Prothrombin activation by platelet-associated prothrombinase proceeds through the prethrombin-2 pathway via a concerted mechanism. *J Biol Chem* 2012;287:38647–55.
17. Bevers EM, Comfurius P, Zwaal RF. The nature of the binding for prothrombinase at the platelet surface as revealed by lipolytic enzymes. *Eur J Biochem* 1982;122: 81–5.
18. Wester J, Sixma JJ, Geuze JJ, Heijnen HF. Morphology of the hemostatic plug in human skin wounds: transformation of the plug. *Lab Invest* 1979;41:182–92.

19. Monroe DM, Hoffman M. The clotting system: a major player in wound healing. *Haemophilia* 2012;18 Suppl 5:11–16.
20. Sixma JJ, Van den Berg A. The haemostatic plug in haemophilia A: a morphological study of haemostatic plug formation in bleeding time skin wounds of patients with severe haemophilia A. *Br J Haematol* 1984;58:741–53.
21. Moroi M, Jung SM, Okuma M, Shinmyozu K. A patient with platelets deficient in glycoprotein VI that lack both collagen-induced aggregation and adhesion. *J Clin Invest* 1989;84:1440–5.
22. Monković DD, Tracy PB. Functional characterization of human platelet-released factor V and its activation by factor Xa and thrombin. *J Biol Chem* 1990;265:17132–40.
23. Drake TA, Morrissey JH, Edgington TS. Selective cellular expression of tissue factor in human tissues: implications for disorders of hemostasis and thrombosis. *Am J Pathol* 1989;134:1087–97.
24. Hoffman M, Colina CM, McDonald AG, et al. Tissue factor around dermal vessels has bound factor VII in the absence of injury. *J Thromb Haemost* 2007;5:1403–8.
25. Bajaj SP, Rapaport SI, Brown SF. Isolation and characterization of human factor VII: activation of factor VII by factor Xa. *J Biol Chem* 1981;256:253–9.
26. Nakagaki T, Foster DC, Berkner KL, Kisiel W. Initiation of the extrinsic pathway of blood coagulation: evidence for the tissue factor dependent autoactivation of human coagulation factor VII. *Biochemistry* 1991;30:10819–24.
27. Østerud B, Rapaport SI. Activation of 125I-factor IX and 125I-factor X: effect of tissue factor and factor VII, factor Xa and thrombin. *Scand J Haematol* 1980;24:213–26.
28. Broze GJ Jr, Warren LA, Novotny WF, et al. The lipoprotein-associated coagulation inhibitor that inhibits the factor VII-tissue factor complex also inhibits factor Xa: insight into its possible mechanism of action. *Blood* 1988;71:335–43.
29. Rao LV, Rapaport SI. Studies of a mechanism inhibiting the initiation of the extrinsic pathway of coagulation. *Blood* 1987;69:645–51.
30. Jamieson GA, Okumura T, Hasitz M. Structure and function of platelet glyocalicin. *Thromb Haemost* 1980;42:1673–8.
31. Vu TK, Hung DT, Wheaton VI, Coughlin SR. Molecular cloning of a functional thrombin receptor reveals a novel proteolytic mechanism of receptor activation. *Cell* 1991;64:1057–68.
32. Kahn ML, Nakanishi-Matsui M, Shapiro MJ, et al. Protease-activated receptors 1 and 4 mediate activation of human platelets by thrombin. *J Clin Invest* 1999;103:879–87.
33. Collier BS, Peerschke EI, Scudder LE, Sullivan CA. A murine monoclonal antibody that completely blocks the binding of fibrinogen to platelets produces a thrombasthenic-like state in normal platelets and binds to glycoproteins IIb and/or IIIa. *J Clin Invest* 1983;72:325–38.
34. Maynard DM, Heijnen HFG, Horne MK, et al. Proteomic analysis of platelet alpha-granules using mass spectrometry. *J Thromb Haemost* 2007;5:1945–55.
35. Morrissey JH, Choi SH, Smith SA. Polyphosphate: an ancient molecule that links platelets, coagulation, and inflammation. *Blood* 2012;119:5972–9.
36. De Candia E, Hall SW, Rutella S, et al. Binding of thrombin to glycoprotein Ib accelerates the hydrolysis of PAR-1 on intact platelets. *J Biol Chem* 2001;276:4692–8.
37. Owen WG, Wagner RH. Antihemophilic factor: separation of an active fragment following dissociation by salts or detergents. *Thromb Diath Haemorrh* 1972;27:502–15.

38. Heemskerk JW, Vuist WM, Feijge MA, et al. Collagen but not fibrinogen surfaces induce bleb formation, exposure of phosphatidylserine, and procoagulant activity of adherent platelets: evidence for regulation by protein tyrosine kinase-dependent Ca²⁺ responses. *Blood* 1997;90:2615–25.
39. Alberio L, Safa O, Clemetson KJ, et al. Surface expression and functional characterization of alpha-granule factor V in human platelets: effects of ionophore A23187, thrombin, collagen, and convulxin. *Blood* 2000;95:1694–702.
40. Van Dieijen G, Van Rijn JL, Govers-Riemslog JW, et al. Assembly of the intrinsic factor X activating complex—interactions between factor IXa, factor VIIIa and phospholipid. *Thromb Haemost* 1985;53:396–400.
41. Ahmad SS, Rawala-Sheikh R, Walsh PN. Comparative interactions of factor IX and factor IXa with human platelets. *J Biol Chem* 1989;264:3244–51.
42. Hoffman M, Monroe DM, Oliver JA, Roberts HR. Factors IXa and Xa play distinct roles in tissue factor-dependent initiation of coagulation. *Blood* 1995;86:1794–801.
43. Monroe DM, Hoffman M, Oliver JA, Roberts HR. A possible mechanism of action of activated factor VII independent of tissue factor. *Blood Coagul Fibrinolysis* 1998;9 (suppl 1):S15–20.
44. Maroney SA, Cooley BC, Ferrel JP, et al. Absence of hematopoietic tissue factor pathway inhibitor mitigates bleeding in mice with hemophilia. *Proc Natl Acad Sci USA* 2012;109:3927–31.
45. Oliver JA, Monroe DM, Roberts HR, Hoffman M. Thrombin activates factor XI on activated platelets in the absence of factor XII. *Arterioscler Thromb Vasc Biol* 1999;19:170–7.
46. Kravtsov DV, Matafonov A, Tucker EI, et al. Factor XI contributes to thrombin generation in the absence of factor XII. *Blood* 2009;114:452–8.
47. Giesen PL, Rauch U, Bohrmann B, et al. Blood-borne tissue factor: another view of thrombosis. *Proc Natl Acad Sci USA* 1999;96:2311–15.
48. Butenas S, Bouchard BA, Brummel-Ziedins KE, et al. Tissue factor activity in whole blood. *Blood* 2005;105:2764–70.
49. Delluc A, Rousseau A, Delluc C, et al. Venous thromboembolism in patients with pancreatic cancer: implications of circulating tissue factor. *Blood Coagul Fibrinolysis* 2011;22:295–300.
50. Falati S, Liu Q, Gross P, et al. Accumulation of tissue factor into developing thrombi in vivo is dependent upon microparticle P-selectin glycoprotein ligand 1 and platelet P-selectin. *J Exp Med* 2003;197:1585–98.
51. Orfeo T, Brummel-Ziedins KE, Gissel M, et al. The nature of the stable blood clot procoagulant activities. *J Biol Chem* 2008;283:9776–86.
52. Seegers WH, Johnson JF, Fell C. An antithrombin reaction to prothrombin activation. *Am J Physiol* 1954;176:97–103.
53. De Agostini AI, Watkins SC, Slayter HS, et al. Localization of anticoagulant active heparan sulfate proteoglycans in vascular endothelium: antithrombin binding on cultured endothelial cells and perfused rat aorta. *J Cell Biol* 1990;111:1293–304.
54. Zhang J, Piro O, Lu L, Broze GJ Jr. Glycosyl phosphatidylinositol anchorage of tissue factor pathway inhibitor. *Circulation* 2003;108:623–7.
55. Heemskerk JW, Siljander P, Vuist WM, et al. Function of glycoprotein VI and integrin alpha2beta1 in the procoagulant response of single, collagen-adherent platelets. *Thromb Haemost* 1999;81:782–92.
56. Heemskerk JWM, Mattheij NJA, Cosemans JMEM. Platelet-based coagulation: different populations, different functions. *J Thromb Haemost* 2013;11:2–16.
57. Esmon NL, Owen WG, Esmon CT. Isolation of a membrane-bound cofactor for thrombin-catalyzed activation of protein C. *J Biol Chem* 1982;257:859–64.

58. Esmon CT, Esmon NL, Harris KW. Complex formation between thrombin and thrombomodulin inhibits both thrombin-catalyzed fibrin formation and factor V activation. *J Biol Chem* 1982;257:7944–7.
59. Fukudome K, Esmon CT. Identification, cloning, and regulation of a novel endothelial cell protein C/activated protein C receptor. *J Biol Chem* 1994;269:26486–91.
60. Dahlbäck B, Villoutreix BO. Molecular recognition in the protein C anticoagulant pathway. *J Thromb Haemost* 2003;1:1525–34.
61. Oliver JA, Monroe DM, Church FC, et al. Activated protein C cleaves factor Va more efficiently on endothelium than on platelet surfaces. *Blood* 2002;100:539–46.
62. Bertina RM, Koeleman BP, Koster T, et al. Mutation in blood coagulation factor V associated with resistance to activated protein C. *Nature* 1994;369:64–7.
63. Hemker HC, Wielders S, Kessels H, Béguin S. Continuous registration of thrombin generation in plasma, its use for the determination of the thrombin potential. *Thromb Haemost* 1993;70:617–24.
64. Hartert H. Blutgerinnungsstudien mit der Thrombelastographie, einem neuen Untersuchungsverfahren. *Klin Wochenschr* 1948;26:577–83.
65. Bouma BN, Griffin JH. Human blood coagulation factor XI: purification, properties, and mechanism of activation by activated factor XII. *J Biol Chem* 1977;252:6432–7.
66. Seligsohn U. Factor XI in haemostasis and thrombosis: past, present and future. *Thromb Haemost* 2007;98:84–9.
67. Endler G, Marsik C, Jilma B, et al. Evidence of a U-shaped association between factor XII activity and overall survival. *J Thromb Haemost* 2007;5:1143–8.