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The Coagulation Cascade: Initiation, Maintenance, and Regulation†

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There are two principal mechanisms to stop the loss of blood in higher organisms following vascular injury. Initially, platelets are activated and adhere to the site of injury. The platelets then aggregate and form a platelet plug that reduces or temporarily stops the loss of blood. The activation of platelets also releases numerous proteins and small molecules that accelerate and increase platelet plug formation and begin the process of tissue repair (Majerus, 1987). Plasma proteins such as von Willebrand factor play an important role in platelet adhesion by forming a bridge between the activated platelet and the subendothelium (Girma et al., 1987; Ruggeri & Zimmerman, 1987). This is accomplished by the binding of von Willebrand factor to specific receptors (glycoprotein Ib/glycoprotein IX) on the surface of the activated platelets as well as to the subendothelium (Lopez et al., 1988; Hickey et al., 1989). In a similar manner, fibrinogen forms a bridge between activated platelets by binding to the surface receptors (glycoprotein IIb/IIIa) on adjacent activated platelets (Bennett et al., 1982; Savage & Ruggeri, 1991). The latter reaction leads to platelet aggregation and platelet plug formation. These reactions also set the stage for the coagulation cascade and fibrin formation by making available negatively charged phospholipids such as phosphatidylserine on the surface of the activated platelets or damaged cell membranes. A series of reactions are then triggered that lead to fibrin formation and the generation of an insoluble fibrin clot that strengthens the platelet plug.

Initiation of the Blood Coagulation Cascade by the Extrinsic Pathway

More than 25 years ago, two similar proposals (Davie & Ratnoff, 1964; MacFarlane, 1964) were made independently that formed the basis for the intrinsic pathway of the coagulation cascade (left side, Figure 1). It was proposed that fibrin formation resulted from a series of stepwise reactions involving only proteins circulating in blood in a precursor or inactive form. Each of the plasma proteins was activated by minor proteolysis and many were converted to serine proteases. The phospholipid was provided by the activated platelets where many of the calcium ion dependent reactions occurred. Most of these proteins were initially identified in patients with bleeding complications whose coagulation was corrected by a protein present in normal plasma. Present evidence suggests that the intrinsic pathway plays an important role in the growth and maintenance of fibrin formation in the coagulation cascade while a second overlapping mechanism, called the extrinsic pathway, is critical in the initiation of fibrin formation (Figure 1, right side with heavy arrows).

The extrinsic pathway requires tissue factor, which is located in the tissue adventitia and comes in contact with blood only after vascular injury (Maynard et al., 1975, 1977; Weiss et al., 1989; Wilcox et al., 1989). Tissue factor apoprotein (M_r 46 000) is an integral membrane glycoprotein (Bach et al., 1981; Broze et al., 1985; Guha et al., 1986) that is tightly associated with phospholipid (Pitlick & Nemerson, 1970). It also has a high affinity for factor VII that circulates in blood (Broze, 1982; Bach et al., 1986; Fair & McDonald, 1987; Sakai et al., 1989). When vascular injury occurs, the two proteins form a one-to-one complex in the presence of calcium ions, and this facilitates the conversion of factor VII to a serine protease (factor VIIa) by minor proteolysis (Nemerson & Repke, 1985; Rao & Rapaport, 1988; Sakai et al., 1989). This is due to the cleavage of a single internal Arg_{152}–Ile peptide bond in the precursor protein (Hagen et al., 1986) (Figure 2). This calcium ion dependent reaction is catalyzed by a trace amount of a protease circulating in blood (such as factor Xa, thrombin, factor VIIa, or factor IXa) or by some as yet unidentified plasma or cellular enzyme (Radcliffe & Nemerson, 1976; Kisiel et al., 1977b; Masys et al., 1982; Wildgoose & Kisiel, 1989; Pedersen et al., 1989). The factor VIIa–tissue factor complex then converts factor X to a serine protease (factor Xa) by the cleavage of a single Arg_{52}–Ile peptide bond in the amino-terminal end of the heavy chain (DiScipio et al.,

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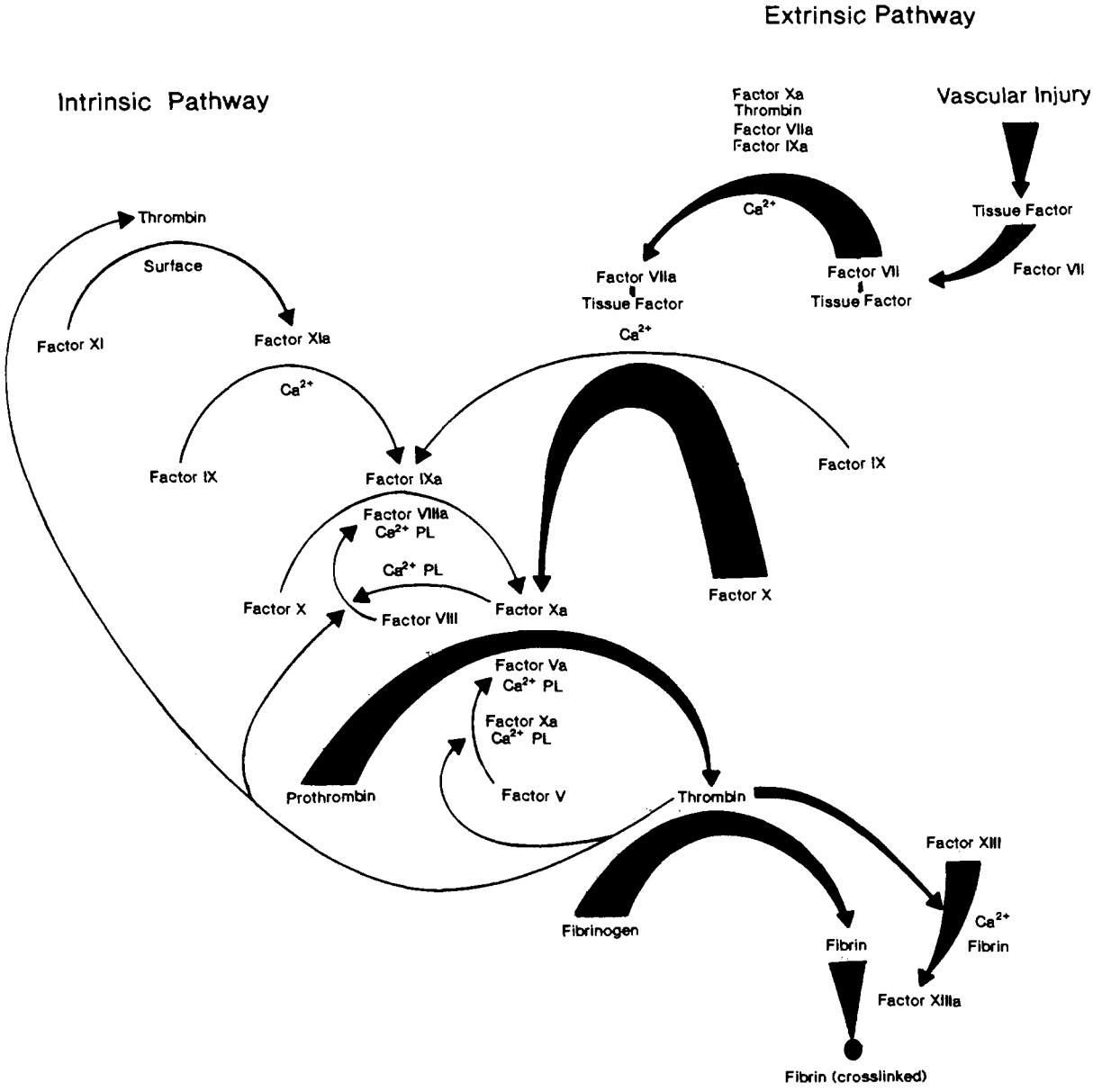

FIGURE 1: Coagulation cascade and fibrin formation by the intrinsic and extrinsic pathways. The initiation of the coagulation cascade occurs following vascular injury and the exposure of tissue factor to the blood. This triggers the extrinsic pathway (right side), shown in heavy arrows. The intrinsic pathway (left side) can be triggered when thrombin is generated, leading to the activation of factor XI. The two pathways converge by the formation of factor Xa. The activated clotting factors (except thrombin) are designated by lowercase a, i.e., IXa, Xa, XIa, etc. PL refers to phospholipid. The phospholipid bound to tissue factor apoprotein is not shown.

1977) (Figure 2). This results in the release of a small activation peptide (52 amino acids) from the precursor protein. Tissue factor functions as a cofactor in the activation of factor VII and factor X and greatly accelerates these two reactions, apparently by causing a conformational change in factor VII or factor VIIa (Nemerson & Gentry, 1986).

The newly generated factor Xa then forms a one-to-one complex with factor Va in the presence of calcium ions and phospholipid (Tracy et al., 1981) (Figure 1). This complex, sometimes referred to as prothrombinase, converts prothrombin to thrombin, a serine protease composed of two polypeptide chains (Figure 2). The activation of prothrombin is due to the hydrolysis of two internal peptide bonds (Arg_{271}–Thr and Arg_{320}–Ile) that reduces the molecular weight of the precursor from 71 600 to 39 000 (Mann et al., 1990). This results in the generation and release of thrombin, a serine protease, from the carboxyl-terminal portion of the precursor, while the Gla- and kringle-containing region from the amino-terminal end of prothrombin remains attached to the phospholipid. Factor Va participates as a cofactor in prothrombin activation by increasing the V_{max} of the reaction about 1000-fold, whereas the phospholipid provided by the activated platelets reduces the K_m for prothrombin about 1400-fold (Krishnaswamy et al., 1987). It is also essential that factor V be activated by partial proteolysis to participate in this reaction (Figure 1). Initially, this activation is probably catalyzed by the newly generated factor Xa in the presence of calcium ions and phospholipid (Monkovic & Tracy, 1990). After thrombin is generated, it will also activate factor V (M_r 330 000) (Nesheim & Mann, 1979; Esmon, 1979, Suzuki et al., 1982). In each case, the activation is due to the cleavage of two internal peptide bonds (Arg_{710}–Ser and Arg_{1545}–Ser) in the single-chain precursor protein (Kane & Davie, 1988) (Figure 2). The cleavage of two internal peptide bonds in the protein causes the release of a large internal connecting region (M_r 170 000) from the precursor and the generation of an amino-terminal heavy chain (M_r 94 000) and a carboxyl-terminal light chain (M_r 69 000). These latter two chains are held together by

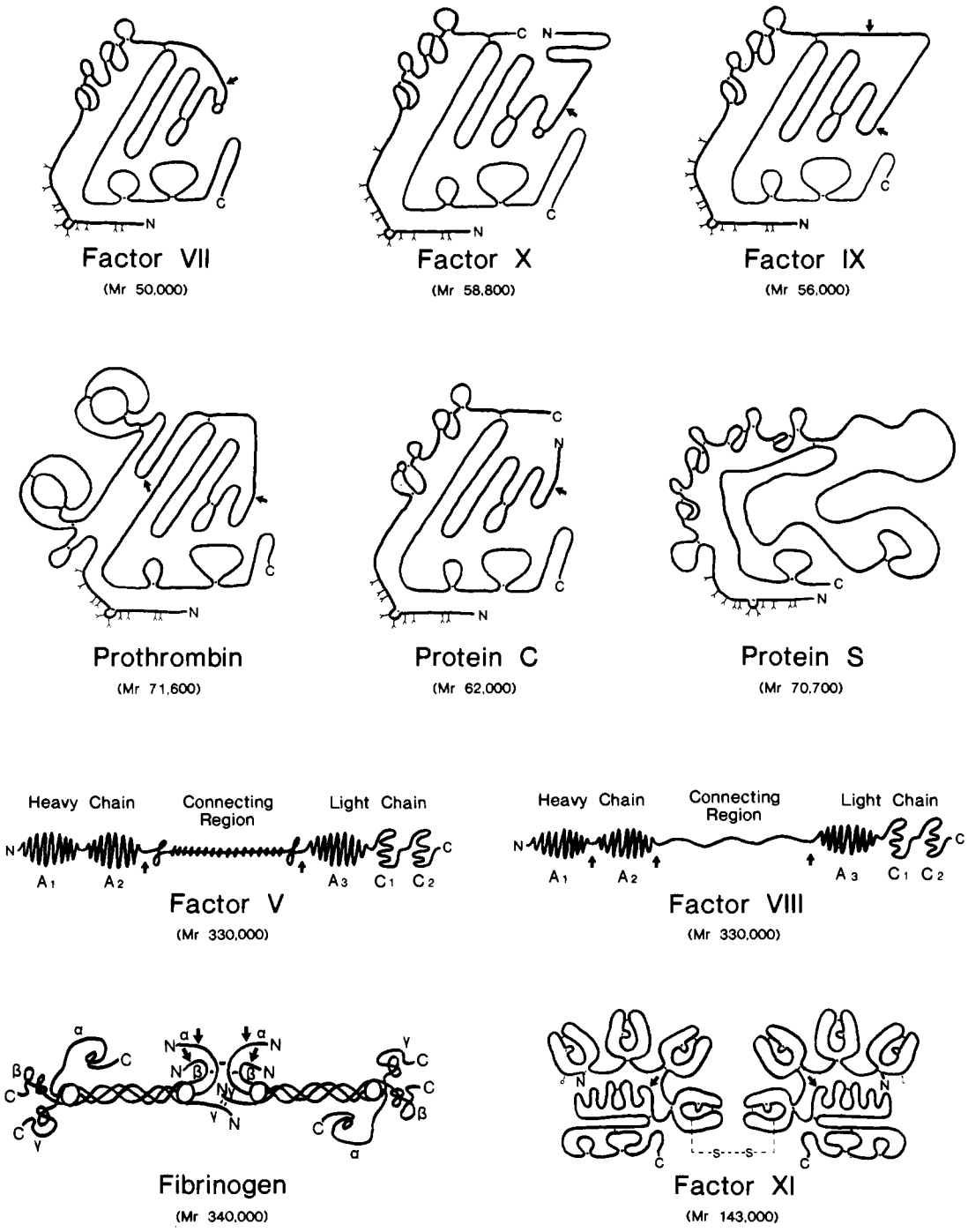

FIGURE 2: Tentative structures of the glycoproteins that participate in the coagulation cascade and its regulation. Each of the six vitamin K dependent proteins (top two rows) contains 9–12 γ-carboxyglutamic acid residues (Gla residues) in the amino terminal region of their molecule (shown by a Y shape). With factors VII, IX, and X and protein C, the Gla region (≈40 amino acids) is followed by two tandem repeats (≈50 amino acids) homologous to epidermal growth factor and a serine protease domain homologous to pancreatic trypsin. In prothrombin, the two growth factor domains are replaced by two kringle domains (≈85 amino acids). Protein S contains a Gla domain followed by four growth factor domains but lacks a serine protease domain. Factors VII and IX, prothrombin, and protein S are single-chain proteins, while factor X and protein C are two-chain proteins with a light chain linked to the heavy chain, or serine protease domain, by a single disulfide bond. Factor X, protein C, and protein S also contain hydroxyaspartic acid, or hydroxyasparagine, in their epidermal growth factor domains. Factor V and factor VIII are large glycoproteins that are present in blood as inactive cofactors. Factor VIII circulates in a complex with von Willebrand factor and is also very susceptible to proteolysis, resulting in fragmented forms of lower molecular weight (M_r 200 000 and 80 000). These fragments results from proteolysis at Arg_{1313}–Ala and Arg_{1648}–Glu during secretion or circulation in blood. Factor V and factor VIII share amino acid sequence identity in their three A domains (≈350 amino acids) and their two C domains (≈150 amino acids). The large connecting regions, between the amino-terminal heavy chains and the carboxyl-terminal light chains in each protein, are rich in carbohydrate and show no amino acid sequence homology. In factor V, the connecting region contains 31 homologous tandem repeats of nine amino acids. Fibrinogen is composed of three pairs of nonidentical polypeptide chains held together by 29 inter- and intrachain disulfide bonds. The α (M_r 66 000), β (M_r 52 000), and γ (M_r 46 500) polypeptide chains form a trinodular structure of approximately 450 × 90 Å. Factor XI contains four apple domains (≈90 amino acids) followed by a serine protease domain in each of two identical subunits. A single disulfide bond in the fourth apple domain holds the two subunits together to form the homodimer. The first apple domain in each subunit of factor XI contains a Cys linked to another single Cys residue by a disulfide bond. The small arrows shown for each protein identify the site of cleavage in the polypeptide chain when the protein precursors are activated during the coagulation cascade. Protein S, however, does not require minor proteolysis prior to its participation as a cofactor with activated protein C.

calcium ions and constitute factor Va (Guinto & Esmon, 1982; Laue et al., 1989).

When thrombin is formed (Figure 1), it converts fibrinogen to fibrin by limited proteolysis (Figure 2). The formation of fibrin is due to the cleavage of a peptide bond in each of the two α chains (Arg_{18}–Gly) and in each of the two β chains (Arg_{16}–Gly) (Blomback & Blomback, 1972). This cleavage results in the release of four fibrinopeptides and gives rise to fibrin monomers with new amino-terminal sequences of Gly-Pro-Arg in the α chains and Gly-His-Arg in the β chains. The newly generated Gly-Pro-Arg residues in the α chain of one monomer then bind to the D domain in the adjacent fibrin monomer, leading to the linear polymerization of fibrin. In a similar manner, the new amino-terminal sequence of Gly-His-Arg from the β chain binds to the D domain of an adjacent fibrin monomer, resulting in the side-by-side polymerization of fibrin (Laudano & Doolittle, 1980). These polymerization reactions generate the insoluble fibrin clot.

When fibrin is formed, it also accelerates the conversion of factor XIII to factor XIIIa by thrombin in the presence of calcium ions (Lorand & Konishi, 1964; Naski et al., 1991) (Figure 1). Factor XIIIa is an enzyme that cross-links fibrin monomers by forming ϵ-(γ-glutamyl)lysine bonds between two adjacent molecules (Folk & Finlayson, 1977). These cross-linking reactions initially involve the γ chains of fibrin followed by cross-linking of the α chains. These covalent cross-links lead to the formation of a very strong fibrin clot. Factor XIIIa will also cross-link other plasma proteins such as fibronectin and α_2-antiplasmin to the α chains of fibrin, resulting in their incorporation into the fibrin clot. Factor XIII (M_r 320 000) is a tetramer composed of two a subunits (M_r 83 000) and two b subunits (M_r 80 000) (Schwartz et al., 1973). The a subunits contain the catalytic domain, while the b subunits stabilize and regulate activation of the a subunits (Lorand, 1986). The activation of factor XIII by thrombin is due to the cleavage of a peptide bond (Arg_{37}–Gly) in each of the two a chains in the tetramer (a_2b_2) (Takagi & Doolittle, 1974). This leads to the formation of a'_2b_2 and the release of two small activation peptides (37 amino acids) from the amino-terminal ends of the two a polypeptide chains (Ichinose et al., 1986). In the presence of calcium ions, the two a′ subunits dissociate from the two b subunits, generating the active enzymes (a'_2) (Curtis et al., 1974; Hornyak & Shafer, 1991).

When the amount of tissue factor is limiting following vascular injury, the activation of factor IX rather than factor X by the factor VIIa–tissue factor complex may play a significant role in the initiation of the extrinsic pathway of coagulation (Osterud & Rapaport, 1977; Bauer et al., 1990) (Figure 1). This is due in part to the fact that the K_m and V_{max} for factor IX and factor X vary with the tissue factor concentration, and at low tissue factor concentrations, factor IX appears to be a better substrate than factor X (Zur & Nemerson, 1980; Komiyama et al., 1990). The activation of factor IX by the factor VIIa–tissue factor complex also involves limited proteolysis during which two internal peptide bonds (Arg_{145}–Ala and Arg_{180}–Val) are cleaved and a small activation peptide (35 amino acids) is released (DiScipio et al., 1978; Yoshitake et al., 1985) (Figure 2). The newly generated factor IXa, composed of a heavy and a light chain, then forms a complex with factor VIIIa in the presence of calcium ions and phospholipid. This complex activates factor X by minor proteolysis by cleaving the same bonds that are hydrolyzed by the complex of factor VIIa and tissue factor. Factor VIIIa, like factor Va, participates as a cofactor that increases the V_{max} of the reaction about 200 000-fold (van Dieijen et al., 1981). A deficiency of factor VIII results in classic hemophilia or hemophilia A (Sadler & Davie, 1987), while a deficiency of factor IX causes hemophilia B (Hedner & Davie, 1987). Factor VIII is activated by minor proteolysis (Arg_{372}–Ser, Arg_{740}–Ser, and Arg_{1689}–Ser) during which a large connecting region (M_r 170 000) is released from the precursor (Toole et al., 1984; Vehar et al., 1984; Eaton et al., 1986) (Figure 2). Factor VIIIa is composed of two amino-terminal fragments (M_r 50 000 and 43 000) and a carboxy-terminal fragment (M_r 69 000); these three fragments are held together by calcium ions. These initial cleavages in factor VIII occur in the presence of factor Xa, calcium ions, and phospholipid (Figure 1) or by thrombin alone once it is generated from prothrombin (Eaton et al., 1986).

One of the unresolved questions concerning the importance of the extrinsic pathway in the initiation and maintenance of blood coagulation is why hemophiliacs bleed in spite of normal plasma levels of factors VII and X and abundant tissue factor at the site of injury. In this regard, the extrinsic pathway is probably short-lived due to the presence in blood of a lipoprotein-associated coagulation inhibitor (LACI) (Broze et al., 1990) or extrinsic pathway inhibitor (EPI) (Rapaport, 1989). This protein inactivates the factor VIIa–tissue factor complex in the activation of factor X by forming a complex with the ternary factor VIIa–tissue factor–factor Xa complex. LACI (M_r 50 000) contains three Kunitz-type inhibitor domains. The first binds to factor VIIa, while the second binds to factor Xa (Girard et al., 1989). Surprisingly, LACI does not block the activation of factor IX by the factor VIIa–tissue factor complex. Thus, the activation of factor X may continue in part via the intrinsic pathway in the presence of LACI by the formation of factor IXa. In addition, recent in vitro studies have shown that the effectiveness of LACI in inhibiting the activation of factor X by factor VIIa–tissue factor is greatly diminished in the presence of plasma levels of factors IX and VIII (Pedersen et al., 1990; Repke et al., 1990). Accordingly, the inhibitory effects of LACI would seemingly be magnified in patients deficient in factor IX or factor VIII.

Growth and Maintenance of Fibrin Formation in the Blood Coagulation Cascade

As the extrinsic pathway for fibrin formation is inhibited by LACI, it is very likely that the intrinsic pathway becomes the primary route for the continued growth of the fibrin clot (Figure 1, left side). Fibrinolysis, however, is initiated when fibrin is formed and the clot begins to lyse within a few hours. The lytic reaction is catalyzed by plasmin, which is generated from plasminogen. The activation of plasminogen is catalyzed by tissue plasminogen activator in the presence of the newly generated fibrin. The formation and slow turnover of fibrin is important as tissue regeneration and remodling occurs. During this very slow turnover of fibrin, it is also likely that the intrinsic pathway maintains clot formation.

The intrinsic pathway of the blood coagulation cascade requires a plasma glycoprotein called factor XI. This coagulation factor also circulates in blood as a precursor of a serine protease (Figure 2). Recently, the activation of factor XI by thrombin in the presence of a negatively charged surface, such as sulfatide, heparin, or dextran sulfate, has been demonstrated (Naito & Fujikawa, 1991). This activation reaction is due to the cleavage of an Arg_{369}–Ile peptide bond in each of the two subunits of factor XI by the newly generated thrombin. This leads to the formation of factor XIa, a serine protease composed of two heavy chains and two light chains; these four chains are held together by three disulfide bonds. Factor XIa is an unusual serine protease in that it contains two active

catalytic sites (Kurachi & Davie, 1977; Bouma & Griffin, 1977). Furthermore, factor XIa will activate factor XI in an autocatalytic manner after trace amounts of factor XIa are generated in the presence of dextran sulfate (Naito & Fujikawa, 1991). Factor XIa also converts factor IX to factor IXa in the presence of calcium ions (DiScipio et al., 1978) (Figure 1). This occurs by the hydrolysis of the same peptide bonds in factor IX as those cleaved by the factor VIIa–tissue factor complex. The newly generated thrombin also stimulates the intrinsic pathway further by activating factor V and factor VIII by minor proteolysis. These two reactions that generate additional factors Va and VIIIa occur in the absence of phospholipid and calcium ions.

The discovery of a mechanism for factor XI activation that is independent of factor XII, plasma prekallikrein, and high molecular weight kininogen helps to clarify the role of factor XI in the intrinsic pathway of blood coagulation. This is important since patients deficient in factor XI often experience bleeding episodes, in contrast to individuals lacking factor XII, plasma prekallikrein, or high molecular weight kininogen. These latter three plasma proteins, however, are involved in the activation of factor XI when plasma comes in contact with a surface such as glass or kaolin in vitro. These reactions, referred to as the contact phase of the intrinsic pathway of blood coagulation, could be of physiological importance, however, when circulating blood comes in contact with an artificial surface, such as a heart valve, or during blood dialysis.

Whether there are other mechanisms that will also activate factor XI under physiological conditions remains to be determined. For instance, blood cells or endothelial cells may also contain a protease that activates factor XI and triggers the intrinsic pathway of blood coagulation.

Regulation of the Coagulation Cascade

As previously stated, LACI, a multivalent Kunitz-type plasma serine protease inhibitor, is capable of shifting much of the coagulation cascade from the extrinsic pathway to the intrinsic pathway. Plasma also contains other protease inhibitors that will slow the generation of fibrin. Antithrombin III is of particular importance in this regard since it is capable of blocking the activity of thrombin (Rosenberg & Damus, 1973), factor IXa (Kurachi et al., 1976), factor Xa (Kurachi et al., 1976), and factor XIa (Kurachi & Davie, 1977). This inhibition is not rapid, however, and consequently the serine proteases in the coagulation cascade have ample opportunity to generate fibrin before they are inactivated. The inhibition by antithrombin III is due to the formation of inactive one-to-one complexes of inhibitor and enzyme. In the presence of heparin or similar sulfated glycosaminoglycans, the reaction of antithrombin III and a serine protease is virtually instantaneous and under these conditions fibrin formation is completely blocked.

Other plasma serine protease inhibitors that may play a significant role in regulating blood coagulation are heparin cofactor II (Tollefsen et al., 1982), α_2-macroglobulin (Sottrup-Jensen, 1987), activated protein C inhibitor (Suzuki et al., 1983), Cl-esterase inhibitor, and α_1-antitrypsin (Heeb & Griffin, 1988). The coagulation cascade is also retarded by the trapping of thrombin in the fibrin matrix as well as by removal and dilution of activated clotting factors by the circulating blood.

Another plasma glycoprotein that is extremely important in the regulation of blood coagulation is activated protein C. This vitamin K dependent protein inactivates both factor Va (Kisiel et al., 1977a; Marlar et al., 1982) and factor VIIIa (Vehar & Davie, 1980) by minor proteolysis in the presence of phospholipid and calcium ions, a reaction stimulated by protein S (Walker, 1980). Protein C (Figure 2) also circulates in blood in a precursor or zymogen form and is converted to a serine protease by thrombin in the presence of a membrane-bound cofactor called thrombomodulin (Esmon & Owen, 1981; Owen & Esmon, 1981; Esmon et al., 1982a).

The activation of protein C is due to the cleavage of a single peptide bond (Arg_{12}–Leu) in the amino-terminal end of the heavy chain of the vitamin K dependent protein (Kisiel, 1979). This results in the release of a small activation peptide (12 amino acids) and the generation of a serine protease (Figure 2). Thrombomodulin (M_r 75000) is a single-chain glycoprotein located on the surface of endothelial cells (Esmon & Owen, 1981; Salem et al., 1984). The amino-terminal or extracellular portion of the molecule contains six growth factor domains (as tandem repeats) followed by a carbohydrate-rich region, a transmembrane domain, and a small cytoplasmic region (Wen et al., 1987; Suzuki et al., 1987; Jackman et al., 1987; Shirai et al., 1988). Thrombomodulin forms a one-to-one complex with thrombin and changes the substrate specificity of the enzyme (Esmon, 1987). Surprisingly, the thrombin which has formed a complex with thrombomodulin now readily activates protein C while losing its platelet-activating activity (Esmon et al., 1983) as well as its protease activity toward other substrates such as fibrinogen or factor V (Esmon et al., 1982b). Thus, thrombin has been changed from a procoagulant to an anticoagulant by its interaction with thrombomodulin. The importance of this pathway is emphasized by the fact that individuals lacking protein C or protein S often have thrombotic complications (Griffin et al., 1981; Comp & Esmon, 1984).

Many of the blood coagulation reactions described above occur on a phospholipid surface. It is also likely that these reactions, e.g., the activation of factor X and prothrombin, often may occur on the surface of the same phospholipid vesicle (Figure 1). In this case, the efficiency of these stepwise reactions is greatly increased since the reactants do not need to dissociate and reassociate from different phospholipid vesicles in order to participate in subsequent reactions. The phospholipid provided by the activated platelets or damaged cellular membranes also restricts the coagulation process to the site of vascular injury and greatly reduces the coagulation process elsewhere in the blood. With the exception of factor VII and protein C (Nelsestuen et al., 1978; Bach et al., 1986; Bom & Bertina, 1990), the Gla region present in each of the vitamin K dependent proteins is particularly important in this regard in that this domain binds to the platelet phospholipid in the presence of calcium ions under physiological conditions. Vitamin K dependent proteins synthesized in the absence of vitamin K or in the presence of antagonists, such as dicumarol, contain few or no Gla residues; consequently, they bind poorly to phospholipid and have little, if any, biological activity (Stenflo & Suttie, 1977).

Future Research in the Field of Blood Coagulation

The coagulation cascade described above has been studied primarily in the test tube. In vivo, however, the endothelium and subendothelium, as well as many other blood cells and proteins, are present and may influence the coagulation process. Accordingly, it seems likely that additional modifications to the scheme shown in Figure 1 will be necessary as our understanding of the role of these cells as well as other proteins is established. Furthermore, little is known about how the coagulation process may differ in arteries and veins, in large vessels and small vessels, or in vessels with rapid or limited

blood flow. Thus, there is still a great deal to be learned about the coagulation of blood under conditions that more closely mimic the physiological state.

The important discovery of γ-carboxyglutamic acid and the presence of 10 residues of this unusual amino acid in prothrombin and 9–12 in the other vitamin K dependent proteins has stimulated considerable research into the role of the vitamin K in the carboxylation of glutamic acid (Stenflo et al., 1974; Magnusson et al., 1974; Nelsestuen et al., 1974). Also, the role of the preproleader sequence, which is typical for the vitamin K dependent proteins present in plasma (Ichinose et al., 1990), has become more evident in that it is important as a secretion signal as well as a recognition signal for the carboxylase (Jorgensen et al., 1987; Foster et al., 1987). Thus, it seems likely that the detailed mechanisms by which vitamin K participates in these reactions will be clarified in the near future.

In recent years, new techniques in molecular biology have made it possible to isolate, characterize, and identify the chromosomal location of the genes for all of the proteins known to participate in the coagulation process (Davie, 1987). Most of these genes are on separate chromosomes, although factors VII and X are adjacent to each other on chromosome 13 while the genes for factors VIII and IX are located on the tip of the long arm of the X chromosome. This research has also opened up many other new and exciting avenues of investigation. For instance, abnormalities in nearly every coagulation protein have been identified by DNA sequence analysis of the genes from patients with various coagulation disorders. This, in turn, has made prenatal diagnoses of many such abnormalities possible, as well as genetic counseling for families known to carry mutant genes. Thus far, nearly every type of genetic abnormality in the coagulation proteins has been identified, including deletion, insertion, or substitution of single nucleotides as well as large segments of DNA. These changes result in various abnormalities, such as replacement, insertion, or deletion of amino acids in the protein, the generation of stop codons, reading frame shifts, errors in mRNA splicing, and abnormal synthesis and secretion of mutant proteins. Occasionally, mutations have also been observed in the regulatory segment of the gene. An especially interesting mutation identified in the promoter region of the gene for factor IX is an A to G conversion in the CCAAT/enhancer protein (C/EBP) binding site (Reitsma et al., 1989; Crossley & Brownlee, 1990). This mutation prevents a nuclear protein from binding to the gene, resulting in hemophilia B in these patients.

Thus far, most of the mutations in the coagulation proteins have been found as expected in all regions of the genes studied. In the case of factor XI deficiency in the Ashkenazi Jewish population, however, more than 95% of the abnormalities have been shown to occur with nearly equal frequency in two places in the gene (Asakai et al., 1989, 1991). These mutations, which result in a stop codon or an amino acid replacement, represent an excellent example of a founder effect in this gene.

The characterization of the genes and cDNAs for the coagulation proteins has also led to the expression of each of the coagulation proteins by means of recombinant DNA techniques. Thus, the availability of plasma proteins, such as factor VIIa (Thim et al., 1988) or factor VIII (Eaton et al., 1987), for replacement therapy in patients is now possible. Such preparations avoid the serious problem of viral contamination that can occur when the coagulation proteins are prepared from pooled human plasma. Expression of these proteins will also make it possible to study and, in many cases, to identify critical or important regions in the coagulation proteins by site-specific mutagenesis. Additionally, it is now possible to study the assembly and processing of these plasma proteins during their biosynthesis.

Characterization of the genes coding for the coagulation proteins also made it possible to examine their regulation at the level of transcription. Acute-phase proteins, such as fibrinogen, are carefully regulated and DNA sequences in the 5′ end or nearby regions of these genes are involved in this regulation. Also, proteins such as tissue factor are induced in monocytes/macrophages or endothelial cells by molecules such as endotoxin (Lerner et al., 1971). Accordingly, a number of potential regions have been identified in the 5′ region of the gene for tissue factor that play an important role in its regulation (Mackman et al., 1989, 1990). An understanding of the regulation of these coagulation proteins is essential in the development of procedures for gene transfer into patients with a deficiency in a specific coagulation protein.

Characterization of the genes for the coagulation proteins has also provided insight into the evolution of these proteins. Clearly, many, such as the vitamin K dependent family of plasma proteins, have evolved from a common ancestor, which is evident from their amino acid sequences as well as from an examination of their gene structure and organization (Patthy, 1985; Davie et al., 1986). Other proteins, such as factor V and factor VIII, share considerable amino acid sequence homology with each other (Kane & Davie, 1988), as well as with ceruloplasmin and mammary epithelial cell surface protein (Stubbs et al., 1990). Accordingly, the suggestion that exon shuffling has played a significant role in the evolution of the genes for these plasma proteins is strengthened. It is also evident that this exon shuffling during evolution has occurred primarily at type I splice junctions in the various genes (Petersen et al., 1990).

These are just a few examples of where basic and clinical research in the future will add to our understanding of the physiology of the blood coagulation cascade and the proteins that participate in the reactions associated with it.

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