1 Overview of hemostasis

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Laboratory data combined with clinical pathology lead to the conclusion that the physiologically relevant hemostatic mechanism is primarily composed of three procoagulant vitamin K-dependent enzyme complexes [which utilize the proteases factor IXa (FIXa), FXa, and FVIIa] and one anticoagulant vitamin K-dependent complex [1,2] (Figure 1.1). These complexes, which include elements of the "extrinsic" pathway, the FIXa–FVIIIa complex [3], and thrombin–thrombomodulin [4], are each composed of a vitamin K-dependent serine protease, a cofactor protein, and a phospholipid membrane, this last provided by an activated or damaged cell. The membrane-binding properties of the vitamin K-dependent proteins are consequence of the post-translational γ-carboxylation of these macromolecules [5]. The cofactor proteins are either membrane binding (FVa, FVIIIa), recruited from plasma, or intrinsic membrane proteins (tissue factor, thrombomodulin). Each catalyst is 10^3- to 10^6-fold more efficient than the individual serine protease acting on its substrate in solution. Membrane binding, intrinsic to complex assembly, also locates catalysis to the region of vascular damage. Thus, a system selective for regulated, efficient activity presentation provides for a regionally limited, vigorous arrest of hemorrhage.

Additional complexes associated with the "intrinsic" pathway are involved in the surface contact activation of blood [3]. However, the association of the contact-initiating proteins [factor XII (FXXa), prekallikrein, high-molecular weight kininogen] with hemorrhagic disease is uncertain [6].

Of equal importance to the procoagulant processes is regulation of anticoagulation by the stoichiometric and dynamic inhibitory systems. The effectiveness of inhibitory functions is far in excess of the potential procoagulant responses. These inhibitory processes provide activation thresholds, which require presentation of a limiting concentration of tissue factor prior to significant thrombin generation [7]. Antithrombin III (AT-III) [8] and tissue factor pathway inhibitor (TFPI) are the primary stoichiometric inhibitors while the thrombin–thrombomodulin–protein C system [4] is dynamic in its function.

The initiating event in the generation of thrombin involves the binding of membrane-bound tissue factor with plasma FVIIa [9]. The latter is present in blood at ~0.1 nmol/L (~1–2% of the FVII concentration of 10 nmol/L) [10]. Plasma FVIIa does not express proteolytic activity unless it is bound to tissue factor; thus, FVIIa at normal blood level has no significant activity toward either FIX or FX prior to its binding to tissue factor. The inefficient active site of FVIIa permits its escape from inhibition by the AT-III present in blood. Vascular damage or cytokine-related presentation of the active tissue factor triggers the process by interaction with activated FVIIa, which increases the catalytic efficiency (k_{cat}) of the enzyme and increases the rate of FX activation by four orders of magnitude [11]. This increase is the result of the improvement in catalytic efficiency and the membrane binding of FIX and FX.

The FVIIa–tissue factor complex (extrinsic factor Xase) (Figure 1.2) catalyzes the activation of both FIX and FX, the latter being the more efficient substrate [12]. Thus, the initial product formed is FXa. Feedback cleavage of FIX by membrane-bound FXa enhances the rate of generation of FIXa in a cooperative process with the FVII–tissue factor complex [13].

The initially formed, membrane-bound FXa activates small amounts of prothrombin to thrombin [14]. This initial prothrombin activation provides the thrombin essential to the acceleration of the hemostatic process by serving as the activator for platelets [15], FV [16], and FVIII [17] (Figure 1.1). Once FVIIIa is formed, the FIXa generated by FVIIa–tissue factor complex combines with FVIIIa on the activated platelet membrane to form the "intrinsic factor Xase" (Figure 1.2), which becomes the major activator of FX. The FIXa–FVIIIa complex is 10^6-fold more active as a FX activator and 50 times more efficient than FVIIa–tissue factor in catalyzing FX activation [18,19] (thus, the bulk of FXa is ultimately produced by FIXa–FVIIIa). As the reaction progresses, FXa generation by the more active "intrinsic factor Xase" complex exceeds that of the extrinsic factor Xase [20]. In addition, the "extrinsic factor Xase" is subject to inhibition by the TFPI [21]. As a consequence, most (>90%) FXa is ultimately produced by the FVIIIa–FIXa complex in tissue factor-initiated hemostatic processes. In hemophilia A and hemophilia B, the "intrinsic factor Xase" cannot be assembled, and amplification of FXa generation does not occur [22]. FXa combines with FVa on the activated platelet membrane receptors, and this FVa–FXa "prothrombinase" catalyst (Figure 1.2a) converts prothrombin to thrombin. Prothrombinase is 300 000-fold more active than FXa alone in catalyzing prothrombin activation [23].

The coagulation system is tightly regulated by the inhibition systems. The tissue factor (TF) concentration threshold for reaction initiation is steep and the ultimate amount of thrombin produced is largely regulated by the concentrations of plasma procoagulants and the stoichiometric inhibitors and the constituents of the dynamic inhibition processes [20]. TFPI blocks

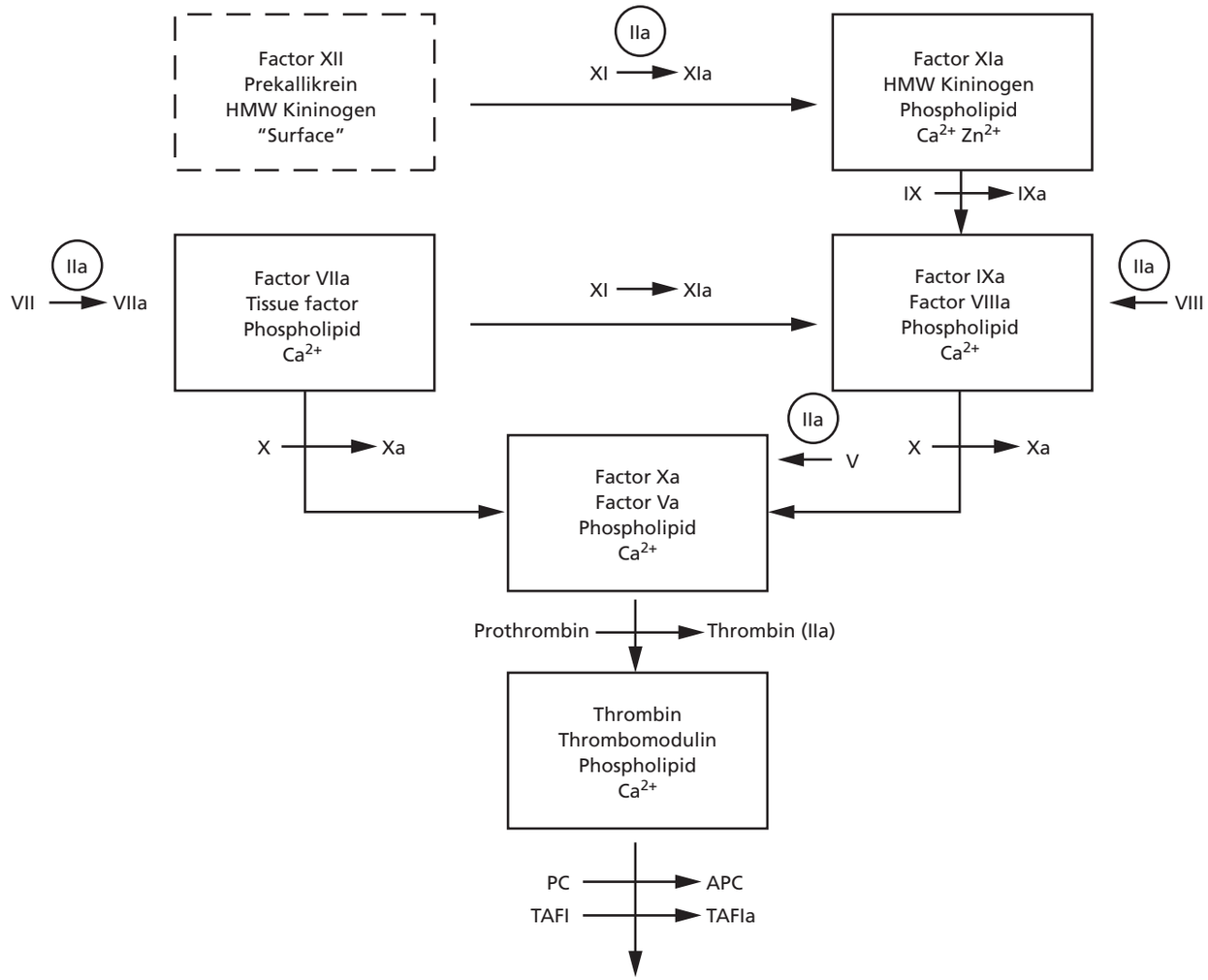

Figure 1.1 A representative map of the various catalysts required to generate the enzymes of the hemostatic system. The outline of the "contact catalyst" of the intrinsic pathway is dashed because of its uncertain contributions to the hemostatic process. The contribution of the contact catalyst to thrombosis is unresolved. The various points at which thrombin catalyzes its own generation by conversion of zymogens and procofactors to the active species required for catalyst formation are illustrated (from Mann KG. *Chest* 2003; **124**: 4–9S, with permission). APC, activated protein C; PC, protein C; TAFI, thrombin activable fibrinolysis inhibitor.

the FVIIa–tissue factor–FXa product complex, thus effectively neutralizing the "extrinsic factor Xase" (Figure 1.2b) [24]. However, TFPI is present at low abundance (~2.5 nmol/L) in blood and can only delay the hemostatic reaction [25]. AT-III, normally present in plasma at twice the concentration (3.2 μmoL) of any potential coagulation enzyme, neutralizes all the procoagulant serine proteases primarily in the uncomplexed state [8].

The dynamic protein C system is activated by thrombin binding to constitutive vascular thrombomodulin (Tm); this complex activates protein C (PC) to its activated species APC (Figure 1.1). APC competes in binding with FXa and FIXa and cleaves FVa and FVIIIa eliminating their respective complexes [16]. The PC system, TFPI, and AT-III cooperate to produce steep tissue factor concentration thresholds, acting like a digital "switch," allowing or blocking thrombin formation [7].

In humans, the zymogen FXI, which is present in plasma and platelets, has been variably associated with hemorrhagic pathology [26]. FXI is a substrate for thrombin (Figure 1.1) and has been invoked in a "revised pathway of coagulation" contributing to FIX activation [27]. The importance of the thrombin activation of FXI is evident only at low tissue factor concentrations [22].

FXII, prekallikrein, and high-molecular-weight kininogen (Figure 1.1) do not appear to be fundamental to the process of hemostasis [28]; the contribution of these contact pathway elements to thrombosis remains an open question and requires further experimentation to resolve this issue.

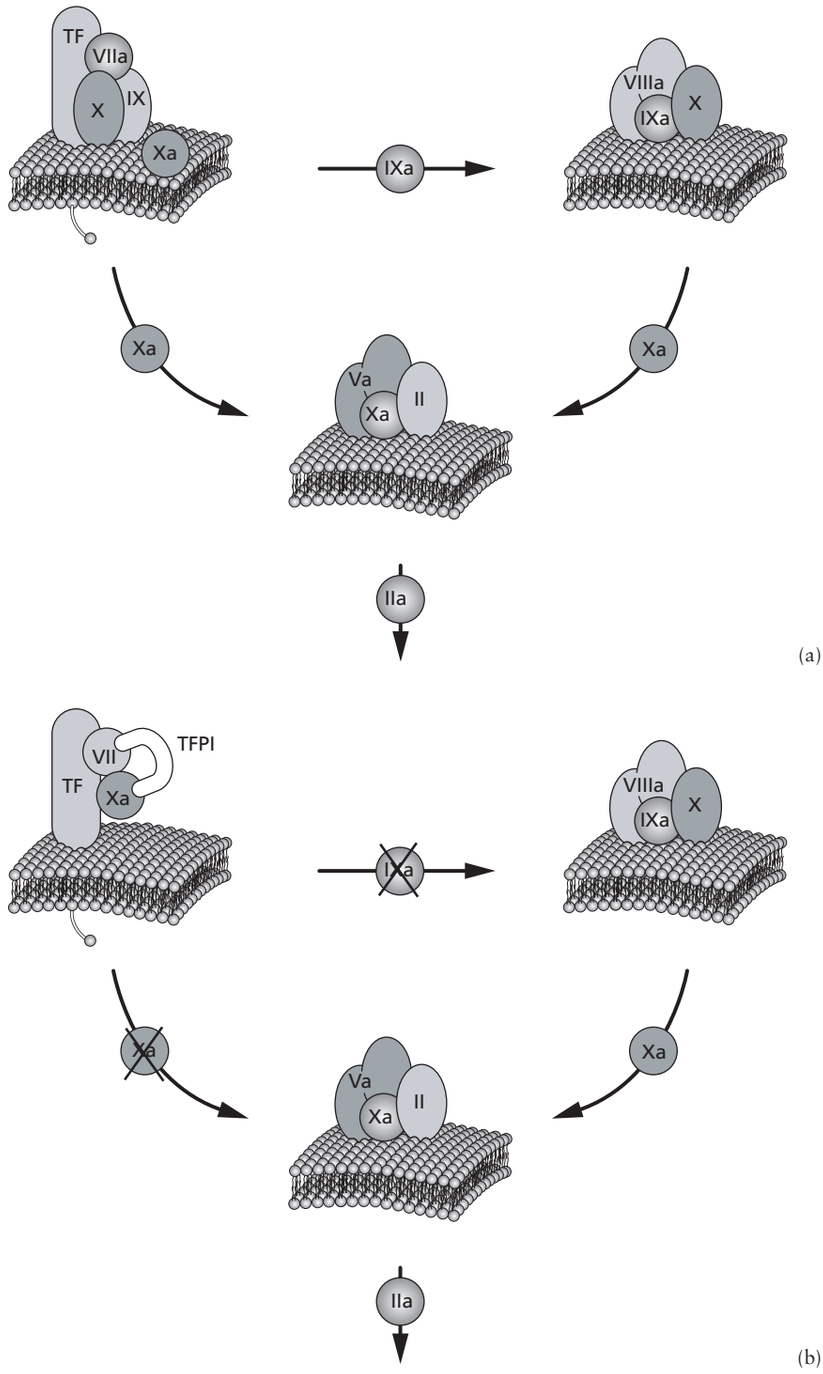

Figure 1.2 (a) The factor Xa (FXa) generated by the factor VIIa (FVIIa)–tissue factor complex activates a small amount of thrombin, which activates factor V (FV) and factor VIII (FVIII), leading to the presentation of the intrinsic factor Xase (FIXa–FVIIIa) and prothrombinase (FXa–FVa). At this point in the reaction, FIXa generation is cooperatively catalyzed by membrane-bound FXa and by FVIIa–tissue factor. The thick arrow representing FXa generation by the intrinsic factor Xase illustrates the more efficient Xa generation by this catalyst. (b) The tissue factor pathway inhibitor, (TFPI) interacts with the FVIIa–tissue factor–FXa product complex to block the tissue factor-initiated activation of both FIX and FX, leaving the FIXaβ–FVIIIa complex as the only viable catalyst for FX activation (from Mann KG. *The Dynamics of Hemostasis*. Burlington, VT: Haematologic Technologies, 2002, with permission).

Summary

Advances in genetics, protein chemistry, bioinformatics, physical biochemistry, and cell biology provide arrays of information with respect to normal and pathologic processes leading to hemorrhagic or thrombotic disease. The challenge for the twenty-first century will be to merge mechanism-based, quantitative data with epidemiologic studies and subjective clinical experience associated with the tendency to bleed or thrombose along with the therapeutic management of individuals with thrombotic or hemorrhagic disease. *In vitro* data and clinical experience with individuals with thrombotic and hemorrhagic disease will ultimately provide algorithms which can combine the art of clinical management with the quantitative

science available to define the phenotype *vis-à-vis* the outcome of a challenge or the efficacy of an intervention.

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