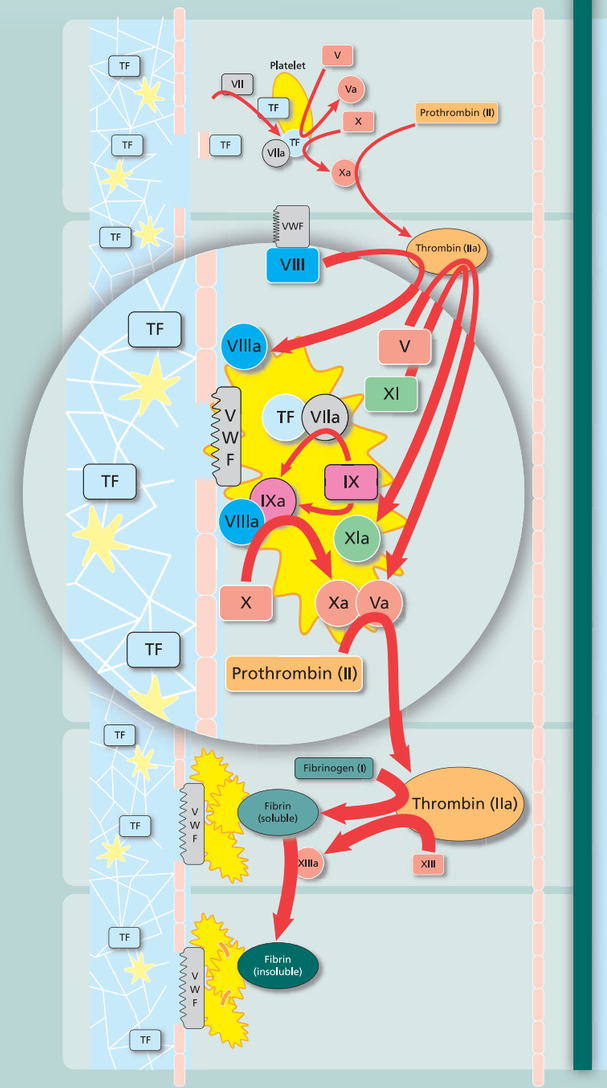
Coagulation

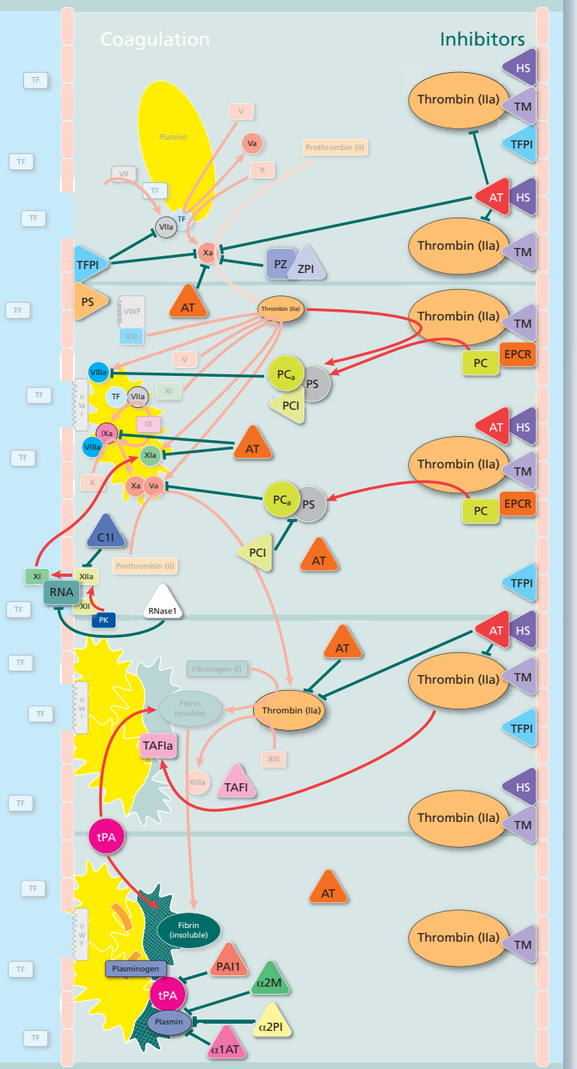
Coagulation Cascade

Summary of Stages



Inhibitor pathways

– including intrinsic anticoagulants and fibrinolytic reactions of coagulation



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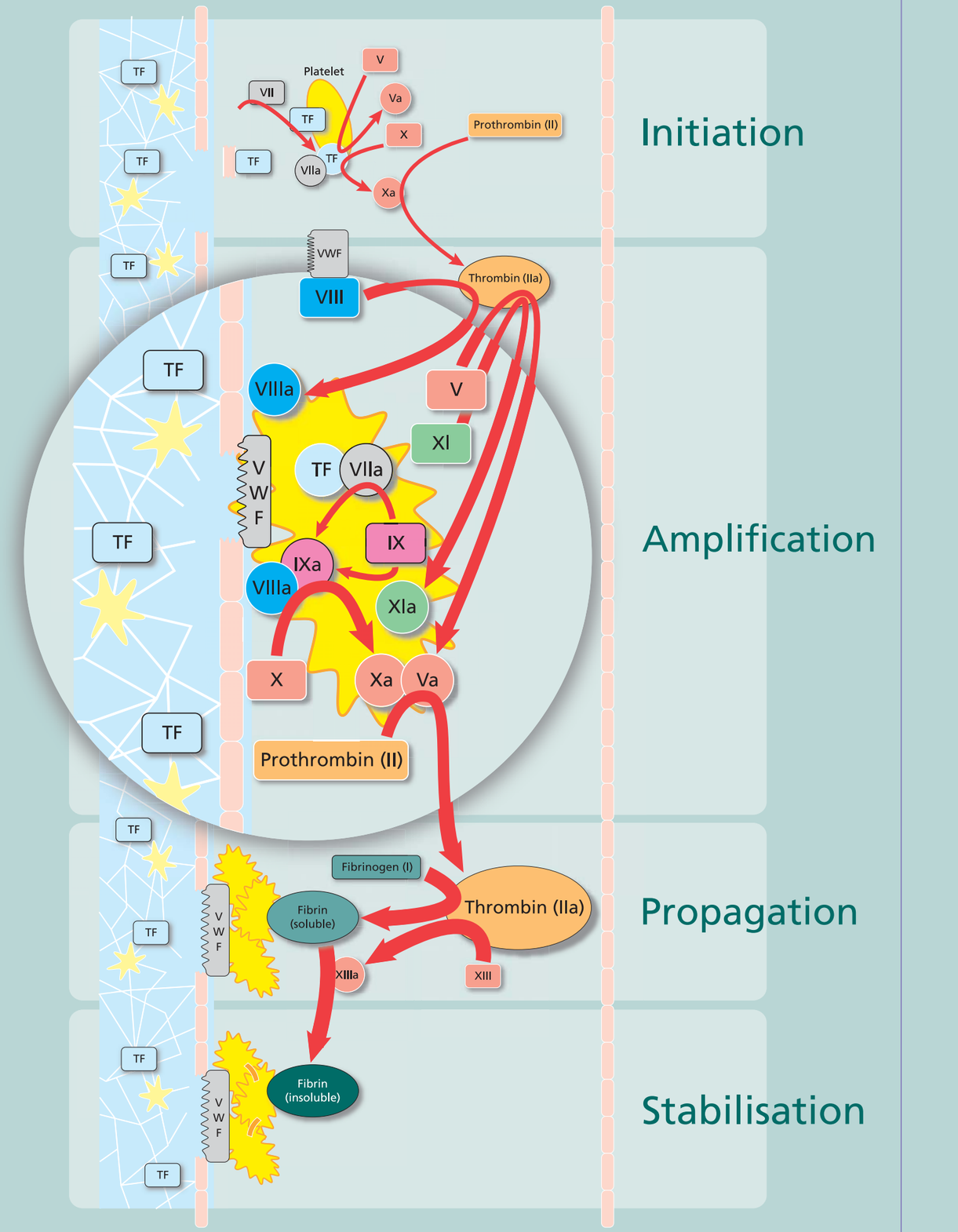
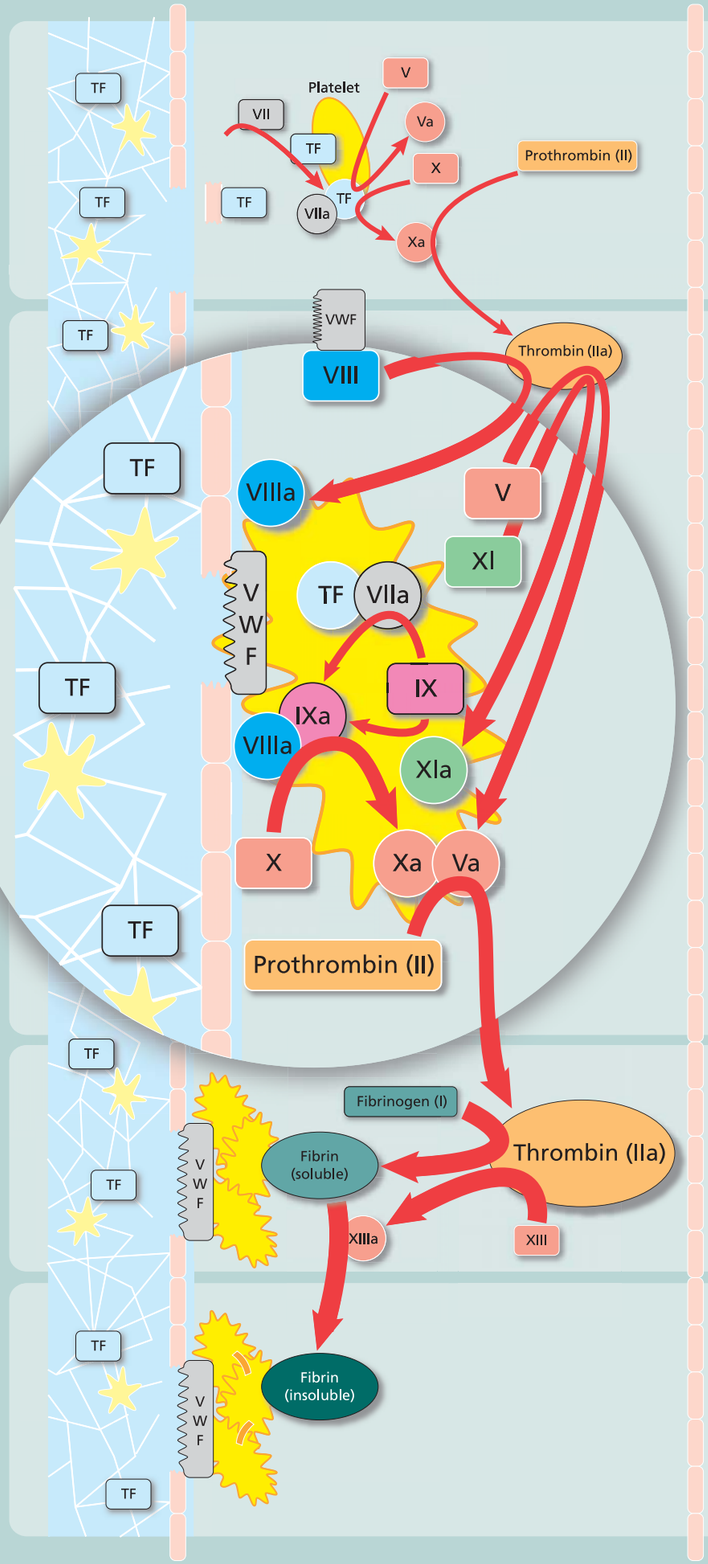




Dear Reader,
This brochure may serve physicians, pharmacists, medical students, nurses and other health care professionals as a short introduction to the complex mechanism of coagulation and its regulation.
We thank Professor Klaus T. Preissner (Giessen, Germany) for his expertise and creativity in developing this innovative educational guide.

Yours sincerely,
CSL Behring Team

Marburg, Germany

Legend to figures

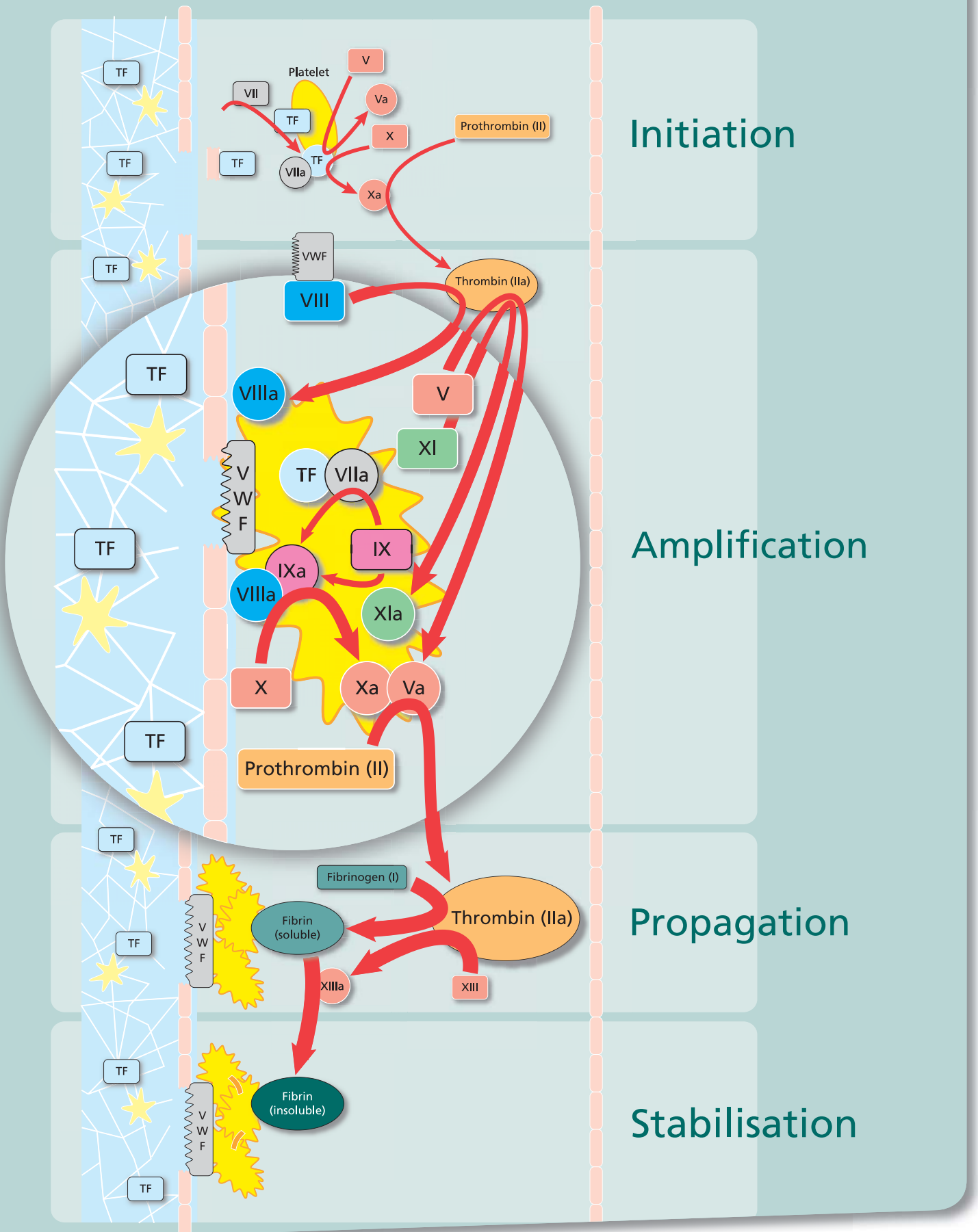
- Quiescent platelet
- Activated platelet
- Vessel wall
- TF — Tissue factor
- Inactive coagulation factor (various colours)
- Activated coagulation factor (various colours)
- VWF — Von Willebrand factor (coiled conformation)
- V W F — Von Willebrand factor (stretched conformation)

General introduction

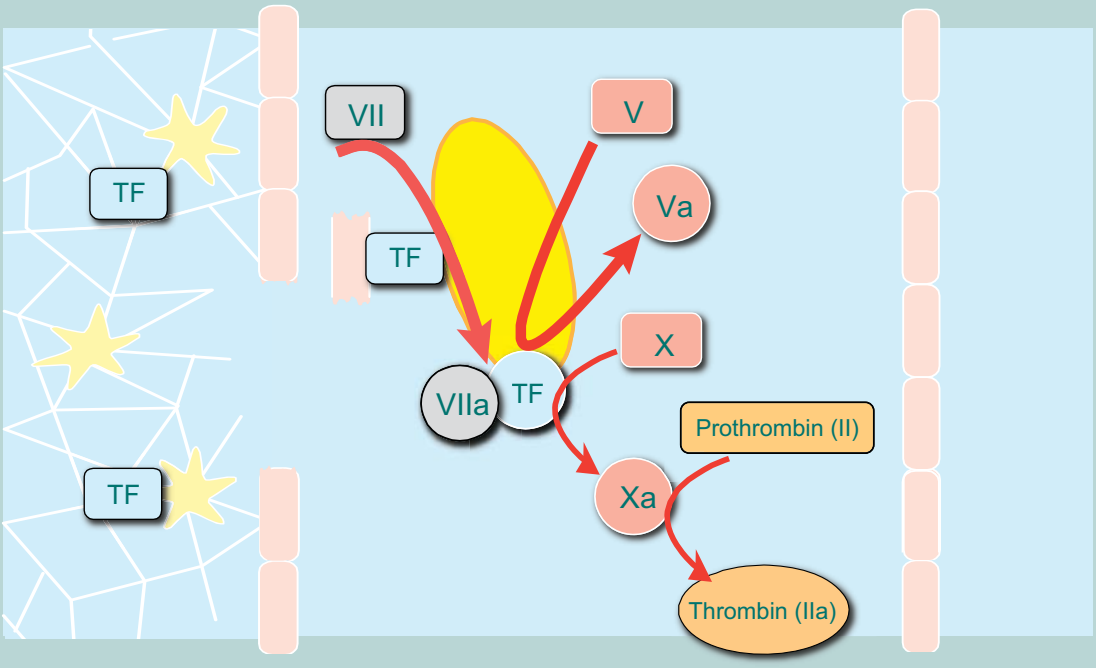
An injury in the vessel wall results in the formation of a haemostatic plug, consisting of a mass of activated platelets interspersed with fibrin, which enables wound sealing even when the vasoconstriction is released.

Following blood vessel injury, subsequent adhesion/aggregation of activated platelets to the sub-endothelial layer (involving von Willebrand factor, VWF) occurs in order to provide a negatively charged phospholipid surface (left side) to which the coagulation proteins become concentrated in a calcium-dependent manner from the flowing blood. In the initial phase of blood coagulation, the key protease thrombin (factor IIa) is formed in only minute amounts via a tissue-factor (TF)-dependent reaction (extrinsic pathway), followed by amplification of thrombin formation in a positive feedback loop, mediated by the protease itself (intrinsic pathway). Once sufficient quantities of thrombin are generated, fibrin is generated at this site to stabilize the generated thrombus, allowing protected wound healing reactions to occur in the injured vessel wall/tissue. If patients have received vitamin K-antagonists, the calcium-binding capacity of coagulation proteins is lowered such that less factors are concentrated on the hemostatic plug with a decrease in the procoagulant response and subsequent fibrin/thrombus formation.

The coagulation cascade



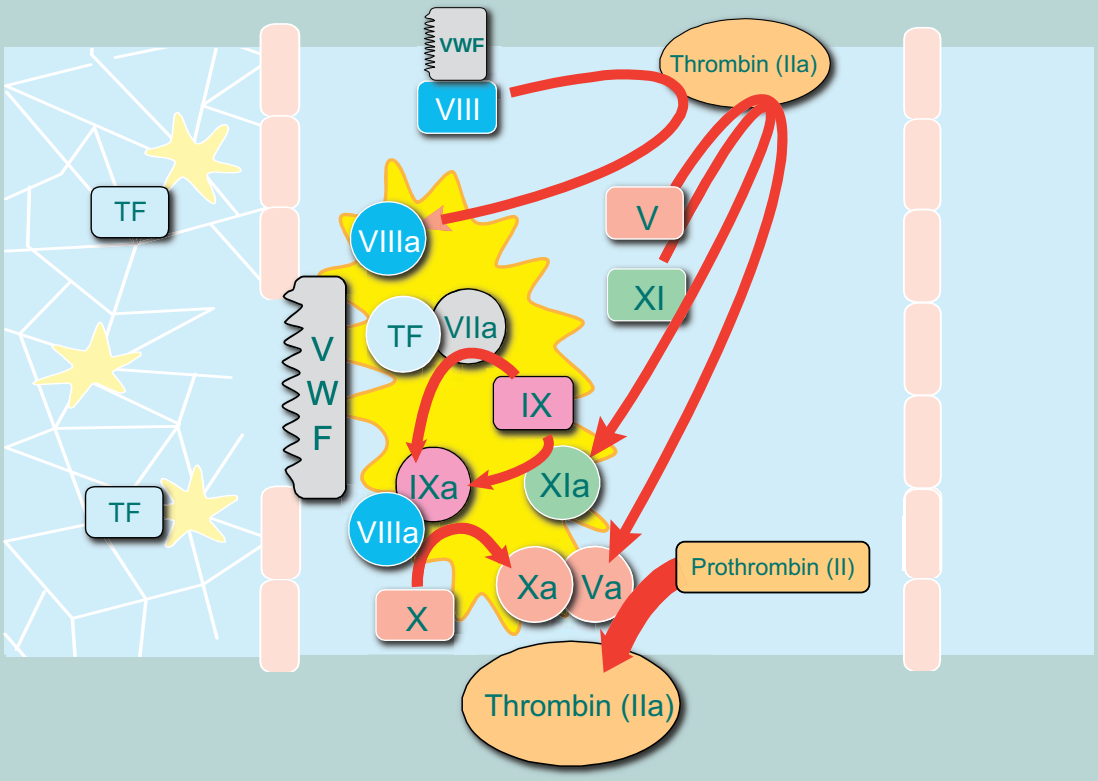
Initiation



Upon vascular injury and tissue damage, haemostasis begins by the initial adherence of blood born platelets to the injury site, mediated by vessel wall and plasma von Willebrand factor particularly under dynamic conditions. The provisional sealing of the wound site by aggregated platelets with their negatively charged phospholipid surface is followed by the process of blood coagulation culminating in fibrin formation in order to stabilize the barrier between the flowing blood and the injured tissue. Blood coagulation can be divided into four overlapping stages: Initiation, amplification, propagation and stabilization of the clot material (Hoffman & Monroe 2001). In the initiation phase, tissue factor (TF) plays a pivotal role as physiological trigger of coagulation when a break in the vessel wall allows plasma to come into contact with TF bearing extravascular cells. Based on the strong binding of plasma coagulation factor VII/VIIa to TF and the presence of phospholipids and calcium ions this complex generates initial amounts of factor Xa (Hoffbrand et al., 2001; Roberts et al., 2004) which in turn will be incorporated into the pro-thrombinase complex to generate initial minute amounts of thrombin. Larger quantities of the TF/VIIa complex may also lead to the generation of factor IXa. The initially generated minute dose of thrombin is insufficient to generate fibrin at this time but is able to induce activation of factor XI and cofactors VIII and V to stimulate its own formation. This enormous spatio-temporal amplification of the intrinsic pathway of blood coagulation in a positive feedback manner will yield high concentrations of thrombin to be sufficient for fibrin generation (Hoffman & Monroe 2001, Obergfell et al. 2007) and factor XIII activation.

Obergfell A, Walter U, Preissner KT. Thromboseprophylaxe und Thrombolytika, in: Estler CJ, Schmidt H. Pharmakologie und Toxikologie, Schattauer 2007; 507–548.
Roberts HR, Monroe DM, Escobar MA. Current concepts of hemostasis. Anesthesiology 2004; 100: 722–730.

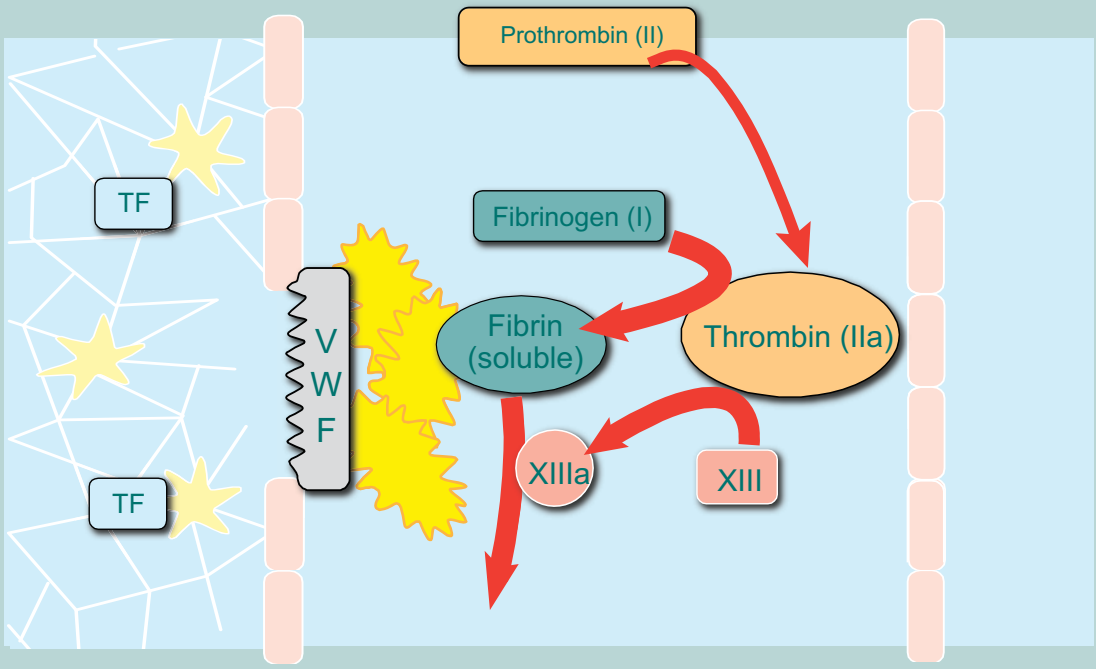
Amplification



Von Willebrand factor (VWF) is the carrier for factor VIII (FVIII) in plasma. The VWF/FVIII complex binds to platelets and is cleaved by thrombin from the initiation phase to activate FVIII and release VWF (Hoffman & Monroe, 2001). FVIIIa remains bound to the surface of the platelet, which is activated by thrombin, while VWF promotes platelet adhesion to thrombogenic surfaces (Hoffman & Monroe, 2001; Federici, 2002).
Thrombin also activates factor XI (FXI) on the surface of platelets (Huntington, 2005). Factor IX (FIX) is then activated by FXIa on the surface of activated platelets (Gailani, 2000) or via an extrinsic pathway involving the TF/FVIIa complex (Hoffman & Monroe, 2001).
During activation, platelets release FV which is then activated by thrombin. FIXa in association with FVIIIa (the tenase complex) increases the activation of FX at the site of vessel injury. FXa then forms a complex with FVa (the prothrombinase complex) (Hoffman & Monroe, 2001), and together, on the phospholipid surface of the activated platelet, they convert prothrombin into thrombin (Butenas & Mann, 2002; Hoffbrand et al., 2001), thereby amplifying the quantities of thrombin that were originally produced during 'initiation'.

Butenas S, Mann KG. Blood coagulation. Biochemistry (Mosc) 2002; 67: 5–15.
Federici AB. The factor VIII/von Willebrand factor complex: basic and clinical issues. Haematologica 2003; 88 (Suppl 9): 3–12.
Gailani D. Activation of factor IX by factor Xia. Trends Cardiovasc Med 2000; 10: 198–204.
Hoffbrand AV, Pettit JE, Moss PAH. Essential haematology. 4th edition. Blackwell Science 2001.
Hoffman M, Monroe III DM. A cell-based model of hemostasis. Thromb Haemost 2001; 85: 958–965.
Huntington JA. Molecular recognition mechanisms of thrombin. J Thromb Haemost 2005; 3: 1861–1872.

Propagation

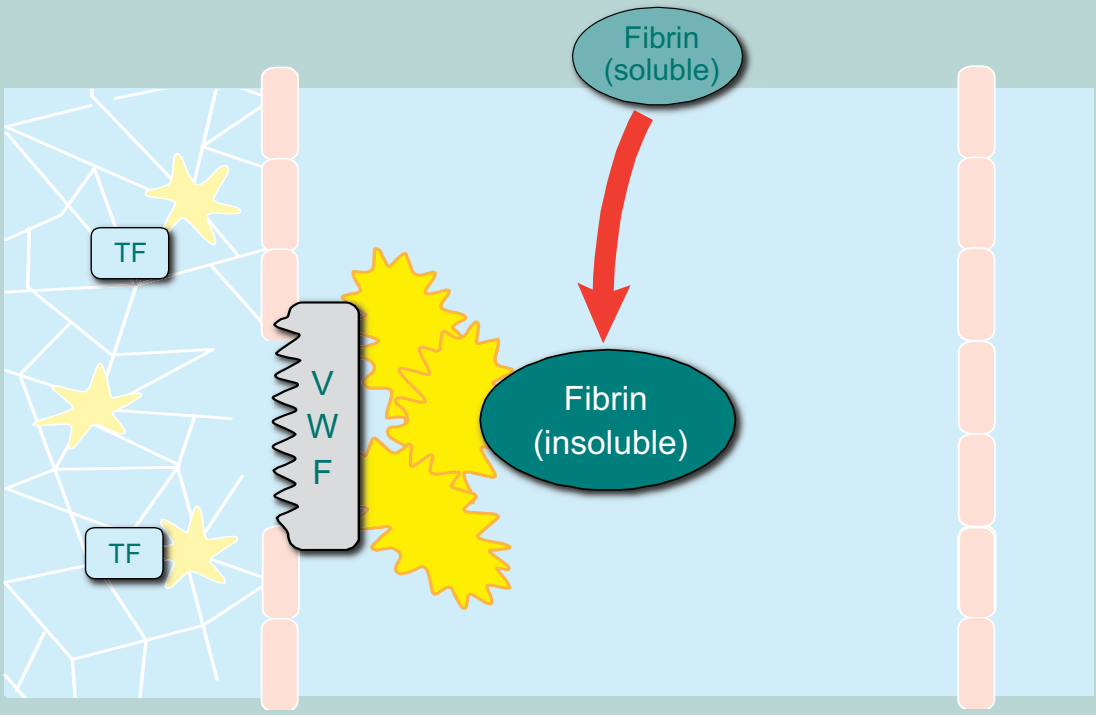


In the propagation/production phase, thrombin cleaves fibrinogen to form soluble fibrin (Huntington, 2005) and thereby releases two pairs A and B of fibrinopeptides per fibrinogen molecule.

Stabilisation of the platelet aggregate occurs through the subsequent polymerisation of fibrin monomers, which are linked by hydrogen bonding. These fibrin polymers subsequently form a nascent insoluble fibrin mesh that stabilises the platelet aggregate.

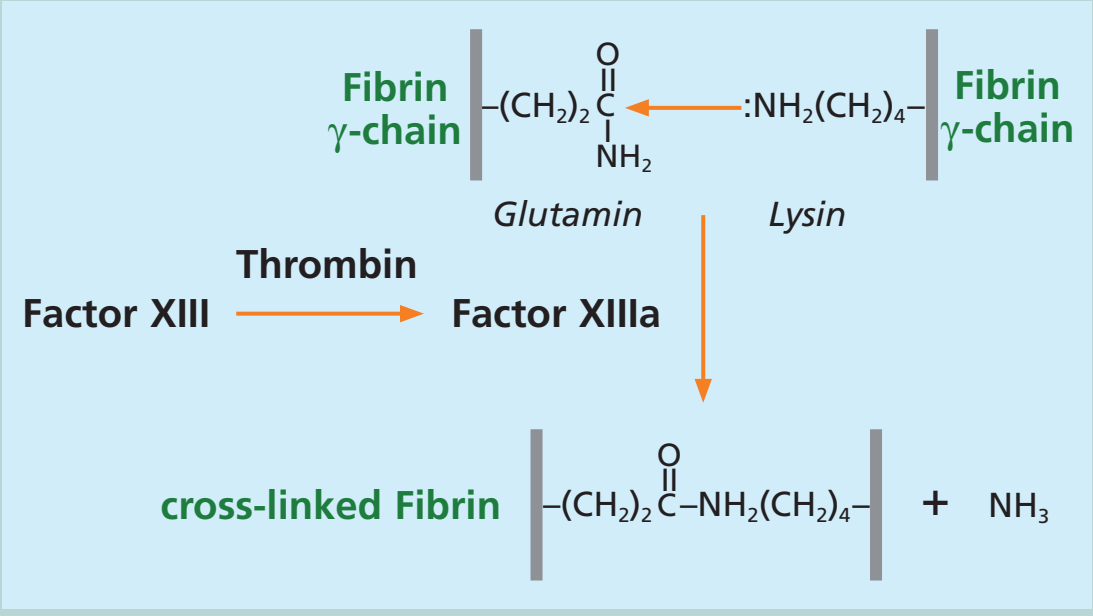
Huntington JA. Molecular recognition mechanisms of thrombin. J Thromb Haemost 2005; 3: 1861–1872.

Stabilisation



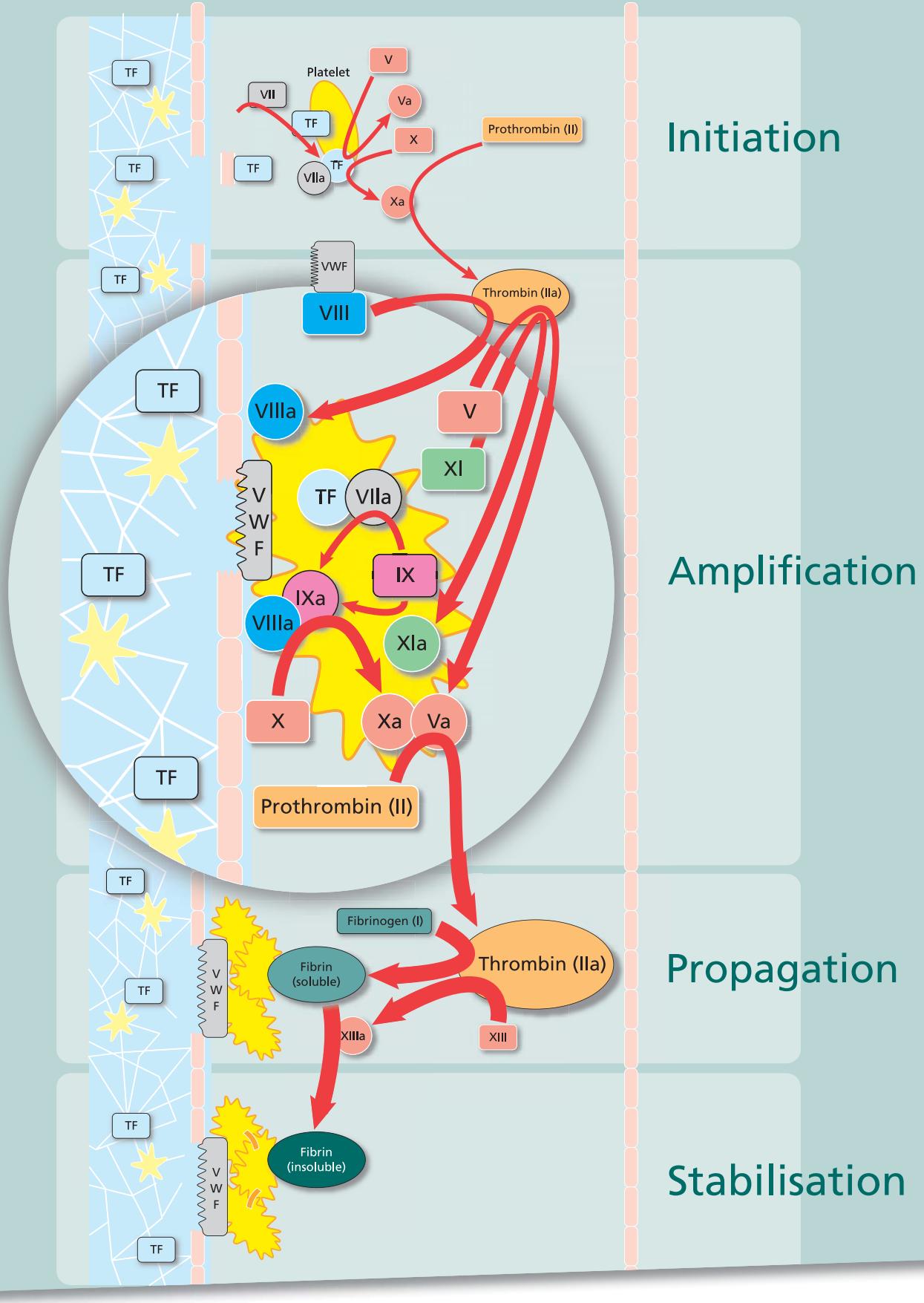
Thrombin helps to further stabilise the fibrin clot by activating FXIII. FXIIIa covalently cross-links adjacent γ-chains of fibrin monomers to stabilise the nascent fibrin clot (Huntington, 2005).

FXIIIa-mediated cross-linking of fibrin

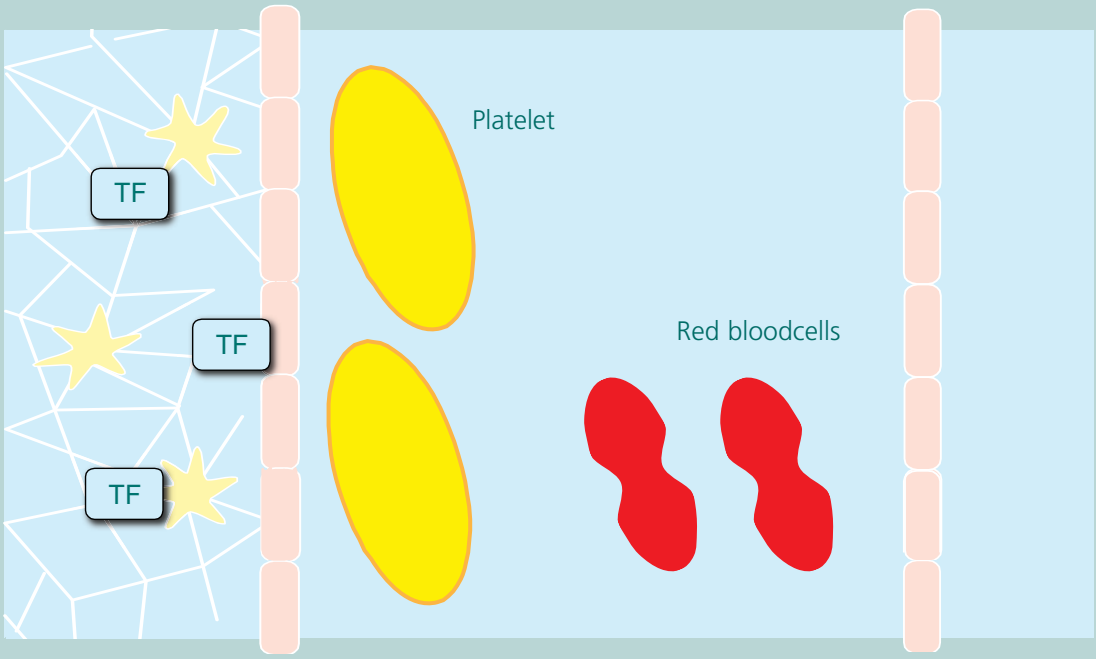


http://tollefsen.wustl.edu/coagulation/ coagulation.html

The coagulation cascade:
Detailed description

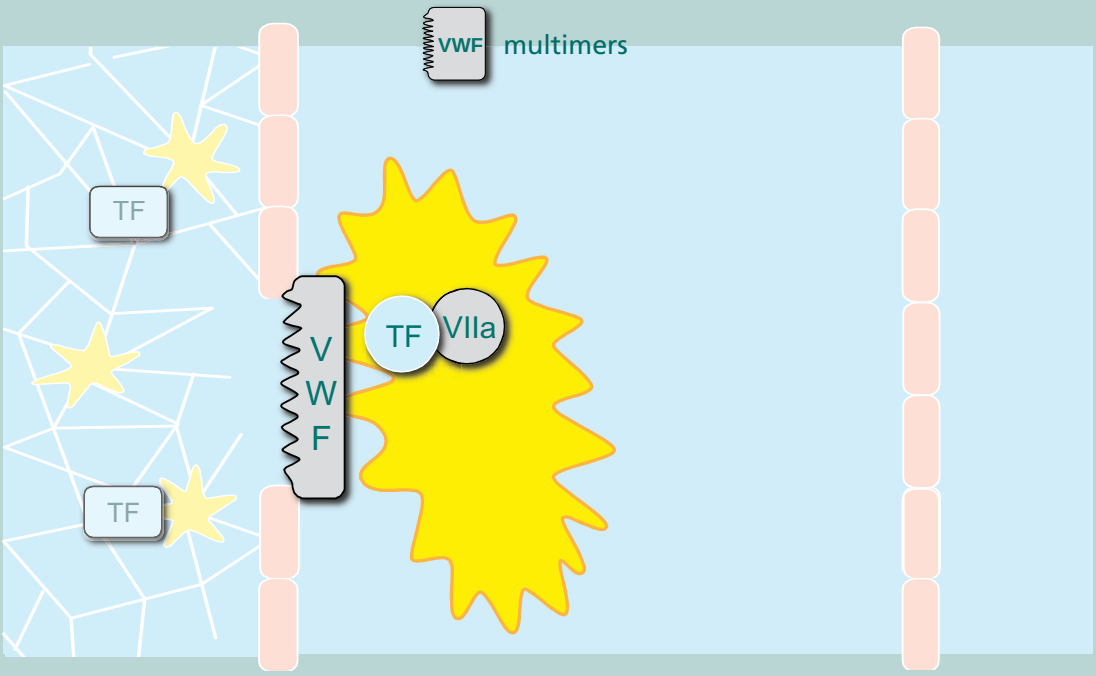


Initiation – 1



Within the normal flowing blood, erythrocytes represent the largest portion of the intravascular cellular mass. Due to the haemodynamic process of axial migration during blood flow, the bulk of erythrocytes passes through the central area of the vascular lumen. Consequently, close to the vessel wall, there is an erythrocyte-free marginal layer that is enriched in platelets. In the marginal layer between the static vessel wall and the flowing blood, the laminar sheer is maximal, and, following vascular in jury, must be overcome during haemostasis.

Initiation – 2

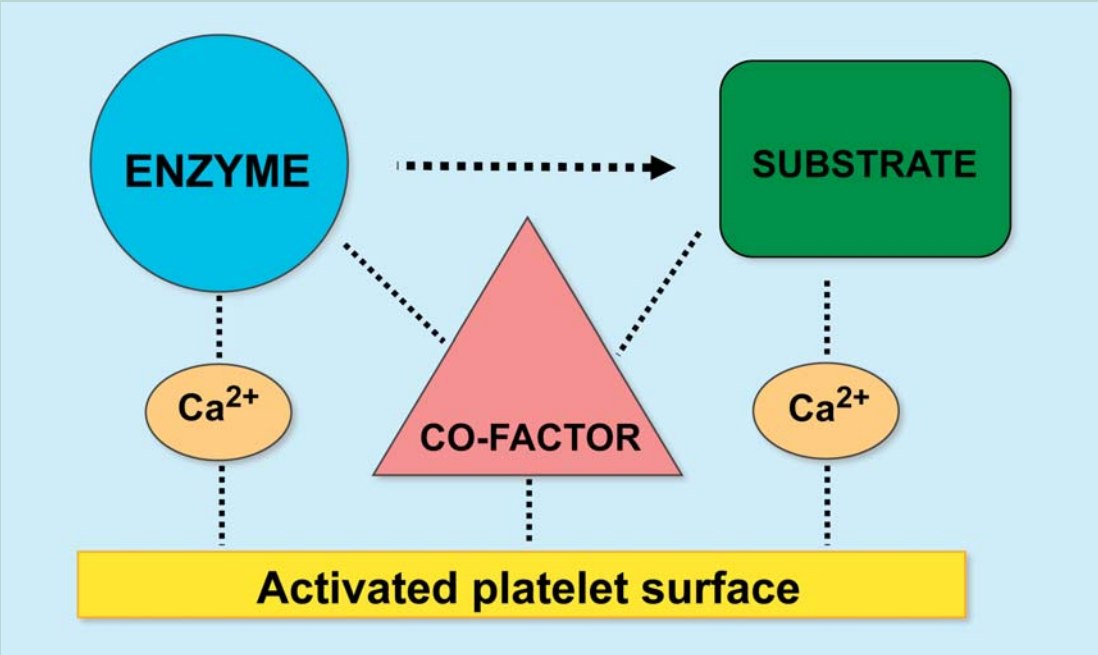


The first physiological reaction to a vessel injury is vasoconstriction – to limit the loss of blood – followed by patelet adhesion and aggregation.

When damage of the vascular surface occurs, coiled VWF forms overcome the high shear stress between the rapidly moving bloodstream and the damaged endothelium by binding immediately to subendothelial collagen and also to damaged endothelial cells. Coiled VWF binds via its exposed A3 domains to collagen in the subendothelium and this triggers the unfolding of the VWF multimers, exposing further A3 domains, as VWF changes its form from a coiled to a stretched conformation. Sufficient VWF A3 binding sites become available and the binding is strong enough to hold the molecule in place. The unfolding of VWF leads to the exposure of the A1 functional domains, which subsequently capture and bind platelets by binding to glycoprotein Ibα receptor (GPIbα) – one of four integral membrane proteins comprising the platelet receptor complex. The circulating platelets are tethered to the injured vessel wall, in a process where they 'roll over' the immobilised VWF, slow down, and become attached. The A1–GPIbα interaction leads to the activation of other platelet contact sites such as the integrin $\alpha_{IIb}\beta_3$. This integrin binds to the Arg-Gly-Asp (RGD) adhesion sequence of the VWF C1 functional domain, resulting in stable platelet adhesion and leading to the activation of the platelet itself. Consequently, immobilised VWF enables platelet adhesion by capturing platelets from the circulating blood under high shear-rate conditions. Subsequent binding of collagen by the platelet receptors GP VI and $\alpha_2\beta_1$ integrin reinforces platelet adhesion.
Collagen exposure and thrombin at the site of the injury cause the adherent platelets to release their granule contents. Released ADP causes platelets to swell and aggregate. After the first layer of platelets has been formed, VWF, together with fibrinogen, functions as a ligand that forms a bridge between $\alpha_{IIb}\beta_3$ integrins on adjacent platelets in the growing thrombus (platelet aggregation). Additional platelets from the circulating blood are drawn to the area of injury, and soon cover the exposed connective tissue (Hoffbrand et al., 2001).

Hoffbrand AV, Pettit JE, Moss PAH. Essential haematology. 4th edition. Blackwell Science 2001.

Multi-component enzyme complexes



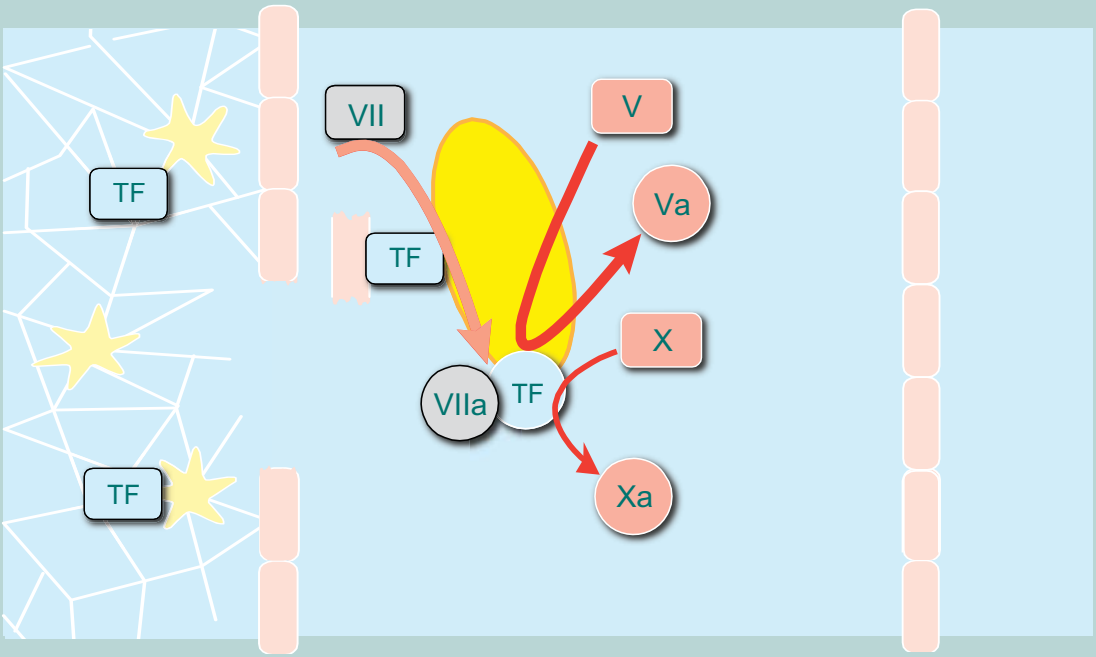
Blood coagulation is a series of coordinated and Ca^{2+}-dependent proenzyme to serine protease conversions likely to be localised on the surfaces of activated cells in vivo.
Most of the enzyme complexes that activate clotting factor zymogens into their respective serine proteases consist of a serine protease and its cofactor bound to activated cell surfaces in an acidic phospholipid- and Ca^{2+}-dependent manner (Ofosu, 2002). The complexes assemble on the phospholipid surface of activated platelets, and it is here that the substrate becomes activated (Obergfell et al., 2007).

Ofosu FA. The blood platelet as a model for regulating blood coagulation on cell surfaces and its consequences. Biochemistry (Mosc) 2002; 67: 47–55.
Obergfell A, Walter U, Preissner KT. Thromboseprophylaxe and Thrombolytika, in: Estler CJ, Schmidt H: Pharmakologie und Toxikologie, Schattauer 2007; 507–548.

Composition of multi-component enzyme complexes

Enzyme	Cofactor	Substrate (Pro-enzyme)
Kallikrein	HMW-Kininogen	Factor XII
Factor IXa	Factor VIIIa	Factor X
Factor VIIa	Tissue factor	Factor X
Factor Xa	Factor Va	Prothrombin
Thrombin	Thrombomodulin	Protein C
Protein Ca	Protein S	Factors Va/VIIIa
tPA	Fibrin	Plasminogen

Initiation – 3



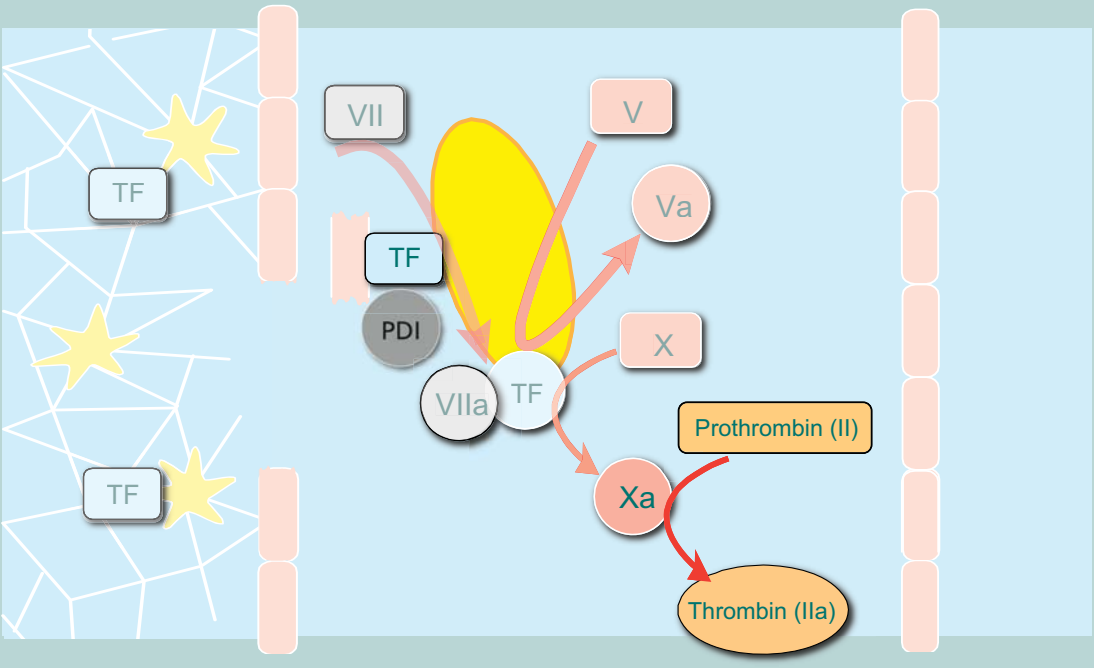
It is generally accepted that the process of coagulation and fibrin formation then occurs in four overlapping stages: Initiation, amplification, propagation and stabilisation of the platelet plug (Hoffman & Monroe, 2001). Tissue factor (TF) is the primary physiologic initiator of coagulation (Hoffman & Monroe, 2001). TF is expressed in deeper cell layers of blood vessels (smooth muscle cells and fibroblasts) as well as in other tissues (Hoffman & Monroe, 2001; Roberts et al., 2004). TF is structurally unrelated to other coagulation proteins – it is an integral membrane protein and remains localised to the membrane of the cell in which it was synthesised (Hoffman & Monroe, 2001).

The initiation stage in normal haemostasis begins when exposure of TF occurs at the site of a vascular injury (Walsh, 2003). A break in the vessel wall allows plasma to come into contact with TF-bearing extravascular cells. TF then binds to coagulation factor VII (FVII) on the TF-bearing cell surface (Hoffbrand et al., 2001; Roberts et al., 2004), forming a complex which is crucial for the auto-activation of FVII as well as the activation of coagulation factors X and IX and subsequent thrombin formation. Alternatively, TF-bearing microparticles in conjunction with platelets promote thrombin formation by the so-called 'intravascular TF pathway' (Engelmann et al., 2003).

The FVIIa/TF complex is known to activate factor X (FX) (Hoffman & Monroe, 2001), which is further involved (probably with low levels of thrombin) in activation of factor V (FV) (Hoffman & Monroe, 2001). Both FXa and FVa remain in the vicinity of the TF-bearing cell and eventually FXa/FVa complexes are formed on the activated platelets (see Amplification – 4) (Roberts et al., 2004).
FXa is rapidly inhibited by tissue factor pathway inhibitor (TFPI) or antithrombin (AT) if it leaves the protected environment of the TF-bearing cell surface (Hoffman & Monroe, 2001).

Hoffman M, Monroe III DM. A cell-based model of hemostasis. Thromb Haemost 2001; 85: 958–965.
Roberts HR, Monroe DM, Escobar MA. Current concepts of hemostasis. Anesthesiology 2004; 100: 722–730.
Engelmann B, Luther T, Müller I. Intravascular tissue factor pathway – a model for rapid initiation of coagulation within the blood vessel. Thromb Haemost 2003; 89: 3–8.
Hoffbrand AV, Pettit JE, Moss PAH. Essential haematology. 4th edition. Blackwell Science 2001.
Walsh PN. Roles of factor XI, platelets and tissue factor-initiated blood coagulation. J Thromb Haemost 2003; 1: 2081–2086.

Initiation – 4



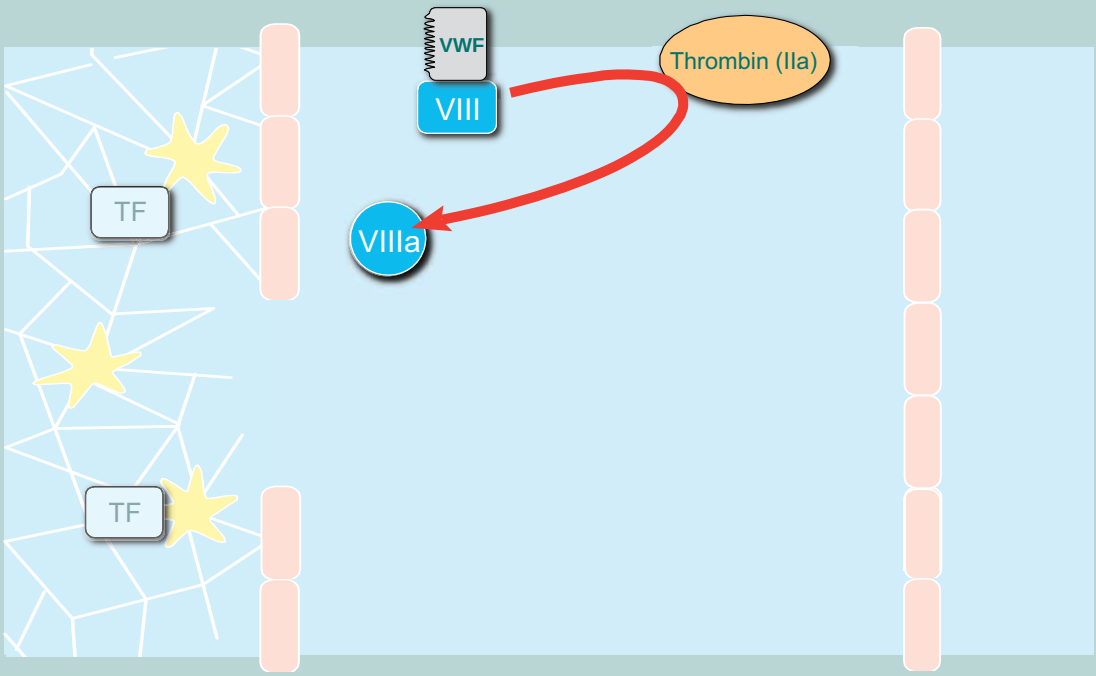
The small quantity of FXa that remains on the cell surface leads to the generation of small amounts of thrombin (Hoffbrand et al., 2001; Hoffman & Monroe, 2001) that are insufficient to promote effective fibrin formation at this stage.
Rather, the thrombin that is formed initially will activate factors XI, VIII, and V to promote and amplify the intrinsic pathway of blood coagulation. Thus, thrombin serves as a priming initiator for subsequent haemostatic events (Roberts et al., 2004). If the procoagulant stimulus is sufficiently strong, enough FXa and thrombin are formed to successfully amplify and propagate the coagulation process (Hoffman & Monroe, 2001).

Hoffbrand AV, Pettit JE, Moss PAH. Essential haematology. 4th edition. Blackwell Science 2001.
Hoffman M, Monroe III DM. A cell-based model of hemostasis. Thromb Haemost 2001; 85: 958–965.
Roberts HR, Monroe DM, Escobar MA. Current concepts of hemostasis. Anesthesiology 2004; 100: 722–730.

Protein disulfide isomerase
Protein disulfide isomerase (PDI) is an ubiquitously expressed oxidoreductase in the body that acts on many different intracellular and extracellular protein substrates by rearranging disulfide bridges. Based on the respective redox environment, PDI can both form or dissociate disulfide bonds, a process that may lead to activation or inactivation of respective proteins. In this regard, vascular cell and platelet derived PDI contributes to the onset of extrinsic pathway activation by promoting disulfide bridge rearrangement in tissue factor, thereby leading to the oxidised de-encrypted form of the extrinsic pathway cofactor. Thus, PDI in the context of vascular injury may be considered as a procoagulant factor.

Manukyan D, von Bruehl ML, Massberg S, Engelmann B. Protein disulfide isomerase as a trigger for tissue factor-dependent fibrin generation. Thromb Res. 2008; 122 Suppl1: S19-22.

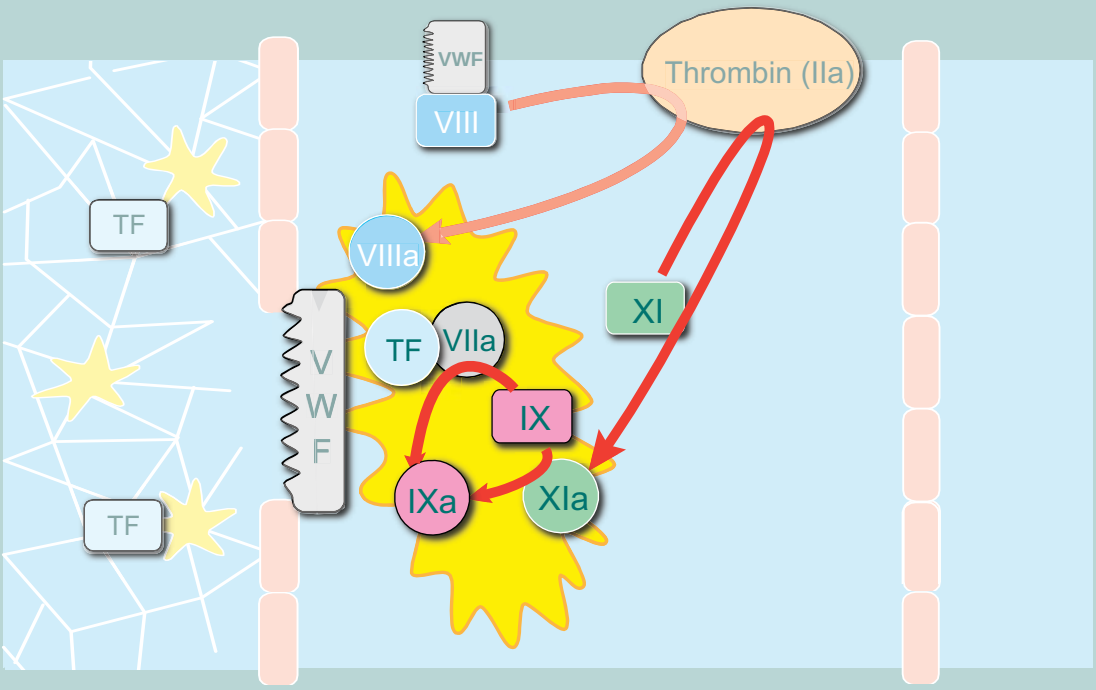
Amplification – 1



Von Willebrand factor (VWF) is the carrier for factor VIII (FVIII) in plasma. Unbound FVIII is characterised by a very short half-life, leading to very low plasma FVIII levels. Bound as a complex, VWF protects the FVIII molecule from rapid proteolysis in plasma thereby prolonging the half-life of this coagulation factor. FVIII is proteolytically activated by the thrombin produced during the initiation phase – VWF also keeps FVIII in a high degree of reactivity, increasing its susceptibility towards activation by thrombin (Federici, 2002).

Federici AB. The factor VIII/von Willebrand factor complex: basic and clinical issues. Haematologica 2003; 88 (Suppl 9): 3–12.

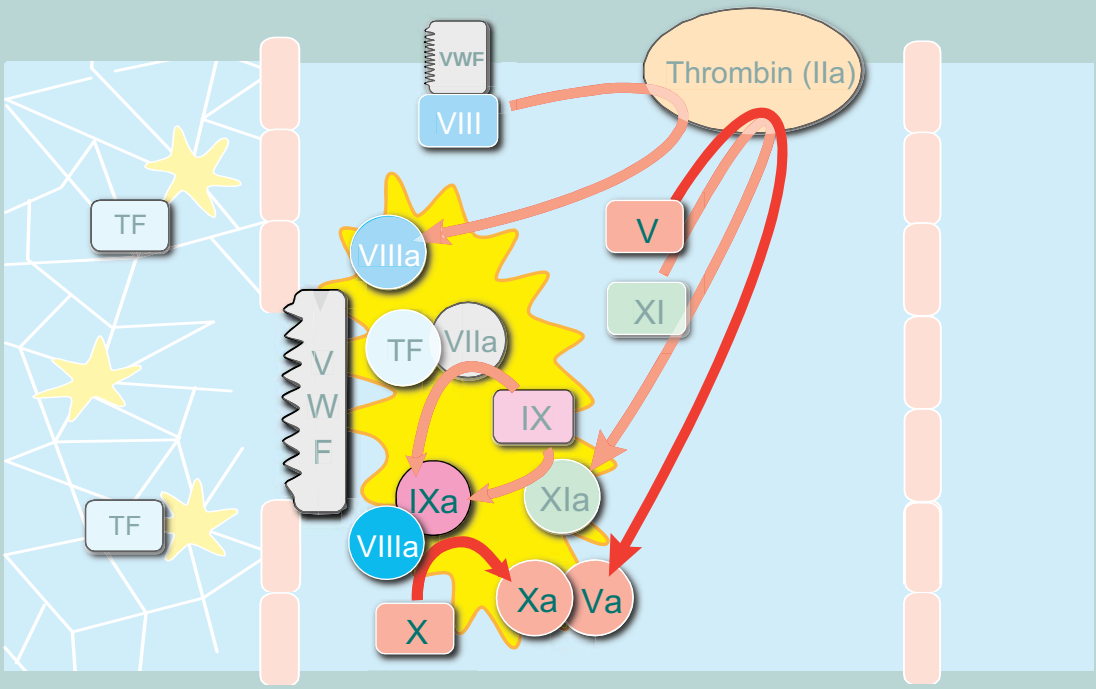
Amplification – 2



Thrombin activates platelets via its protease-activated receptors (PARs) (Hoffman & Monroe, 2001; Roberts et al., 2004). The VWF/FVIII complex binds to platelets and is efficiently cleaved by thrombin to activate FVIII and release VWF. FVIIIa remains bound to the surface of the activated platelet, while the endothelial cell-derived VWF in the subendothelium promotes platelet adhesion to thrombogenic surfaces (Hoffman & Monroe, 2001; Federici, 2002).
Factor XI (FXI) is activated by thrombin, which cleaves it in multiple places to release a central active component (FXIa). This activation occurs on the surface of platelets where thrombin and FXI are colocalised (Huntington, 2005).
Factor IX (FIX) is activated by two distinct mechanisms – it can be activated by FXIa on the surface of activated platelets (Gailani, 2000) or via an extrinsic pathway involving the TF/FVIIa complex (Hoffman & Monroe, 2001) if generated at higher doses. This second pathway is independent of FXIa (Østerud & Rapaport, 1977). Activation through FVIIa/TF occurs early in the course of fibrin clot formation, whereas activation by FXIa appears to be important for maintaining the integrity of the clot over time (Gailani, 2000).

Gailani D. Activation of factor IX by factor Xia. Trends Cardiovasc Med 2000; 10: 198–204.
Hoffman M, Monroe III DM. A cell-based model of hemostasis. Thromb Haemost 2001; 85: 958–965.
Huntington JA. Molecular recognition mechanisms of thrombin. J Thromb Haemost 2005; 3: 1861–1872.
Østerud B, Rapaport SI. Activation of factor IX by the reaction product of tissue factor and factor VII: additional pathway for initiating blood coagulation. Proc Natl Acad Sci USA 1977; 74: 5260–5264.
Hoffman M, Monroe III DM. A cell-based model of hemostasis. Thromb Haemost 2001; 85: 958–965.
Roberts HR, Monroe DM, Escobar MA. Current concepts of hemostasis. Anesthesiology 2004; 100: 722–730.

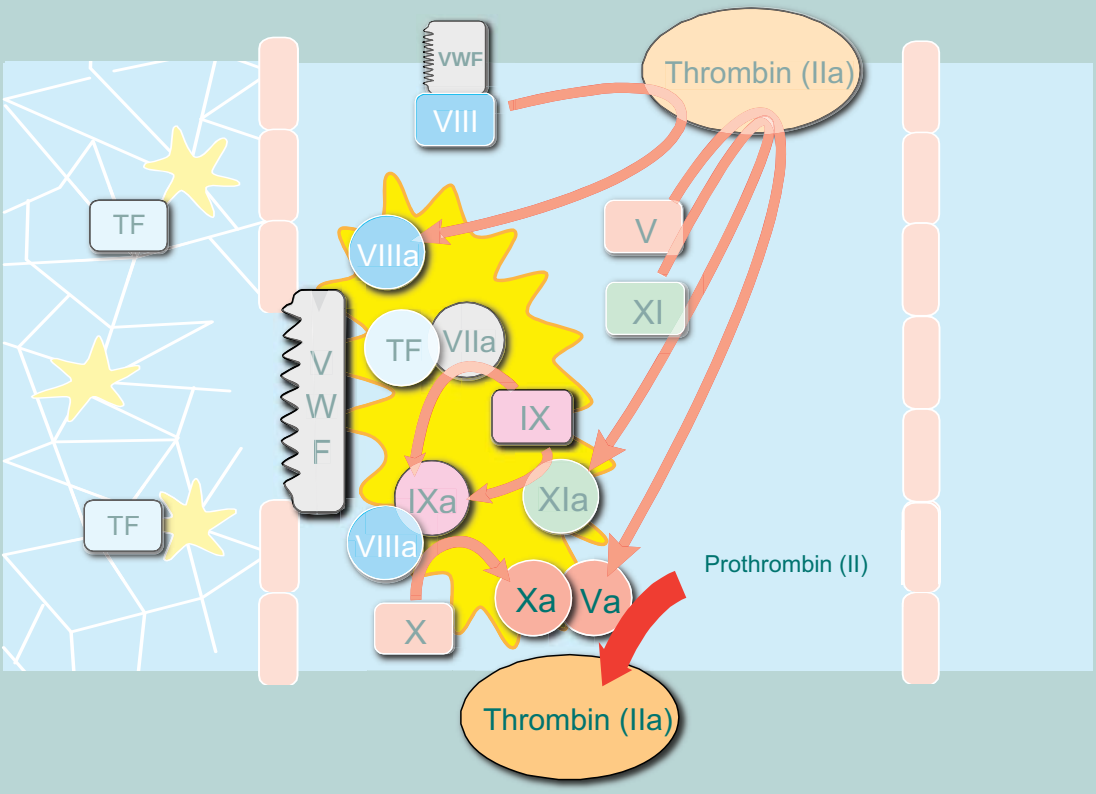
Amplification – 3



During activation, platelets release partially-activated FV from α-granules onto their surfaces (Hoffman & Monroe, 2001). Together with FXI, this FV is then fully activated by thrombin, which cleaves it in multiple places to release the central B domain from the active component (FVa). FVIIIa and FVa are required for activation of the tenase and the prothrombinase complexes, respectively (Huntington, 2005). Activated platelets serve to bind the cofactors FVIIIa and FVa first, followed by their respective enzymes, FIXa and FXa (Roberts et al., 2004).
The physiological function of activated FVIII is to act as an accelerator, increasing by several thousand-fold the activation of FX by activated factor IX (FIXa) at the site of vessel injury. The activation complex, also know as the 'tenase' complex, (FVIIIa/FIXa) assembles when FIXa reaches the platelet surface, and activates FX, which then moves directly into a complex with its cofactor, FVa (Hoffman & Monroe, 2001), the prothrombinase complex.
Subsequent events leading to further thrombin generation, as well as amplification of the intrinsic pathway, take place on the surface of activated platelets (Roberts et al., 2004).

Hoffman M, Monroe III DM. A cell-based model of hemostasis. Thromb Haemost 2001; 85: 958–965.
Huntington JA. Molecular recognition mechanisms of thrombin. J Thromb Haemost 2005; 3: 1861–1872.
Roberts HR, Monroe DM, Escobar MA. Current concepts of hemostasis. Anesthesiology 2004; 100: 722–730.

Amplification – 4



FXa, in association with its cofactor FVa on the phospholipid surface of the activated platelet, converts prothrombin into thrombin (Butenas & Mann, 2002; Hoffbrand et al., 2001). Thrombin is generated from its zymogen (inactive precursor), prothrombin, through cleavage at two sites by the prothrombinase complex (Huntington, 2005). The prothrombinase complex is capable of converting large amounts of prothrombin to thrombin (Roberts et al., 2004) hence amplifying the quantities of thrombin that were originally produced during 'initiation'. Thrombin feeds back to stimulate its own formation by activating cofactors FV and FVIII, and by activating FXI (Huntington, 2005). Thrombin is the only coagulation enzyme that can diffuse away from the site of clot formation, whereby about 5% of the formed thrombin is sufficient to mediate fibrin generation, whereas more than 95% are involved in the thrombomodulin – protein C anticoagulant pathway (see page 29).

Butenas S, Mann KG. Blood coagulation. Biochemistry (Mosc) 2002; 67: 5–15.
Hoffbrand AV, Pettit JE, Moss PAH. Essential haematology. 4th edition. Blackwell Science 2001.
Huntington JA. Molecular recognition mechanisms of thrombin. J Thromb Haemost 2005; 3: 1861–1872.
Roberts HR, Monroe DM, Escobar MA. Current concepts of hemostasis. Anesthesiology 2004; 100: 722–730.

Amplification – 5

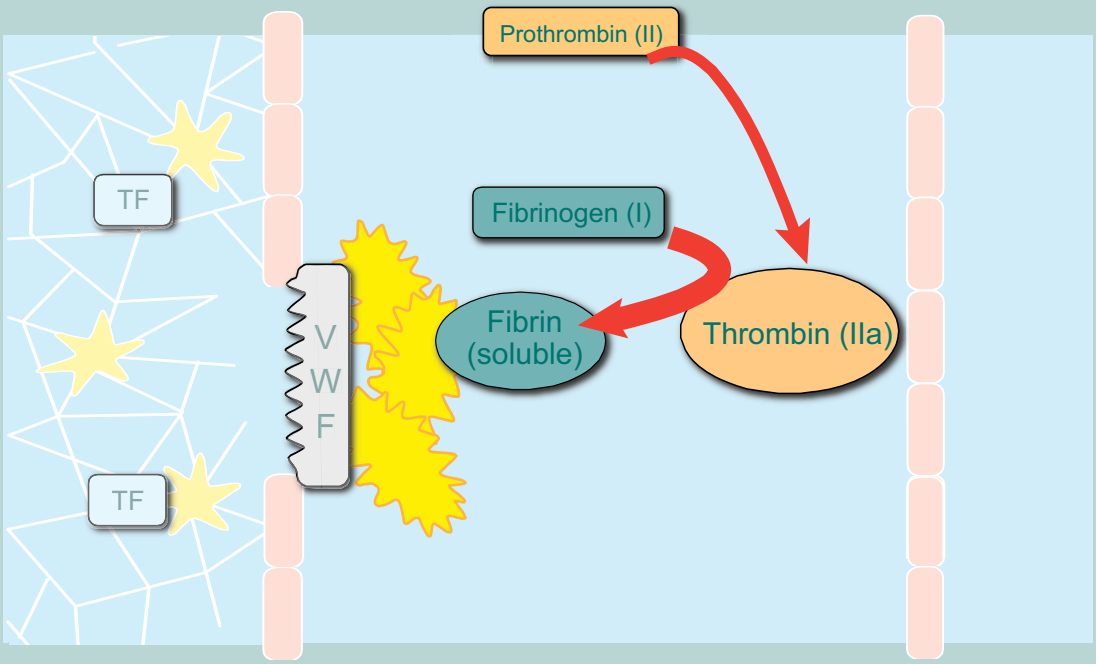
Amplification of blood coagulation reactions by assembly of multi-component enzyme complexes

	Components	Relative rate of thrombin generation
(1)	Prothrombin, factor Xa, Ca^{2+}	1
(2)	Prothrombin, factor Xa, phopholipids, Ca^{2+}	50
(3)	Prothrombin, factor Xa, factor Va, Ca^{2+}	350
(4)	Prothrombin*, factor Xa*, factor Va, phospholipids, Ca^{2+}	<1000
(5)	Prothrombin, factor Xa, factor Va, phospholipids, Ca^{2+}	19,000
(6)	Prothrombin, factor Xa, factor Va, platelets, Ca^{2+}	300,000

* Proteins in Vitamin K absence (PIVKA), due to medication with Vitamin K-antagonist (acquired) or due to lack in Ca-binding as result of a mutation, have a substantially decreased potency to assemble in multi-component enzyme complexes.

http://tollefsen.wustl.edu/coagulation/ coagulation.html

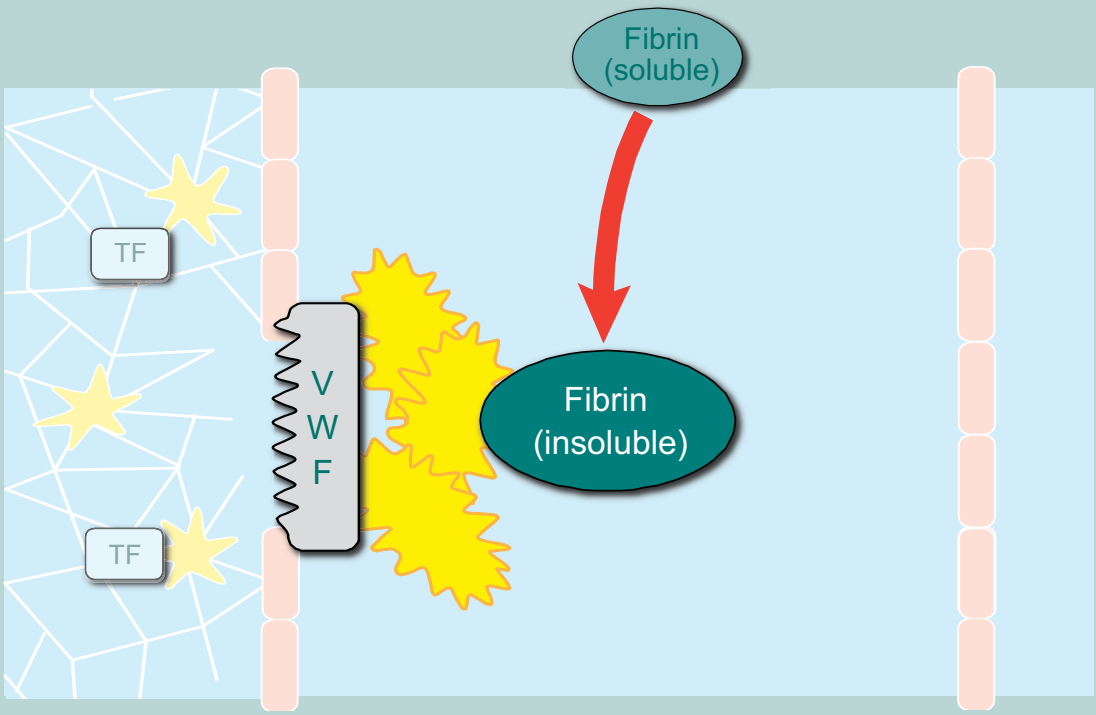
Propagation



Following its formation, thrombin rapidly cleaves fibrinogen to soluble fibrin (Huntington, 2005). Cleavage occurs at the N-termini of the α- and β-chains of the dimeric fibrinogen molecule and results in the release of fibrinopeptides A and B leading to formation of a fibrin monomer capable of linear and lateral self-association, resulting in an insoluble fibrin clot (Huntington, 2005).

Huntington JA. Molecular recognition mechanisms of thrombin. J Thromb Haemost 2005; 3: 1861–1872.

Stabilisation



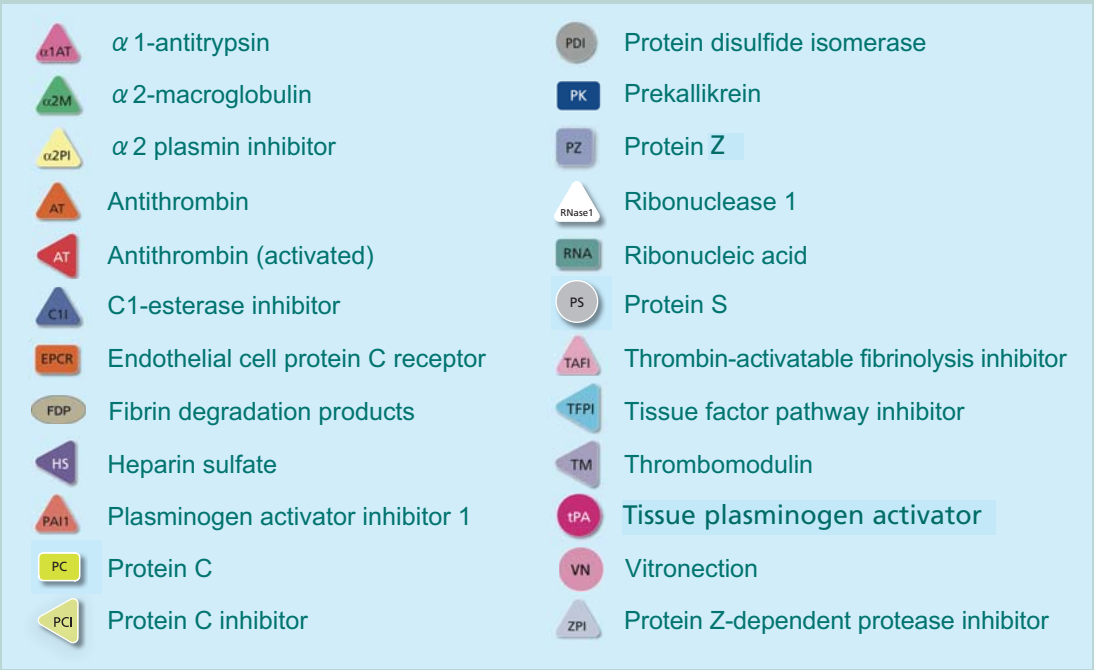
Fibrin monomers link spontaneously by hydrogen bonding to form a loose, insoluble fibrin polymer. Consequently, during the events of haemostasis, local activation and amplification of the clotting cascade results in the formation of an insoluble fibrin mesh that further stabilises the platelet aggregate.

Thrombin helps to further stabilise the fibrin clot by activating the transglutaminase FXIII (Huntington, 2005) in the presence of calcium ions (Hoffbrand et al., 2001).
FXIII is a heterotetrameric zymogen composed of two catalytic A subunits and two carrier B subunits (Huntingdon, 2005). Cleavage by thrombin occurs at a single position in the A chains and results in the release of the B-chains and exposure of the catalytic site. FXIIIa covalently cross-links glutamine residues with lysines on adjacent γ-chains of fibrin monomers to stabilise the nascent fibrin clot (Huntingdon, 2005).

Hoffbrand AV, Pettit JE, Moss PAH. Essential haematology. 4th edition. Blackwell Science 2001.
Huntington JA. Molecular recognition mechanisms of thrombin. J Thromb Haemost 2005; 3: 1861–1872.

Notes

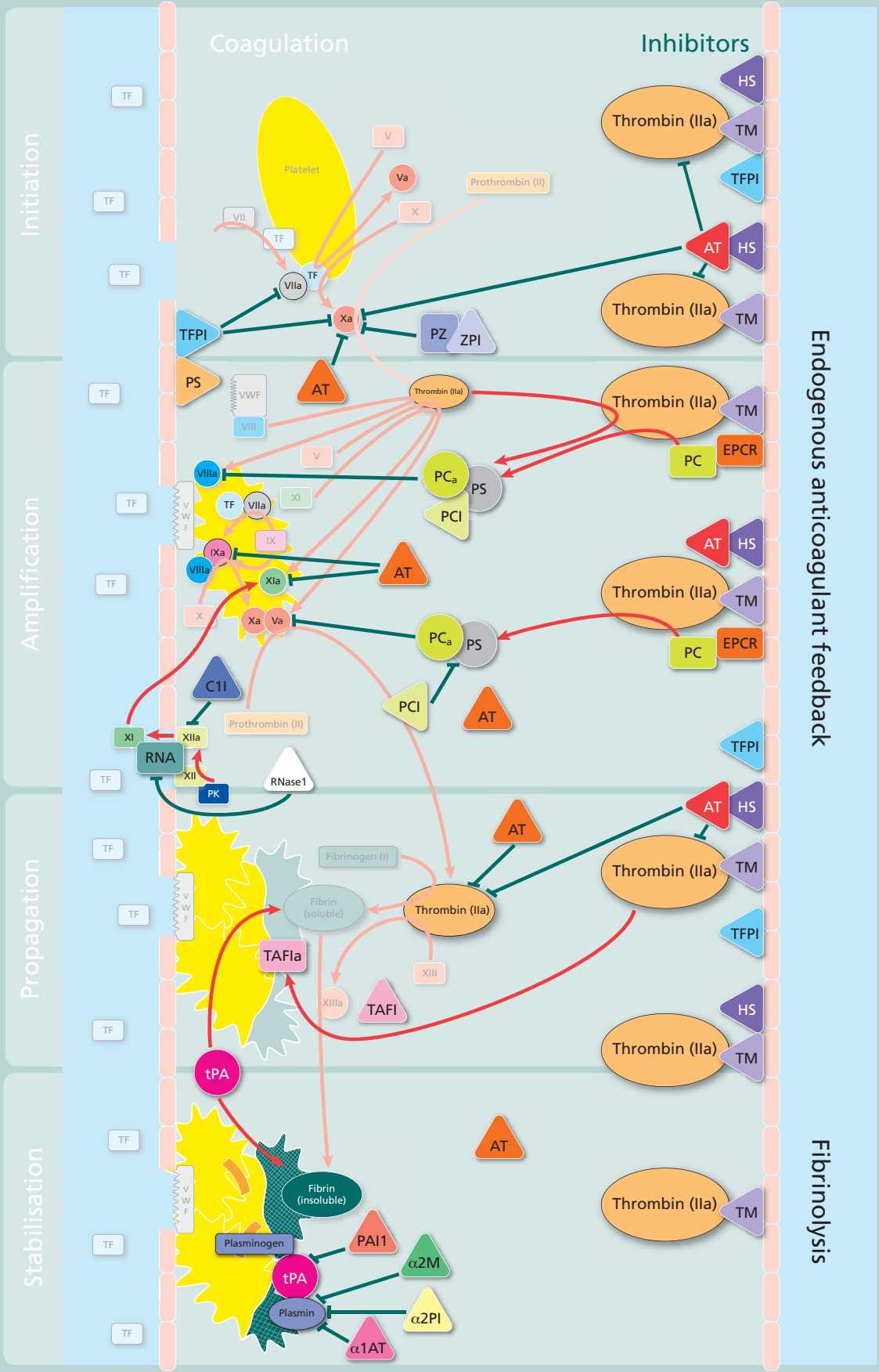
Components of the inhibitory and anticoagulant pathways



Following blood vessel injury, subsequent adhesion/aggregation of activated platelets to the sub-endothelial layer (involving von Willebrand factor, VWF) occurs in order to provide a temporary patelet plug presenting negatively charged phospholipid surface (left side) to which the coagulation proteins become concentrated in a calcium-dependent manner from the flowing blood. In the initial phase of blood coagulation, the key protease thrombin (factor IIa) is formed in only minute amounts via a tissue-factor (TF)-dependent reaction (extrinsic pathway), followed by amplification of thrombin formation in a positive feedback loop, mediated by the protease itself (intrinsic pathway). Once sufficient quantities of thrombin are generated, fibrin is generated at this site to stabilize the generated thrombus, allowing protected wound healing reactions to occur in the injured vessel wall and the underlying tissue.

If patients have received vitamin K-antagonists, the calcium-binding capacity of coagulation proteins is lowered such that less factors are concentrated on the haemostatic plug with a decrease in the procoagulant response and subsequent fibrin/thrombus formation.

Inhibitory and anticoagulant pathways

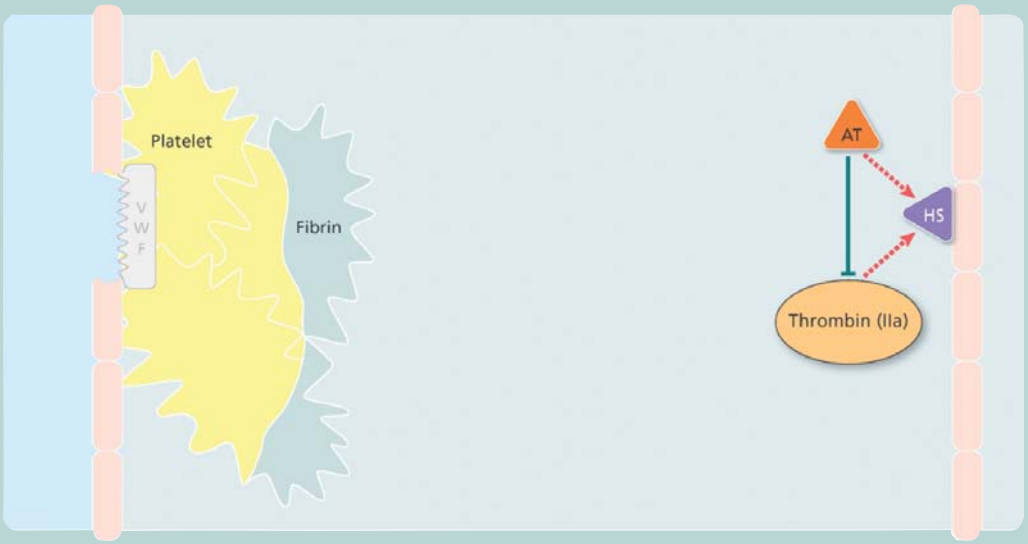


Thrombin-thrombomodulin (T-TM)

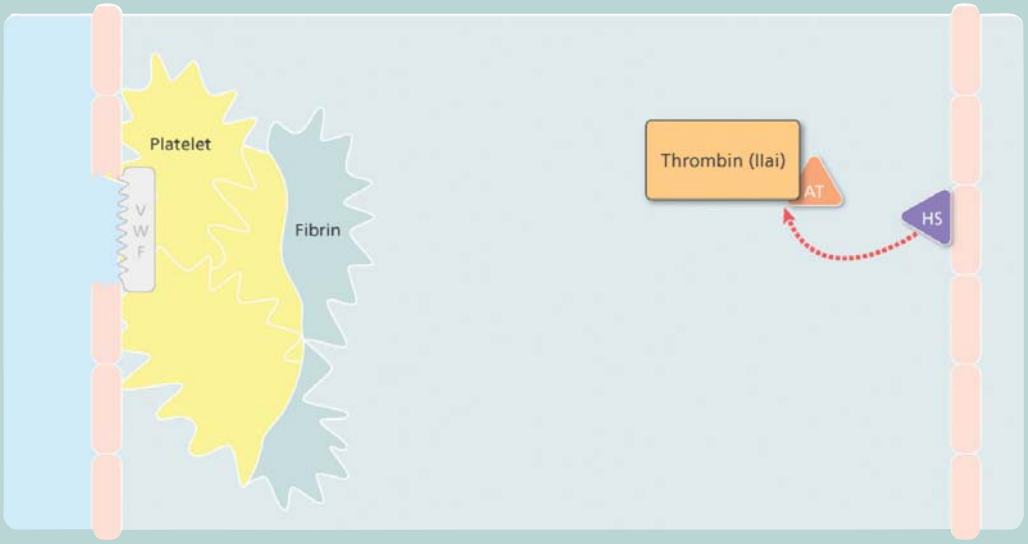
Only about 5% of the generated thrombin is sufficient to generate a fibrin clot, whereas > 90% of thrombin is drifted away from the site of injury and becomes tightly bound to the intact vascular endothelium via thrombomodulin (TM, right side). Together with the substrate/enzymogen protein C (bound to its receptor EPCR in large vessels) an endogenous anticoagulant feedback mechanism is initiated, resulting in the effective production of activated protein C (PCa). All procoagulant activities of thrombin are blocked in complex with TM. Subsequently, PCa (in association with its cofactor protein S, PS) will bind to the haemostatic plug and induce proteolytic inactivation of cofactors VIIIa and Va in order to limit further thrombin generation. Deficiency of PC or PS will significantly decrease the anticoagulant capacity of this pathway and is associated with thromboembolic complications. Additional anticoagulant reactions (also including antithrombin) are available to control the activity of fluid phase coagulation proteases, including factors Xa and IIa.

Esmon CT. Regulation of blood coagulation. Biochim Biophys Acta. 2000; 1477: 349-360.
Esmon CT. Inflammation and the activated protein C anticoagulant pathway. Semin Thromb Hemost. 2006; 32 Suppl1: 49-60.
Weiler H. Multiple receptor-mediated functions of activated protein C. Haemostaseologie. 2011; 31: 185-195.

Thrombin-antithrombin (T-AT)



In addition to the activated protein C pathway, circulating antithrombin (AT) provides another important endogenous anticoagulant mechanism, relevant for irreversible inactivation of coagulation proteases in a slow progressive manner.



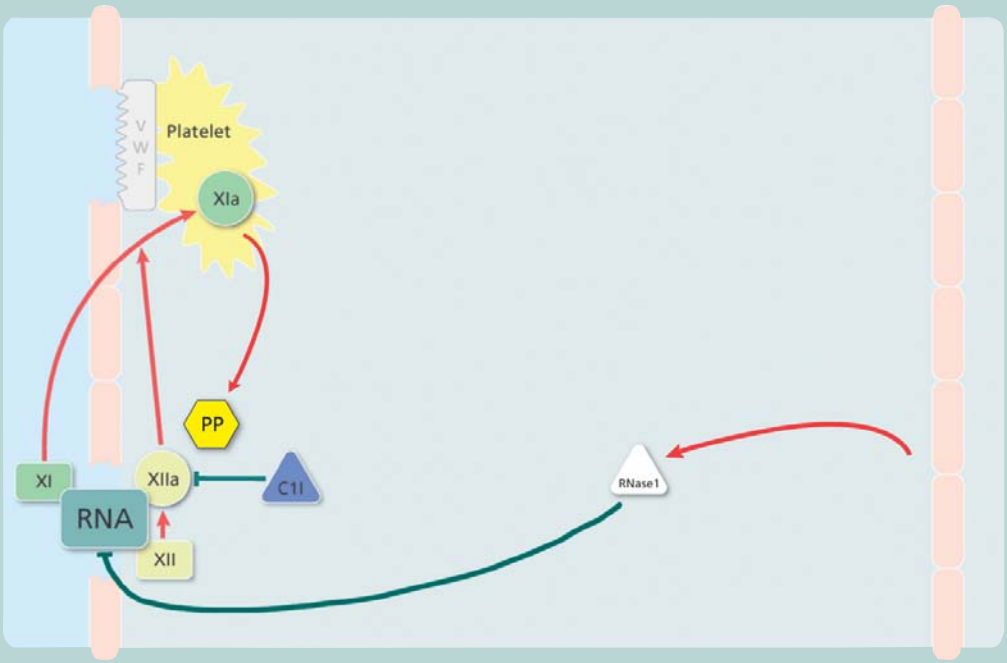
In particular, thrombin is inhibited by AT in a slow progressive fashion, whereby endothelial cell heparan sulphate (HS) proteoglycanes can significantly enhance enzyme inhibition, similar to the catalytic action of exogenously administered heparin. Once the covalent inactive thrombin-AT complex has been formed, it dissociates from the catalytic HS-template. A deficiency of AT can have substantial effects on the anticoagulant control of thrombin and is associated with thromboembolic complications.

Tissue factor pathway inhibitor (TFPI)

Upon initiation of the extrinsic pathway, the outcome of tissue factor (TF)-mediated reactions is controlled by intact tissue factor pathway inhibitor (TFPI), present on the endothelium, on circulating cells and in association with lipoproteins. Through multiple interactions with factors VIIa and Xa via its Kunitz-type domains, TFPI serves as a natural threshold to balance the onset of coagulation by forming stoichiometric complexes with the indicated proteases. Protein S (PS) may work in concert with TFPI to support its anticoagulant activity. Degradation of TFPI by neutrophil elastase or cathepsin G may limit the inhibitory potential of the inhibitor, indicative for the procoagulant activity of these neutrophil proteases.

Broze GJ. Tissue factor pathway inhibitor. Thromb Haemost. 1995; 74: 90-93.
Crawley JT, Lane DA. The haemostatic role of tissue factor pathway inhibitor. Arterioscler Thromb Vasc Biol. 2008; 28: 233-242.
DelGiudice LA, White GA. The role of tissue factor and tissue factor pathway inhibitor in health and disease states. J Vet Emerg Crit Care. 2009; 19: 23-29.
Kasthuri RS, Glover SL, Boles J, Mackman N. Tissue factor and tissue factor pathway inhibitor as key regulators of global hemostasis: measurement of their levels in coagulation assays. Semin Thromb Hemost. 2010; 36: 764-771.

Contact phase, C1-inhibitor



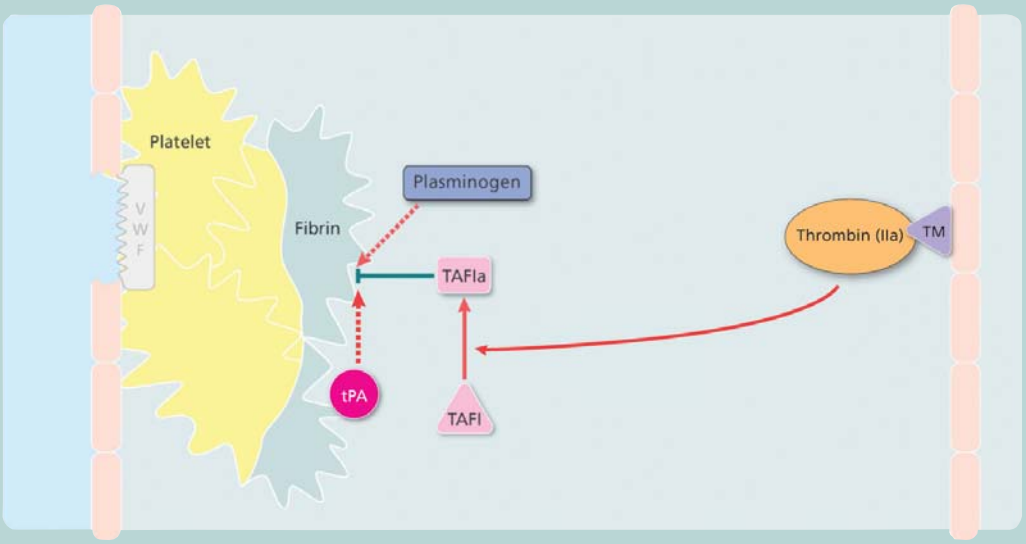
The intrinsic pathway may be triggered in vivo by polyanionic molecules such as extracellular RNA or polyphosphates (PP) that induce factor XII (auto-)activation and amplification of the "contact phase" system, leading to enhanced thrombin formation.

C1 inhibitor not only blocks the activity of complement proteases C1r and C1s, but also serves as a main anticoagulant protein to limit the activity of the "contact phase" proteases, including factor XIIa and kallikrein. Thus, not only procoagulant reactions are controlled, but also kallikrein-mediated cleavage of high molecular weight kininogen and subsequent generation of the vasodilator bradykinin is limited by C1-inhibitor. Consequently, deficiency of C1-inhibitor results in increased vascular permeability, associated with massive edema formation.

Ribonuclease1 (RNase1)
RNase1, which is a well-known hydrolytic enzyme in the digestive tract, is also present in the vasculature where it is produced and secreted mainly by endothelial cells. Due to the high ribonucleolytic activity and the broad substrate specificity of this extremely stable enzyme, different types of circulating or surface-bound extracellular RNA may be digested. Based on the fact that extracellular RNA may promote the initiation of intrinsic blood coagulation by direct auto-activation of contact phase proteins, RNase1 is considered as an anticoagulant factor that may limit thrombus formation and vessel occlusion, as demonstrated in different experimental thrombosis models.

Zeerleder S. C1-inhibitor: more than a serine protease inhibitor. Semin Thromb Hemost. 2011; 37: 362-374.
Davis AE, Lu F, Mejia P. C1 inhibitor, a multi-functional serine protease inhibitor. Thromb Haemost. 2010; 104: 886-893.
Deindl E, Fischer S, Preissner KT. New directions in inflammation and immunity: the multifunctional role of the extracellular RNA/RNase system. Indian J Biochem Biophys. 2009; 46: 461-466.

Thrombin-activated fibrinolysis inhibitor (TAFI)

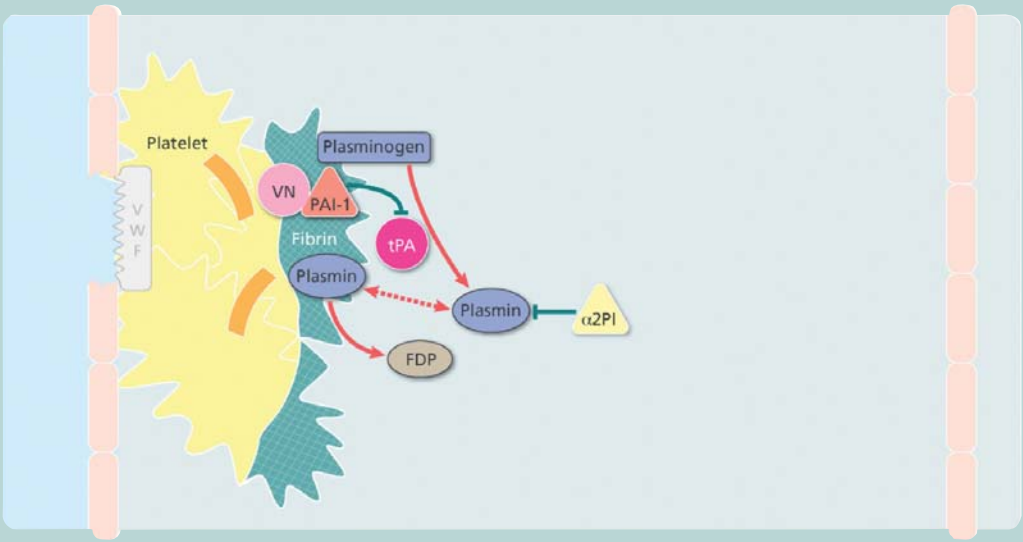


In a fibrin-specific manner, involving the action of tissue plasminogen activator (tPA, derived from endothelial cells) and fibrin-bound plasminogen, the generated thrombus is dissolved in a later stage of wound healing, once the repair process in the vessel wall has been completed.

Thrombin-associated fibrinolysis inhibitor (TAFI) is a circulating procarboxy-peptidase, which becomes activated through proteolytic cleavage by thrombin (particularly in complex with TM). The generated TAFIa removes carboxy-terminal amino acid residues (including lysines) from protein substrates like fibrin, such that the binding of tPA and plasminogen to this modified fibrin is hampered and hence, fibrinolysis at a premature stage is prevented. This allows fibrin clot stabilisation in the early phase of thrombus formation.

Willemse JL, Heylen E, Nesheim ME, Hendriks DF. Carboxypeptidase U (TAFIa): a new drug target for fibrinolytic therapy? J Thromb Haemost. 2009; 7: 1962-1971.
Declerck PJ. Thrombin activatable fibrinolysis inhibitor. Haemostaseologie. 2011; 31: 165-173.

Plasminogen activator inhibitor-1 (PAI-1)

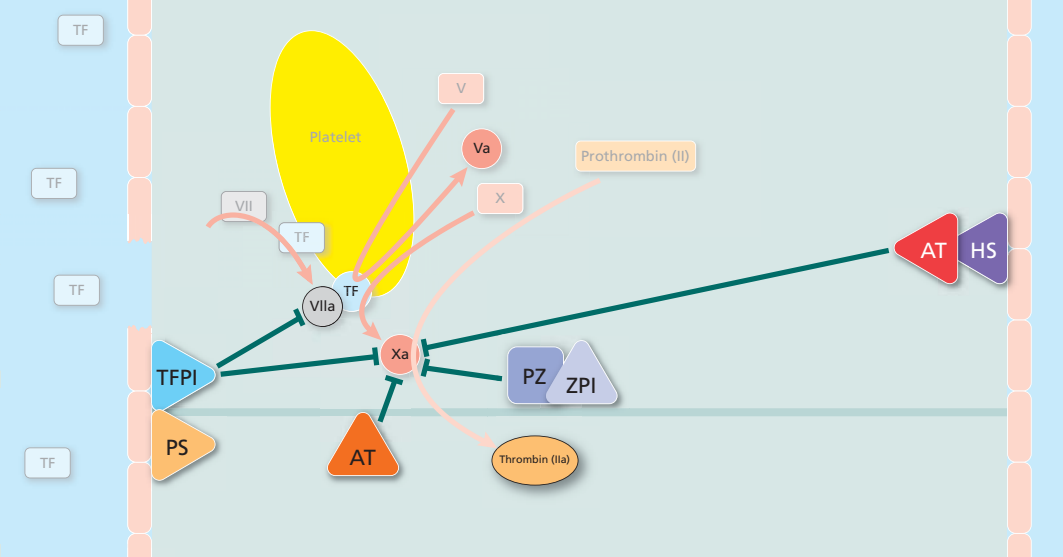


Plasminogen activator inhibitor-1 (PAI-1) in blood is mostly derived from activated platelets, and circulates in its active form in a complex with vitronectin (VN). Stabilized PAI-1 serves as the primary inhibitor of several proteases, including tPA or urinary-type plasminogen activator. At the early phase of platelet plug/fibrin formation, tPA is blocked by PAI-1 in order to limit fibrinolysis at that stage and to warrant early clot stabilisation. Conversely, elevated levels of PAI-1 are associated with an increased risk of atherothrombosis due to prevention of tPA-mediated thrombolysis.

α_2-Plasmin inhibitor (α_2-PI) is the fastest serine protease inhibitor in blood to limit the activity of soluble plasmin in a spatio-temporal manner. In contrast, fibrin-bound plasmin degrades the clot material (with the generation of fibrin degradation products, FDP) and is largely protected against inactivation by α_2-PI. This allows efficient fibrinolysis to occur and prevents unwanted side reactions such as fibrinogenolysis.

Loskutoff DJ, Samad F. The adipocyte and hemostatic balance in obesity: studies of PAI-1. Arterioscler Thromb Vasc Biol. 1998; 18: 1-6.
Gils A, Declerck PJ. Plasminogen activator inhibitor-1. Curr Med Chem. 2004; 11: 2323-2334.
Dellas C, Loskutoff DJ. Historical analysis of PAI-1 from its discovery to its potential role in cell motility and disease. Thromb Haemost. 2005; 93: 631-640.
Preissner KT, Reuning U. Vitronectin in vascular context: facets of a multitalented matricellular protein. Semin Thromb Hemost. 2011; 37: 408-424.

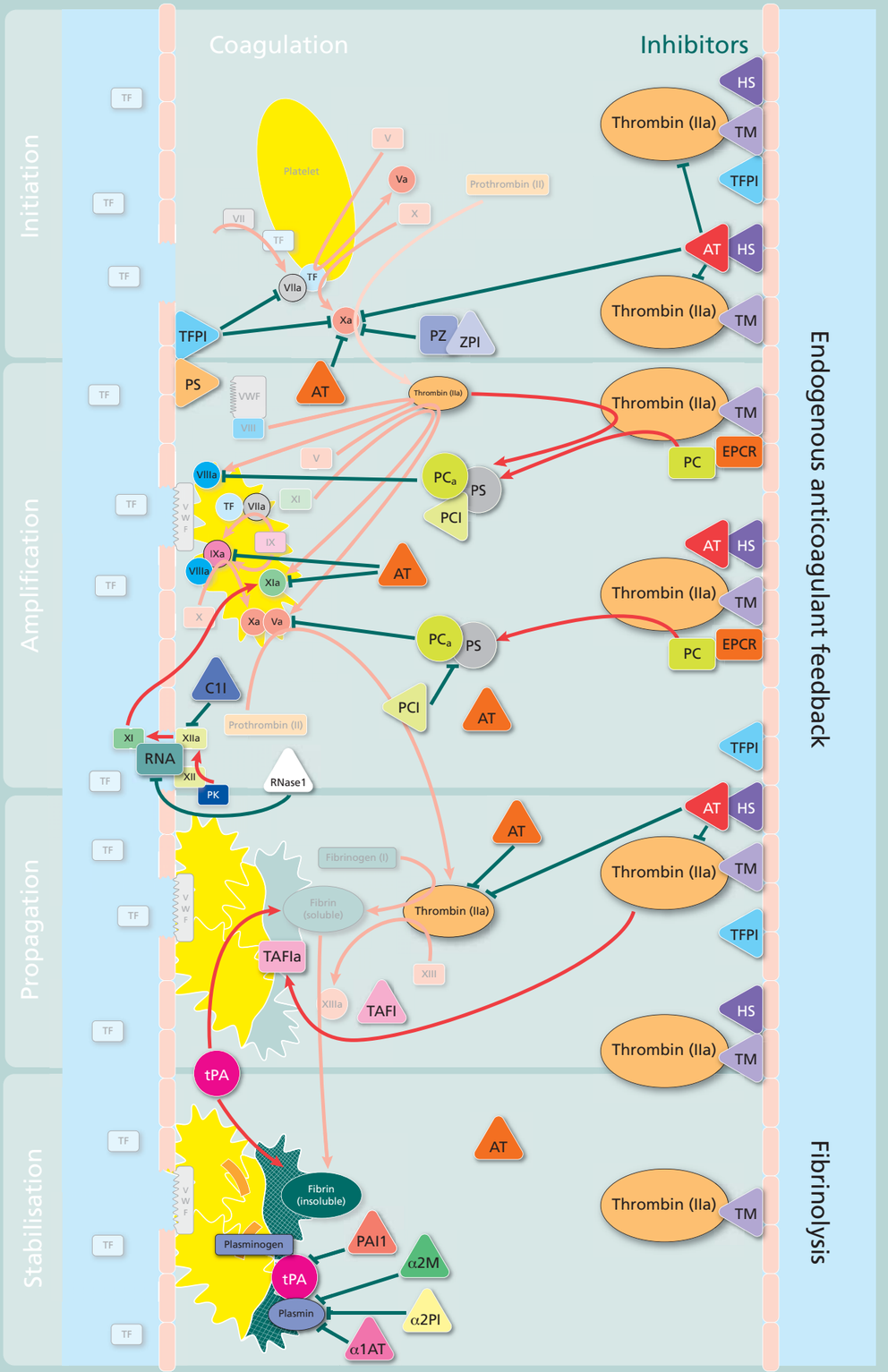
Protein Z (PZ)/Protein Z inhibitor (PZI)

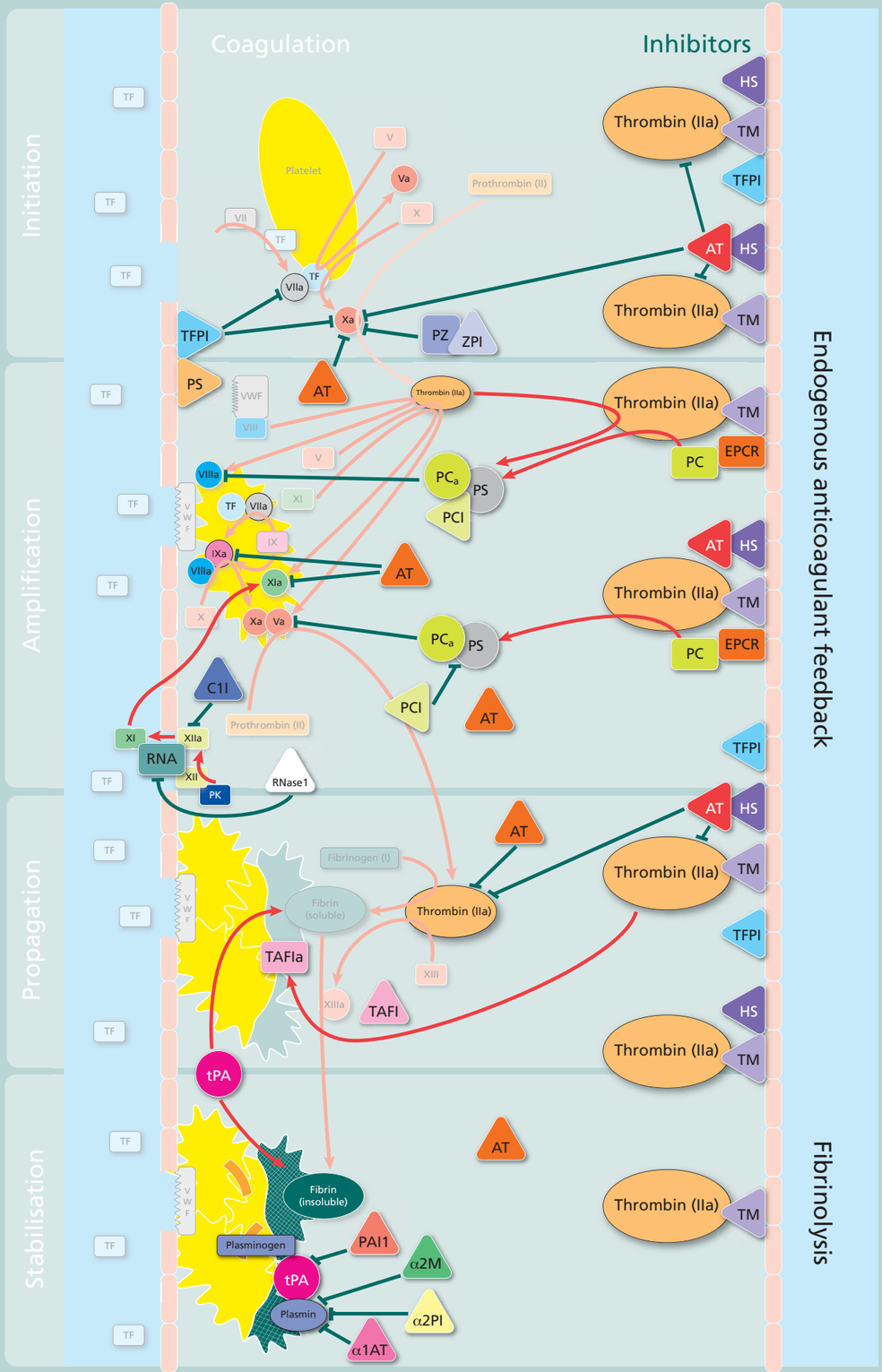


Protein Z is a vitamin-K-dependent anticoagulant protein that presents structural homology to protein C and protein S. Unlike activated protein C (PCa) protein Z does not contain any enzymatic activity, but enhances the anti-factor Xa function of the protein Z-dependent protein inhibitor (PZI). Since the PZ/PZI complex is involved in the intrinsic anticoagulation, protein Z-deficiency appears to contribute to the occurrence of cardio-vascular diseases. In particular, PZ-deficiency could increase the risk of established thrombotic risk factors such as factor V-Leiden, the G20210A-prothrombin mutation or hyperhomocysteinemia.

Al-Shanqeeti A, van Hylckama Vlieg A, Berntorp E, Rosendaal FR, Broze GJ. Protein Z and protein Z-dependent protease inhibitor. Determinants of levels and risk of venous thrombosis. Thromb Haemost. 2005; 93: 411-413.
Vasse M. Protein Z, a protein seeking a pathology. Thromb Haemost. 2008; 100: 548-556.

Notes


Coagulation
Inhibitors
Initiation
Amplification
Propagation
Stabilisation
Fibrinolysis
Endogenous anticoagulant feedback
TF
VII
VIIa
Platelet
V
Va
X
Prothrombin (II)
Xa
TFPI
PZ
ZPI
AT
PS
VWF
VIII
Thrombin (IIa)
HS
TM
EPCR
PC
PCa
PCI
VIIIa
IXa
IX
XI
XIa
C1I
XIIa
XII
RNA
PK
RNase1
Fibrinogen (I)
Fibrin (soluble)
TAFIa
XIII
XIIIa
TAFI
tPA
Fibrin (insoluble)
Plasminogen
PAI1
α2M
Plasmin
α2PI
α1AT



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