Blood Biochemistry
BCH 471
Blood clotting

Blood clotting

- It is the conversion, catalyzed by thrombin, of the soluble plasma protein fibrinogen (factor I) into polymeric fibrin, which is deposited as a fibrous network in the primary thrombus.
- Thrombin (factor IIa) is a serine proteinase that cleaves small peptides from fibrinogen. This exposes binding sites that spontaneously allow the fibrin molecules to aggregate into polymers.
- Subsequent covalent cross-linking of fibrin by a transglutaminase (factor XIII) further stabilizes the thrombus.

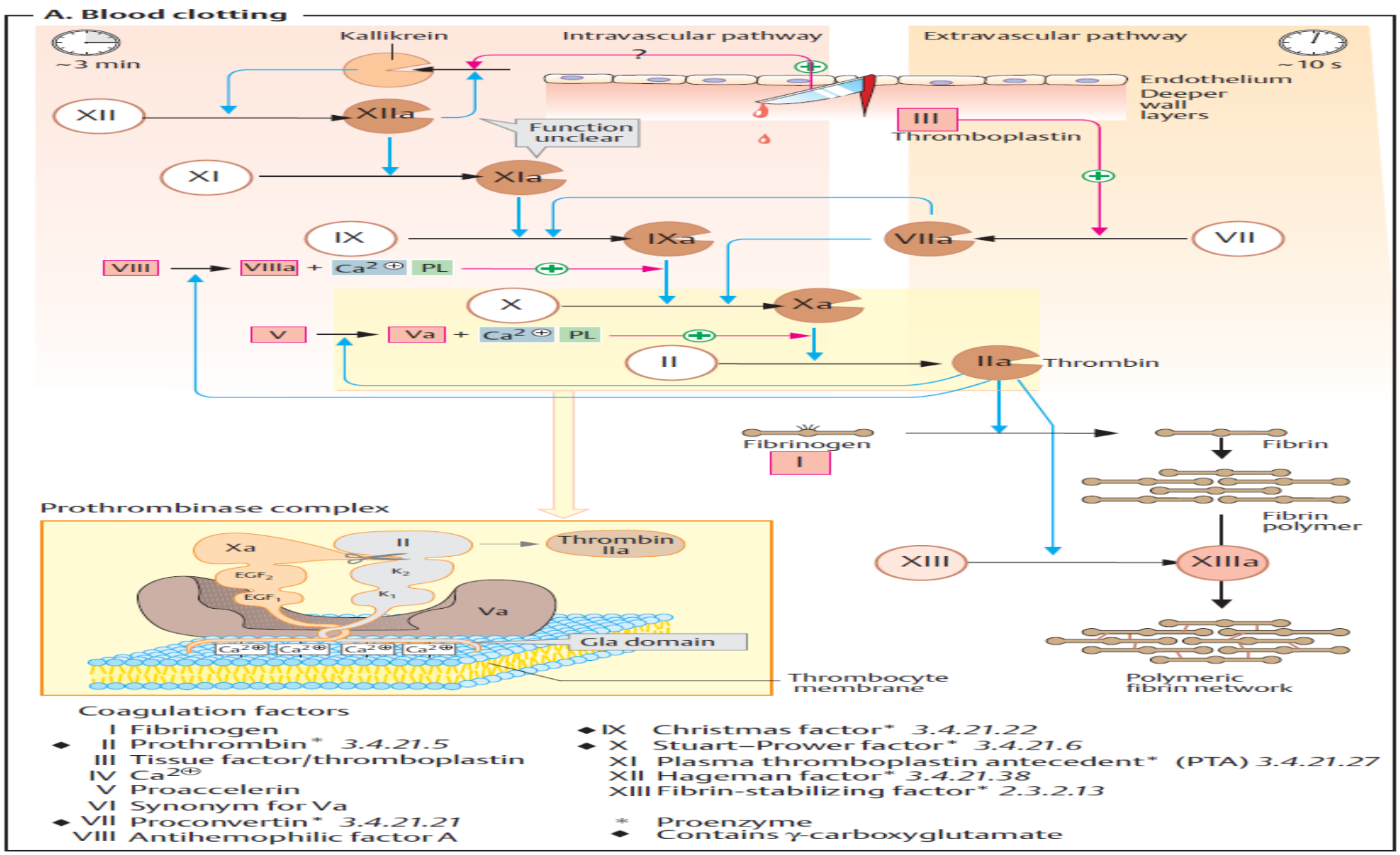
- Thrombin is present in the blood as an inactive proenzyme. Prothrombin is activated in two different ways, both of which represent cascades of enzymatic reactions in which inactive proenzymes are proteolytically converted into active proteinases.
- The proteinases activate the next proenzyme in turn, and so on. Several steps in the cascade require additional protein factors (factors III, Va and VIIIa) as well as anionic phospholipids and Ca2+ ions. Both pathways are activated by injuries to the vessel wall.

Extravascular pathway:

Tissue thromboplastin (factor III), a membrane protein in the deeper layers of the vascular wall, activates coagulation factor VII. The activated form of this (VIIa) autocatalytically promotes its own synthesis and also generates the active factors IXa and Xa from their precursors. With the aid of factor VIIIa, PL, and Ca2+, factor IXa produces additional Xa, which finally—with the support of Va, PL, and Ca2+—releases active thrombin.

The intravascular pathway:

- Triggered by vascular injuries.
- It leads in five steps via factors XIIa, XIa, IXa, and Xa to the activation of prothrombin. The significance of this pathway in vivo has been controversial since it was found that a genetic deficiency in factor XII does not lead to coagulation disturbances.
- Both pathways depend on the presence of activated thrombocytes, on the surface of which several reactions take place.


A. Blood clotting
~3 min
Kallikrein
Intravascular pathway
?
Extravascular pathway
~10 s
Endothelium
Deeper wall layers
XII
XIIa
Function unclear
III
Thromboplastin
XI
XIa
IX
IXa
VIIa
VII
VIII
VIIIa + Ca2⊕ PL
X
Xa
V
Va + Ca2⊕ PL
II
IIa
Thrombin
Fibrinogen
I
Fibrin
Fibrin polymer
XIII
XIIIa
Polymeric fibrin network
Prothrombinase complex
Xa
II
Thrombin IIa
EGF2
EGF1
K2
K1
Va
Gla domain
Ca2⊕
Thrombocyte membrane
Coagulation factors
I Fibrinogen
◆ II Prothrombin* 3.4.21.5
III Tissue factor/thromboplastin
IV Ca2⊕
V Proaccelerin
VI Synonym for Va
◆ VII Proconvertin* 3.4.21.21
VIII Antihemophilic factor A
◆ IX Christmas factor* 3.4.21.22
◆ X Stuart–Prower factor* 3.4.21.6
XI Plasma thromboplastin antecedent* (PTA) 3.4.21.27
XII Hageman factor* 3.4.21.38
XIII Fibrin-stabilizing factor* 2.3.2.13
* Proenzyme
◆ Contains γ-carboxyglutamate

The prothrombinase complex forms when factors Xa and II, with the help of Va, bind via Ca^{2+} ions to anionic phospholipids in the thrombocyte membrane. For this to happen, factors II and X have to contain the non-proteinogenic amino acid γ-carboxyglutamate (Gla), which is formed in the liver by post-translational carboxylation of the factors. The Gla residues are found in groups in special domains that create contacts to the Ca^{2+} ions. Factors VII and IX are also linked to membrane phospholipids via Gla residues.

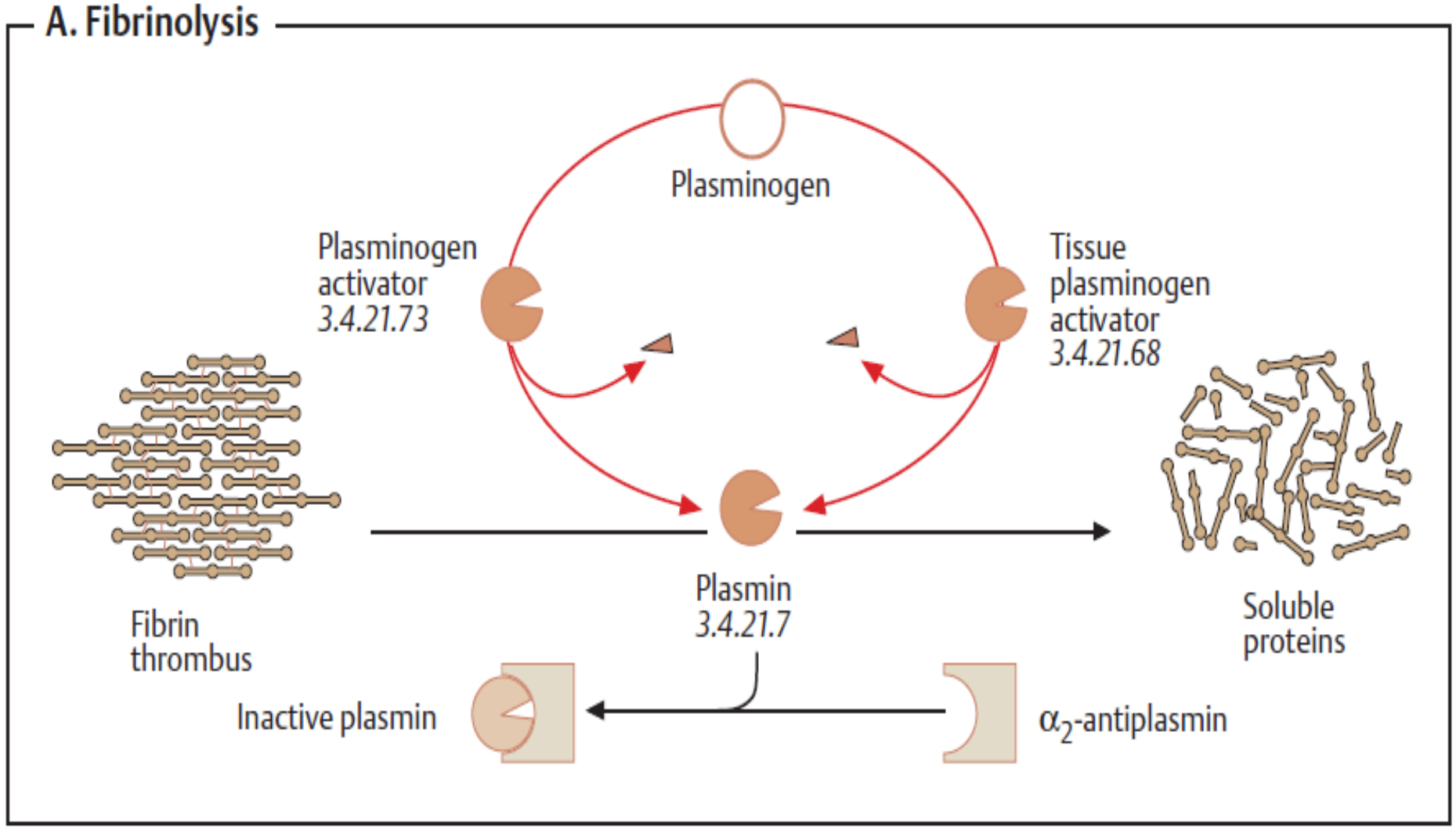
Substances that bind Ca2+ ions (e. g., citrate) prevent Gla-containing factors from attachingto the membrane and therefore inhibit coagulation. Antagonists of vitamin K, which is needed for synthesis of the Gla residues also have anticoagulatory effects. These include dicumarol, for example. Active thrombin not only converts fibrinogen into fibrin, but also indirectly promotes its own synthesis by catalyzing the activation of factors V and VIII. In addition, it catalyzes the activation of factor XIII and thereby triggers the cross-linking of the fibrin.

Regulation of blood clotting:

To prevent the coagulation reaction from becoming excessive, the blood contains a number of anticoagulant substances, including highly effective proteinase inhibitors. For example, antithrombin III binds to various serine proteinases in the cascade and thereby inactivates them. Heparin, an anticoagulant glycosaminoglycan, potentiates the effect of antithrombin III. Thrombomodulin, which is located on the vascular endothelia, also inactivates thrombin. A glycoprotein known as Protein C ensures proteolytic degradation of factors V and VIII. As it is activated by thrombin, coagulation is shut down in this way.

Fibrinolysis:

The fibrin thrombus resulting from blood clotting is dissolved again by plasmin, a serine proteinase found in the blood plasma. For this purpose, the precursor plasminogen first has to be proteolytically activated by enzymes from various tissues. This group includes the plasminogen activator from the kidney (urokinase) and tissue plasminogen activator (t-PA) from vascular endothelia. By contrast, the plasma protein α2-antiplasmin, which binds to active plasmin and thereby inactivates it, inhibits fibrinolysis. Urokinase, t-PA, and streptokinase, a bacterial proteinase with similar activity, are used clinically to dissolve thrombi following heart attacks. All of these proteins are expressed recombinantly in bacteria.


A. Fibrinolysis
Plasminogen
Plasminogen activator 3.4.21.73
Tissue plasminogen activator 3.4.21.68
Fibrin thrombus
Plasmin 3.4.21.7
Soluble proteins
Inactive plasmin
α_2-antiplasmin