Blood coagulation

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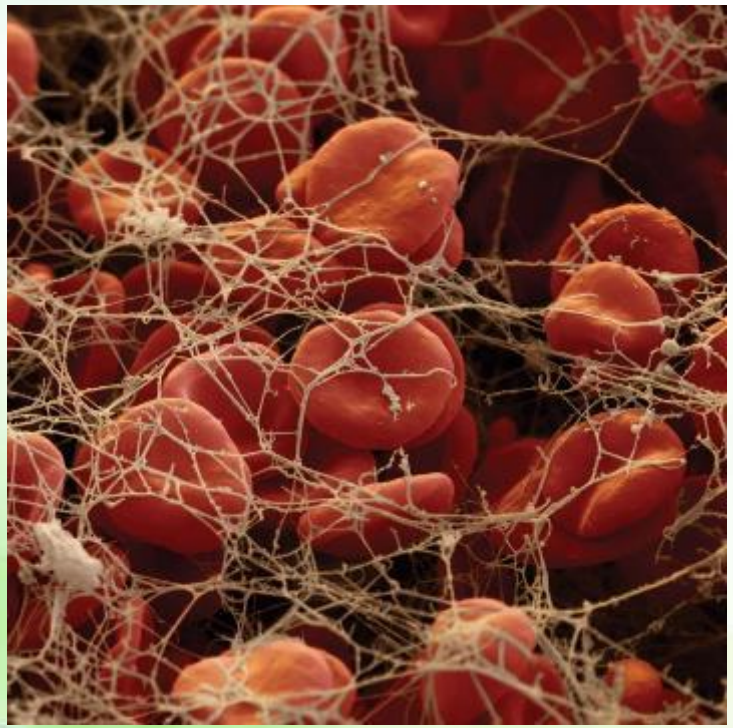
Hematopoietic-lymphatic system

Resources

- This lecture
- Harper's Medical Biochemistry, 31st edition, Chapter 55
- Mark's Basic Medical Biochemistry, 7th edition, Chapter 43

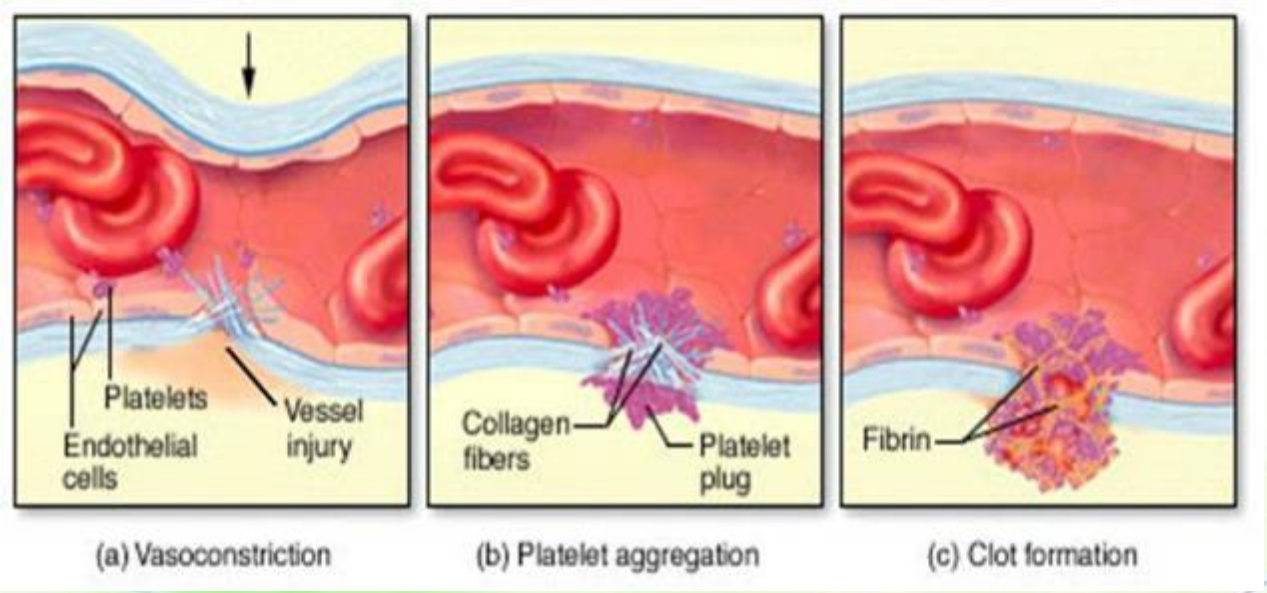
What is blood coagulation (clotting)?

- It is an *orchestrated*, biochemical process that is initiated as a result of vascular injury where a small area blood of surrounding injury changes from liquid to gel, forming a clot made of fibrin, which results in hemostasis (the cessation of blood loss) followed by clot dissolution and repair.



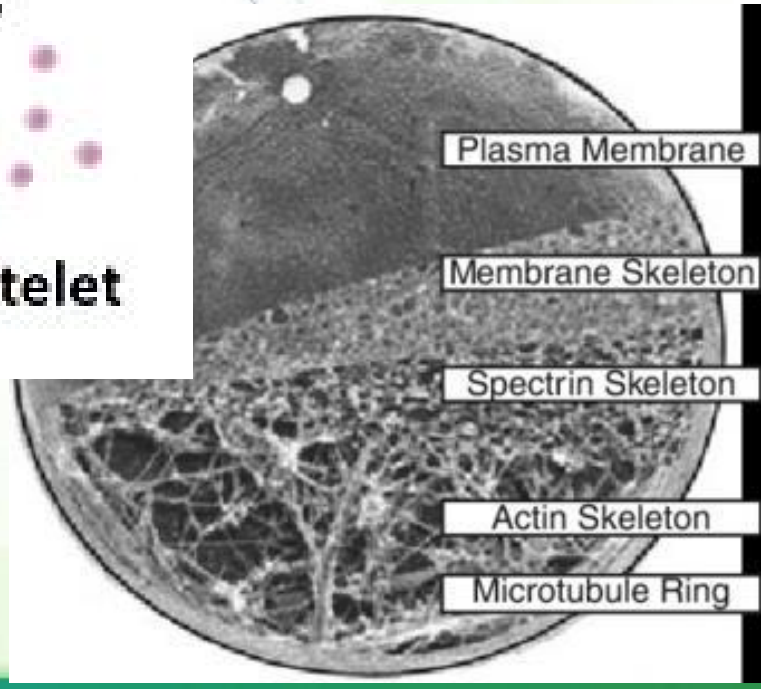
Steps of hemostasis and thrombosis

- Vascular constriction limiting blood flow to the area of injury
- Activation then aggregation of platelets at the site of injury, forming a loose platelet plug
- Formation of a fibrin mesh to entrap the plug
- Dissolution of the clot in order for normal blood flow to resume following tissue repair



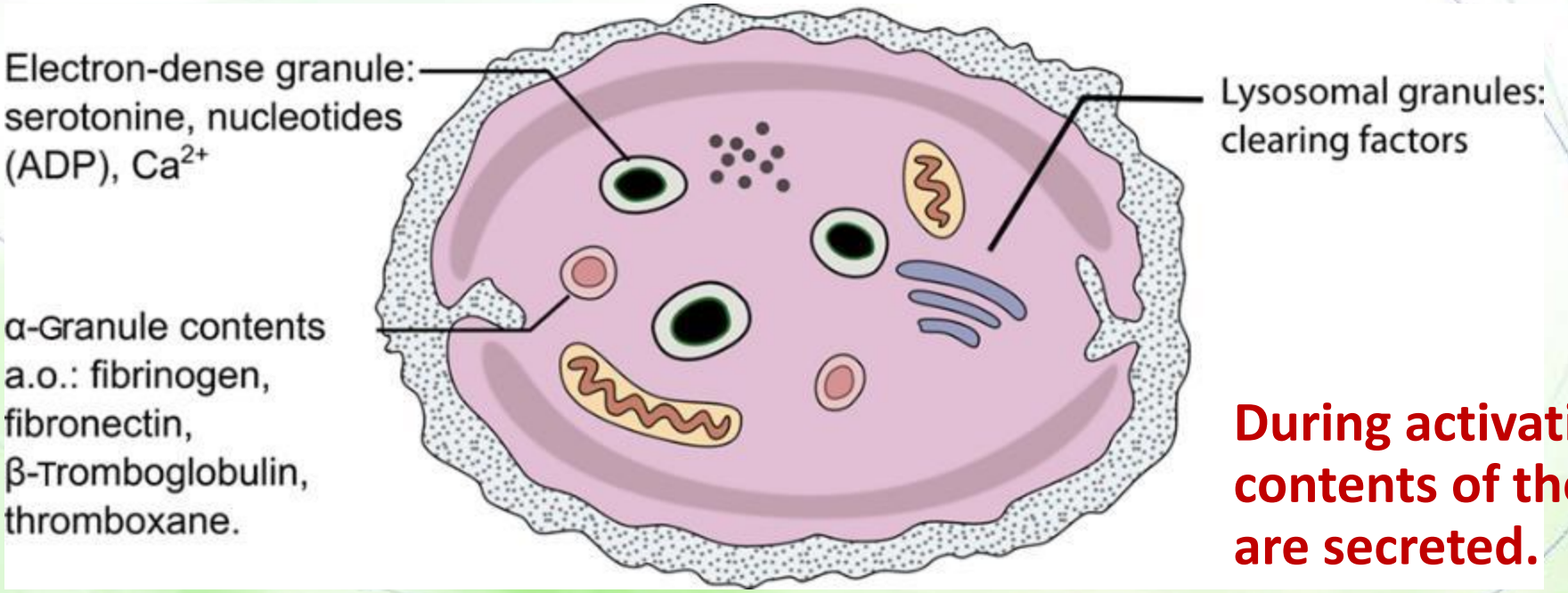
Platelets are a major player

- Small anuclear cell fragments produced from the megakaryocytes.
- Platelets have numerous kinds of surface receptors.
- Platelets also have actin filaments and myosin, which change the shape of the platelet upon activation.
- They also have three types of granules that store substances that are released upon platelet activation.

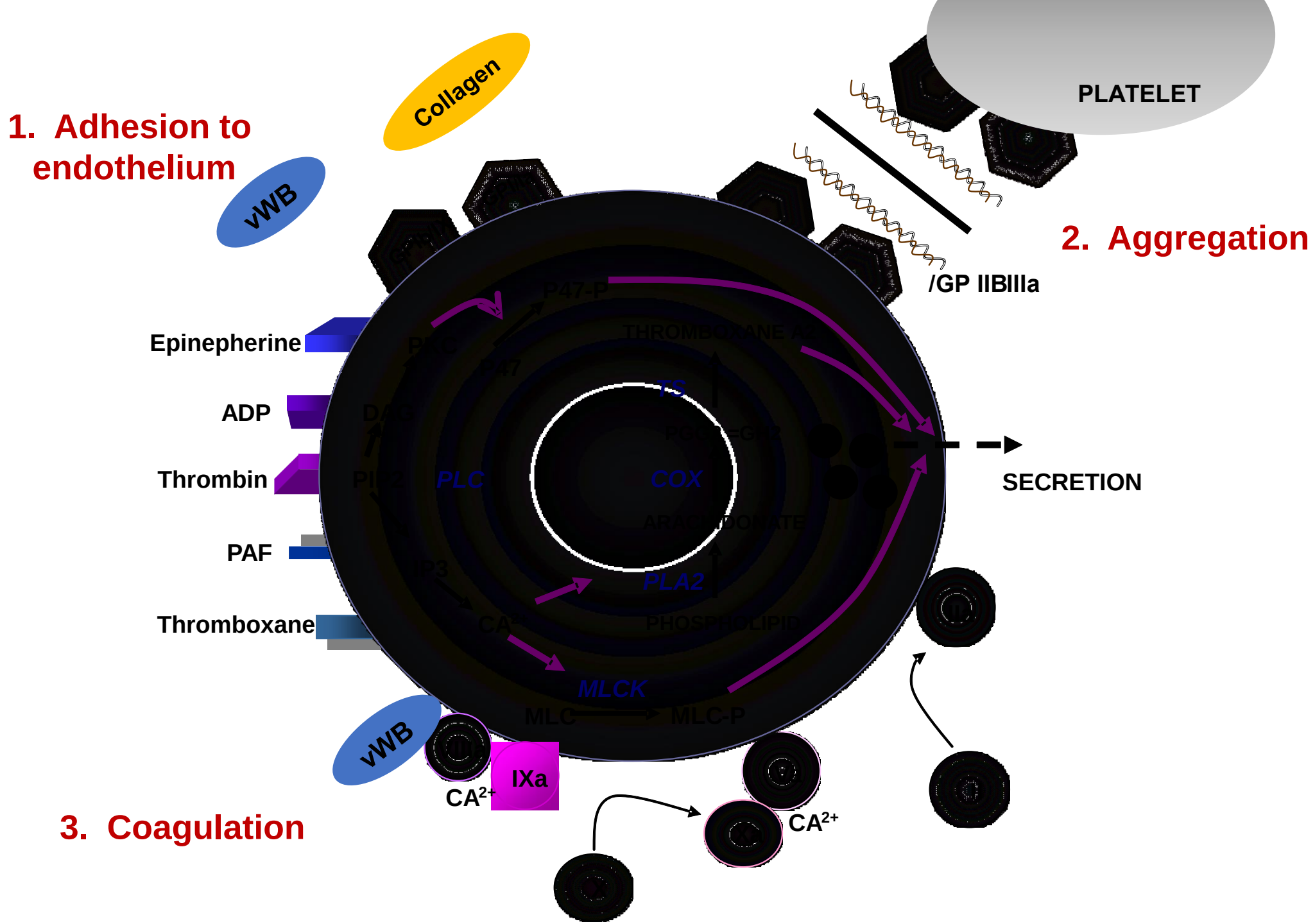


The granules

- Electron-dense granules (calcium ions, ADP, ATP, serotonin)
- α-granule (a heparin antagonist, platelet-derived growth factor, fibrinogen, von Willebrand factor (vWF), clotting factors)
- Lysosomal granules (hydrolytic enzymes)



During activation, the contents of these granules are secreted.

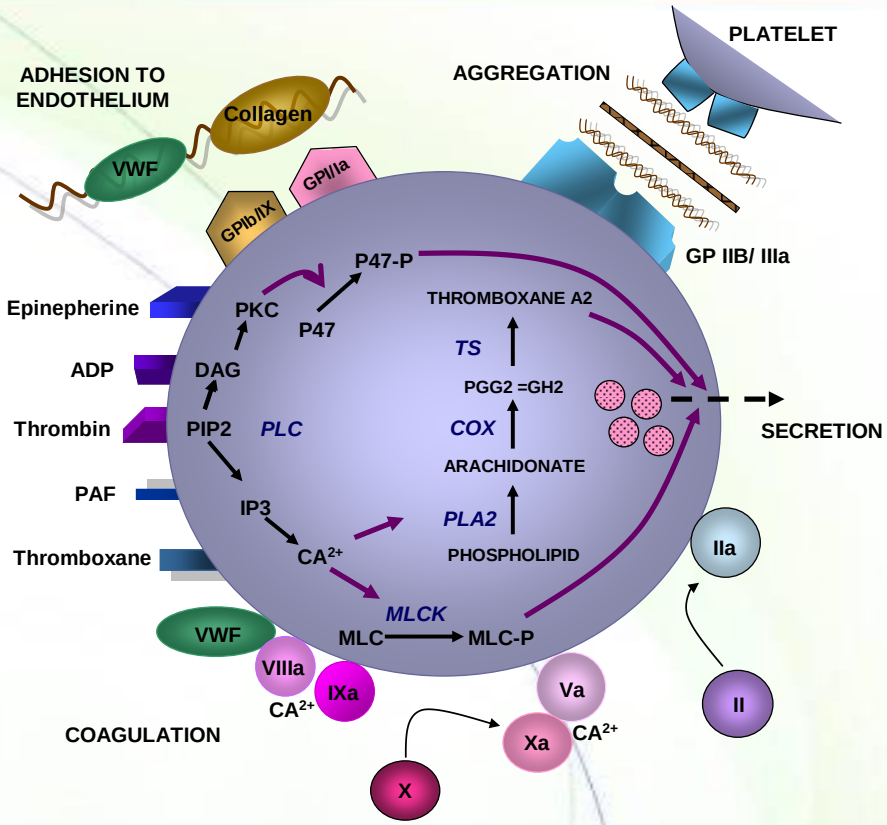

1. Adhesion to endothelium
Collagen
vWB
PLATELET
2. Aggregation
/GP IIBIIIa
Epinepherine
ADP
Thrombin
PAF
Thromboxane
P47-P
THROMBOXANE A2
TS
PLC
COX
ARACHIDONATE
PLA2
PHOSPHOLIPID
MLCK
MLC
MLC-P
SECRETION
vWB
IXa
CA2+
CA2+
3. Coagulation

Adhesion

- The endothelial von Willebrand factor (vWF) protein and exposed collagen bind to the platelet glycoproteins (GP).
- Some platelets release substances from the granules:
  - ADP
  - Serotonin
  - Factor V
  - ATP
  - Calcium
  - Fibrinogen
  - vWF
  - Thrombin
  - Thromoxane

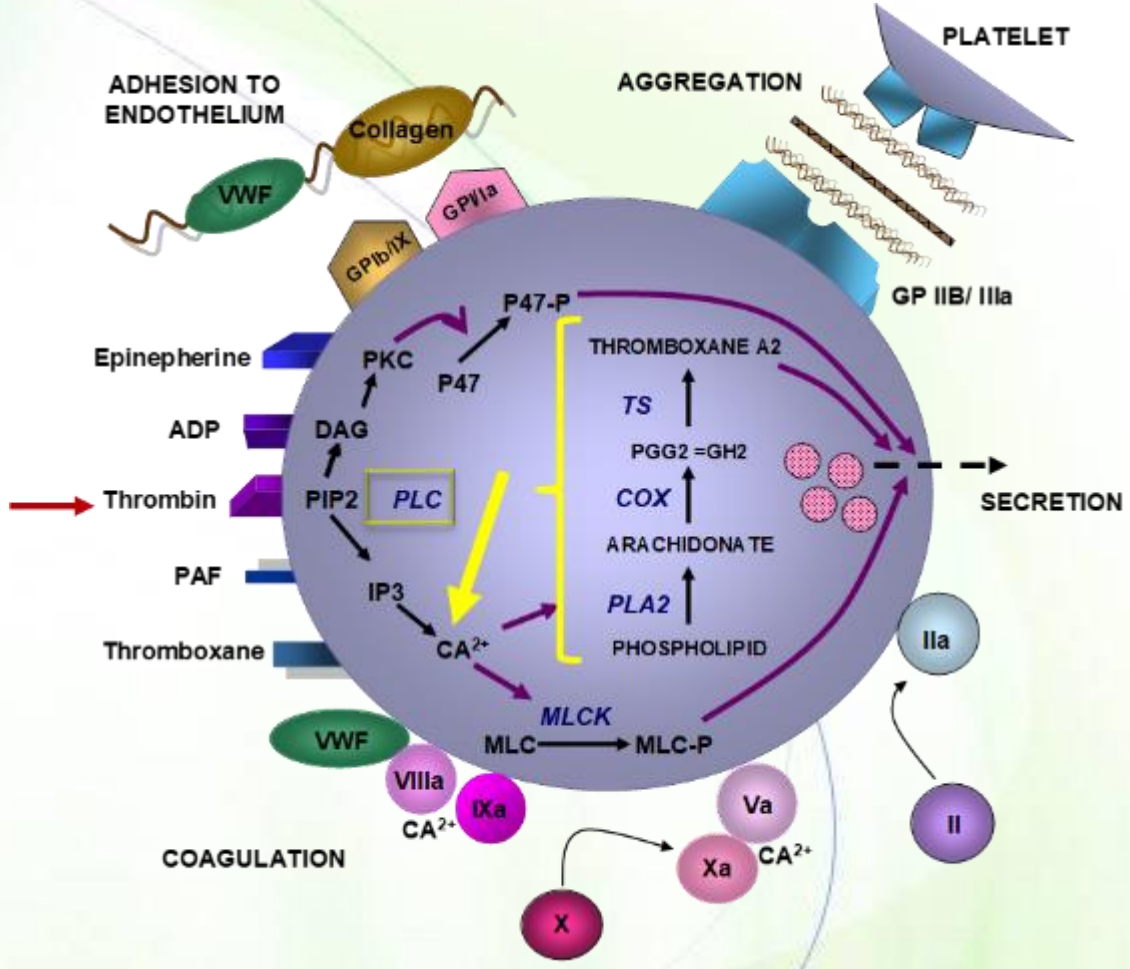
Bind to receptors

- Platelets also change shape allowing for more platelet-platelet interaction and aggregation.



Thrombin receptor

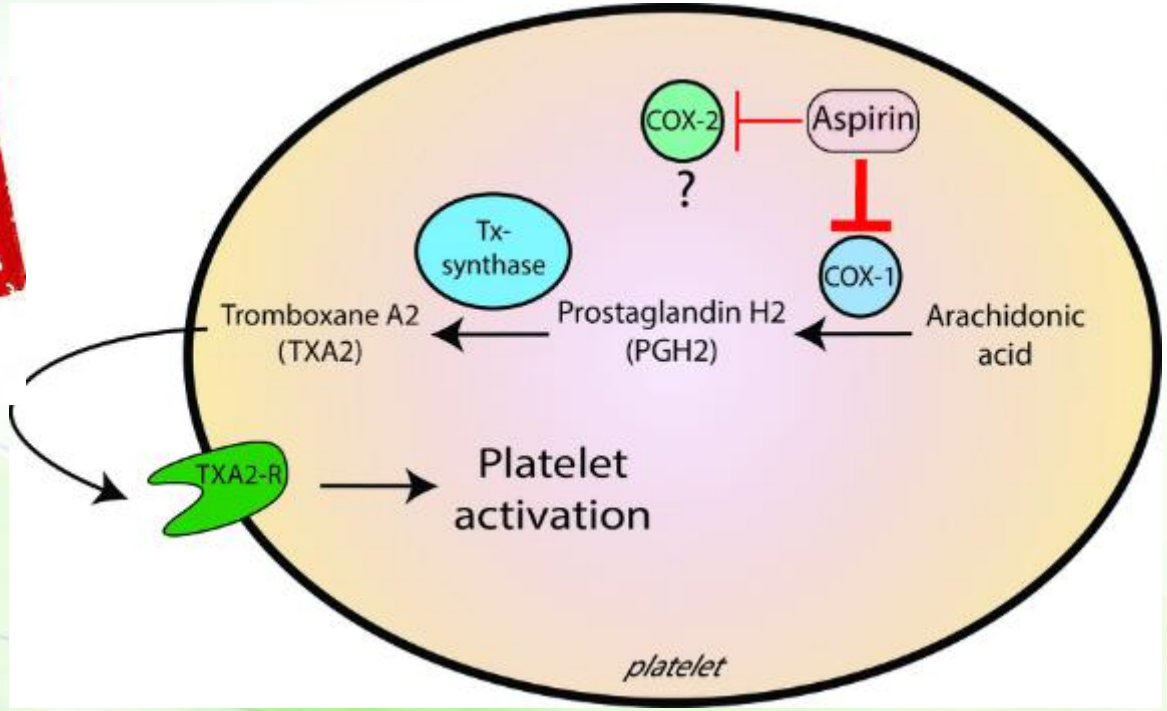
- Thrombin receptor activates a G-protein that activates phospholipase C-β (PLC-β).
- PLC-β hydrolyzes phosphatidylinositol-4,5-bisphosphate (PIP2) into inositol trisphosphate (IP3) and diacylglycerol (DAG).
- IP3 induces the release of intracellular Ca2+ stores, and DAG activates protein kinase C (PKC).
- Calcium triggers liberation of arachidonic acid from membrane phospholipids by the enzyme phospholipase A2.
- Arachidonate is converted by cyclooxygenase to prostaglandins, which are then converted by thromboxane synthetase to thromboxane A2.
  - Thromboxane is as vasoconstrictor and a further inducer of PLC-β activity (and platelet aggregation).
  - It acts in autocrine and paracrine manners.



- ***Serotonin is also a vasoconstrictor.***
- ***PDGF stimulates proliferation of endothelial cells to reduce blood flow.***

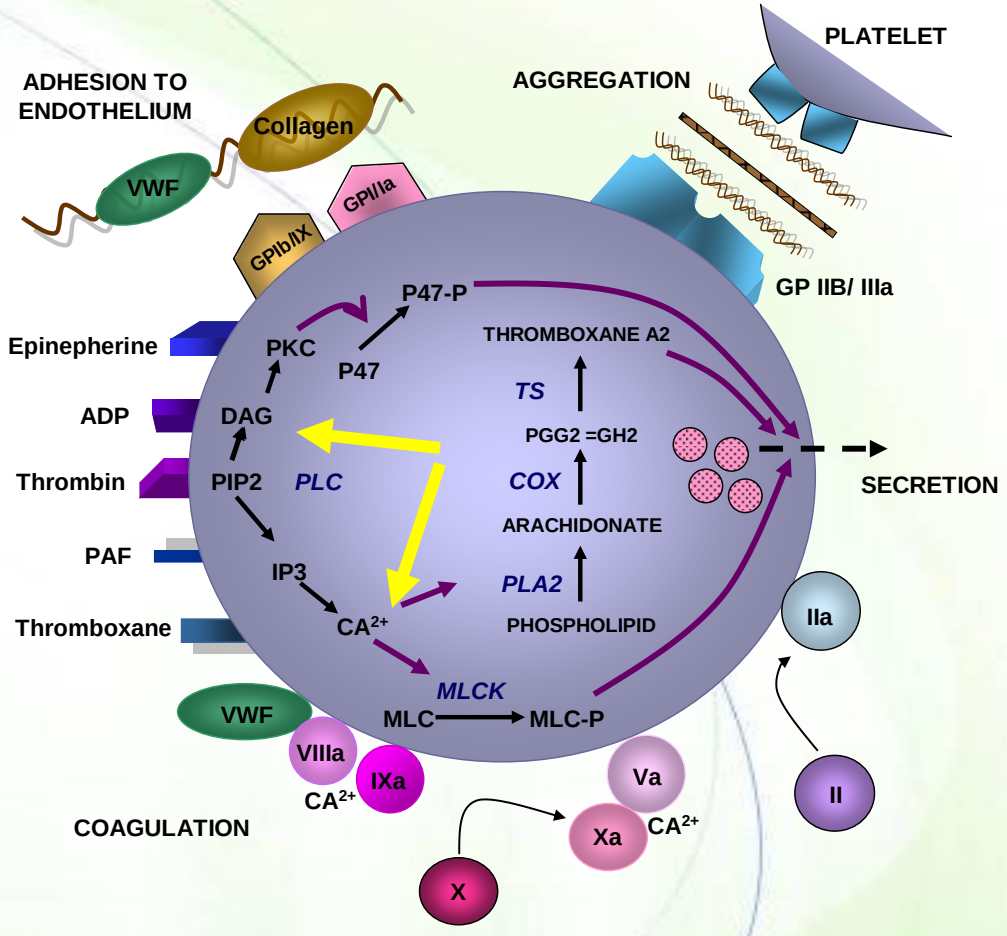
NSAID

- Non-steroidal anti-inflammatory drugs inhibit the enzyme cyclooxygenase, accounting for their anticoagulant effects.
  - Aspirin also inhibits production of endothelial prostacyclin, which opposes platelet aggregation and is a vasodilator, but unlike platelets, these endothelial cells regenerate cyclooxygenase within a few hours. Thus, the overall balance between TxA2 and PGI2 can be shifted in favor of the latter.



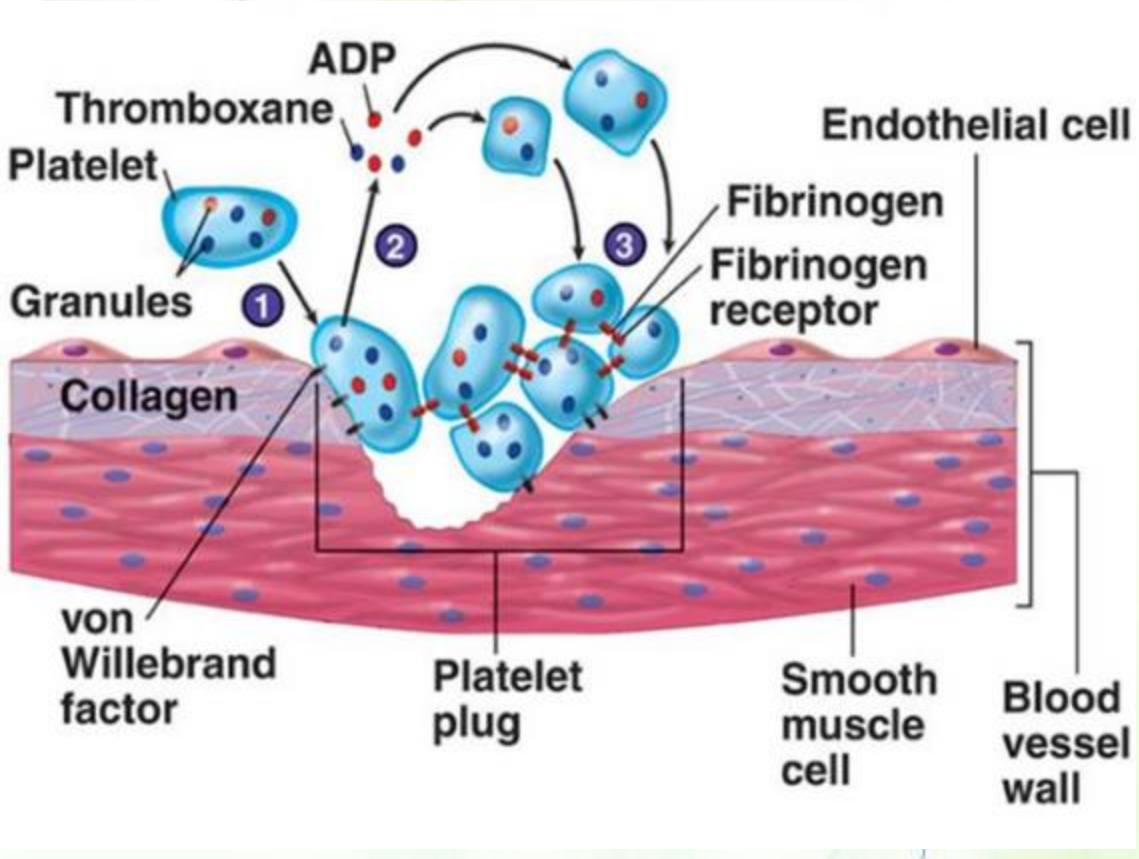
More release of granular contents

- Ca2+ ions activate myosin light chain kinase (MLCK), which phosphorylates the light chain of myosin allowing it to interact with actin and resulting in altered platelet morphology, induced motility, and release of granules.
- DAG activates PKC, which phosphorylates and activates specific platelet proteins that induce the release of platelet granule contents including ADP.



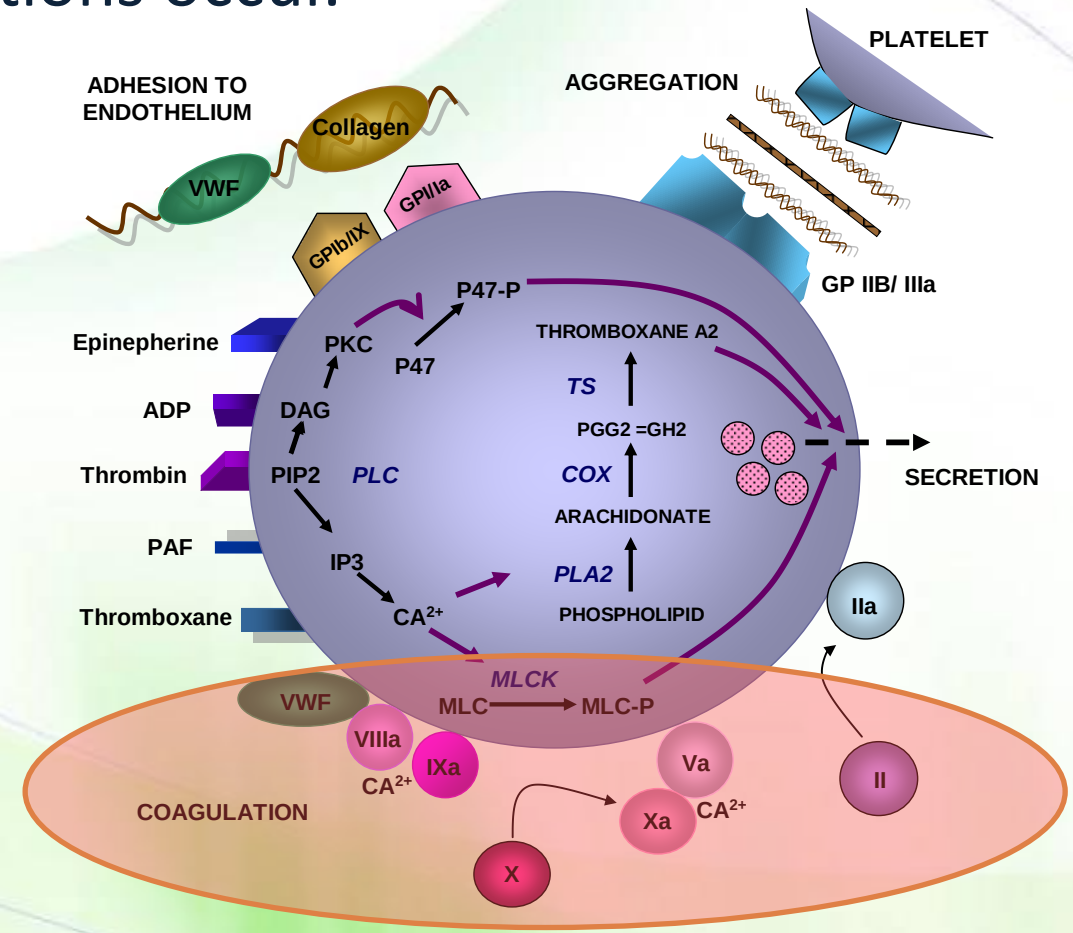
Role of ADP

- ADP is a platelet activator that binds to its receptor and modifies the platelet membrane allowing fibrinogen to adhere to platelet surface glycoproteins resulting in fibrinogen-induced platelet aggregation, called platelet plug.



Role of platelet cell surface

- The accumulated platelet plug provides an important surface on which coagulation reactions occur.



Biochemistry of coagulation

Components of coagulation

- An organizing surface (platelets)
- Proteolytic zymogens (prekallikrein, prothrombin, and factors VII, IX, X, XI, XII, and XIII)
  - These are mainly serine proproteases released from hepatocytes.
  - The subscript "a" designates the activated form of a factor
    - e.g., “XIII" is versus "XIIIa"
- Anti-coagulants (protein C, protein S)
- Non-enzymatic protein cofactors (factors VIII, V, and tissue factor)
- Calcium ions
- Vitamin K
- Fibrinogen

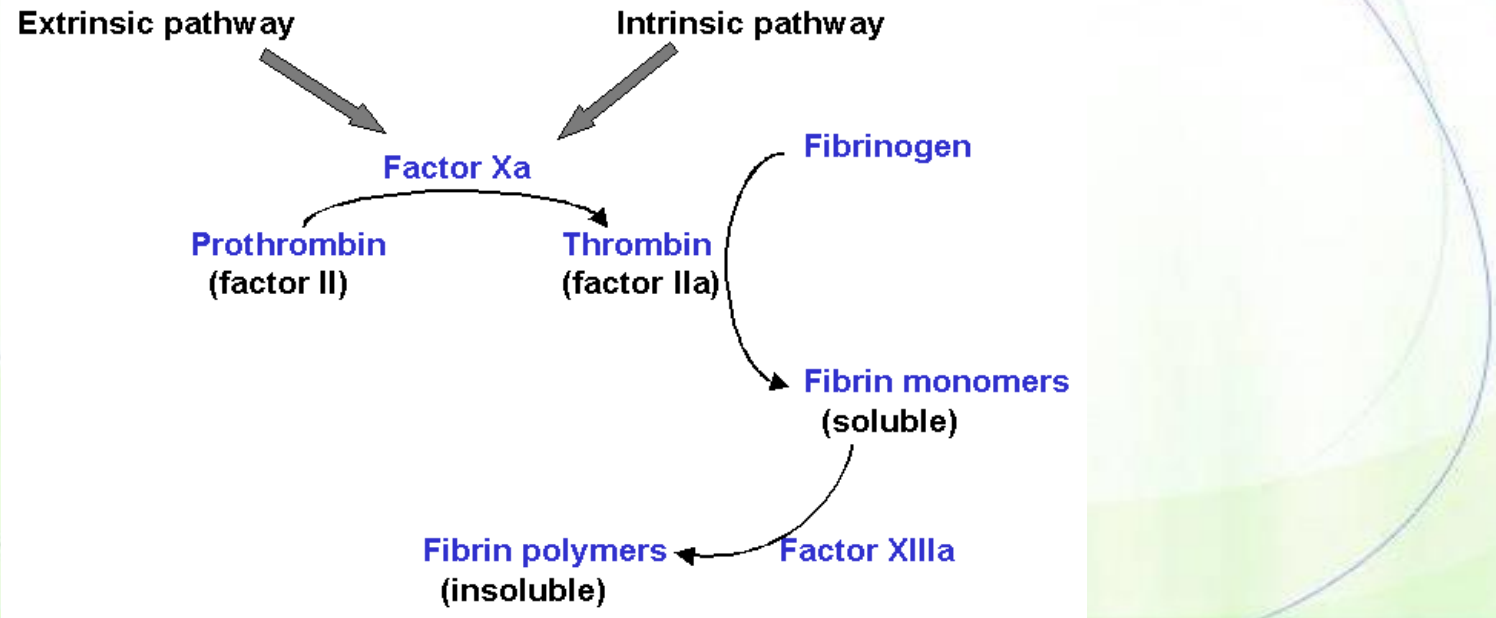
Molecular components of coagulation

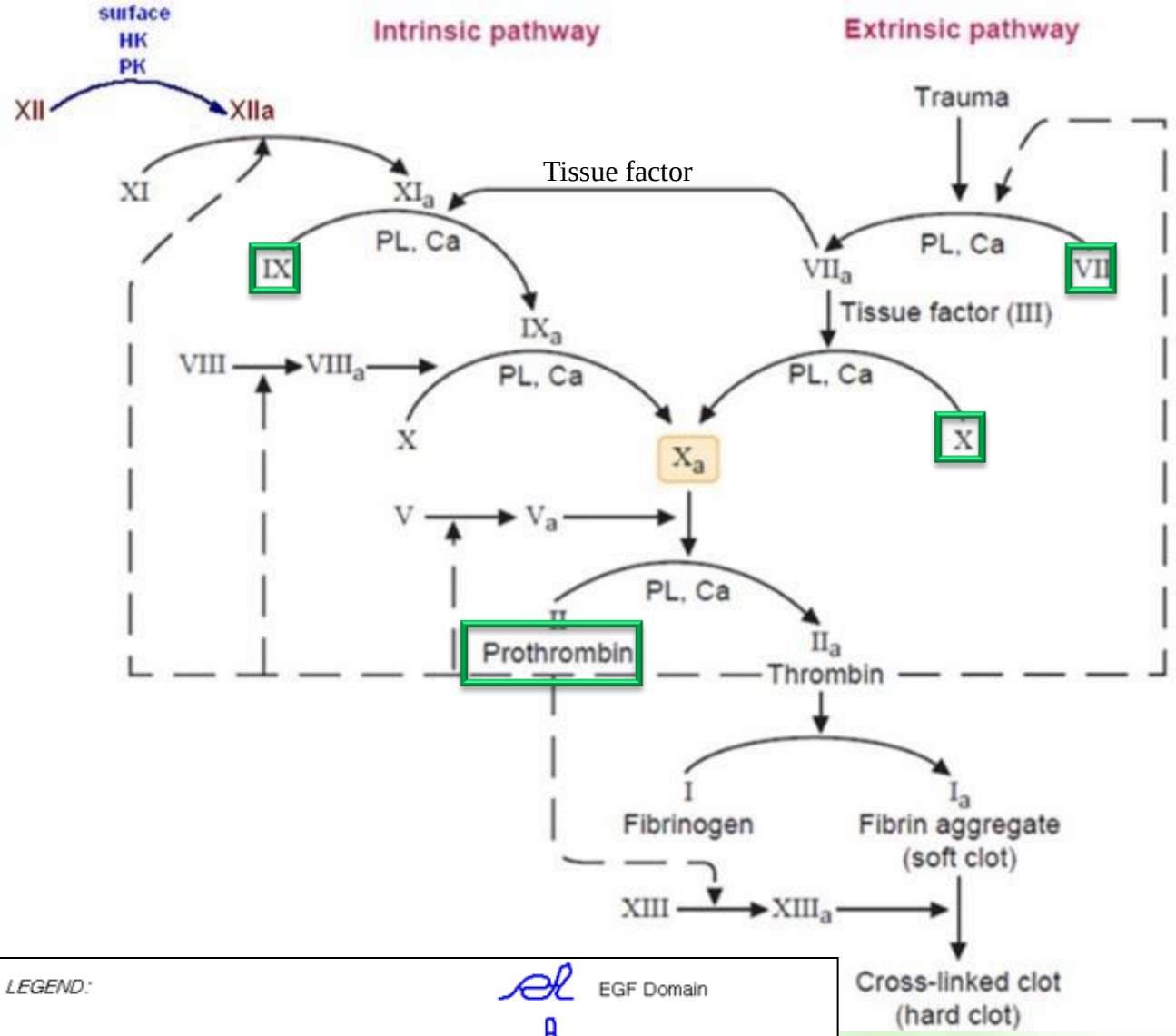
Clotting factor number	Clotting factor name	Function	Plasma half-life (h)
I	Fibrinogen	Clot formation	90
II	Prothrombin	Activation of I, V, VII, VIII, XI, XIII, protein C, platelets	65
III	TF	Co factor of VIIa	-
IV	Calcium	Facilitates coagulation factor binding to phospholipids	-
V	Proacclerin, labile factor	Co-factor of X-prothrombinase complex	15
VI	Unassigned		
VII	Stable factor, proconvertin	Activates factors IX, X	5
VIII	Antihaemophilic factor A	Co-factor of IX-tenase complex	10
IX	Antihaemophilic factor B or Christmas factor	Activates X: Forms tenase complex with factor VIII	25
X	Stuart-Prower factor	Prothrombinase complex with factor V: Activates factor II	40
XI	Plasma thromboplastin antecedent	Activates factor IX	45
XII	Hageman factor	Activates factor XI, VII and prekallikrein	
XIII	Fibrin-stabilising factor	Crosslinks fibrin	200
XIV	Prekallikerin (F Fletcher)	Serine protease zymogen	35
XV	HMWK- (F Fitzgerald)	Co factor	150
XVI	vWf	Binds to VIII, mediates platelet adhesion	12
XVII	Antithrombin III	Inhibits IIa, Xa, and other proteases	72
XVIII	Heparin cofactor II	Inhibits IIa	60
XIX	Protein C	Inactivates Va and VIIIa	0.4
XX	Protein S	Cofactor for activated protein C	

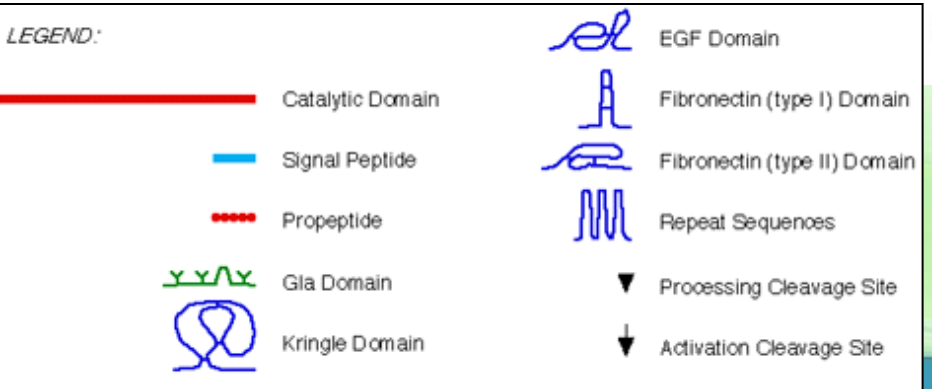
HMWK – High molecular weight kininogen; vWf – Von Willebrand factor; TF – Tissue factor

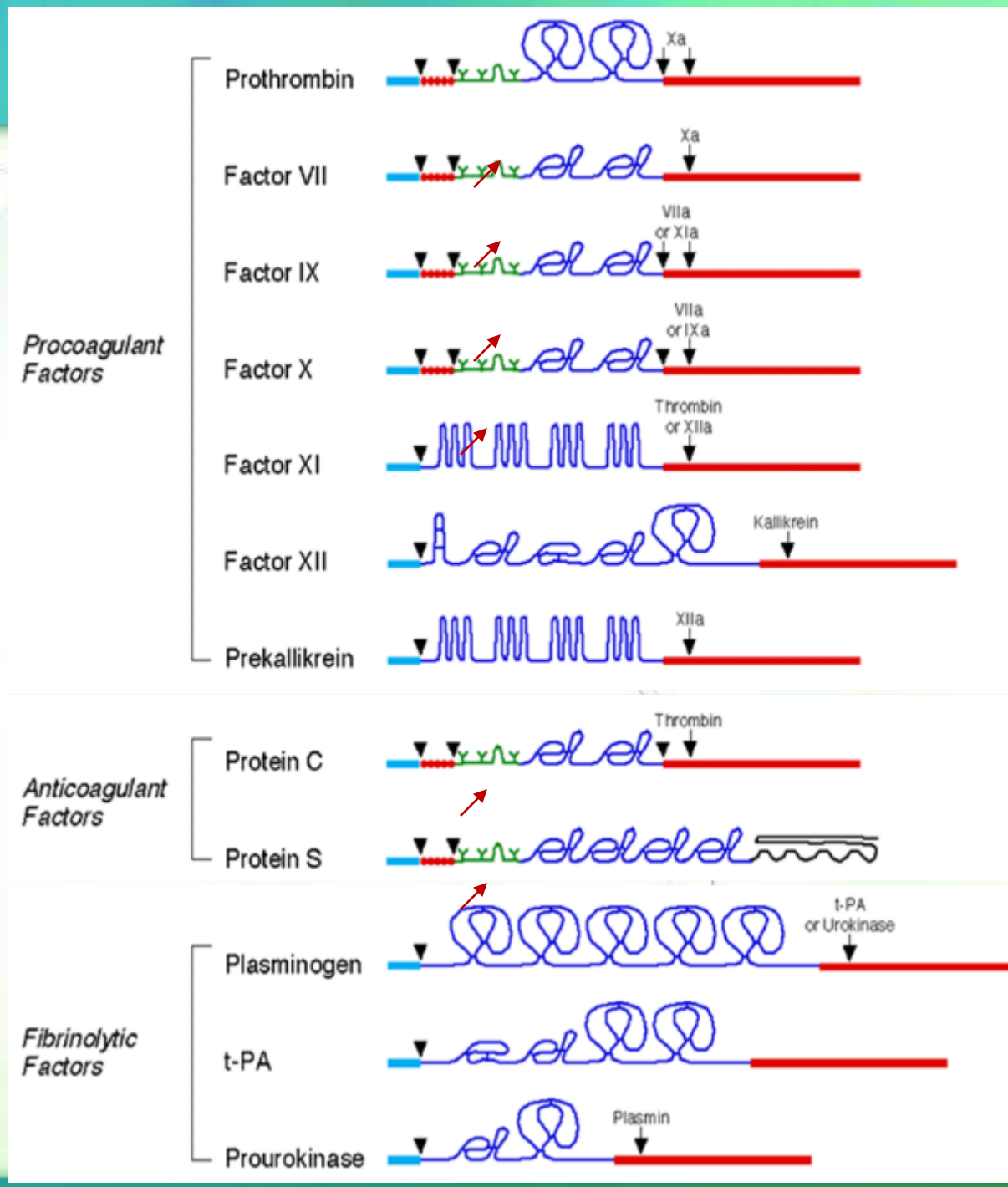
The two pathways

- The intrinsic pathway is initiated when subendothelial surface (i.e., collagen) is exposed.
- The extrinsic pathway is initiated in response to tissue injury.
  - Tissue factor (TF) protein is released.
- However, the two pathways converge on a common pathway.



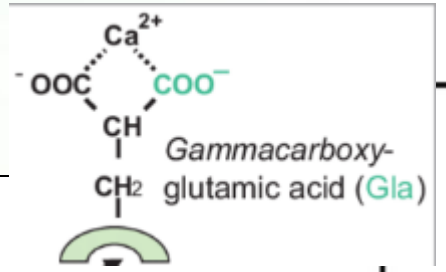

surface
HK
PK
Intrinsic pathway
Extrinsic pathway
XII
XIIa
Trauma
Tissue factor
XI
XIa
PL, Ca
IX
VIIa
PL, Ca
VII
Tissue factor (III)
IXa
VIII
VIIIa
PL, Ca
PL, Ca
X
Xa
X
V
Va
PL, Ca
II
Prothrombin
IIa
Thrombin
I
Fibrinogen
Ia
Fibrin aggregate
(soft clot)
XIII
XIIIa
Cross-linked clot
(hard clot)

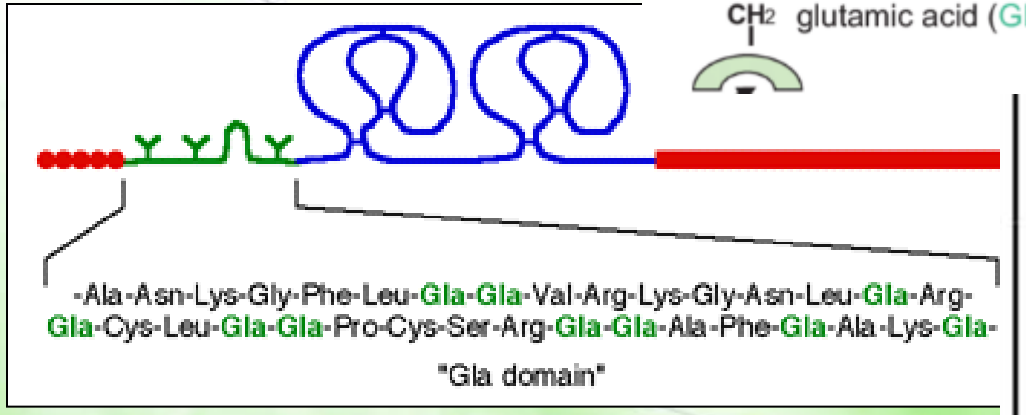

LEGEND:
Catalytic Domain
Signal Peptide
Propeptide
Gla Domain
Kringle Domain
EGF Domain
Fibronectin (type I) Domain
Fibronectin (type II) Domain
Repeat Sequences
Processing Cleavage Site
Activation Cleavage Site

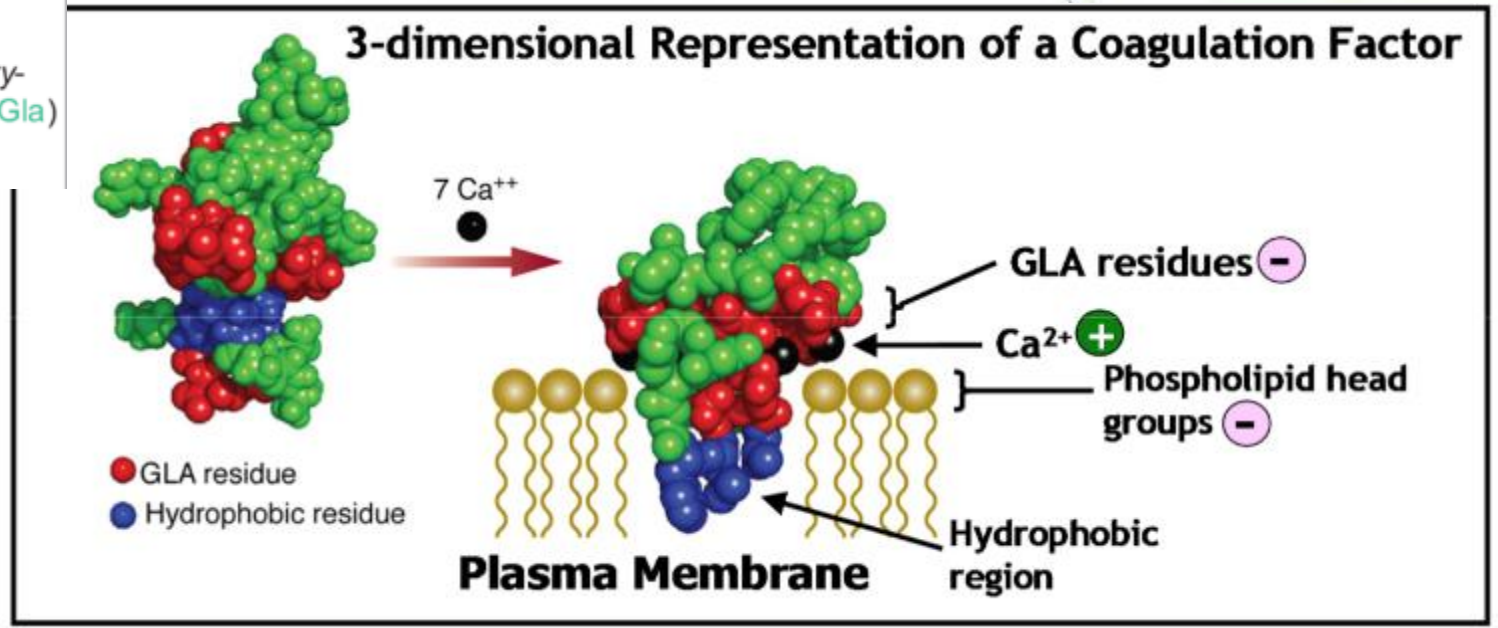

Procoagulant Factors
Prothrombin
Xa
Factor VII
Xa
Factor IX
VIIa or XIa
Factor X
VIIa or IXa
Factor XI
Thrombin or XIIa
Factor XII
Kallikrein
Prekallikrein
XIIa
Anticoagulant Factors
Protein C
Thrombin
Protein S
Fibrinolytic Factors
Plasminogen
t-PA or Urokinase
t-PA
Prourokinase
Plasmin

Gla domain

- An ER/Golgi carboxylase binds to prothrombin and factors IX, VII, and X and converts 10≥ glutamate (Glu) residues to γ-carboxyglutamate (Gla), followed by a small (10 a.a.) hydrophobic region.
- The Gla residues bind calcium ions and are necessary for the activity of these coagulation factors and formation of a coordinated complex with the charged platelet surface to localize the complex assembly and thrombin formation to the platelet surface.

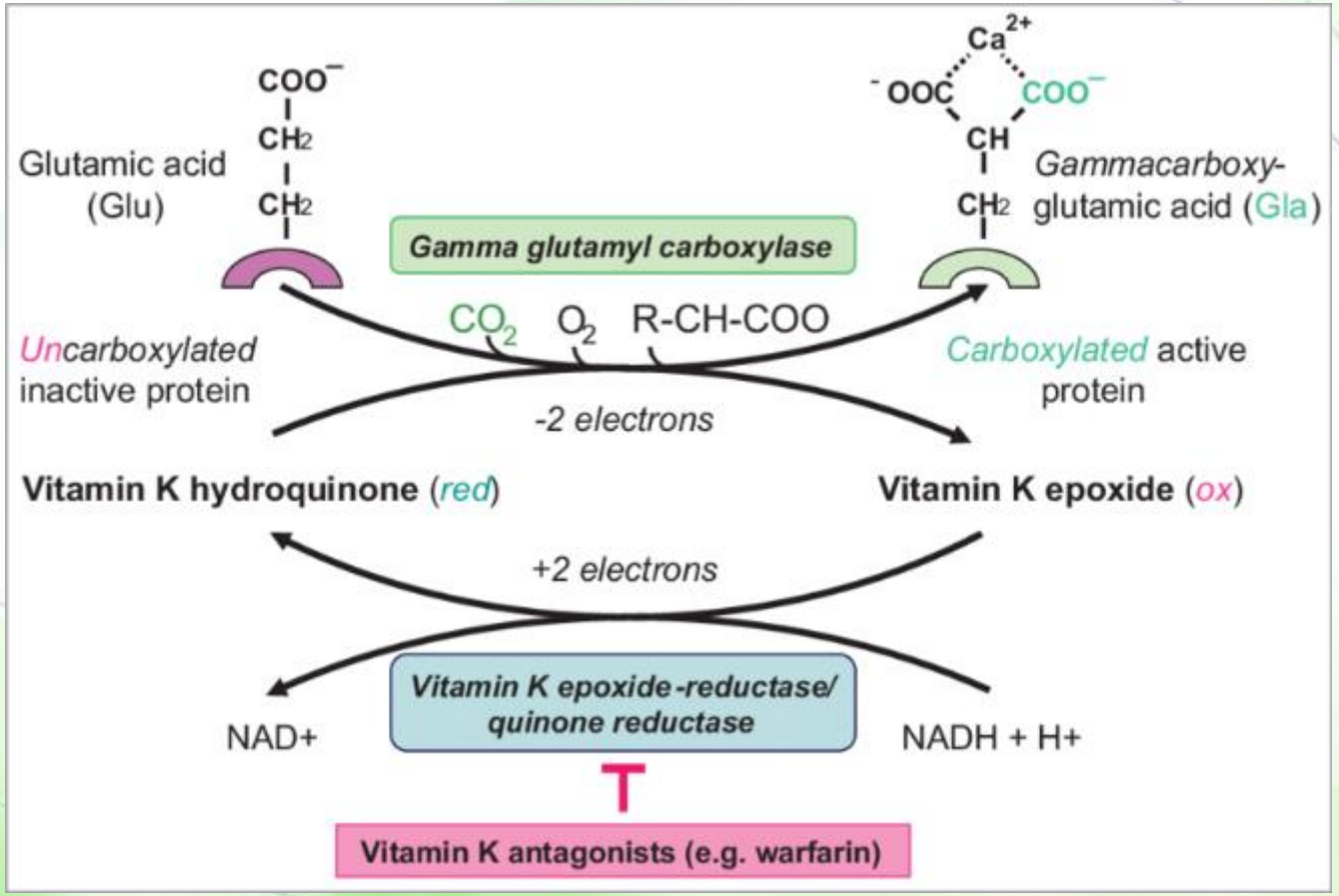






The role of vitamin K

- Vitamin K participates in conversion of Glu to γ-Gla.
- Vitamin K becomes oxidized and must be regenerated.

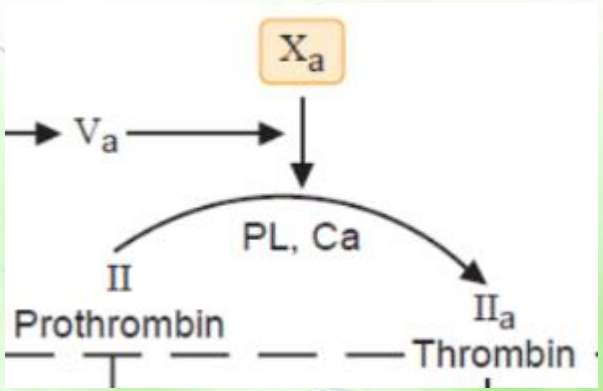


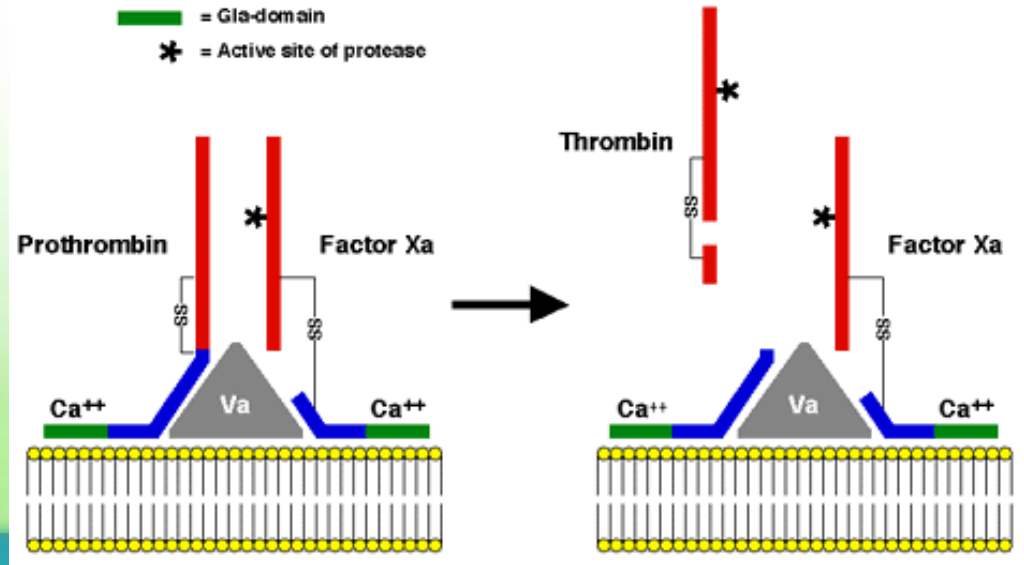
Newborns and vitamin K deficiency

- Newborns are at risk for early vitamin K deficiency bleeding. Why?
  - The placenta is a poor passage channel for fat-soluble compounds, including vitamin K.
  - Neonates are born with an immature liver that impairs coagulation factor synthesis and GLA modifications.
  - Breast milk is a poor source of vitamin K.
  - Intestinal flora, the main source of vitamin K, is not established yet.

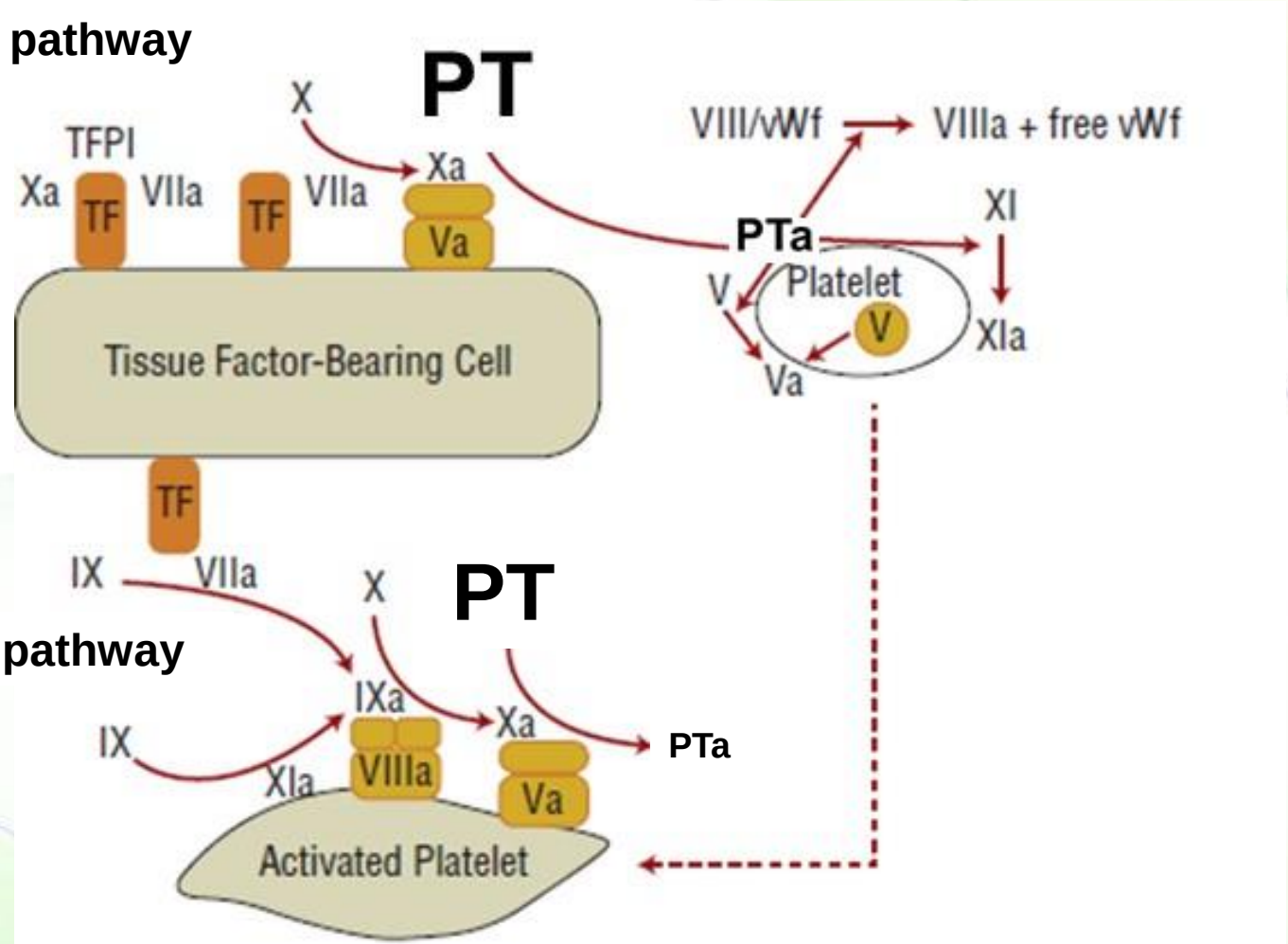
Prothrombin activation

- The complex of factor Xa/Va/Ca^{2+} is the “prothrombinase complex”.
- Factor Xa converts prothrombin to thrombin, which is accelerated by Va, platelets (or phospholipids), and calcium ions.
- Binding of calcium alters the conformation the Gla domains of these factors, enabling them to interact with a membrane surface of platelets.
- Aggregated platelets provide the surface upon which prothrombin activation occurs .





Extrinsic pathway



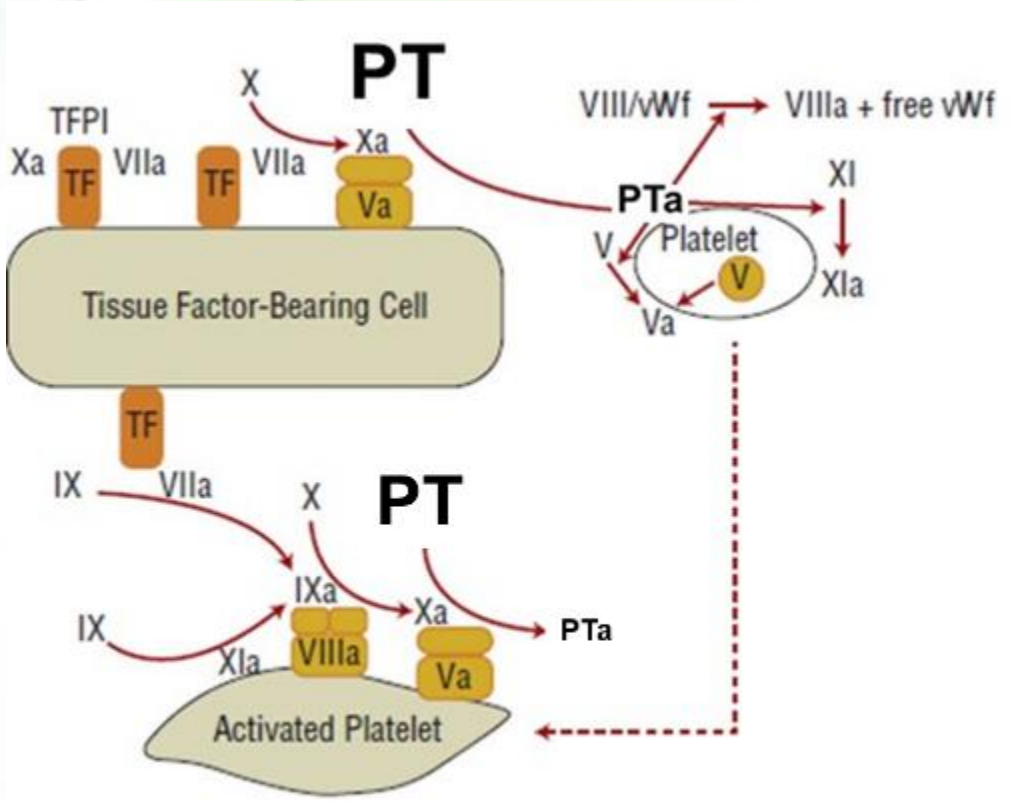
Intrinsic pathway

Source: DiPiro JT, Talbert RL, Yee GC, Matzke GR, Wells BG, Posey LM: *Pharmacotherapy: A Pathophysiologic Approach, 8th Edition*: www.accesspharmacy.com

PT: prothrombin

The tenase complexes

- The activating complexes of factor X are called the “tenase” complexes.
- The intrinsic tenase complex contains the active factor IX (IXa), its cofactor factor VIII (VIIIa), and Ca^{2+}.
- The extrinsic tenase complex is made up of tissue factor, factor VIIa, and Ca^{2+}.
  - Tissue factor and factor VIIa also activate factor IX in the intrinsic pathway.
- Va and VIIIa are cofactors that increase the proteolytic efficiency of Xa and IXa, respectively.
  - Both factors V and VIII are activated by thrombin via a feedback mechanism



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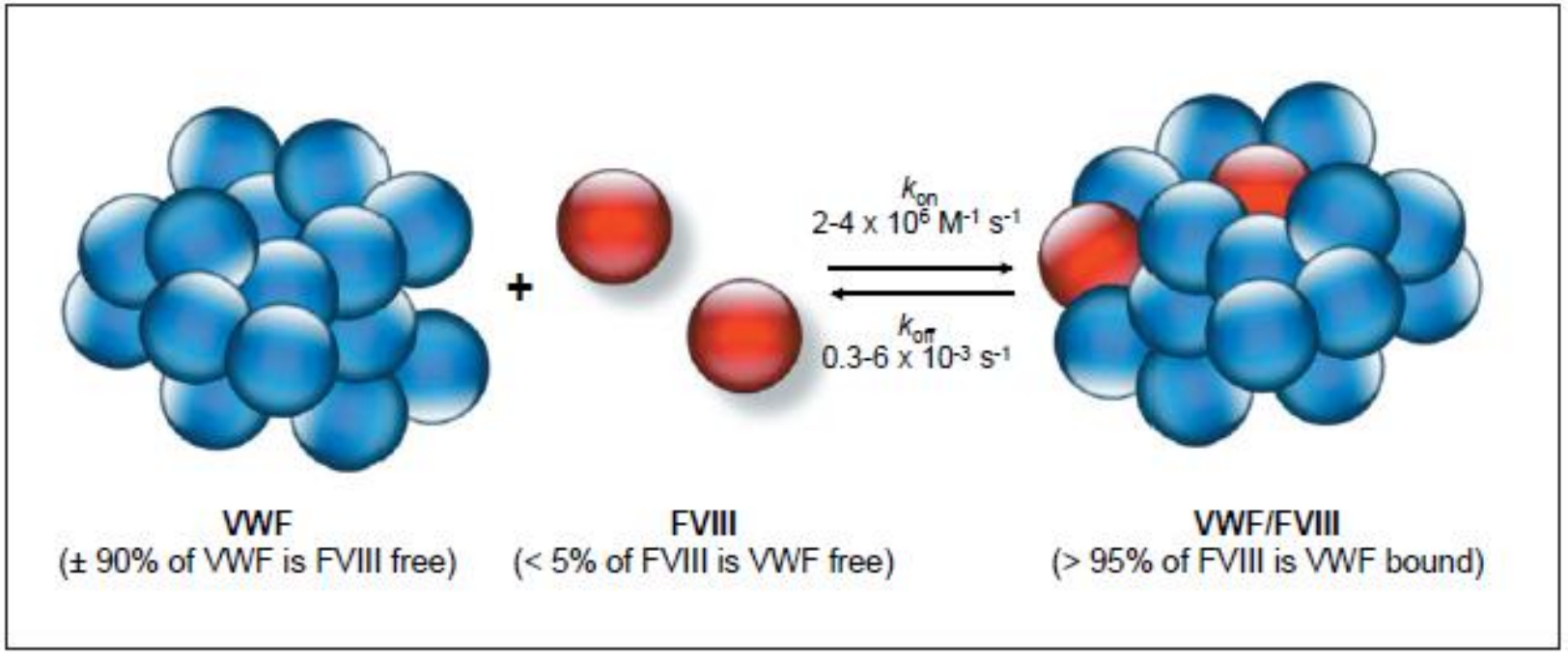
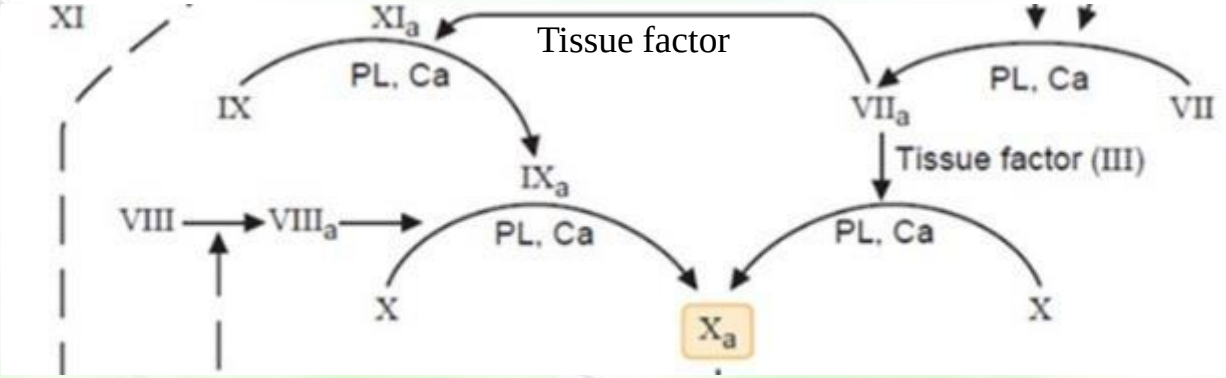
von Willebrand factor deficiency

- Factor VIII circulates in plasma bound to von Willebrand factor, which increases VIII half-life, and, when released, it gets activated.
  - von Willebrand factor deficiency is associated decrease in the plasma concentration of factor VIII.

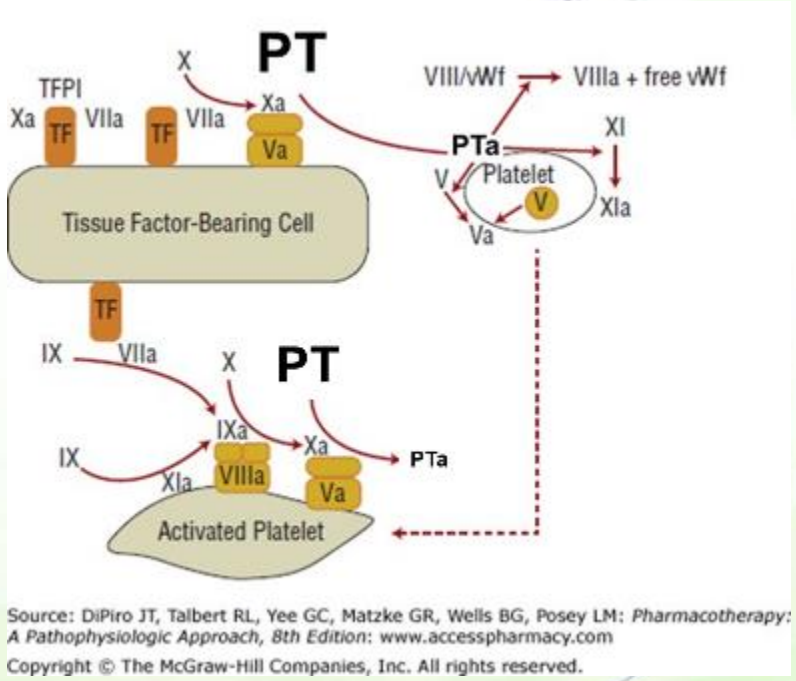


Tissue factor

- Tissue factor is an integral membrane protein that is expressed on the surface of "activated" monocytes, subendothelial cells, and other cells.
- Tissue factor increases the proteolytic efficiency of VIIa.



Exposure of tissue factor initiates the coagulation cascade.
TF/VIIa complex is the “initiation complex”.



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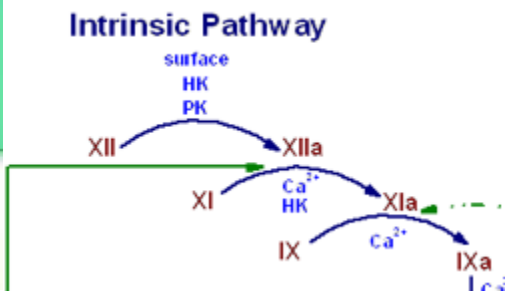
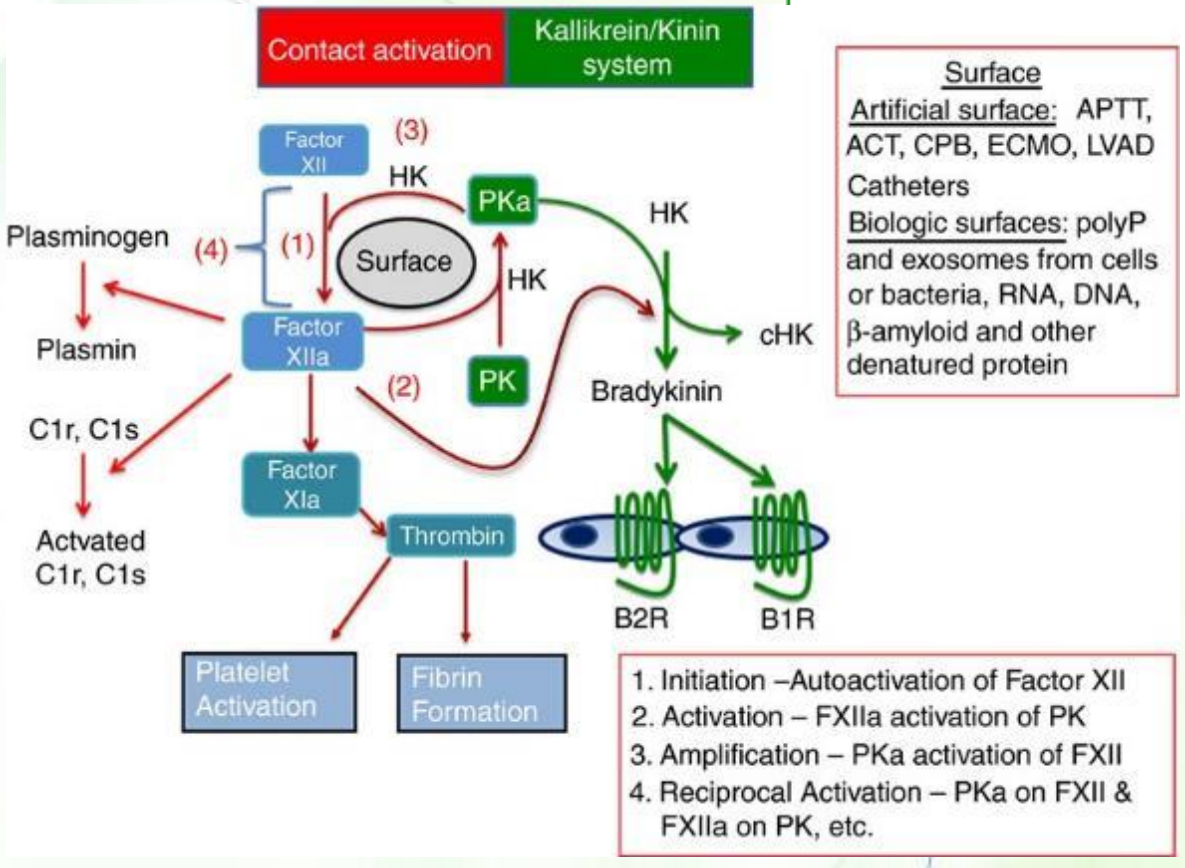
Initiation of the intrinsic pathway



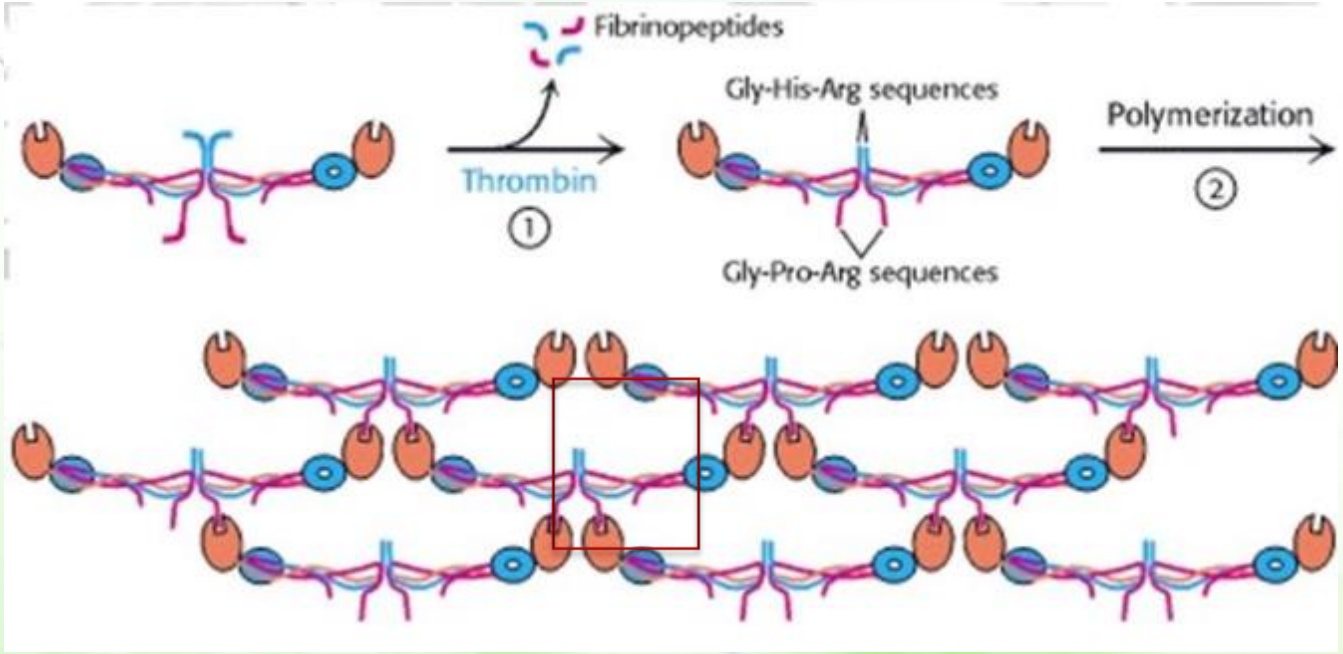
- Prekallikrein, HMW kininogen, factors XII and XI are exposed to a negatively charged activating surface.
- Factor XII is autoactivated to XIIa, which has several substrates:
  1. Kallikrein from prekallikrein (note the positive feedback activation loop).
  2. factor XI, which activates factor IX.
  3. HMW kininogen releasing bradykinin (a peptide with potent vasodilator action).
     - Bradykinin is also generated by kallikrein.
  4. Other substrates: plasminogen (fibrinolysis) and complement system proteins.

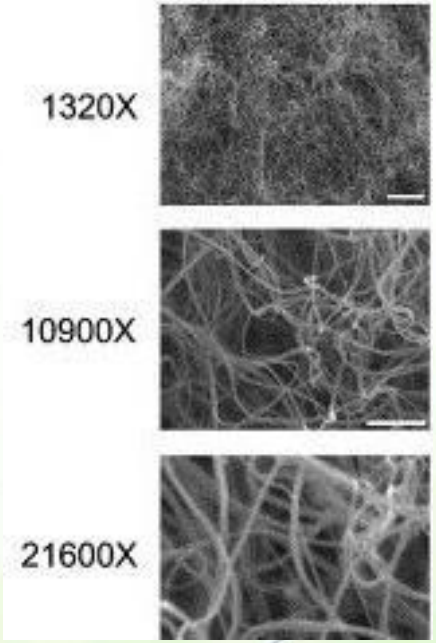


HK, intact high-molecular-weight kininogen; HKc, cleaved high-molecular-weight kininogen; PK, prekallikrein; PKa, plasma kallikrein; polyP, polyphosphate

Formation of a fibrin clot

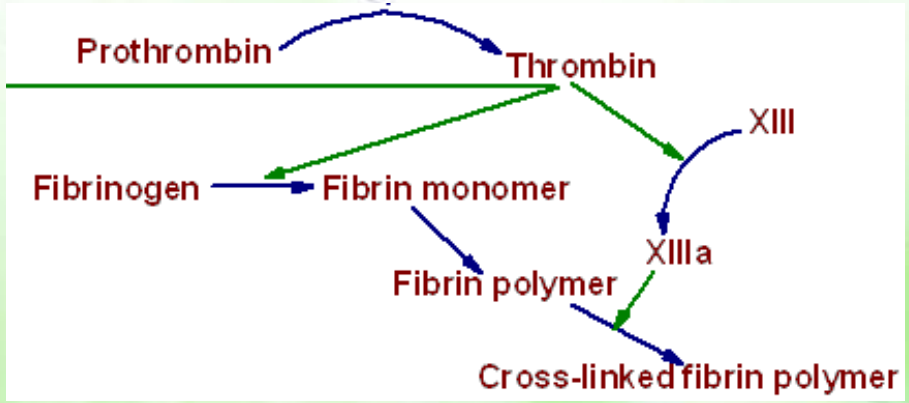
- Thrombin cleaves fibrinogen releasing fibrinopeptides.
  - Fibrinogen is a two triple-stranded helical protein held together by disulfide bonds.
- Fibrin molecues create electrostatic attractions between the central domain and the end domains facilitating the aggregation of the monomers into a gel consisting of long polymers.
- The clot resulting from aggregation of fibrin monomers is referred to as the "soft clot“.

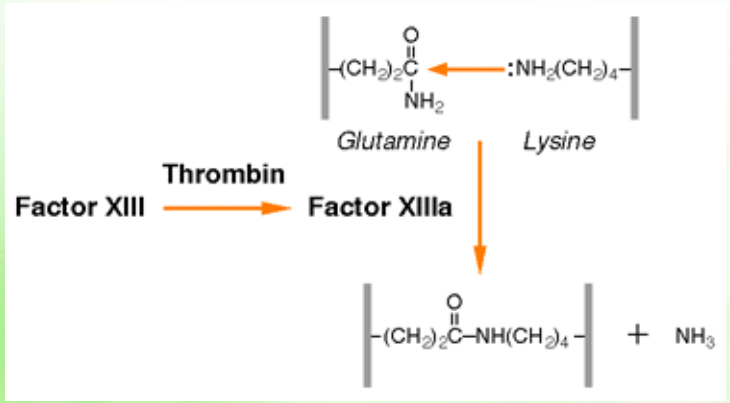


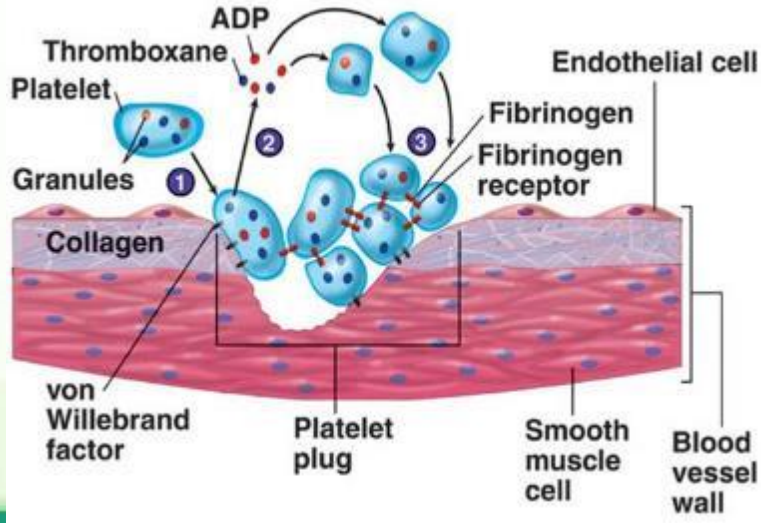


Factor XIII

- Factor XIII is a transglutaminase that is activated by thrombin.
- Factor XIIIa catalyzes a transglutamination reaction that catalyzes covalent cross-linking reaction between a glutamine of one fibrin monomer to a lysine of an adjacent fibrin monomer.
  - It also cross-links the fibrin clot to adhesive proteins on the endothelial tissue and to the platelet surfaces strengthening the platelet plug.
  - The cross-links strengthen the fibrin mass, forming the "hard clot“

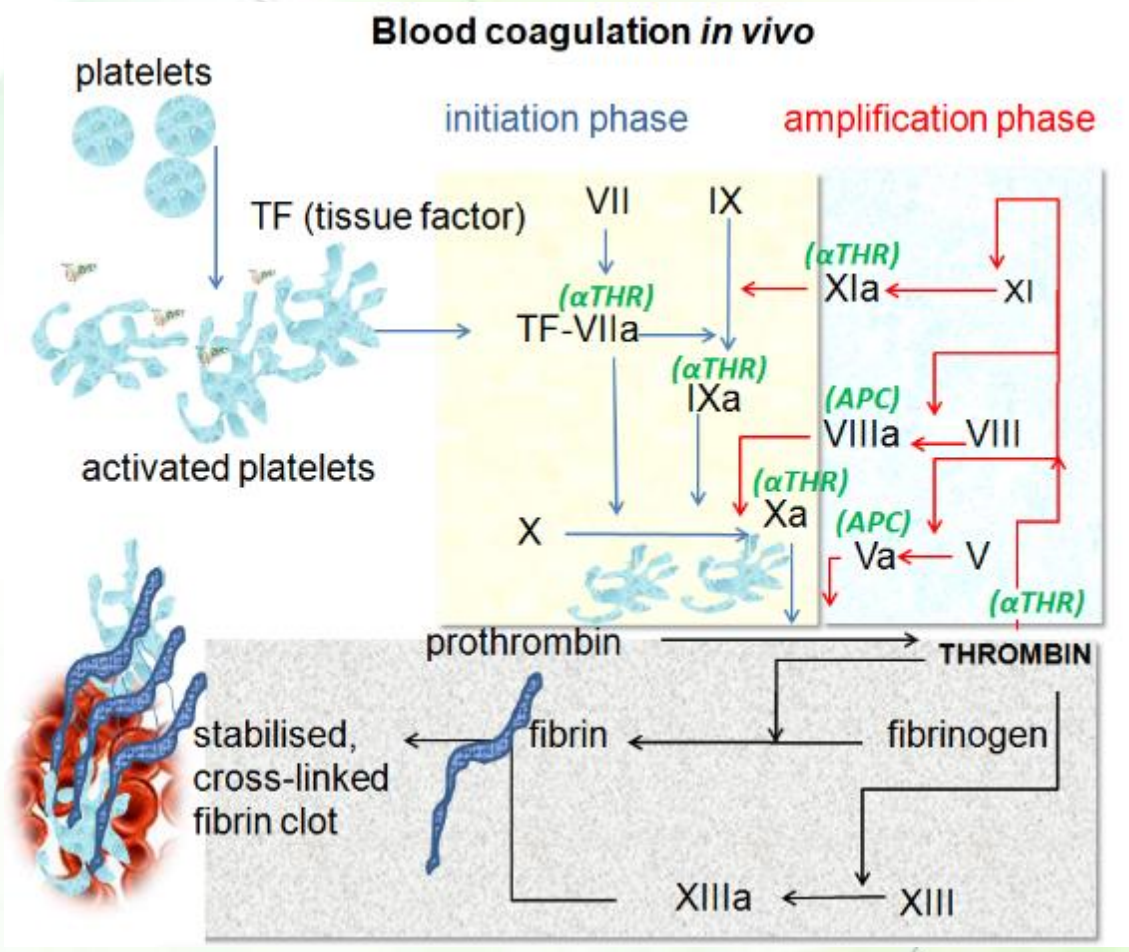






Amplification of coagulation reactions

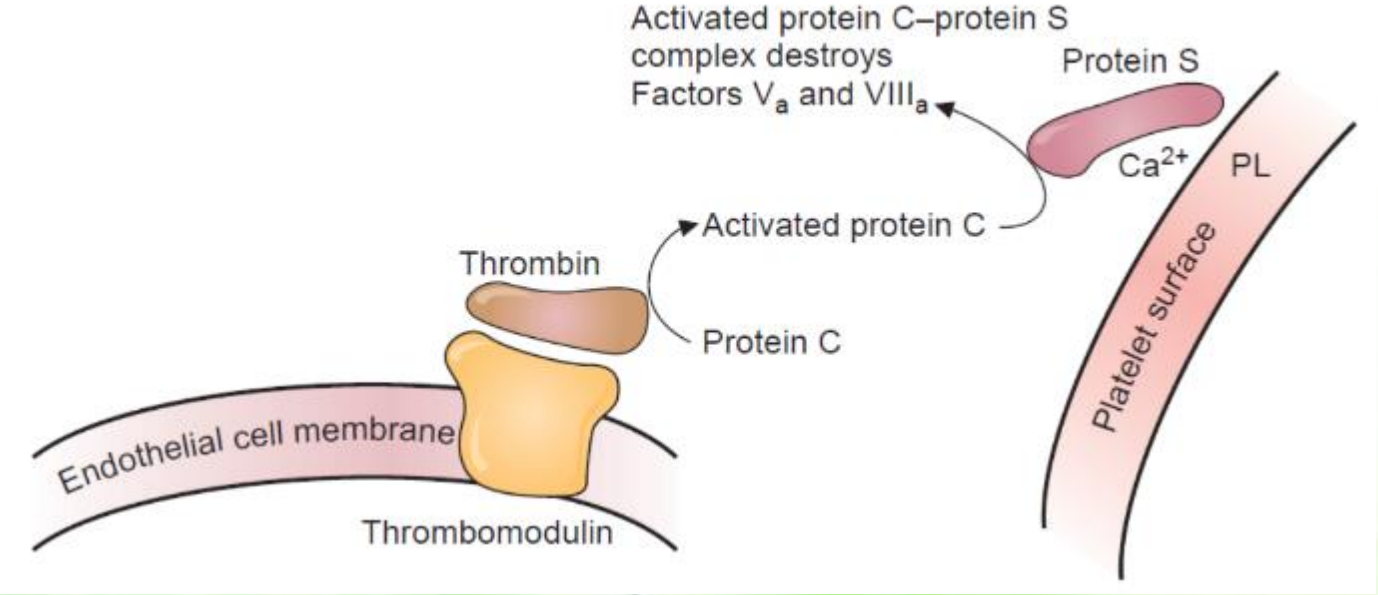
- The sequential enzymatic activation allows for amplification.
- Amplification also results from positive feedback reactions.
- These include activation of V, VII, VIII, and XI by thrombin.

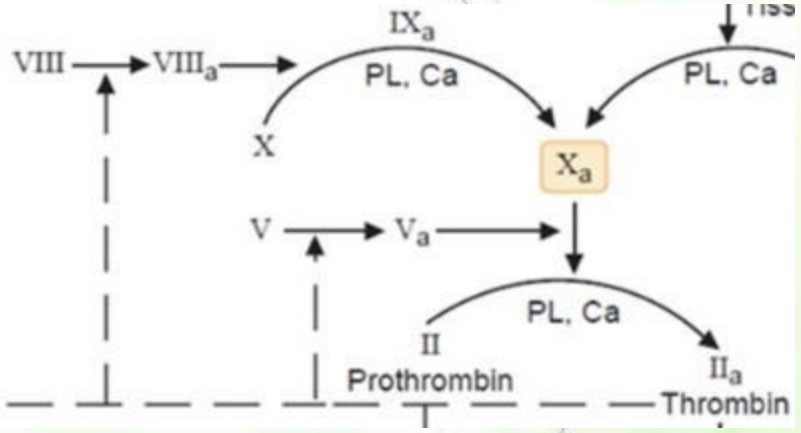


Anti-clotting factors

Protein C and protein S

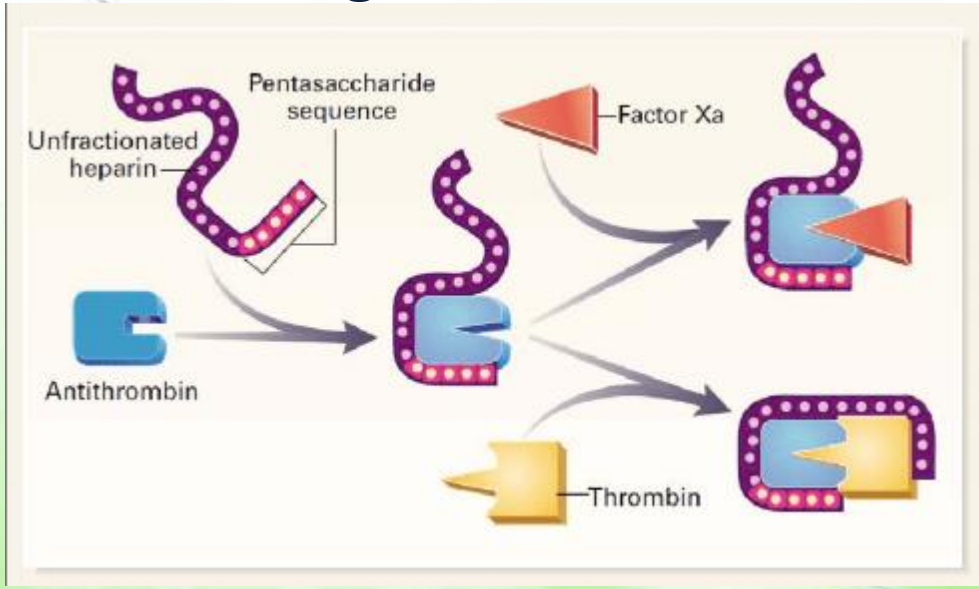
- Thrombin binds to thrombomodulin in the surface of endothelial cells.
- Thrombin can then activate protein C, which forms a complex with protein S, both of which are vitamin K-dependent cofactors.
- The complex degrades factors V and VIII.

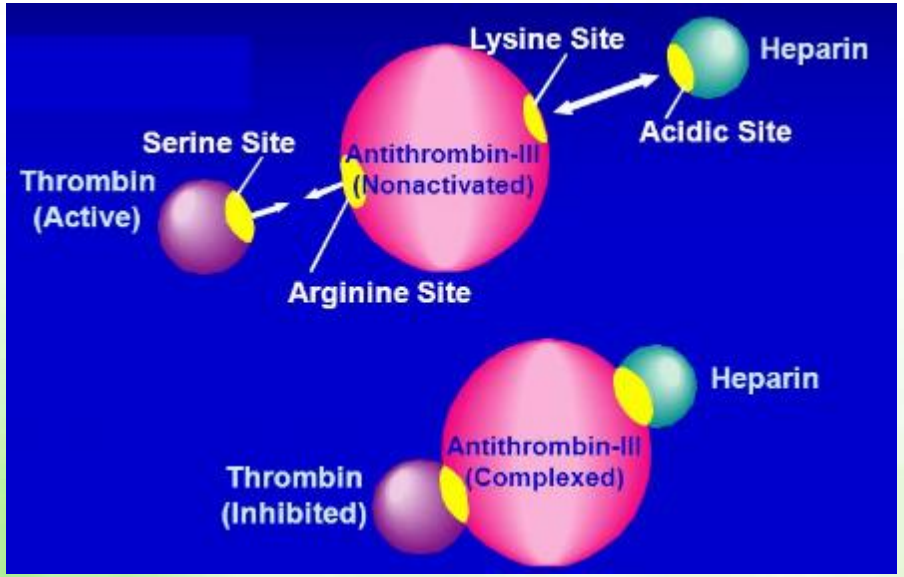




Antithrombin III

- Antithrombin III is a serine protease inhibitor of thrombin as well as other clotting factors (IXa, Xa, XIa, XIIa, and VIIx when complexed with TF).
- Heparin sulfate, a polysaccharide synthesized by mast cells and present on surface of endothelial cells, binds to antithrombin III, promoting binding to its substrates.

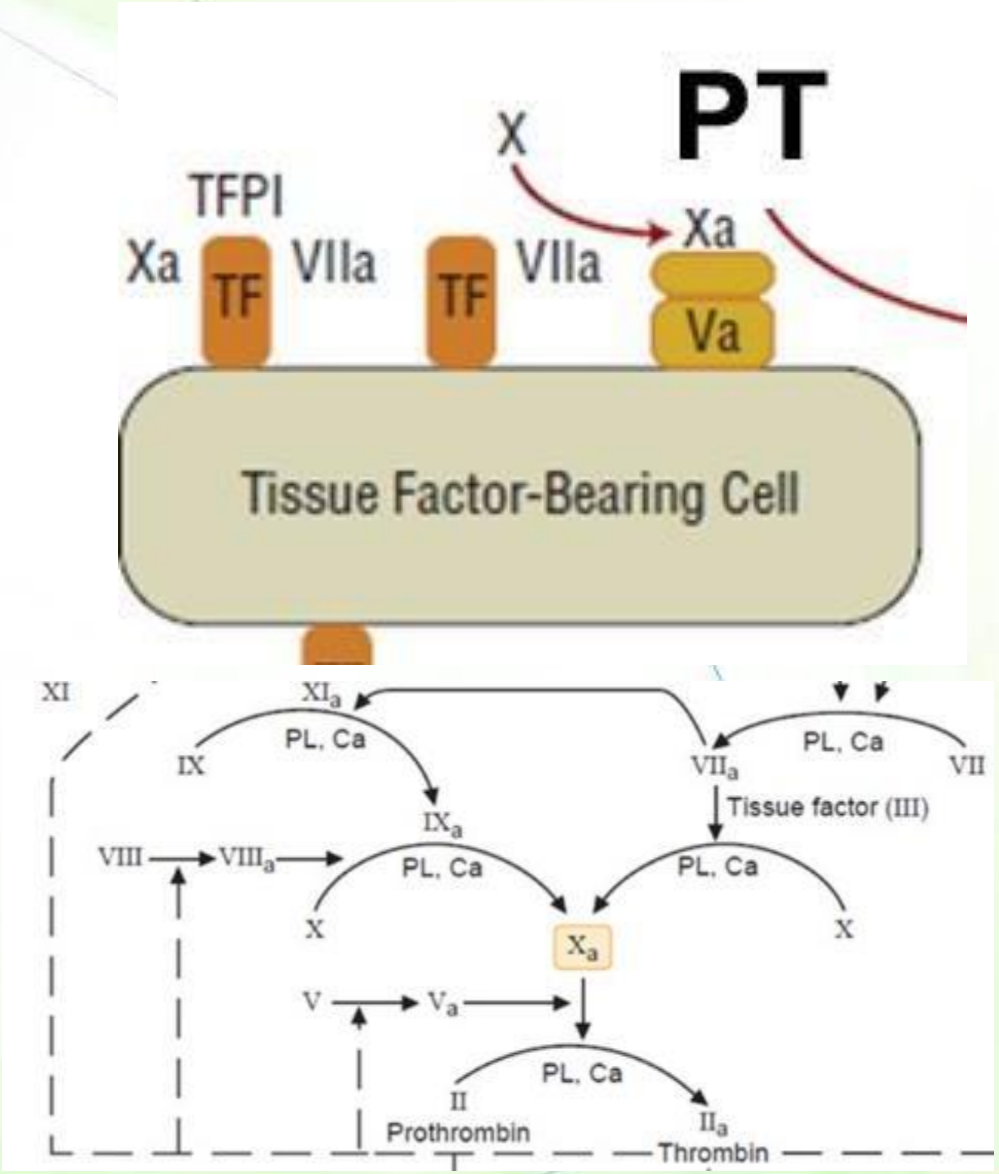




In the clinic, phlebotomy tubes are often treated with heparin in order to inhibit clot formation.

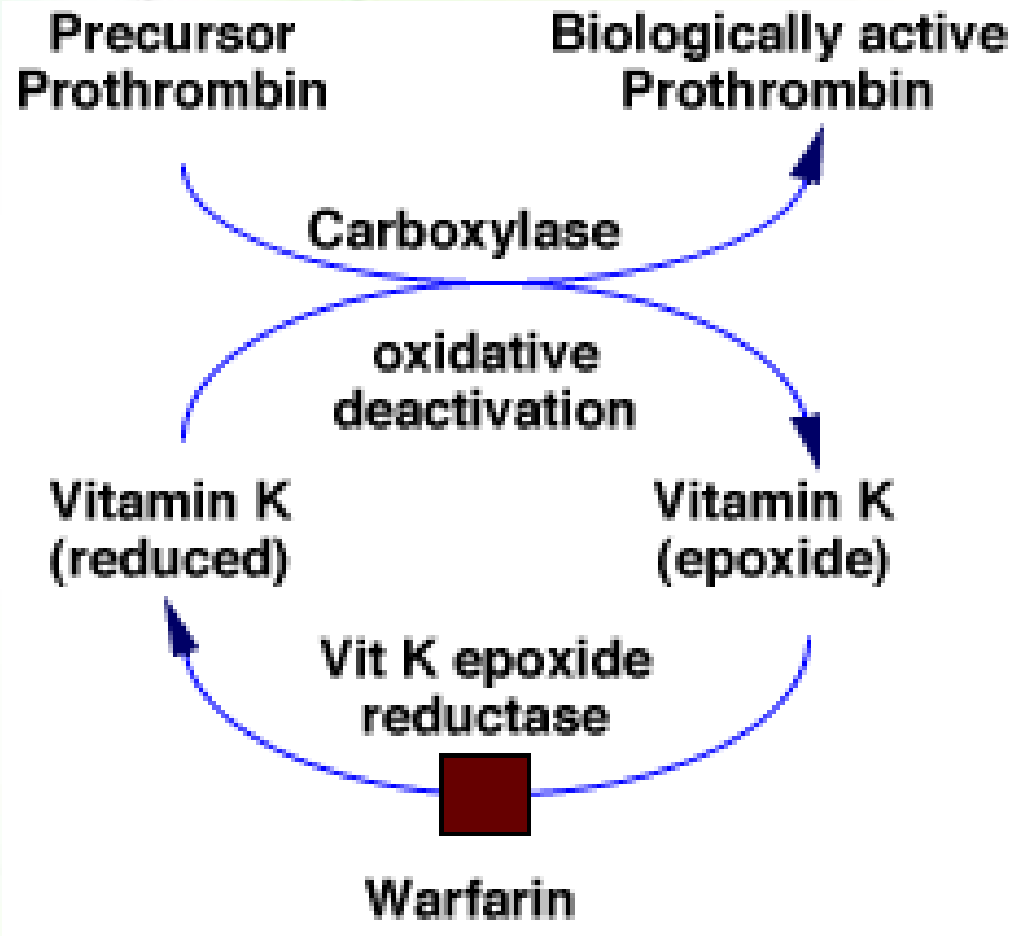
Tissue Factor pathway inhibitor

- Tissue factor pathway inhibitor (TFPI) is a protein found in plasma lipoproteins and bound to the vascular endothelium.
  - It binds to and inhibits factor Xa.
  - The Xa-TFPI complex then interacts with the tissue factor-VIIa complex and inhibits its activation of factors X and IX.
  - Protein S binds to TFPI localizing it to membrane surfaces and enhancing the inhibition of Xa.
- TFPI is also able to inhibit Xa-activated Va resulting in inhibition of the pro-thrombinase complex.



Anti-coagulants

- Blood clotting can be prevented by addition of Ca2+ chelators and vitamin K antagonists such as the anticoagulant drug warfarin, which inhibits reduction of vitamin K and thereby prevents synthesis of active prothrombin and factors VII, IX, and X.



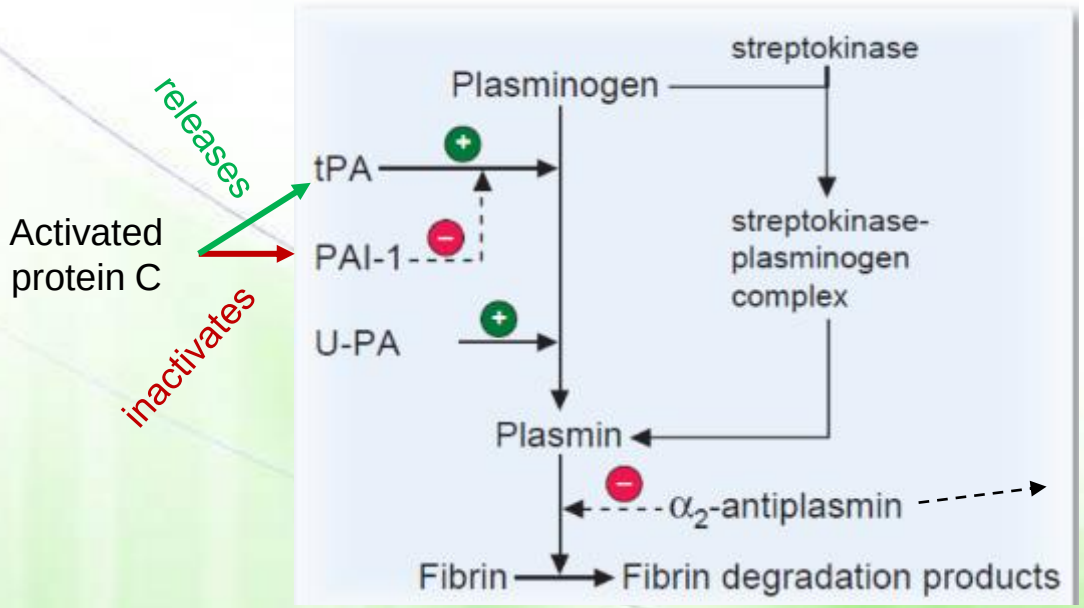
Degradation of the fibrin clot

Clot dissolution

- It is important to prevent clot formation when not needed by anti-clotting factors and to dissolve a clot when formed.
- Clot dissolution starts concomitant with its formation.

The fibrinolytic system

- Plasmin, a serine protease formed from plasminogen, is responsible for fibrinolysis where it catalyzes the hydrolysis of fibrin and fibrinogen to degradation products.
- Plasminogen has a high affinity for fibrin clot.

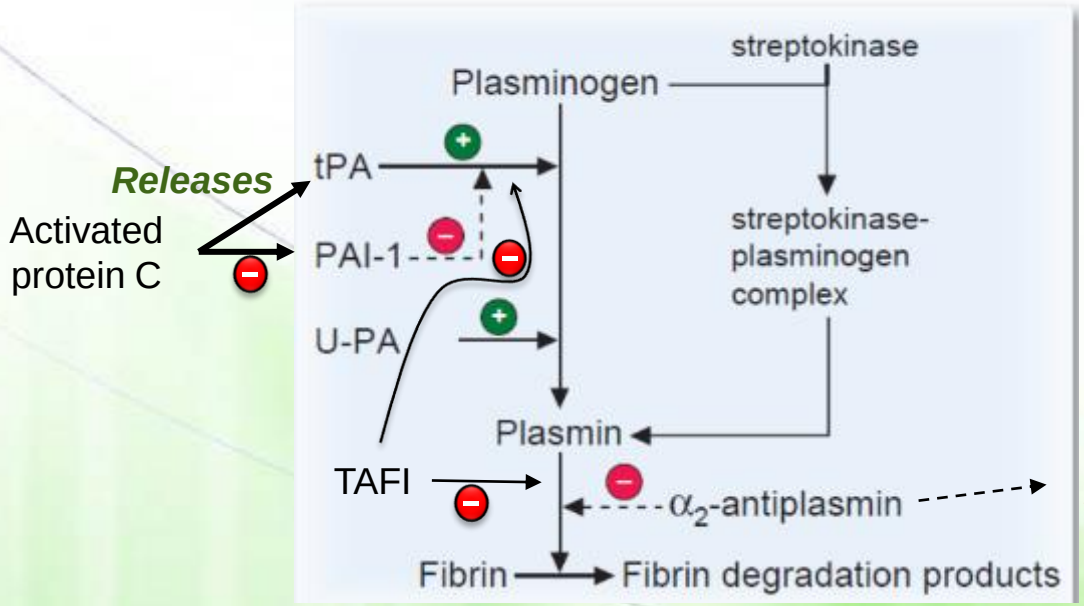


Streptokinase, a regulatory protein isolated from streptococci, can activate circulating plasminogen to form plasmin in blood, resulting in degradation of fibrinogen as well as fibrin.

but not when plasminogen/plasmin are clot-bound

The fibrinolytic system

- Plasmin, a serine protease formed from plasminogen, is responsible for fibrinolysis where it binds to the lysine residues of fibrin and catalyzes the its hydrolysis.
  - Plasminogen has a high affinity for fibrin clot.
- Thrombin activatable fibrinolysis inhibitor (TAFI) is a carboxypeptidase that removes terminal lysine residues and prevent fibrinolysis.

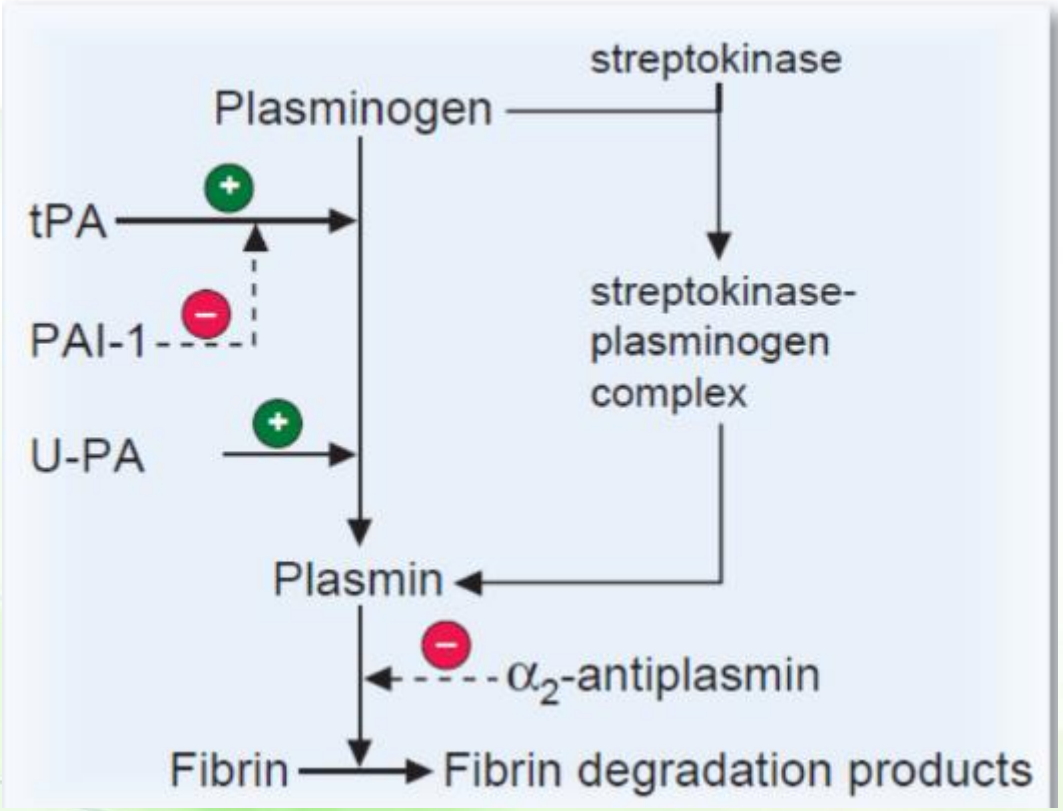


Streptokinase, a regulatory protein isolated from streptococci, allows autoactivation of plasminogen in blood, resulting in degradation of fibrinogen as well as fibrin.

but not when plasminogen/plasmin are clot-bound

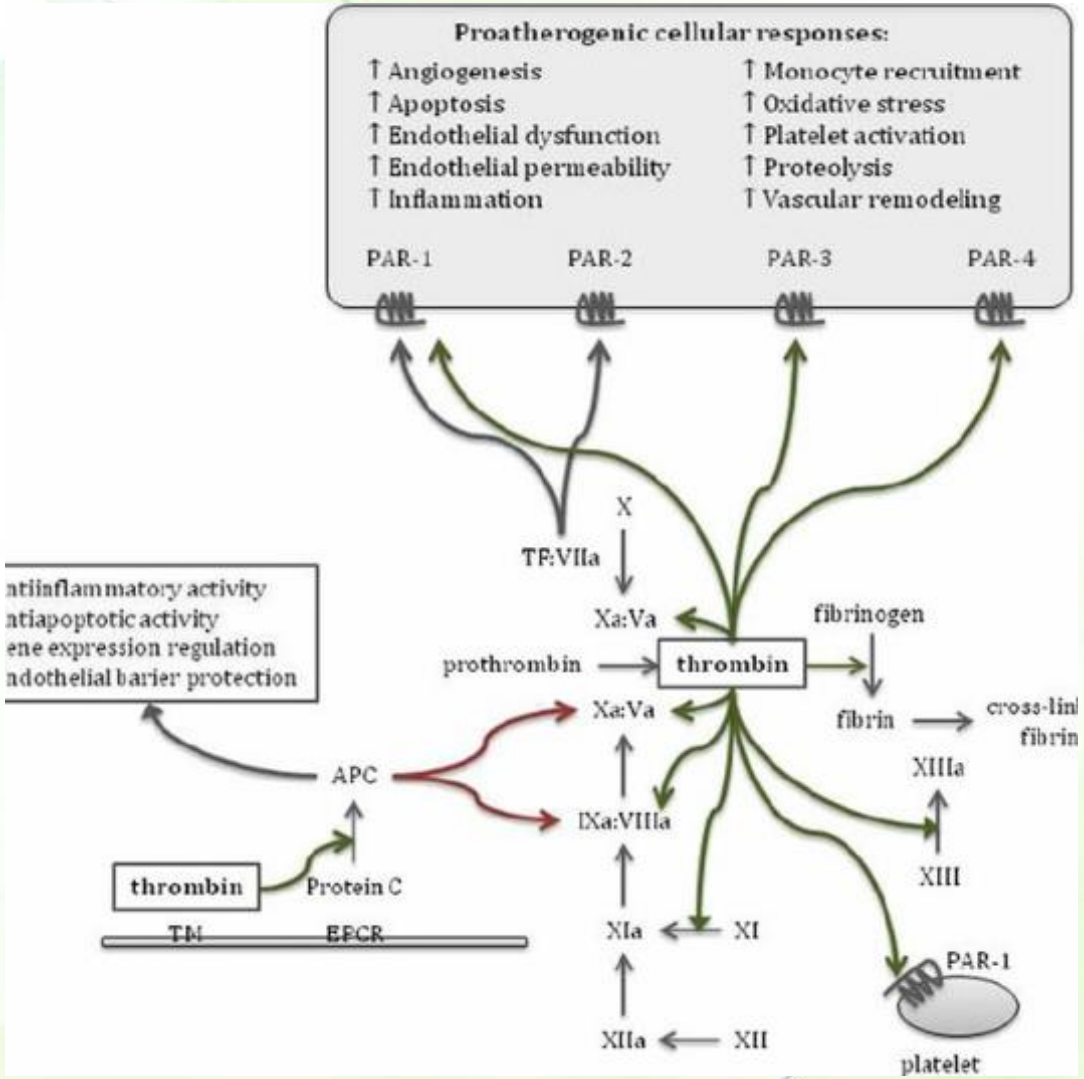
Urokinase

- Urokinase, a serine protease is formed from the zymogen pro-urokinase
- It is a potent plasminogen activator, and is used clinically

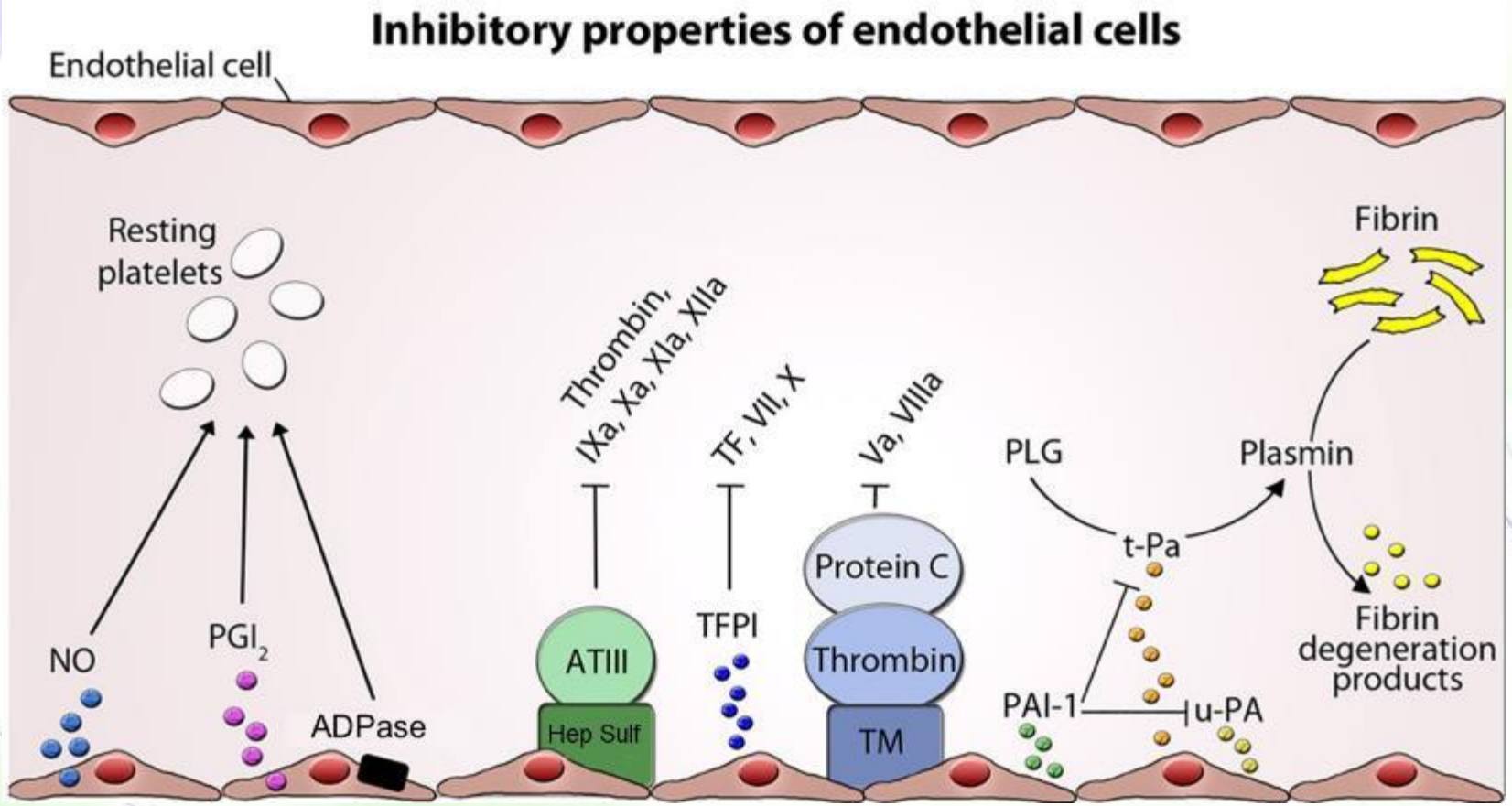


Roles of thrombin

- Platelet recruitment
- Amplification of the coagulation complex
- Formation of soft clot
  - Proteolytic cleavage of fibrinogen
- Formation of hard clot
  - Activation of factor XIII
- Attenuation of its own activity
  - Activation of protein C
- Other actions
  - Binding to its receptor on surface of platelets induces vascular remodeling (e.g. angiogenesis) and inflammation.



Role of endothelial cells in coagulation



- ECs release NO, prostacyclin (PGI2), and ADPase, which inhibit platelet adhesion and aggregation.
- Membrane-bound heparin sulfate bind to antithrombin III (ATIII) inactivating several coagulation factors.
- ECs express tissue factor pathway inhibitor (TFPI), which inhibits tissue factor (TF) and, consequently, factors VII and X.
- Thrombomodulin (TM) binds thrombin activating protein C and degrades factors Va and VIIIa.
- ECs balance fibrin accumulation and lysis by releasing plasminogen activators, t-PA and u-PA, and their inhibitor (PAI)

Neubauer and Zieger. Endothelial cells and coagulation. Cell and Tissue Research (2021)

It is a symphony played by an orchestra