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BIOCHEMISTRY

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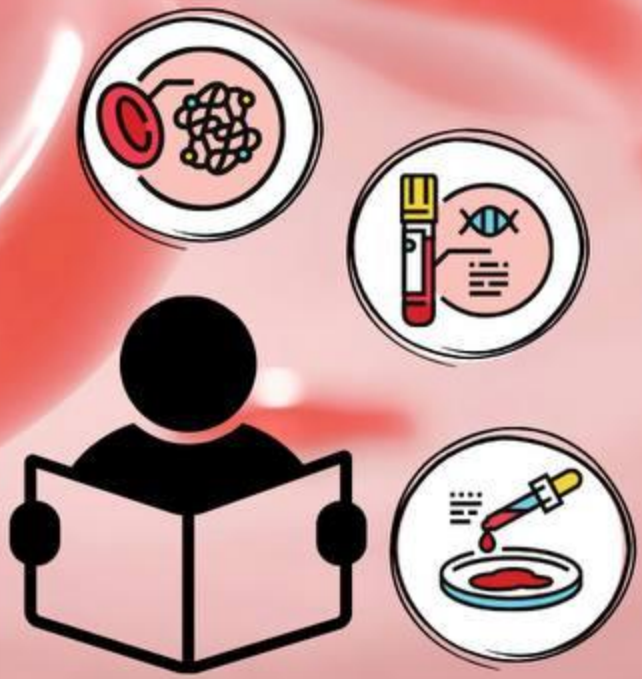
تدقيق: تم التدقيق

الدكتور: مأمون أهرام



Blood coagulation

Prof. Mamoun Ahram

Hematopoietic-lymphatic system

Resources

This lecture

Harper's Medical Biochemistry, 31st edition, Chapter 55

Mark's Basic Medical Biochemistry, 7th edition, Chapter 43

Color code

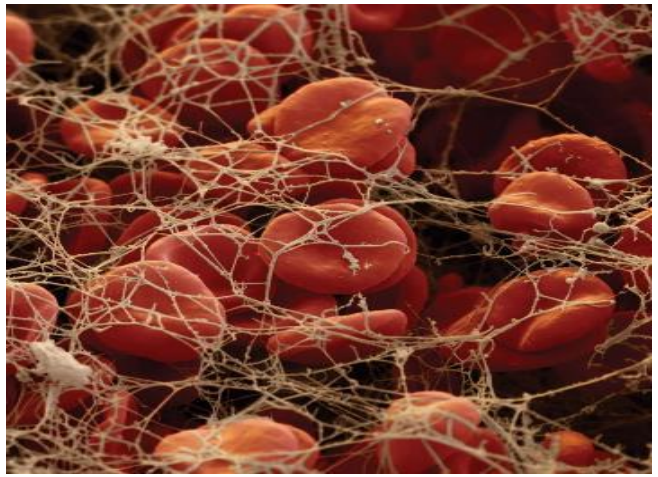
Slides
Doctor
Additional info
Important

We covered blood coagulation in our physiology lectures, and now we'll approach it from a biochemical perspective.

What is blood coagulation (clotting)?

- It is an **orchestrated** **(harmonious)**, biochemical process that is initiated as a result of external or internal 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.

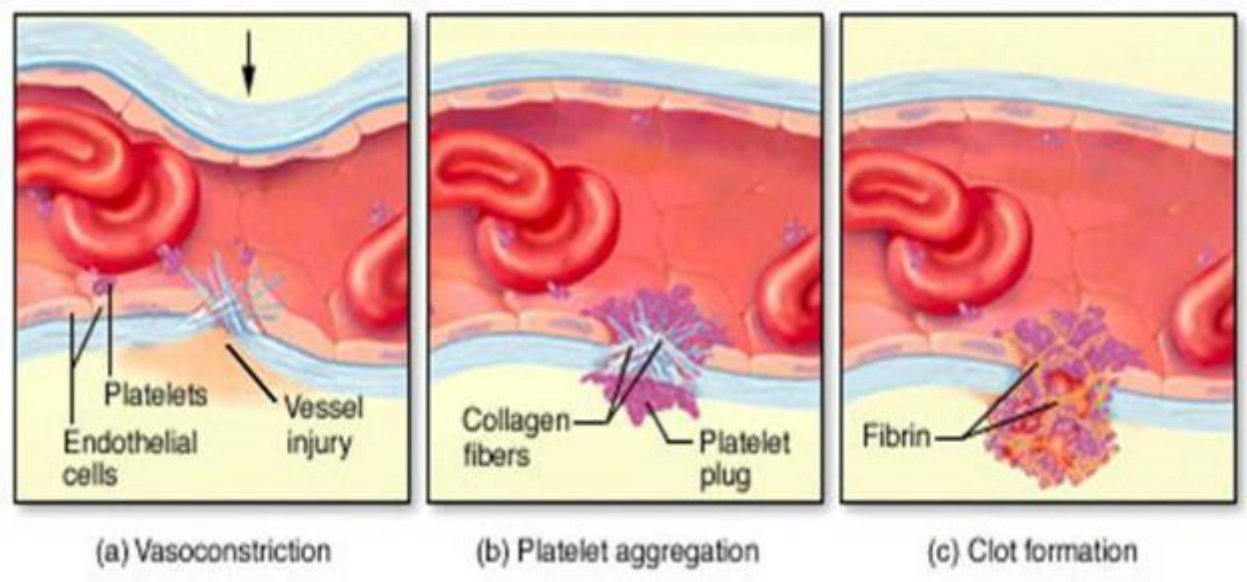
Many cells and proteins work together to regulate blood coagulation. This process is essential for our bodies to repair injuries, and afterward, the clot will dissolve.



Steps of hemostasis and thrombosis

It formed by four steps, respectively:

1. Vascular constriction limiting blood flow to the area of injury
2. Activation then aggregation of platelets at the site of injury, forming a loose gelly platelet plug
3. Formation of a fibrin mesh to entrap the plug (coagulation)
4. Dissolution of the clot in order for normal blood flow to resume following tissue repair

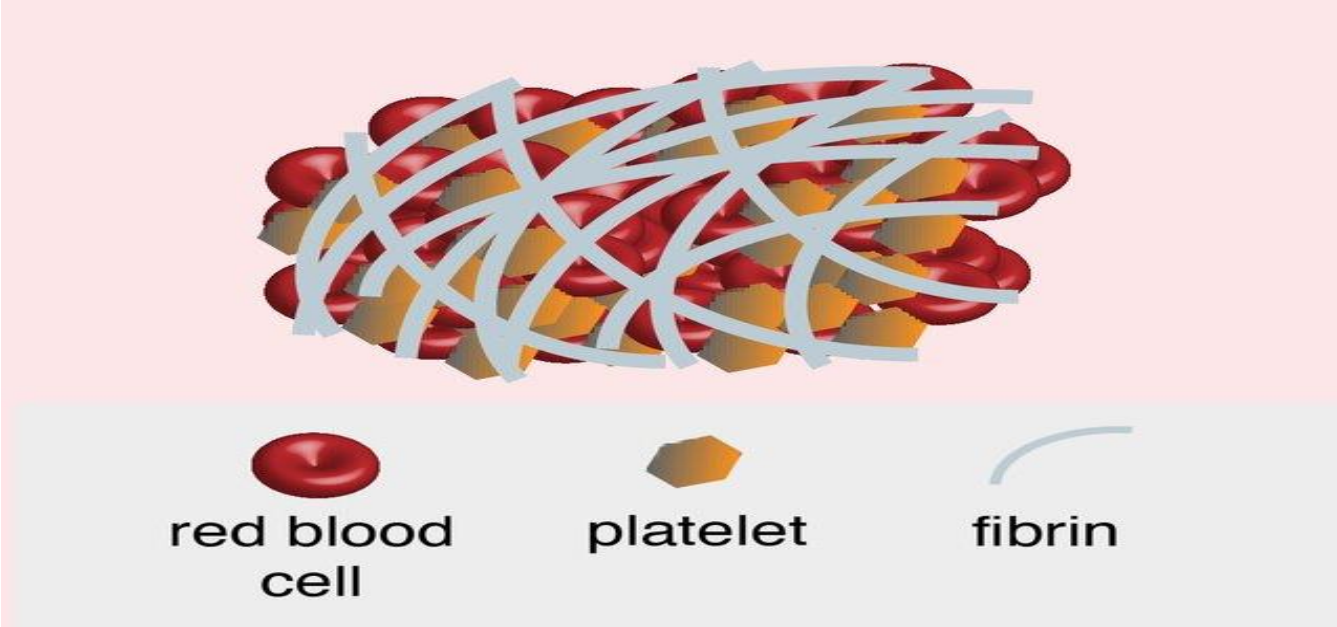


Composition of a clot

The main components of blood clot are:

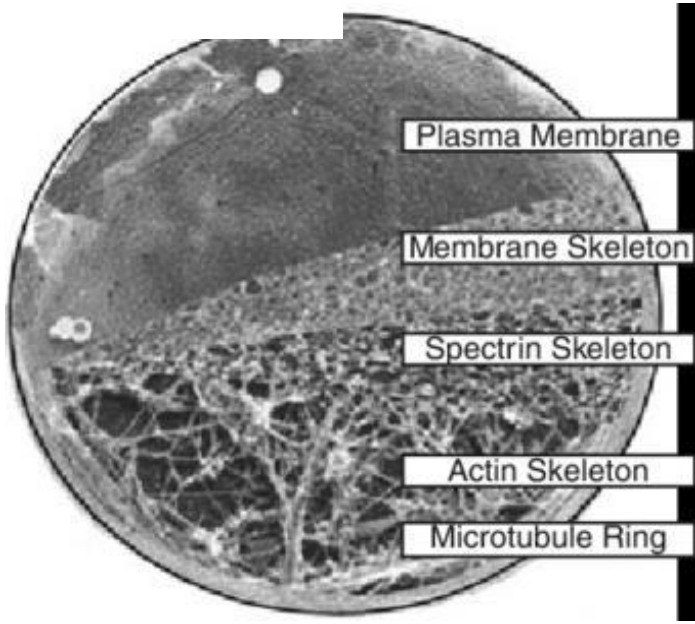
1) RBCs
2) Platelets
3) Fibrin is a protein that envelops cells and molecules to form a clot.

The proportion of red blood cells and platelets varies depending on the type of injury.



Platelets are a major player

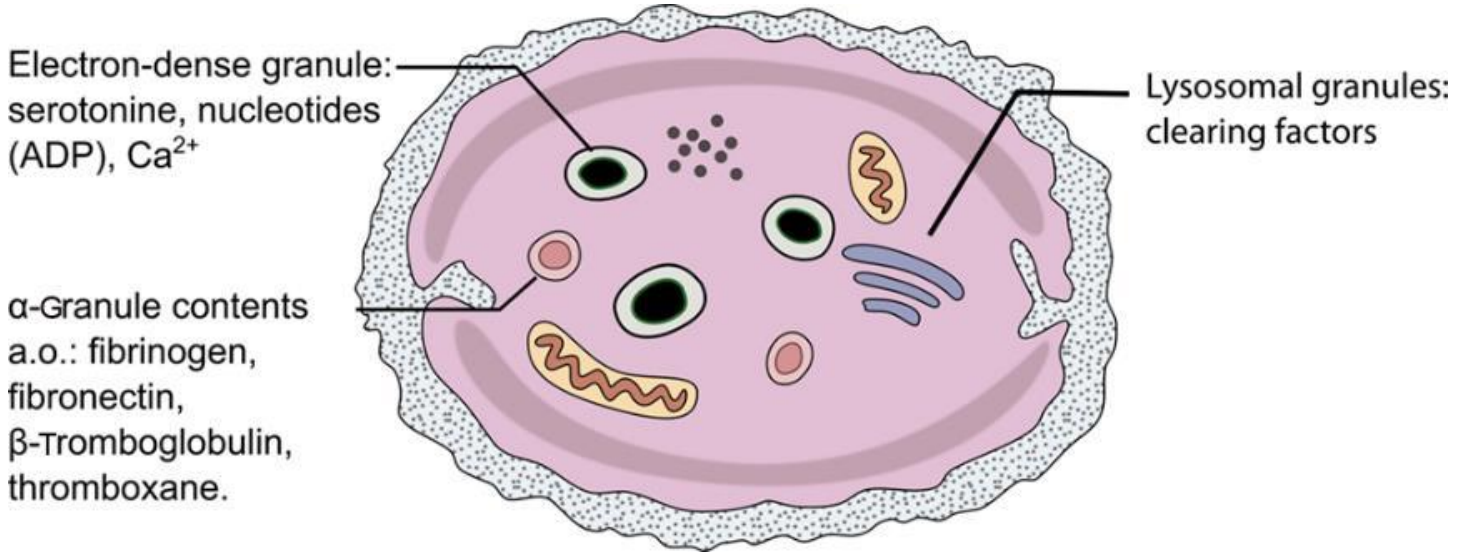
- Small anuclear cell fragments produced from the megakaryocytes.
- Platelets have numerous kinds of surface receptors and many of the ligands that bind to these receptors are released from the platelets themselves.
- Platelets also have actin filaments and myosin, which change the shape of the platelet upon enabling them to aggregate and form a plug.
- Platelets also have three types of granules that store substances that are released upon platelet activation.



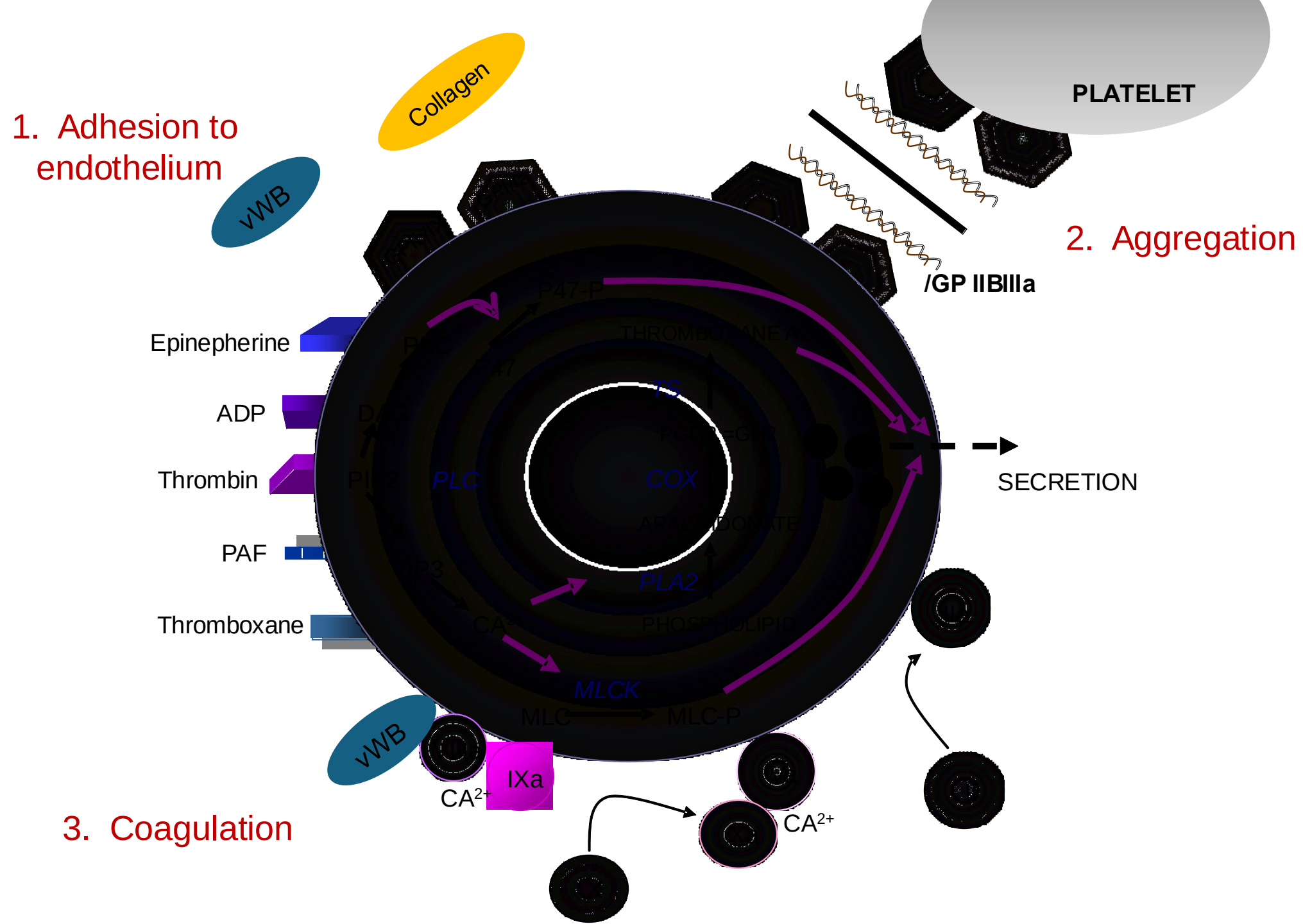
Types of granules:

It’s not necessary to memorize every molecule in each granule.

- **Electron-dense granules** contain signaling molecules as (calcium ions, ADP, ATP, serotonin)
- **alpha-granule** (a heparin antagonist, platelet-derived growth factor, fibrinogen, von Willebrand factor (vWF), clotting factors)
- **Lysosomal granules** (hydrolytic enzymes, those enzymes are needed at the end of process to clear the clot)



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
PLA2
PHOSPHOLIPID
MLCK
MLC
MLC-P
SECRETION
vWB
IXa
CA2+
CA2+
3. Coagulation

At the surface of platelets, there are specific receptors for various ligands as ADP has its own receptor, e.g..

The functions of glycoproteins on the surface of platelets :

A) In cases of endothelial injury, collagen fibers in the endothelial matrix are exposed to the glycoproteins on the platelet surface and interact together result in platelets activation.

B) Glycoproteins facilitate platelet-platelet interactions, promoting aggregation.

Recall, there are three steps of hemostasis:

1) Adhesion to endothelium
2) Platelets aggregations
3) Coagulation

Adhesion

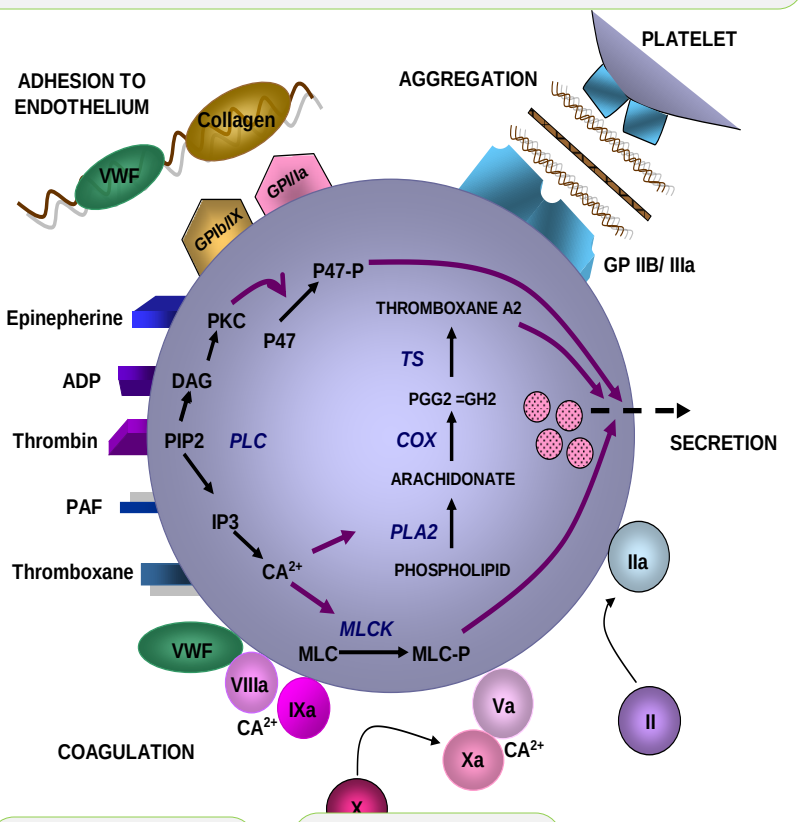
- The endothelial von Willebrand factor (vWF) protein and exposed collagen bind to the platelet glycoproteins (GP).

When the endothelium is injured, collagen becomes exposed

- Some platelets release substances from the granules:
  - ADP
  - Serotonin
  - Factor V
  - ATP
  - Calcium
  - Fibrinogen
  - vWF
  - Thrombin
  - Thromoxane

Bind to receptors

VWF is synthesized in endothelium and is also found inside platelets



Resting platelets

Activated platelet

Platelet plug

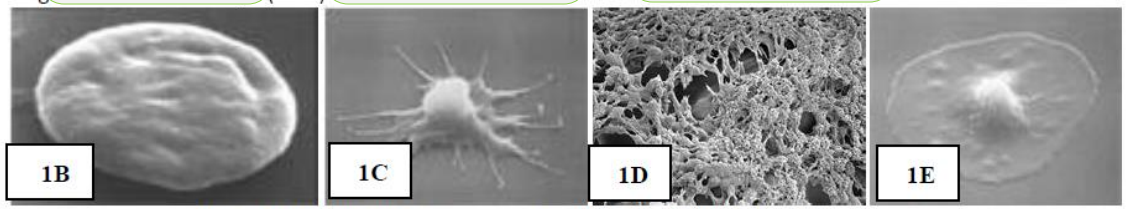
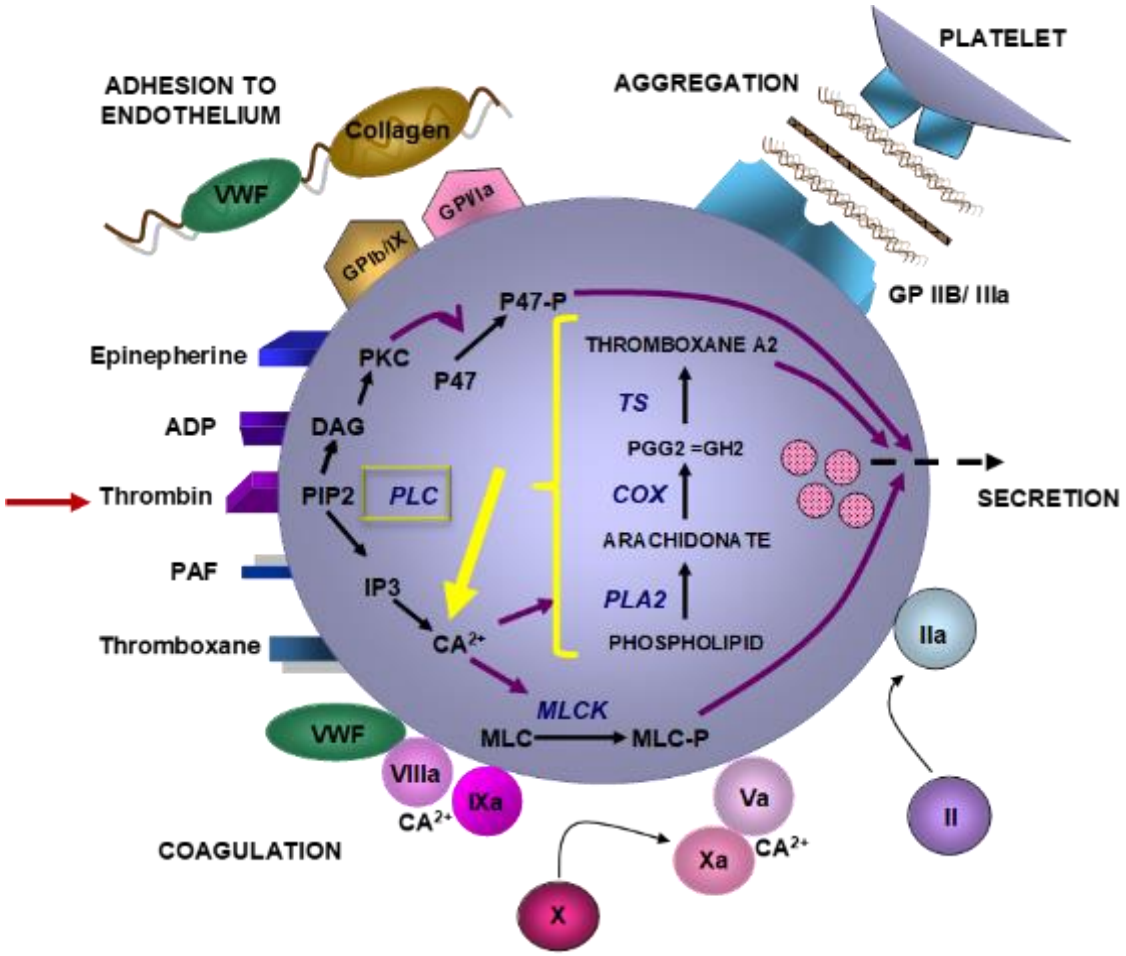


Figure 1 [(**B**) Platelet in resting mode (**C**) Activated platelets change into a pseudopodia shape (**D**) Aggregated platelets (**E**) Platelet spreading]

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

Thrombin receptor

- Thrombin binds to its receptor activates a G protein that activates phospholipase C-B (PLC-B).
- PLC-B hydrolyzes phosphatidylinositol-4,5-bisphosphate (PIP2) into inositol trisphosphate (IP3) and diacylglycerol (DAG).
- IP3 induces the release of intracellular Ca^{2+} stores in platelets, and DAG activates protein kinase C (PKC).
- Ca^{2+} triggers the release of arachidonate from membrane phospholipids by 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-B 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.*

1) Arachidonic acid is a precursor for eicosanoids synthesis, while eicosanoids are the signalling molecules that regulate various physiological processes.

2) TXA2 and PG stimulate the release of other molecules, as well as enhancing their own release.

3) Thromboxane activates PLC through feedback activation and acting in an endocrine manner to promote its own activation and in a paracrine manner to activate other platelets.

4) PDGF stimulates the proliferation of endothelial cells, leading to a thickening of the vascular surface to reduce blood flow.

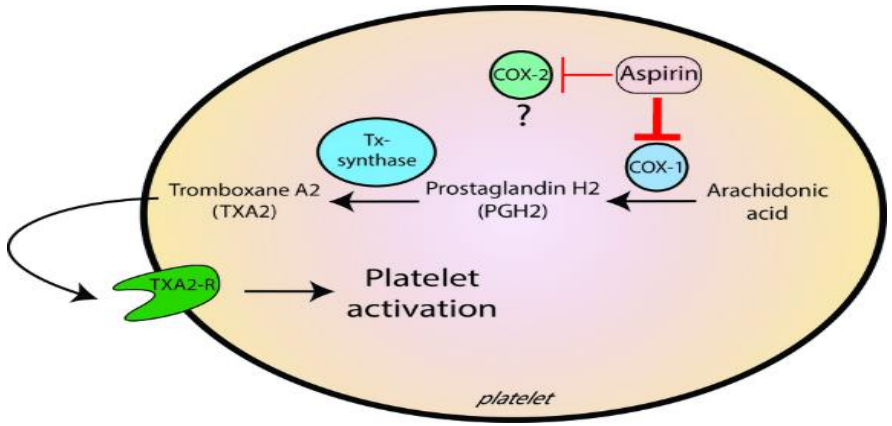
5) COX is responsible of eicosanoids production as thromboxanes and PGs.

6) COX has three isoenzymes 1,2 and 3 and as we known those enzymes are a target of NSAIDs.

NSAID

- Non-steroidal anti-inflammatory drugs inhibit the 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.

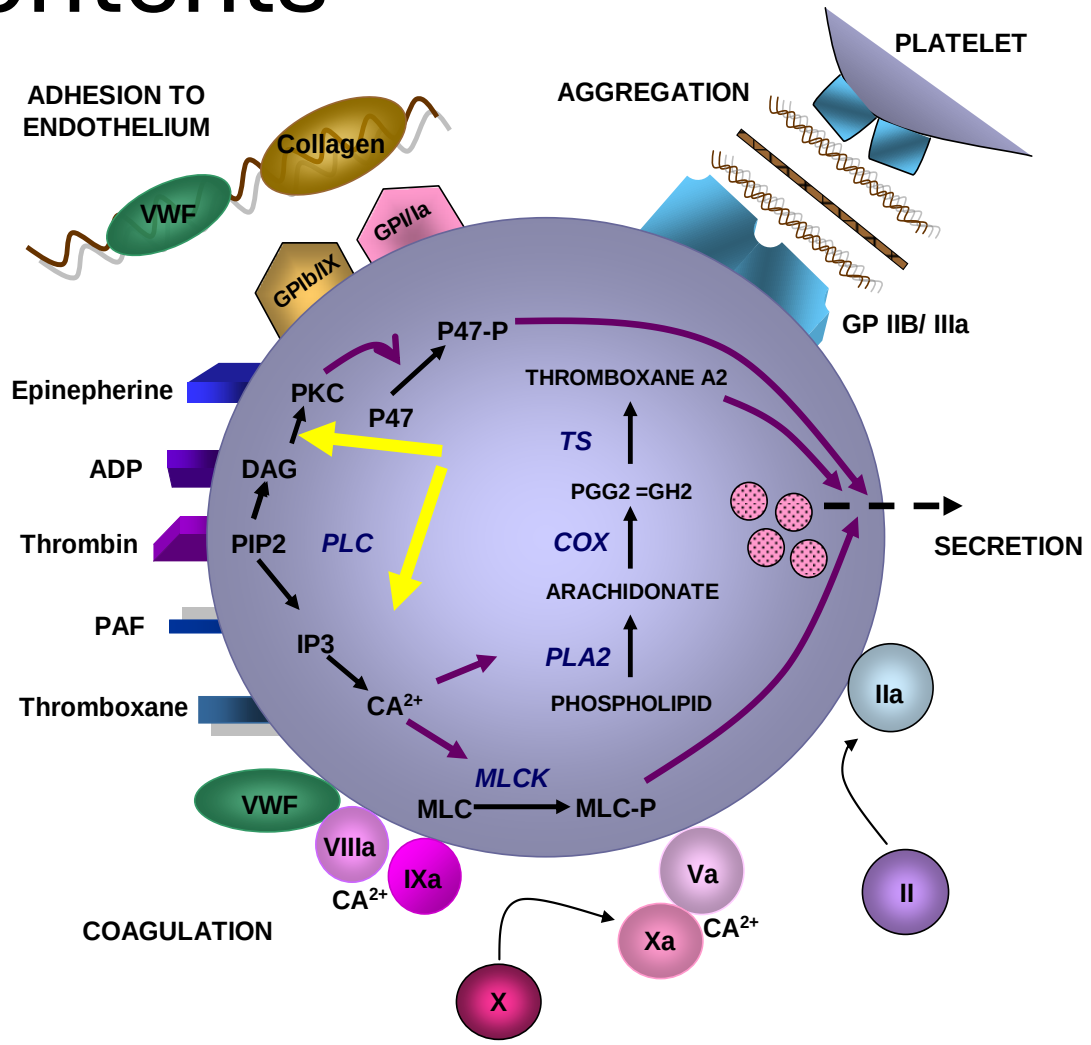


In the early stages of blood coagulation, thromboxanes and PGs are predominant. At the end stage, prostacyclin begins to appear to reduce inflammatory effects and blood aggregation.

Aspirin provides cardiovascular benefits. However ,it carries a high risk of excessive bleeding, especially in elderly people over 60 who are vulnerable to fall, making them more susceptible to internal bleeding.

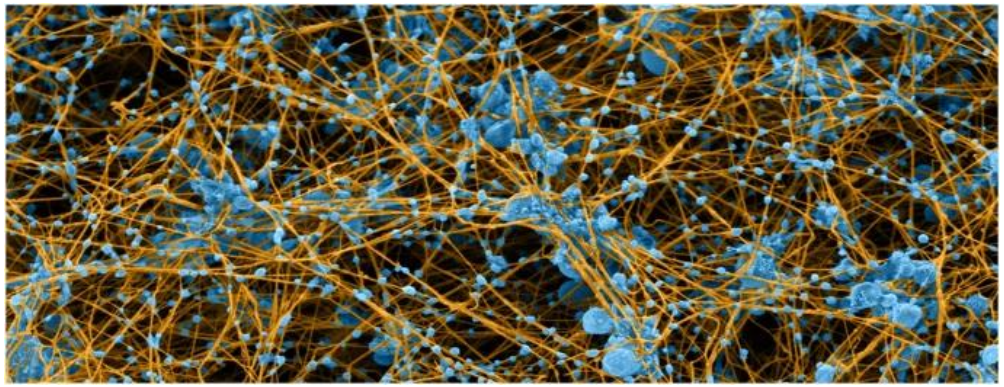
More release of granular contents

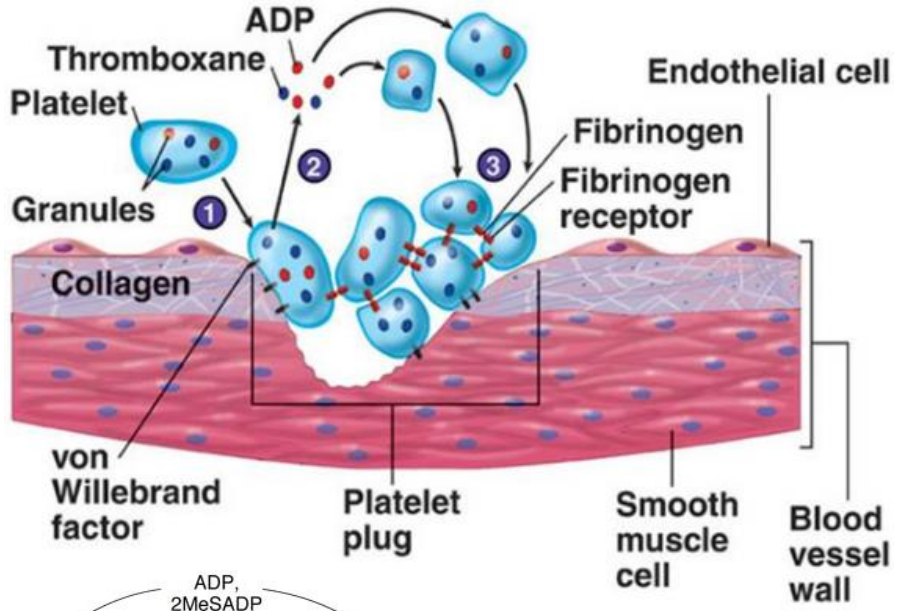
- Ca^{2+} activates myosin light chain kinase (MLCK), which phosphorylates the light chain of myosin allowing it to interact with actin and resulting in altered platelet morphology by modulating actin cytoskeleton, induced motility, and release of granules.
- DAG activates PKC, which phosphorylates and activates specific platelet proteins that induce the release of platelet granule contents including ADP.

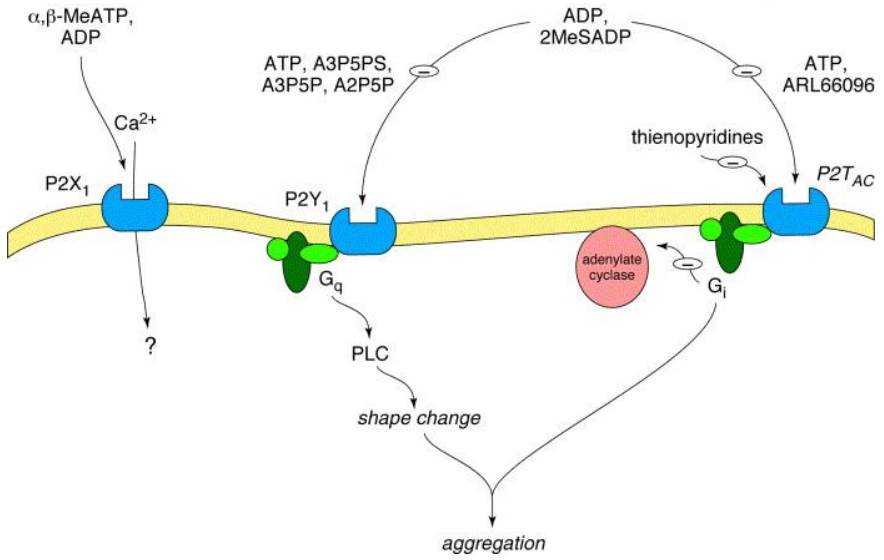


ADP drives the formation of platelet plug

- 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.



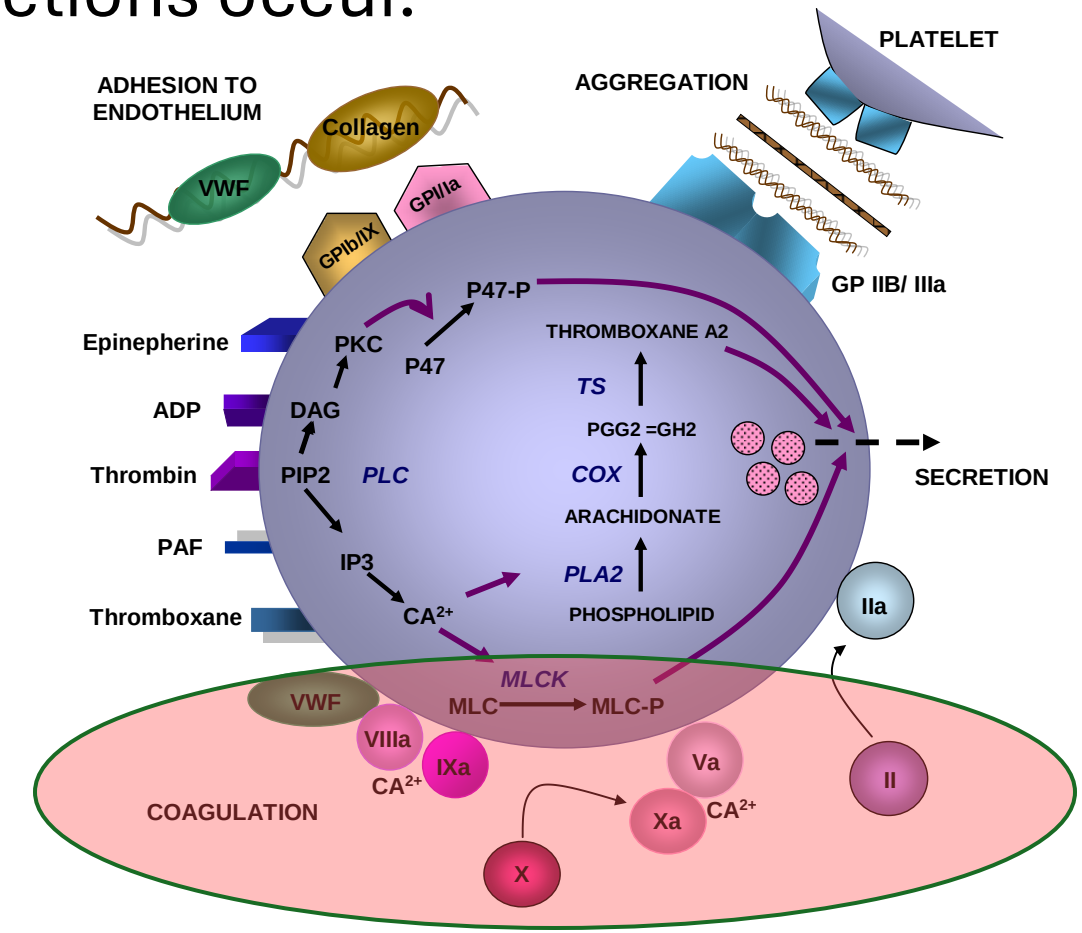




Role of platelet cell surface

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

We will have platelet aggregation and coagulation on the surface of the endothelium



Biochemistry of coagulation

Components of coagulation

- An organizing surface (platelets)
- Proteolytic zymogens -inactive enzymes undergo proteolytic degradation to be active- (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" ("a" stands to active form)
- Anti-coagulants (protein C, protein S)
- Non-enzymatic protein cofactors (factors VIII, V, and tissue factor)
- Calcium ions
- Vitamin K
- Fibrinogen

Table 3: illustrates the engagement and detailed explanation of coagulation factors, that aid in the blood coagulation cascade

Factor	Name	Source	Pathway	Description	Function
I	Fib	Liver	Common	Plasma glycoprotein; Molecular Weight (MW)= 340 kilodaltons (kDa)	Adhesive protein which aids in fibrin clot formation.
II	Prothrombin	Liver	Common	Vitamin K-dependent serine protease; MW= 72 kDa	Presence in the activated form and the main enzyme of coagulation
III	Tissue factor	Secrete by the damaged cells and platelets	Extrinsic and Intrinsic	Known as thromboplastin; MW= 37 kDa	Lipoprotein initiator of the extrinsic pathway
IV	Calcium ions	Bone and gut	Entire process	Required for coagulation factors to bind to phospholipid (formerly known as factor IV)	Metal cation which is important in coagulation mechanisms
V	Proaccererin / Labile factor	Liver and platelets	Intrinsic and extrinsic	MW = 330 kDa	Cofactor for the activation of prothrombin to thrombin (prothrombinase complex)
VII	Proconvertin (stable factor)	Liver	Extrinsic	MW = 50 kDa; vitamin K-dependent serine protease	With tissue factor, initiates extrinsic pathway (Factor IX and X)
VIII	Antihemophilic factor A (cofactor)	Platelets and endothelium	Intrinsic	MW = 330 kDa	Cofactor for intrinsic activation of factor X (which it forms tenase complex)
IX	Christmas factor / Antihemophilic factor B (plasma thromboplastin component)	Liver	Intrinsic	MW = 50 kDa; vitamin K-dependent serine protease	Activated form is enzyme for intrinsic activation of factor X (forms tenase complex with factorVIII)
X	Stuart-Prower factor (enzyme)	Liver	Intrinsic and extrinsic	MW = 58.9 kDa; vitamin K-dependent serine protease	Activated form is the enzyme for final the common pathway activation of prothrombin (forms prothrombinase complex with factor V)
XI	Plasma thromboplastin antecedent	Liver	Intrinsic	MW = 160 kDa; serine protease	Activates intrinsic activator of factor IX
XII	Hageman factor	Liver	Intrinsic; (activates plasmin)	MW = 80 kDa; serine protease	Initiates activated partial thromboplastin time (aPTT) based intrinsic pathway; Activates factor XI, VII and prekallikrein
XIII	Fibrin stabilizing factor	Liver	Retards fibrinolysis	MW = 320 kDa; Crosslinks fibrin	Transamidase which cross-links fibrin clot

This table is highly important except for the MWs. Memorize the function of each

Molecular components of coagulation

Notes:

1. Names and symbols
2. Pathway
3. Sources
4. Functions
5. Do not worry about MW

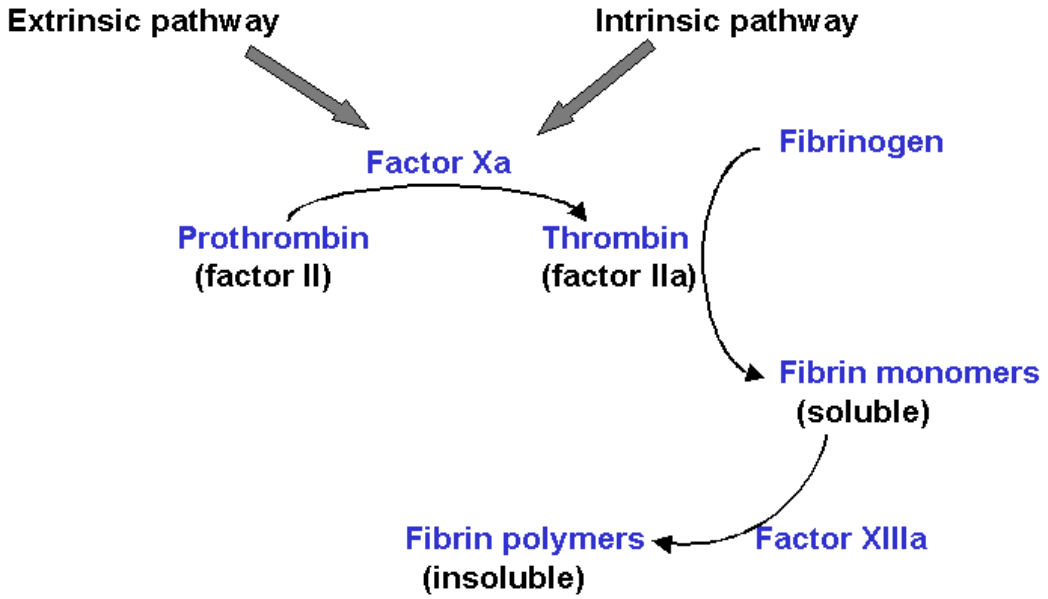
Focus that the liver is the main source

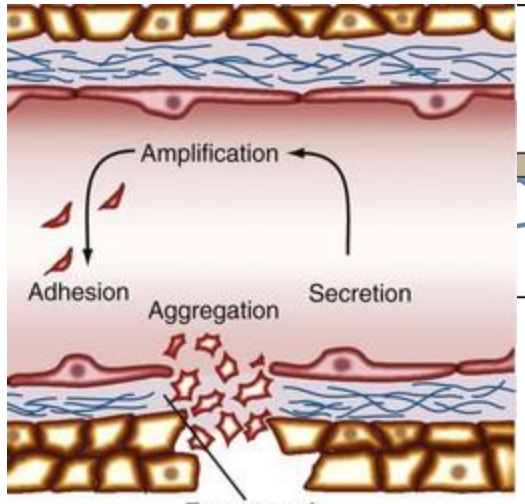
The two pathways

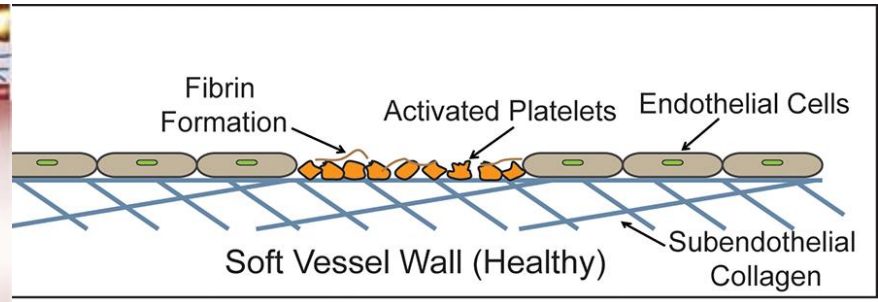
- The intrinsic pathway is initiated when subendothelial surface (i.e., collagen) is exposed.
- The extrinsic pathway is initiated in response to tissue injury.
  - Tissue factor (TF) protein is released.

External cause for tissue injury

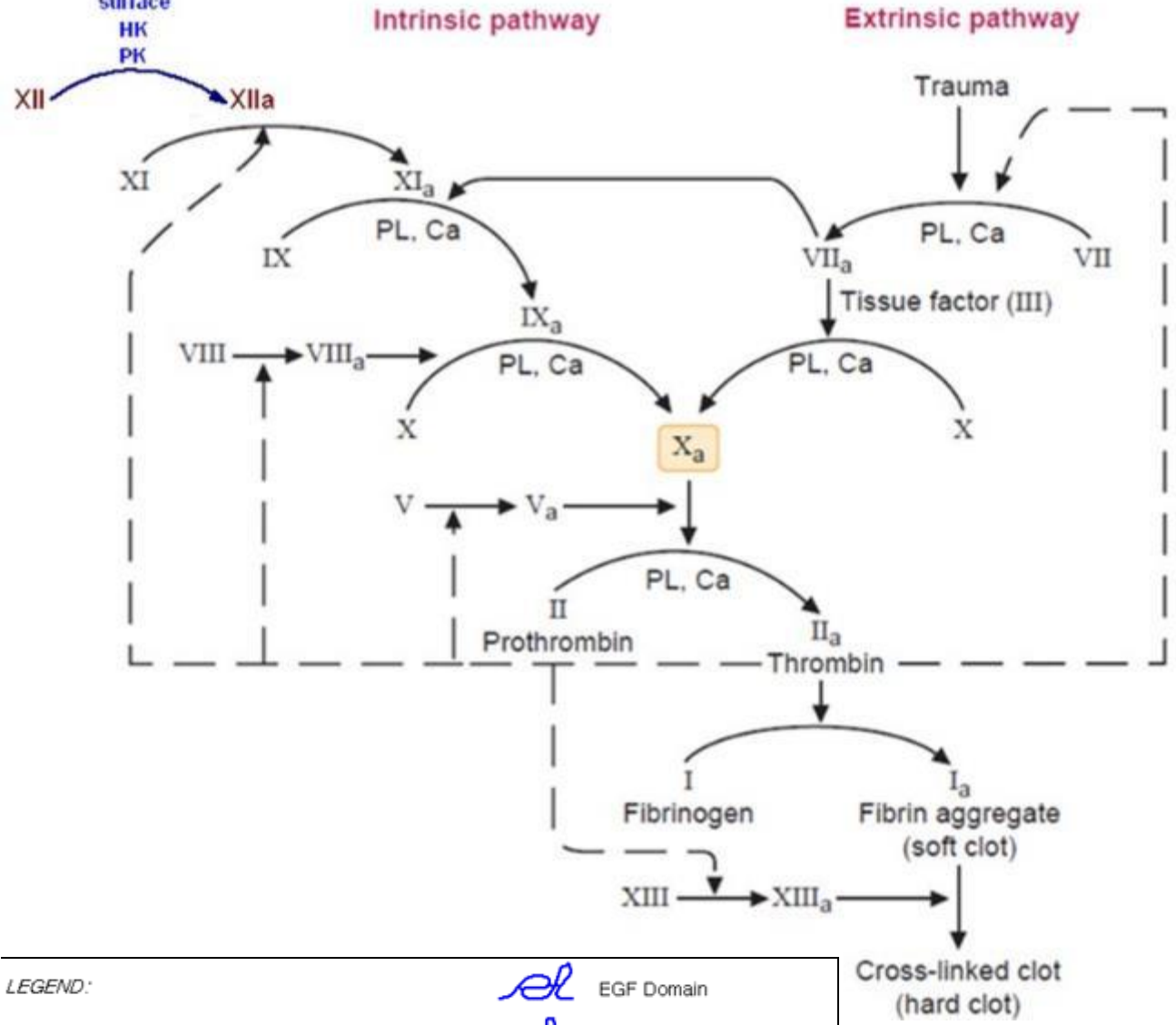
- However, the two pathways converge on a common pathway.

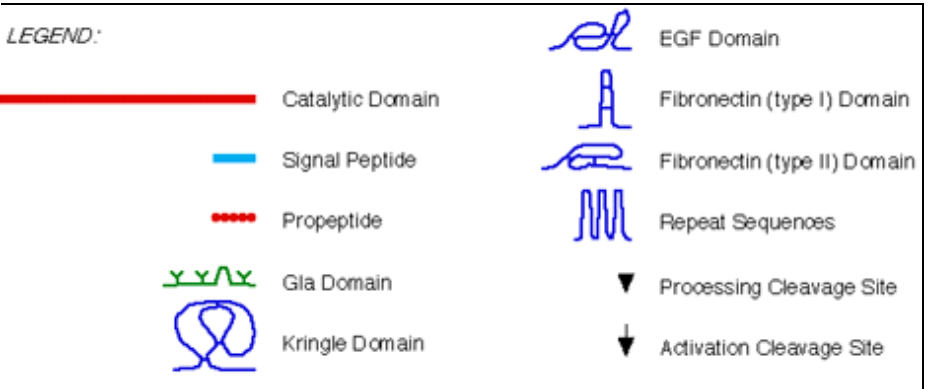


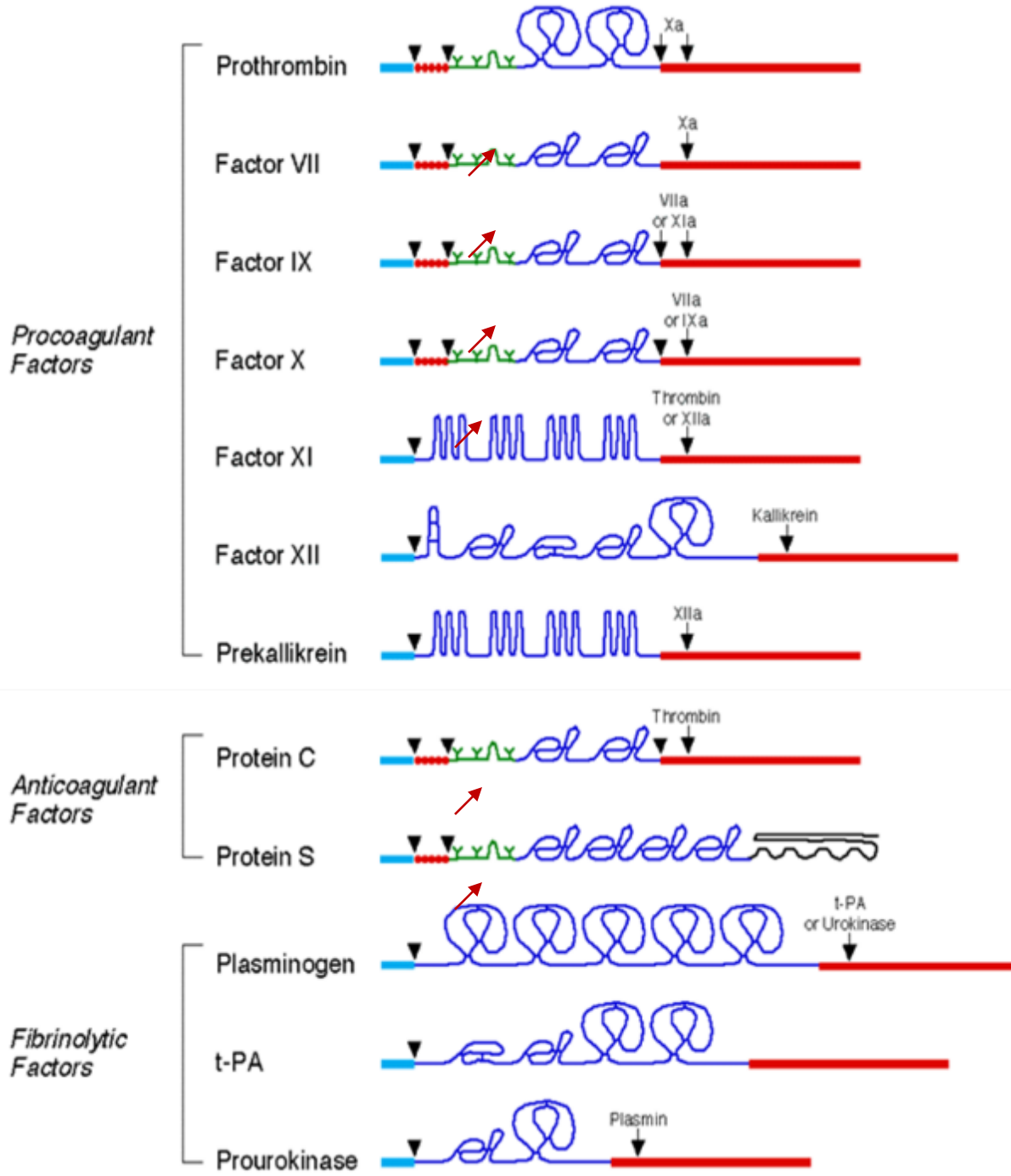




- We have two pathways controlling coagulation in our bodies, intrinsic and extrinsic pathways , according to the type of damage (internal or external). However, there is a connection between the two pathways.
- Intrinsic pathway is initiated when subendothelial surface (especially the collagen) is exposed, while extrinsic pathway is initiated when the tissues are injured, in this case the tissue releases TF (Tissue factor).


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

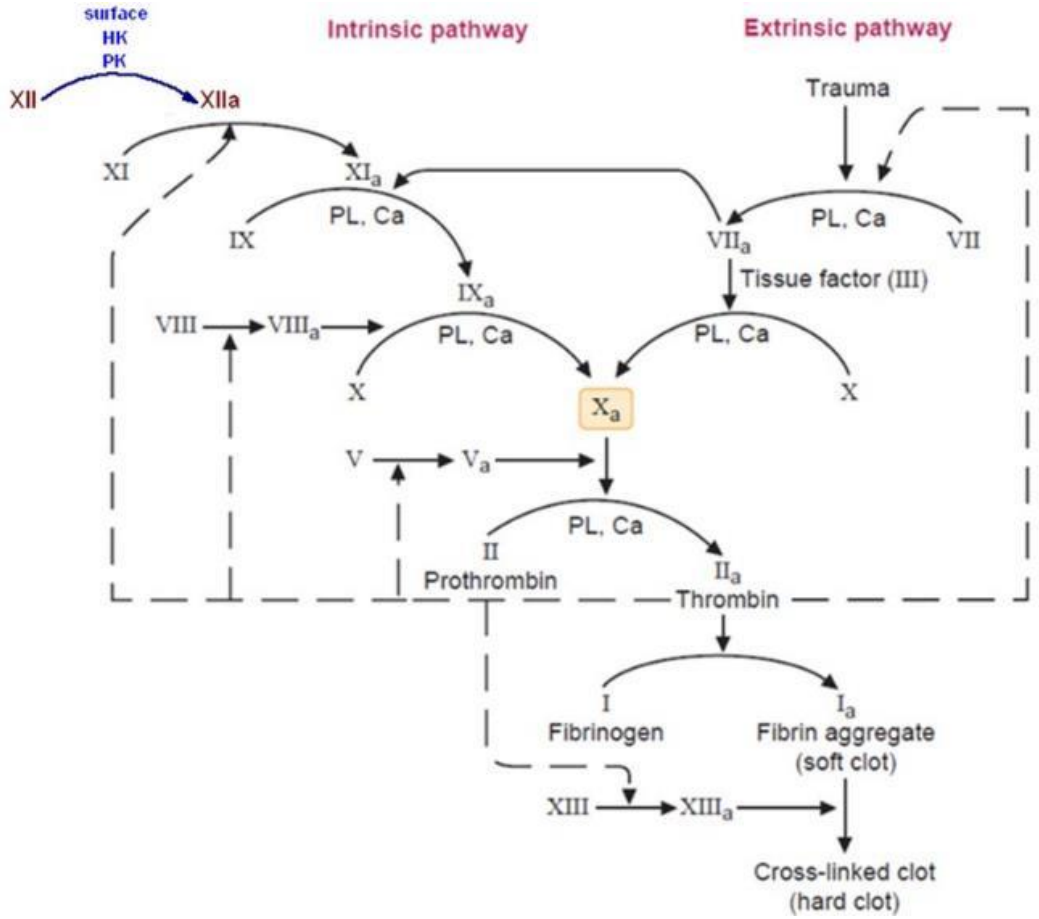

LEGEND:
Catalytic Domain
Signal Peptide
Propeptide
Gla Domain
Kringle Domain
EGF Domain
Fibronectin (type I) Domain
Fibronectin (type II) Domain
Repeat Sequences
Processing Cleavage Site
Activation Cleavage Site

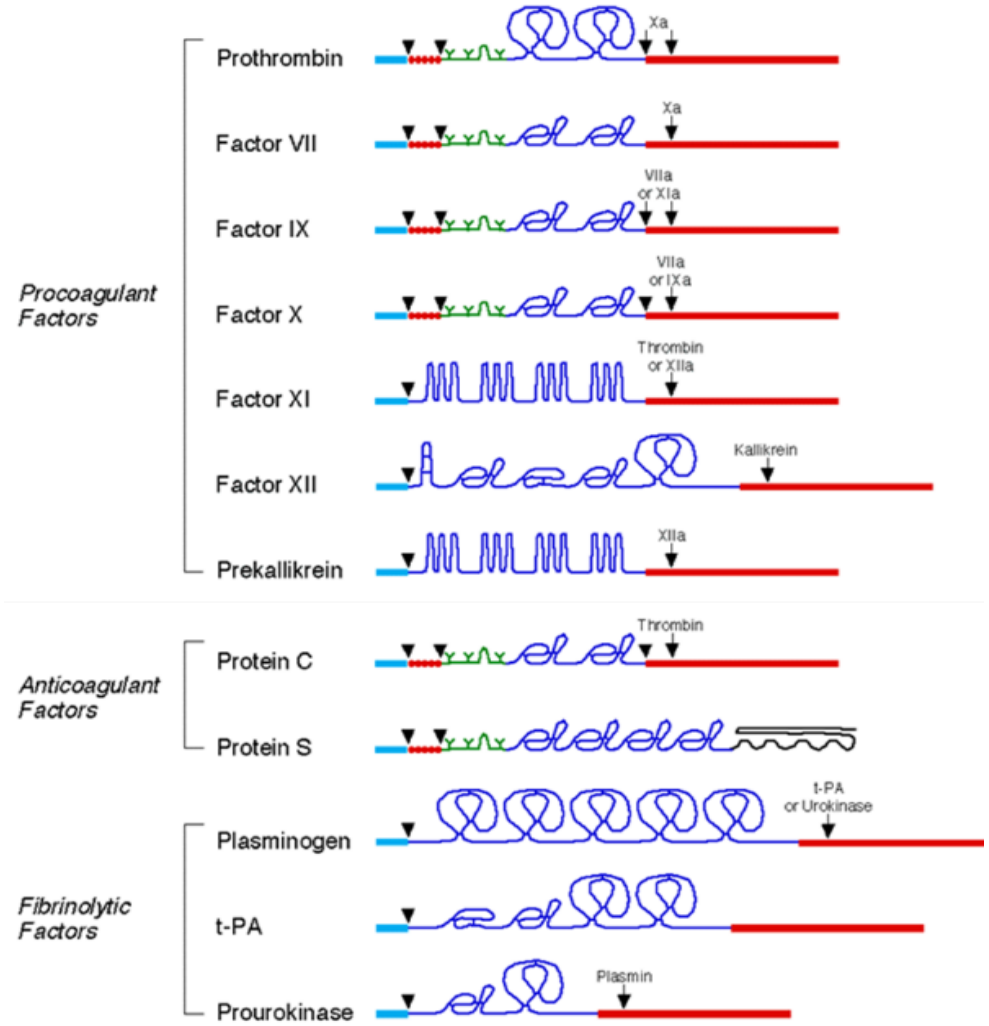

Procoagulant Factors
Prothrombin
Xa
Factor VII
Xa
Factor IX
VIIa or XIa
Factor X
VIIa or IXa
Factor XI
Thrombin or XIIa
Factor XII
Kallikrein
Prekallikrein
XIIa
Anticoagulant Factors
Protein C
Thrombin
Protein S
Fibrinolytic Factors
Plasminogen
t-PA or Urokinase
t-PA
Prourokinase
Plasmin

- Look at the diagram, it shows the intrinsic and extrinsic pathways of blood coagulation, notice that they meet on factor X activation. When factor X is activated, it converts prothrombin into thrombin, leading to activation of thrombin and the coagulation continues the common pathway.

- Remember that zymogen (inactive form of enzyme) are often named either by adding –ogen suffix (like pepsinogen), or by adding Pro- prefix (like prothrombin).

- Then thrombin converts fibrinogen (soluble) into fibrin (insoluble, so fibrin molecules aggregate with each other forming soft clot, soft clot is converted into hard clot by covalent linkage between fibrin monomers.

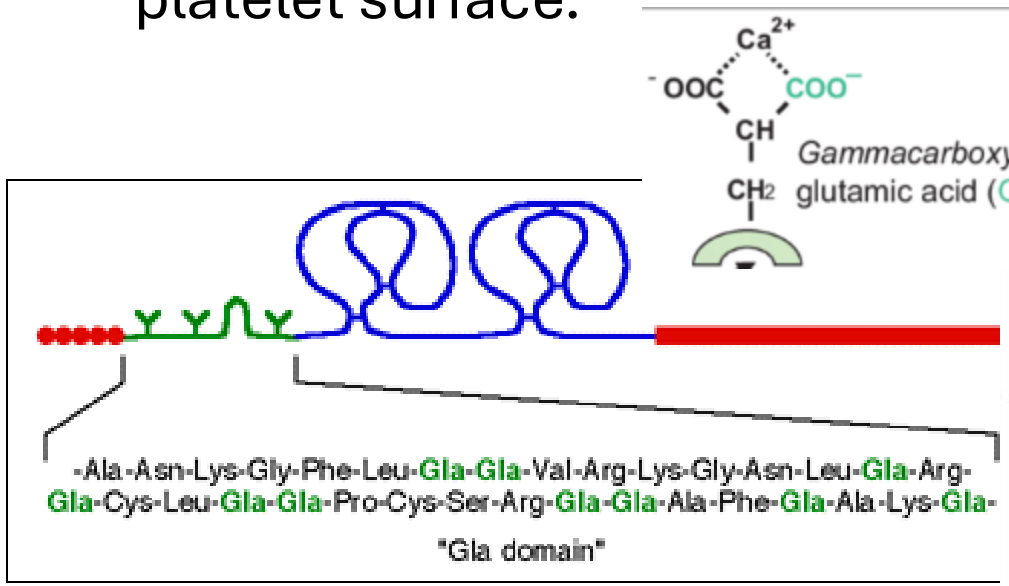


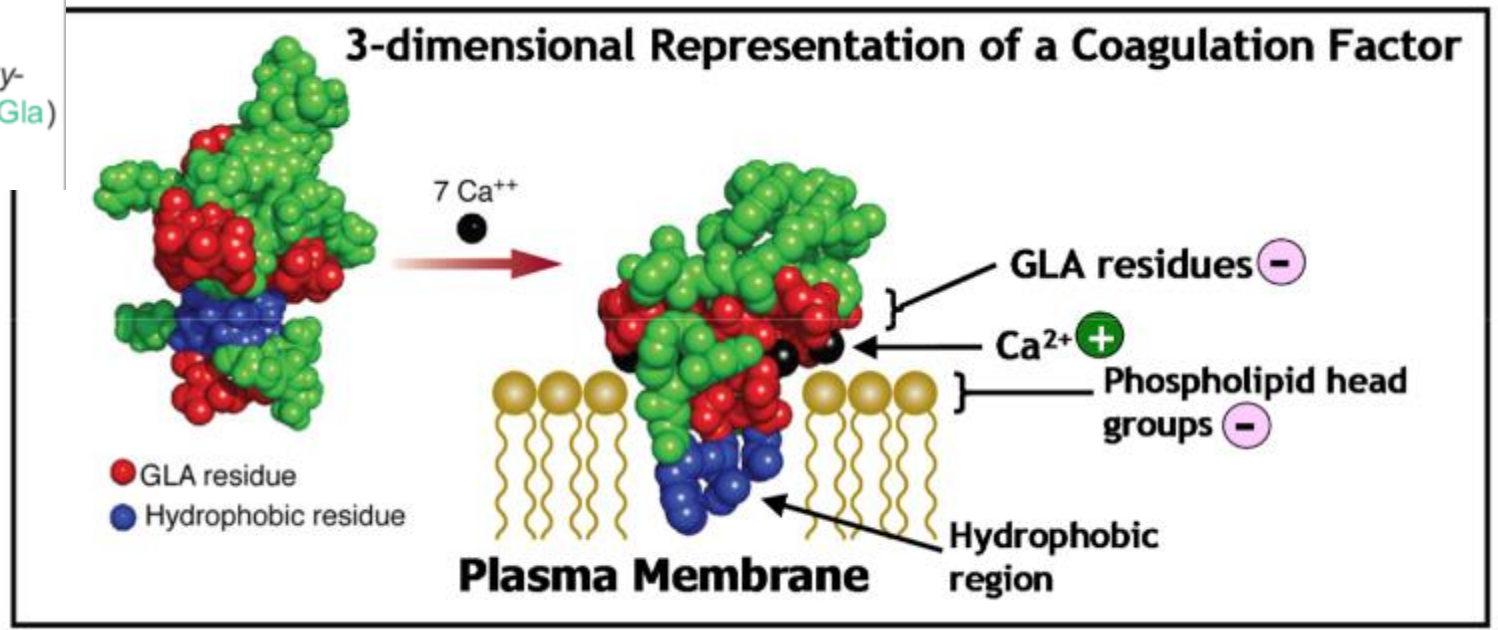


- These are the structures of different domains of clotting factors, we will focus on Glutamate domain. (coloured green at the diagram)

Gla domain

- An ER/Golgi carboxylase binds to prothrombin and factors IX, VII, and X and converts 10≥ glutamate (Glu) residues to gamma-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.





- Glutamate domain, is a sequence of amino acid in primary structures where we have a lot of glutamate amino acids. This sequence is very important because the Gla undergoes carboxylation. Gla domain already has a carboxyl group, and another carboxyl group is added on its gamma -carbon, forming gamma-carboxyglutamate. The biochemical relevance of carboxyl group is that it makes cross linking with Ca+2 when it is released from platelets.

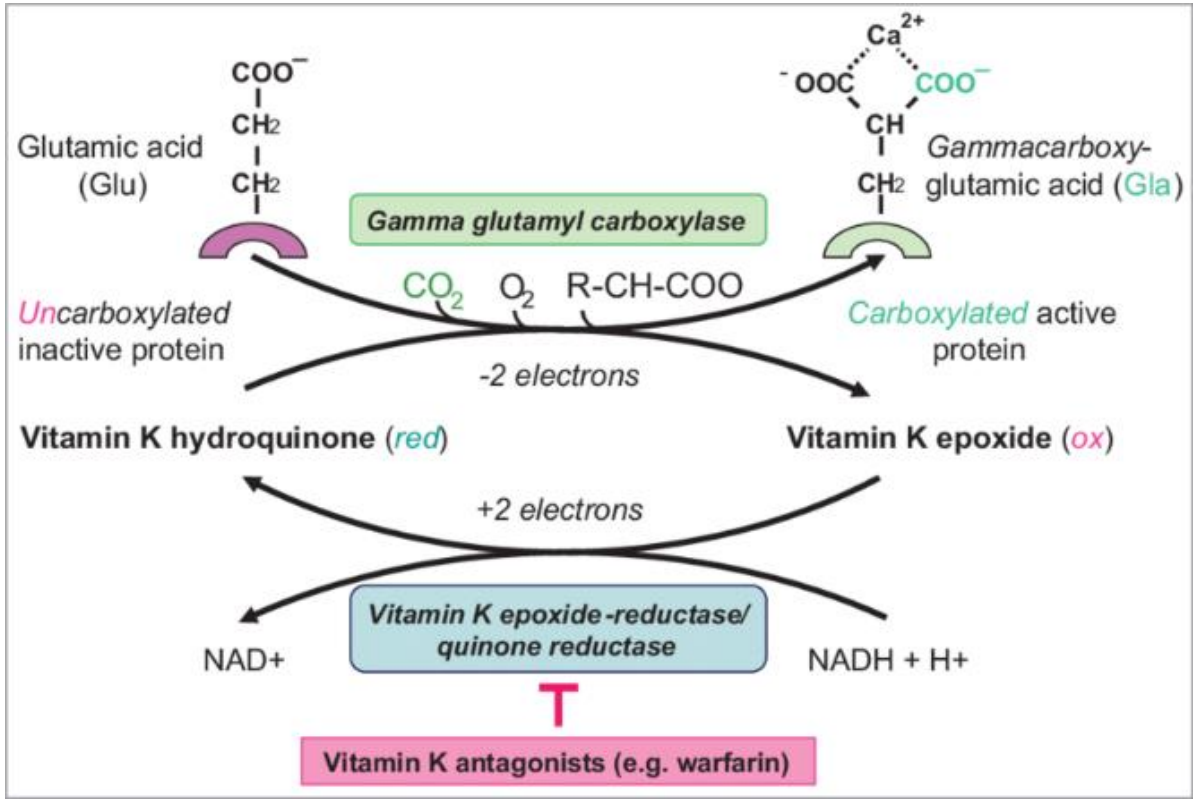
- Ca2+ ions make interactions with phospholipids on platelets surface because of phospholipids negative charge, and calcium ions positive charge, there will be interactions between proteins that contain glutamate and phospholipids, mediated by calcium ions. Why Our bodies do this to put factors together? in order to activate each other “through compartmentalization”.

- Compartmentalization means to put the enzyme and its substrate on cells surface to increase their chance to find each other. Gla domain is found on factors IX, VII and X.

- The carboxylation of Gla is catalyzed by a carboxylase enzyme (Gamma glutamyl carboxylase), this enzyme needs vitamin K to work. So, vitamin Kis responsible of carboxylation reaction.For vitamin K to work again, it needs regeneration through reductase enzyme.

The role of vitamin K

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

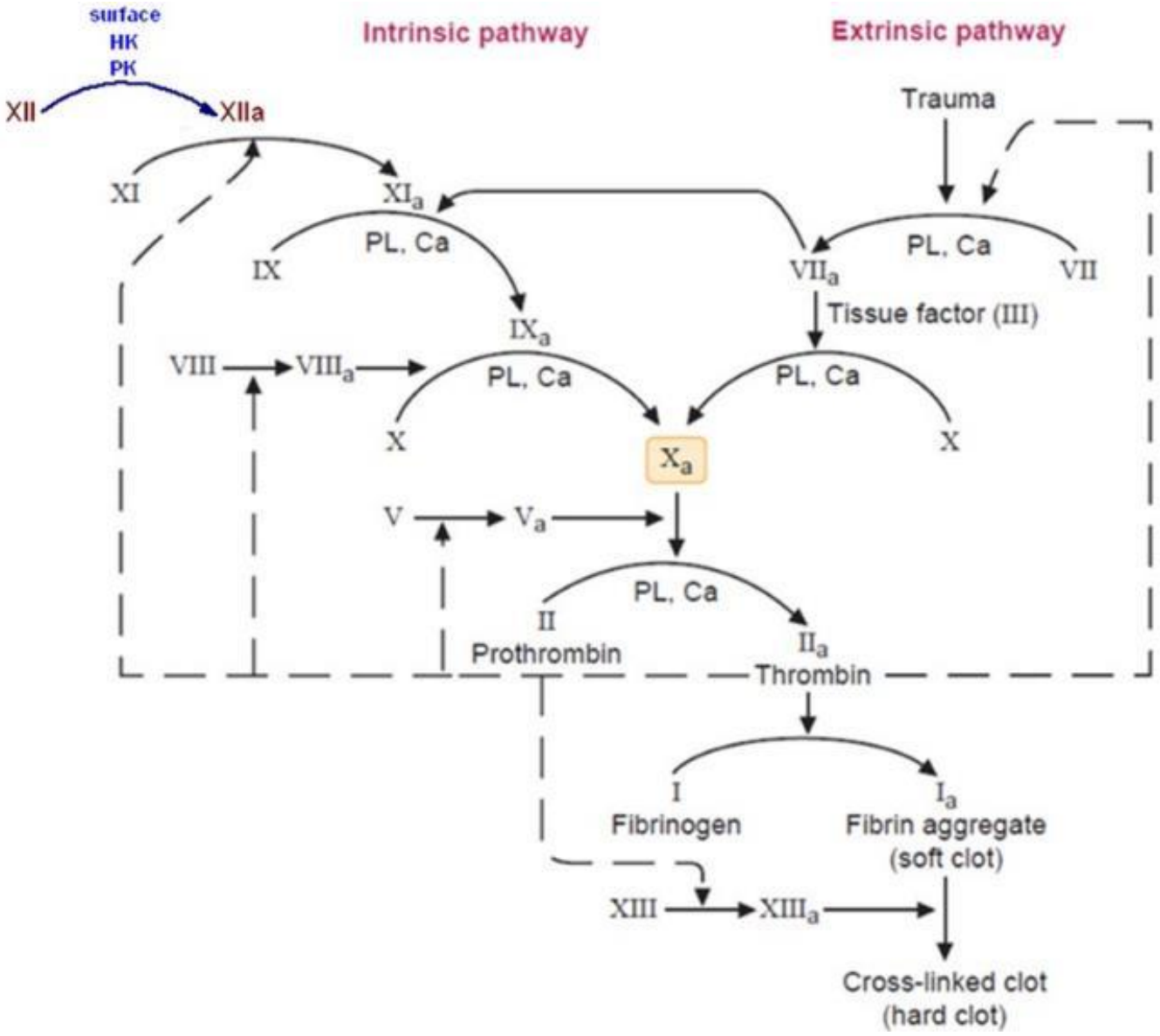


- Glutamate carboxylation catalysed by carboxylase.
- Vitamin K is required for the activation of the carboxylate.
- For vitamin K to work again, it needs to be regenerated through reduction by a reductase & NADH.
- This reductase is important since it is targeted by drugs specially warfarin.

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.

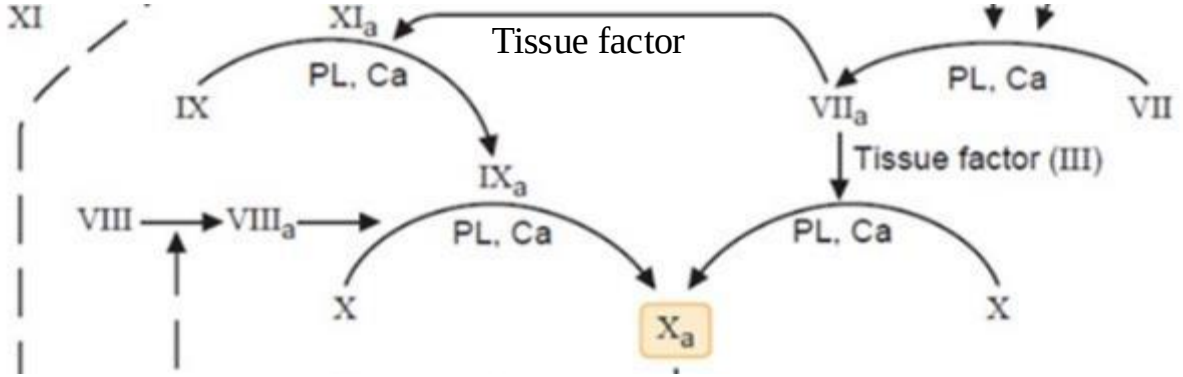
Vitamin K is generated at the intestine That why it's hard for adult to have deficient vitamin K

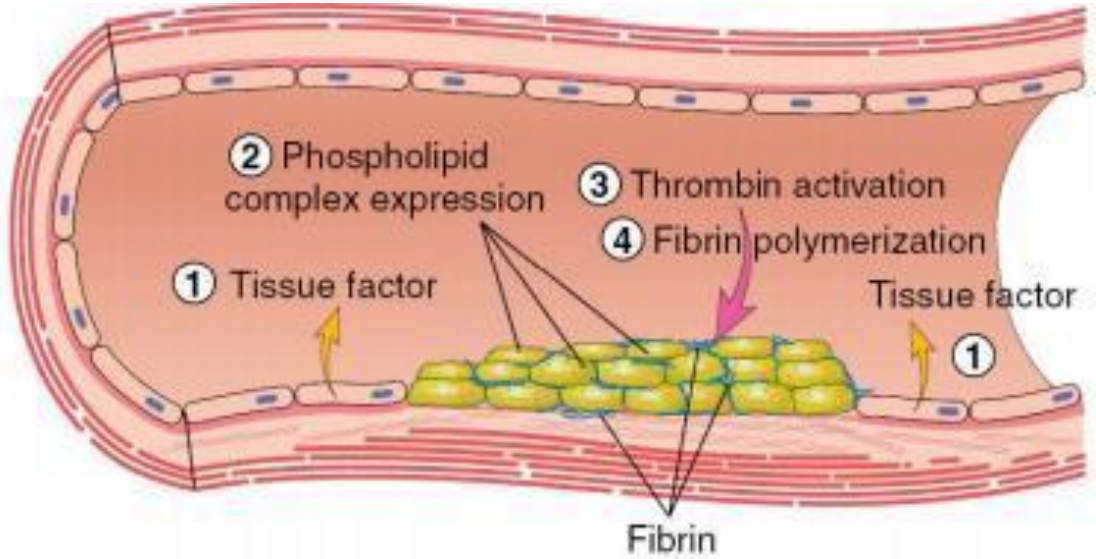


- TF (Tissue factor) is protein released from damaged cells, it does an interaction with factor VII, which increases its proteolytic activity (factor VII). This complex between TF and factor VII is called initiation complex. Notice that the initiation complex activates the intrinsic pathway as well.
- Calcium ions and phospholipids are needed for the function of TF and VII(this happens on surface of platelets). So, the extrinsic pathway starts from TF and VII arriving to factor X.

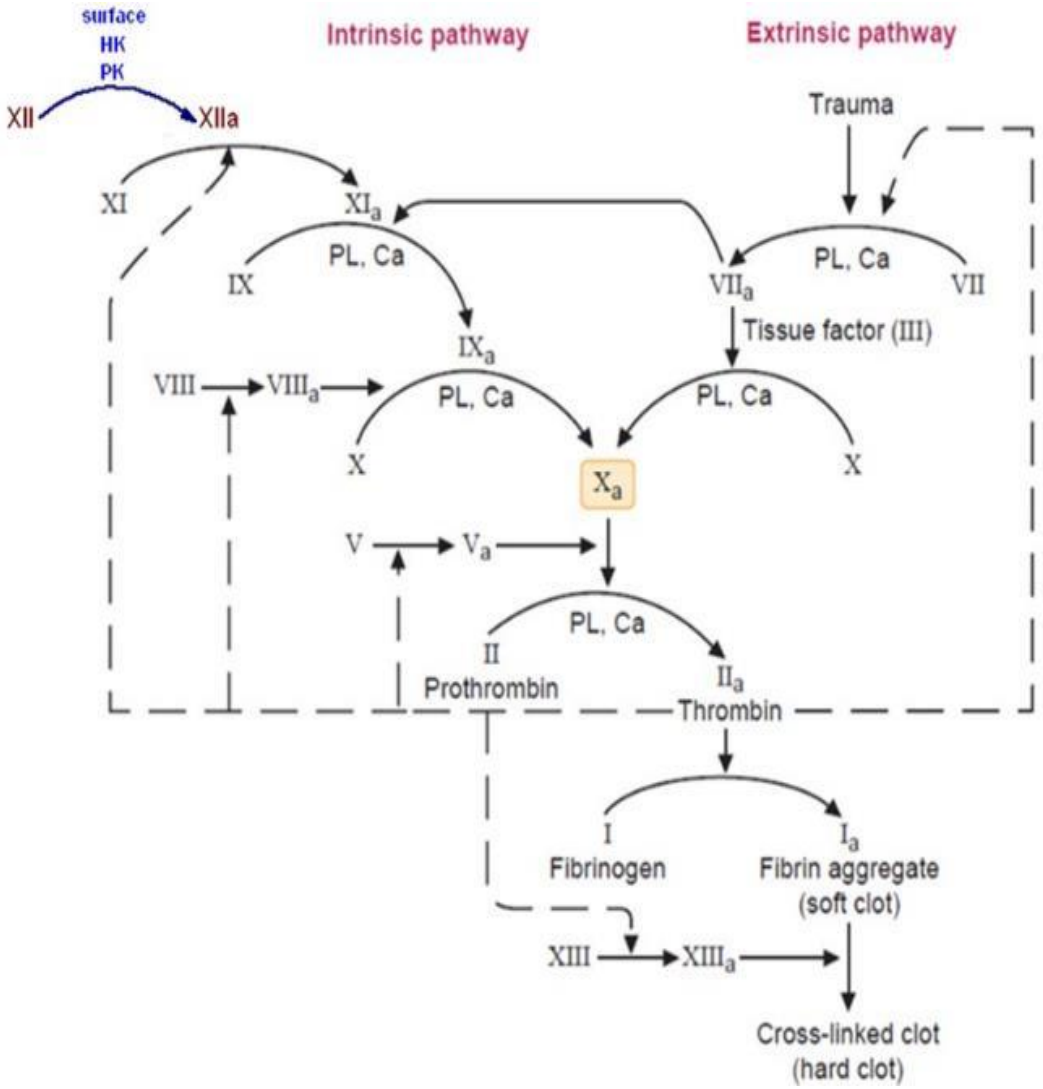
Tissue factor

- TF is an integral membrane protein that is expressed on the surface of "activated" monocytes, subendothelial cells, and other cells.
- It is the primary initiator of coagulation and is not exposed to blood until disruption of the vessel wall.
- It increases the proteolytic efficiency of VIIa.





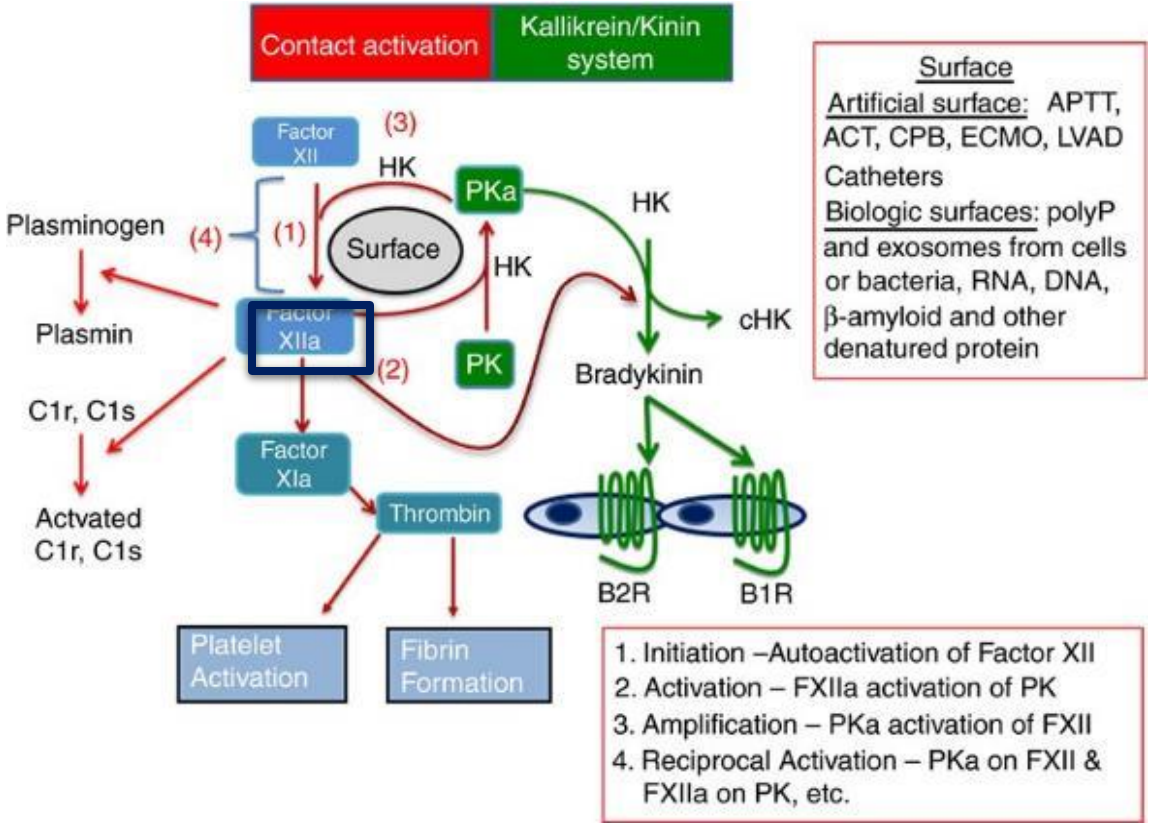
Exposure of tissue factor initiates the coagulation cascade.
TF/VIIa complex is the “initiation complex”.



- We said that TF binds to VII on platelets surface which activates factor X.
- Moreover, TF-VII complex activates factor IX (intrinsic pathway), which is already being activated by factor XI which was activated by factor XII.
- Factor XII is activated by prekallikrein on the surface of platelets.
- In extrinsic pathway: factor X is activated by TF-VII complex.
- In intrinsic pathway: factor X is activated by complex IXa.

Initiation of the intrinsic pathway

- Prekallikrein, HMW kininogen, factors XII and XI are exposed to a negatively charged activating surface.(the platelets)
- Factor XII is autoactivated into XIIa, which has several substrates:
  1. factor XI, which activates factor IX.
  2. Kallikrein from prekallikrein (note the positive feedback activation loop).
  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.



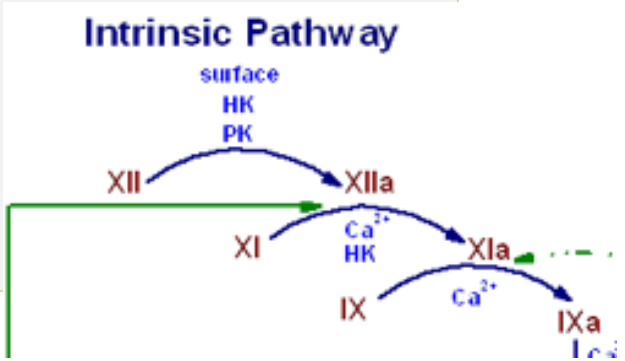
HK, intact high-molecular-weight kininogen; HKc, cleaved high-molecular-weight kininogen; PK, prekallikrein; PKa, plasma kallikrein; polyP, polyphosphate

- We have different components until reaching factor X (Prekallikrein, HMW (high molecular weight) kininogen, factors XII and XI) which are exposed to negatively charged activating surface (platelets).
- factor XII has many functions:

1. It activates factor XI
2. Factor XI activates factor IX
3. factor IX with VIII will activate factor X
4. Factor X activates thrombin

Prekallikrein is activated to kallikrein, notice that kallikrein has feedback ACTIVATION on factor XII.

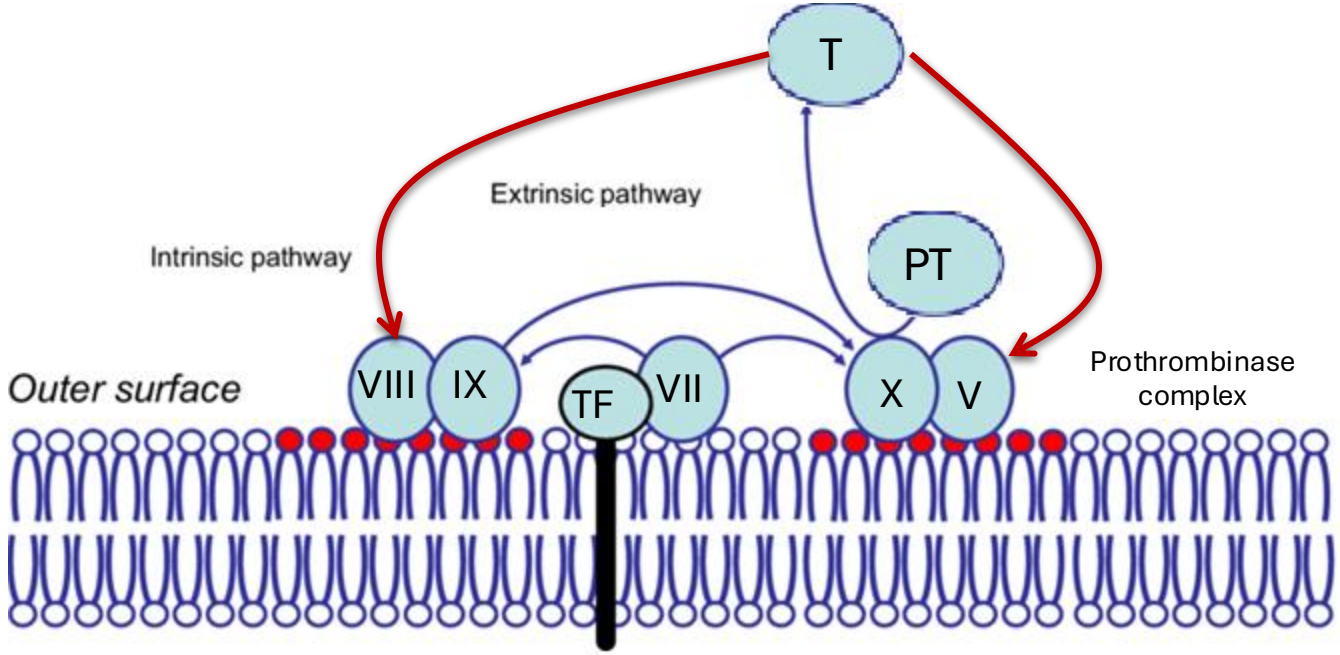
Activated prekallikrein (PKa) activates HMW kininogen to bradykinin (vasodilator).
Note: factor XII activates HMW kininogen as well.



The tenase complexes

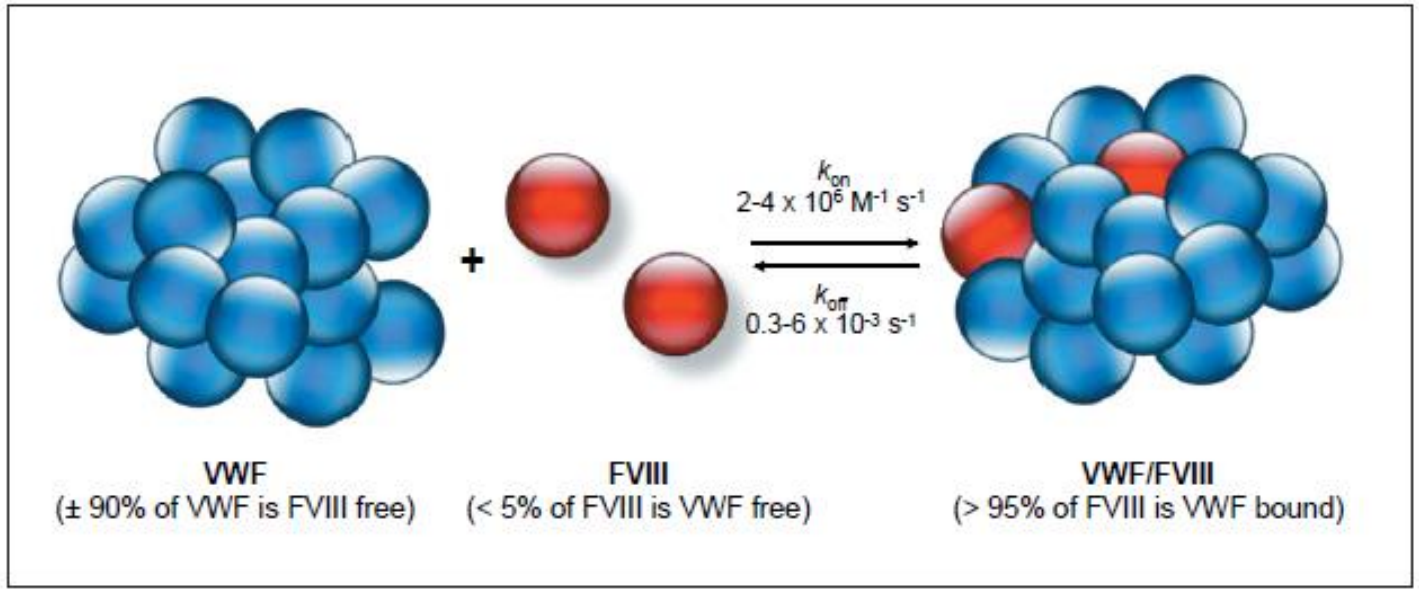
- For factor X activation, it should be part of two complexes called the tenase complexes.“Tenase: ten (10) / ase (enzyme)“
- The activating complexes of factor X are called the “tenase” complexes.
  - The extrinsic tenase complex is made up of tissue factor, factor VIIa, and Ca^{2+}.
  - The intrinsic tenase complex contains the active factor IX (IXa), its cofactor factor VIII (VIIIa), and Ca^{2+}.
    - Tissue factor and factor VIIa also activate factor IX in the intrinsic pathway.

- Va and VIIIa are cofactors (not enzymes)that increase the proteolytic efficiency of Xa and IXa, respectively.
  - Both factors V and VIII are activated by thrombin via a feedback mechanism.

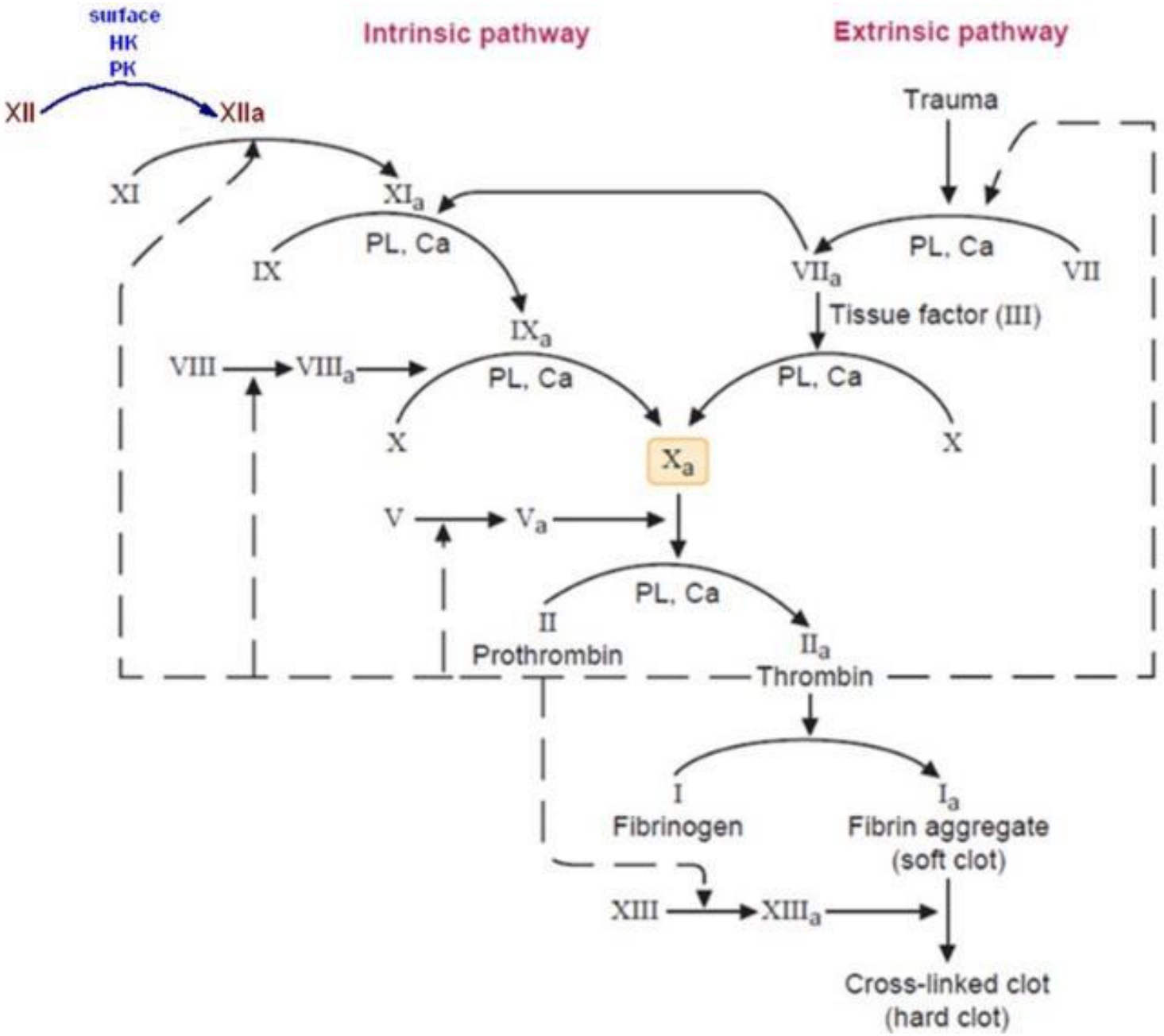


von Willebrand factor deficiency

- 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.



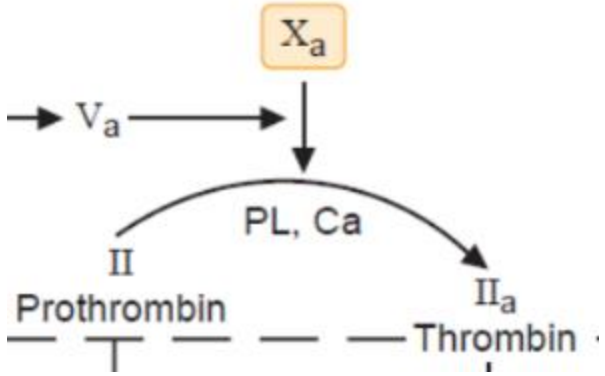
- When von Willbrand factor is released from endothelial cells and platelets it binds with factor VIII on cell surface, as result it increases VIII half-life. When von Willbrand factor is not being released (deficiency) , VIII half-life will be decreased.

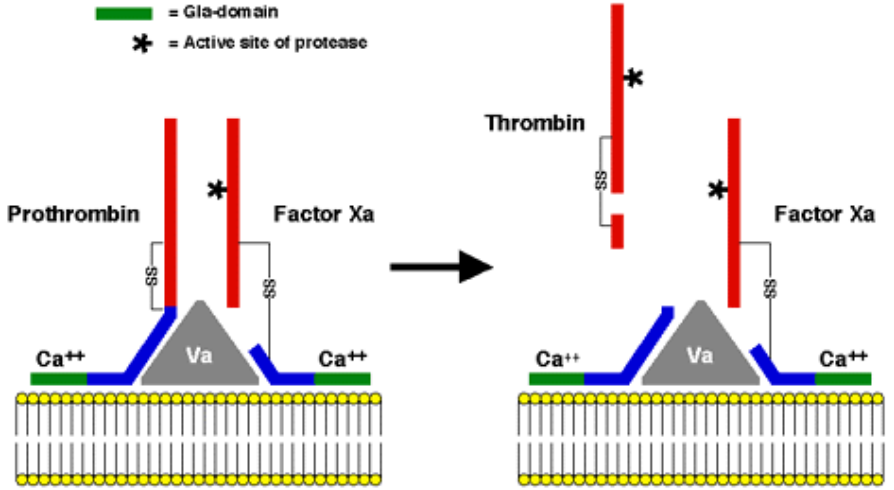


- Factor X needs factor V to activate prothrombin.

Prothrombin activation

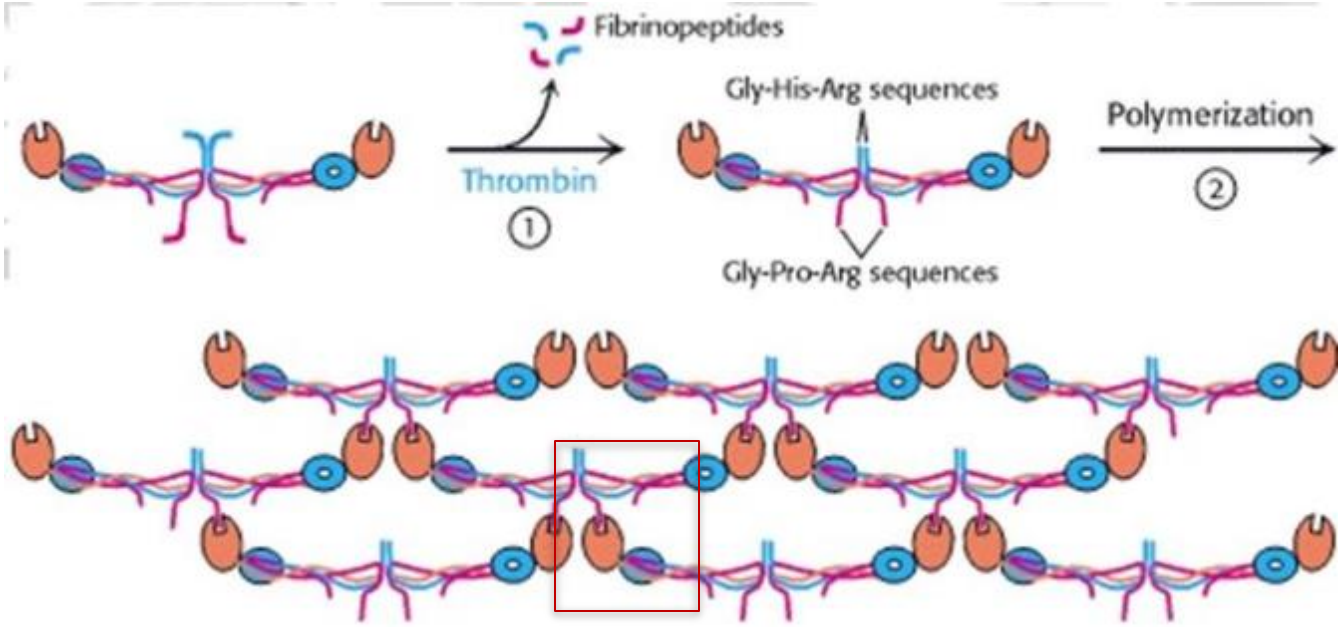
- The complex (on the surface of platelet)of factor Xa/Va/Ca^{2+} and phospholipids is the "prothrombinase complex".
- Factor Xa converts prothrombin to thrombin, which is accelerated by Va, platelets (or phospholipids), and calcium ions.
- Binding of calcium alters the conformation the Gla domains of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

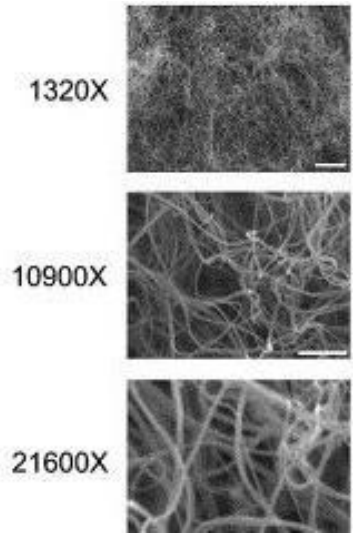




Formation of a *soft* fibrin clot

- Thrombin cleaves fibrinogen releasing fibrinopeptides.
  - Fibrinogen is a two triple-stranded helical protein held together by disulfide bonds.
- Fibrin molecules create electrostatic attractions among each other 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“ (non covalent interactions – electrostatic interactions-).



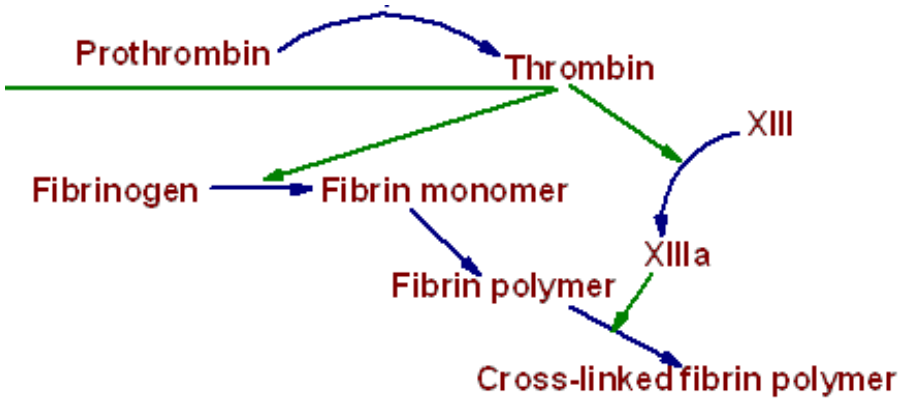


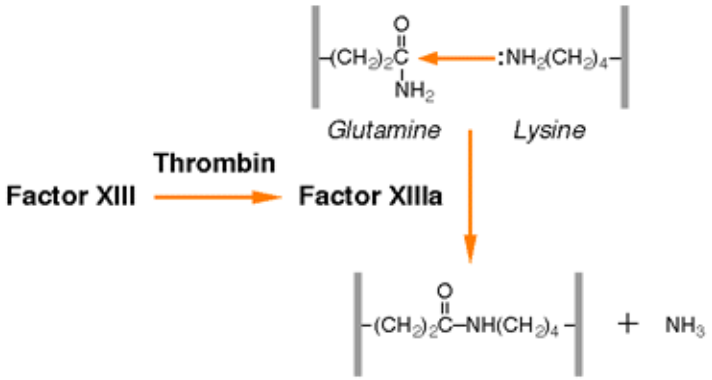
Electron microscope image (like a network).

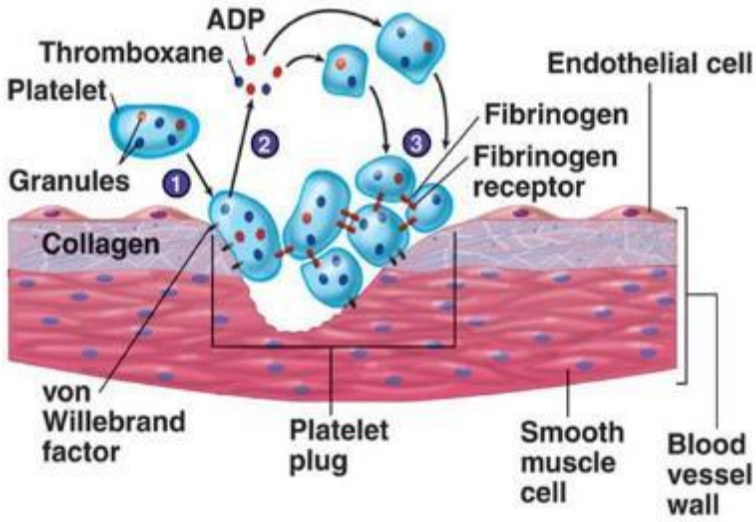
Fibrinogen is composed of 3 strands; (triple stranded molecule) those strands are separated molecules (the do not have interactions between each other) because of fibrinopeptides. What thrombin does is removing these peptides converting fibrinogen to fibrin.

The formation of a hard clot by factor XIII

- Factor X also converts factor XIII into its active form.
- Factor XIII is a transglutaminase that is activated by thrombin.
- Factor XIIIa catalyzes a transglutamination reaction that causes a 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“

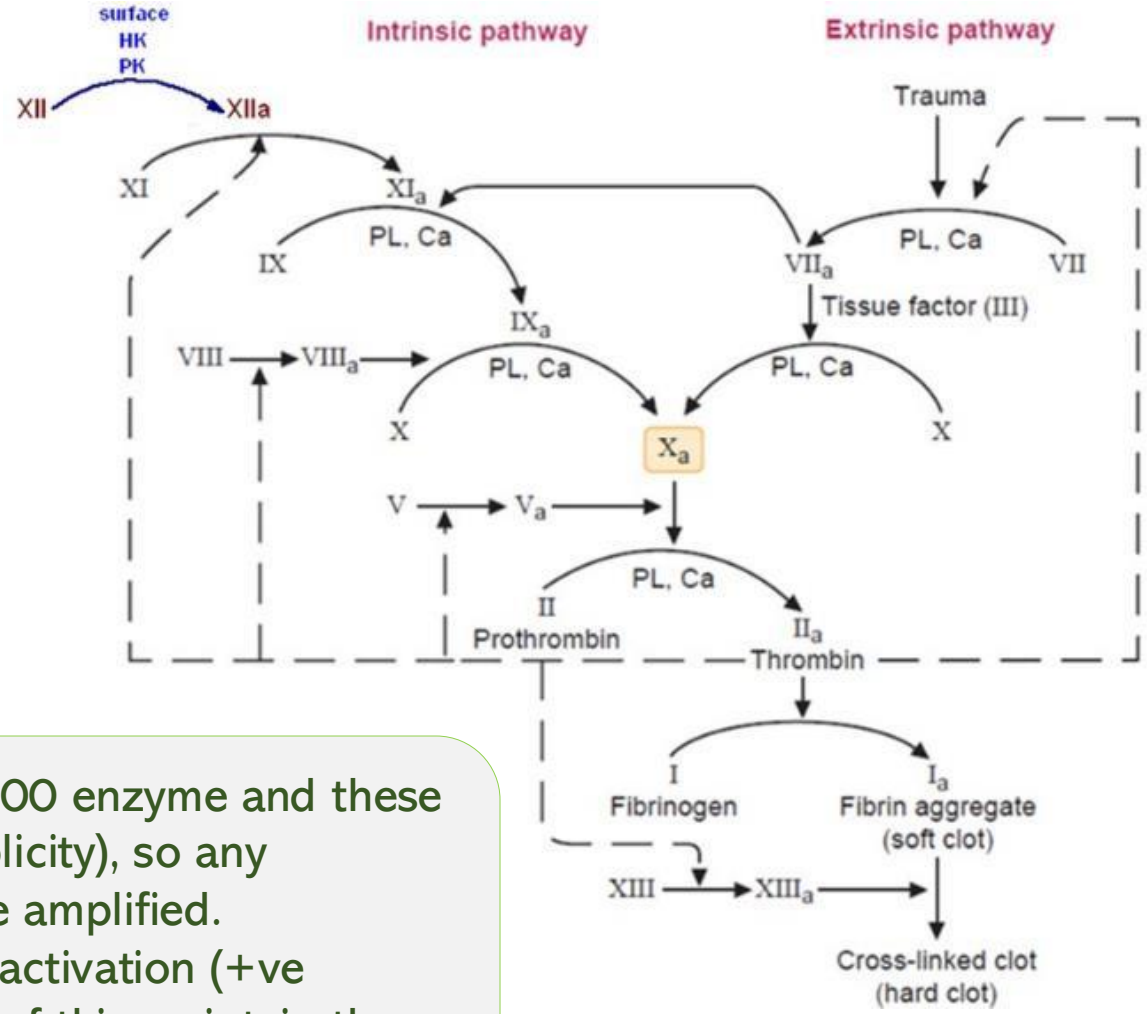






Amplification of coagulation reactions

- 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.



All these processes are amplified, one enzyme activate 100 enzyme and these 100 enzymes will activate 100 each (numbers are for simplicity), so any enzymatic activation in intrinsic or extrinsic pathway can be amplified.

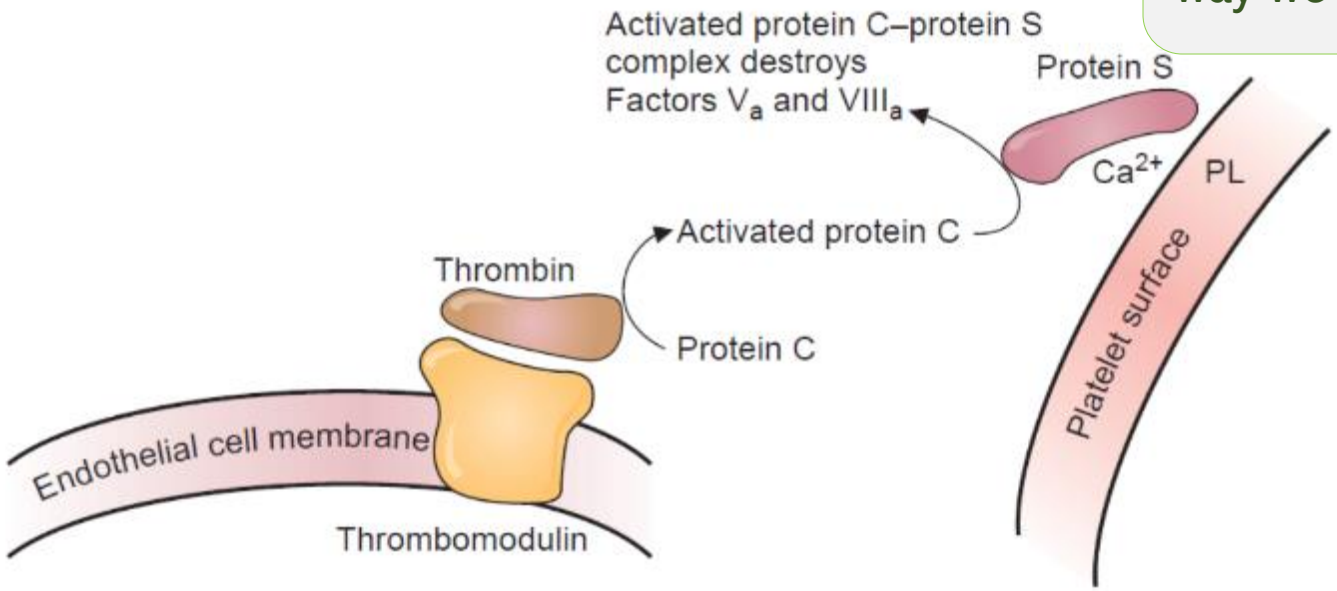
Also, thrombin plays a role in amplification, by feedback activation (+ve feedback) to different factors like factor XI, VIII, V & VII. All of this maintain the hard clot

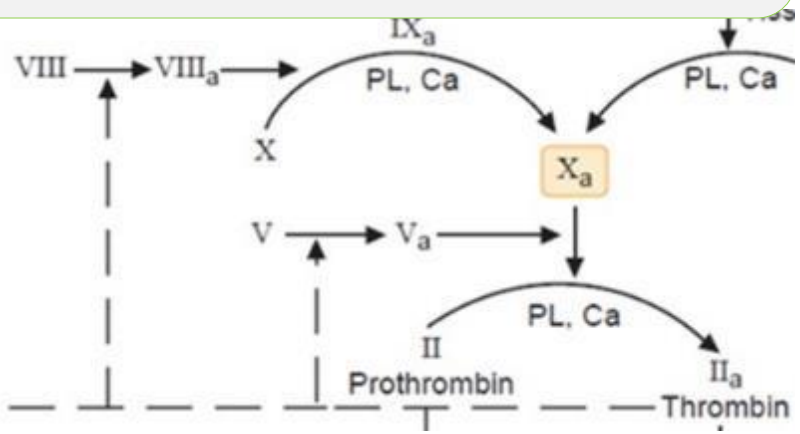
Anti-clotting factors

Protein C and protein S

- Thrombin binds thrombomodulin (modulin means modulate, or change)on 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.

The activation of protein X is decreased, so prothrombin—> thrombin will decrease, in this way we slow down the activation of the pathway.

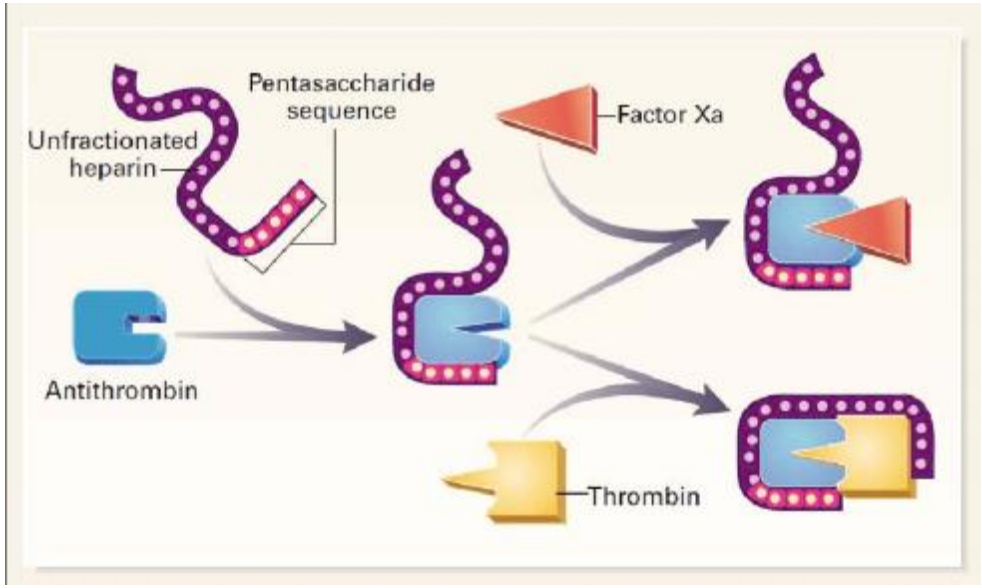


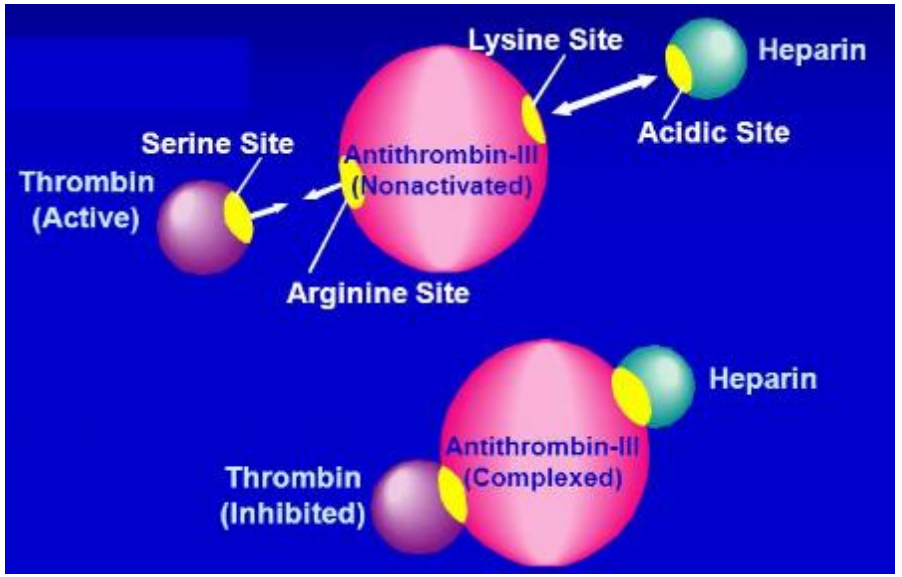


Antithrombin III

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

Heparin activate antithrombin III inhibition to thrombin, so heparin is an anticoagulant



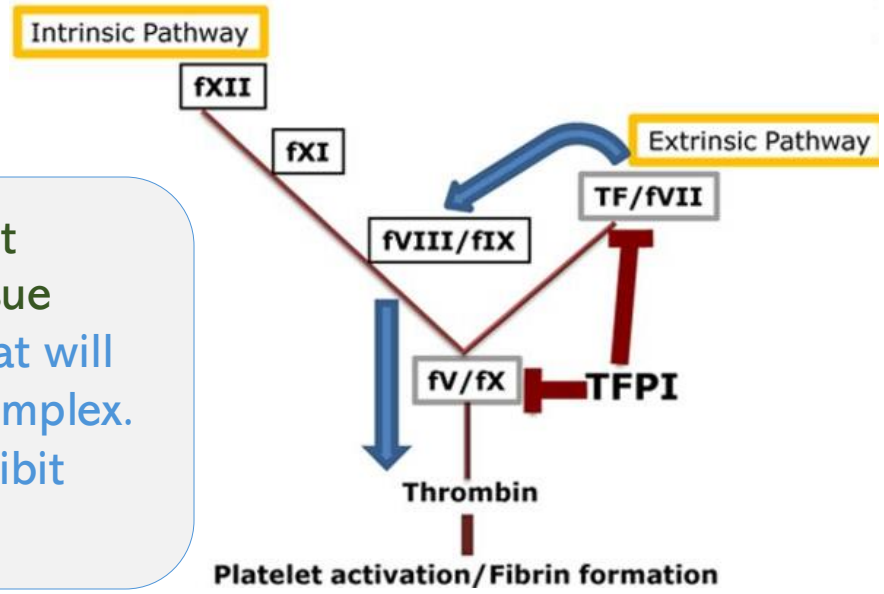


In the clinic, phlebotomy tubes are often treated with heparin to inhibit clot formation.

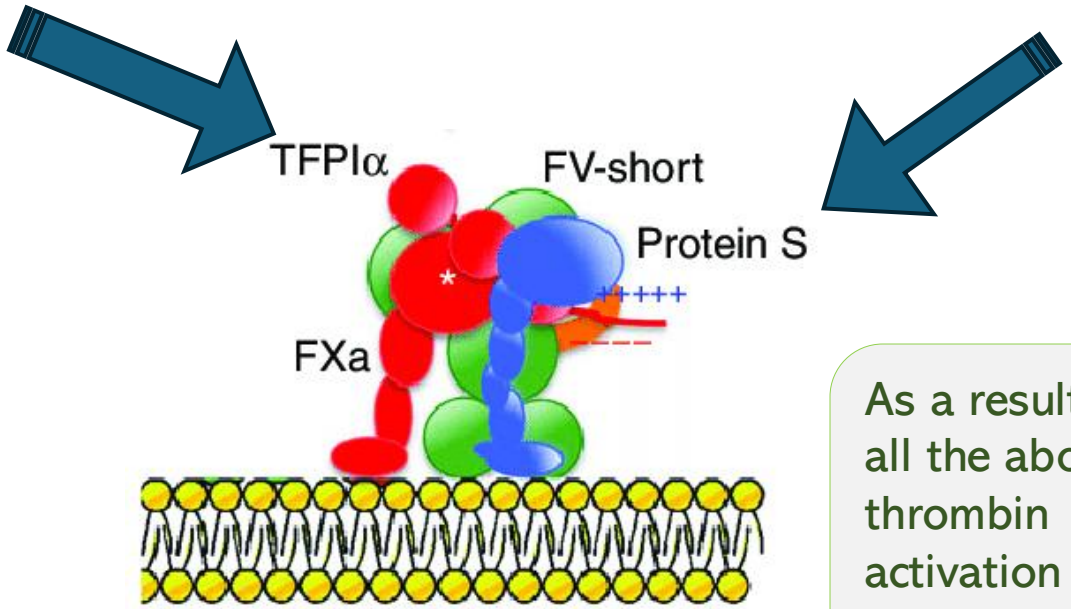
Heparin is widely used in phlebotomy tubes, these tubes contain a gel in the bottom, this is heparin, which is used to inhibit the coagulation by antithrombin III, so the blood remain fluidic because when blood is exposed to air, it'll coagulate.

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 TF-VIIa complex and inhibits it and its activation of factors X and IX which is in intrinsic pathway
  - TFPI also inhibits Xa-activated complexed with Va resulting in inhibition of the pro-thrombinase complex.
  - Protein S binds to TFPI localizing it to membrane surfaces of platelets and enhancing the inhibition of Xa., inhibiting



What TFPI do is it interact with Tissue Factor (TF) so that will inhibit TF/VIIa complex. And TFPI will inhibit Xa/Va complex.

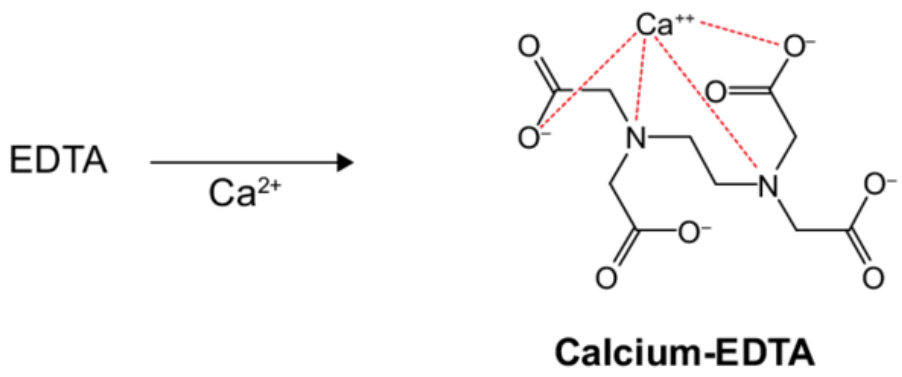


As a result of all the above, thrombin activation will be inhibited

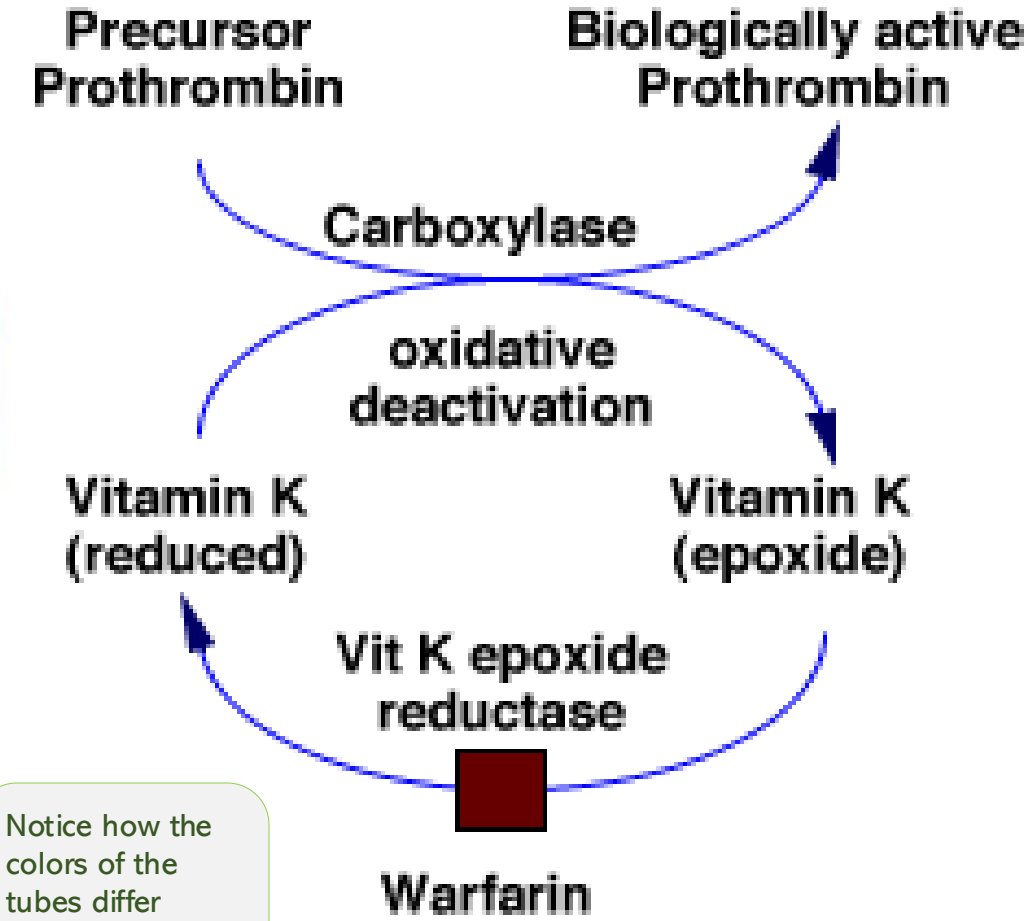
These are non-enzymatic inhibitors of blood coagulation :

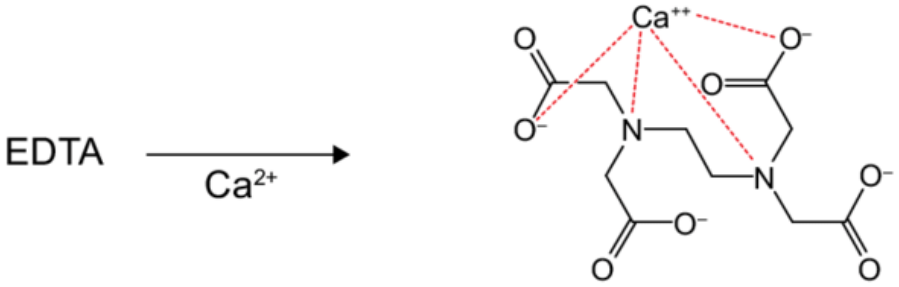
Ca^{2+} chelators and vitamin K antagonists

- Blood clotting can be prevented by addition of Ca^{2+} chelators like EDTA and vitamin K antagonists such as the drug warfarin, which inhibits the reduction of vitamin K and thereby prevents the synthesis of active prothrombin and factors VII, IX, and X.

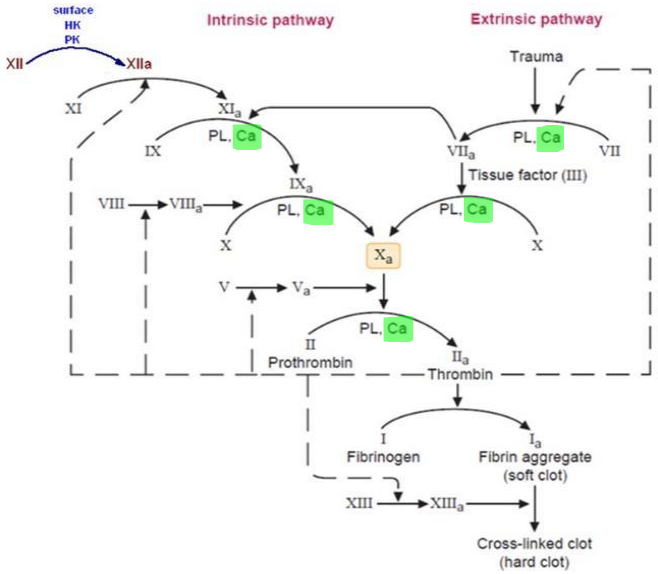


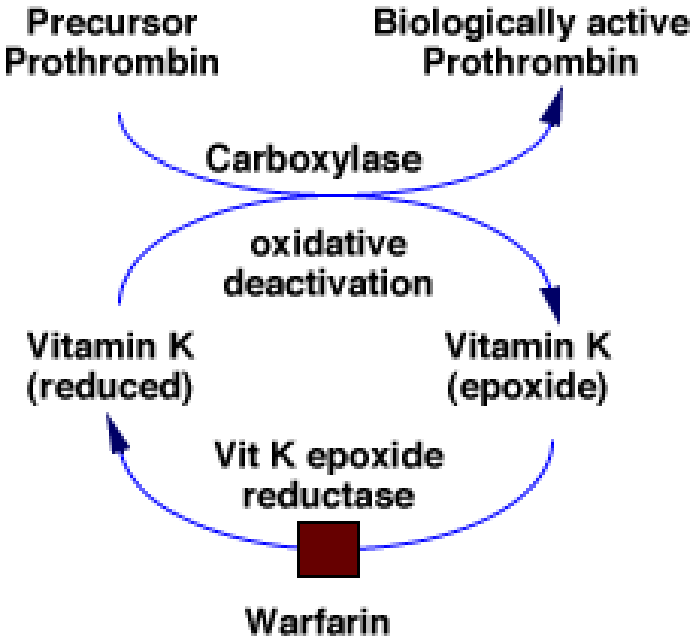
Notice how the colors of the tubes differ according to the purpose of each tube





The Ca2+ chelators like EDTA will bind with Calcium and pull it from blood to prevent the coagulation. Remember that calcium is needed in almost every step in platelets activation and coagulation except some. (Green highlight)





This is mentioned earlier briefly, vitamin k is needed in the coagulation, it's needed in the carboxylation of some AAs, after that the vitamin will become inactive, it should be reduced in order to back active by reductase. Warfarin drug target this reductase enzyme and inhibit it, so vitamin k can't be regenerated (or reactivated) so carboxylase enzymes doesn't function, so coagulation process will be inhibited by inhibiting the carboxylation of glutamate, so warfarin is vitamin K antagonist.

Degradation of the fibrin clot

When healing process is finished, how the clot is degraded ?

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.

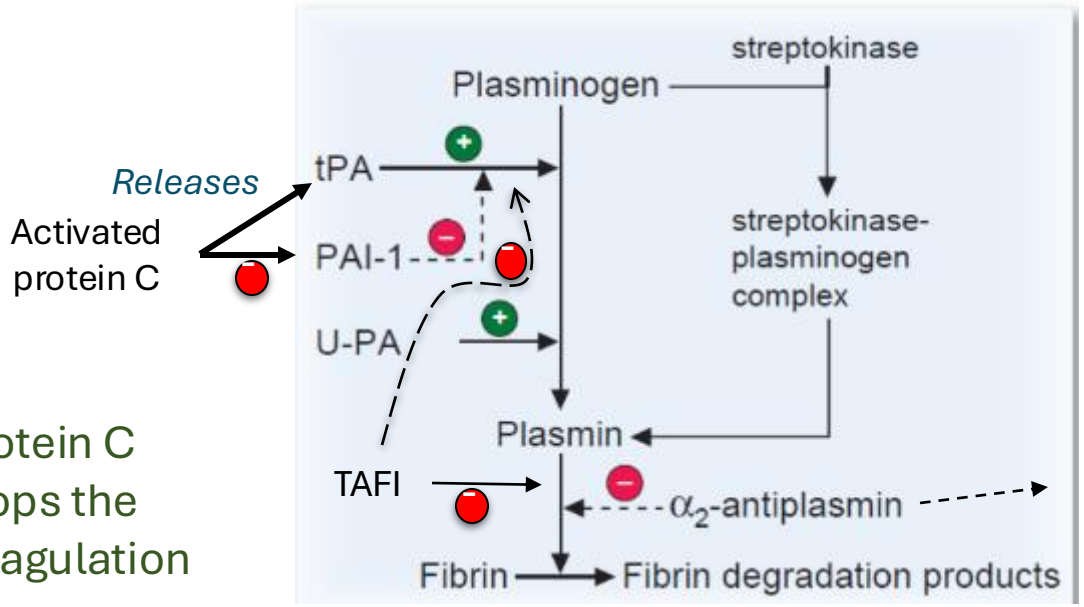
It's important to remove the clot, because the clot prevent the blood from moving

The clot must be dissolved, not only removed, because if it is removed only and moves freely, a plug could be formed in another site

How does it dissolute ? By proteases, there's an important group of proteases for clot dissolution

The fibrinolytic system

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



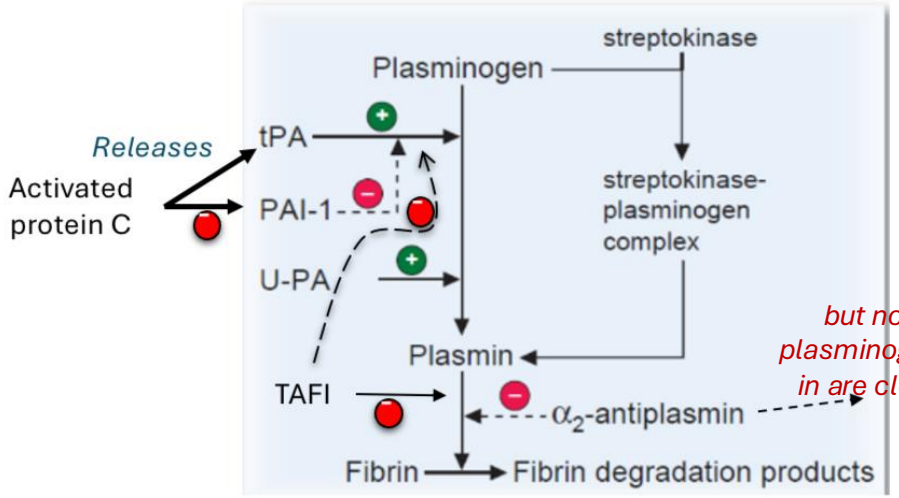
Protein C stops the coagulation

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

Removal of fibrin clot is highly organised, the most important enzyme in removing the clot is plasmin, when it's activated (proteolytic activation) from plasminogen (a zymogen) it'll degrade fibrin clot

Note: Plasminogen won't be activated unless it binds to fibrin clot (so as long as it's free in blood, it won't be activated)



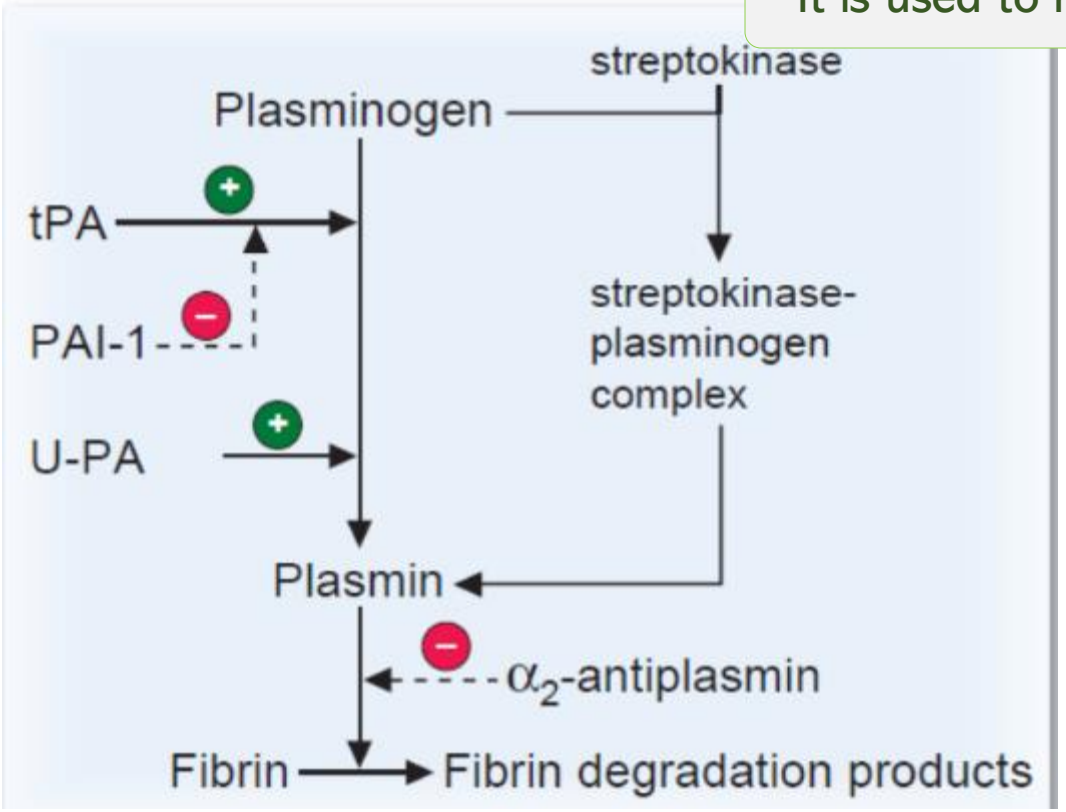
- Plasminogen is activated by tPA (tissue Plasminogen Activator) and inhibited by PAI-1
- Protein C enhance the activation process, so it activate tPA and deactivate PAI to facilitate the activation process.
- U-PA (Urokinase-Plasminogen Activator) is another activator for plasminogen (discussed in the next slide)
- TAFI (Thrombin activatible fibrinolysis inhibitor) inhibit the process of activation by tPA so it prevent fibrinolysis, also it inhibit plasmin degradation of fibrin. (Note the figure)
- a2-antiplasmin: when plasmin is released from clot, antiplasmin is released and interacts with plasmin and inhibit it, why after plasmin is released? In order not to function on something other than plasmin, so we don't need it to function on platelet or erythrocyte or to damage surrounding tissues or proteins in blood

Streptokinase: isolated from streptococci, allows autoactivation of plasminogen in blood to plasmin, and helps in removing clots. It's used clinically

Urokinase

- Urokinase (plasminogen activator) is a protease that is formed from the zymogen pro-urokinase.
- It is a potent plasminogen activator and is used clinically.

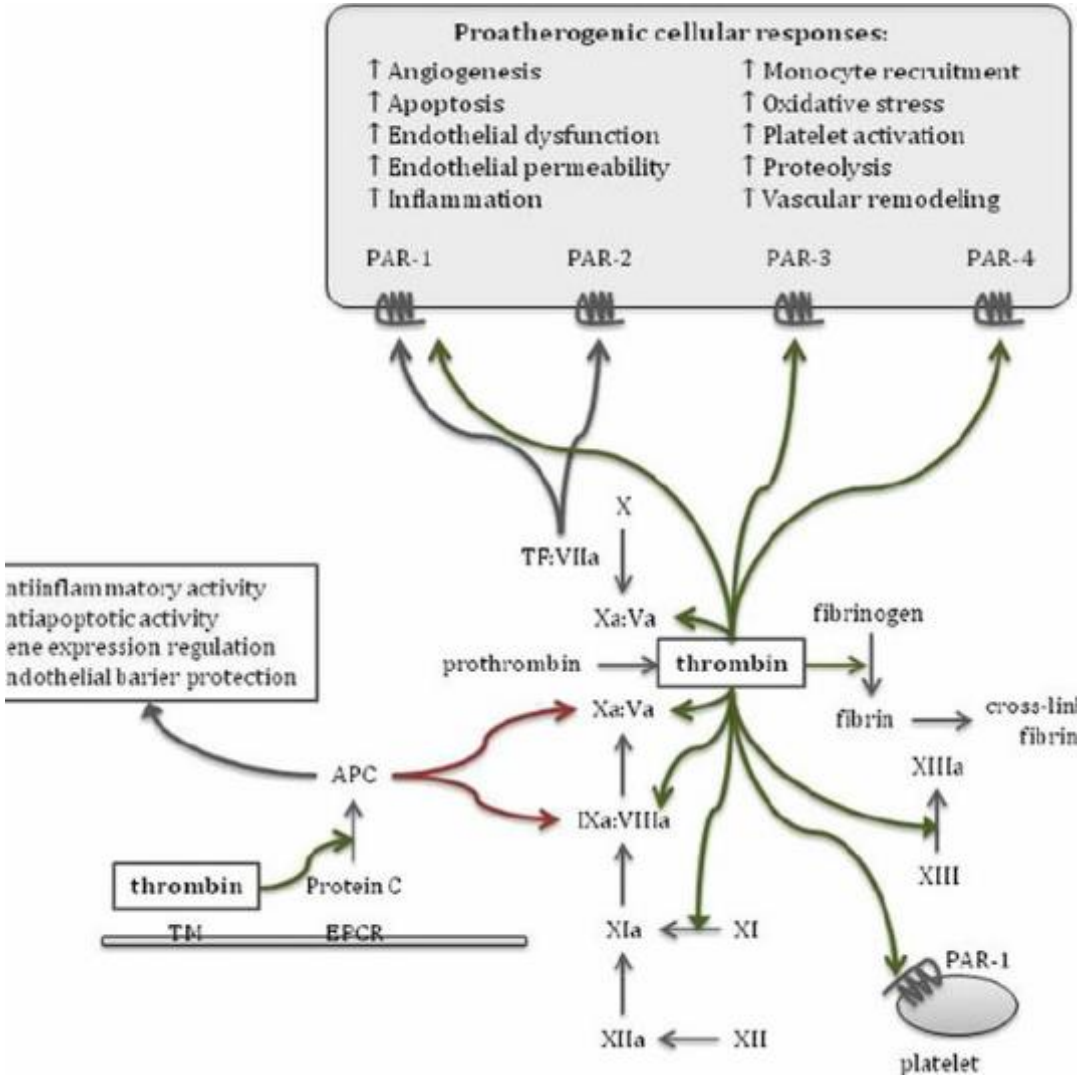
It is used to remove clots in certain patients



Thrombin is multi-functional

Roles of thrombin

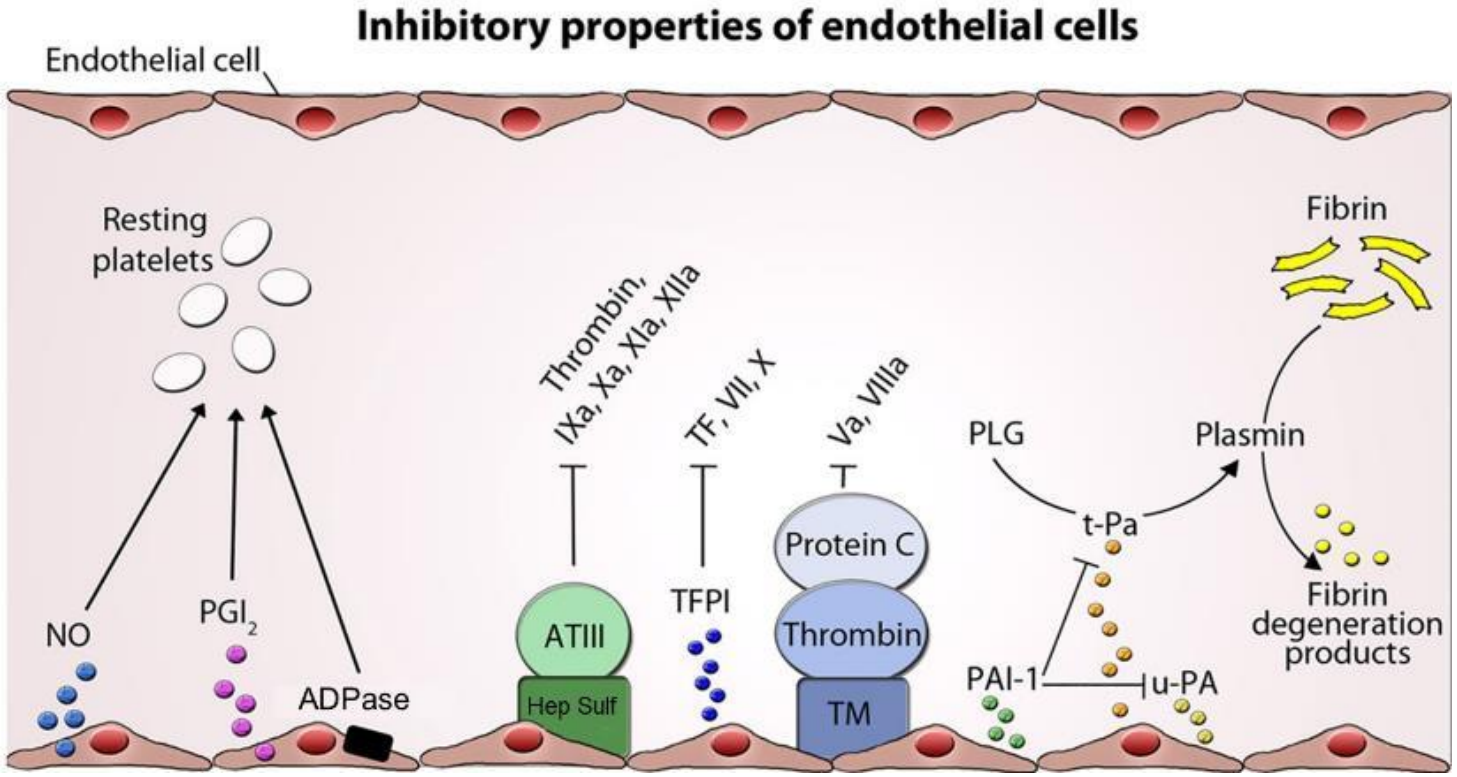
- Platelet recruitment Signaling pathway, by binding to receptor on platelet and activation of PLC
  Change in cytoskeletal structure of platelets and release of granular contents.
- Amplification of the coagulation complex feedback activation
- Formation of soft clot
  - Proteolytic cleavage of fibrinogen
- Formation of hard clot
  - Activation of factor XIII
- Attenuation of its own activity slowing down the process of coagulation
  - Activation of protein C
- Other actions
  - Binding to its receptor on the surface of platelets induces vascular remodeling (e.g. angiogenesis) and inflammation.
    Angiogenesis and inflammation actions aren't mentioned in this lecture



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

Role of endothelial cells in coagulation

NO = nitric oxide



NO & PGI2 are vasodilators

Intrinsic & Extrinsic pathways

- ECs release NO, prostacyclin (PGI2), and ADPase, which inhibit platelet adhesion and aggregation.
- Membrane-bound heparin sulfate binds to (activate) antithrombin III (ATIII) inactivating several coagulation factors like thrombin & others
- ECs express tissue factor pathway inhibitor (TFPI), which inhibits tissue factor (TF) and, consequently, factors VII, IX, and X.
- Express Thrombomodulin (TM), binds thrombin activating protein C, which degrades when complexed with protein S factors Va and VIIIa.
- ECs balance fibrin accumulation and lysis by releasing plasminogen activators, t-PA and u-PA, and their inhibitor (PAI).
  Balance things in terms of clot formation and removal.

It is a symphony played by an orchestra.

"و اعلموا أن مهمتكم ليست ورقة تنالونها، إنما أمة تحيونها"

Additional sources

1. Book pages
2. Youtube videos
3. Webpages...etc

VERSIONS	SLIDE #	BEFORE CORRECTION	AFTER CORRECTION
V1→ V2			
V2→V3			

امسح الرمز و شاركنا بأفكارك لتحسين أدائنا !!