Biochemistry - HLS

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Blood coagulation

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Hematopoietic-lymphatic system

Listen to Vivaldi while studying this lecture & connect the music to the biological processes
Enjoy :)
- Dr. Mamoun

Resources

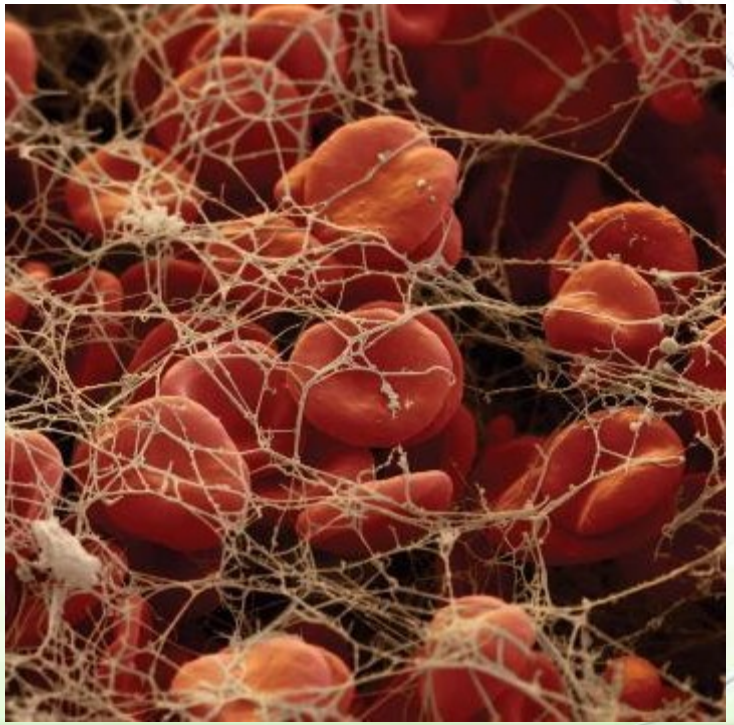
- This lecture
- Harper's Medical Biochemistry, 31st edition, Chapter 55
- Mark's Basic Medical Biochemistry, 7th edition, Chapter 43

Grab a cup of coffee and let's start!
And don't count the slides :)

What is blood coagulation (clotting)?

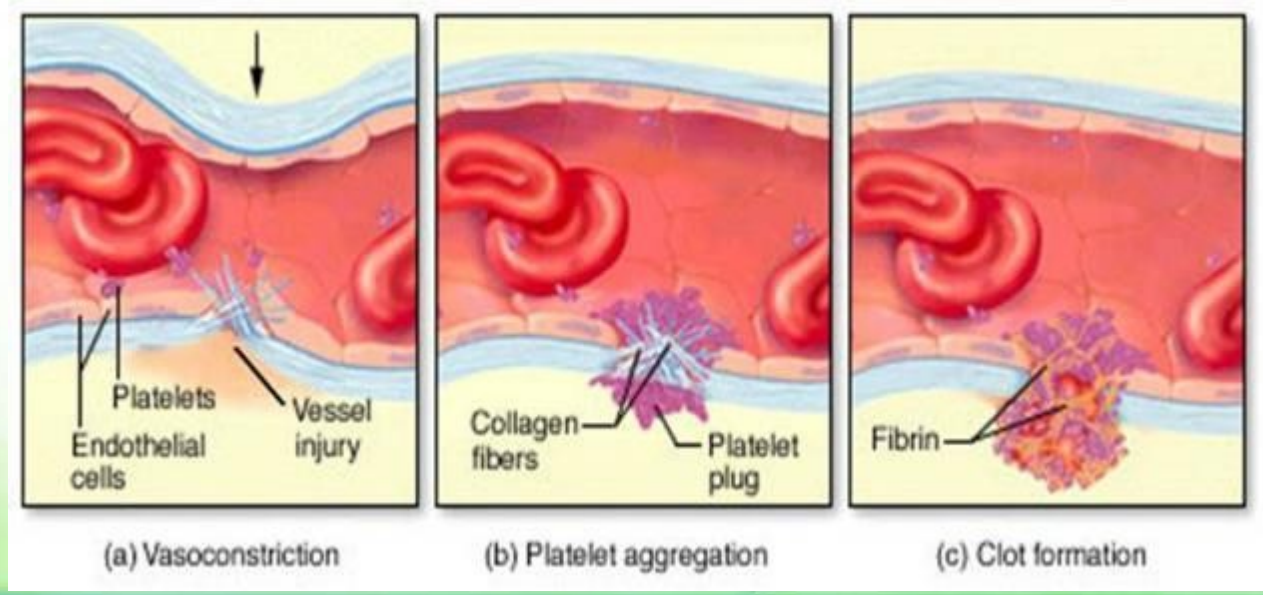
- It is an *orchestrated*, biochemical process that is initiated as a result of vascular injury where a small area blood of surrounding injury changes from liquid to gel, forming a clot made of fibrin, which results in hemostasis (the cessation of blood loss) followed by clot dissolution and repair.

The word "orchestrated" was chosen because as you go through blood coagulation, it is almost like music, it's like a classical symphony of Beethoven or Mozart, it goes slow and then it goes up and up until it reaches a climax and then you see the music going down until you get to the end.



Steps of hemostasis and thrombosis

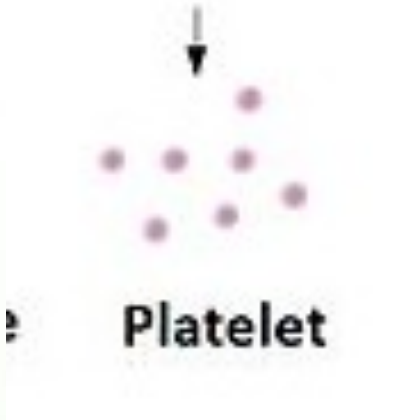
- Vascular constriction limiting blood flow to the area of injury (physiological effect)
- Followed by a chemical process (cellular and biochemical): Activation then aggregation of platelets at the site of injury, forming a loose platelet plug
- This platelet plug is loose, but it becomes solidified and hard, forming a hard clot, via Formation of a fibrin mesh to entrap the plug (A network of fibers surrounding & entrapping the platelets
- Dissolution of the clot in order for normal blood flow to resume following tissue repair



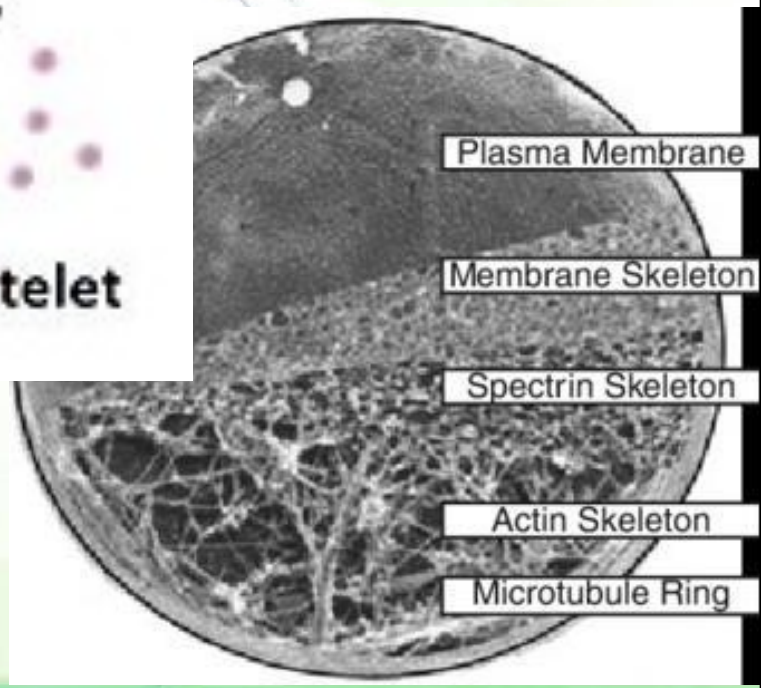
Platelets are a major player

- 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.

Megakaryocyte



Platelet

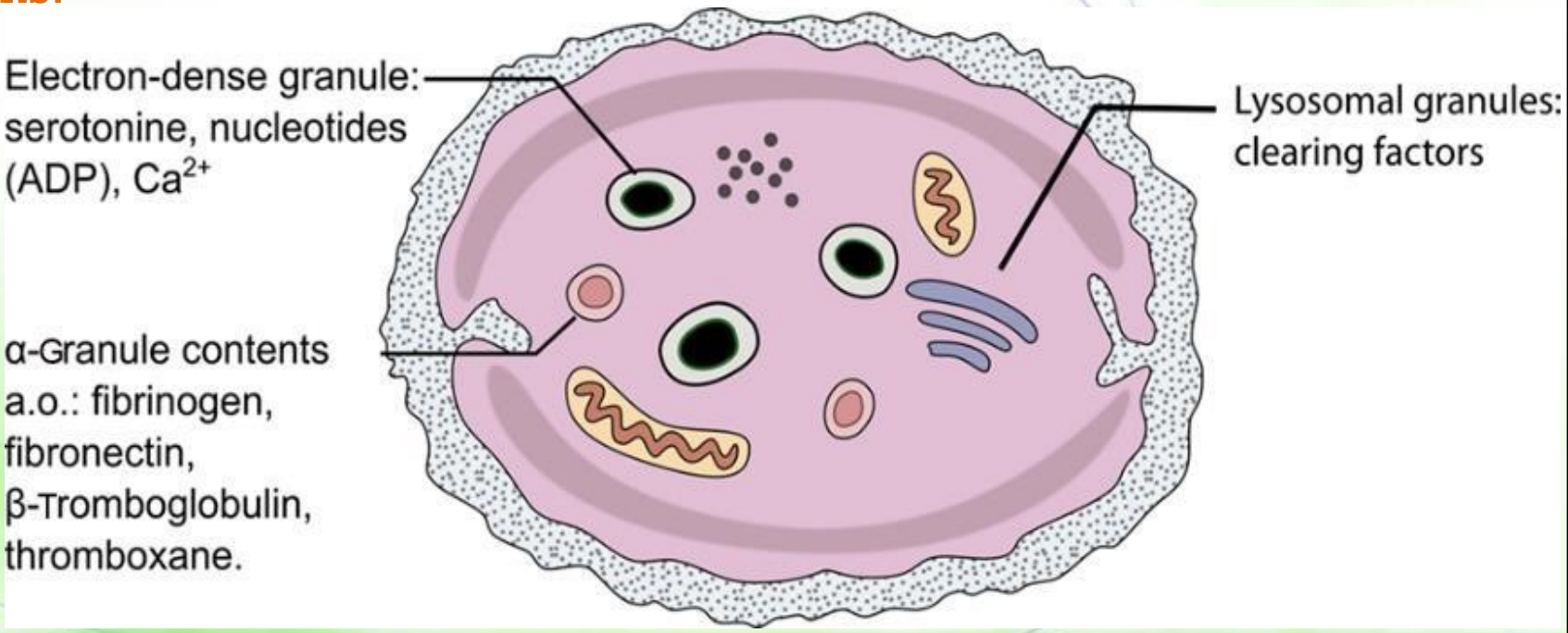


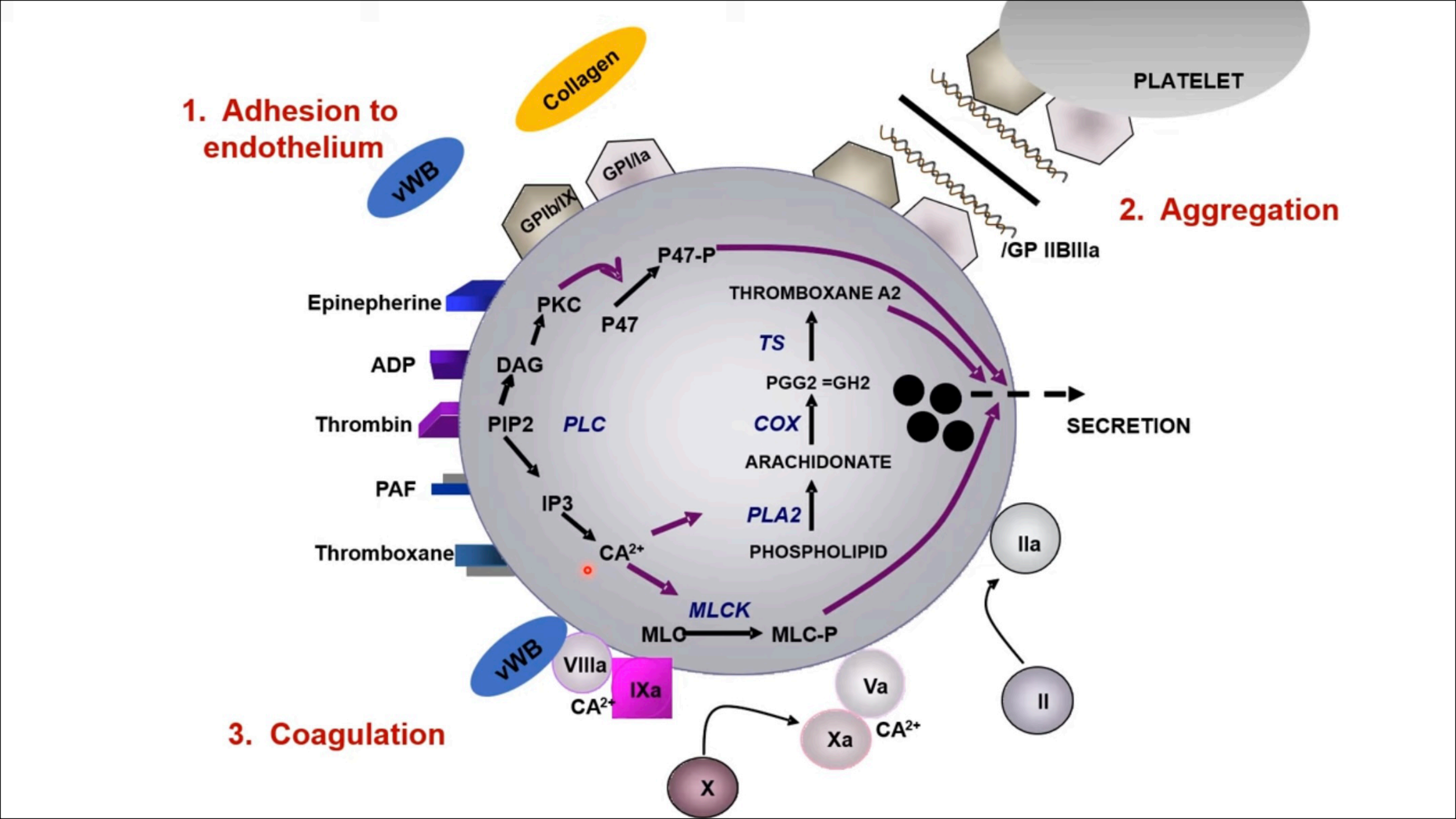
The granules

- Electron-dense granules (calcium ions, ADP, ATP, serotonin) (ADP and ATP inside these vesicles are not really used as sources of energy, rather, they are used as signaling molecules)
- α-granule (a heparin antagonist, platelet-derived growth factor, fibrinogen, von Willebrand factor (vWF), clotting factors)
- Lysosomal granules (hydrolytic enzymes) necessary for removal of the clot & activation of different proteins.

During activation, the contents of these granules are secreted.

Once platelets are activated in response to an injury, these granules fuse with the plasma membrane releasing their contents




1. Adhesion to endothelium
Collagen
vWB
GPIb/IX
GPI/Ia
PLATELET
2. Aggregation
/GP IIBIIIa
Epinepherine
ADP
Thrombin
PAF
Thromboxane
PKC
P47
P47-P
DAG
PIP2
PLC
IP3
CA2+
THROMBOXANE A2
TS
PGG2 =GH2
COX
ARACHIDONATE
PLA2
PHOSPHOLIPID
SECRETION
MLCK
MLC
MLC-P
IIa
II
vWB
VIIIa
IXa
CA2+
Va
Xa
CA2+
X
3. Coagulation

There are numerous receptors on the cell surface
For example, receptor for epinephrine, receptor for ADP, receptor for thrombin and so on,

There are also glycoproteins (namely glycoprotein Ib and glycoprotein IIb-IIIa) on the cell surface:

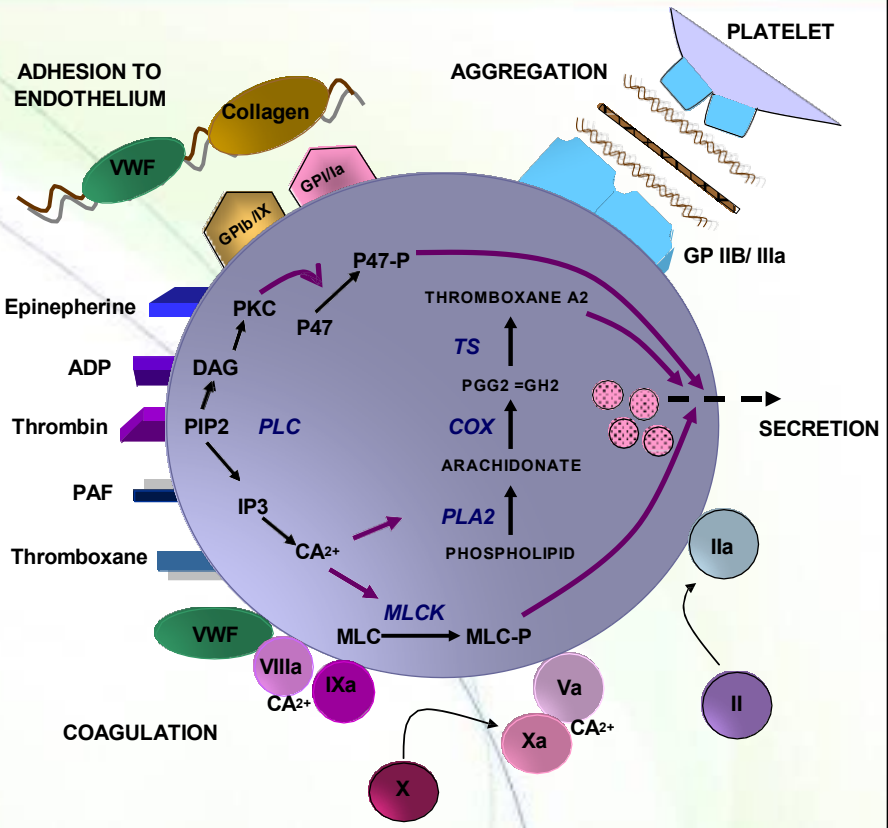
- important for interacting with collagen, von Willebrand factor, and fibrinogen
- important for forming aggregation of platelets at the site of injury

Also, on the membrane of platelets, there's the process of blood coagulation going on

Adhesion

- The first thing that happens whenever there's a vascular injury, von Willebrand factor is exposed, then platelet bind to this factor causing the activation of the platelets, that would cause a series of signaling reactions inside the cell and that leads to secretion of factors.
- 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

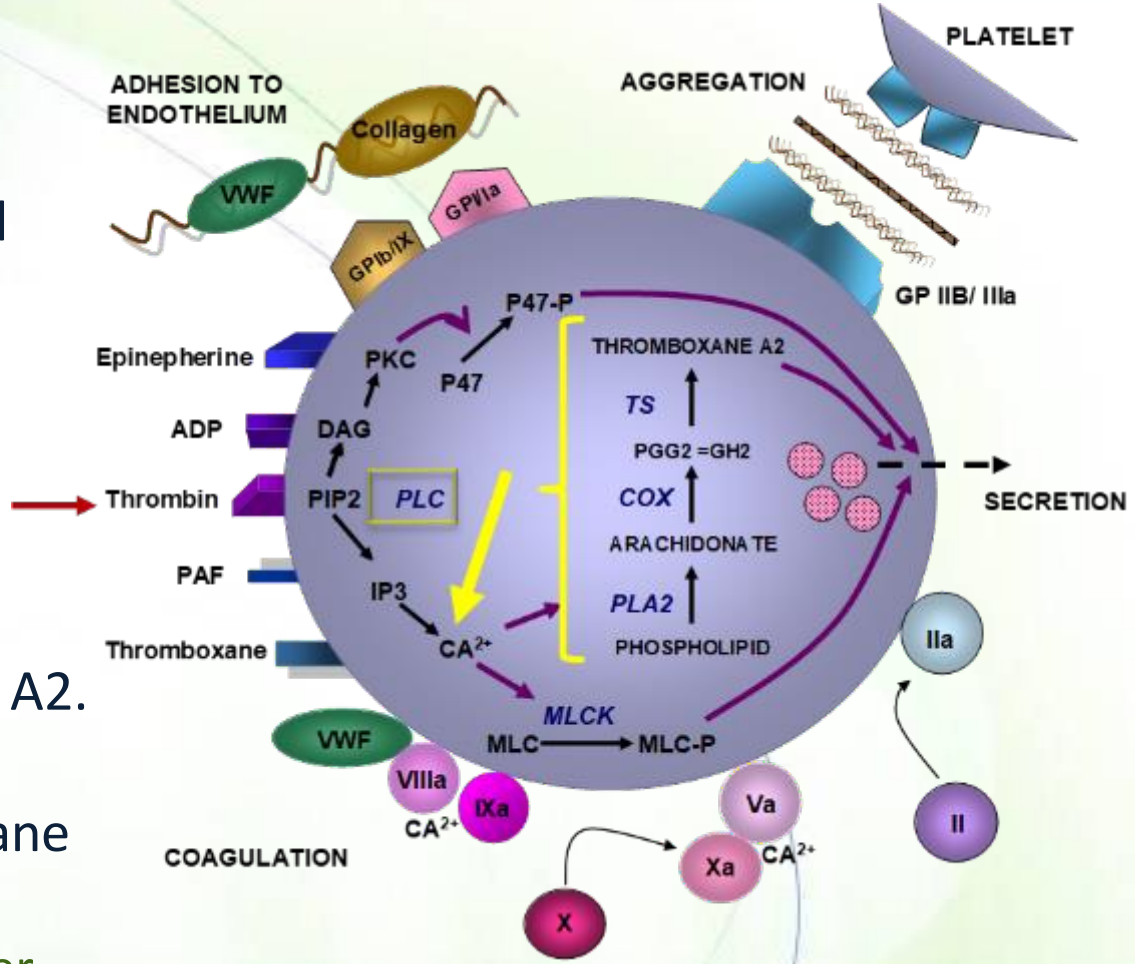


- Once platelets are activated, Platelets also change shape allowing for more platelet-platelet interaction and aggregation.

Thrombin receptor

Thrombin is a major player in blood coagulation

- 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 activates 2 enzymes: phospholipase A2 (PLA2) & myosin light chain kinase (MLCK)
- 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 A2 is as vasoconstrictor and a further inducer of PLC-β activity (and induces platelet aggregation).
  - It acts in autocrine and paracrine manners. (it acts on the same cell that releases it and on neighboring cells)



- ***Serotonin is also a vasoconstrictor*** released from platelets
- ***PDGF*** (platelet derived growth factor(***stimulates proliferation of endothelial cells to reduce blood flow.***

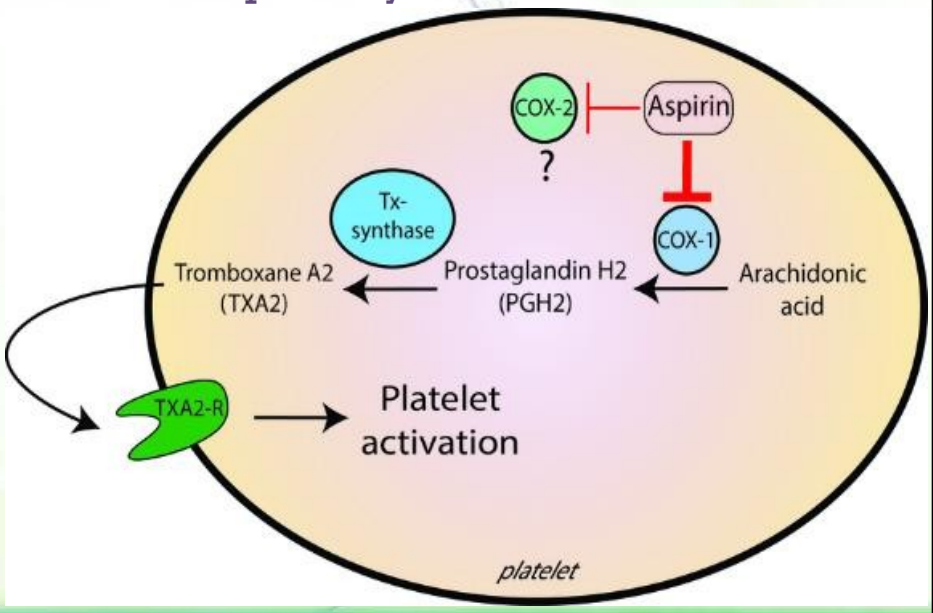
NSAID

- Non-steroidal anti-inflammatory drugs inhibit the enzyme cyclooxygenase, accounting for their anticoagulant effects.
  - Aspirin inhibits the enzyme cyclooxygenase preventing the production of prostaglandins and thromboxanes. So, aspirin is beneficial in reducing the incidence of myocardial infarction because it reduces the platelet aggregation and vasoconstriction
  - 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.

There is a balance between molecules that have opposing actions.
For example, thromboxane is a vasoconstrictor, but aspirin inhibits the production of prostacyclin preventing platelet aggregation and it is also a vasodilator. So, we have a vasoconstrictor in thromboxanes and we have a vasodilator in prostacyclin.

Which molecule does win the competition? Eventually, prostacyclins because once cyclooxygenase is inhibited, platelets cannot regenerate cyclooxygenase and as a result they cannot produce more from thromboxane. On the other hand, the source of prostacyclin is endothelial cells so if cyclooxygenase is inhibited, there should be no problem because these cells can synthesize more cyclooxygenase

We have to be cautious about prescribing aspirin especially to the elderly. Yes, it's quite beneficial in reducing incidence of myocardial infarction. However, the results of two clinical trials were published in 2018 pointed out that aspirin can actually be harmful especially to the elderly where it causes excessive bleeding and hemorrhage in many cases



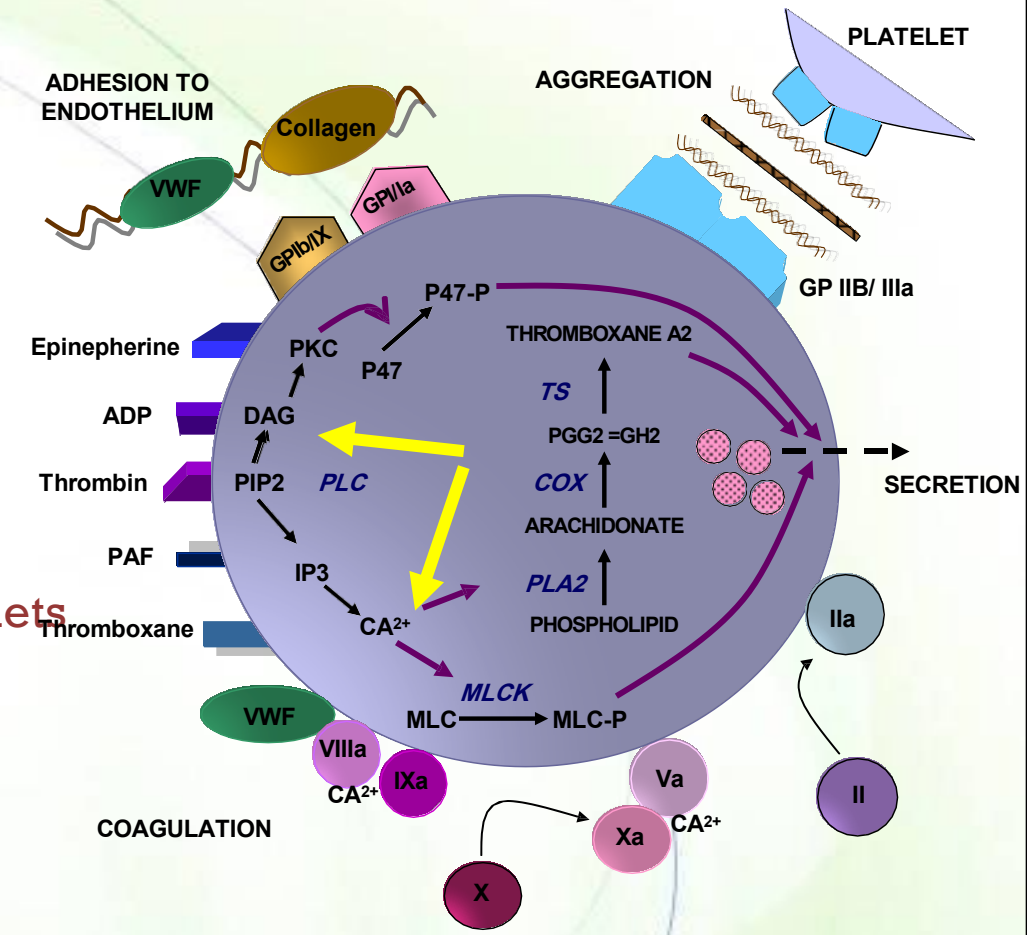
More release of granular contents

We said Ca^{2+} activates of phospholipase A_2 and triggering the release of arachidonate from phospholipids. Additionally,

- 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.

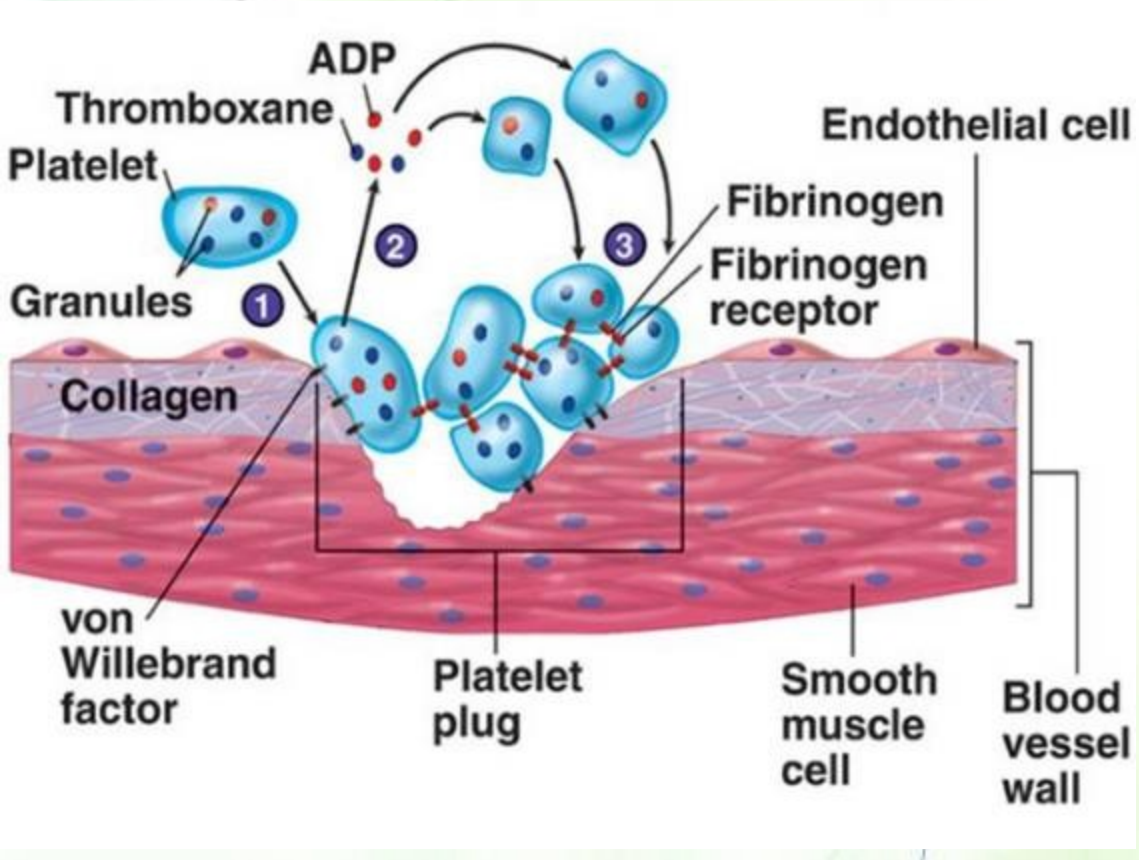
The phosphorylated myosin light chain has several effect:
1. It stimulates further fusion of platelets granules with the plasma membrane releasing of their contents
2. Modifies the actin cytoskeleton so it can induce motility of platelets and It also changes platelet morphology leading to more aggregation of platelets.

- DAG (The second product of hydrolysis of PIP_2) activates PKC, which phosphorylates and activates specific platelet proteins (including protein 47) that induce the release of platelet granule contents including ADP.



Role of ADP

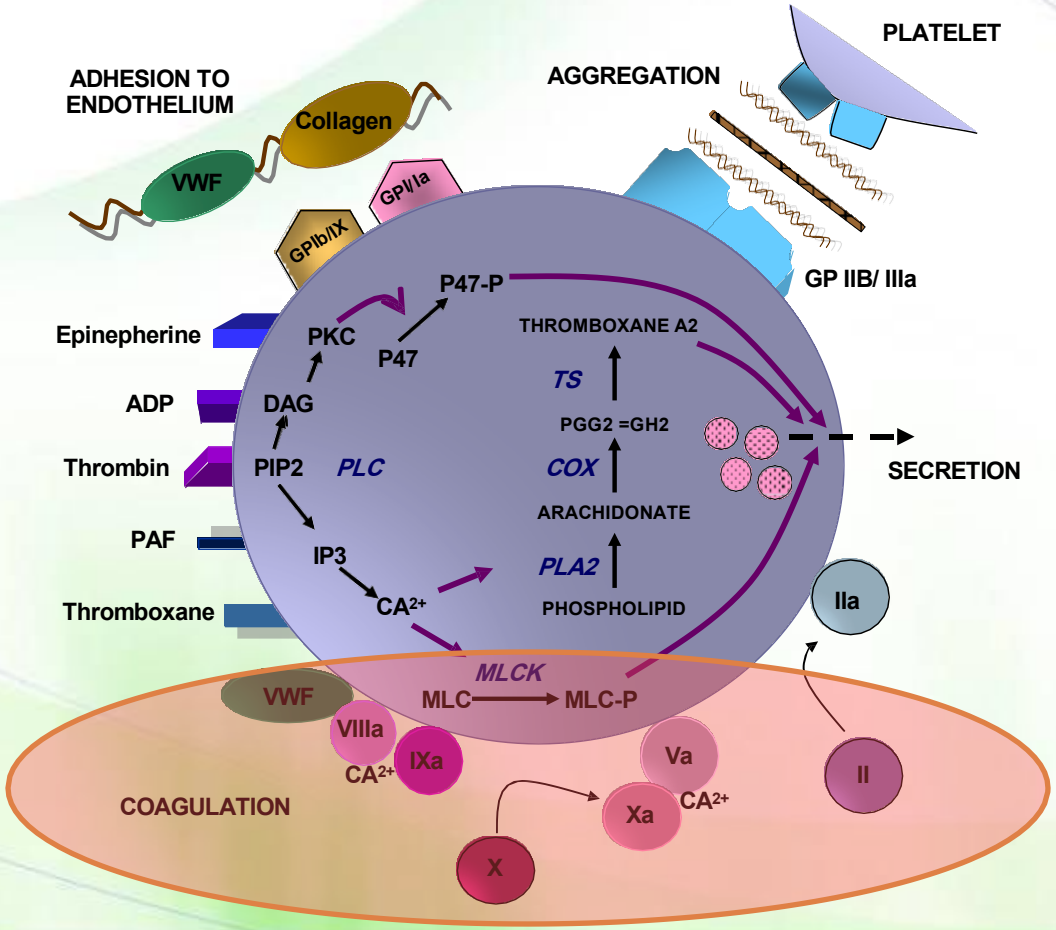
- ADP is a platelet activator that binds to its receptor and modifies the platelet membrane allowing fibrinogen to adhere to platelet surface glycoproteins resulting in fibrinogen-induced platelet aggregation, called platelet plug.



Role of platelet cell surface

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

The surface of platelets is the theater for the blood coagulation process, the biochemical reactions that lead to blood coagulation (we will take about blood coagulation in the coming slides)



Biochemistry of coagulation

Coagulation: the biochemical reactions that take place in order to form blood clots.

Components of coagulation

There are several players in the process of blood coagulation, some are small like Ca^{2+} and vitamin K, others are large like platelets themselves and you have molecules in between.

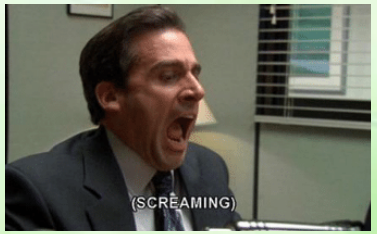
- An organizing surface (platelets)
- Proteolytic zymogens (prekallikrein, prothrombin, and factors VII, IX, X, XI, XII, and XIII)
  - Remember: “zymogens” means enzymes that require proteolytic cleavage in order for them to be active, e.g.: trypsinogen, chymotrypsinogen, proelastase, which require proteolytic modification generating the active enzymes, trypsin, chymotrypsin, elastase.
    - 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 (inhibitors of blood coagulation) (protein C, protein S)
- Non-enzymatic protein cofactors (factors VIII, V, and tissue factor)
- Calcium ions
- Vitamin K
- Fibrinogen (forms the fibrin network)

Molecular components of coagulation

These are the different clotting factors, their names and what their functions are.

As you go through this lecture, you can go back to this slide to summarize what the process is.

Note that:
factor II is prothrombin,
factor I is fibrinogen,
however, factor IV is calcium ions, so these factors do not necessarily indicate a protein.



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	

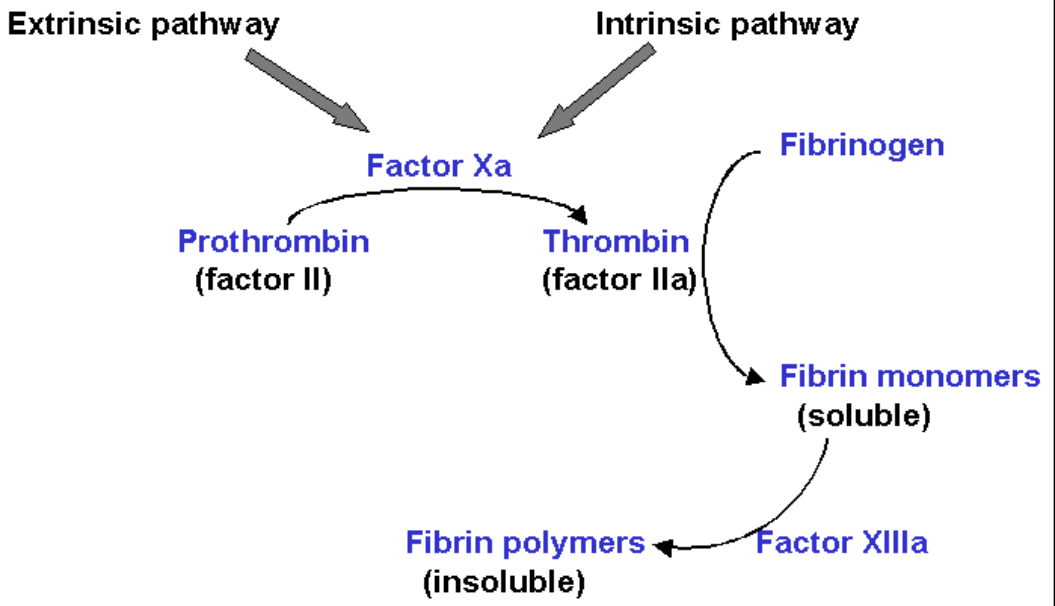
HMWK – High molecular weight kininogen; vWf – Von Willebrand factor; TF – Tissue factor

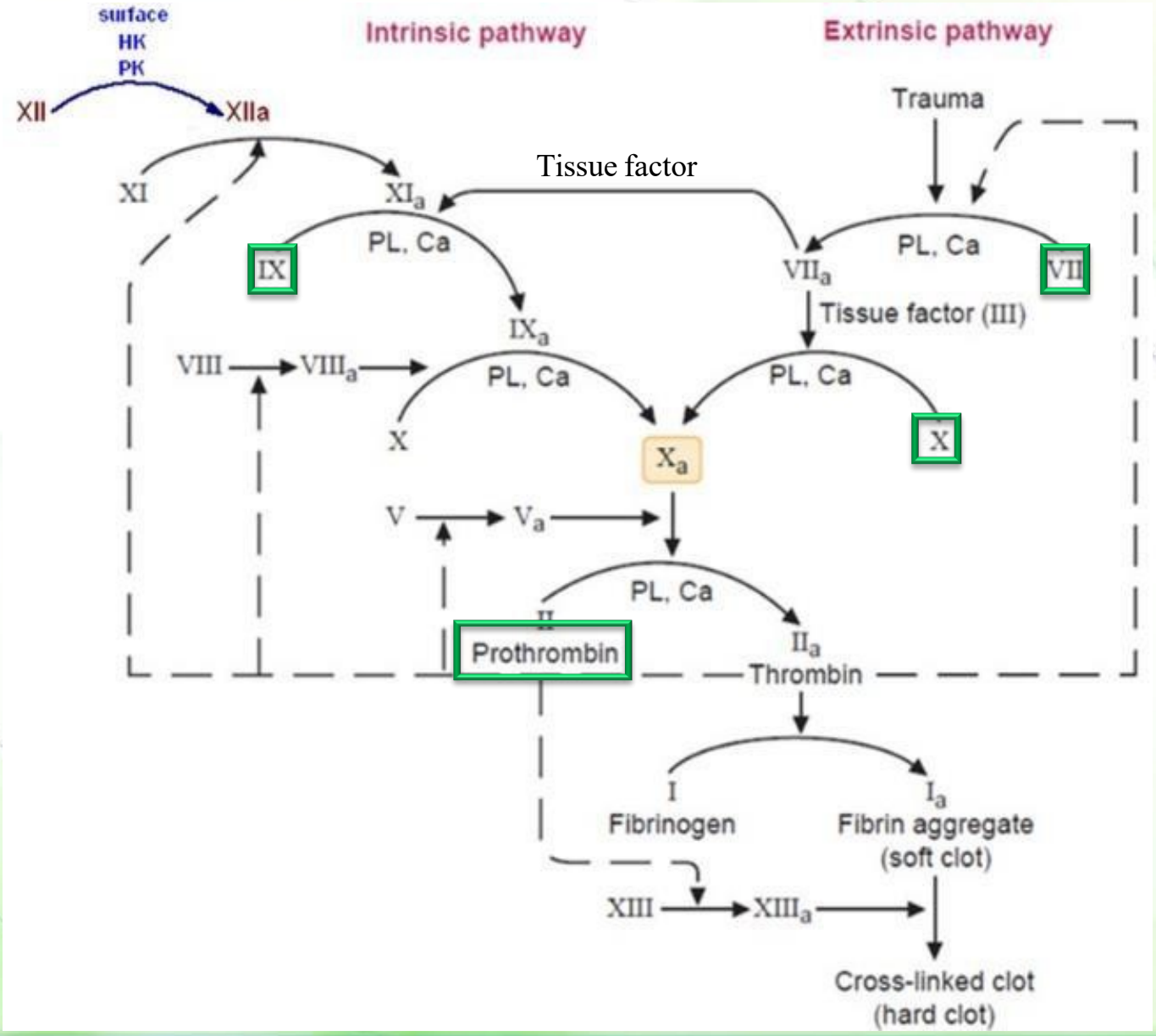
The two pathways

Classically, blood coagulation has been classified into two pathways that were thought to be independent

- The intrinsic pathway is initiated when subendothelial surface (i.e., collagen) is exposed. The intrinsic pathway is activated as a result of an internal effect like inflammation.
- The extrinsic pathway is initiated in response to tissue injury.
  - Tissue factor (TF) protein is released.
- It has been found that these 2 pathways are not totally independent, rather, there is a bridge that connects both pathways to each other and when the extrinsic pathway is activated you have activation of the intrinsic pathway and vice versa, so they are really interconnected and not totally independent of each other.
- However, the two pathways converge on a common pathway.

The two pathways converge at a single point which is the activation of factor X, and factor Xa is responsible for activating factor II (prothrombin) to form factor IIa (thrombin), then thrombin can form the fibrin polymers/mesh.





CHECK NEXT SLIDE

The extrinsic pathway is activated when there's a vascular injury,
vascular injury results in exposure of collagen as well as von Willebrand factor,
this exposure results in the activation of factor VII (stable factor) → Vlla,
then, with the help of tissue factor, factor VIIa activates factor X (Stuart-Prower factor) → Xa.

In the intrinsic pathway, the tissue factor also plays an important role, as it forms a bridge whereby factor Vlla can activate factor IX (Christmas factor) → IXa,
then factor IXa can activate factor X (Stuart-Prower factor).
In case of inflammation, you have activation of the kallikrein pathway, in which activates factor XII (Hageman factor) → XIIa , which in turn activates factor XI → XIa, that activates factor IX (Christmas factor) → IXa

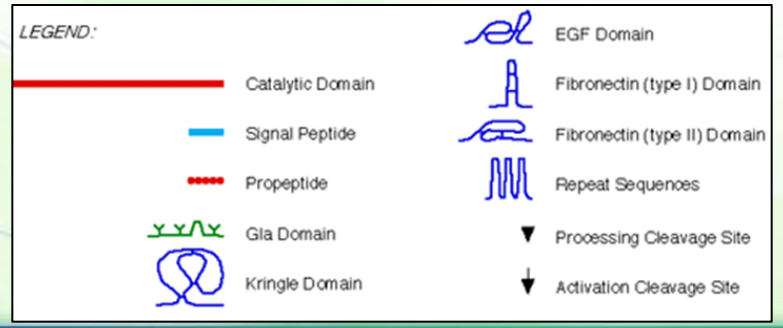
Then both pathways converge with the activation of factor X → Xa, then, factor Xa activates prothrombin (factor II) → thrombin (factor IIa) which then forms the fibrin network.

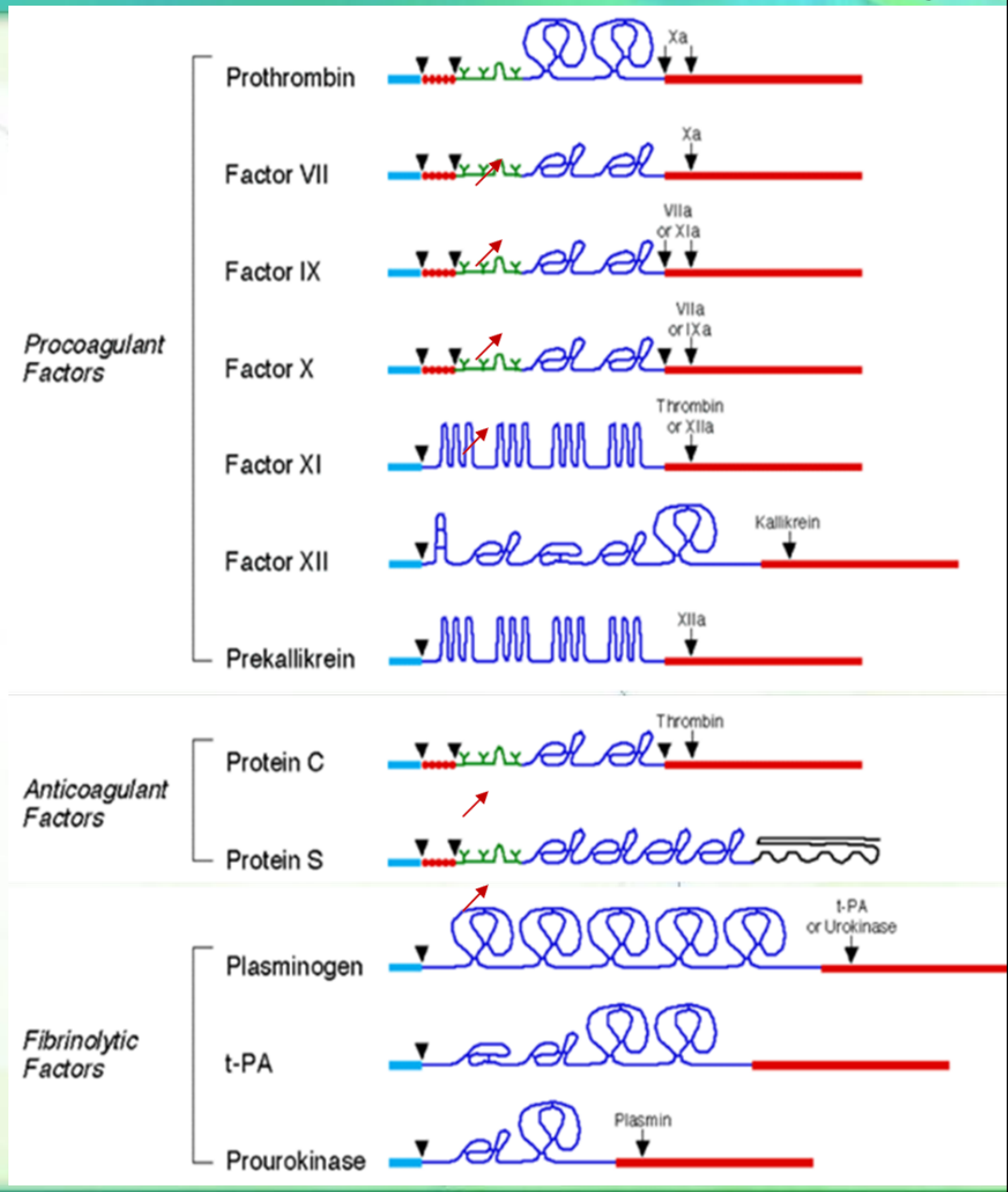
There are several co-factors that play an important role in this process, non-enzymatical co-factors, including tissue factor, factor VIII, factor V and so on.
Also, there's the involvement of phospholipids that are at the surface of platelets and calcium ions in the process of activation

Gla domain

The figure on the right is an illustration of the different domains of the different proteins of the clotting cascade

What we need to know is the presence of a domain known as the Gla domain (glutamate domain).
This glutamate domain is present in prothrombin (Factor II), factor VII, factor IX, factor X as well as protein C and protein S which are anticoagulants.

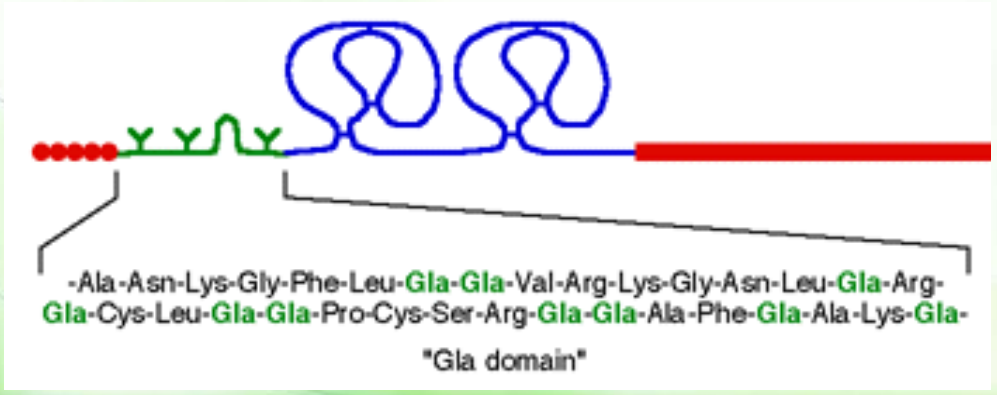


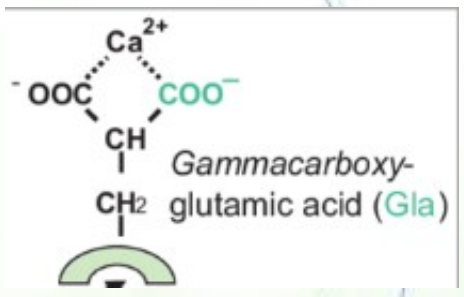


Gla domain

- 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.

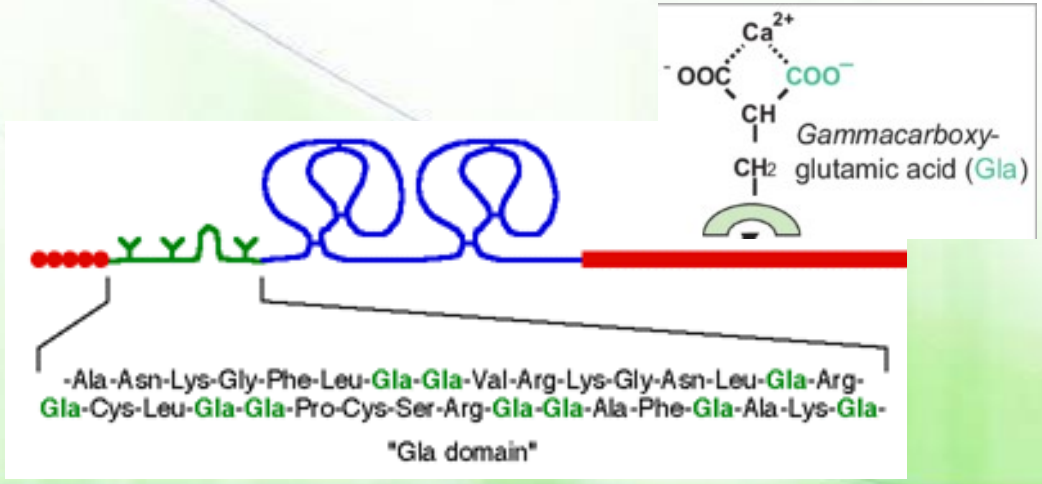
- Gla domain (glutamate domain) is part of the primary structure (amino acid structure) of the proteins we mentioned (prothrombin (Factor II), factor VII, factor IX, factor X as well as protein C and protein S), this domain is rich with glutamate residues (about 9 to 12 glutamate residues), these glutamate residues are the substrates for carboxylase enzyme which adds another carboxyl group, forming a glutamate with two carboxyl groups known as γ-carboxyglutamate, γ-carboxyglutamate is highly negatively charged, these charges facilitate interaction between γ-carboxyglutamate with the positively charged calcium ions

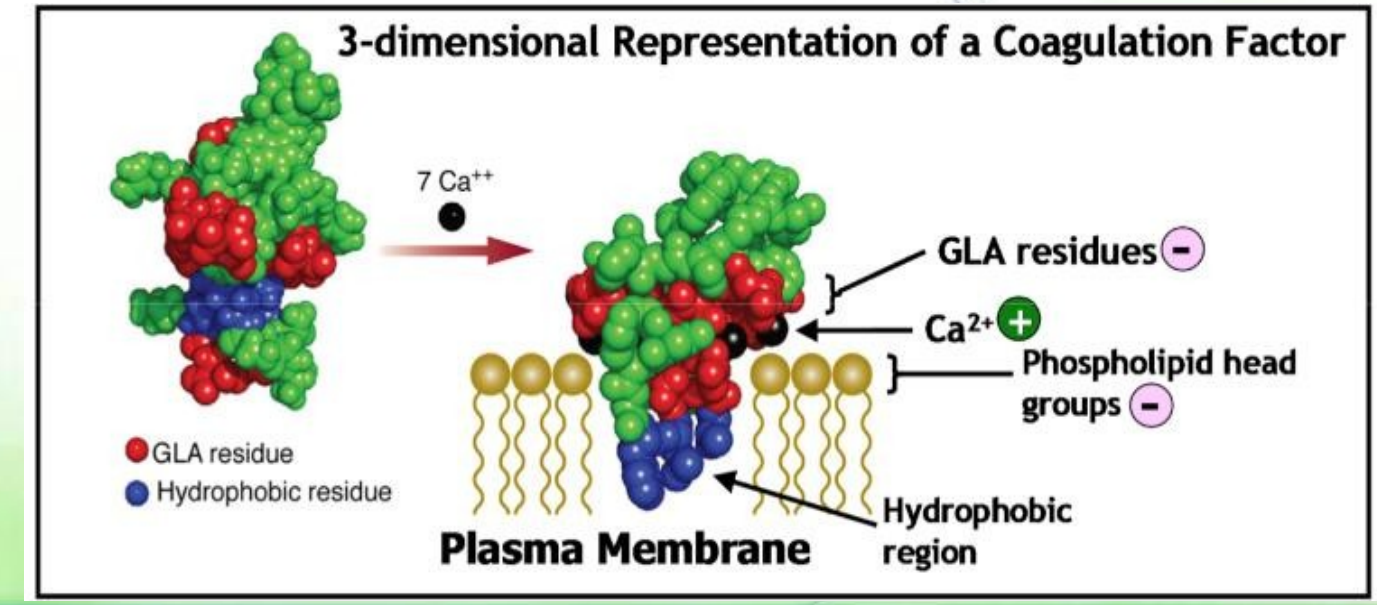




Gla domain

- The interaction between γ-carboxyglutamate and the Ca^{2+} helps in binding of the proteins (prothrombin (Factor II), factor VII, factor IX, factor X as well as protein C and protein S) with the plasma membrane of platelets. In the plasma membrane of the platelets, the phospholipid head groups are negatively charged, and Ca^{2+} is positively charged, so Ca^{2+} mediates the interaction of these proteins with the plasma membrane.
- The insertion of these proteins with plasma membrane is also solidified by having a hydrophobic region in these proteins, so that helps inserting the proteins into the plasma membrane and that what makes the coagulation take place on the surface of platelets
- 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

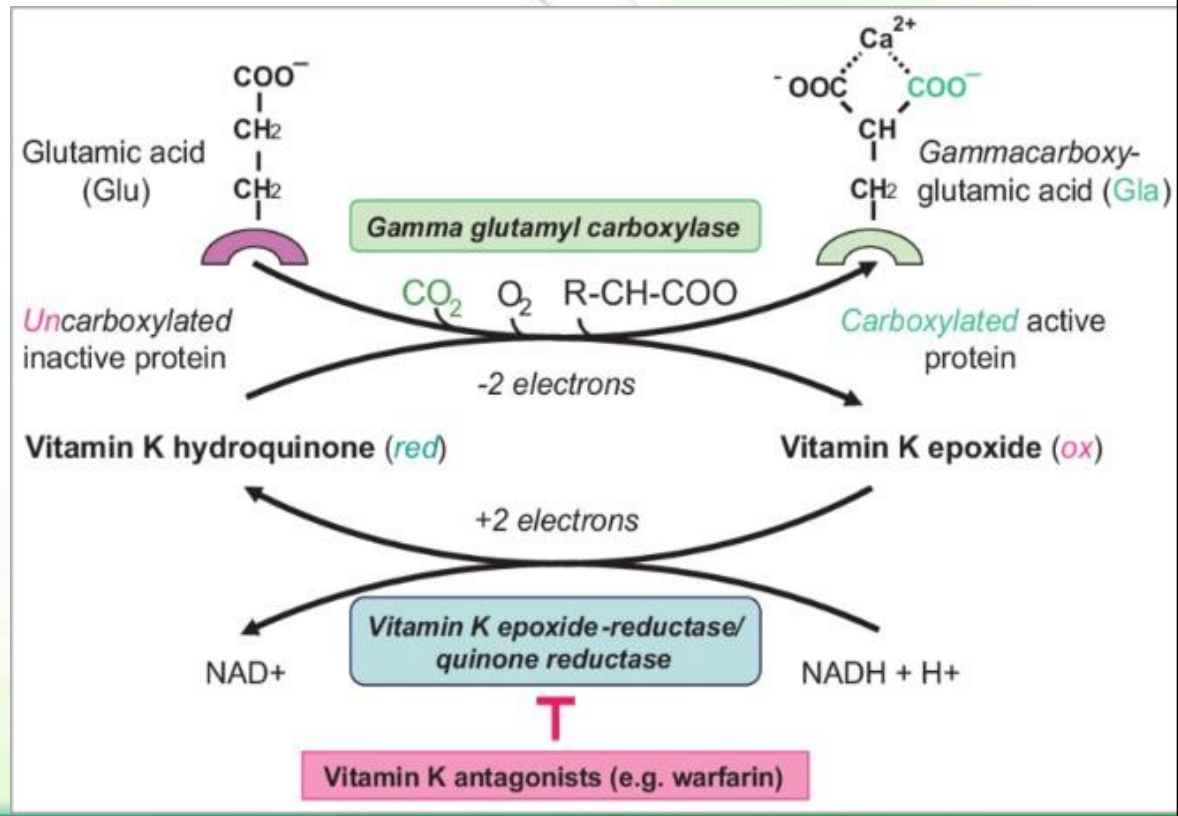
- Vitamin K participates in conversion of Glu to γ-Gla.

  Carboxylation of glutamate residues requires vitamin K as a source of electrons.
  Vitamin K is oxidized into vitamin K epoxide and the carboxylation of glutamate happens.

- Vitamin K becomes oxidized and must be regenerated.

  It's important to reduce vitamin K epoxide to regenerate the active form of vitamin K (vitamin K hydroxyquinone) and this happens enzymatically through vitamin K epoxide reductase enzyme, vitamin K epoxide reductase requires NADH as a source of electrons

Vitamin K epoxide reductase is the target of an antagonist known as Warfarin, which inhibits regeneration of Vitamin K, thus inhibiting synthesis of factors II, VII, etc. and preventing clotting



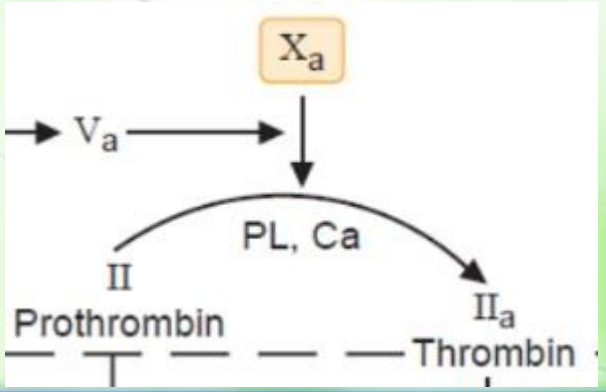
Newborns and vitamin K deficiency

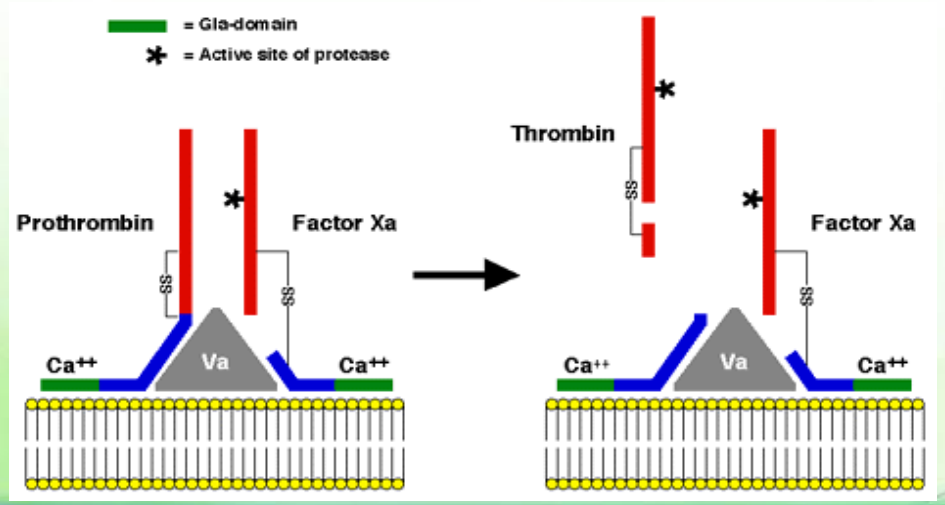
- Newborns are at risk for early vitamin K deficiency bleeding. Why?
  - The placenta is a poor passage channel for fat-soluble compounds, including vitamin K.
  - Neonates are born with an immature liver that impairs coagulation factor synthesis and GLA modifications.
  - Breast milk is a poor source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

- How do we know that these newborns have vitamin K deficiency?
  There are several signs or manifestations, such as:
  1. the gum bleeding
  2. formation of bruises

Prothrombin activation

- Once factor X is activated, it forms a complex with factor Va with interacting with Ca^{2+}
  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.
- Factor V brings both factor Xa and prothrombin close to each other, and then factor Xa cleaves prothrombin forming the active enzyme thrombin
- 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 .





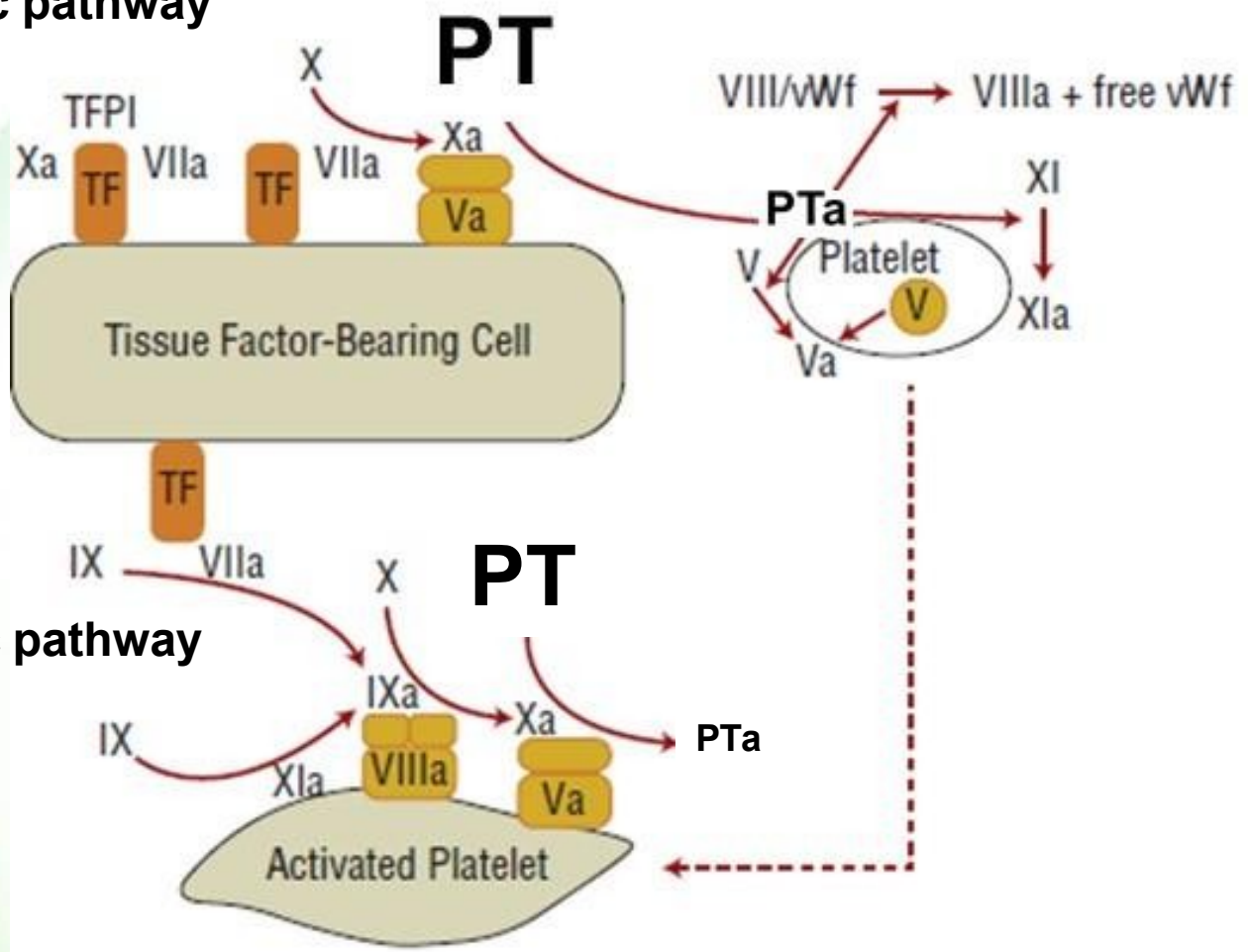
There's the involvement of other several factors in the extrinsic pathway and the intrinsic pathway

In the extrinsic pathway, prior to activation of factor X, you have activation of factor VII with the help of tissue factor.

In the intrinsic pathway, there's also the involvement of tissue factor which activates factor IX, and factor IX can activate factor X by factor VIII.

PT: prothrombin

Extrinsic pathway



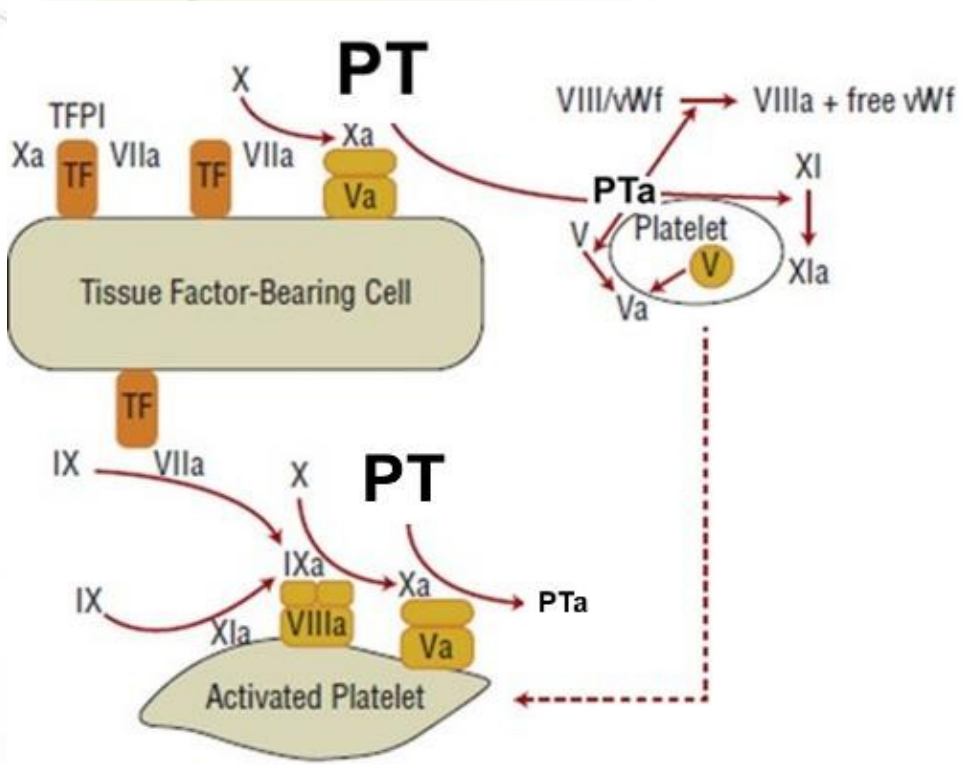
Source: DiPiro JT, Talbert RL, Yee GC, Matzke GR, Wells BG, Posey LM: *Pharmacotherapy: A Pathophysiologic Approach, 8th Edition*: www.accesspharmacy.com

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) (that sits on the plasma membrane of platelets), and Ca^{2+}.

  This complex activates factor X.
- The extrinsic tenase complex is made up of tissue factor, factor VIIa, and Ca^{2+}.

  This complex activates factor X.
  - 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
  - These are docking molecules; they stabilize enzymes on the cell surface



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

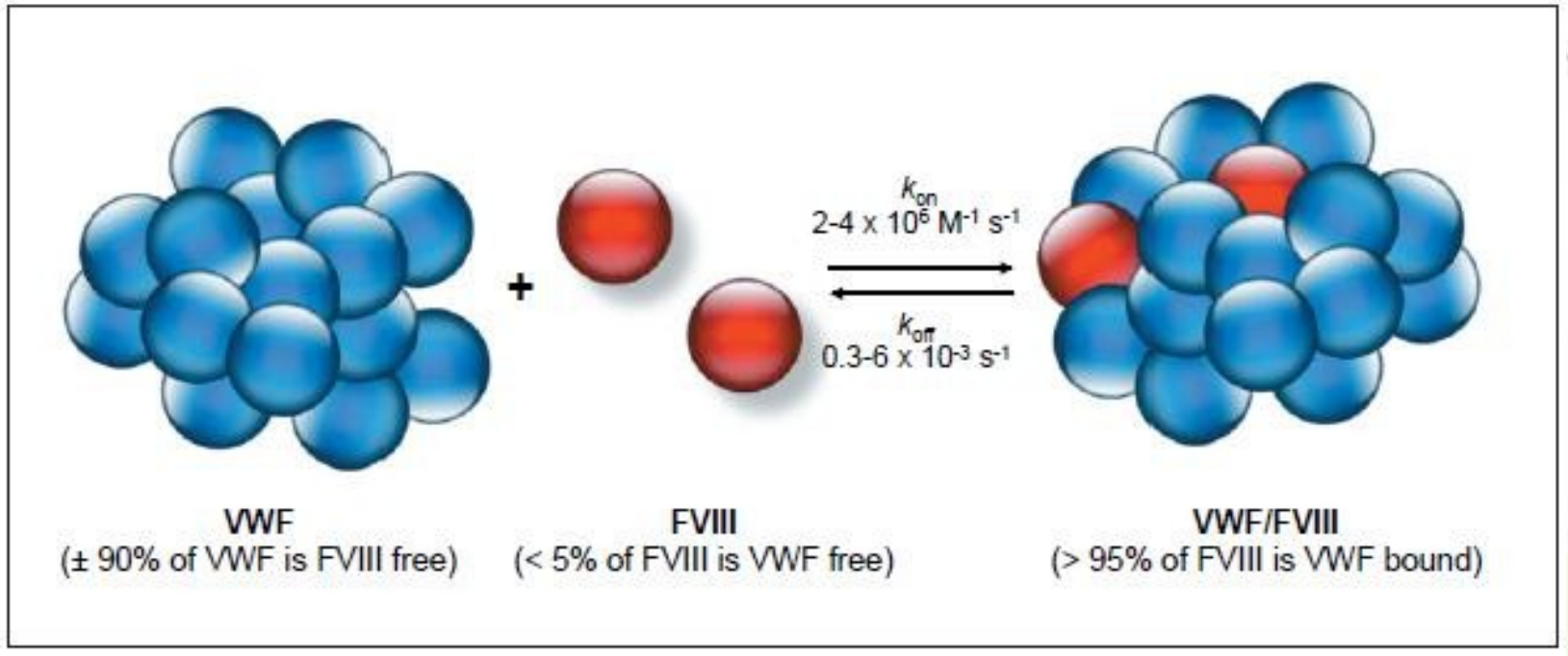
von Willebrand factor deficiency

Factor VIII has a very short life if it circulates by itself in the blood

- Factor VIII circulates in plasma bound to von Willebrand factor, which increases VIII half-life, and, when released, it gets activated.

VWF is synthesized and secreted by endothelial cells and platelets

- von Willebrand factor deficiency is associated decrease in the plasma concentration of factor VIII. Patient with von Willebrand factor deficiency suffer from excessive bleeding



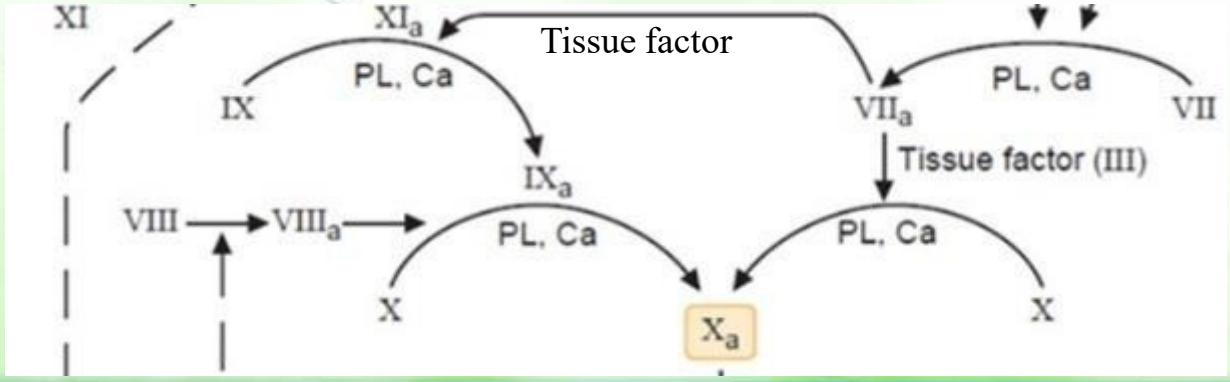
Tissue factor

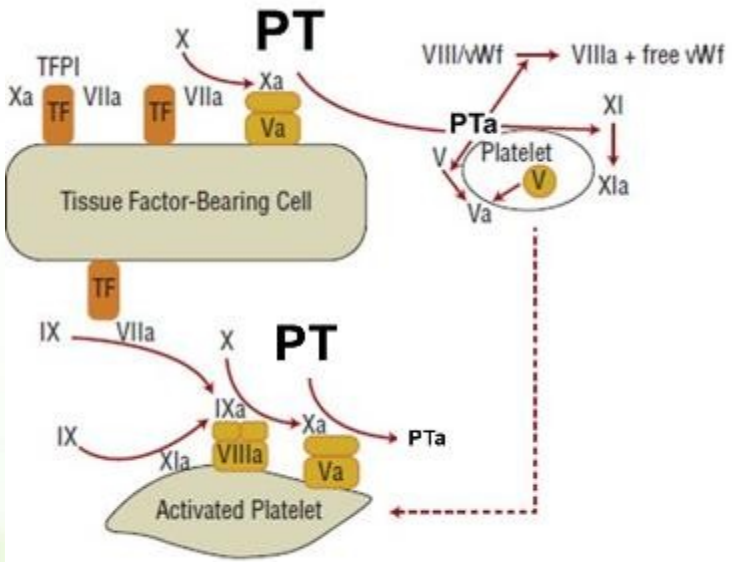
- Tissue factor is an integral membrane protein that is expressed on the surface of "activated" monocytes, subendothelial cells, and other cells.
- Tissue factor increases the proteolytic efficiency of VIIa.

Platelets are not exposed to the subendothelial cells unless there is tissue injury then platelets bind to tissue factor. Once this interaction occurs, you can have the activation of factor X in the extrinsic pathway.
In the intrinsic pathway, there's the involvement of tissue factor, it is important for activating factor IX which activates factor X.

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

The complex of tissue factor with factor VIIa is known as the initiation complex because both the intrinsic and the extrinsic pathways are activated



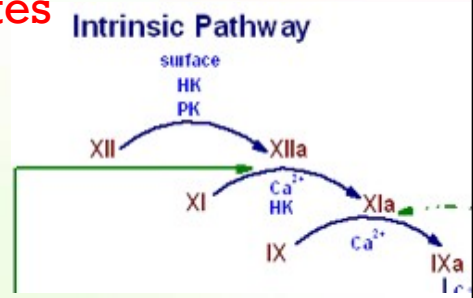


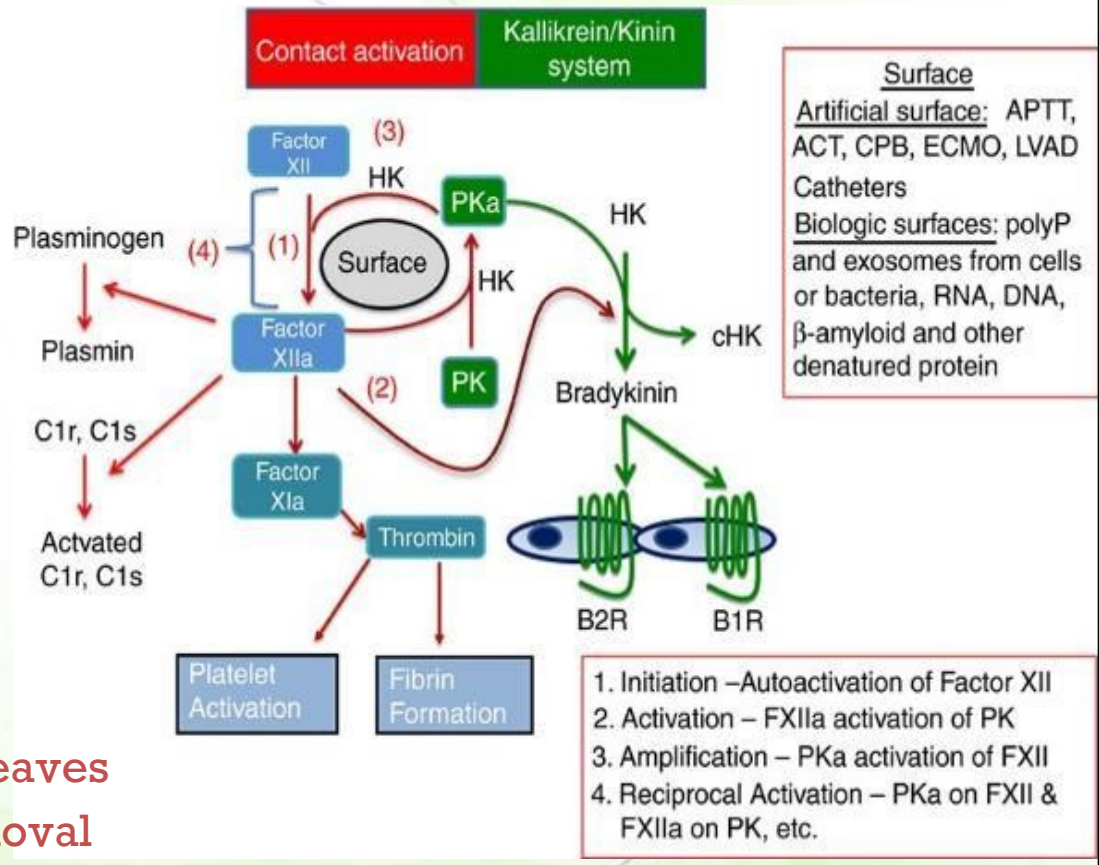
Initiation of the intrinsic pathway

The major player in initiating the intrinsic pathway is factor XII, and factor XII has several substrates

- 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. Prekallikrein is activated by factor XII into kallikrein which can then activate more factor XII) note the positive feedback activation loop).
2. factor XI, which activates factor IX (activates factor X).
3. HMW kininogen releasing bradykinin. factor XIIa cleaves a protein known as high molecular weight kininogen into bradykinin which is **a peptide with potent vasodilator action**, & it can bind to receptors on the cell surfaces including the endothelial cells & muscle cells and it causes vasodilation allowing for recruitment of more cells including platelets.
   - Bradykinin is also generated by kallikrein.
4. Other substrates: plasminogen (fibrinolysis) (factor XIIa cleaves plasminogen into plasmin which is involved in fibrinolysis/removal of the fibrin clot) and complement system proteins.



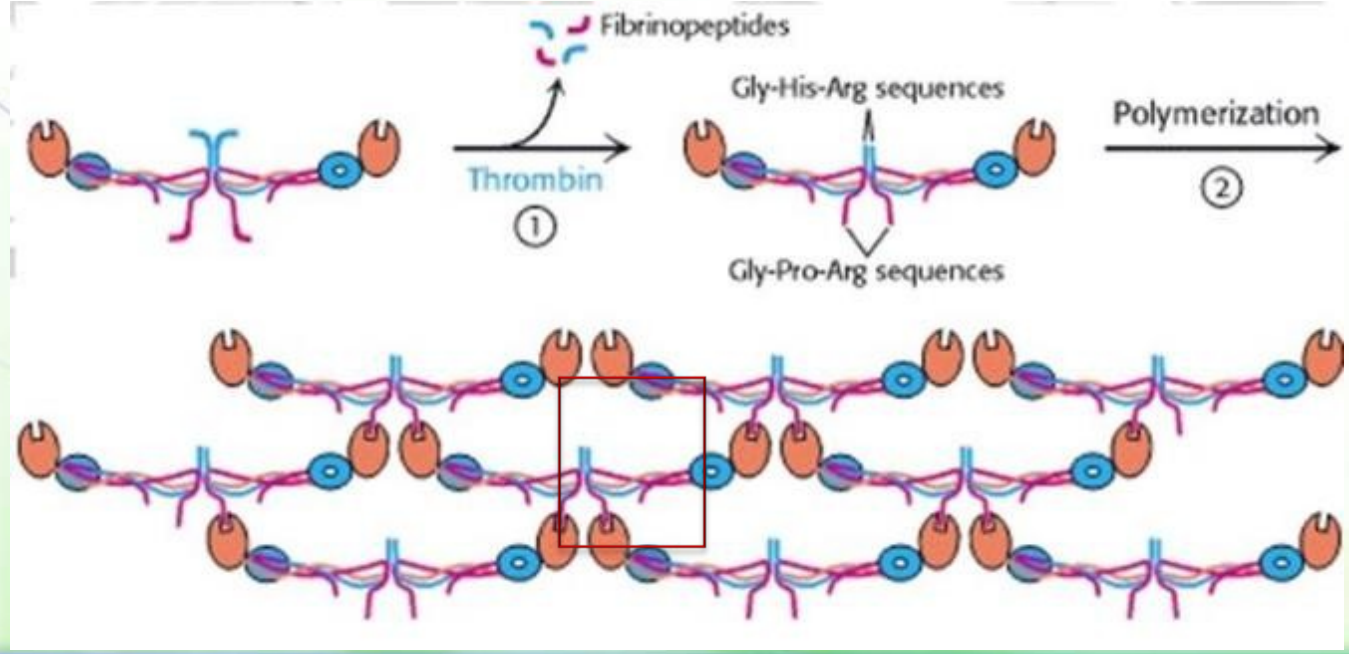


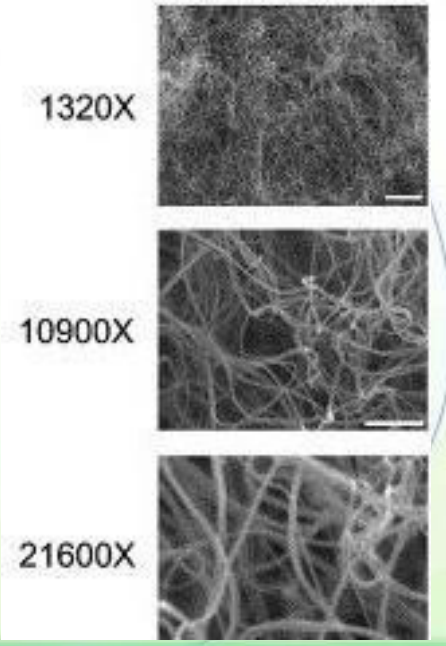
HK, intact high-molecular-weight kininogen; HKc, cleaved high-molecular- weight kininogen; PK, prekallikrein; PKa, plasma kallikrein; polyP, polyphosphate

Formation of a fibrin clot

After the activation of factor X, factor Xa activates prothrombin (factor II) into thrombin (factor IIa)

- Thrombin cleaves fibrinogen into fibrin 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 (between the head and the tails of two other molecules) facilitating the aggregation of the monomers into a gel consisting of long polymers (a clot).
- The clot resulting from aggregation of fibrin monomers is referred to as the "soft clot" because the interactions between all these molecules are based on electrostatic interactions which are non-covalent.

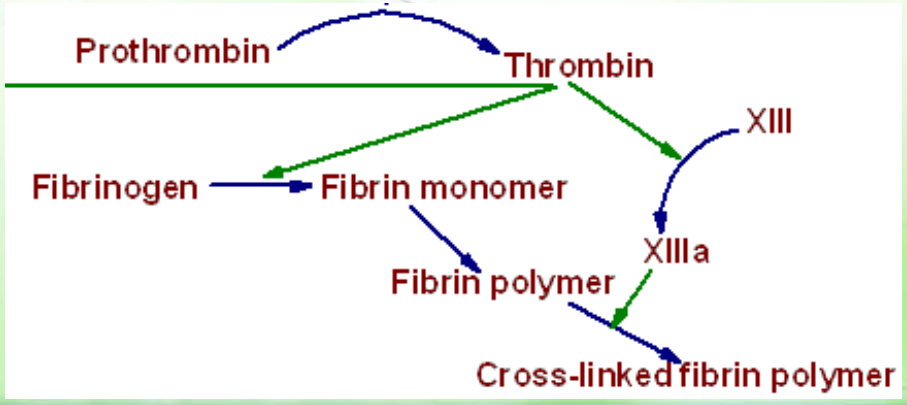


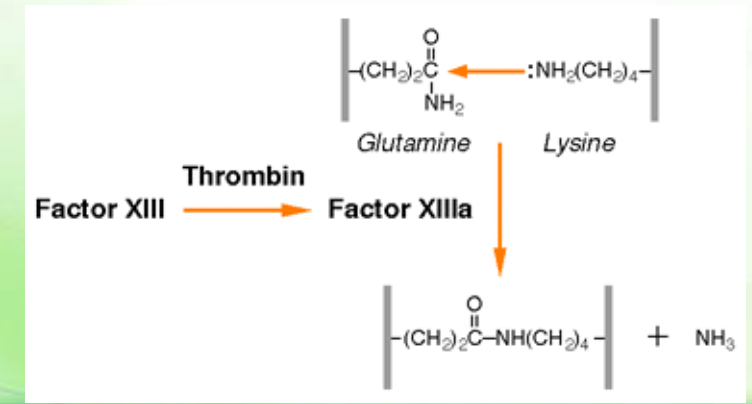


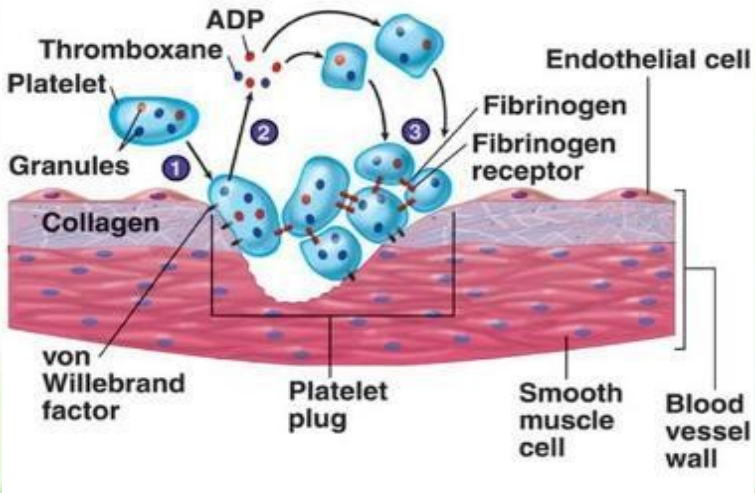
Factor XIII

Thrombin can also activate factor XIII → factor XIIIa

- 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. This clot traps platelets inside it forming platelet plug
  - The cross-links strengthen the fibrin mass, forming the "hard clot“ because of the covalent cross-linking between the fibrin molecules





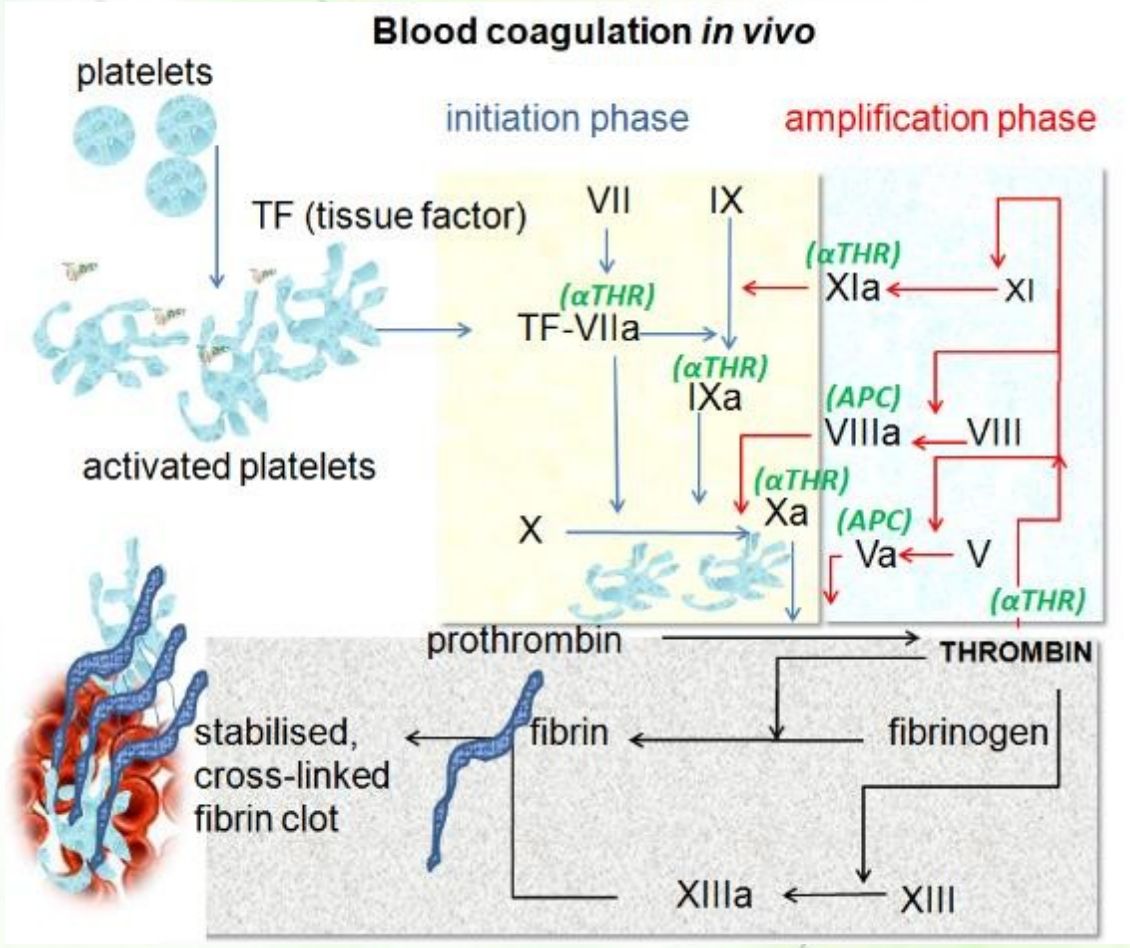


Amplification of coagulation reactions

- 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.

Thrombin acts back on all these factors, so it activates more factor V, more of factor VIII, more of factor XI, and it can also act on factor VII. (feedback activation)

So more amplification and activation of these zymogens, getting more factor X that activates more and more prothrombin to thrombin.

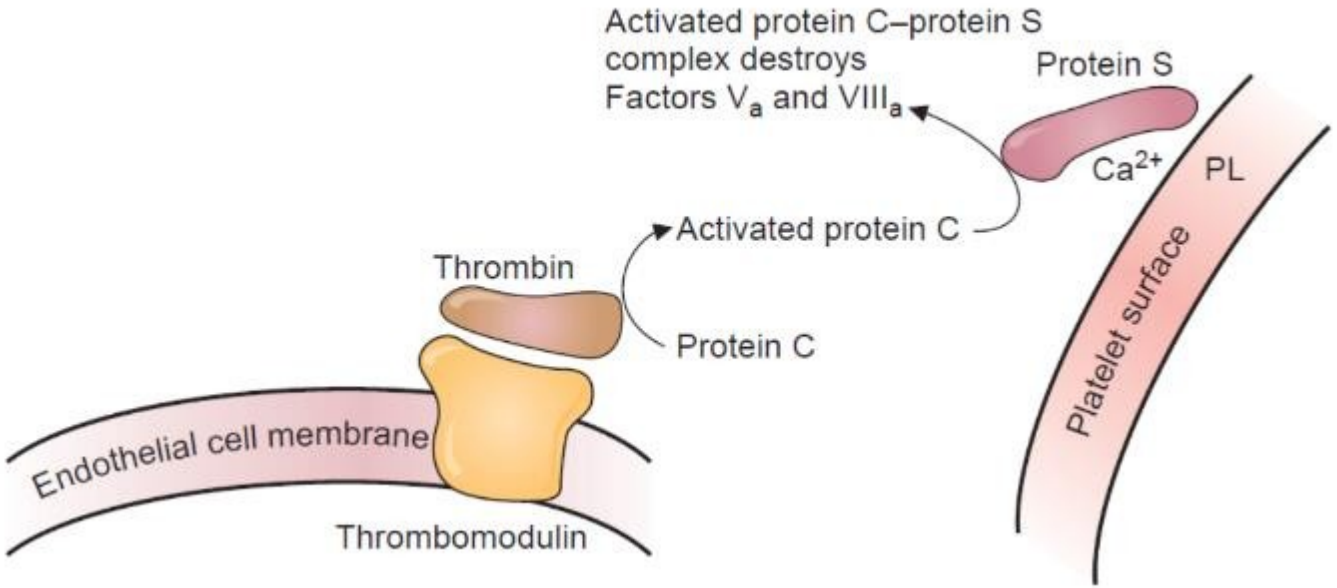


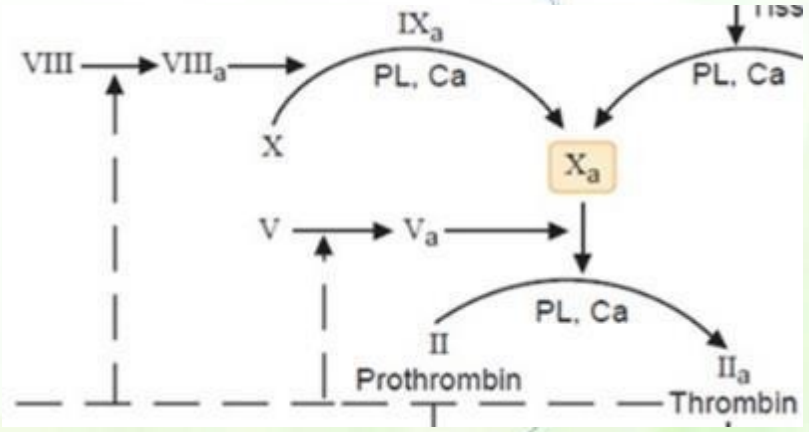
Anti-clotting factors

Protein C and protein S

- Thrombin binds to thrombomodulin in the surface of endothelial cells.
  This thrombomodulin brings thrombin closer to a protein C
- Thrombin can then activate protein C, which forms a complex with protein S
  (protein S is present on the surface of platelets)

  Both of which are vitamin K-dependent cofactors.
- The complex degrades factors V and VIII so termination of activation of prothrombin and factor X.





Antithrombin III

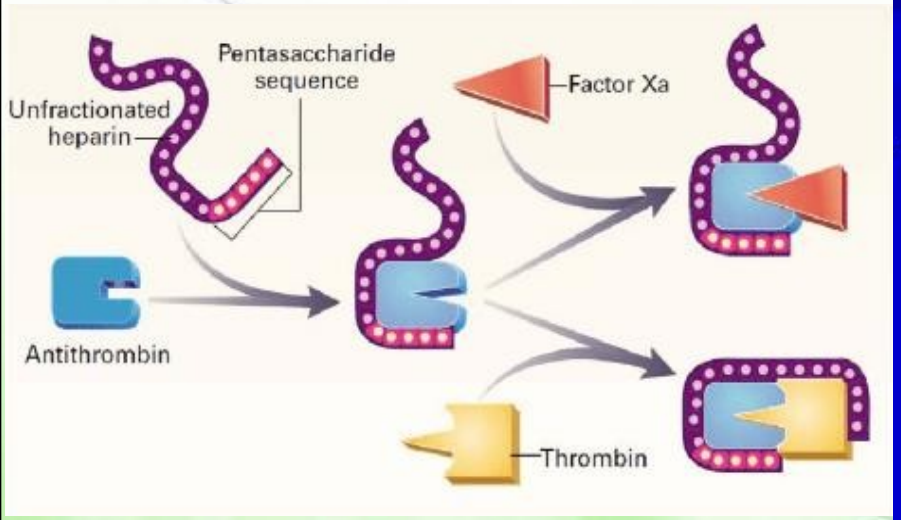
A major inhibitor of thrombin

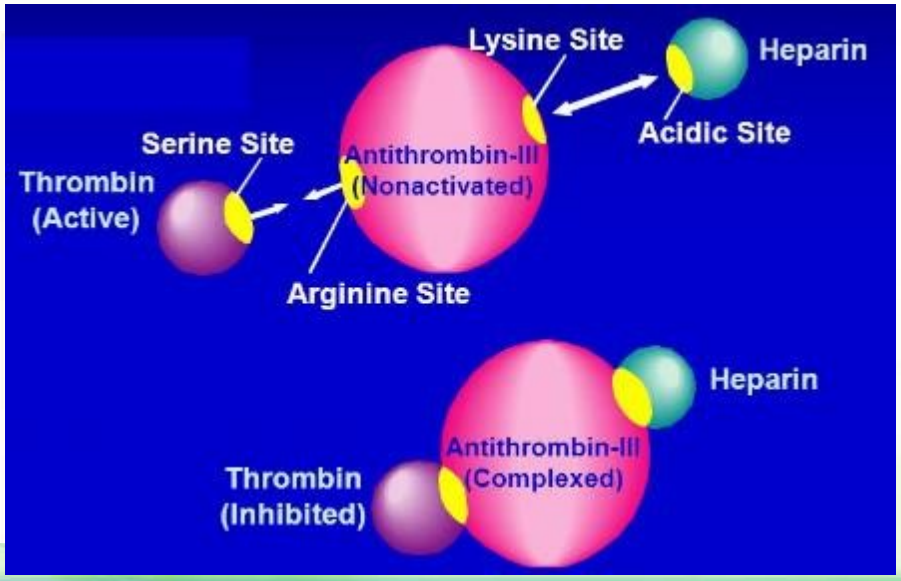
- Antithrombin III is a serine protease inhibitor of thrombin as well as other clotting factors (IXa, Xa, XIa, XIIa, and VIIx when complexed with TF).

  In order for antithrombin to function, it has to bind to heparin (glycosaminoglycan).

  The positively charged amino acid lysine residues in antithrombin III interacts with the acidic site of heparin sulfate, then the structure of antithrombin III changes allowing it to bind to thrombin inactivating it.

- Heparin sulfate, a polysaccharide synthesized by mast cells and present on surface of endothelial cells, binds to antithrombin III, promoting binding to its substrates.



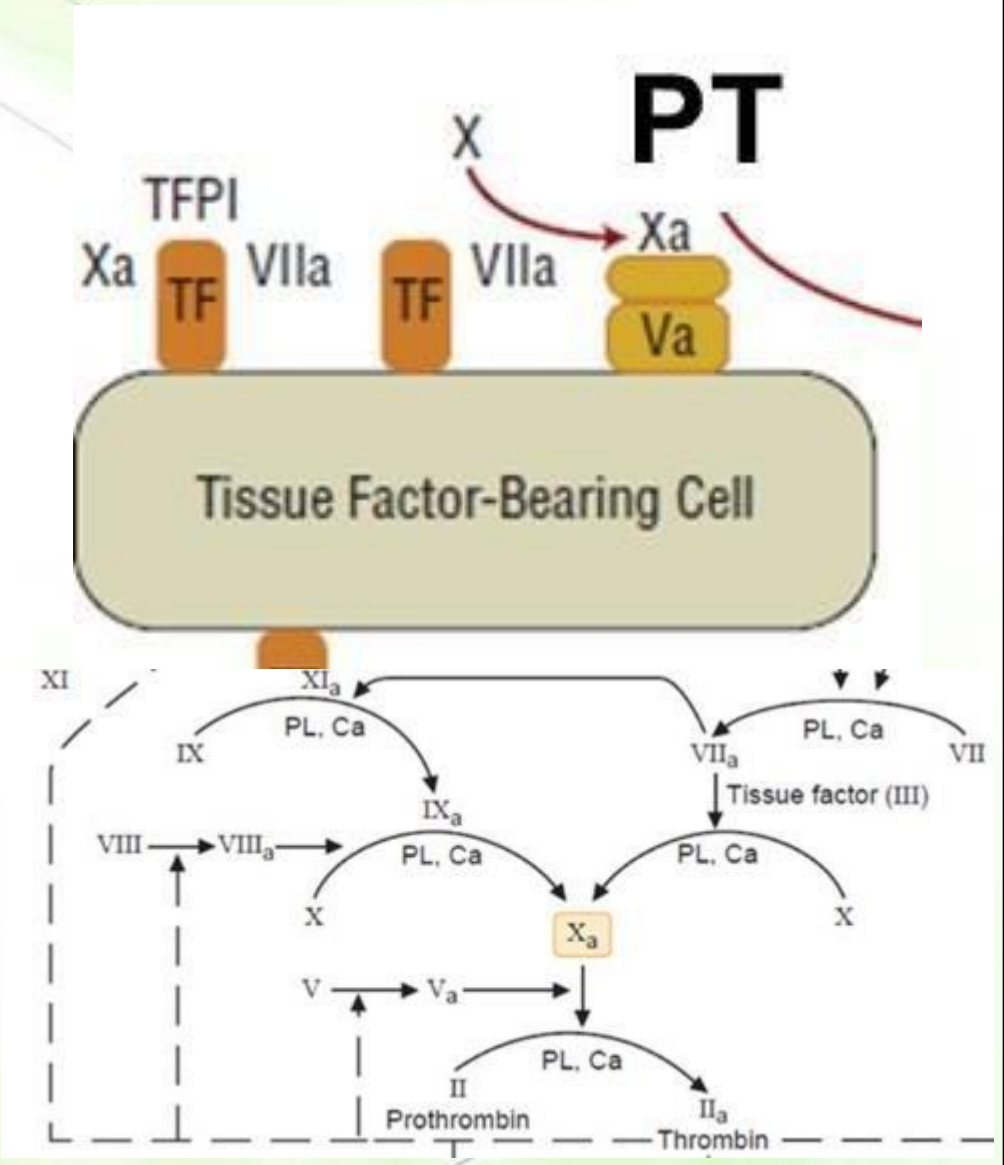


In the clinic, phlebotomy tubes are often treated with heparin in order to inhibit clot formation.

heparin is important as an anticoagulant; it is used in laboratories and in tubes when blood is collected, it prevents blood coagulation.

Tissue Factor pathway inhibitor

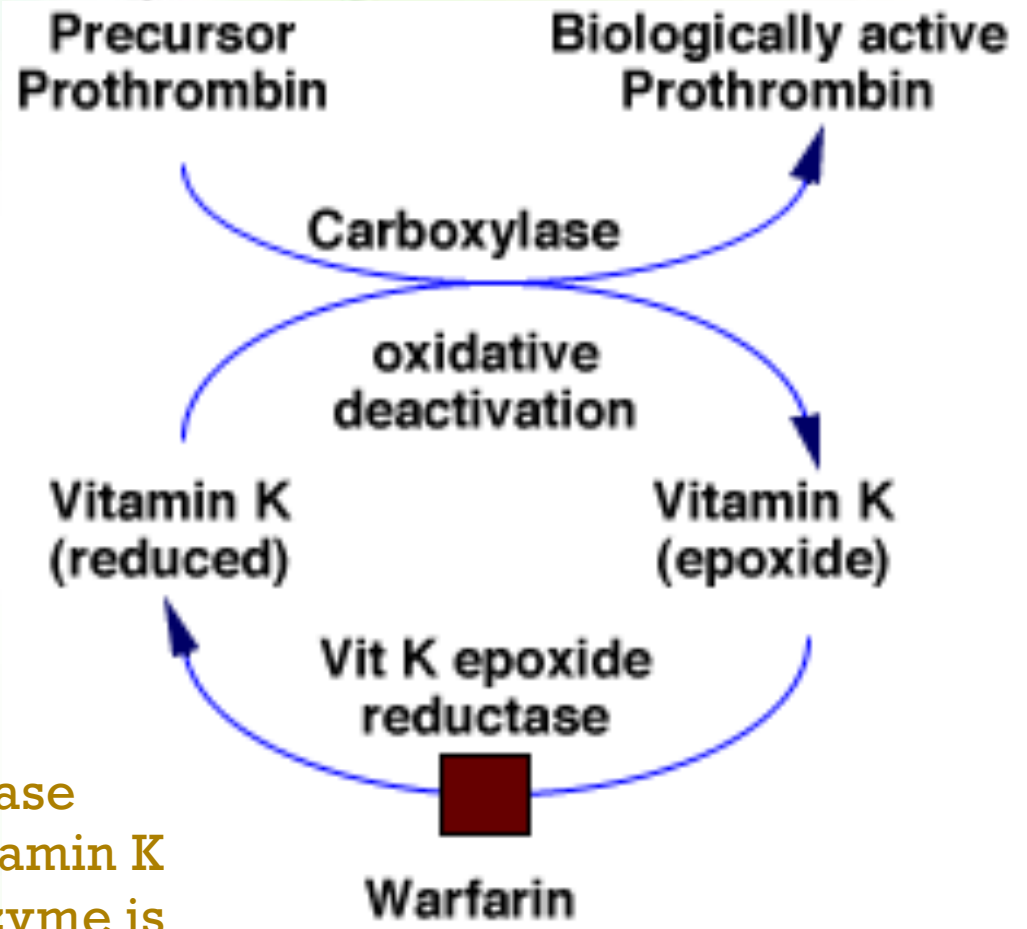
- Tissue factor pathway inhibitor (TFPI) is a protein found in plasma lipoproteins and bound to the vascular endothelium.
  - It binds to and inhibits factor Xa.
  - The Xa-TFPI complex then interacts with the tissue factor-VIIa complex and inhibits its activation of factors X and IX.
  - Protein S binds 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

Ca^{2+} chelators & vitamin K antagonists

- 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.



Ca^{2+} is important for the process of coagulation, so by using chelators that absorb calcium ions, it compromises the efficiency of blood coagulation

Warfarin drug inhibits the vitamin K epoxide reductase preventing the regeneration of the active form of vitamin K which is the reduced form, then the carboxylase enzyme is unable to carboxylate glutamate, and this compromises the interaction of proteins with the surface of platelets.

Degradation of the fibrin clot

Now there's a clot, there’s a cessation of bleeding, and there’s renewal of cells so the clot must be removed

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.

Activation of the clot removal happens simultaneously with the activation of blood coagulation, so that things do not go out of control.

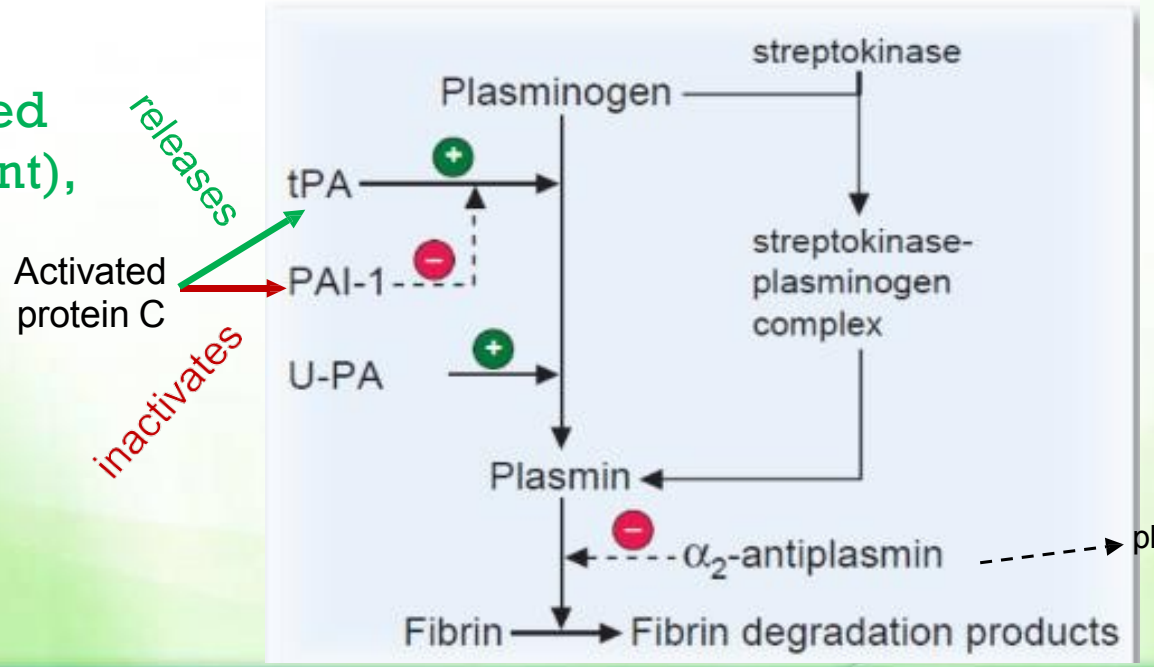
The fibrinolytic system

The dissolution of blood clots is enzymatically dependent; it's done by a protease known as plasmin

- 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.

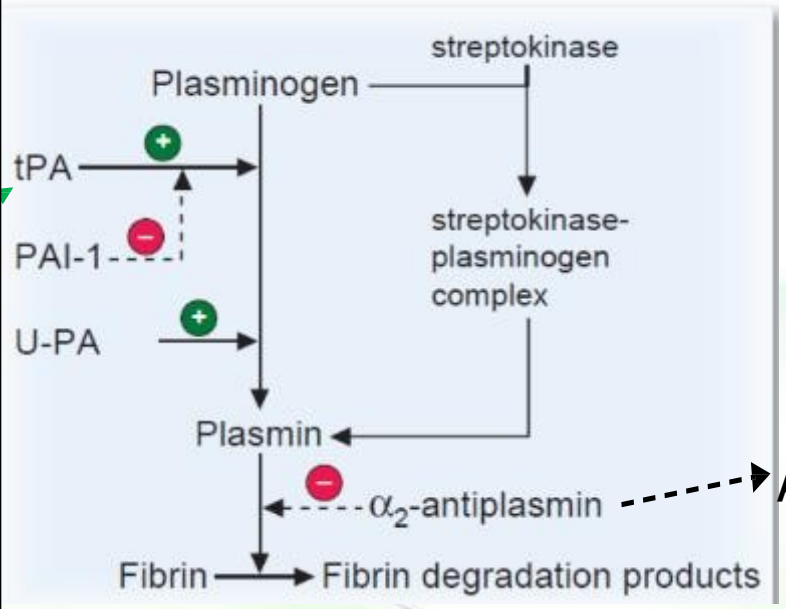
Once plasmin is activated, it can degrade fibrin, this activation process is induced by tissue plasminogen activator (tPA), and by urokinase plasminogen activator (UPA).

The tissue plasminogen activator is activated by the activated protein C (the anticoagulant),
At the same time, activated protein c inactivates the inhibitor of plasminogen activator (PAI)
The result would be stimulation of the fibrinolysis process.



The fibrinolytic system

Also, there's an inhibitor known as α_2-antiplasmin and this antiplasmin keeps things in check; it inhibits plasmin but not when plasminogen/plasmin are clot-bound



If you have a plasmin that is soluble and is outside the clot, it is immediately inhibited by antiplasmin but as long as it is bound to the clot degrading/removing and it's active, it cannot be inhibited by the antiplasmin. This ensures the removal of the clot without affecting the surrounding tissue and surrounding proteins

Plasmin stays bound & restricted inside the fibrin clot,
but once it gets released it gets inactivated by α_2-antiplasmin

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.

So, it induces the removal of the blood clot

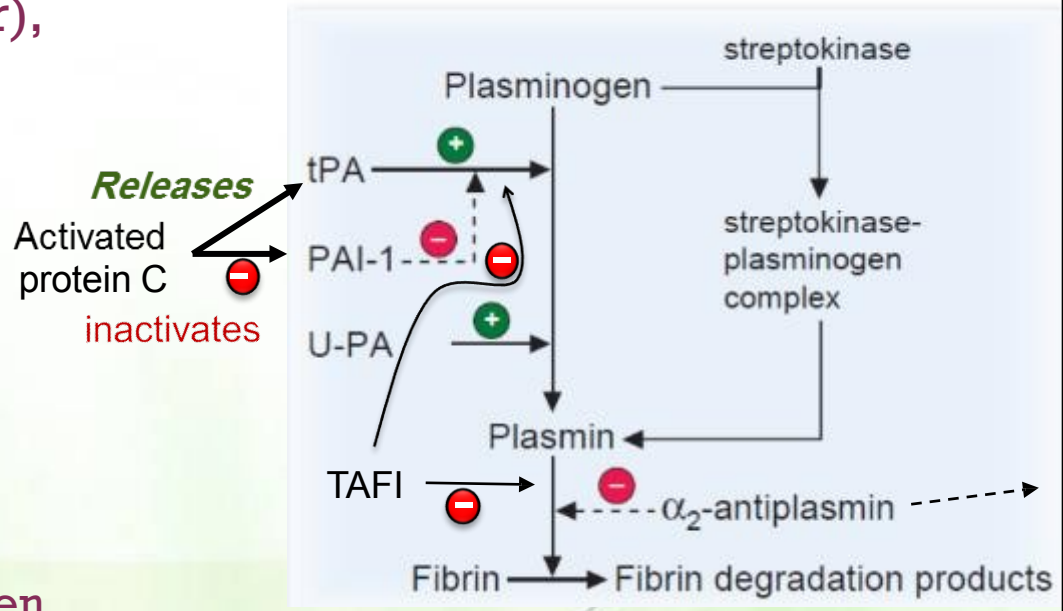
It can be used as a treatment.

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

Tissue plasminogen activator (tPA) & urokinase plasminogen activator (UPA) are regulated by inhibitors and there are two types of inhibitors:

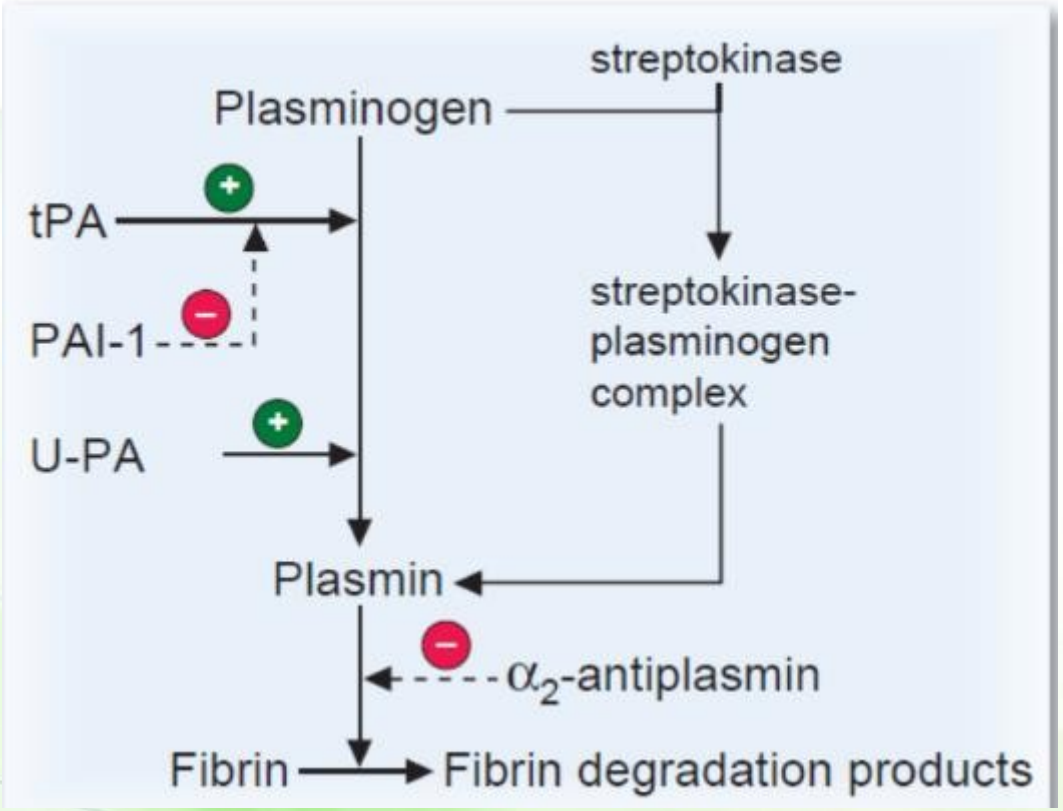
1. Plasminogen activator inhibitor (PAI) (A specific inhibitor), it targets both the plasminogen activator tissue and urokinase
2. Thrombin activatable fibrinolysis inhibitor (TAFI), it removes the N-terminal lysine residues in fibrin (lysine residues are important for the binding of plasmin to the fibrin clot) so when these lysine residues are removed, plasmin cannot bind to fibrin, and it gets released and inhibited right away by antiplasmin.
   Also, it directly inhibits the tissue plasminogen activator.



And, as we said, Activated protein C can activate tissue plasminogen activator and it inhibits plasminogen activator inhibitor

Urokinase

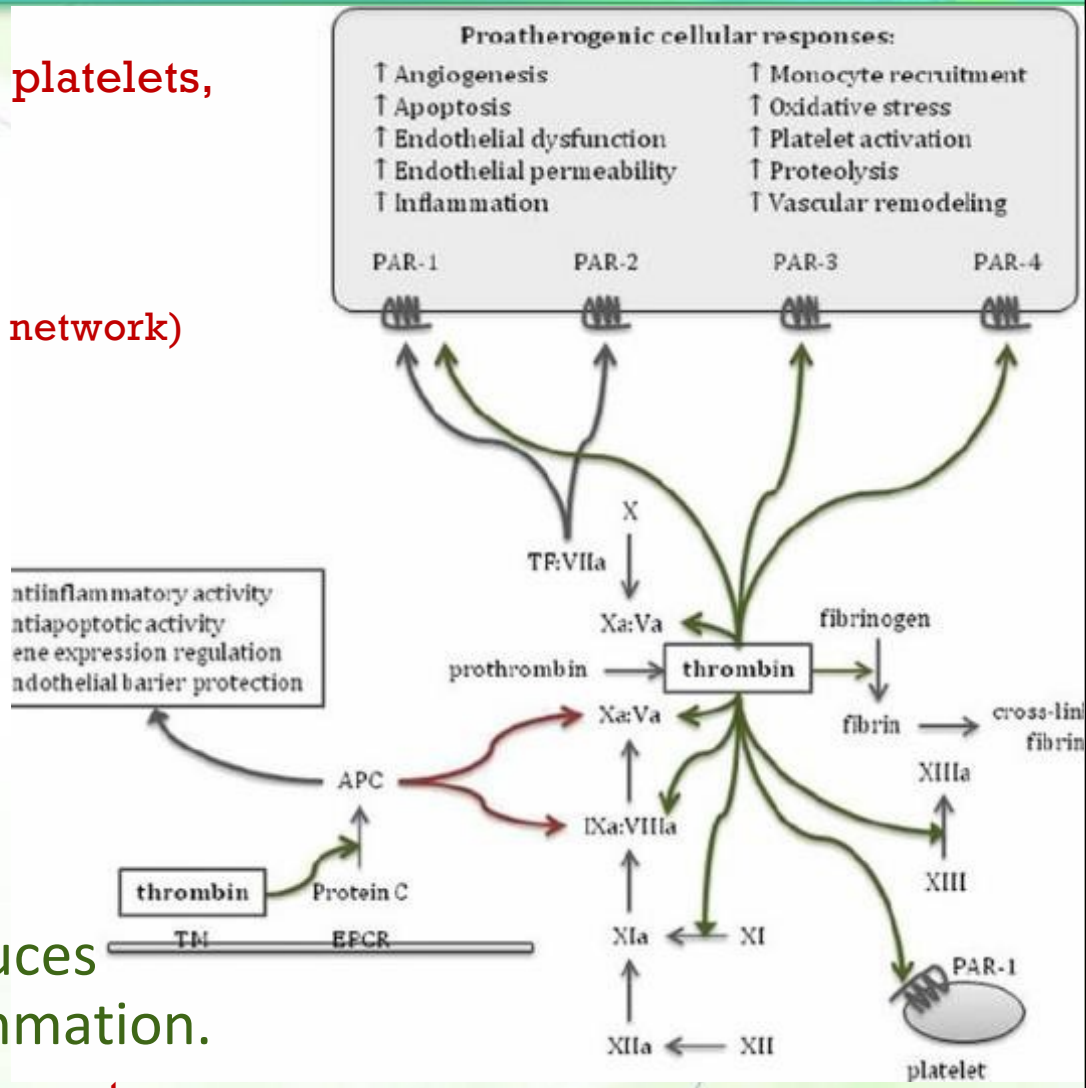
- Urokinase, a serine protease is formed from the zymogen pro-urokinase
- It is a potent plasminogen activator, and is used clinically



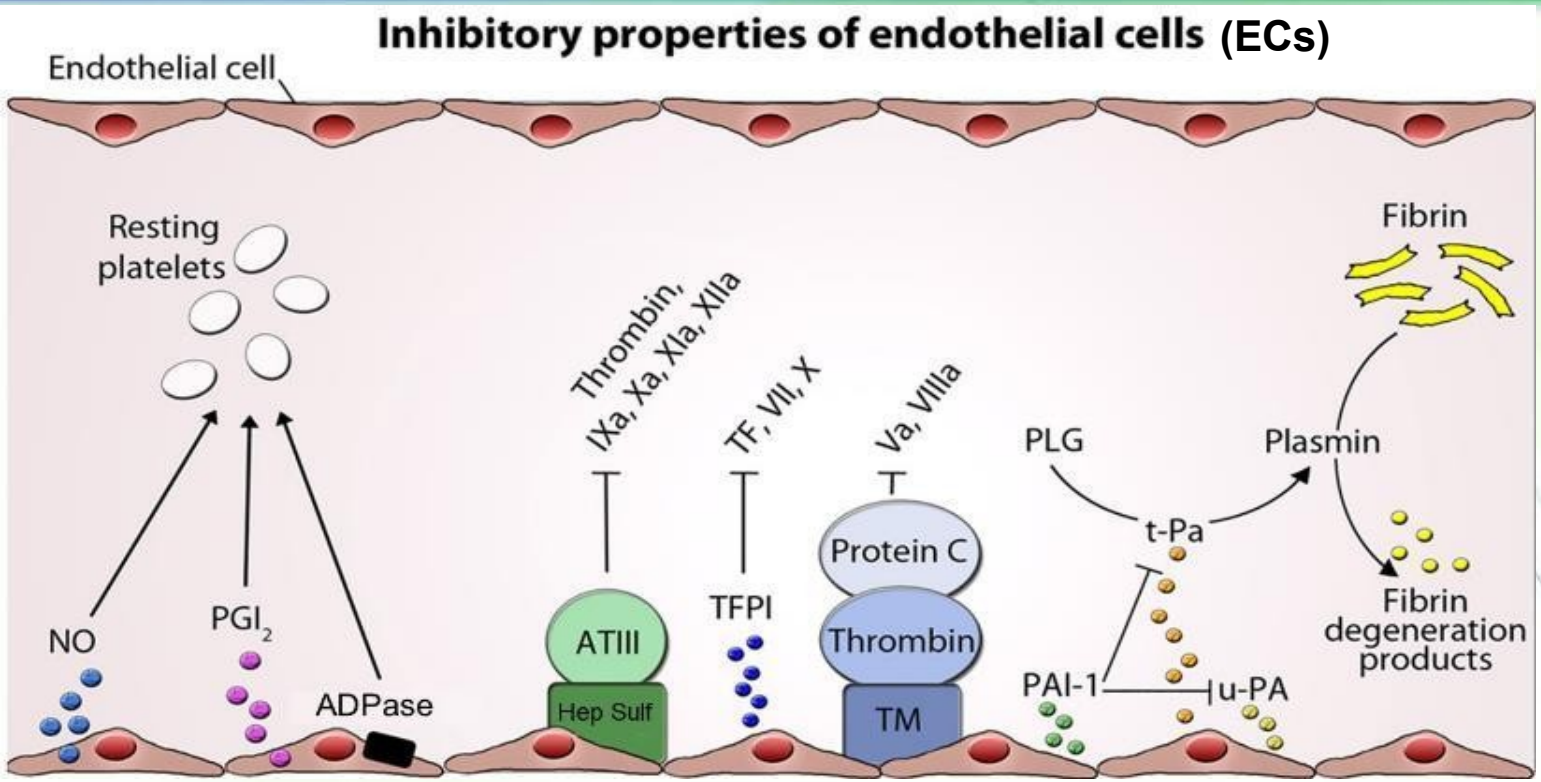
Roles of thrombin

- Platelet recruitment (thrombin has its own receptor on platelets, this receptor recruits platelets to the site of injury)
- Amplification of the coagulation complex
- Formation of soft clot (with the fibrin monomers forming a network)
  - 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.

Thrombin can bind to protease activated receptors, these receptors are present on the several cells' surfaces including the endothelial cells and they can induce vascular remodeling (angiogenesis) as well as inducing inflammation.



Role of endothelial cells in coagulation

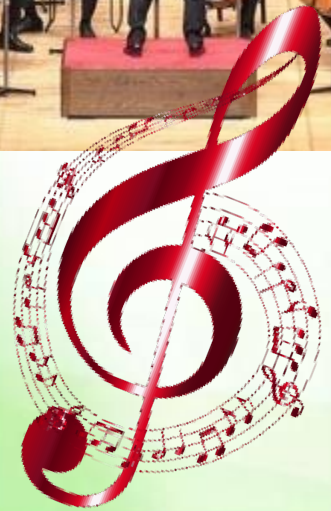


- ECs release NO, prostacyclin (PGI2), and ADPase, which inhibit platelet adhesion and aggregation.
  (so endothelial cells would prevent the formation of clots)
- Membrane-bound heparin sulfate on ECs bind to antithrombin III (ATIII) inactivating several coagulation factors.
- ECs express tissue factor pathway inhibitor (TFPI), which inhibits tissue factor (TF) and, consequently, factors VII and X.
- Thrombomodulin (TM) on ECs' surface 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

So is thrombin the maestro? or it could be endothelial cells? ;)



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Get some rest, you've earned it :)