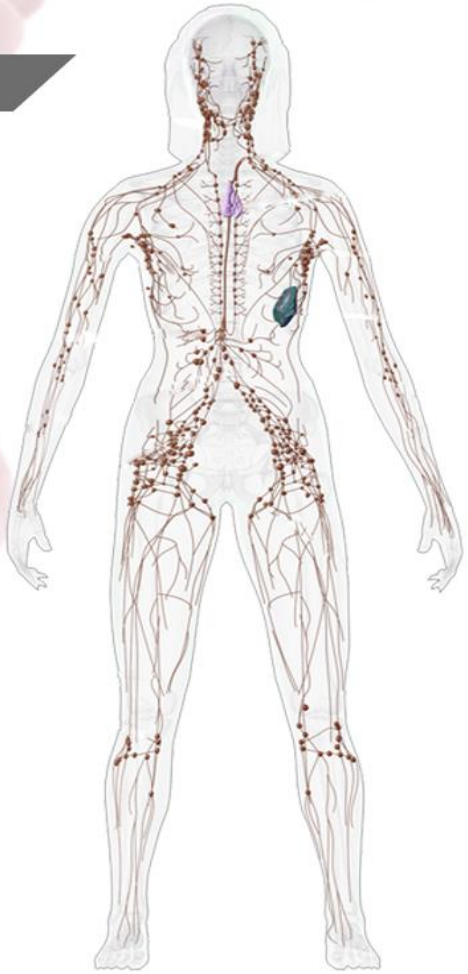
Sheet 6

Hematology and Lymphatic system



Subject | Biochemistry

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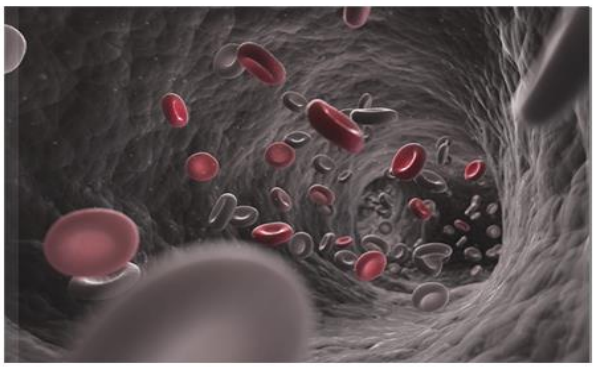


Blood coagulation

(Without pictures, this sheet would be 10 pages). Today we will discuss:

- *Platelet contents*
- *Hemostasis*
- *Coagulation cascade*
- *Prothrombin and Factor X activation*
- *Factor VIII and vWf*
- *Kallikrin-Kinin system*
- *Amplification of coagulation*
- *Anti-coagulants*
- *Fibrin clot formation*
- *Anti-clotting factors*
- *Degradation of blood clot*

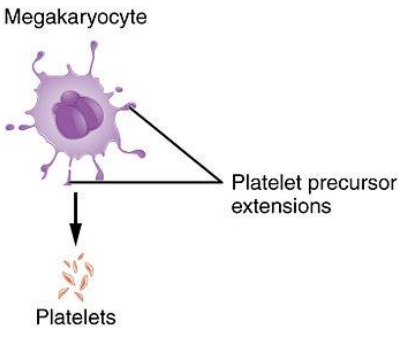
Blood coagulation is a coordinated, biochemical process that is initiated as a result of vascular injury where a small area blood of surrounding injury changes from liquid to gel then this gel solidifies, forming a clot made of fibrin, which results in hemostasis (the cessation of blood loss) followed by clot dissolution and repair of injury site.

The blood clotting process involves a complex array of compounds, such as:

Blood cells, inflammatory cells, cell fragments (Platelets), blood proteins (structural and enzymatic), enzymes, enzyme cofactors, inhibitors, tissue glycoproteins, surface glycoproteins on the surface of endothelial cells & platelets, lipids (phospholipids), ions (specifically Ca^{+2}) / vasoactive compounds. (we will talk about these compounds in details later in this sheet).

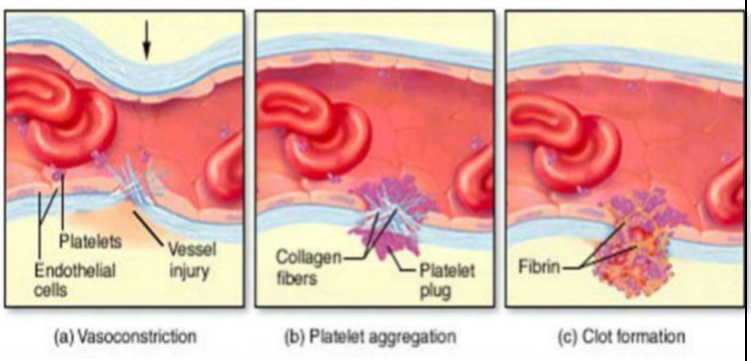
Firstly, we should talk about the main component in blood coagulation which are Platelets:

Platelets are small anuclear cell fragments produced from the megakaryocytes. They have:



1. Numerous kinds of surface receptors.
2. Actin filaments, actin regulatory proteins and myosin, which change the shape of the platelet upon activation.
3. Three types of granules that store substances, and upon platelet activation it fuses with platelets' surface releasing its content:
   a. Electron-dense granules: contains Ca^{+2} ions, ADP, ATP, serotonin.
   b. Alpha-granule: contains a heparin antagonist, platelet-derived growth factor PDGF (stimulates proliferation of endothelial cells to reduce blood flow & close the wound), fibrinogen, von Willebrand factor (vWF), clotting factors.
   c. Lysosomal granule: contains hydrolytic enzymes.

So, what are the steps of hemostasis??



1. Vascular constriction limiting blood flow to the area of injury to stop bleeding.

2. Activation then aggregation of platelets at the site of injury, forming a loose platelet plug to close the wound.

3. Formation of a fibrin mesh to entrap the plug.

4. Dissolution of the clot in order for normal blood flow to resume following tissue repair.

Now, we will talk about them in details:

1. Adhesion to endothelial cells and matrix proteins.

Blood vessels are surrounded with collagen. So once injured, collagen and the endothelial von Willebrand factor (vWF) protein will be exposed to platelets and bind to platelets' surface receptors which are glycoproteins (a type of integrins). Then platelets get activated and granules fuse with the plasma membrane releasing its contents. So, some platelets release substances from the granules such as:

*ADP

*ATP

*Serotonin
*Factor V (5)

*Calcium

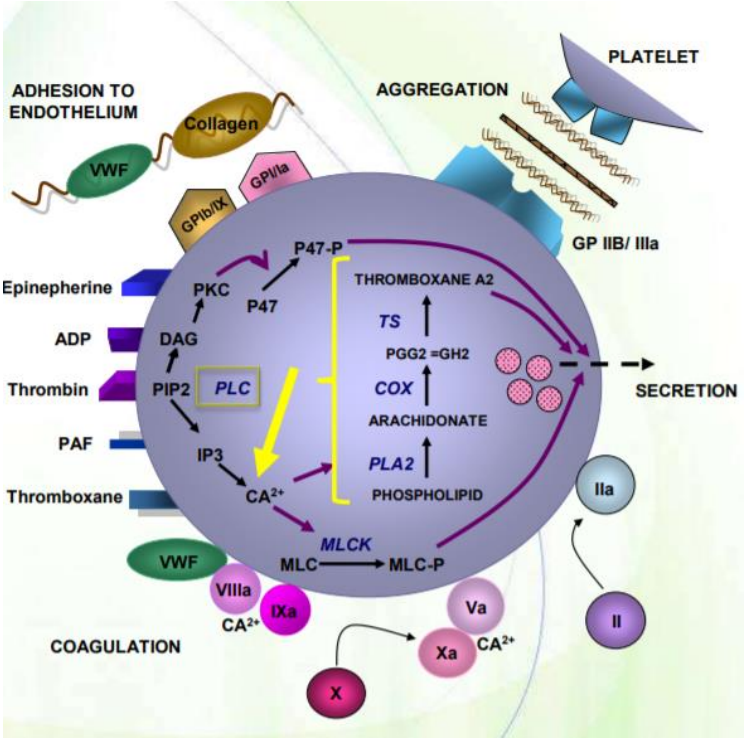
*Fibrinogen

*vWF (producing amplification of the first step)

*Thrombin

*Thromoxane

-These substances bind to receptors on platelets' surface and have a role in platelet coagulation which will be shown in details.



Upon activation, actin cytoskeleton also gets modified so that the shape of the platelets ghange from rounded to structures with phylopodia (extensions which contain more receptors) allowing for more platelet-platelet interaction and aggregation.

Thrombin:

This released thrombin binds its receptor which activates a G-protein that activates phospholipase C-γ (PLC-γ), this PLC-γ hydrolyzes phosphatidylinositol 4,5-bisphosphate (PIP2) into inositol trisphosphate (IP3) and diacylglycerol (DAG).

1. IP3 induces the release of intracellular Ca2+ stores, which has many effects:

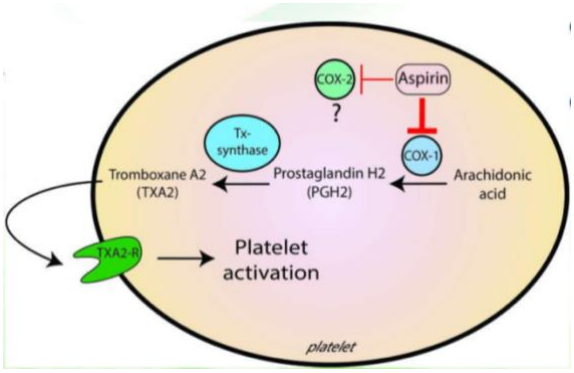
   *It triggers liberation of arachidonic acid from membrane phospholipids by the enzyme phospholipase A2. Arachidonate is converted by cyclooxygenase to prostaglandins, which are then converted by thromboxane synthetase to thromboxane A2.

   TXA2 is a potent vasoconstrictor to slow down blood flow and inducer of platelet aggregation.

   * Intracellular Ca2+ stores acts on myosin light chain kinase (MLCK), which phosphorylates the light chain of myosin MLC converting it into MLC-P allowing it to interact with actin resulting in altered platelet morphology (platelet shape), induced motility and facilitating release of granules, which induces more platelet activation.

2. DAG activates protein kinase C (PKC), which phosphorylates and activates specific platelet proteins that induce the release of platelet granule contents including ADP.

Note: Non-steroidal anti-inflammatory drugs (e.g. aspirin) inhibit the enzyme cyclooxygenase, inhibiting the inflammatory response and clot formation accounting for these drugs anticoagulant effects.



Serotonin:

a vasoconstrictor acting on different receptors on epithelial and mucus cells.

ADP:

ADP is a platelet activator that binds to its receptor and modifies the platelet membrane (changes structure) allowing fibrinogen to adhere to platelet surface glycoproteins resulting in fibrinogen-induced platelet aggregation which induces more platelet aggregation.

2. Aggregation of platelets (platelet-platelet interaction and clustering):

Now, the accumulated platelet plug provides an important surface on which coagulation reactions occur.

3. Coagulation: **the enzymatic process that takes place on the surface of platelets.**

Components of coagulation:

1. An organizing surface (platelets)… Remember that everything happens on their surface.
2. Proteolytic zymogens (prekallikrein, prothrombin, and factors XII, XI, IX, VII, and X)

Note: zymogens are inactive enzymes that can be activated by proteolytic cleavage of part of it

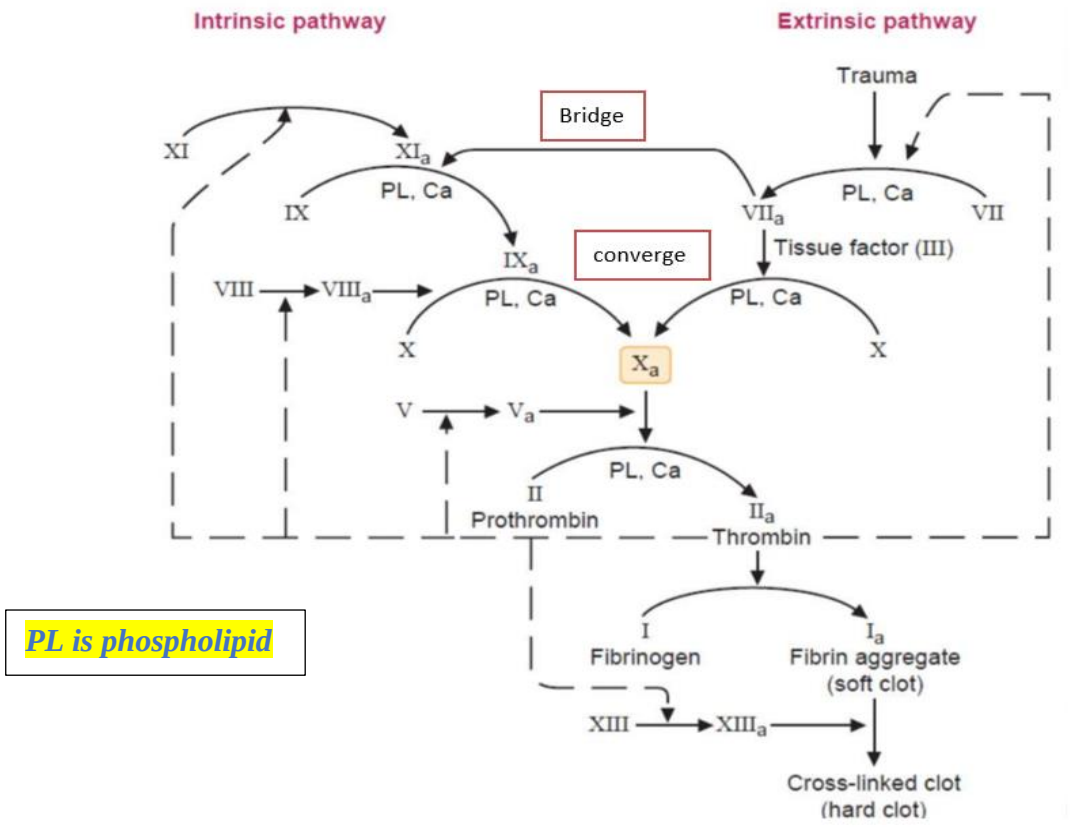
-These are mainly inactive serine proteases produced and released from hepatocytes.

-The subscript "a" designates the activated form of a factor (e.g., “XIII" is inactive while "XIIIa" is active)

3. Anti-coagulant (protein C, protein S)
4. Non-enzymatic protein cofactors that activates enzymes (factors VIII, V, and III (tissue factor)
5. Calcium ions
6. Thrombin, it originates as a zymogen (prothrombin) that's cleaved to form thrombin.
7. Fibrinogen, the protein that forms the clot itself.

When pathways of blood coagulation were first studied, they initially thought that there are 2 pathways independent of each other. But they've discovered that there's a bridge that links these 2 pathways, and they also converge (meet) at a point of factor Xa which's commonly activated by both pathways. These 2 pathways are separated by the source of injury:

1. The intrinsic pathway is initiated when subendothelial surface (i.e., collagen) is exposed, so the injury is inside the body.
2. The extrinsic pathway is initiated in response to tissue injury, so the injury is caused by an extrinsic factor (e.g. trauma).In this pathway tissue factor (TF) protein is released.



PL is phospholipid

Steps of blood coagulation:

1. We will start with the intrinsic pathway:
   a- kallikrein can activate factor XII *(Kallikren-Kininogen system will be discussed in details later; page 10)*
   b- Factor XII_a activates factor XI.
   c- Factor XI_a activates factor IX
   d- Factor IX_a activates factor X with the need for the cofactor $VIII_a$.

2. In the extrinsic pathway:
   a- trauma activates factor VII.
   b- Factor VII_a can bridge the 2 pathways to each other and activate factor IX thus it can continue in the intrinsic pathway, or activate factor X directly with the need for tissue factor (TF or factor III).

3. Here, the 2 pathways converge at the point where they activate factor X.
   -Factor X activation needs phospholipids on platelets' surface (PL) for the reaction to occur on, as well as Ca^{+2} ions to bind to the surface and localize the reaction on the surface (role of Ca^{+2} ions will be discussed in details later in this sheet).

4. Then the 2 pathways continue in the same way:
   a- Factor X_a catalyzes prothrombin activation and the release of thrombin from the surface with the need of activated factor V as a cofactor, phospholipids on platelets' surface (PL) and Ca^{+2} ions.
   b- Thrombin catalyzes the conversion of fibrinogen into fibrin but with electrostatic interactions which forms a soft clot.
   c- This soft clot's fibrin polymers cross links by covalent interactions forming a hard clot, and this last interaction is catalyzed by activated factor XIII ($XIII_a$).

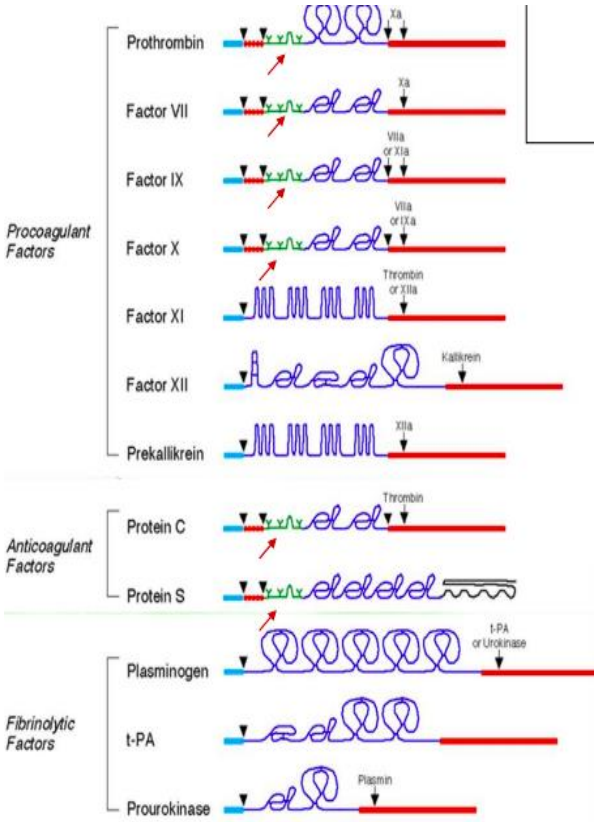
Thrombin has many effects:

1. Facilitates platelet adhesion. *(Remember? at the beginning)*
2. Activates some factors needed in the coagulation pathway such as factor V, VIII, XI, and XIII.
3. Catalyzes the conversion of fibrinogen into fibrin. *(Will be discussed later)*
4. Ends coagulation. *(Will be discussed later)*

Different domains exist in coagulation factors, and there are common domains such as the catalytic domain *(in red)* that exists at the C-terminus. Another example is the GLA domain which we will focus on.

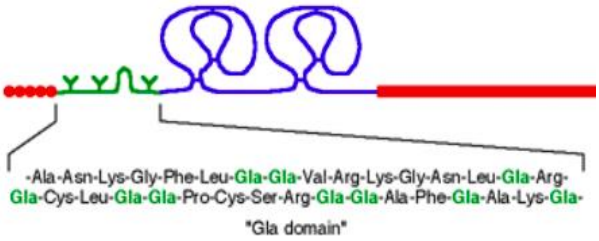
GLA Domain

- This domain is a sequence of amino acids rich with glutamate. These glu-residues get **<u>carboxylated</u>** forming gamma carboxyglutamate (GLA), this compound has two carboxyl groups since glutamate contains a COO- group

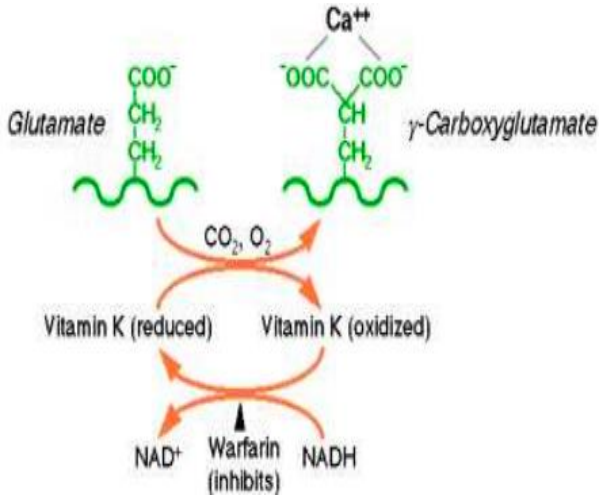


- **In details:** An ER/Golgi carboxylase in hepatocytes binds to and converts 10 ≥ glutamate (Glu) residues to gamma-carboxyglutamate (Gla) forming the GLA domain. This carboxylation process requires Vitamin K where it becomes oxidized requiring regeneration. *(k for koagulation in German)*

- The GLA residues bind calcium ions **<u>chelating</u>** them between two carboxylic acid residues. Why is this important? The +ve charged Ca can now interact with the -ve charged phospholipids on platelets surface. Calcium ions induce conformational changes in the GLA domain that are necessary for the proper folding of the GLA domain to mediate interactions with the platelet surface only.
  - ➔ The GLA domain is present in prothrombin, coagulation factors (IX, VII, X), and anticoagulant factors (C, S).
  - ➔ Thus, activation of these factors containing the GLA domain and the formation of thrombin is contained and **<u>localized to the platelet surface only.</u>** It doesn't happen anywhere in the blood, only at the site of injury.



- *Chelation is when 2 groups interact with a common ion like Ca++*



- **In summary:** the GLA domain is a domain that contains post-translational modifications of many glutamate residues by **<u>Vitamin K - dependent carboxylation</u>** to form γ-carboxyglutamate (Gla). The Gla residues are responsible for the high-affinity binding of calcium ions. Binding of calcium alters the conformation of the Gla domains of the factors, enabling them to interact with the phospholipids platelets surface.

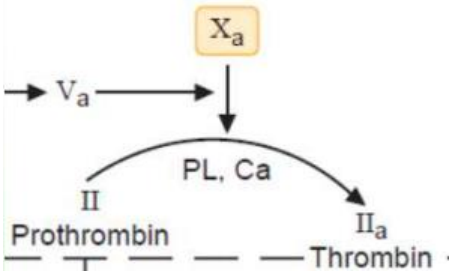
Vitamin K:

- Facilitates the carboxylation rxn
- Vit K must be reduced in order to activate carboxylase enzyme. It also needs to be regenerated by a reductase
- The reductase enzyme is the target of Warfarin; no regeneration, no carboxylation, no coagulation = anti-coagulant *(used especially pre-op)*

Prothrombin Activation

The active form of PT {thrombin} is important in forming the hemostatic plug over a wound site.

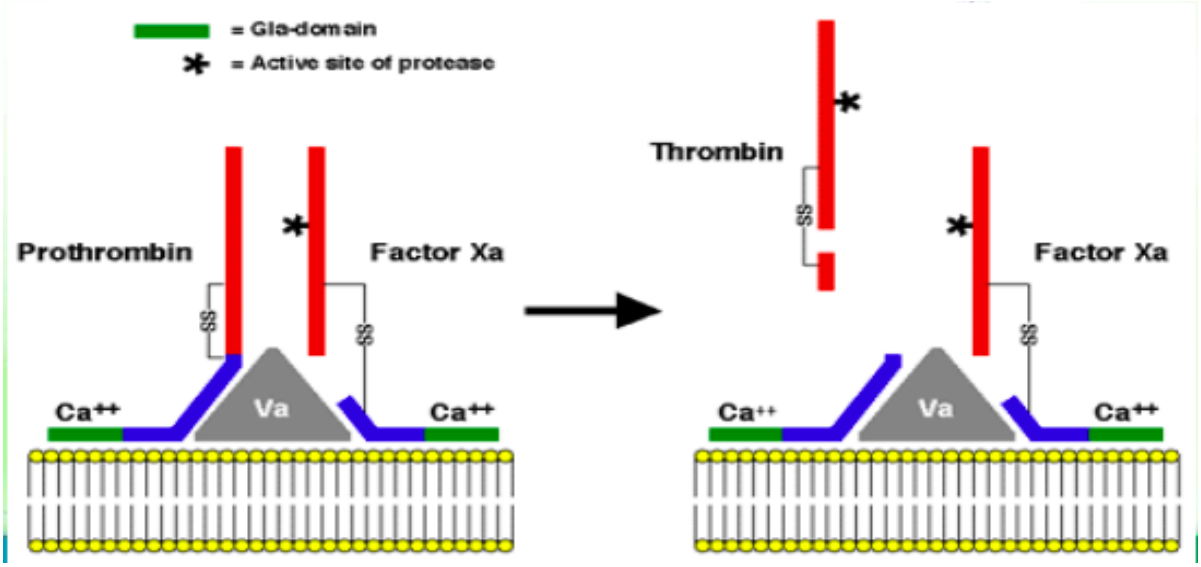
Aggregated platelets provide the surface upon which prothrombin activation occurs and later the coagulation cascade. As mentioned, both extrinsic and intrinsic pathways **end up by activating factor X** which cleaves prothrombin into thrombin. Again, this takes place on surface of platelets.



For factor Xa to activate prothrombin it requires:

1. Ca: for chelation of GLA and associate all these proteins to the surface of platelet.
2. Phospholipids : to interact and bind Ca.
3. Factor Va: a cofactor *not an enzyme*

Steps: Factor Va is bound to the surface of platelets, it binds both Factor Xa and Prothrombin making them close to each other. Factor Xa then cleaves prothrombin and thrombin is released in the active form. Factor V is a cofactor that increase the proteolytic efficiency of Xa.



So how did Factor X get activated ?

PT activation is where the two pathways converge. The step just before this activation is different bw the two pathways;

Activation of factor X

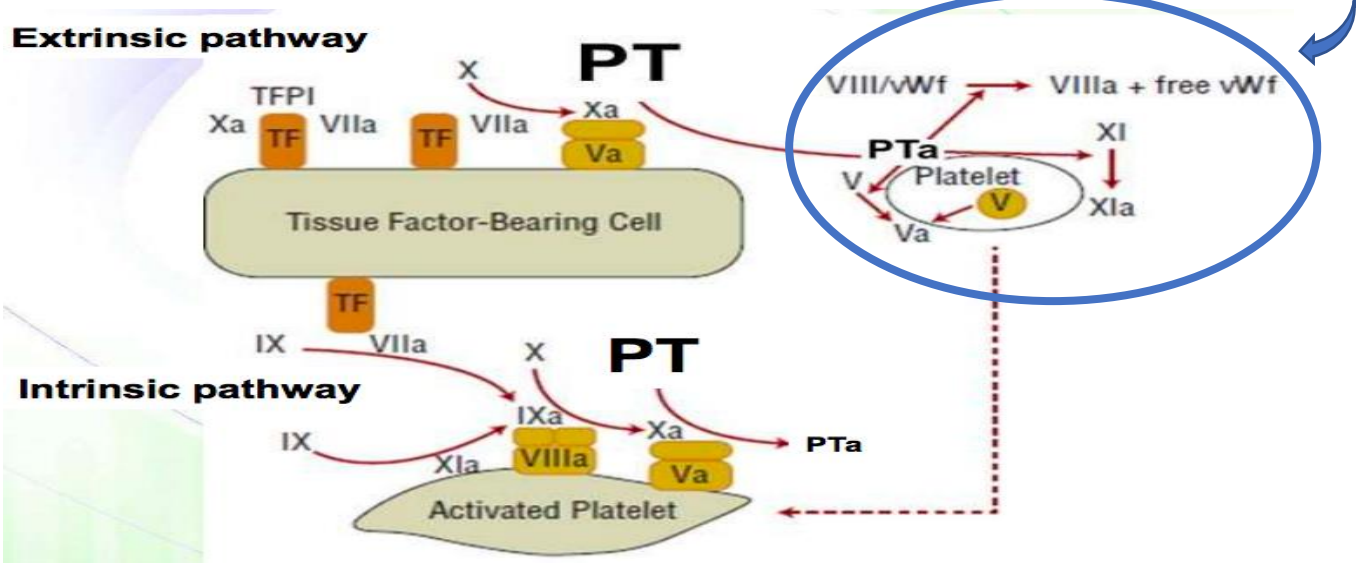
- **Intrinsic pathway:** Factor IX after being activated by Factor XI, associates with Factor VIII and helps in the activation of Factor X. Then, Factor X binds to Factor V.
- **Extrinsic pathway:** Tissue Factor is involved in the extrinsic pathway. Factor VIIa activates Factor X with the help of TF. VIIa is also involved in the intrinsic pathway where it activates XI which further activates X.
- The idea here is that it takes place on the surface of platelets with the presence of Ca ions.

Where did Factor VIII come from ?

Thrombin releases Factor VIII from VWF. When released, it gets activated.

Role of VWf: In addition to being present in the matrix and activating platelets, VWF binds to Factor VIII in the plasma to prolong its life (*Without VWF, Factor VIII has a very short life and gets degraded rapidly).*

Deficiency of VWF → limited amount of Factor VIII is available and as a result, a limited activation of coagulation process. the person will suffer from bleeding.



Thrombin Interaction with coagulation Factors

Factors **VII**(VII is mentioned in Lippincott)**, VIII (**in intrinsic pathway), **XI, XIII** (forms hard clot), and Factor **V** are activated by thrombin. *(it may be a bit confusing that Factor V is required for thrombin activation while at the same time thrombin activates Factor V)* **EXPLANATION:** Thrombin activates more V into Va and other factors in the intrinsic pathway for further **amplification** of thrombin formation.

Factors V and VIII are cofactors that increase the proteolytic efficiency of Xa and IXa respectively.

Activation of the Extrinsic and Intrinsic Pathways

- The activation of these pathways occurs at the surface of platelets.

  Intrinsic pathway: it is activated when there is exposed collagen due to vessel damage.

  Extrinsic pathway: it is activated when there is vessel damage.

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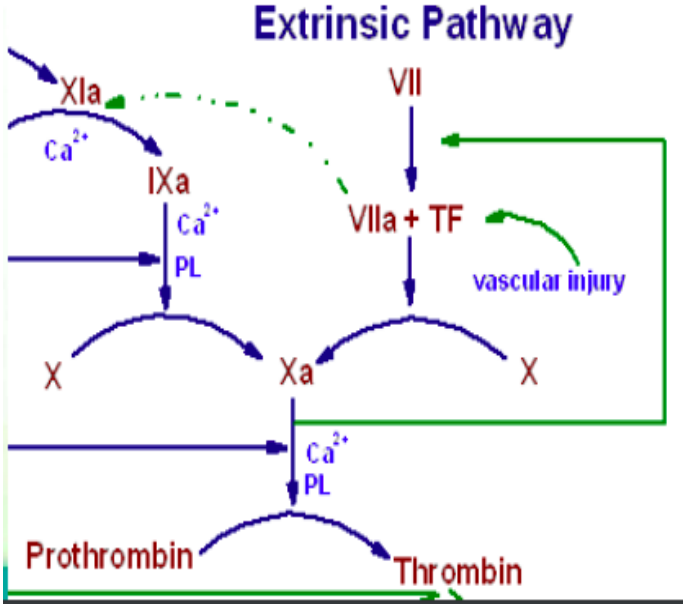
Is there a link between the two pathways ?

Tissue factor: is an integral membrane protein that is expressed on the surface of "activated" monocytes, endothelial cells exposed to various cytokines, and other cells. It greatly increases the proteolytic efficiency of VIIa in. TF is involved in the extrinsic pathway.

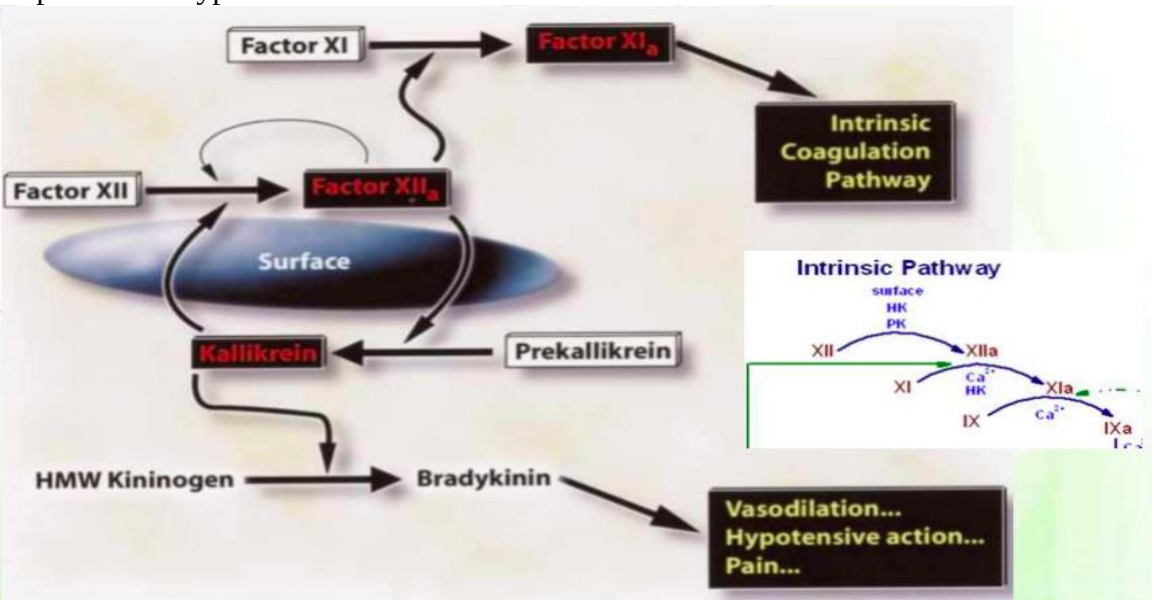
➔ It associates with Factor VIIa activating Factor X. This complex is also linked to the activation of Factor IX by Factor XI in the INTRINSIC pathway. So that is the **link** bw the two pathways.

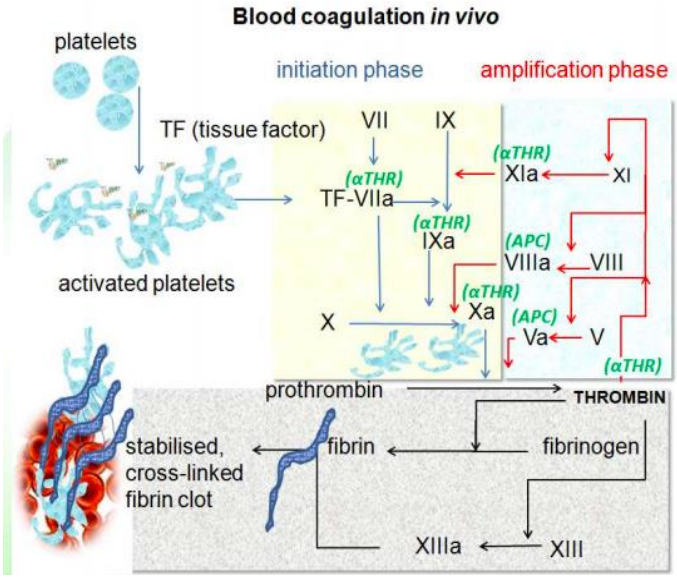


The Kallikrein-Kinin System

- Kallikrein is an enzyme produced as a zymogen “prekallikrein”
- Kinin is produced from a peptide (not an enzyme) called kininogen.
  - On the surface of endothelial cells, when there is an injury, prekallikrein becomes kallilrein.
  - Factor XII binds to exposed collagen at site of vessel wall injury and is activated by kallikrein.
  - Factor XII activates Factor XI which **activates the intrinsic pathway**.
  - Factor XII further converts prekallikrein into kallikrein resulting in **amplification** of the process.

- Kallikrein can also act on kininogen to produce **bradykinin**, which is a short peptide that acts on endothelial cells **inducing vasodilation**. Vasodilation is important because many inflammatory cells are recruited to the site of injury.
- This system is also important in the **Renin-Angiotensin** system which means that it is important for hypertension.





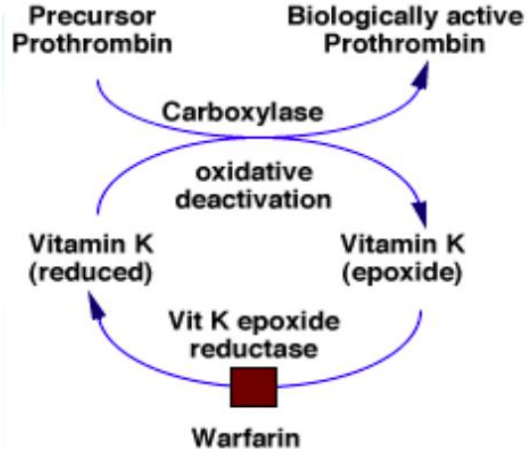
Amplification of Coagulation Reactions

The **sequential enzymatic activation** allows amplification. So one enzyme can activate let's say 1000 enzymes, each one of these 1000 can activate another 1000 enzymes and so on…

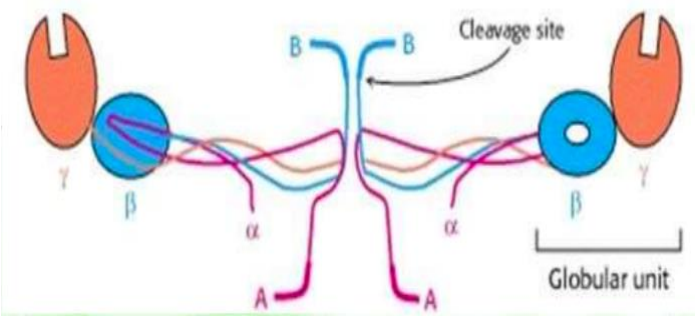
Amplification also results from **positive feedback reactions**. As mentioned previously, thrombin not only can it act on fibrinogen, but also it can go back in a process of feedback activation to activate Factors V, VIII, and XI. This results in amplification of the cascade itself.

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. *(mentioned previously)*



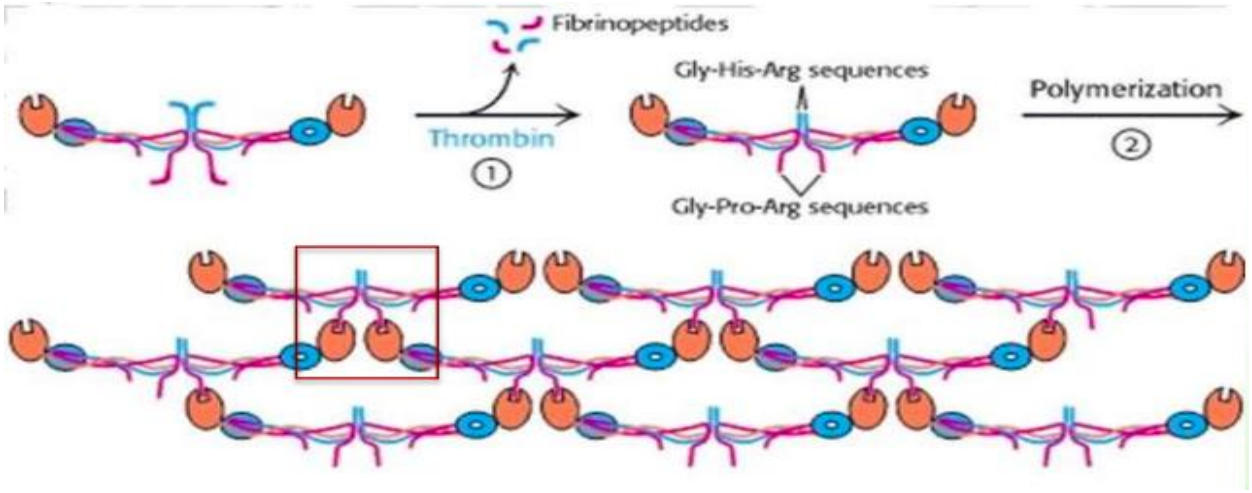
Formation of Fibrin Soft Clot



Fibrinogen is a matrix protein present in the blood. It is a two triple-stranded helical protein held together by disulfide bonds (fibrinogen monomer).

{Refer to slide 27 to see fibrin fibers on EC}

Steps: Thrombin cleaves fibrinogen at the cleavage site releasing fibrinopeptides forming a fibrin monomer and releasing fibrinopeptides *(not imp.)* → electrostatic attractions are formed between the central domain of a fibrin monomer and the globular head of another monomer→ this crosslinking results in the polymerization of fibrin forming a gel-like clot = SOFT CLOT *(because the interactions are non-covalent (i.e. electrostatic))*



How can we make it a hard clot ?

Factor XIII

Factor XIII is an enzyme that is NOT a serine protease. All the previous factors (VII, VIII, IX, X, X11 and XII) are serine proteases just like chymotrypsin and trypsin. It is a transglutaminase that is required for adequate clot strength and normal wound healing in vivo.

Covalent cross-linking of fibrin polymers can be formed by activated factor XIII (XIIIa),

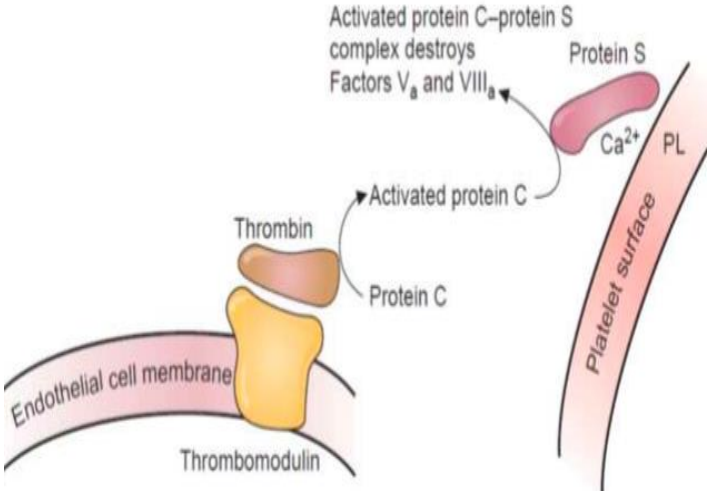
Thrombin activates factor XIII producing XIIIa. *(so, thrombin accelerates the formation of fibrin monomers and also, activates a transglutaminase to form the hard clot)*

Enzymatic mechanism: REFER TO SLIDES 28, 29 FOR PICTURES; IMPORTANT !

- Factor XIIIa catalyzes a transglutamination reaction that catalyzes a cross-linking reaction that covalently attaches a **glutamine** side chain of one fibrin monomer to a **lysine** side chain of an adjacent fibrin monomer. The cross-links strengthen the fibrin mass, forming the "HARD clot".
- Factor XIIIa also cross-links the fibrin clot to **adhesive proteins** on the *endothelial tissue* and to the **platelets surface** strengthening the platelet plug.

The process has to stop somewhere, how?

Anti-Clotting Factors



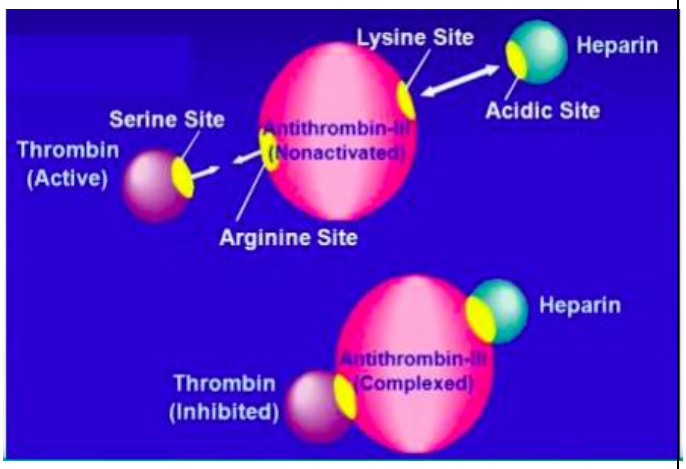
- **thrombomodulin**

  There are two anti-clotting factors; protein C and protein S. They contain GLA domains and therefore are bound to platelet's surface.

  What happens is that once you have thrombin in the system, it binds to a protein called **thrombomodulin** present on the surface of endothelial cells.

  Once bound to thrombomodulin→thrombin convert and activates protein C →protein C forms a complex with protein S → the complex is an enzyme that degrades Factors V and VIII→ activation process stops all together :→: **no more activation of Factor X**

  (so, thrombin induce its own inhibitors)

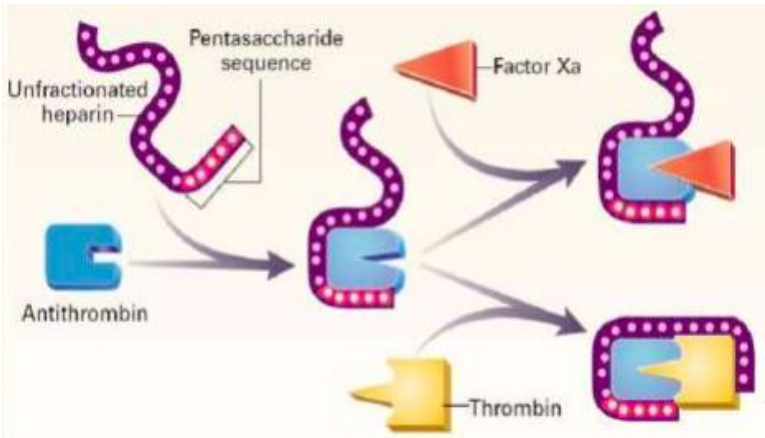


- **Antithrombin III**

  heparin is a sulphated polysaccharide that keeps on swimming in the blood. It binds to antithrombin III, activates and makes it easier for thrombin to bind to the complex→ thrombin inhibited.

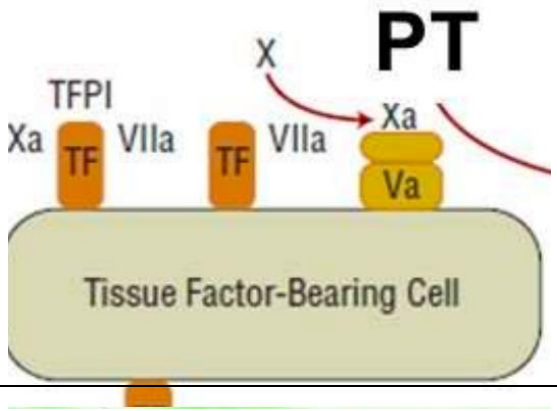
  Antithrombin III is a SerPIn that binds and inhibits the activity of thrombin as well other clotting factors (IXa, Xa, XIa, and XIIa). The interaction is via the "Ser" of the active site.

  Application→ phlebotomy tubes are often treated with heparin in order to inhibit clot formation.



- **Tissue Factor pathway inhibitor**

  It inhibits the factor VIIa-Xa complex.



It is important to prevent clot formation when not needed by anti-clotting factors and to dissolve a clot when formed.

Degradation of Blood Clot

- Clot dissolution starts concomitant with its formation.

The Fibrinolytic System

Plasmin, a serine protease formed from its zymogen "plasminogen", is responsible for fibrinolysis where it catalyzes the hydrolysis of fibrin and fibrinogen to degradation products. *More details?*

Plasminogen has a high affinity for fibrin clot → when it gets activated, it does so within the clot. This allows it to degrade the fibrin into fibers and the clot dissolves

Regulation of plasminogen: 1) by an activator: plasminogen activator, two types:

A. Tissue PA produced by endothelial cells, tPA

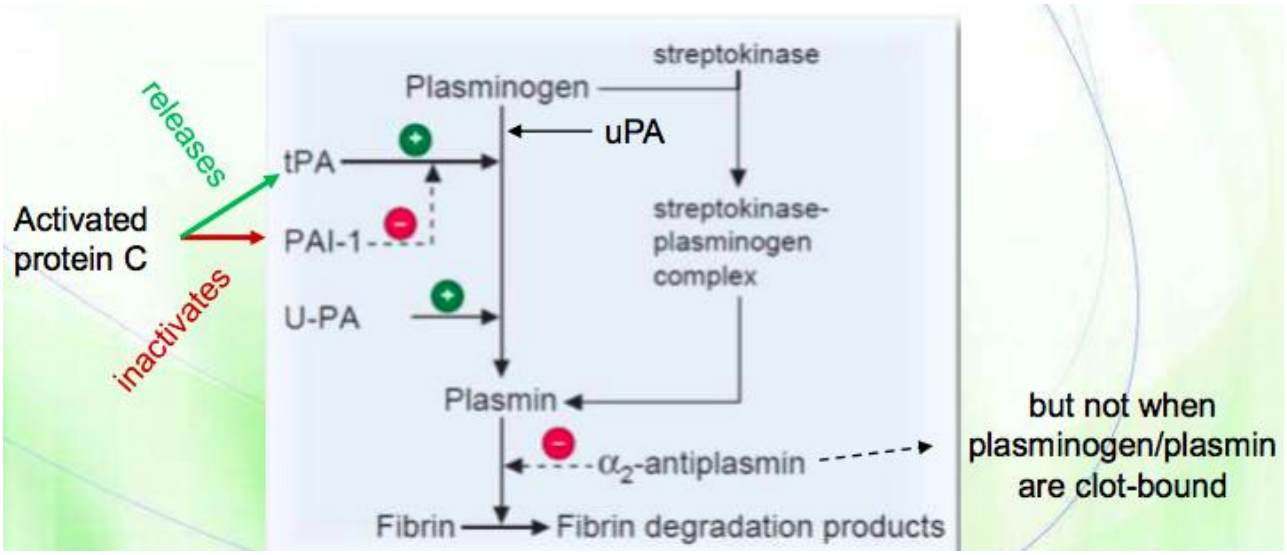
B. Urokinase produced by many cells, uPA

2) by an inhibitor: plasminogen activator inhibitor, PAI

3) protein C: inhibits PAI + activates PA

(an Inhibitor of the inhibitor and an activator of the activator :: the inhibitor is inhibited by prot C. In addition, prot C activates the activator which in turn activates plasminogen)

Regulation of plasmin: 1) inhibitor of plasmin: anti-plasmin



Streptokinase

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.

Application→ old patient pre-surgery, bedbound for a long period, small clots would form here and there. So, streptokinase (or Warfarin) is given to prevent formation of clots. The amount of streptokinase given must be well-controlled, if you give too much, this will cause massive bleeding = dangerous.

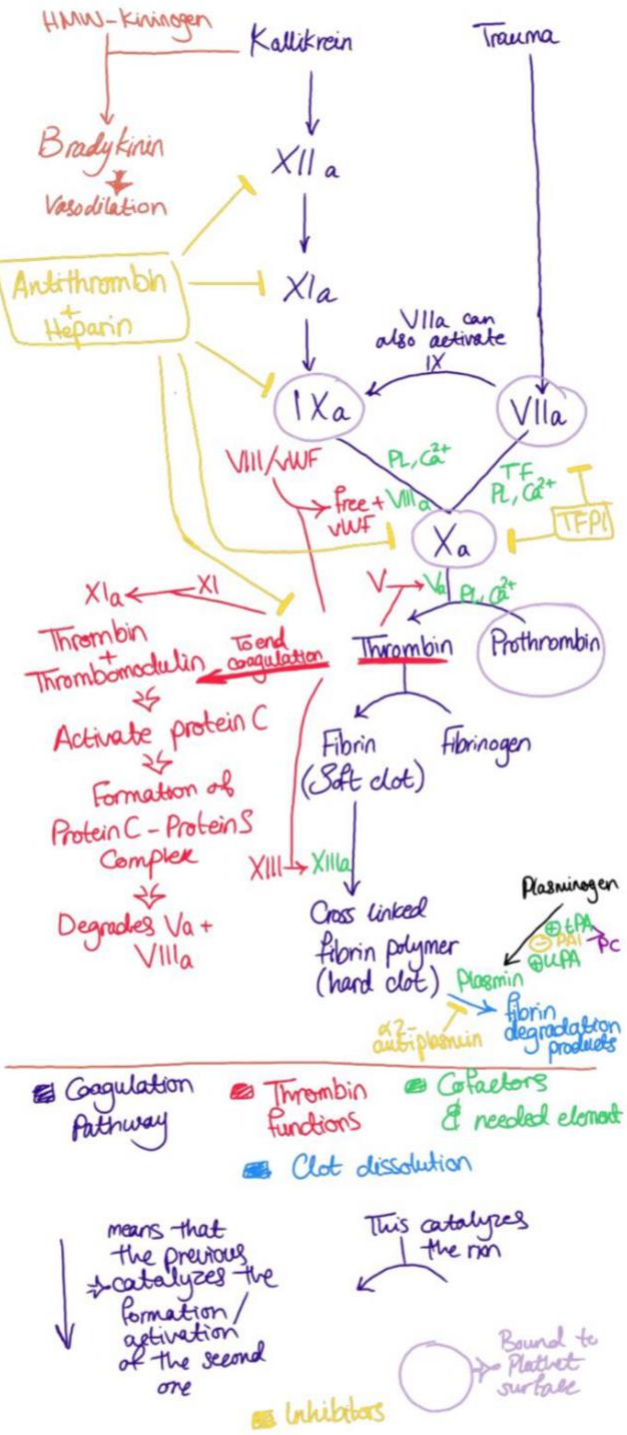
Urokinase ((from slides, Dr didn't mention it))

Urokinase, a serine protease is formed from the zymogen pro-Urokinas.

It is synthesized in the kidney and in remodeling tissues.

It is a potent plasminogen activator, and is used clinically.

This is a summary of coagulation cascade (enjoy ☺):



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