Sheet No.

5

Biochemistry

hematolymphatic system

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Welcome back warriors! :D

In this lecture we will be talking about blood coagulation from a biochemistry point of view. Focusing on the structure of the coagulation factors, their reactions with each other, and, most importantly, making sense of it all!

Professor Mamoun recommended that you listen to The Four Seasons by Vivaldi while studying this lecture :)

https://www.youtube.com/watch?v=4rgSzQwe5DQ

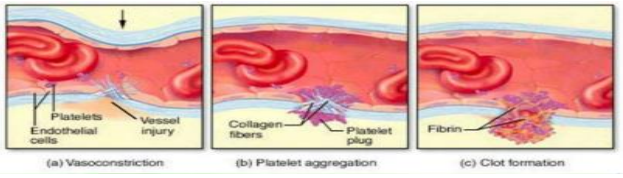
So, let's start simple...

What is blood coagulation?

It is an orchestrated, biochemical process that is, despite its complicity, very beautiful.

- It goes as follows:

1. **Vascular injury.**
2. **Vasoconstriction:** Limiting blood flow to the area of injury.
3. **Platelet plug formation:** Activation then aggregation of platelets at the site of injury forming a loose platelet plug.
4. **Clot formation:** Formation of a fibrin mesh to entrap the plug in place, while also catching RBCs, plasma proteins and other blood components; preventing them from getting out of the circulation.
5. **Dissolution of the clot and repair:** In order for normal blood flow to resume following tissue repair.

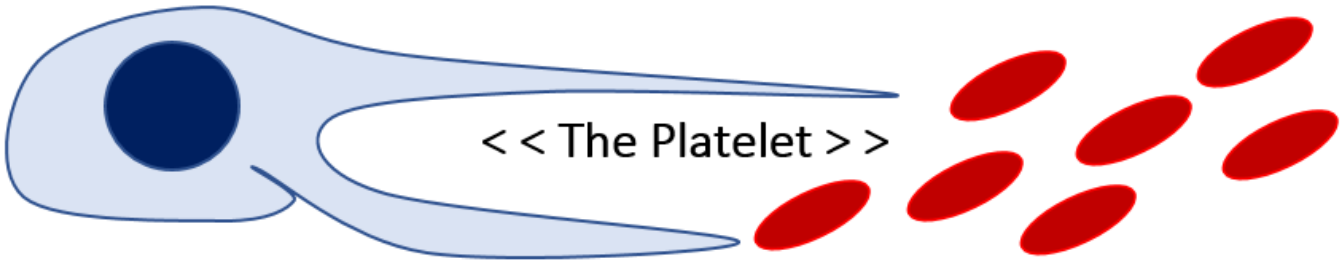


In other words, it is initiated as a result of vascular injury. Where a small amount of blood surrounding injured tissue changes from liquid to gel, forming a clot made out of fibrin, which results in hemostasis followed by clot dissolution and repair.

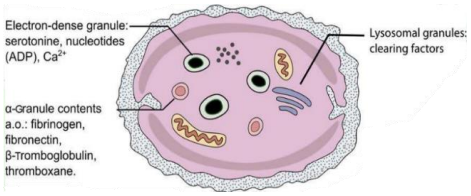
The clot: Is a meshwork of fibrin protein monomers.

Hemostasis: Cessation and preventing blood loss.

It seems that platelets play a major role in blood coagulation.



- Characteristics of platelets:
  - It's a small, anuclear, biconvex cell fragments produced from megakaryocytes.
  - Platelets have numerous surface receptors.
  - Have actin and myosin filaments which change the shape of platelets upon activation and help them secrete granules.
  - Platelets have 3 types of granules released upon activation. (Alpha, Delta, and Lambda as we took in histology)



What are the platelet granules?

Electron-dense granules (delta granules) contains S.A.C:

*Serotonin *ATP

*ADP *Ca^{+2}

Alpha granules (alpha→ strong and has proteins):

*Platelet-derived growth factor (PDGF)

*Fibrinogen *Heparin *Clotting factors

*von Willebrand factor (vWF)

Lysosomal granules (lambda granules) contains:

*Lysozymes.

As mentioned before, the previous granules will be released upon activation. How would that happen?

Adhesion (activation, degranulation) → Aggregation → Coagulation

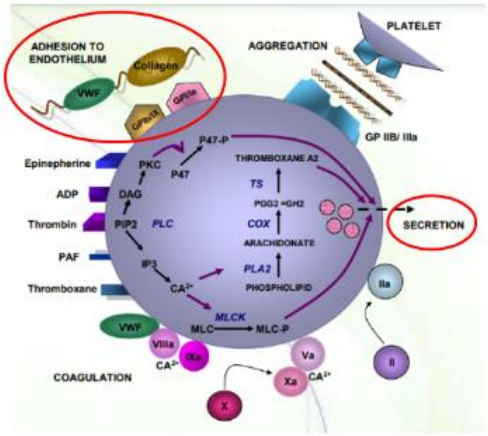
1- Adhesion:

Adhesion occurs when the vWF or the exposed, negatively charged subendothelial collagen (S.E.C) bind to the platelet glycoproteins (GP).

Upon adhesion the platelet gets activated, inducing:

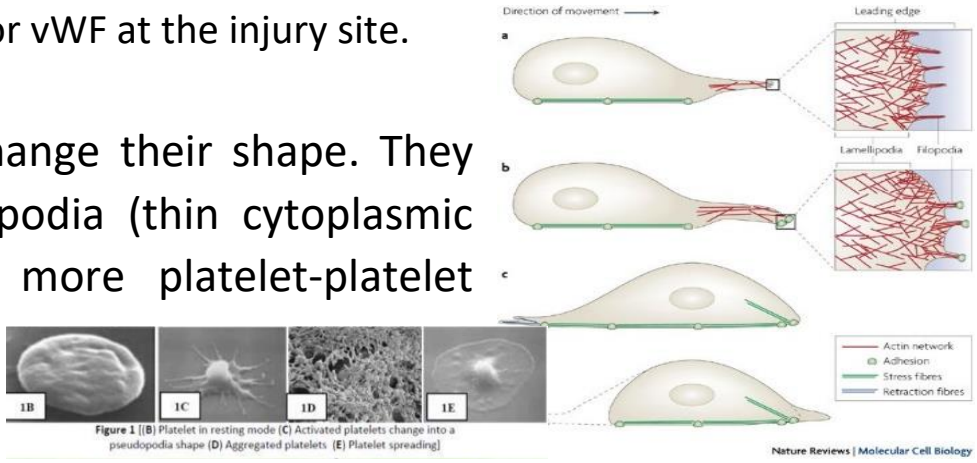
- The release of some substances from the granules, like:

*Serotonin (a vasoconstrictor)
*ADP
*ATP
*Ca^{+2}
*Factor V (proaccelerin)
*Fibrinogen (factor I)
*Thrombin (factor II_a)
*vWF
*Thromboxane A_2 (TXA_2)
*Platelet-derived growth factor (PDGF): Stimulates proliferation of endothelial cells to reduce blood flow.



The release of these substances would result in the activation and aggregation of other platelets that where not in direct contact with the exposed collagen or vWF at the injury site.

- Platelets also change their shape. They give rise to filopodia (thin cytoplasmic extensions) for more platelet-platelet interaction and aggregation.



2- Aggregation:

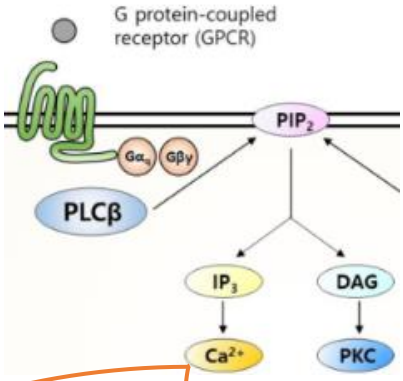
We mentioned that the release of certain granular molecules would cause activation of other platelets. We will go in detail on two of those molecules: Thrombin and ADP.

A-Thrombin: (in alpha granules)

Platelet released thrombin would bind to thrombin receptor (which is a G-protein coupled receptor) that would activate the G- protein. The activated G-protein then goes on to activate phospholipase C-β (PLC-β).

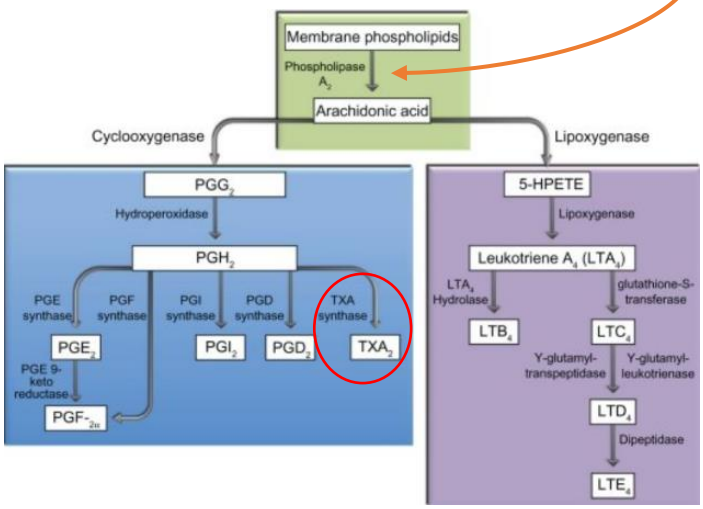
PLC-β hydrolyzes phosphatidylinositol-4,5-bisphosphate (PIP2) into inositol triphosphate (IP3) and diacylglycerol (DAG).

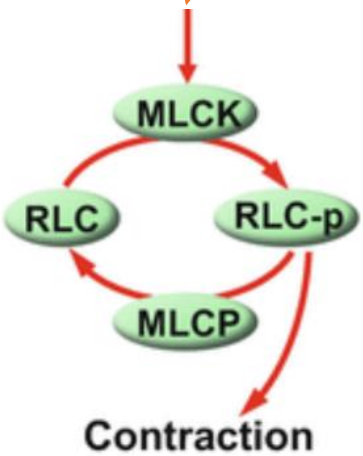
Thrombin→Thrombin receptor→activated G-protein→hydrolysis of PIP2 by PLC-β into→IP3, DAG



-IP3: induces the release of intracellular Ca^{+2}

which in turn:





1- 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 (TXA2).

Features of TXA2:

-It is a vasoconstrictor

-Promotes platelet aggregation

-An inducer of PLC- β

-Acts in an autocrine and paracrine manners (on itself and on neighboring platelets)

⚠ CAUTION

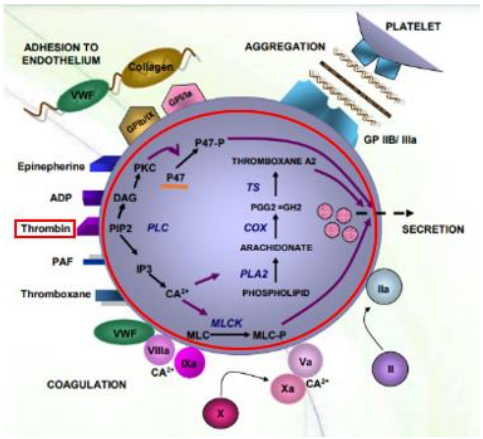
>Side Note<

Non-steroidal anti-inflammatory drugs (NSAIDs) inhibit the cyclooxygenase, accounting for their anticoagulant effects. For example, **Aspirin** irreversibly inhibits cyclooxygenase. Aspirin inhibits cyclooxygenase in the epithelium (mainly produce prostacyclin) as well as ones in platelets (mainly produce thromboxane A2). Platelets cannot regenerate new enzymes. However, epithelial cells can in a few hours. Thus prostacyclin is still being produced while thromboxane A2 is not. Which is especially dangerous in the elderly, because they are prone to injuries such as falling causing internal bleeding. If thromboxane is not present, these injuries could be fatal.

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, induced motility, and release of granules.

-DAG: activates protein kinase C (PKC).

PKC phosphorylates and activates specific platelet proteins such as (P47) that induce the release of platelet granule contents including ADP. What is the role of ADP??



B- ADP: (in delta granules)

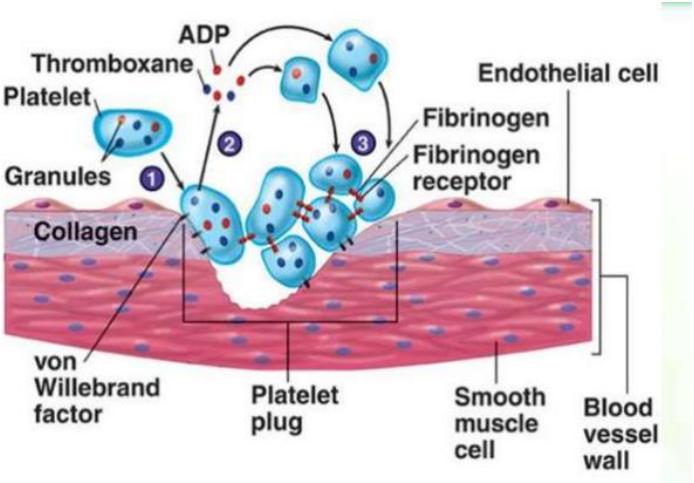
ADP is a platelet activator that binds to its receptor (a G-protein coupled receptor) and modifies the platelet membrane allowing fibrinogen to adhere to platelet surface glycoproteins (GP) resulting in fibrinogen-induced platelet aggregation, called platelet plug.

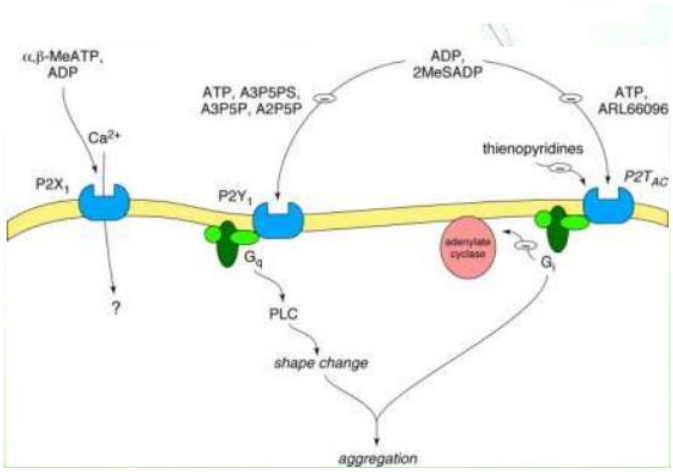
**Additional info:

*The platelet that is involved in platelet adhesion is (GPIb).

*The one that is involved in (platelet-fibrinogen-platelet) interaction (aggregation) is (GPIIb/IIIa).

((The professor didn't mention these but we took them in pathology))

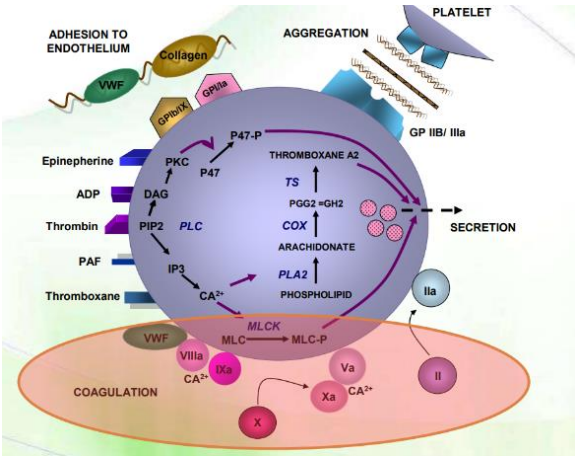




Up to this moment, we have formed the platelet plug that provides an important surface on which the coagulation reaction and clot formation occurs.

3- Coagulation:

The coagulation pathway or cascade has a series of factors, each activating the other by proteolytic cleavage happening on platelet surfaces. We will start this section of the lecture by talking about the components of this pathway abstractly.



➢ Components of coagulation:

-An organizing surface (platelet plasma membrane)

-Proteolytic zymogens (prekallikrein, prothrombin, and factors VII, IX, X, XI, XII, and XIII)

*These are mainly serine proteases released from hepatocytes in the liver.

*The subscript "a" designates the activated form of a factor. (XI_a is the activated form of the coagulation factor XI)

-Anti-coagulants (protein C, protein S)

-Non-enzymatic protein cofactors (factors VIII, V, and tissue factor (factor III))

-Calcium ions (factor IV)

-Vitamin K

-Fibrinogen (factor I)

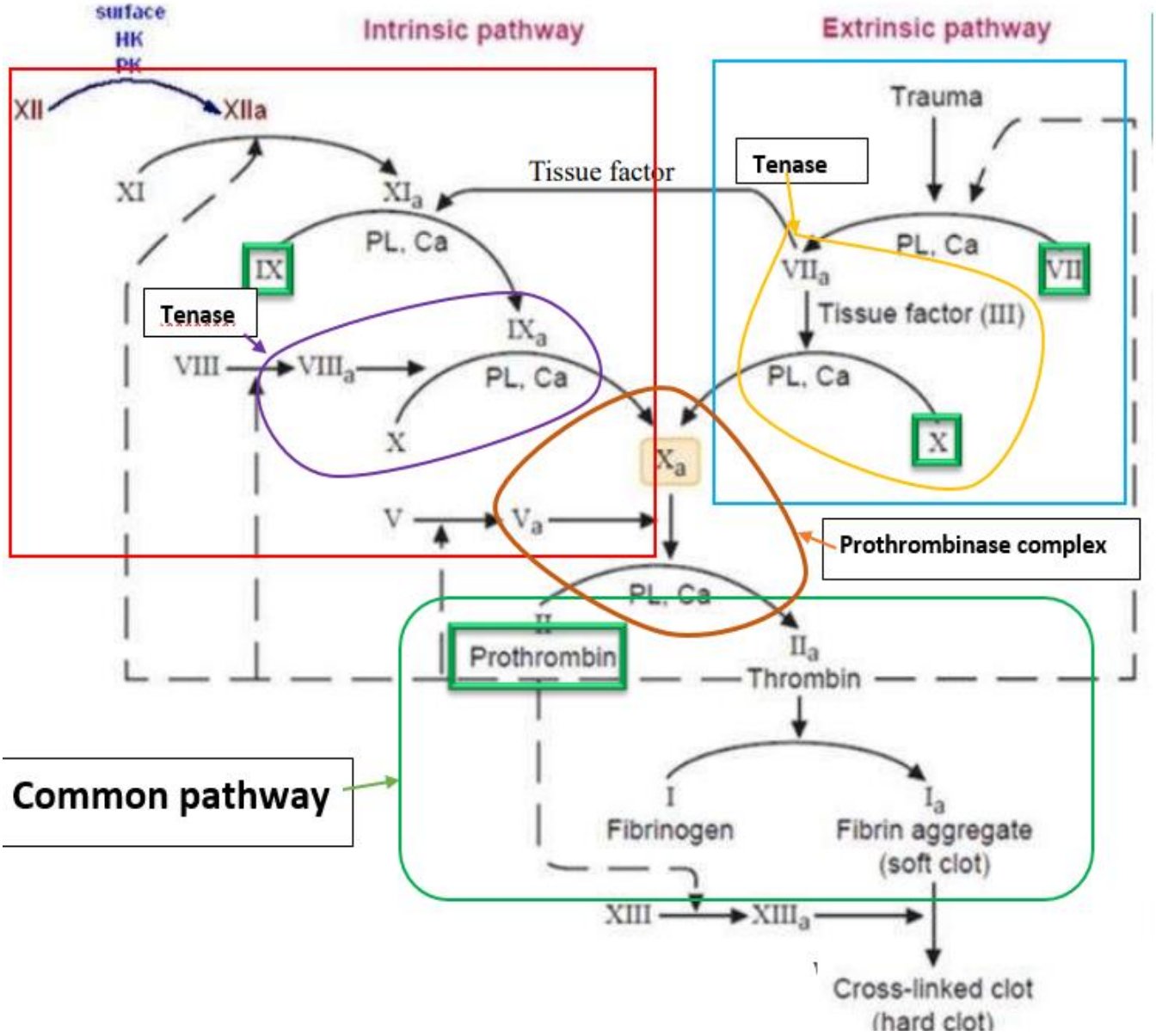
Zymogen: Is an inactive form of an enzyme that needs cleavage to get activated.

*There are different types of proteases depending on the amino acid that is found in its active site.

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

(The previous table is required, however the professor said to not bother with the MW)



There are two main "pathways" for the coagulation cascade:

1- The intrinsic pathway: is initiated when the negatively charged sub endothelial collagen gets exposed.
2- The extrinsic pathway: is initiated in response to tissue injury. (injured tissue release tissue factor(factor III))

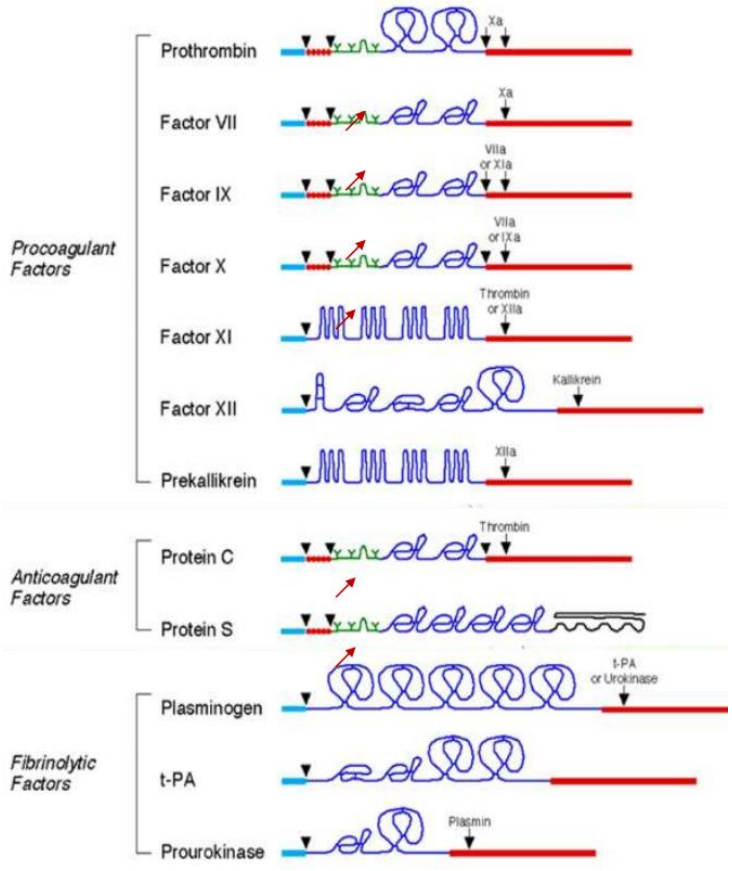
The two pathways then converge on a common pathway.

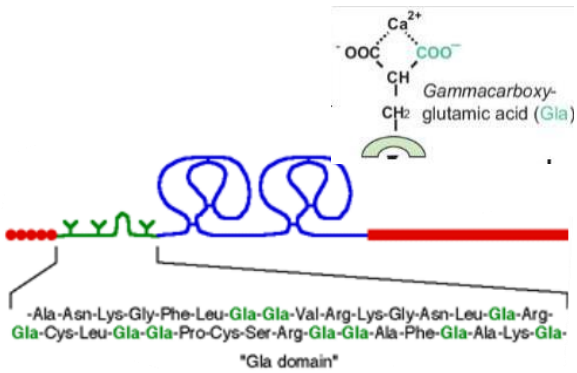
We will go in slight detail here discussing a domain called (The Gla domain), and the importance of vitamin K. This part will help you make sense of things we took in physiology like the importance of Ca^{+2} and vitamin K dependent factors. It might seem a little complicated but it is really not. + I believe in you :)

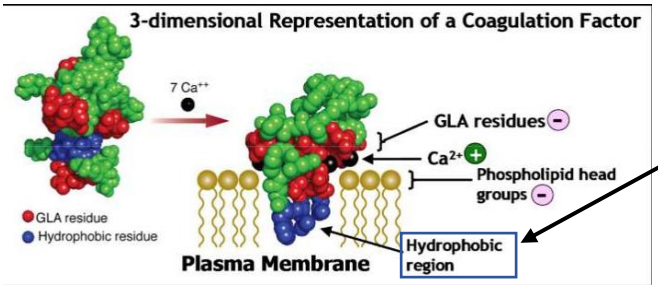
Gla domain:

An ER/Golgi carboxylase binds to prothrombin and factors IX, VII, and X and converts more or equal to 10 glutamate (Glu) residues to γ-carboxyglutamate (Gla) (((in a reaction that is mediated by vitamin K))) this will make it able to bind Ca^{+2} which is necessary for the activity of these coagulation factors and formation of a coordinated complex with the negatively charged plasma membrane that of the platelet's surface to localize the complex assembly and thrombin formation to the platelet surface.

The Gla residues is followed by a small (10 AA) hydrophobic region which will make the complex anchored into the plasma membrane.





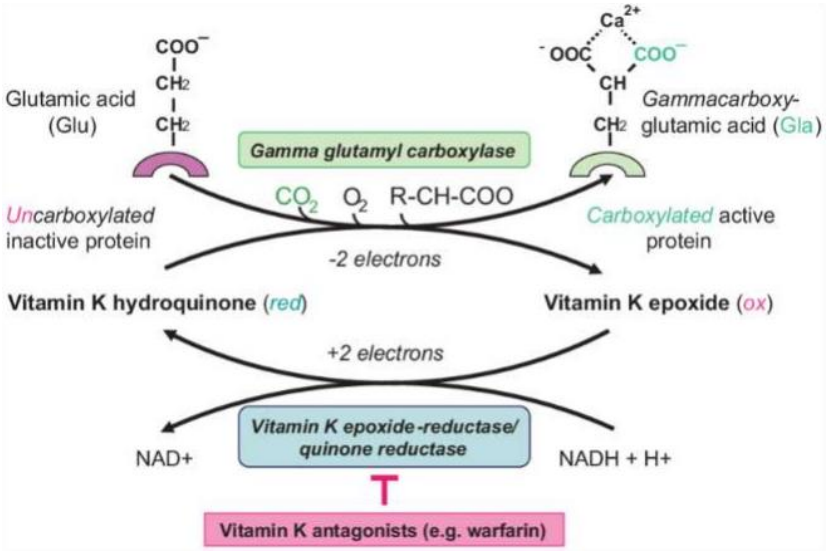


Hydrophobic region after Gla domain.

These factors are complexed together on the surface of platelets to speed up the reaction between them. Having them in proximity is beneficial for the cascade propagation.

➢ The role of Vitamin K

Vitamin K participates in conversion of Glu to γ-Gla in a reaction that is catalyzed by Gamma glutamyl carboxylase.



Vitamin K becomes oxidized and must be regenerated using an e^- from NADH in a reaction that is catalyzed by vitamin K epoxide-reductase which is inhibited by warfarin.

Notes:

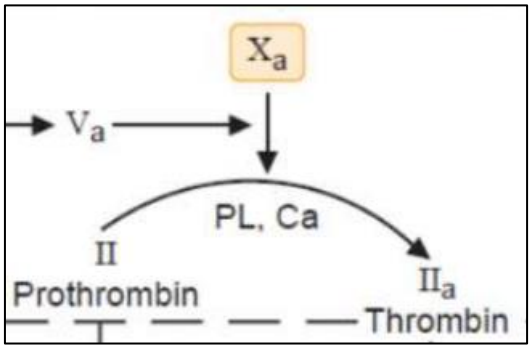
- Warfarin is used clinically as an anticoagulant and it has different effects on different patients due to the different genetic makeup of cyt P450 therefore it could cause excessive bleeding in some patients.
- The vitamin K related coagulation factors are the ones having Gla domains such as (II, VII, IX, and X).

Newborns are at risk for early vitamin K deficiency bleeding. **Why?**

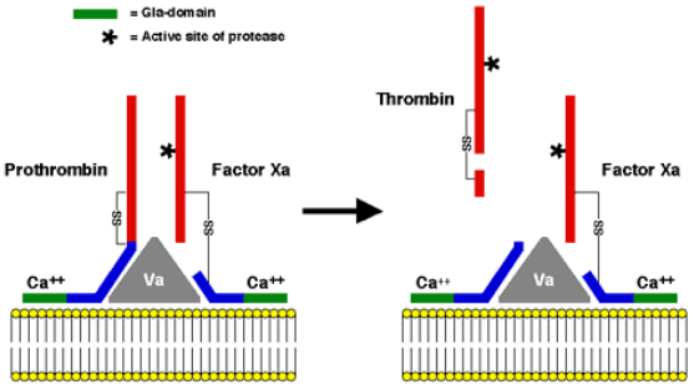
- The placenta is a poor passage channel for fat-soluble compounds, including vitamin K.
- Neonates are born with an immature liver that impairs coagulation factor synthesis and GLA modifications.
- Breast milk is a poor source of vitamin K.
- Intestinal flora, the main source of vitamin K, is not established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

So now that we got those points out of the way, let's get back to the pathway!

Prothrombin activation

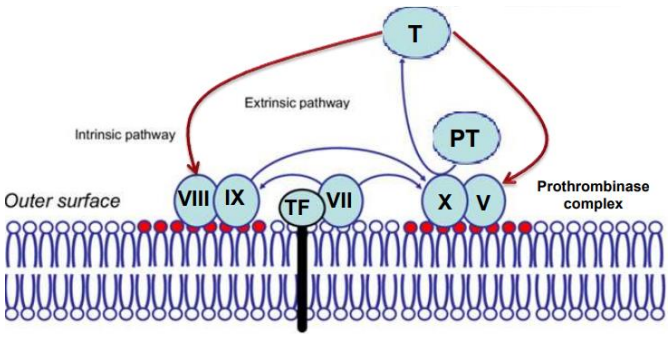


The complex of factor $\underline{X_a/V_a/Ca^{+2}}$ on the surface of plasma membrane is the "**prothrombinase complex**". Factor X_a is the major one here, it converts prothrombin (II) to thrombin (II_a), which is accelerated by V_a (proaccelerin), platelets plasma membranes (or phospholipids), and 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 (platelet plug) provide the surface upon which prothrombin activation occurs.



Factor X activation

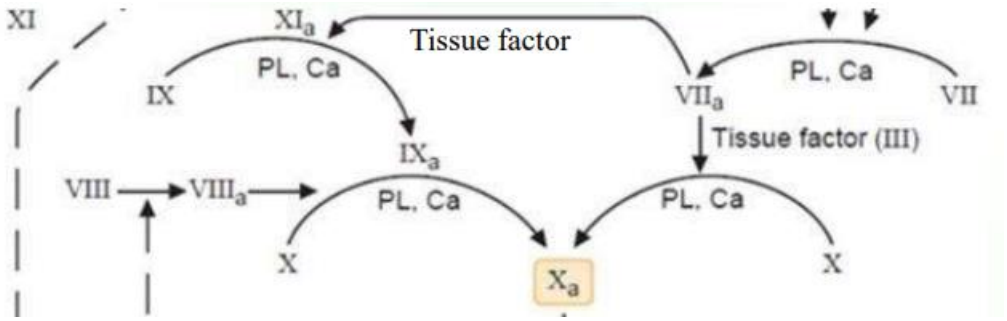
Factor X is the key to activate reach the common pathway from the intrinsic or the extrinsic pathway. Both path ways has its own complex to activate factor X and it's called **the tenase complex**.



The extrinsic tenase complex is made up of tissue factor (III), factor VII_a, and Ca^{+2}. **The intrinsic tenase complex** contains the active factor IX (IX_a), its cofactor factor VIII ($VIII_a$), and Ca^{+2}.

Intrinsic, extrinsic interaction

There is an interaction between the intrinsic and extrinsic pathways, where tissue factor (III) and factor VII_a also activate factor IX in the intrinsic pathway.

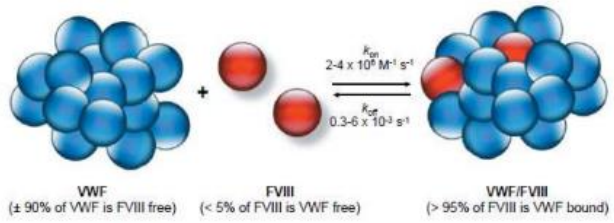


V_a and $VIII_a$ are cofactors that increase the proteolytic efficiency of X_a and IX_a, respectively. Both factors V and VIII are activated by thrombin via a feedback mechanism.

vWF

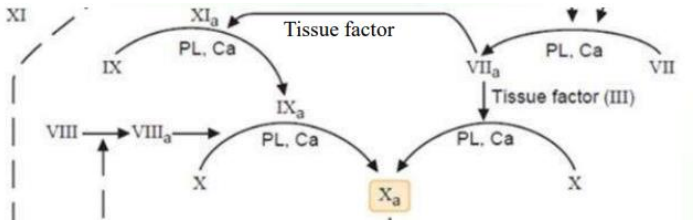
Factor VIII circulates in plasma bound to von Willebrand factor, which increases VIII half-life, and, when released, it gets activated.

von Willebrand factor deficiency is associated decrease in the plasma concentration of factor VIII.



Factor III

Tissue factor (III) 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 VII_a.

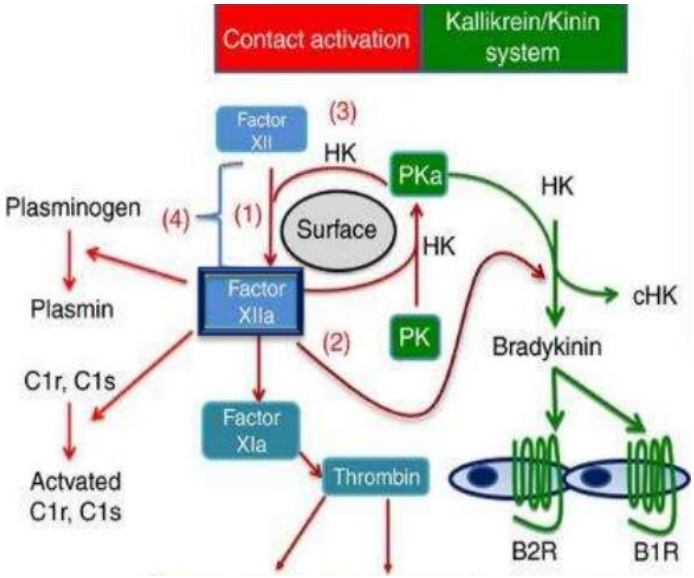


Exposure of tissue factor initiates the coagulation cascade. **TF/VIIa complex is the "initiation complex"** as factor III and VII can also activate the intrinsic pathway as well.

Intrinsic pathway initiation

Prekallikrein, HMWK (high molecular weight kininogen), factors XII and XI are exposed to a negatively charged activating surface (S.E.C).

Factor XII is auto activated to XII_a. It has its active site blocked by one of its chains. The factor, upon interacting with the S.E.C, autocleaves its own part, freeing the active site of the enzyme thus autoactivating itself.



>> Factor XII has several substrates:

1. **Kallikrein from prekallikrein.** Kallikrein activates plasminogen into plasmin which has a fibrinolytic activity.
   ("removing the clot" (fibrinolysis) pathway is initiated at the beginning of the "forming the clot" (coagulation) pathway).
2. **Factor XI**, which activates factor IX. (continuing the intrinsic pathway)
3. **HMW kininogen** releasing bradykinin by the action of kallikrein which was activated in the first place by HMWK. (Bradykinin is a peptide with potent vasodilator action).
4. **Other substrates:** plasminogen (fibrinolysis) and complement system proteins (for pathogen elimination that entered the blood by the injury that caused all of this mesmerizingly beautiful mess).

To summarize:

- Factor XII needs HMWK to be autoactivated.
- HMWK also activates prekallikrein to kallikrein.
- Prekallikrein accelerates the activation of factor XII.
- Kallikrein has two main actions:
  1- Activates plasminogen into plasmin to lyse the clot
  2- Converts HMWK into bradykinin which a vasodilator to antagonize the clotting.

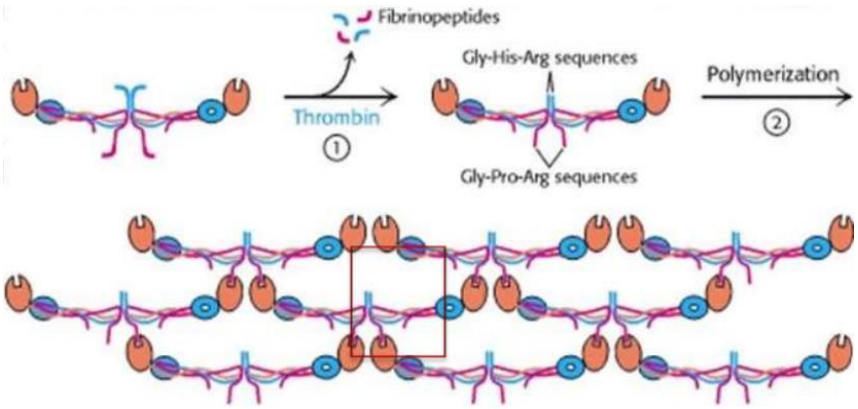
Formation of the fibrin clot:

Fibrinogen is composed of two triple stranded helical protein held together by disulfide bonds.

It is cleaved by thrombin releasing fibrinopeptides as by products and fibrin molecules that interact with each other by electrostatic

attractions (non-covalent) between the central domains and the end domains facilitating the aggregation of the monomers into a gel consisting of long polymers.

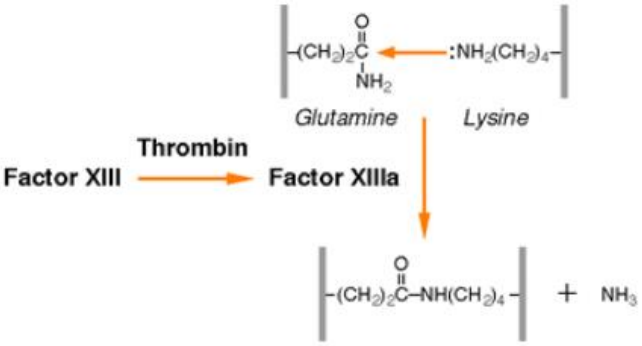
The clot resulting from aggregation of fibrin monomers is referred to as the "soft clot". Which is stabilized by factor XIII (fibrin stabilizing factor).



Factor XIII

Factor XIII is a transglutaminase that is activated by thrombin.

Factor $XIII_a$ 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.

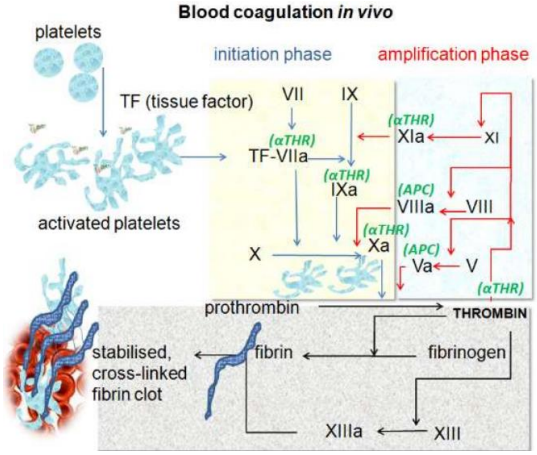


It also cross-links the fibrin clot to adhesive proteins on the endothelial tissue and to the platelet surfaces strengthening the platelet plug.

The cross-links strengthens the fibrin mass, forming the "hard clot".

Amplification of the pathway:

The sequential enzymatic activation allows for amplification. One activated zymogen cleaves and activates multiple factors not only one.



Amplification also results from positive feedback reactions as thrombin exhibits. Thrombin activates factors V, VII, VIII, and XI.

It was a tough one. But it's done. Try to sketch the pathway yourself it will make all of this a lot easier! Believe me on that one.

Good job and good luck!!