Mechanisms of Hemostasis

APPROVED

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she is a clinical pathologist - she does blood banking/ laboratory hematology, in case you were wondering

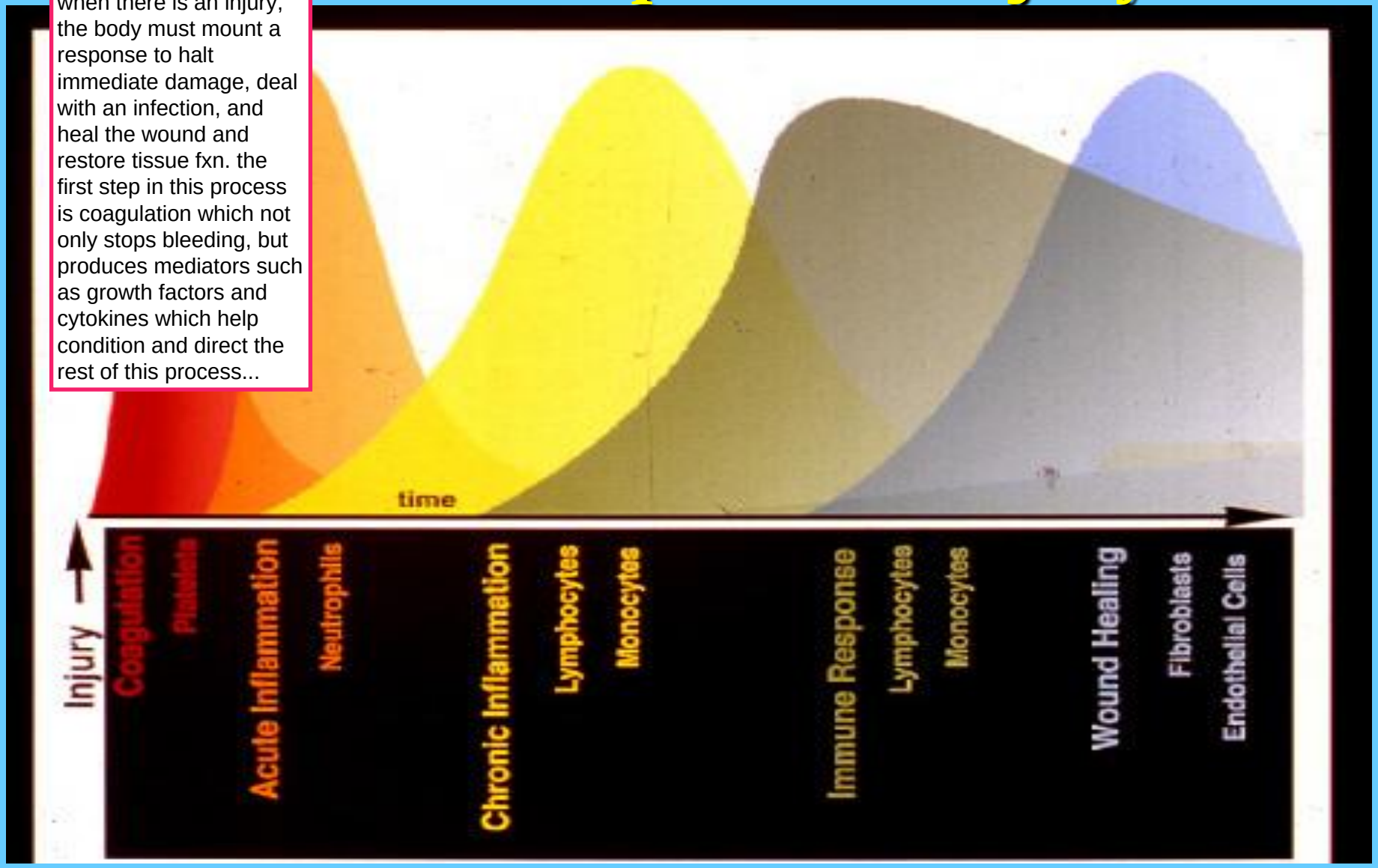
Objectives

- Understand how hemostasis relates to the body's response to tissue injury
- Differentiate the newer cell-mediated model from the classic cascade model
- Describe the basic coagulation tests and how they relate to the clotting cascade

we will talk about the coagulation cascade and compare and contrast this with newer cellular models of how coagulation works in the body.
we will also highlight some defects/ things that can go wrong in hemostasis.

Coagulation:
Host Response to Injury

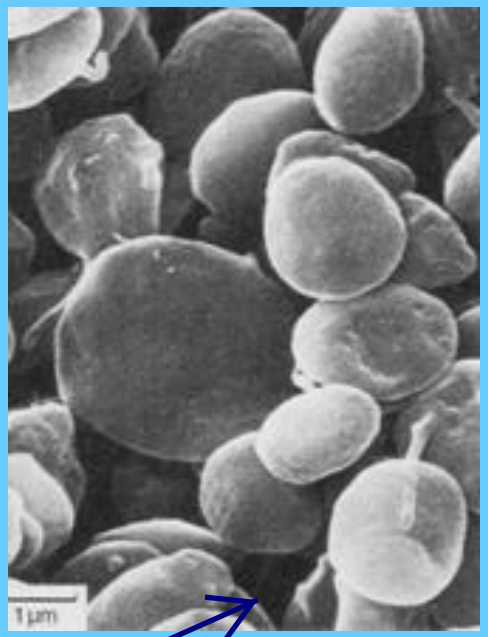
when there is an injury, the body must mount a response to halt immediate damage, deal with an infection, and heal the wound and restore tissue fxn. the first step in this process is coagulation which not only stops bleeding, but produces mediators such as growth factors and cytokines which help condition and direct the rest of this process...



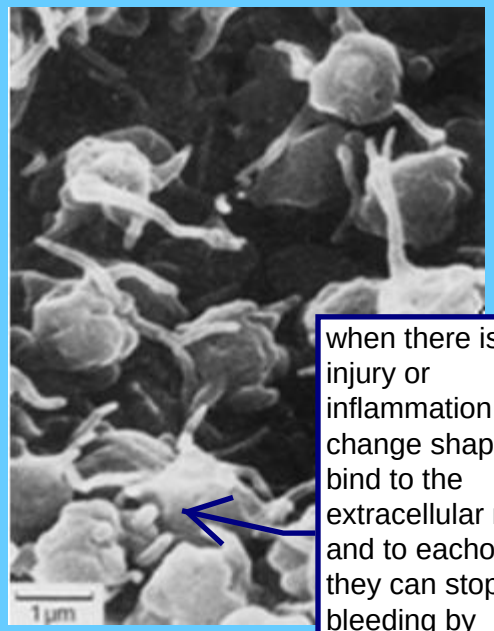
Primary Hemostasis

first step in hemostasis involves platelets

Platelets Adhere & Activate at Sites of Injury



platelets are anuclete fragments of cells that circulate in the blood and are normally disc shaped. in this form they are not responsive and not sticky.

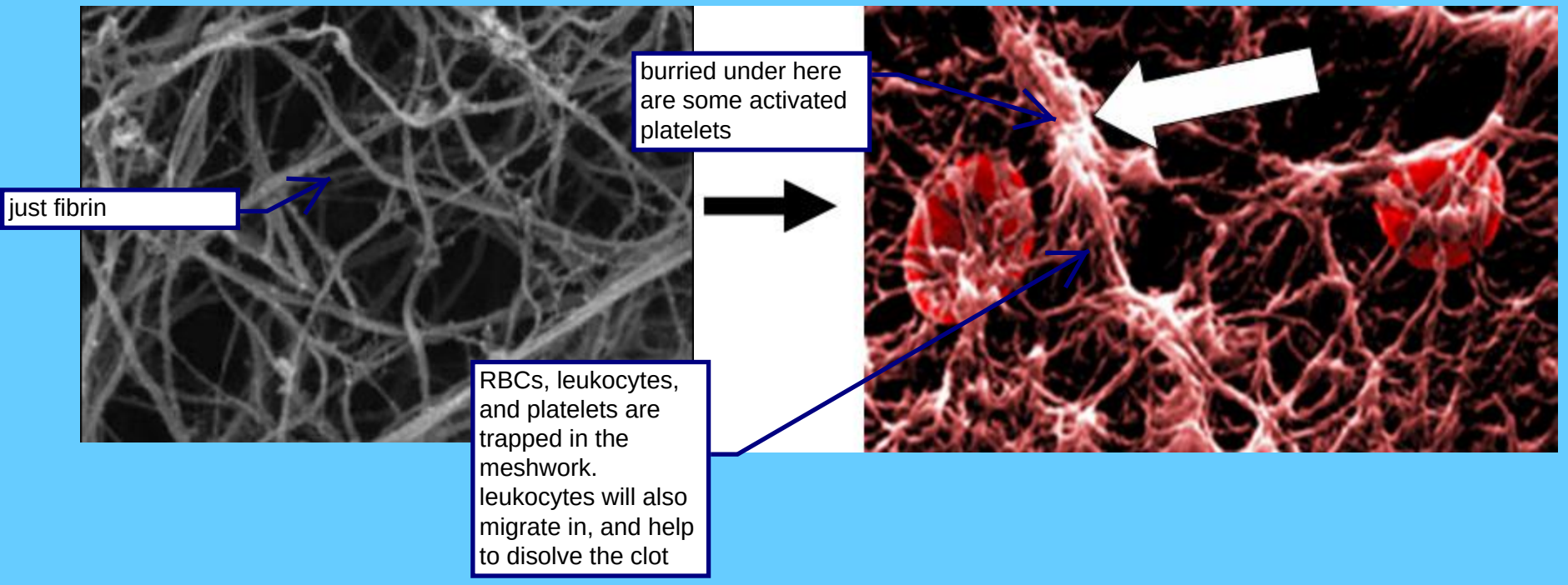


when there is an injury or inflammation, they change shape and bind to the extracellular matrix and to eachother. they can stop bleeding by themselves, and they can express lipids on their surface upon activation that provide a good site for the coagulation reactions to take place

Secondary Hemostasis

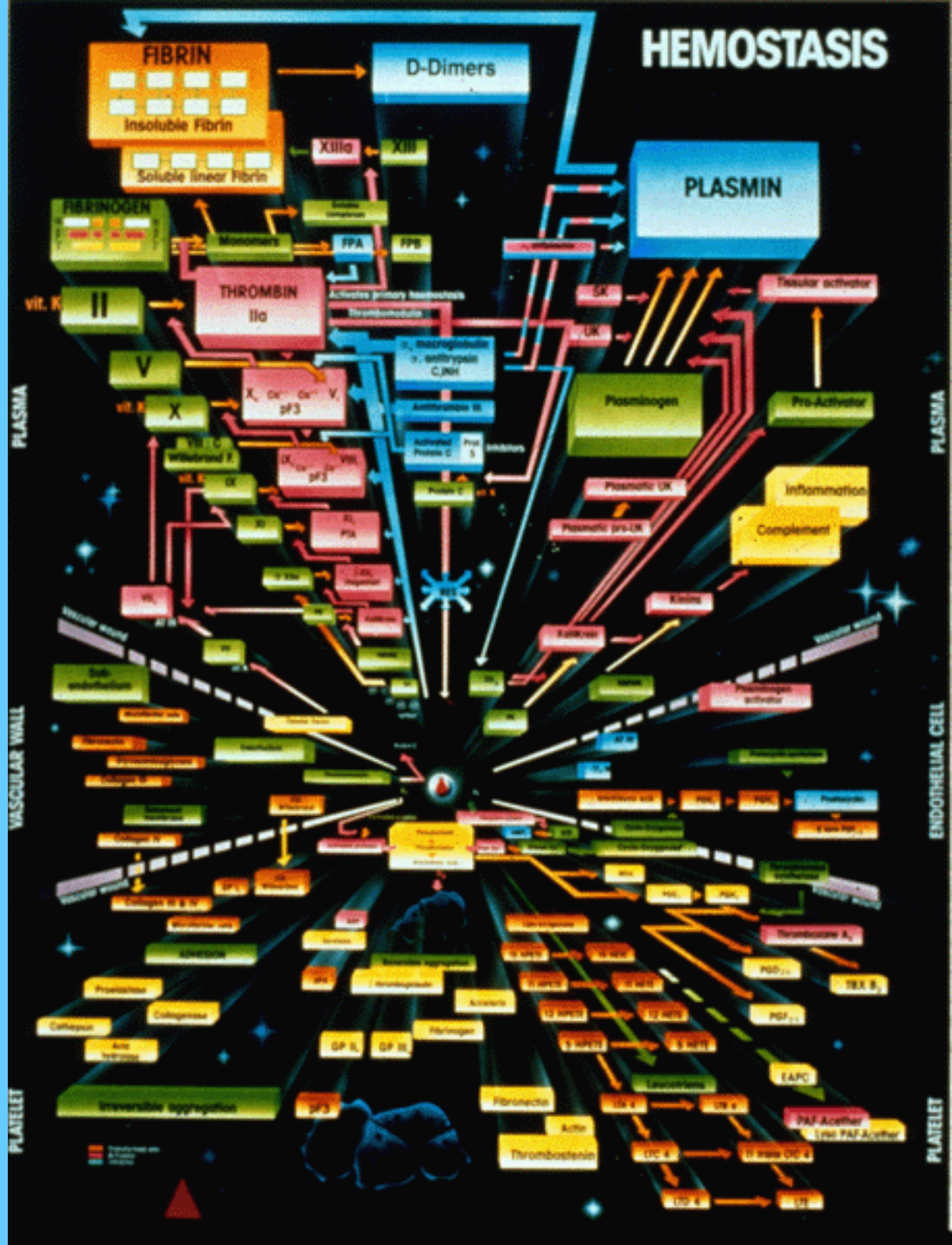
point of secondary hemostasis is to consolidate platelet plug in a fibrin meshwork

Coagulation proteins act on platelet surfaces to form fibrin, which stabilizes the platelet plug

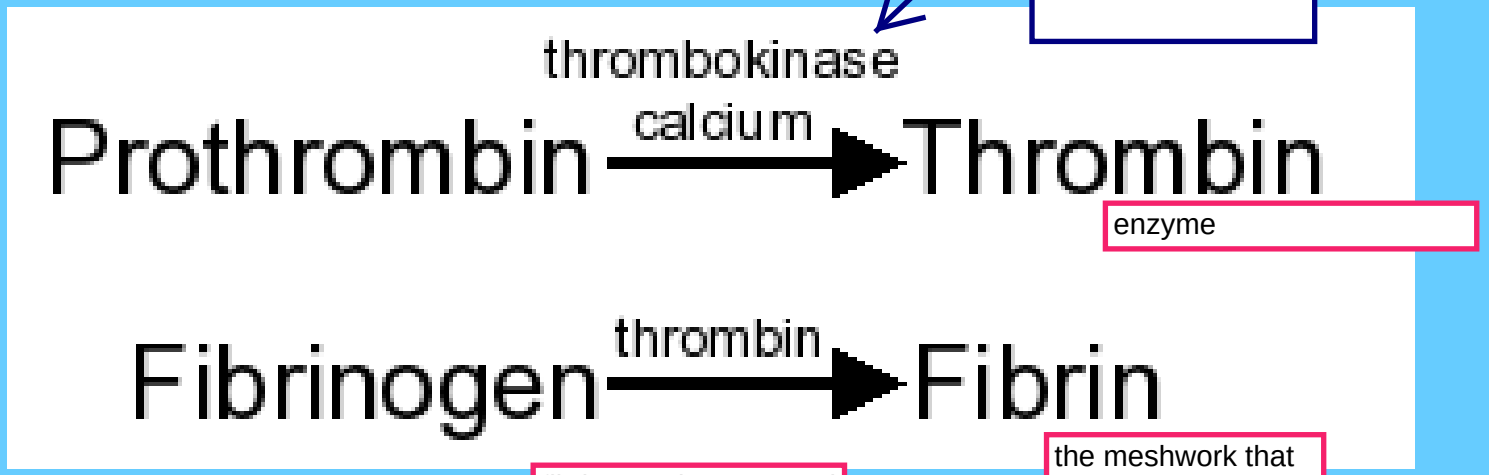


How can we make sense of hemostasis?

its complicated, but we will try to highlight key features that will help us make sense of things that happen in our pts



In 1904 Paul Morawitz proposed a model of coagulation



Morawitz, P. Beiträge zur Kenntniss der Blutgerinnung Dtsch Arch klin Med 1904;79:1-28

More and more factors were discovered and named different things, and it all went down hill from there…..

hemostasis was well studied because of hemophelia in royal families

fibrinogen
prothrombin
accelerator (AC-) globulin
Antihemophiliac Factor
Antihemophilic Factor B
Antihemophilic Globulin (AHG)
Antihemophilic Globulin A
Autoprothrombin I
Autoprothrombin II
Autoprothrombin III
Beta cothromboplastin
Christmas Factor
Contact Factor
Cothromboplastin
Facteur Antihemophilique A
Fibrin Stabilizing Factor
Thromboplastic Plasma Component
Thromboplastinogen
Hageman Factor
Hemophilia A factor
Hemophilia B Factor
Hemophilia C factor
Labile Factor
Laki-Lorand Factor
Pavlovsky Factor
Plasma Thromboplastic Factor
Plasma Thromboplastic Factor A
Plasma Thromboplastin Antecedent (PTA)
Plasma Thromboplastin Component
Plasmakinin
Platelet Cofactor
Proaccelerin
Proconvertin
Prothrombokinase
Protransglutamidase
Prower Factor
Robbins Factor
Serum Factor
Serum Prothrombin Conversion Accelerator (SPCA)
Stable Factor
Stuart Factor
Stuart-Prower Factor
Thrombokatalysin

In 1958 the International Society on Thrombosis and Hemostasis convened a conference to standardize the nomenclature

That's how we got all those roman numerals

at this point though, we still didn't know how it worked - this is why the roman numerals are not in the right order

something in the tissue outside the blood that promotes clotting - we don't really call it factor III anymore. we know now that it is a specific protein called **tissue factor**

agulation Proteins

Factor	Synonyms	Function
I	Fibrinogen	polymer unit
II	Prothrombin	protease
III	Tissue thromboplastin, tissue factor	cofactor
IV	Calcium	
V	Accelerator globulin, proaccelerin, labile factor	cofactor
VII	Proconvertin, stable factor	protease
VIII	Antihemophilic factor or globulin	cofactor

we don't call Calcium factor IV either...

released from platelets in a partially active form, which was known as factor VI, but we don't call it that

Factor	Synonyms	Function
IX	Christmas factor, plasma thromboplastin component	protease
X	Stuart factor, Stuart-Prower factor	protease
XI	Plasma thromboplastin antecedent	protease
XII	Hageman factor	protease
XIII	Fibrin stabilizing factor, fibrinoligase	Fibrin crosslinker
----	Prekallikrein (Fletcher factor)	protease
----	High-molecular-weight kininogen (Fitzgerald factor)	cofactor

unless otherwise specified, we still use the factor name and number to identify parts of the cascade. :)

Factor VI was at one time used to designate activated Factor V.

…… but nobody really knew how all those factors interacted to turn liquid plasma into a solid fibrin clot

That's why the roman numerals aren't in order in the coagulation cascade - thus making it is hard for us to remember

In the 1960's the coagulation factors were organized into a "cascade" or "waterfall" model. This evolved into the current cascade model …

the idea of a sequence of proteases, acting as a biological amplifier (apoptosis and complement also work this way - but the coagulation cascade was described first - this was a groundbreaking idea)

1. Macfarlane RG. An enzyme cascade in the blood clotting mechanism, and its function as a biological amplifier. *Nature*. 1964;202:498-499.
2. Davie EW, Ratnoff OD. Waterfall sequence for intrinsic blood clotting. *Science*. 1964;145:1310-1312

Eventually the many coagulation factors were organized into a cascade model…

originally they thought it looked like this - only one pathway - but this isn't right

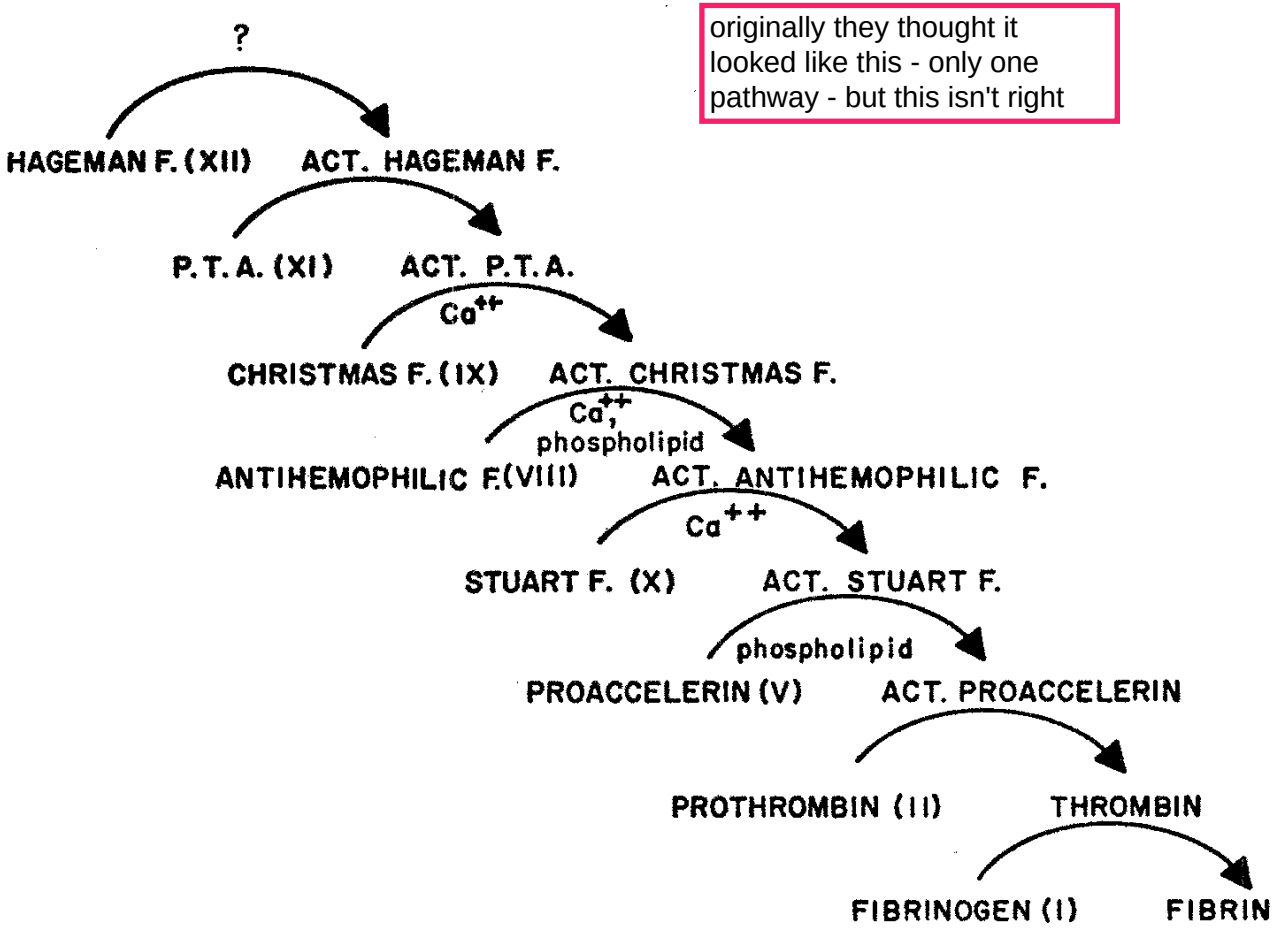


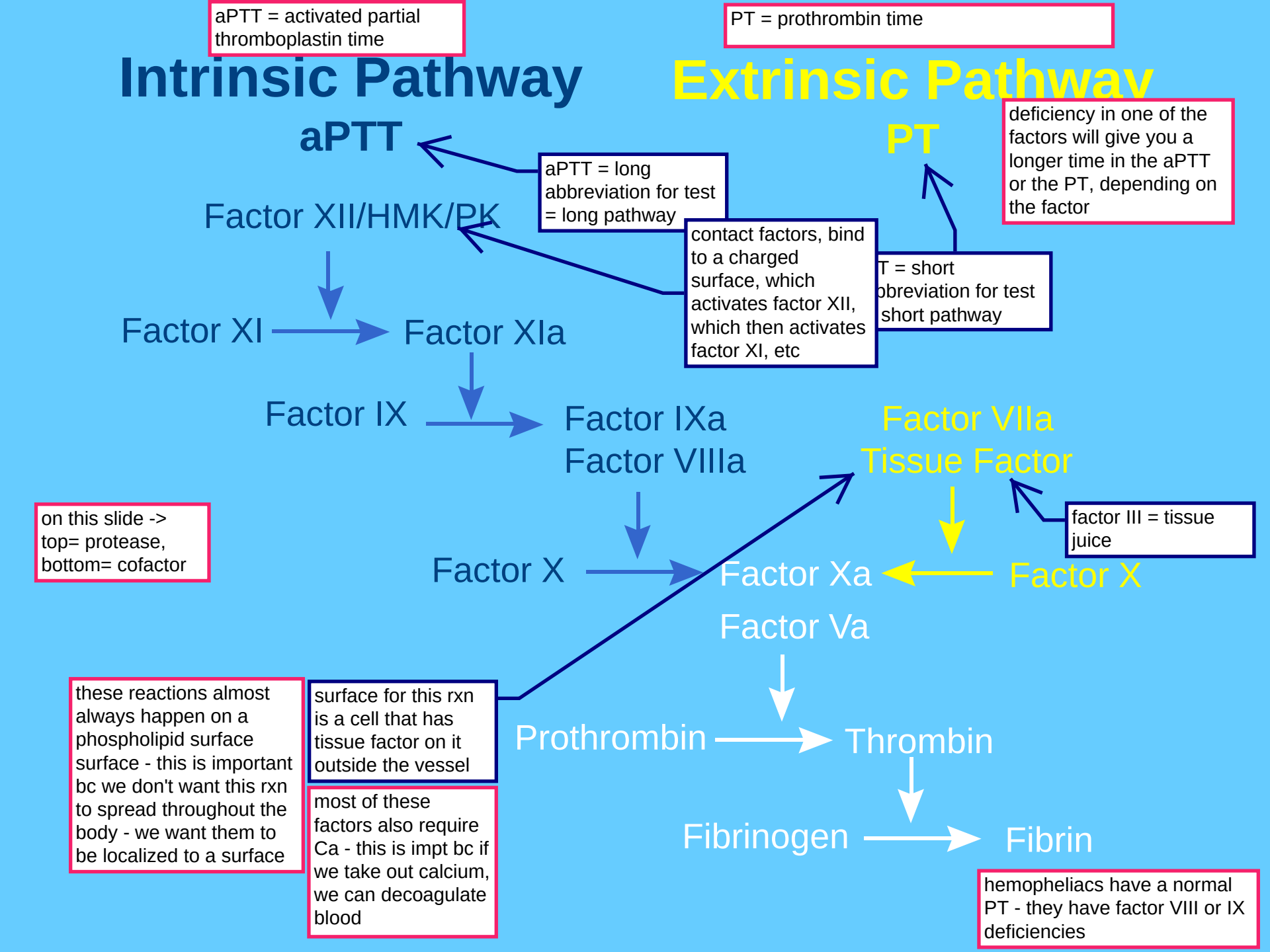
Fig. 1. Tentative mechanisms for the initiation of blood clotting in mammalian plasma in the intrinsic system. Abbreviations: F., factor; Act., activated; P.T.A., plasma thromboplastin antecedent. The term "Act. Proaccelerin" is probably a misnomer but was used in this figure instead of accelerin or prothrombin converting principle. Accelerin refers to a thrombin-modified form of proaccelerin; prothrombin converting principle, a term we have used elsewhere, does not identify the precursor of this enzyme. Hageman factor, Christmas factor, and Stuart factor are clotting factors named after the patients who were among the first observed in which the clotting deficiency was seen. This scheme does not represent all views held on the mechanism of blood coagulation (*32*).

Landmark description of coagulation as a biological amplifier

Earl W. Davie
Oscar D. Ratnoff

Science 1964; 145:1310-1312

The “cascade” model evolved into what my generation of medical students was taught ….


aPTT = activated partial thromboplastin time
PT = prothrombin time
Intrinsic Pathway
Extrinsic Pathway
aPTT
PT
deficiency in one of the factors will give you a longer time in the aPTT or the PT, depending on the factor
aPTT = long abbreviation for test = long pathway
Factor XII/HMK/PK
contact factors, bind to a charged surface, which activates factor XII, which then activates factor XI, etc
T = short obreviation for test short pathway
Factor XI
Factor XIa
Factor IX
Factor IXa
Factor VIIIa
Factor VIIa
Tissue Factor
factor III = tissue juice
on this slide -> top= protease, bottom= cofactor
Factor X
Factor Xa
Factor X
Factor Va
these reactions almost always happen on a phospholipid surface surface - this is important bc we don't want this rxn to spread throughout the body - we want them to be localized to a surface
surface for this rxn is a cell that has tissue factor on it outside the vessel
Prothrombin
Thrombin
most of these factors also require Ca - this is impt bc if we take out calcium, we can decoagulate blood
Fibrinogen
Fibrin
hemopheliacs have a normal PT - they have factor VIII or IX deficiencies

she basically read this slide:

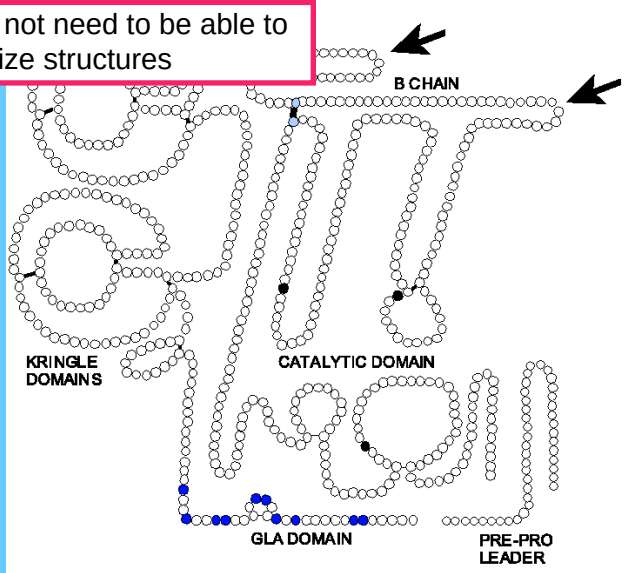
Homologous Coagulation Factors

- Vitamin K-Dependent Serine Proteases:
  - Factors II, VII, IX & X
  - Structurally similar
  - Circulate as inactive zymogens
  - Activated by proteolysis
  - Work best in complex with a protein cofactor on lipid surface containing phosphatidyl serine
  - Activity is calcium dependent

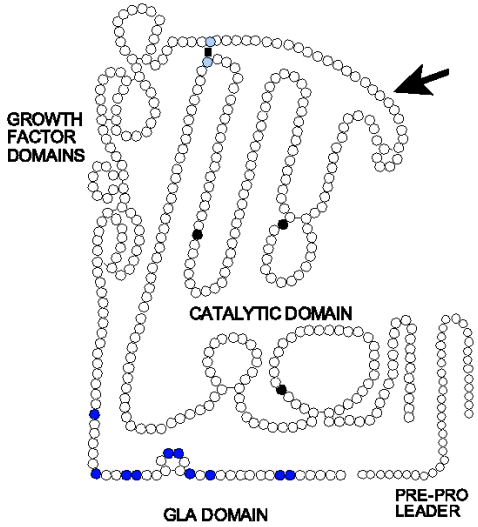
these guys are all very closely related- probably arose by gene duplication

prothrombin

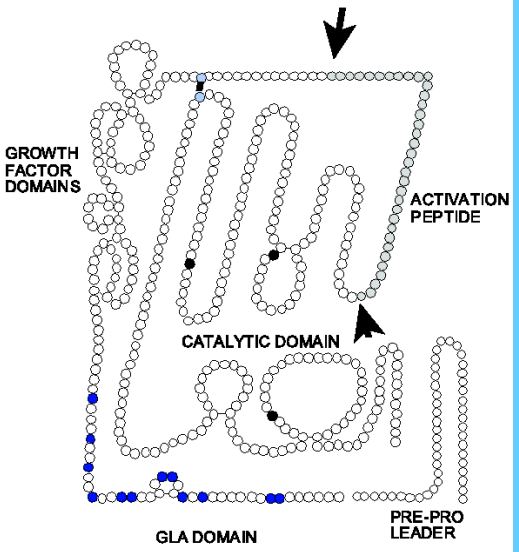
you do not need to be able to recognize structures



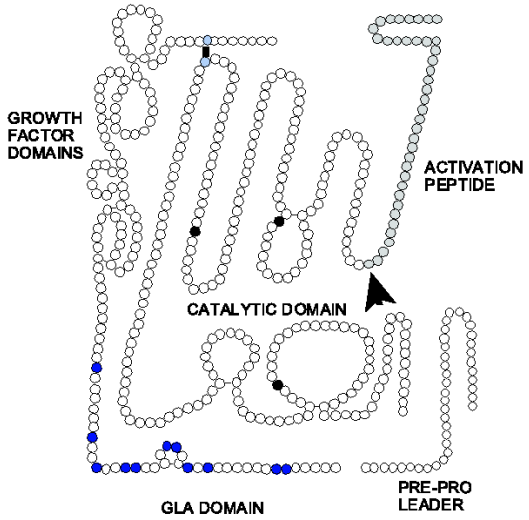
Prothrombin



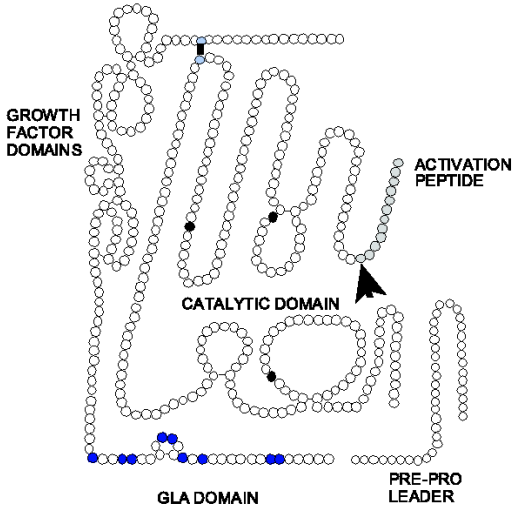
Factor VII



Factor IX



Factor X



Protein C

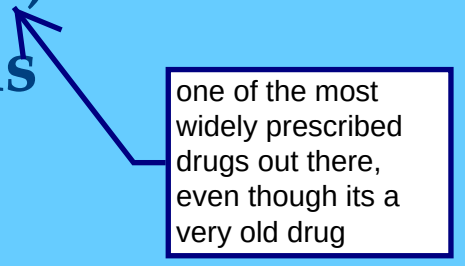
some are activated by one cleavage, others are activated by two cleavages - these cleavages allow them to rearrange into an active conformation

Roberts, Monroe & Hoffman: Molecular Biology and Biochemistry of the Coagulation Factors and Pathways of Blood Coagulation. In William's Hematology 7th ed, 2005

Why should I care about the biochemistry of coag factors?

Why should I care about the biochemistry of coag factors?

- It helps explain some things that are very useful
  - How does Coumadin (Warfarin) work?
  - How do calcium chelators act as anticoagulants?



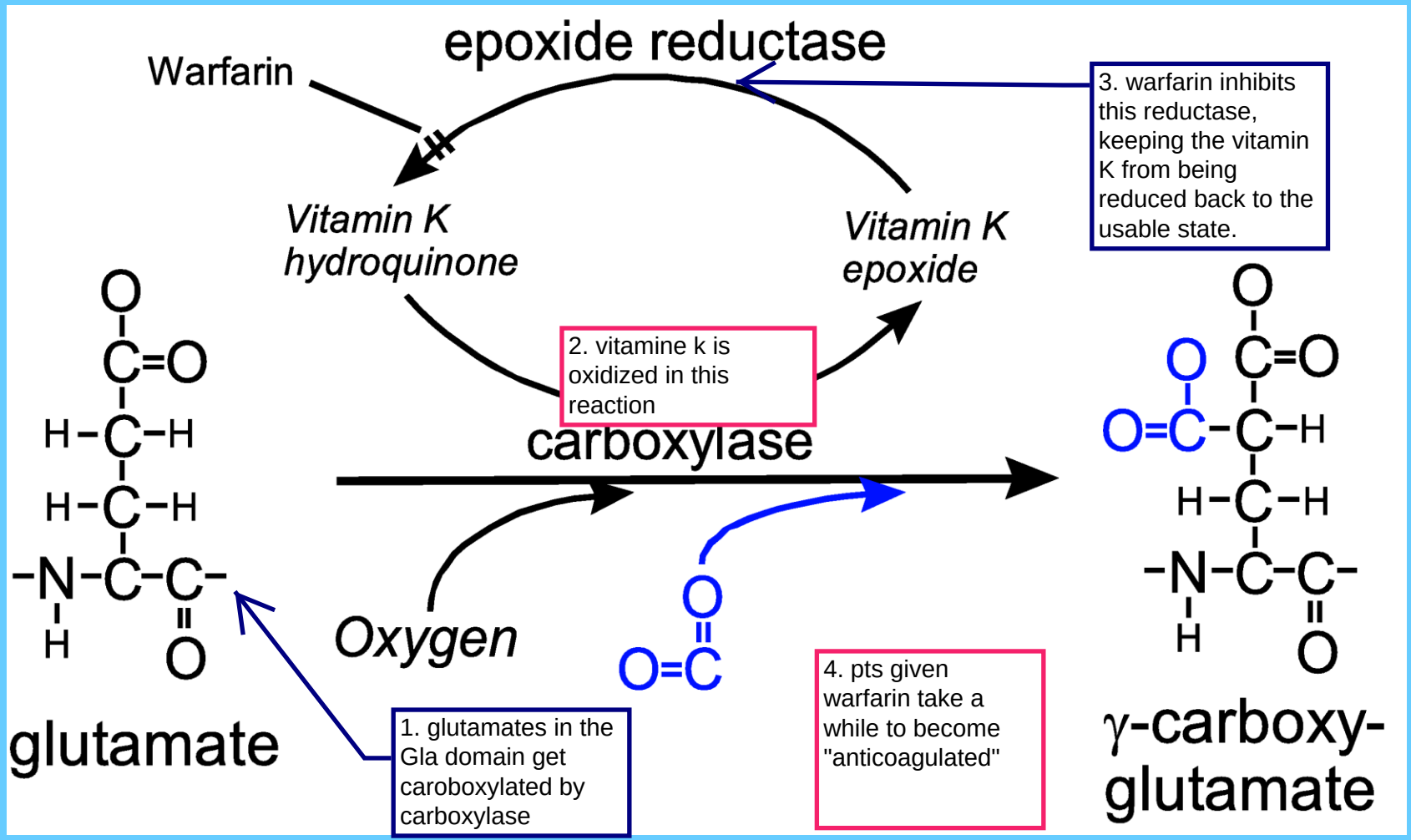
Things that are necessary for coagulation proteases to work

- Post-translational modification to produce gamma-carboxy glutamic acid (Gla) residues, which is vitamin K – dependent
- Calcium to bind to Gla's and hold the protein in the right conformation
- Phospholipid surface for the proteases to bind to along with their cofactors

which gives them a negative charge

active

Vitamin K-dependent factors contain Gla-residues



Warfarin: Commonly Used Oral Anti-Coagulant

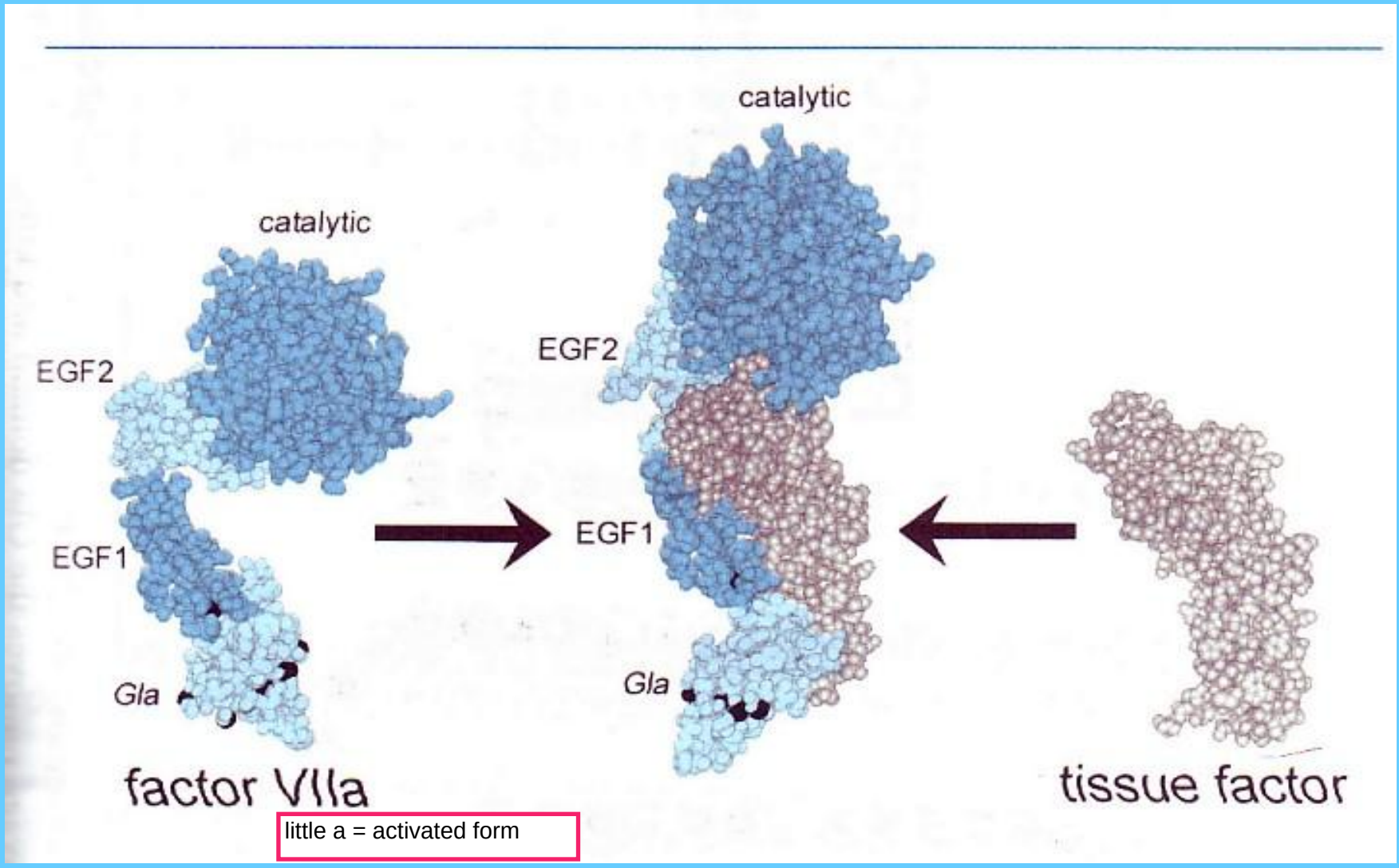
- Warfarin alters synthesis of vitamin K-dependent factors by preventing vitamin K-dependent carboxylation of
  - Factors II, VII, IX, X
  - Protein C & Protein S
- **Result**: no longer bind calcium
- New proteins must be synthesized to overcome the warfarin effect

these proteins are actually anticoagulants and also get messed up by warfarin

so - they aren't in the right conformation and they can't bind phospholipids

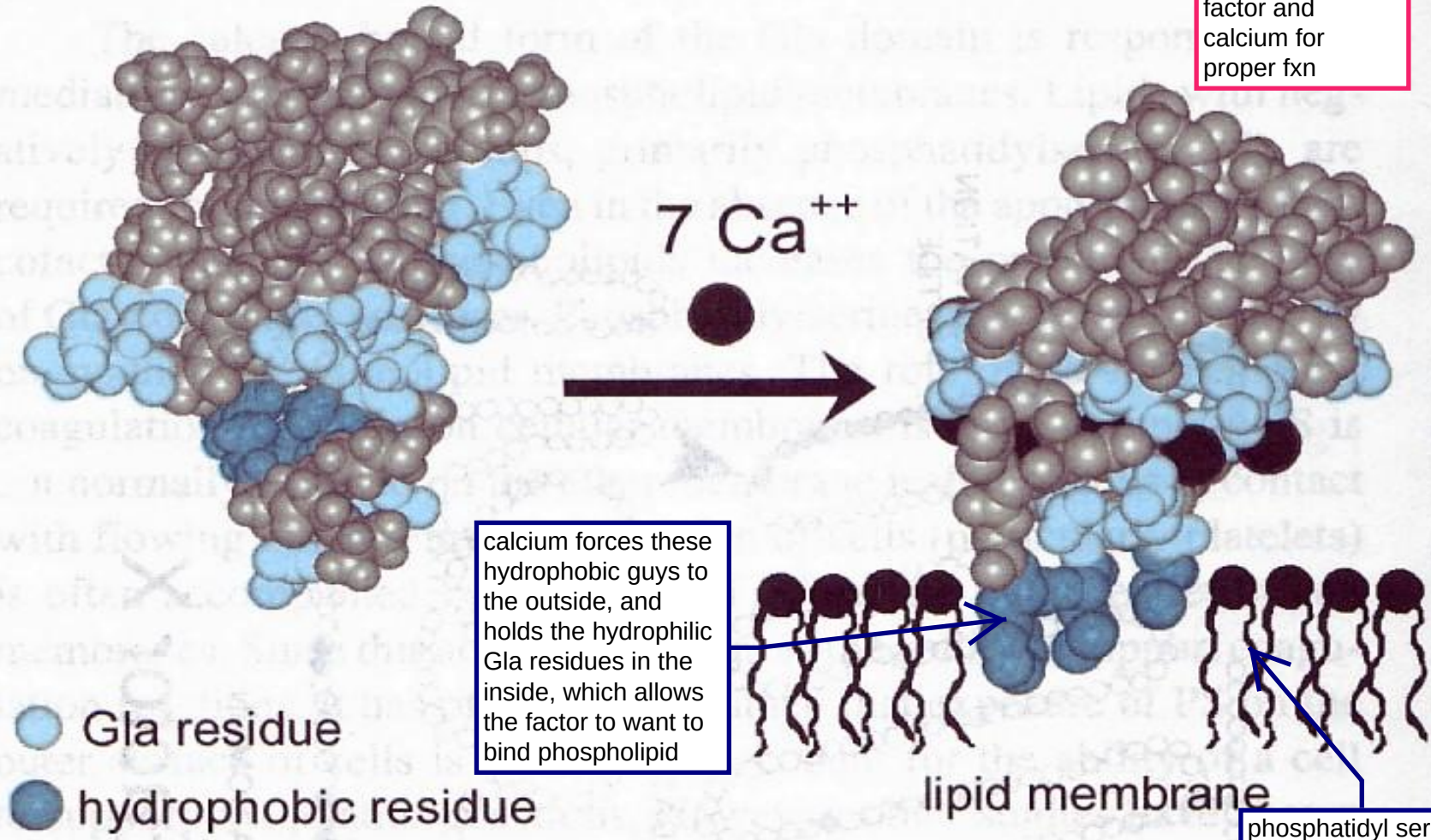
when people need their warfarin reduced what do we do? - give them plasma

Coag proteins work as protease/cofactor complexes



little a = activated form

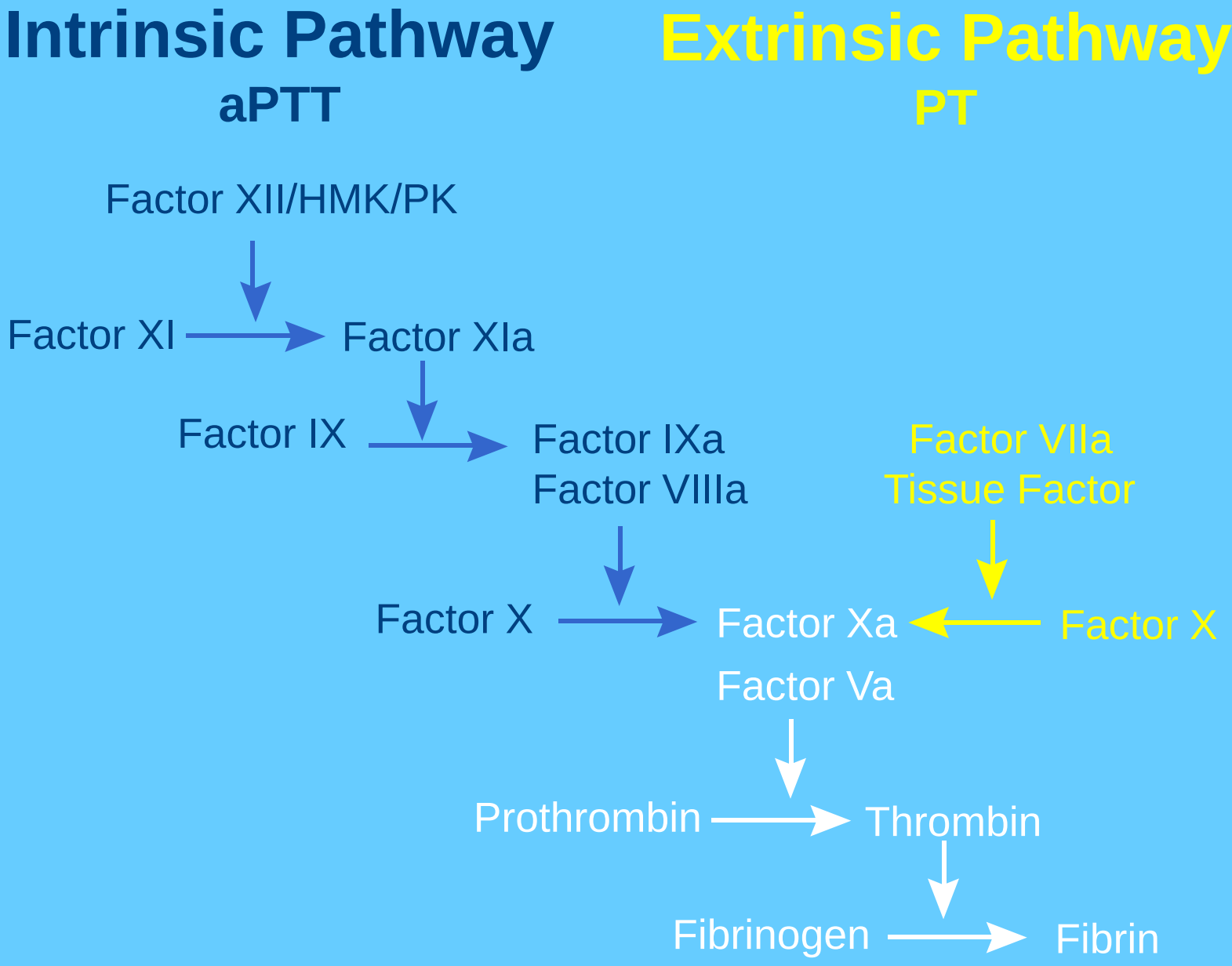
Vitamin K-dependent proteases (FII, VII, IX and X)



Roberts, Monroe & Hoffman: Molecular Biology and Biochemistry of the Coagulation Factors and Pathways of Blood Coagulation. In William's Hematology 7th ed, 2005

The Coagulation Cascade

- Helps us interpret clinical laboratory tests
  - Prothrombin time (PT)
  - Activated Partial Thromboplastin Time (aPTT)

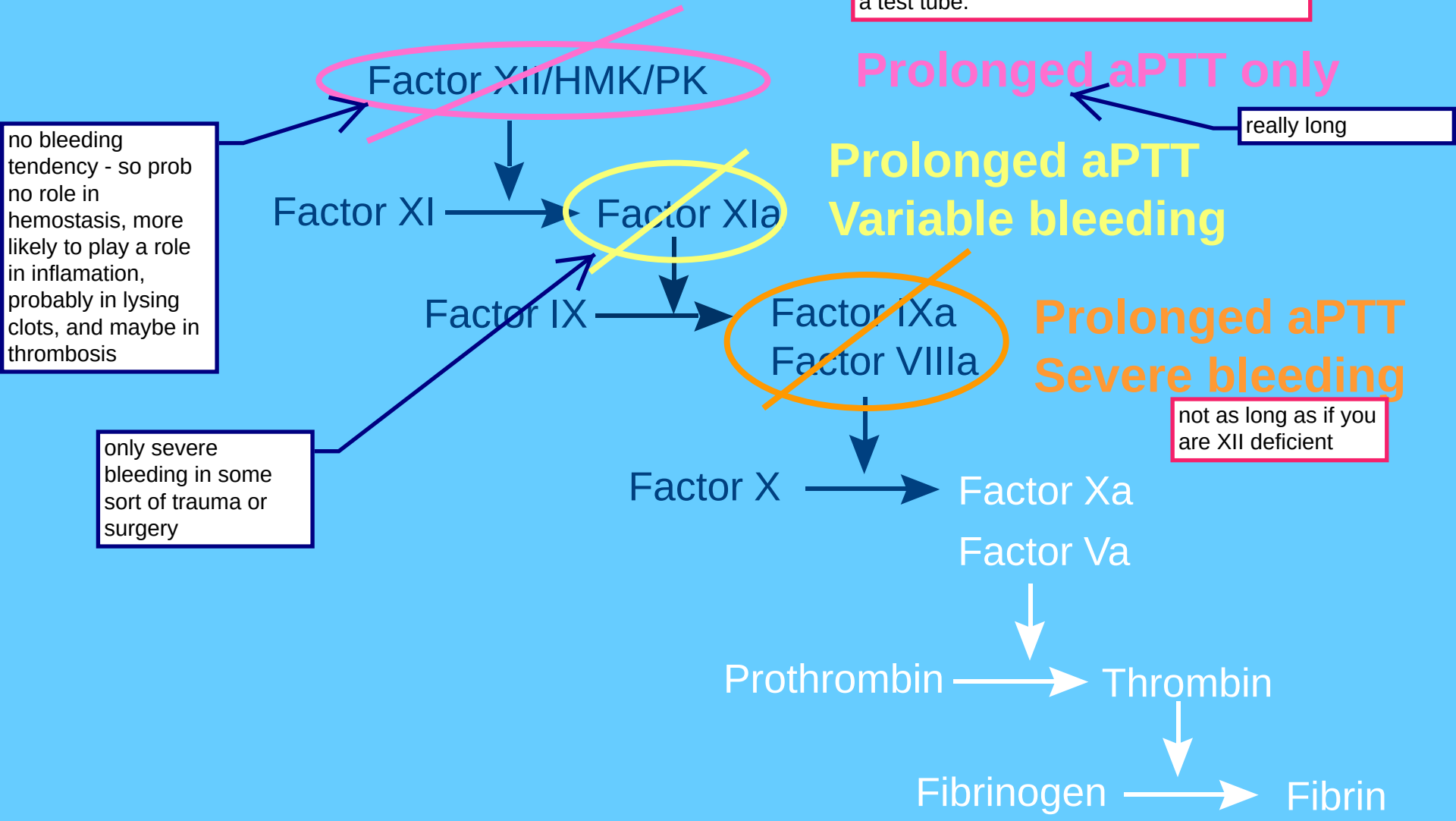

Intrinsic Pathway
aPTT
Extrinsic Pathway
PT
Factor XII/HMK/PK
Factor XI
Factor XIa
Factor IX
Factor IXa
Factor VIIIa
Factor VIIa
Tissue Factor
Factor X
Factor Xa
Factor X
Factor Va
Prothrombin
Thrombin
Fibrinogen
Fibrin

The Coagulation "Cascade" Doesn't Explain How Blood Clots *in vivo*

- Patients lacking FXII, HMK, or PK have a long aPTT but no bleeding
- Patients lacking FXI have a long aPTT and may or may not have bleeding
- Patients lacking FVIII or FIX have an equally long aPTT and serious bleeding

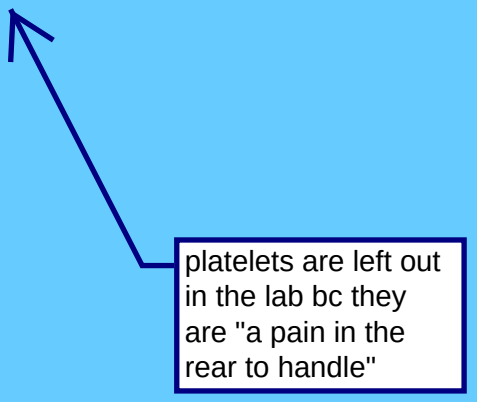
Intrinsic Pathway

aPTT



How does it really work in the body?

Cells are important in the body, but aren't included in the coagulation cascade or the clinical lab tests.



platelets are left out in the lab bc they are "a pain in the rear to handle"

bc we donit have cells or platelets, only some phospholipids and the factors in lab tests - they are probably not a very good model of reality

Hemostasis Occurs on Two Surfaces: TF-bearing Cells and Platelets

1. Initiation

tissue factor bearing cell initiates the coagulation process by making a little bit of thrombin, which then activates platelets stuck down at the site of injury

IIa

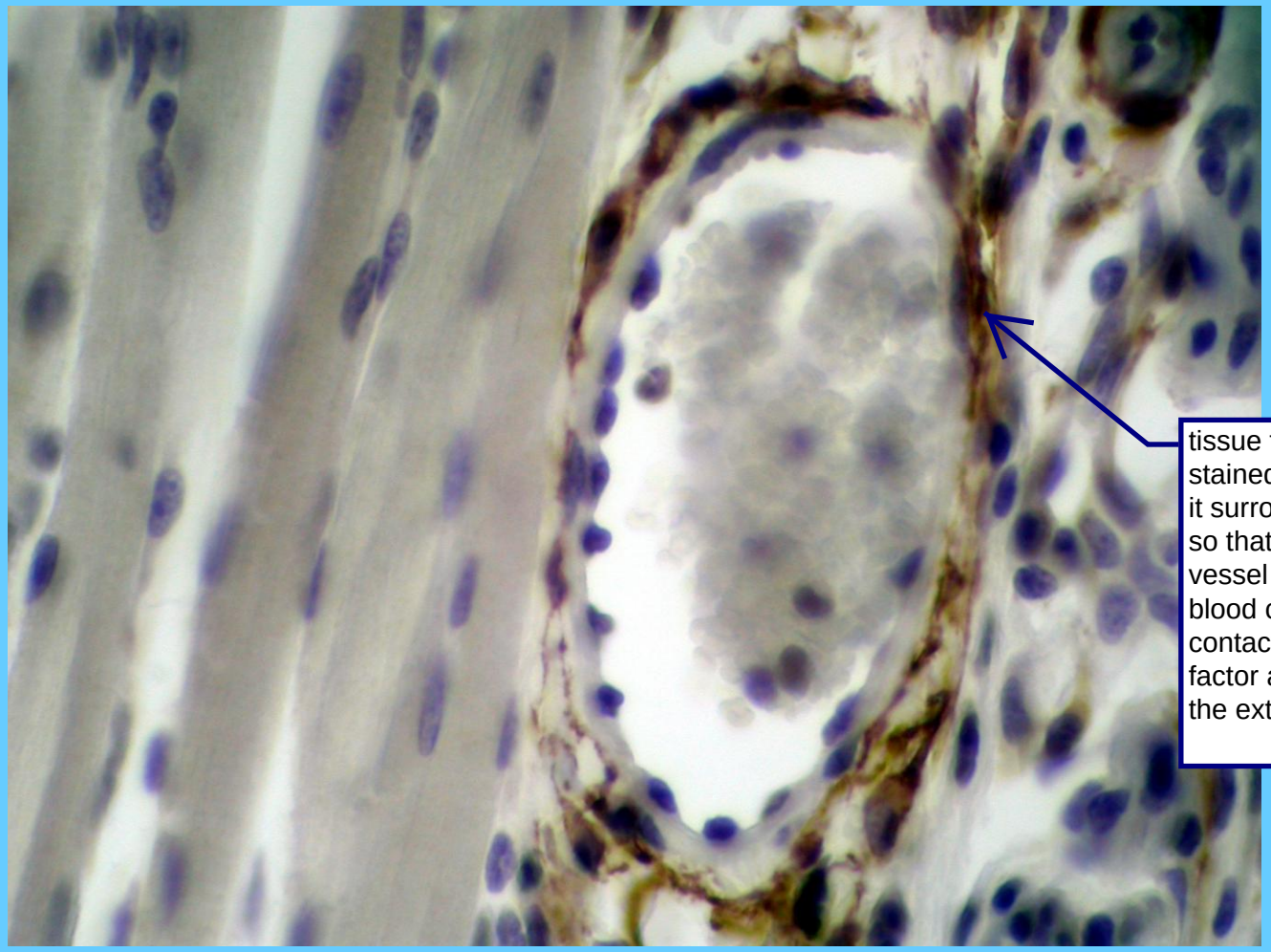
2. Amplification

3. Propagation

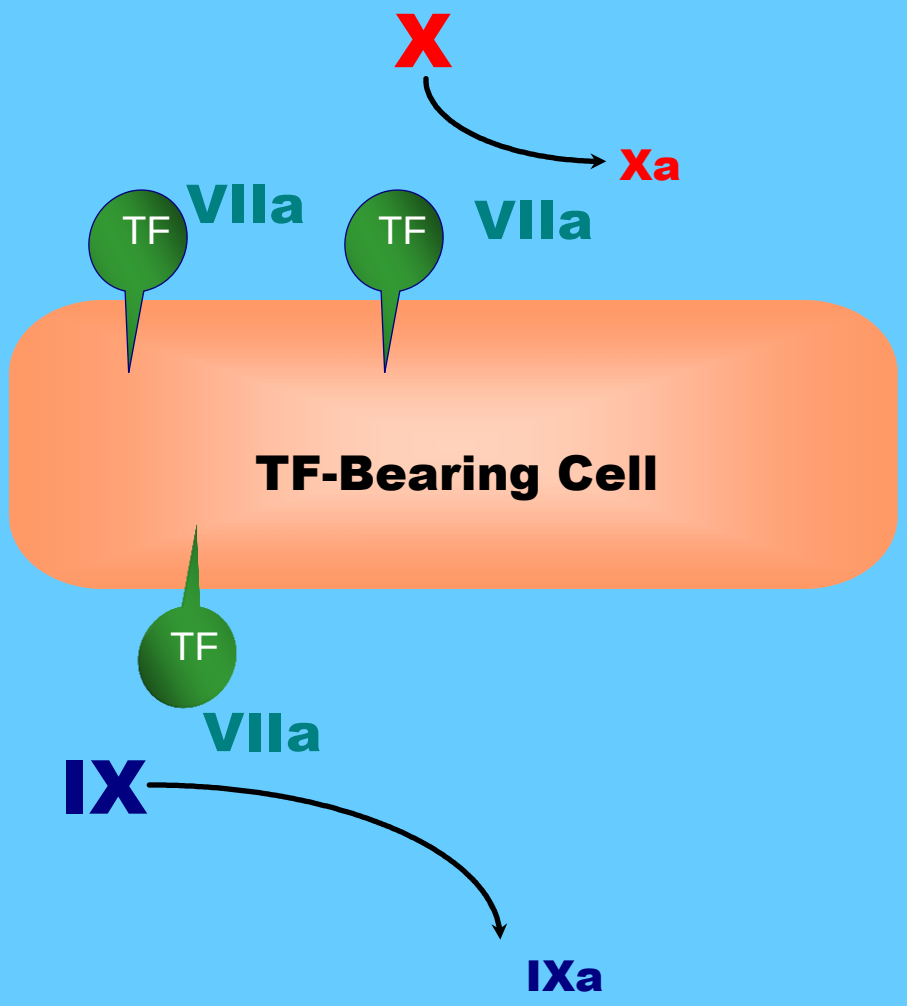
IIa

platelets generate lots of thrombin - the bulk of thrombin responsible for forming the fibrin clot

TF forms a “hemostatic envelope” around the vessel



Initiation

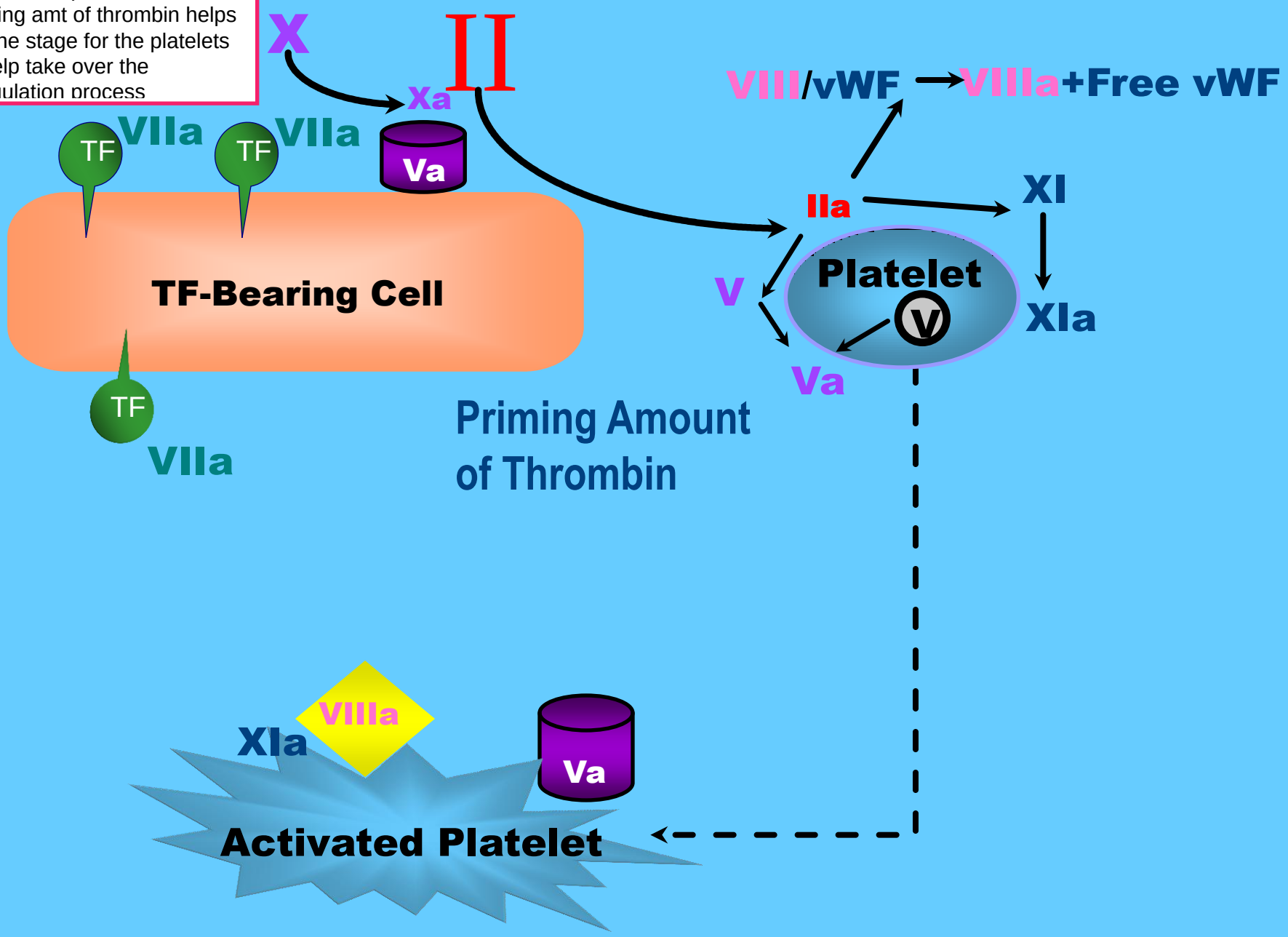


Tissue factor bearing cell can activate factor X and also factor IX, which generates a little thrombin but that doesn't do much normally

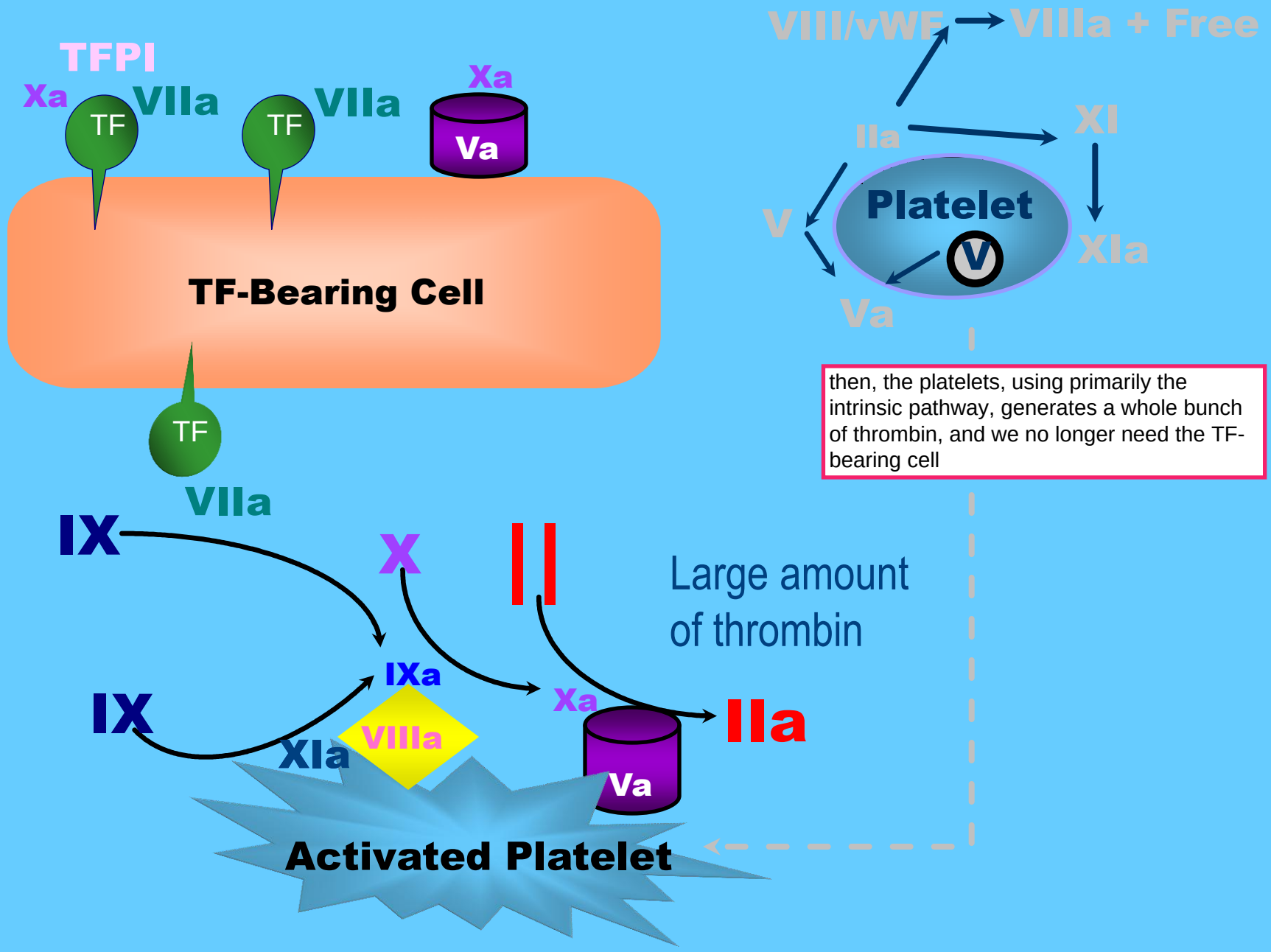
Hoffman M, Monroe DM: A Cell-Based Model of Hemostasis. *Thromb Haemostas*, 85:958-65, 2001

however, when factors are there, the thrombin can help activate the platelets. this priming amt of thrombin helps set the stage for the platelets to help take over the coagulation process

Amplification



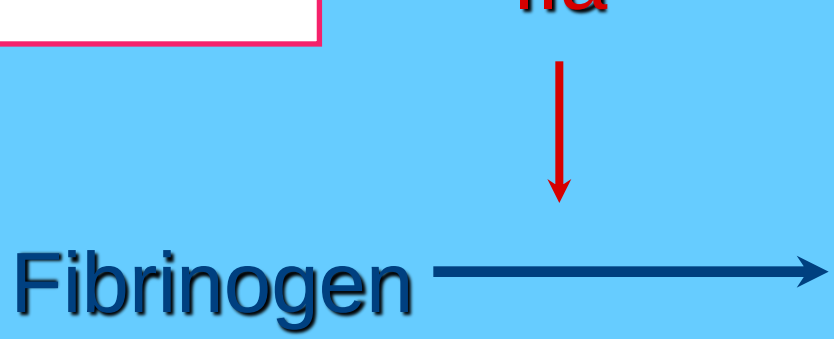
Propagation

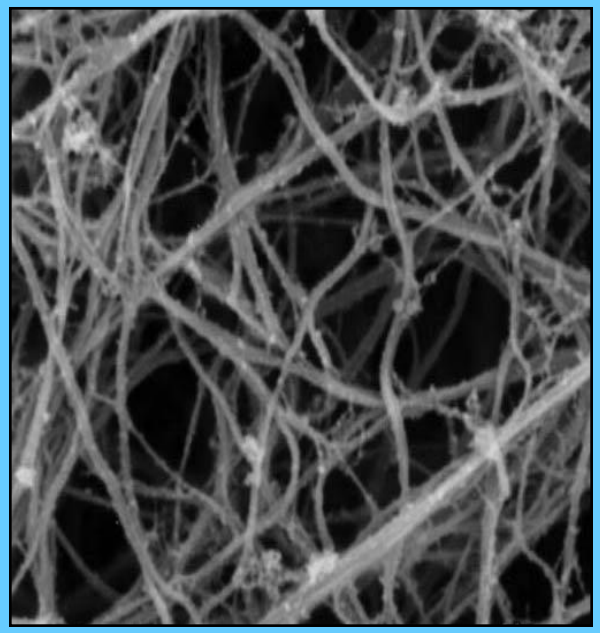


Fibrin Clot Formation

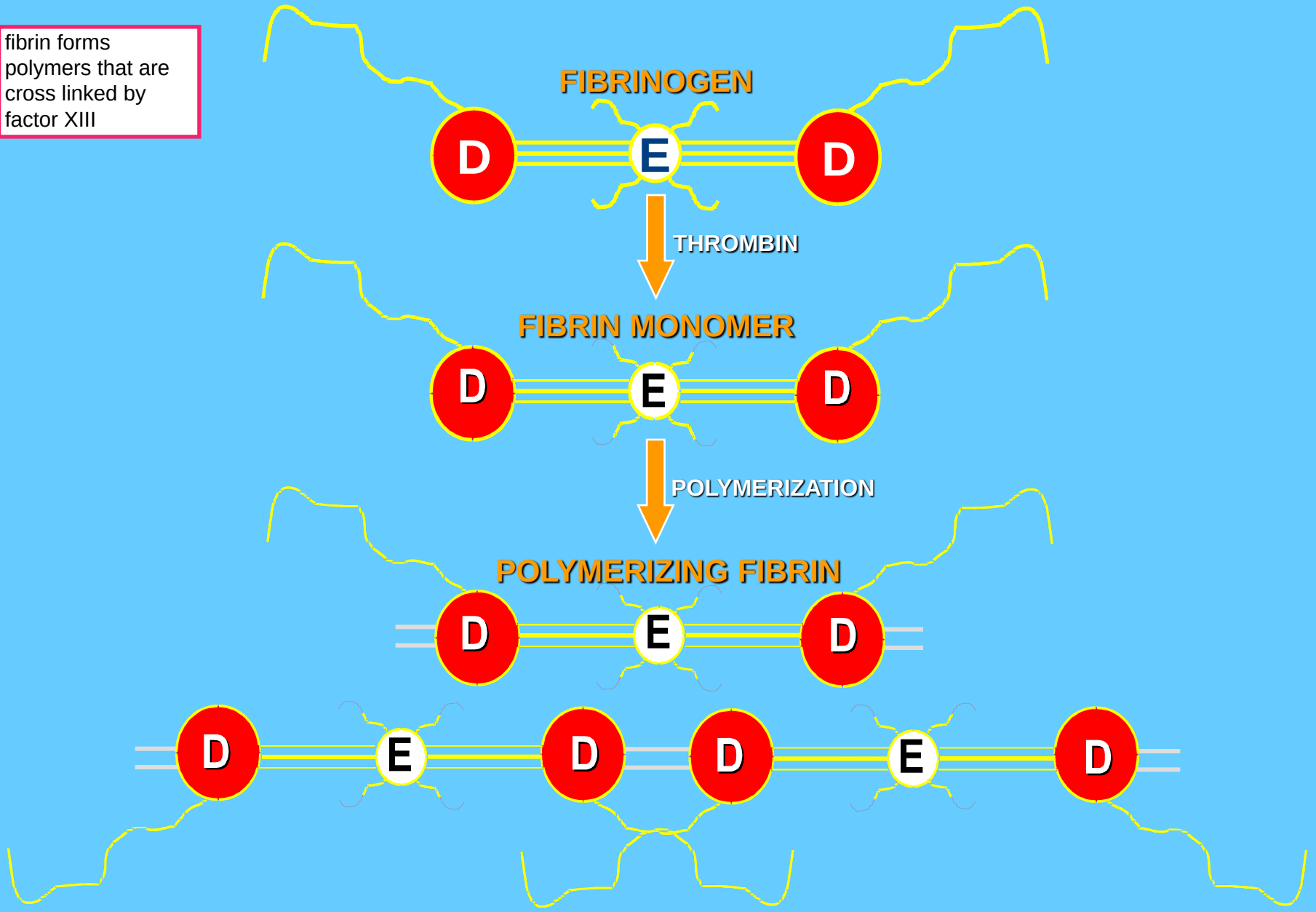
then that thrombin converts fibrinogen to fibrin

IIa





fibrin forms polymers that are cross linked by factor XIII

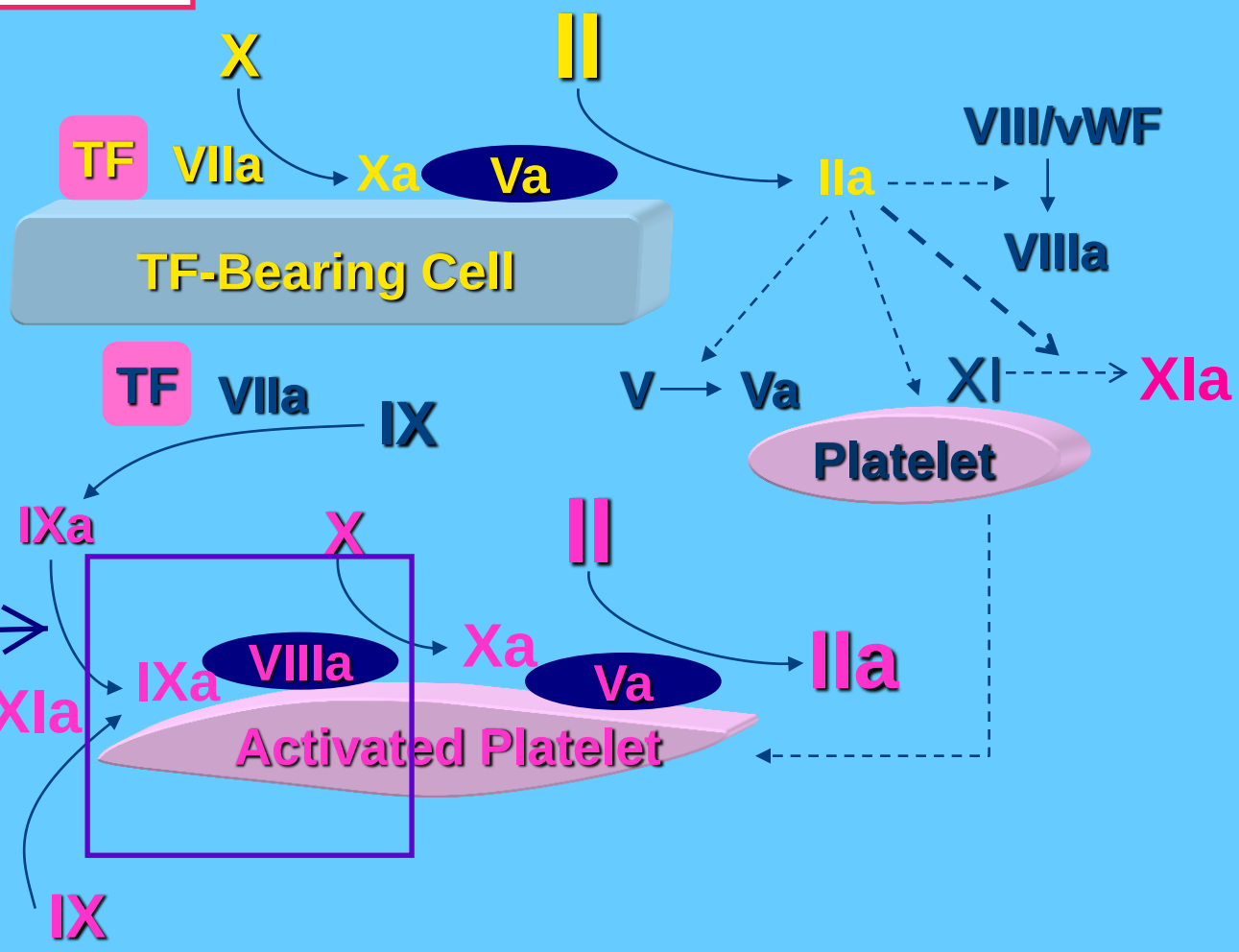


Factor XIII

- Activated by thrombin during coagulation
- Has transglutaminase activity
- Covalently crosslinks fibrin strands to stabilize the clot

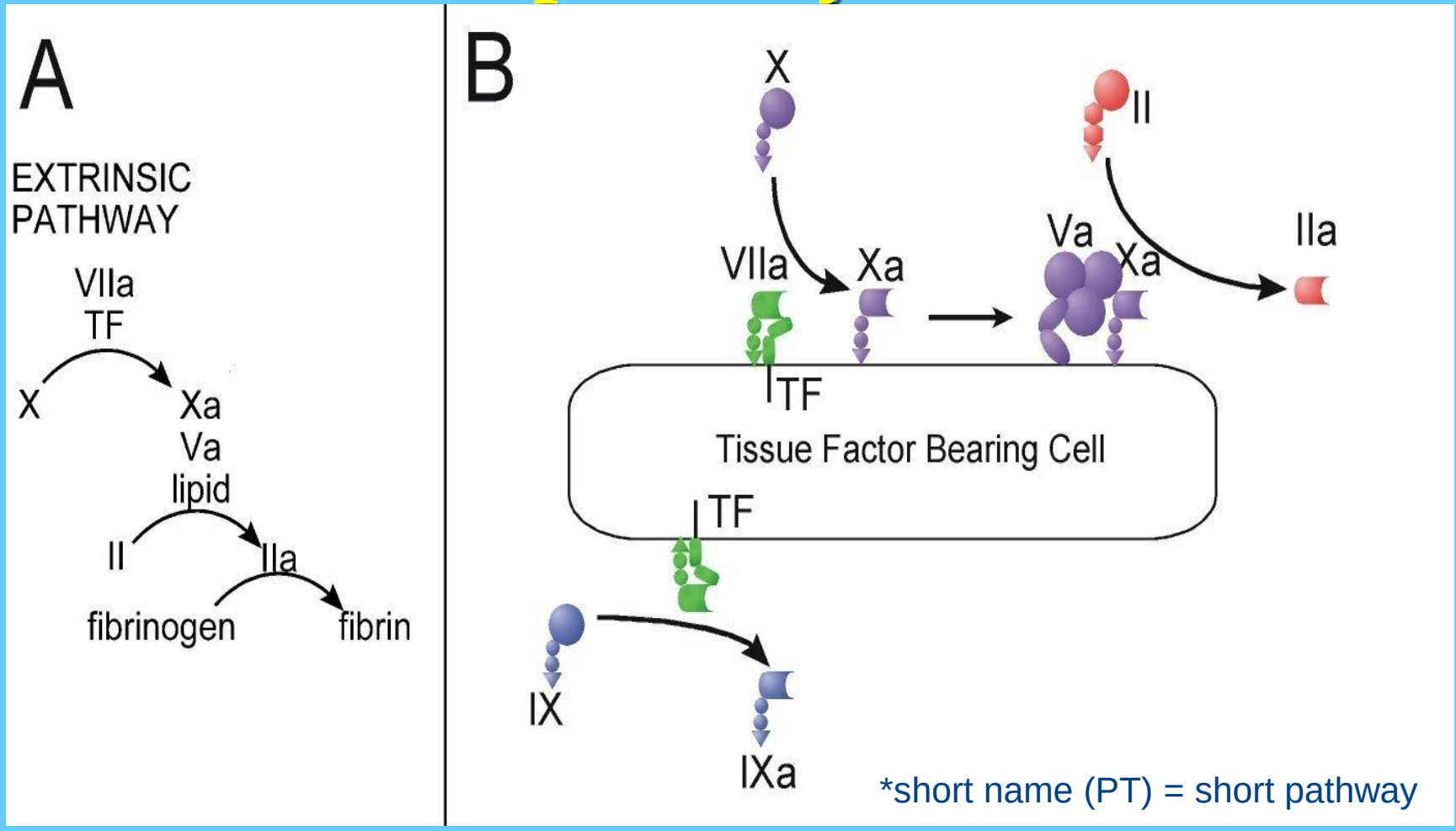
A Cell-Based Model of Hemostasis

same thing we just saw, except now in a pink cartoon version:



Hoffman M, et al. *Blood Coagul Fibrinolysis*. 1998;9(suppl 1):S61-S65.

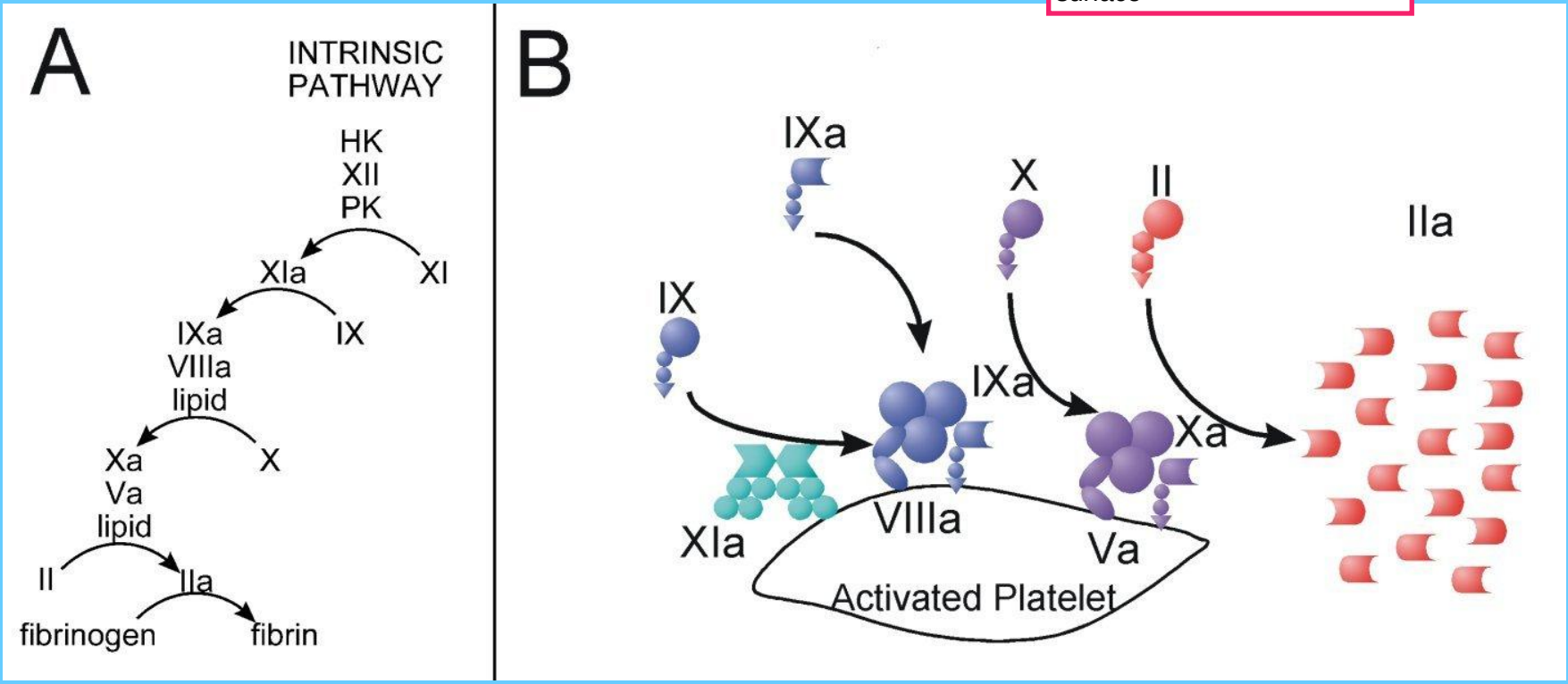
PT: measures extrinsic/initiation pathway*



Monroe, DM and Hoffman, M: What does it take to make the perfect clot? *Arterio Thromb Vasc Biol* 26:41-48, 2006

aPTT: measures intrinsic/platelet pathway*

aPTT measures the pathway that happens on the platelet surface



*long name (aPTT) = long pathway

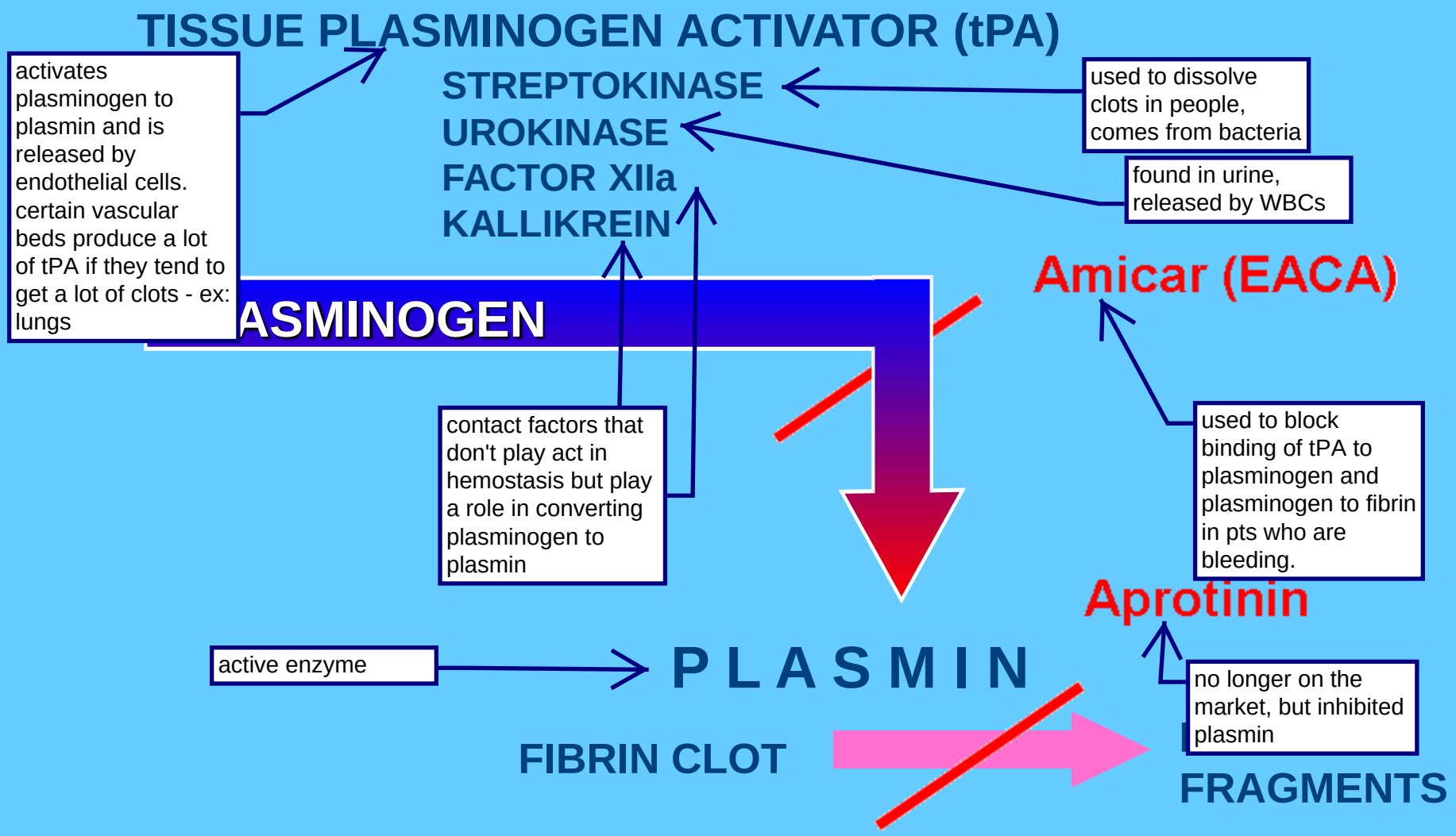
Monroe DM and Hoffman, M: What does it take to make the perfect clot? *Arterio Thromb Vasc Biol* 26:41-48, 2006

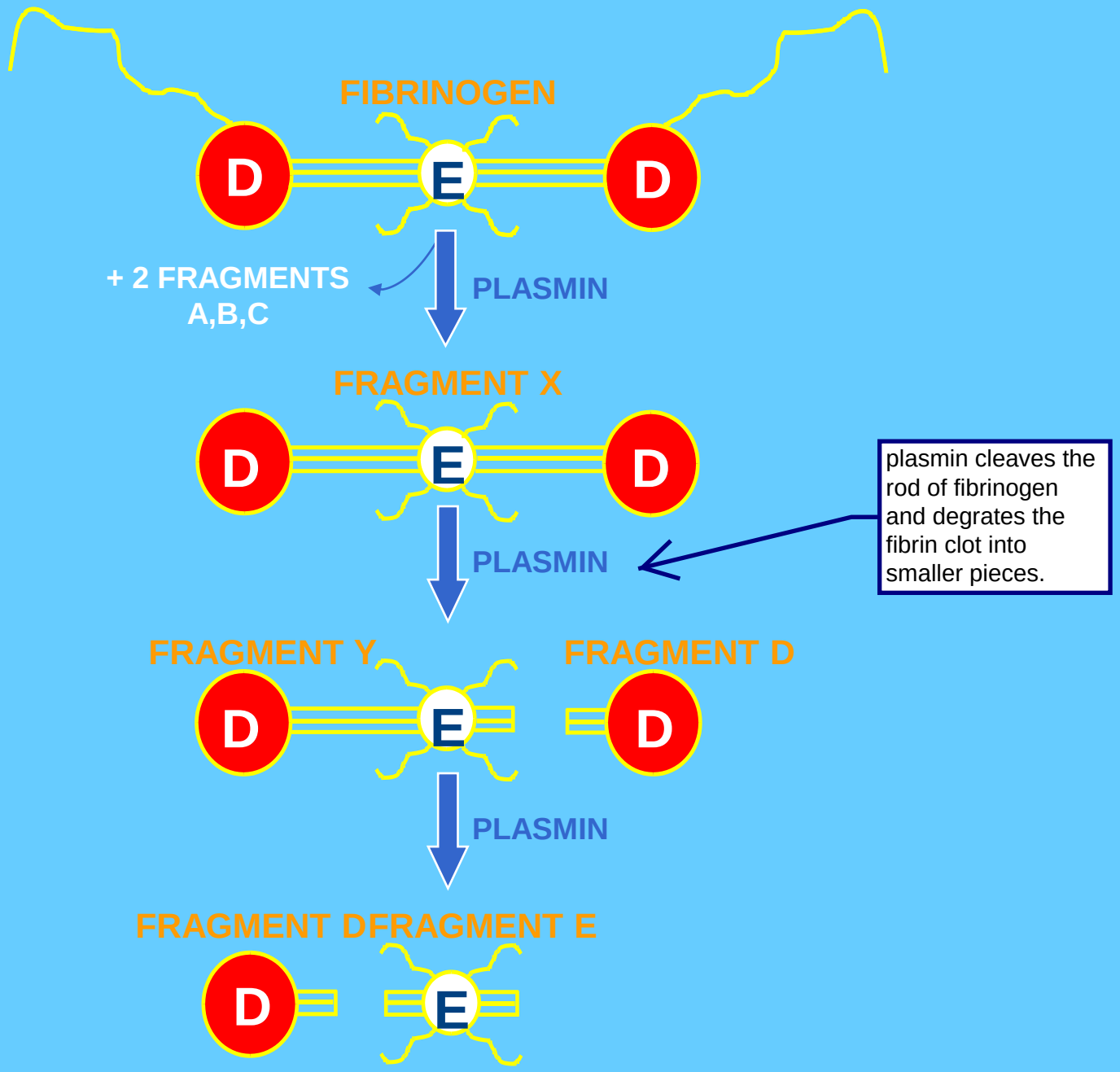
The intrinsic and extrinsic pathways are not redundant, but have distinct roles in hemostasis *in vivo*

so if your patient is missing components of either pathway, they can have a bleeding problem

when the wound heals and we have to dissolve the fibrin clot = fibrinolysis

Fibrinolysis




FIBRINOGEN
D
E
D
+ 2 FRAGMENTS
A,B,C
PLASMIN
FRAGMENT X
D
E
D
PLASMIN
plasmin cleaves the rod of fibrinogen and degrates the fibrin clot into smaller pieces.
FRAGMENT Y
FRAGMENT D
D
E
D
PLASMIN
FRAGMENT DFRAGMENT E
D
E

Control of Clot Formation

Separation of Initiation & Propagation

extrinsic pathway and platelet are separated by the vessel wall - only get large scale thrombin generation if these interact

in the modern world, we are a lot more likely to clot to death than to bleed to death... so its important to know how to deal with clots that are forming in the wrong places.

Presence of plasma coagulation inhibitors

- Antithrombin (AT or ATIII)
  - Activity increased by heparin & LMWH
- Tissue Factor Pathway Inhibitor (TFPI)

inhibits almost all of the coagulation proteases to some degree

people deficient in AT have thrombosis, people deficient tissue factor inhibitor aren't born - knockout mice dont survive if they are born

Anti-thrombotic mechanisms on healthy vascular endothelial cells

- Thrombomodulin (TM)/Protein C/S system
- Heparan sulfates that bind AT

vitamin K dependent - degrade activated coagulation factors so thrombin is not made on healthy endothelial cells

Hemostasis Sets the Stage for

Inflammatory Cell Influx & Effective Wound Healing

Thrombin has many biological activities

set the stage for healing

Fibrin is the matrix for healing

make scaffold for healing to occur on

Platelets release cytokines & growth factors

Hemostatic defects can lead to defective wound healing

Bleeding Disorders

- We are only going to talk about inherited (not acquired) disorders
- Platelet problems
- Coagulation factor problems

Clinical Bleeding

- Platelet Problem
  - Petechiae & purpura
  - Mucocutaneous bleeding
- Coagulation Factor Problem
  - Bruises (ecchymoses)
  - Soft tissue hemorrhage

small areas of bleeding

big areas of bleeding

"for your reference: platelets have all sorts of stuff in them"

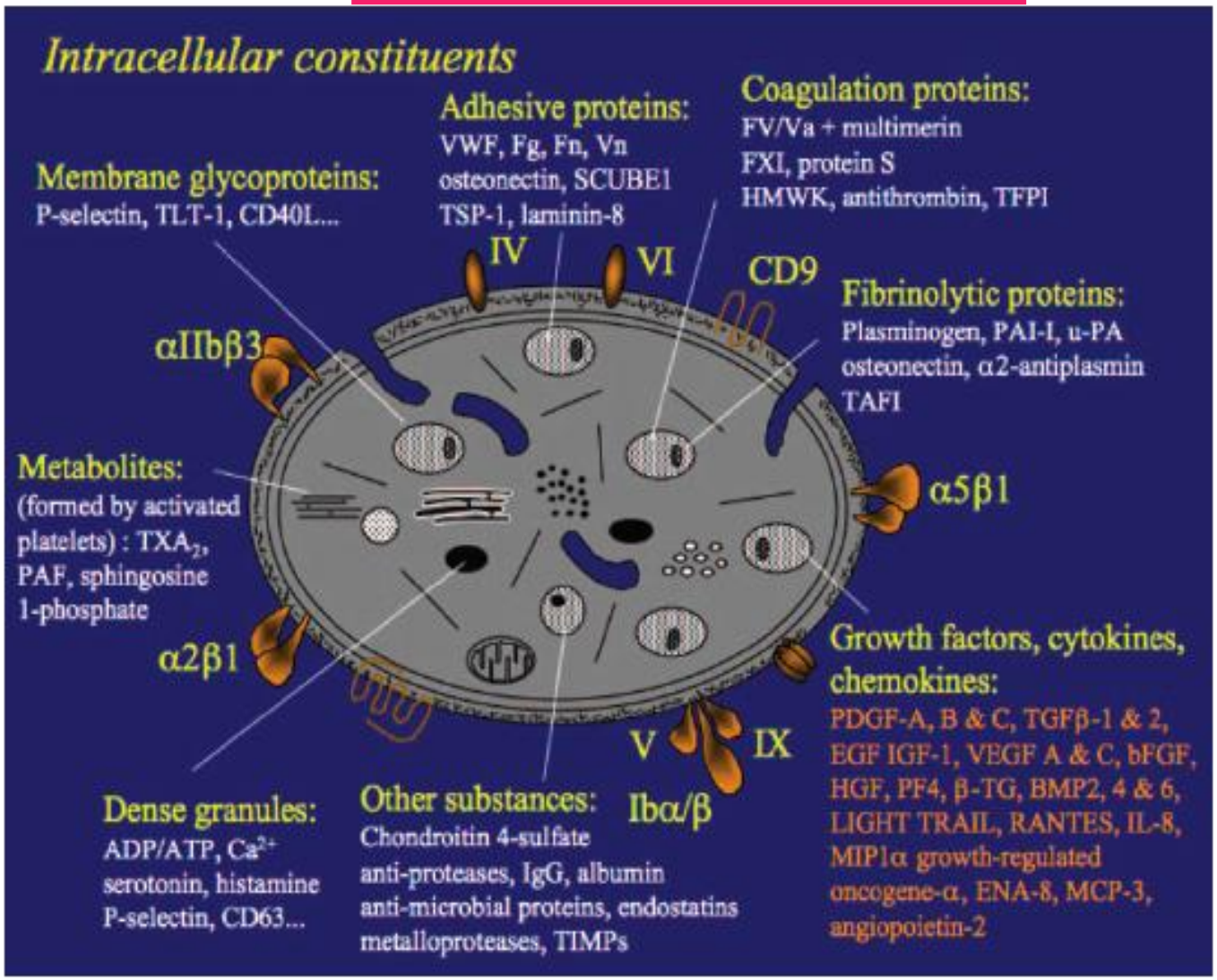


FIG. 1. Platelet storage organelles. Predominant are the α-granules of which there are upwards of 50 per platelet. A large number of proteins are stored and released from these organelles; in the figure proteins are grouped by category for convenience, and this is not meant to signify a physiological storage organization. There is, however, some evidence that subpopulations of α-granules may contain discrete populations of proteins (14).

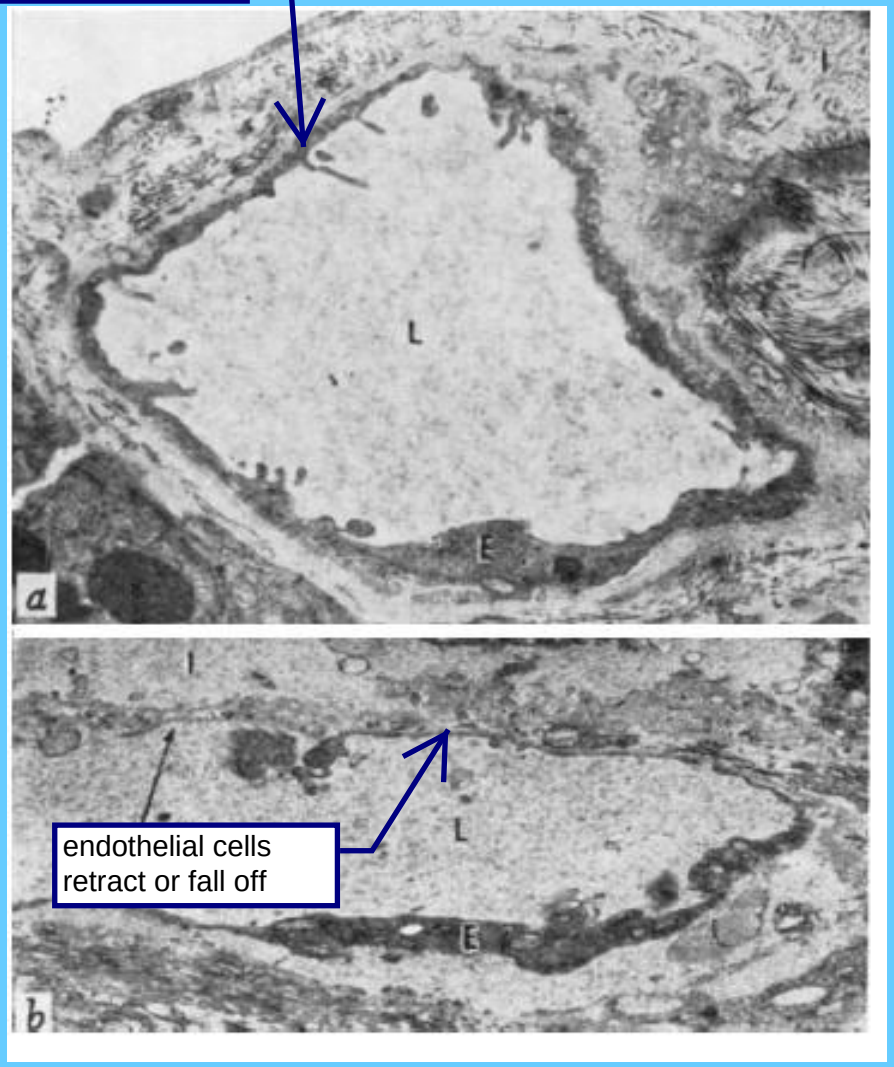
Descriptors of Bleeding

- Petichiae are < 3 mm, Purpura are 0.3-1 cm, Ecchymoses are > 1 cm

http://www.uptodate.com

Thrombocytopenia leads to endothelial changes

endothelial cells need platelets to keep them happy and healthy



endothelial cells retract or fall off

The top shows an EM of a capillary from a thyroid perfused with PRP for 5h

PRP = platelet rich plasma

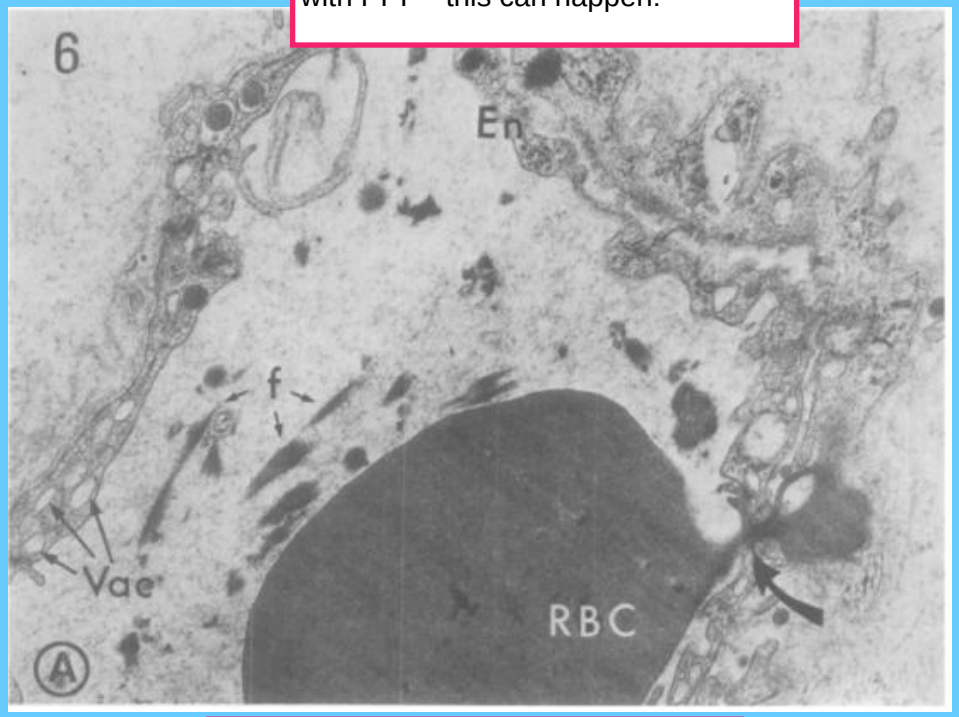
The bottom shows a capillary from a thyroid perfused with PPP for 5h. Note the disruption in the endothelium.

PPP = platelet poor plasma

- Gimbrone et al: Preservation of vascular integrity in organs perfused *in vitro* with a platelet-rich medium. *Nature*, 222:13-4, 1969

Thrombocytopenia leads to endothelial changes

with PPP - this can happen:



this does NOT happen in hemophilia or some other coagulation factor abnormality

This picture shows a RBC extravasating from a capillary of a thrombocytopenic mouse (arrow)

RBC appear to traverse small channels in the endothelial cells.

- Aursnes & Pedersen: Petechial hemorrhage in the ciliary process of thrombocytopenic rabbits. An EM study. *Microvascular Res*, 17:12-21, 1979

Factor Deficiencies: General Considerations

Deficiencies of each of the following exist:

- Factor VIII (hemophilia A)
- Factor XI
- Prothrombin
- Factor V
- Fibrinogen
- Factor IX (hemophilia b)
- Factor VII
- Factor X
- Factor XII
- Factor XIII

deficiency of tissue factor does not exist - you can't survive without tissue factor. vascular system will not develop normally in the absence of TF or its inhibitor

Factor Deficiencies: General Considerations

- Inheritance: Most are inherited as autosomal recessive disorders
- Factors VIII and IX are encoded on the X chromosome and their deficiencies are sex-linked recessive
- While bleeding is the hallmark of these disorders, its severity and pattern vary depending on the involved factor

Congenital Bleeding Disorders

- vonWillebrand Disease
- Hemophilia A & B
- FXI deficiency

vonWillebrand Disease

vonWillebrand Disease

we will see this. it has a wide range of clinical manifestations. may not show up until surgery, trauma, or if the pt begins taking an anticoagulant. can also be very severe bleeding

- Autosomal
- Most common inherited bleeding disorder
- vWF mediates platelet adhesion under high shear - bleeding is typical of platelet defects

petichiae

- vWF is the carrier for FVIII - FVIII level may be reduced and aPTT may be prolonged
- Subdivided into several types based on multimer pattern and antigen level

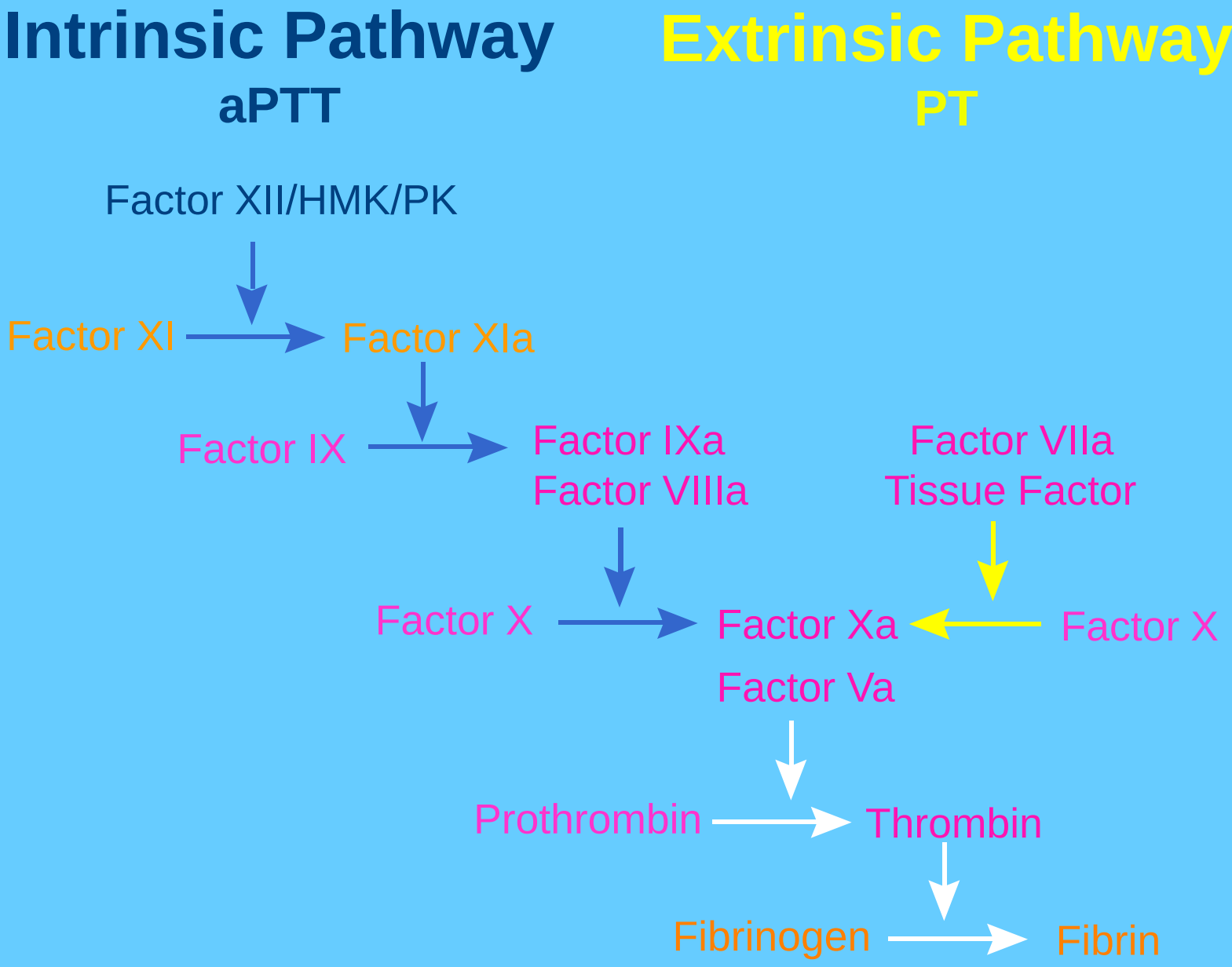
Hemophilia A & B

Deficiency of FVIII or FIX

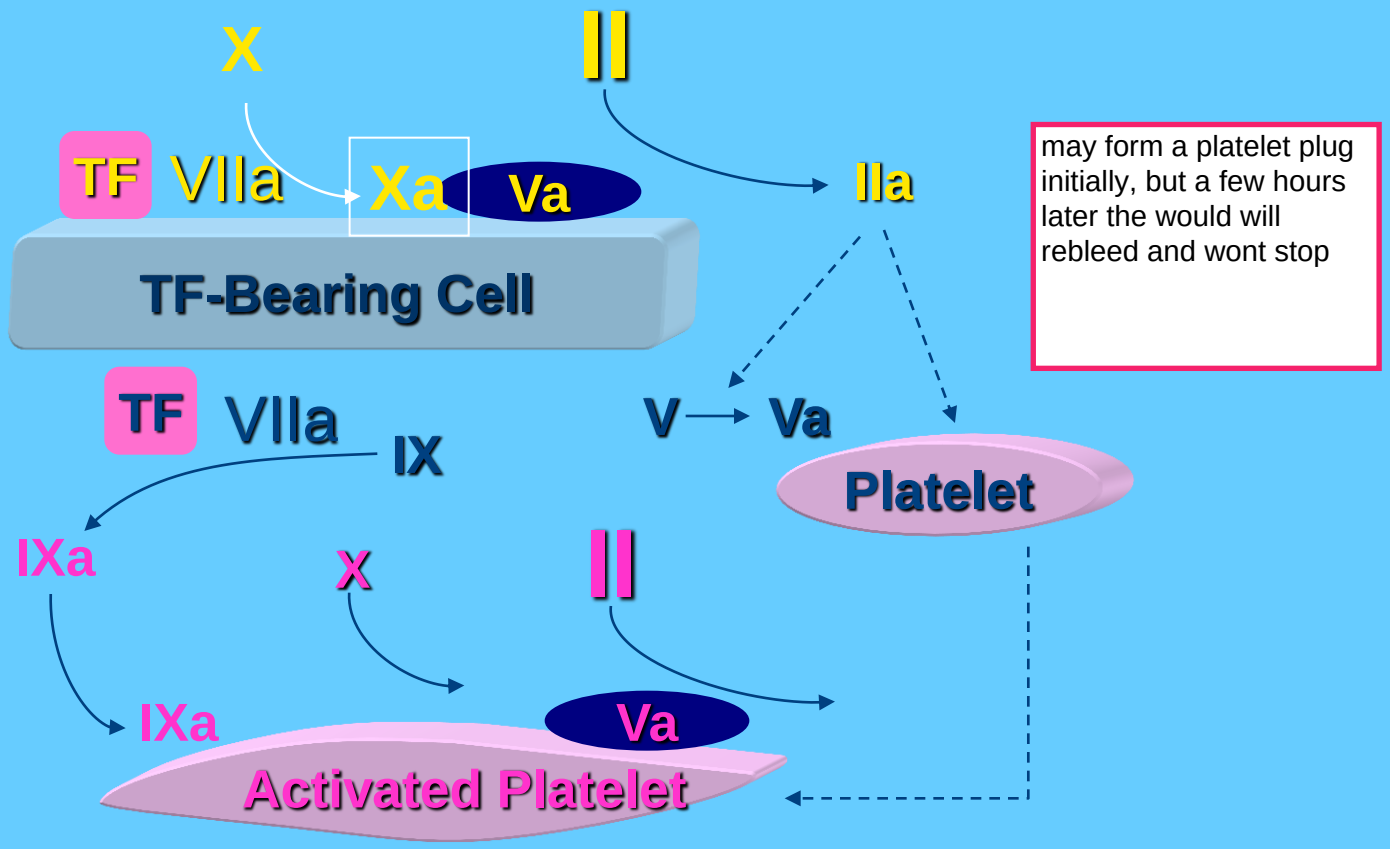
Hemophilia A and B

- X-linked
- Up to 30% from de novo mutation i.e. no family history
- Mild, moderate and severe forms
- Dysfunctional molecules – Cross-reacting material positive (CRM+)
- Reduced level of a normal molecule - Cross-reacting material negative (CRM-)

especially true with factor IX

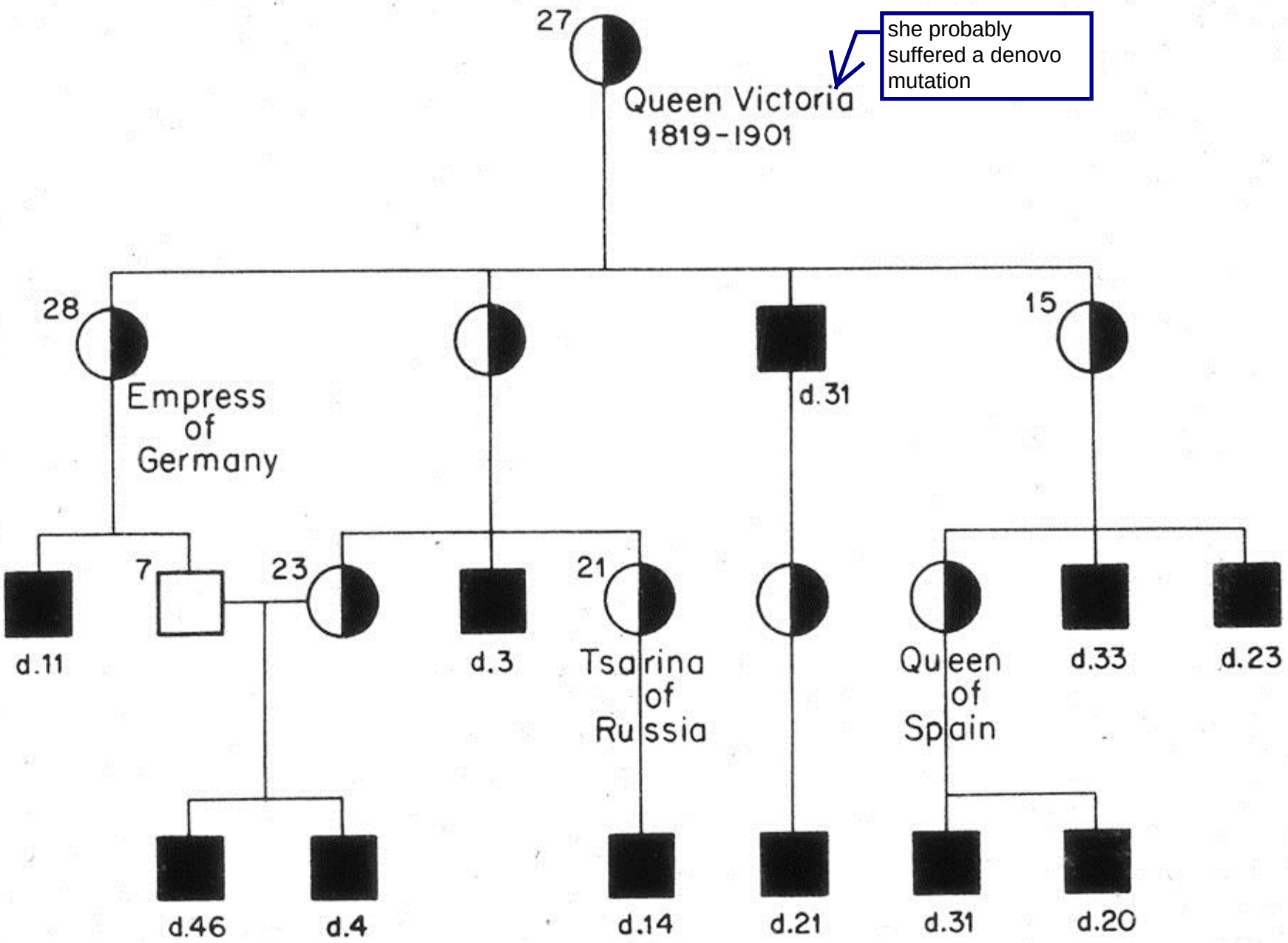

Intrinsic Pathway
aPTT
Extrinsic Pathway
PT
Factor XII/HMK/PK
Factor XI
Factor XIa
Factor IX
Factor IXa
Factor VIIIa
Factor VIIa
Tissue Factor
Factor X
Factor Xa
Factor X
Factor Va
Prothrombin
Thrombin
Fibrinogen
Fibrin

Hemophilia Is a Failure of Platelet Surface Thrombin Generation



Hoffman M, et al. *Blood Coag Fibrinolys.* 1998;9(suppl 1):S61-S65.

Much early coagulation research was driven by the presence of hemophilia in the royal families of Europe


27
Queen Victoria
1819-1901
she probably suffered a denovo mutation
28
Empress of Germany
d.31
15
d.11
7
23
d.3
21
Tsarina of Russia
Queen of Spain
d.33
d.23
d.46
d.4
d.14
d.21
d.31
d.20

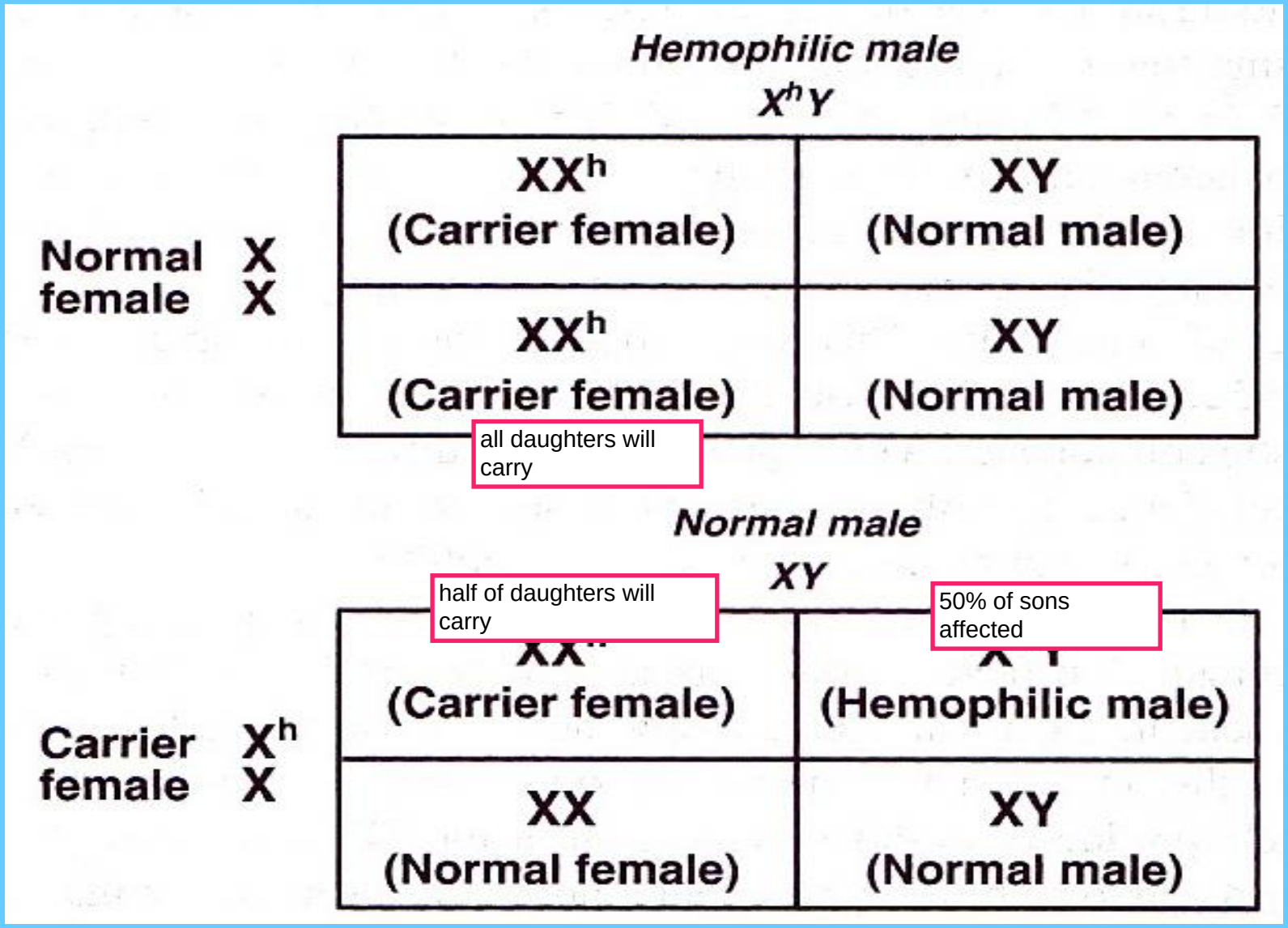
Inheritance of Hemophilia

- **Hemophilia A (FVIII deficiency) 1 in 10,000 live male births**
- **Hemophilia B (FIX deficiency) 1 in 30,000 live male births**
- **Inherited as sex linked recessive traits**

An affected male will produce only normal males and carrier females (with a normal female)

A carrier female will produce offspring of which half the females are carriers and half the males are affected (with a normal male)

Inheritance of Hemophilias



Hemophilia A and B

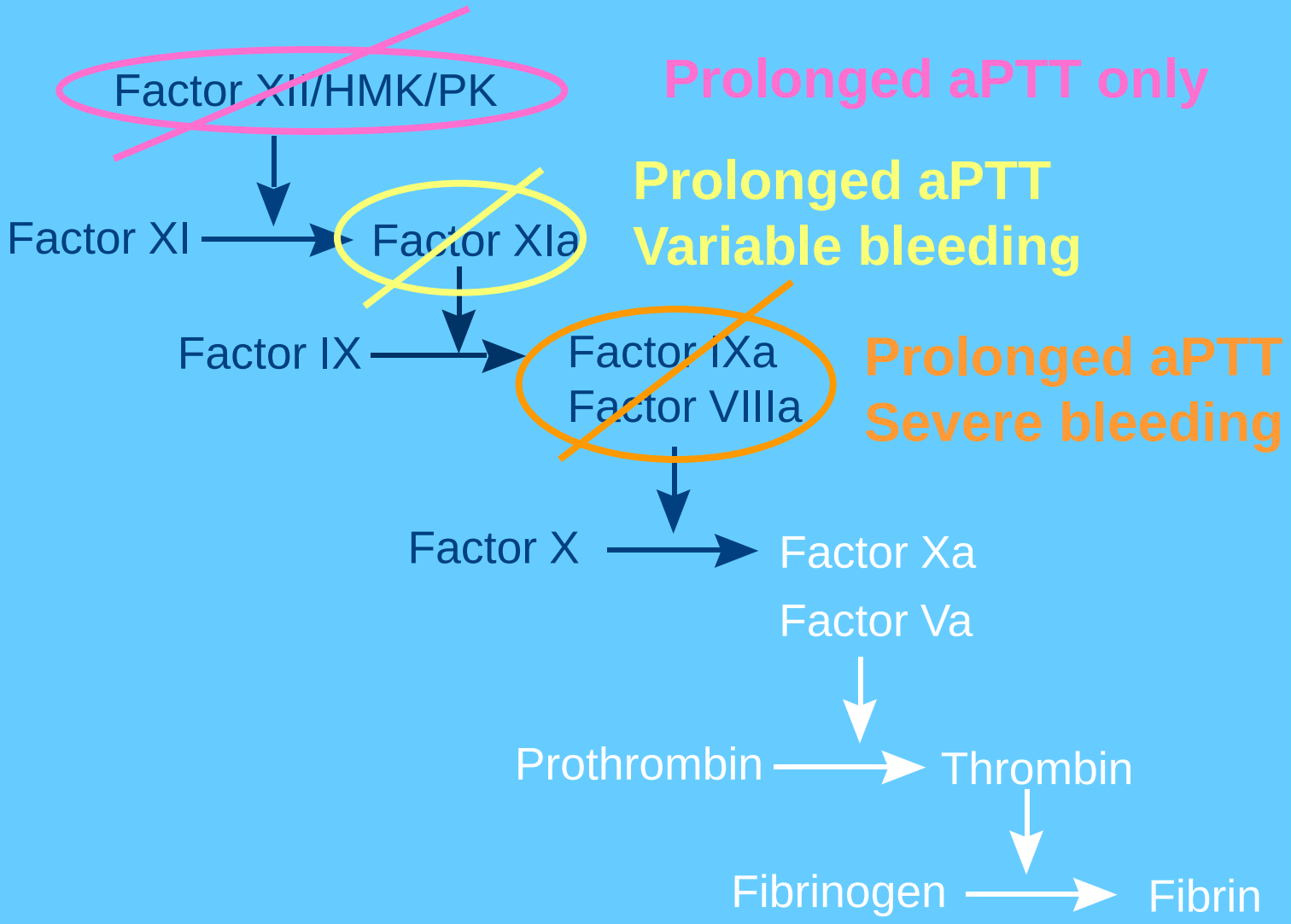
- Clinical picture is identical in A & B
- Prolonged aPTT in both, need factor assays to distinguish
- Severely affected have spontaneous soft tissue and joint hemorrhage
- Severely deficient may develop antibody inhibitors

FXI deficiency

long aPTT but bleeding isn't as bad as hemophilia

Intrinsic Pathway

aPTT



FXI Deficiency

populations where you have consanguinity bc that has a tendency to concentrate recessive genes

- Autosomal
- Common in certain populations - Ashkenazi Jews, some Arab populations

a specific factor X deficieny has been traced to consanguinity in the mountains of North Carolina.

- Bleeding with trauma or surgery, especially if on aspirin

otherwise its usually mild

- Bleeding risk not predictable from aPTT or FXI level