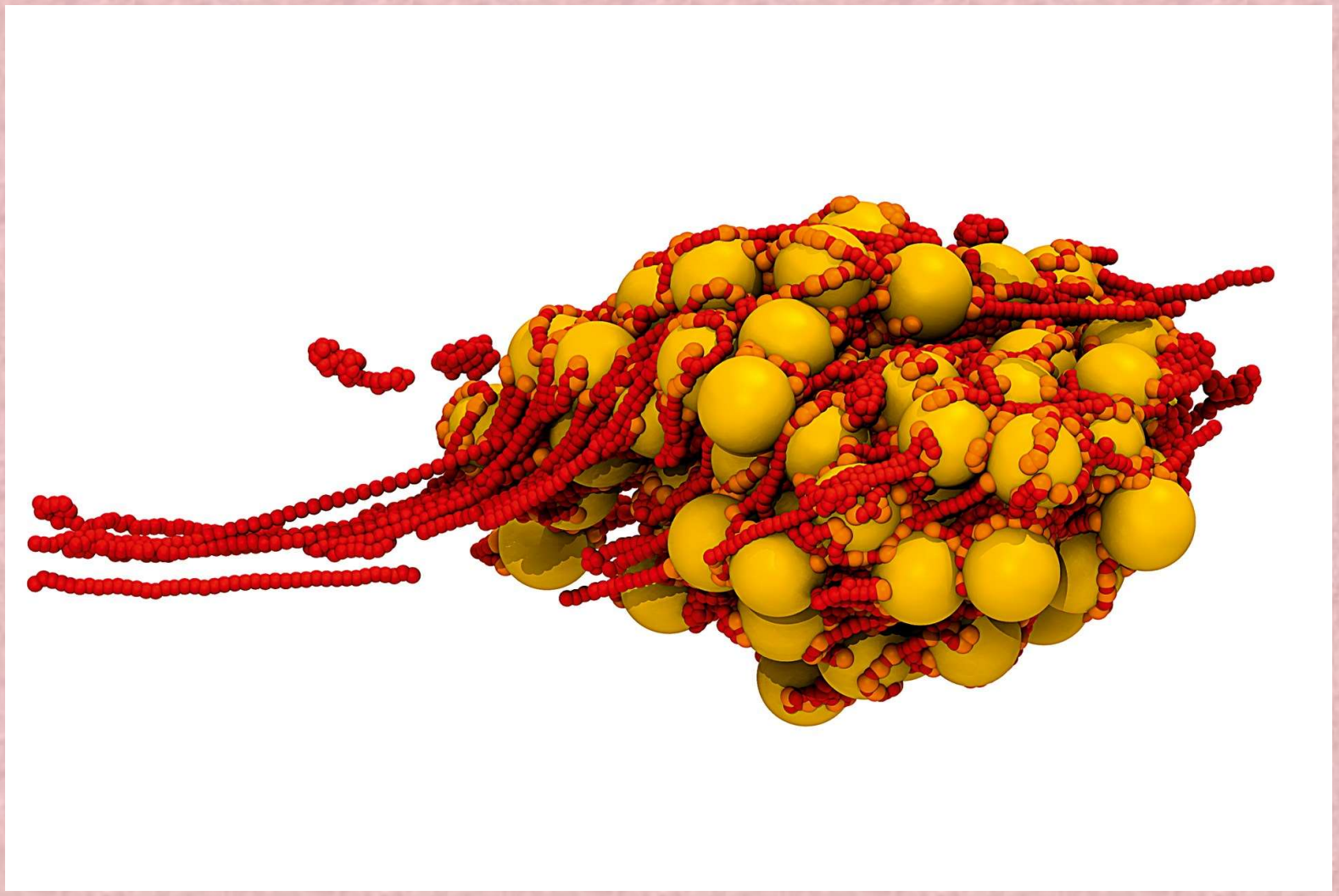
Hemostasis and its disorders

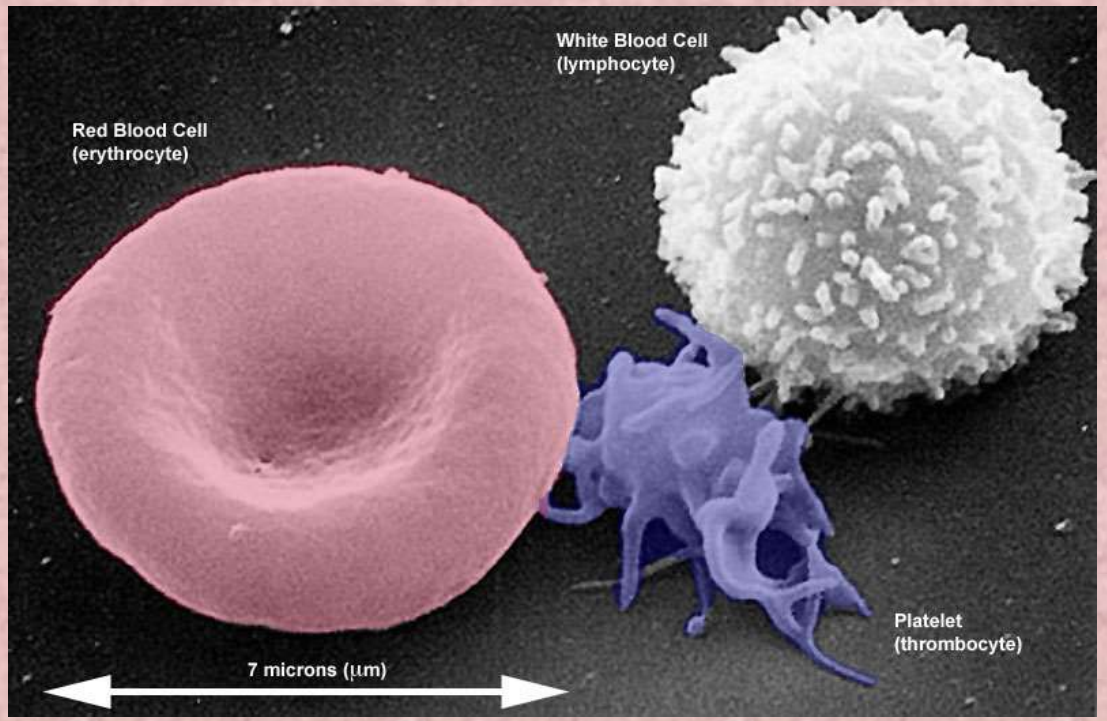


Primary and secondary hemostasis

- Primary (platelet aggregation and activation)
  - Formation of platelet plug
  - Vasoconstriction molecules are released
  - It is important to stop bleeding from capillaries, arterioles and venules
- Secondary (coagulation)
  - Series of reactions of coagulation factors ending by fibrin formation
  - Crucial for large vessels and to prevent protracted bleeding
  - Primary and secondary hemostasis can be studied as separate processes in vitro
  - They are interconnected in vivo

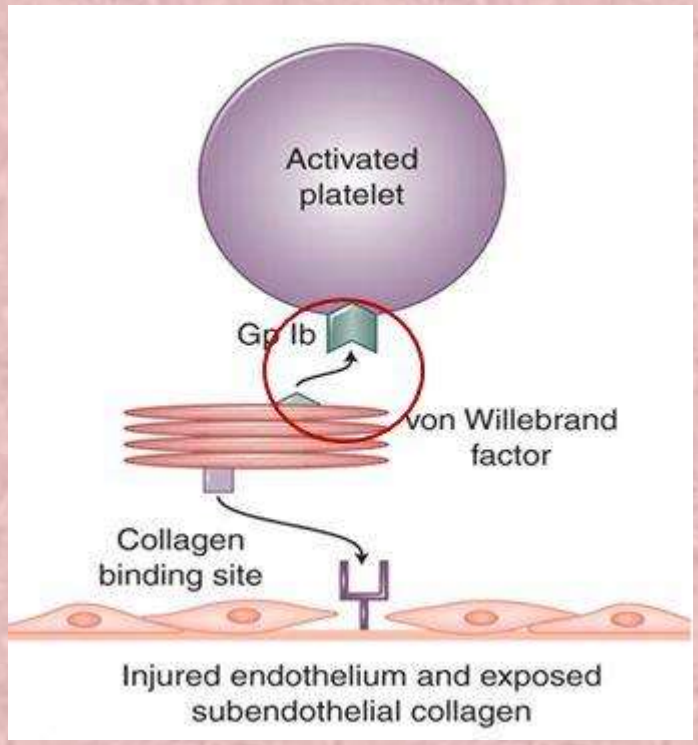
Platelet function during hemostasis

- Adhesion to damaged vessel wall
- Storing and releasing ADP, eicosanoids and proteins
- Aggregation with other platelets
- Surface for coagulation reactions



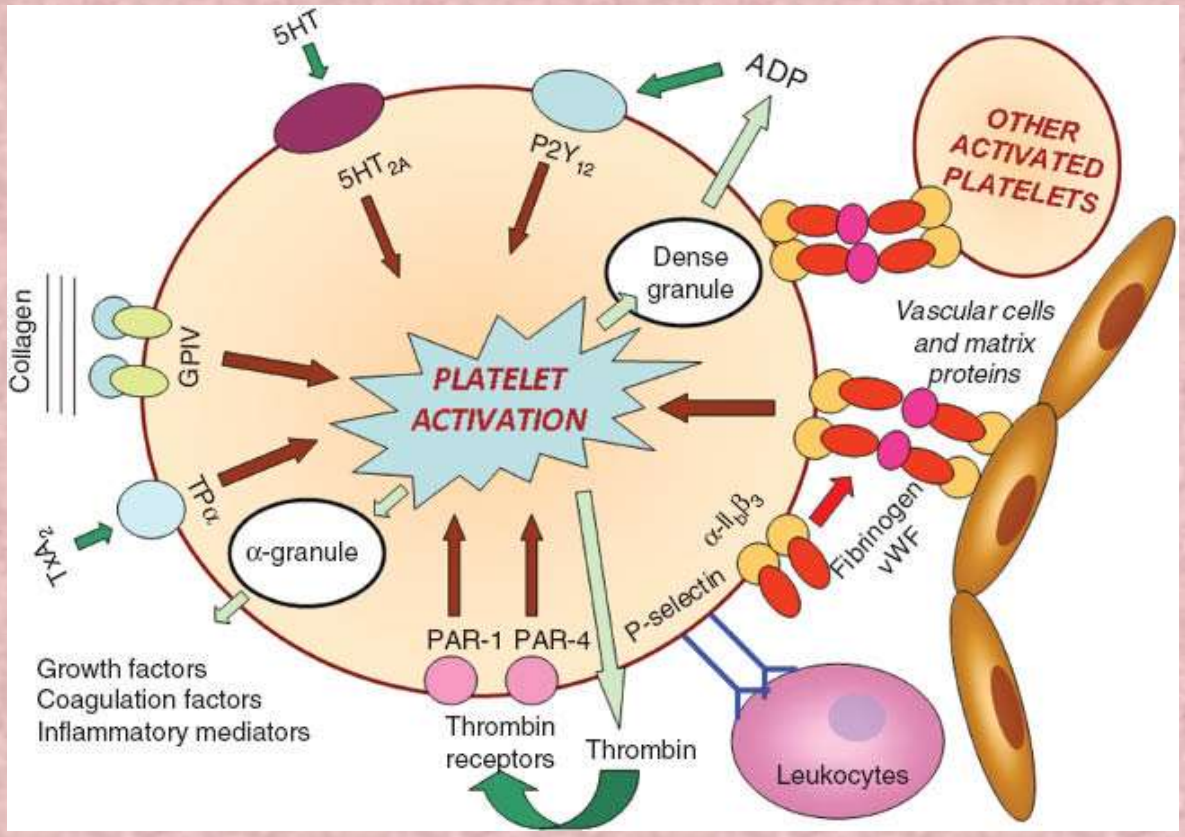
Primary hemostasis – trombocyte adhesion

- The injury of vessel wall leads into the exposure of collagen
- Collagen binds von Willebrand factor (vWF)
- After binding collagen, vWF changes conformation and is able to bind platelet receptor glycoprotein 1b (GP 1b)



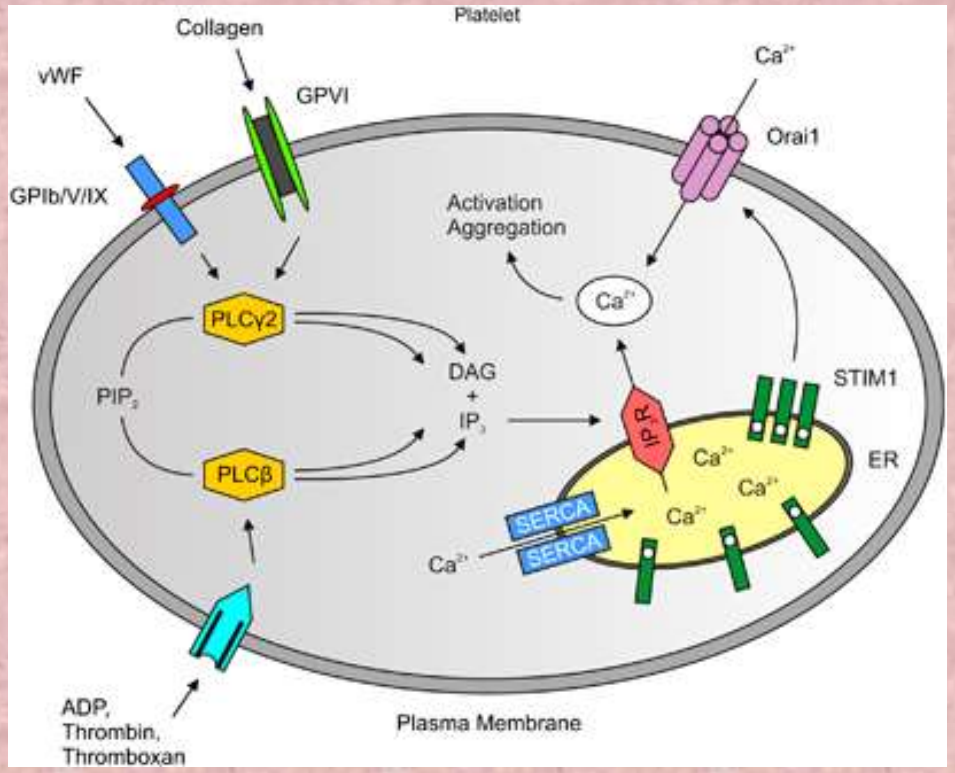
Platelet activation – surface receptors and their ligands

- GP 1b - vWF
- P2Y receptor family – ADP
- P2X - ATP
- PAR receptors - thrombin (strongest platelet activator)
- TP receptors - thromboxane A2
- Different glycoproteins - collagen



Thrombocyte activation – post receptor cascade

- Most often phospholipase C (PLC) activation → cleaves PIP_2 into IP_3 and DAG
- Ca^{2+} release from endoplasmic reticulum
- Some receptors (P2X) are linked with Ca^{2+} membrane channels
- ↑Ca^{2+} in the cytoplasm
- Ca^{2+} comes from both endoplasmic reticulum and extracellular fluid



Inhibition of thrombocyte activation

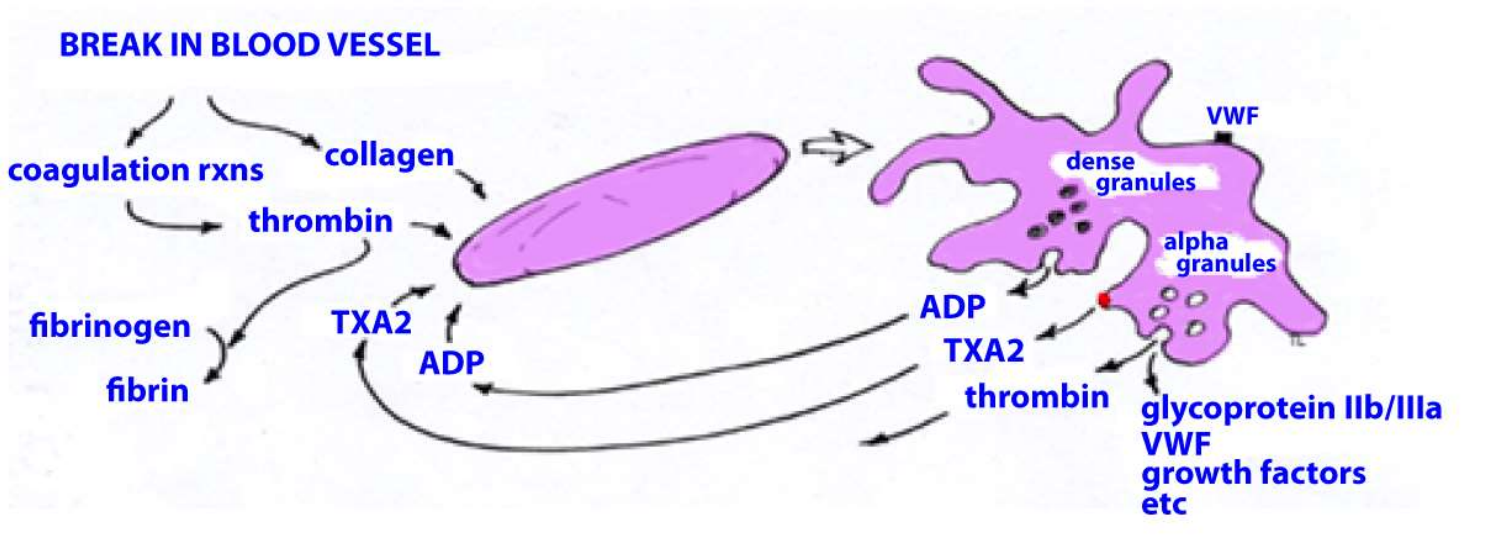
- Induced by ↑cAMP or ↑cGMP
- They activate specific protein kinases(PKA and PKG)
- PKA induces Ca^{2+} transport from cytoplasm into microsomes and extracellular space
- PKA and PKG also inhibit Ca^{2+} release from endoplasmic reticulum (IP3-mediated)
- cAMP and cGMP are formd using cyclases and degraded using phosphodiesterases

Activators and inhibitors of adenylyl cyclase

- Activators (i.e. anti-aggregation)
  - prostaglandins D2, I2
  - adenosine
- Inhibitors (i.e. pro-aggregation)
  - prostaglandin E2
  - catecholamines (via α2 receptors)
  - ADP

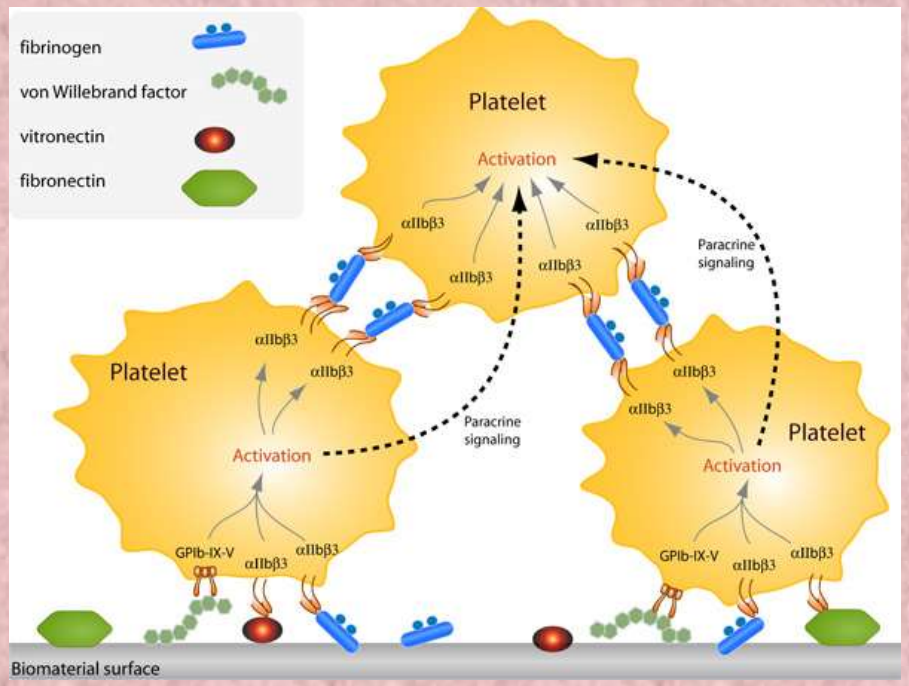
Activated thrombocyte

- Increase of intracytoplasmic Ca^{2+} leads into platelet shape change and degranulation. ADP, thrombin and other vasoconstrictor molecules are released from the granules
- Via COX activation (both isoforms), TXA2 is synthetized (platelet activator and strong vasoconstrictor)
- Ca^{2+} also induces the change of glycoprotein IIb/IIIa conformation into activated state (Gp IIb/IIIa is the most abundant receptor on platelet surface)
- Activated thrombocyte also expose negatively charged phosphatidylserine, which supports coagulation cascade



Aggregation

- Activated GP IIb/IIIa (= integrin αIIbβ3) bind circulating fibrinogen molecules
- Fibrinogen serves as a „glue“ between two platelets
- Alternative (less important) pathway uses vWF as a „glue“ (especially its multimers - TTP)

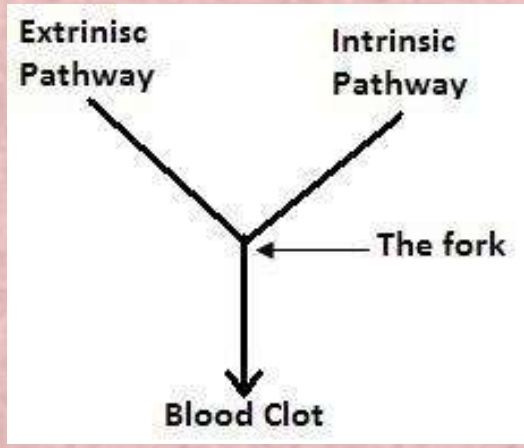


Aggregation tests

- Non-specific: bleeding time (in vivo)
- Platelet count in µl of blood
- Light transmission aggregometry (LTA) vs. impedance
- Either the maximum aggregation rate or its integral per 1 minute is measured (↑ number = ↑ aggregation) after adding an agent
  - Arachidonic acid
  - ADP
  - Thrombin receptor agonist
  - Gp1b agonist
  - collagen

Coagulation

- Can be basically started by two mechanisms:
  1) Tissue factor („extrinsic pathway“)
  2) Contact with negatively charged surface („intrinsic pathway“)



The two pathways are well defined in vitro, but not in vivo (they are useful for diagnosis but do not correspond with physiology)

Reactants and catalyzers of coagulation reactions

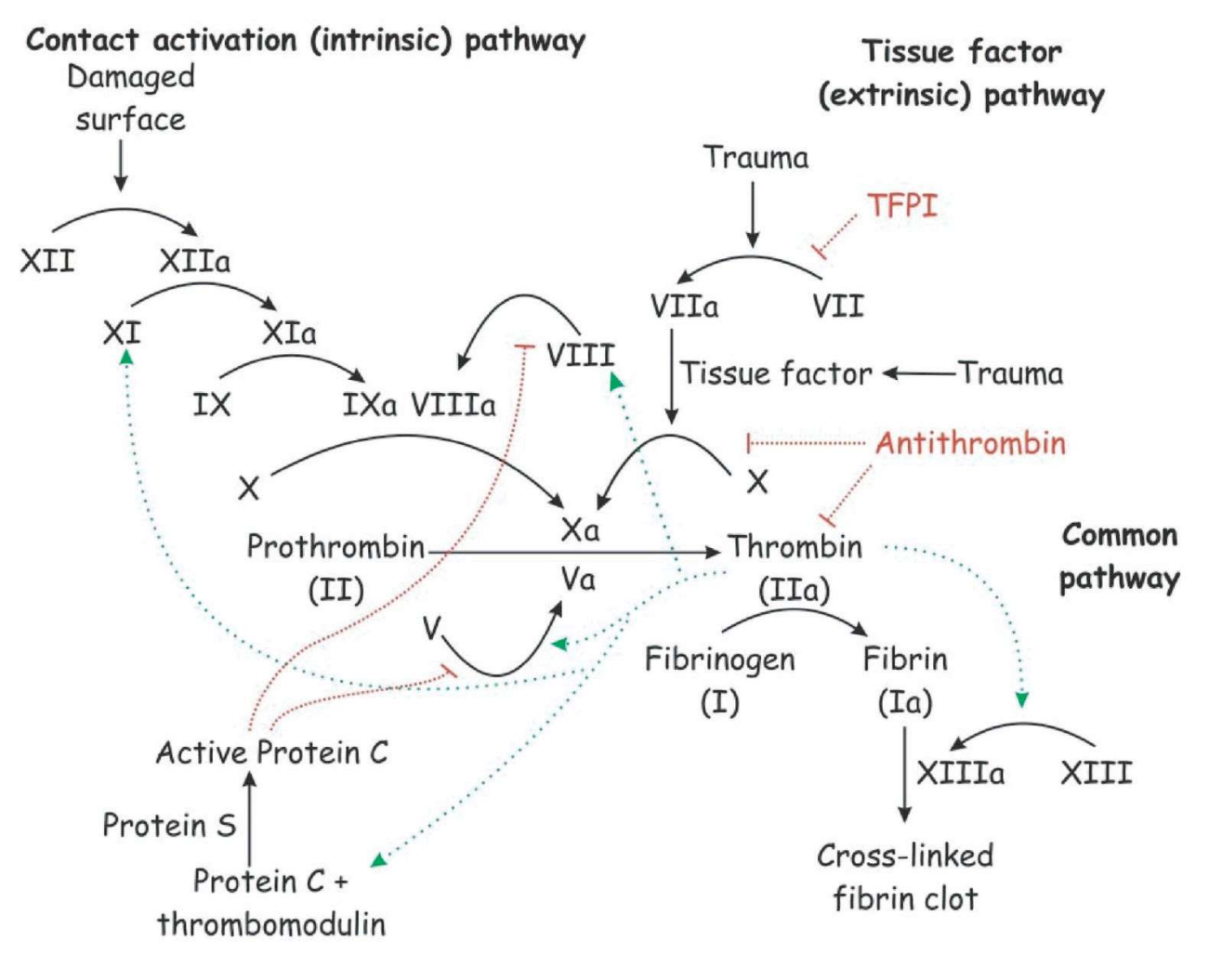
Most coagulation reactions have following components:

1) Activated enzyme – serine protease (IIa, VIIa, IXa, Xa, XIa, protein C, plasmin, tPA)
2) Cofactor – puts together the enzyme and the substrate (TF, Va, VIIIa, protein S, TM, fibrin)
3) Ca^{2+}
4) Negatively charged surface (fastens the reaction by increasing reactant concentration)
5) Substrate (other factor)

Exception: thrombin

- does not need a cofactor for most reactions

Coagulation cascade in vitro



Coagulation factors are usually serine proteases or their co-factors

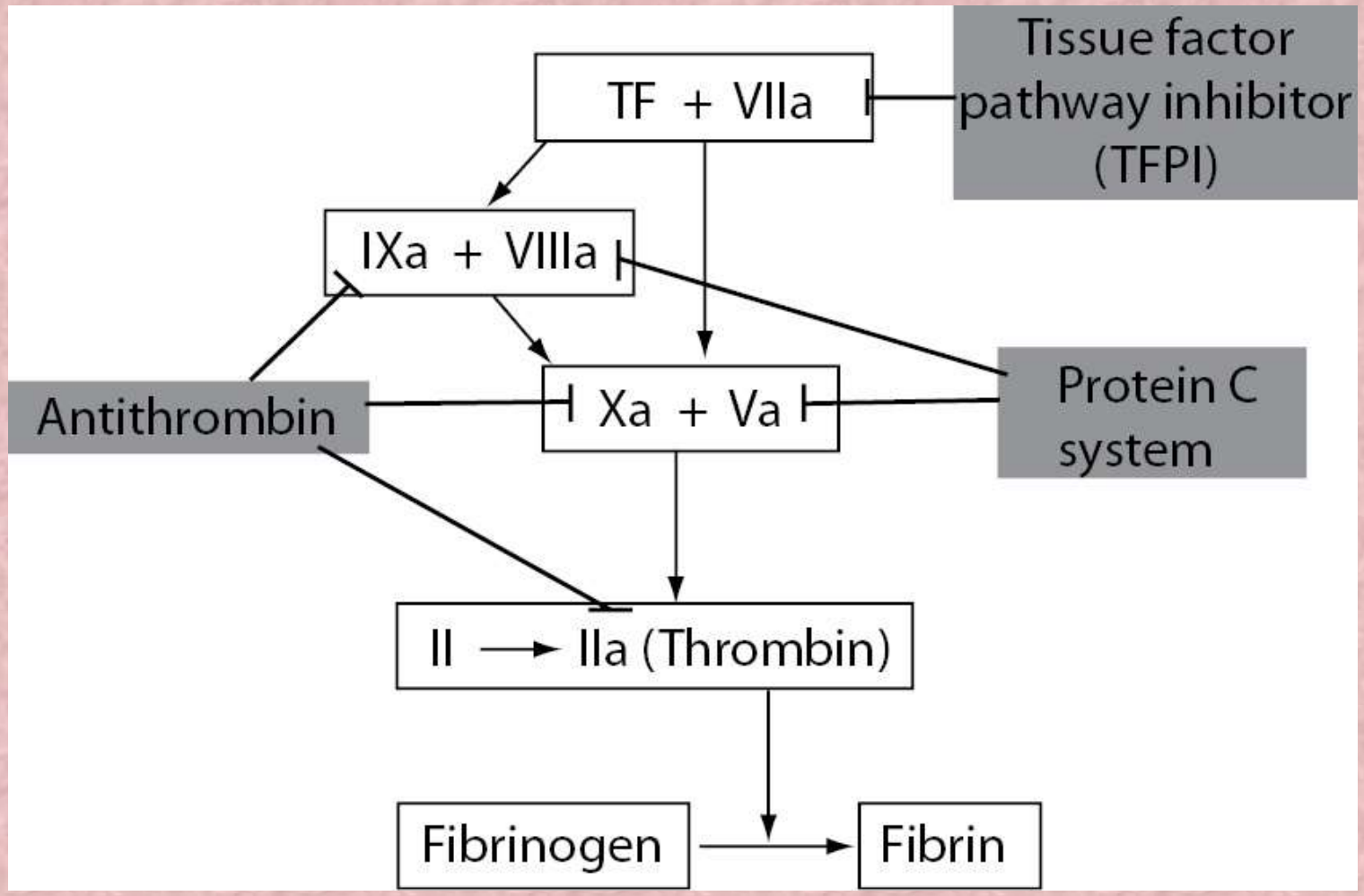
But: contact system is not necessary for the coagulation (but coagulation factors of intrinsic pathway starting by XI are)

Factor XII has ambivalent function – it also promotes fibrinolysis through plasmin activation

Contact system (HMWK, factor XII) also participates in the inflammatory response

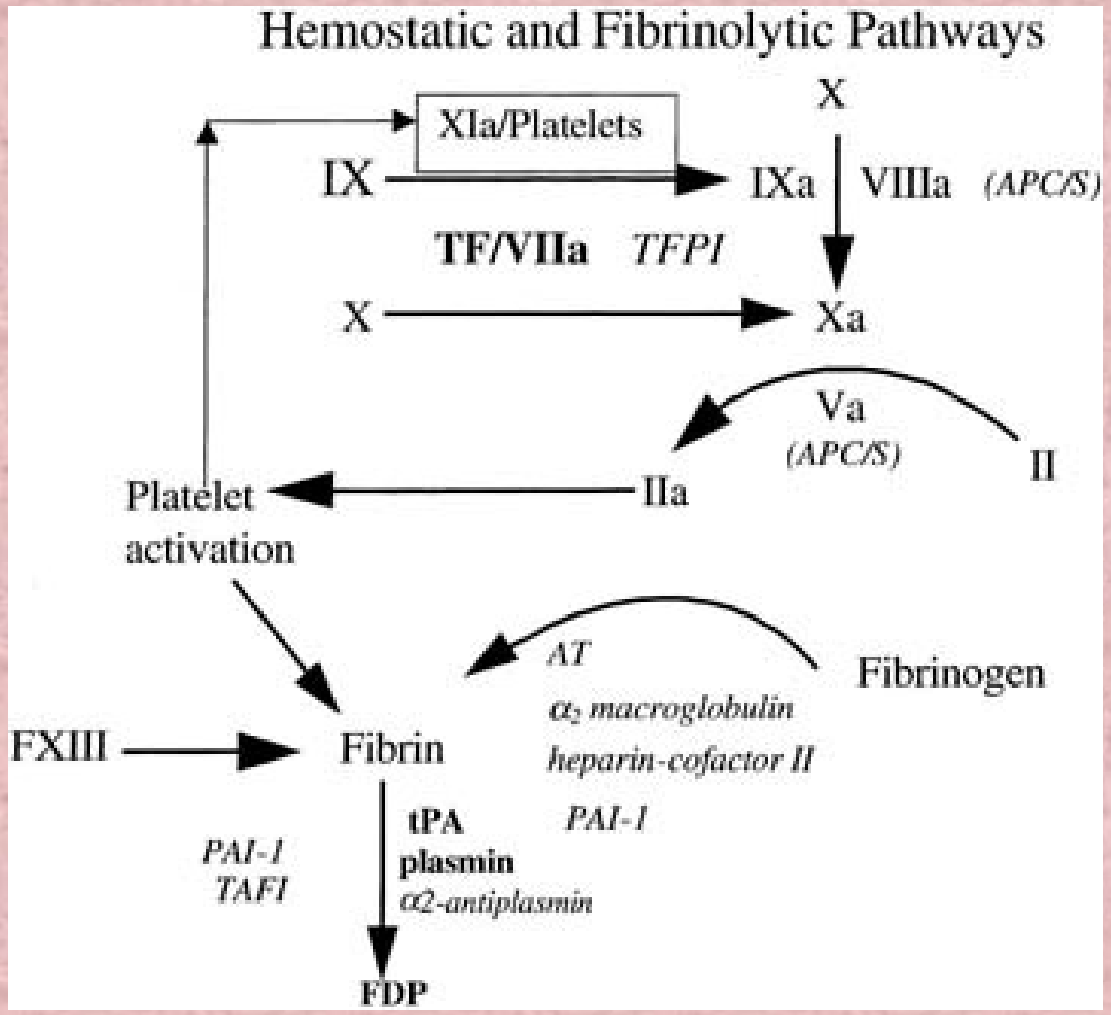
Current model in vivo

(cascade initiation)



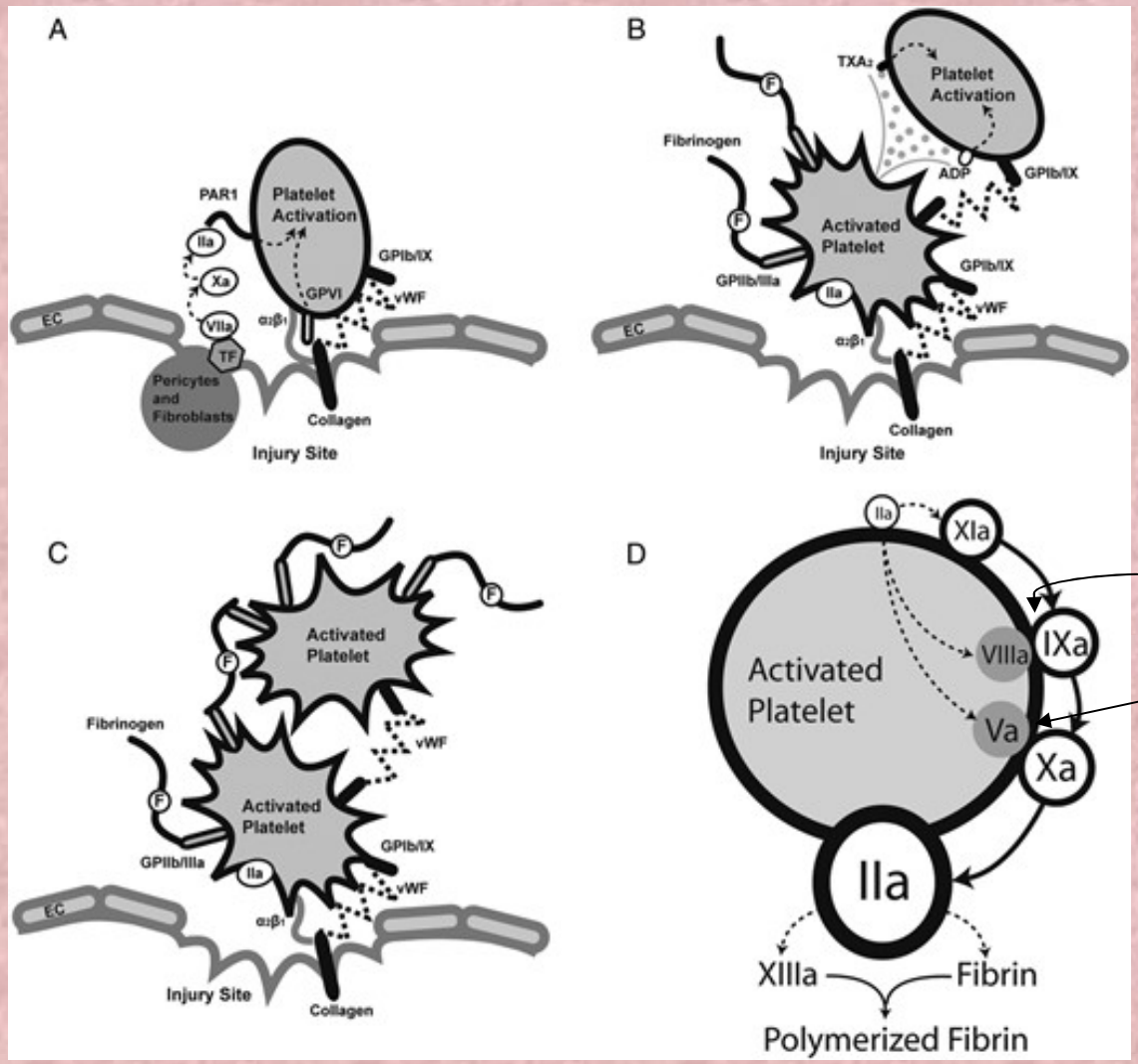
Role of factors VIII, IX and XI

(amplification/propagation phase)



Platelets in coagulation

initiation of coagulation and platelet activation



Activation of neighbouring platelets, thrombin formation

platelet aggregation

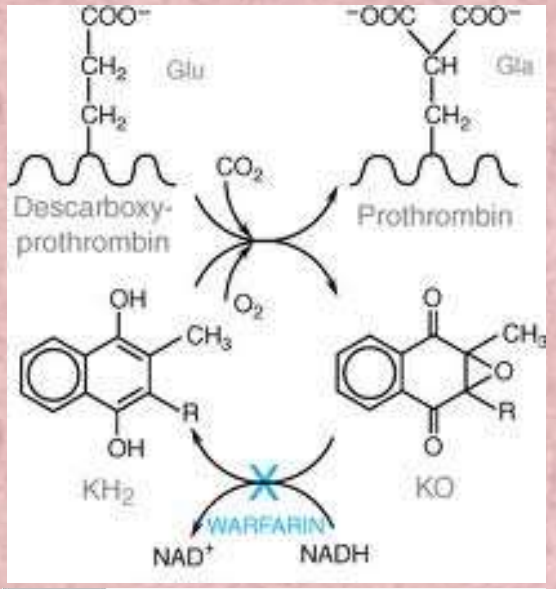
propagation of cogulation

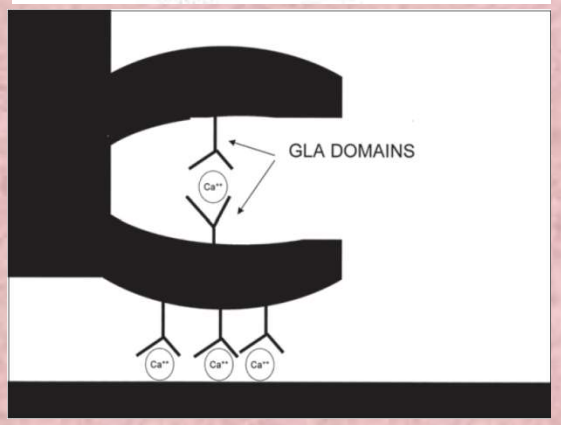
Tissue factor (TF, factor III)

- Membrane protein occuring in all cell types excluding the endothelium and circulating blood cells
- In normal conditions the TF does not come into a contact with coagulation cascade
- After endothelial damage (trauma, bacterial toxins…) TF reacts with factor VII and coagulation cascade is started
- To start the reaction, a small amount of activated factor VIIa is necessary (present in the circulation), which, when bound in a complex with TF, catalyses an activation of more VII-TF complexes
- Thromboplastin = TF + phospholipids; partial thromboplastin = phospholipids only

K-dependent factors and Ca^{2+} binding

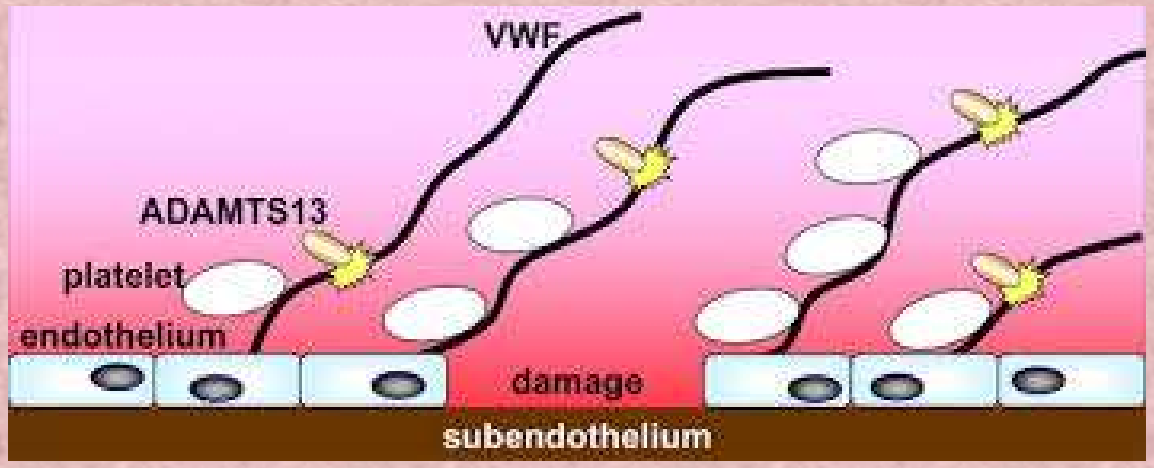
- Factors II, VII, IX, X and protein C possess gama-karboxyglutamic acid (Gla) domains at their N-terminus
- Gla is formed from glutamate using vitamin K as the oxidative agent
- Gla domains act as chelates and bind Ca^{2+}
- They bind the negatively charged phospholipid membranes and change the protein conformation





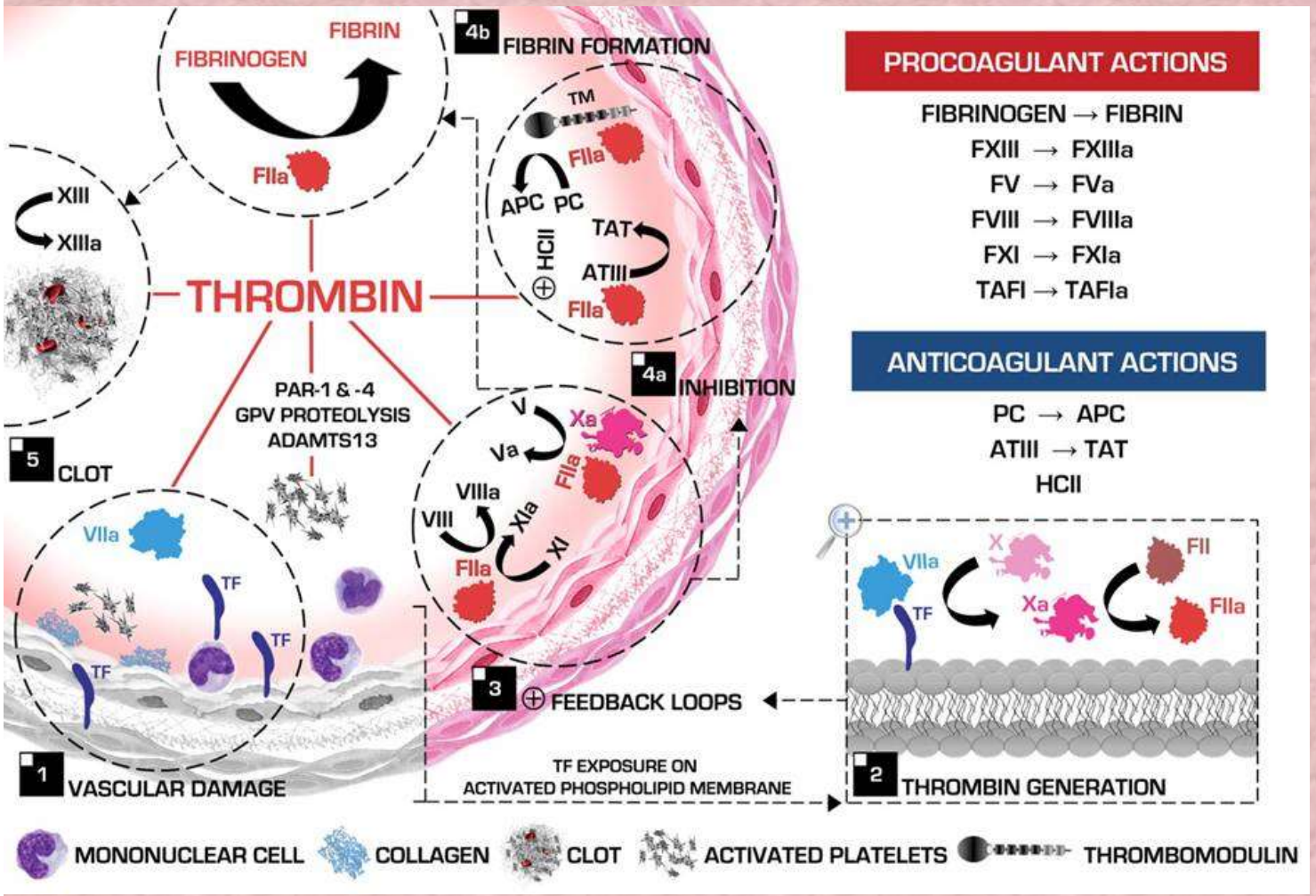
Factor VIII and vWF

- vWF is responsible for platelet adhesion
- It also serves as a plasmatic carrier of factor VIII, that is otherwise quickly degraded



ADAMTS 13 – protease cleaving vWF multimers

Thrombin functions



PROCOAGULANT ACTIONS

FIBRINOGEN → FIBRIN
FXIII → FXIIIa
FV → FVa
FVIII → FVIIIa
FXI → FXIa
TAFI → TAFIa

ANTICOAGULANT ACTIONS

PC → APC
ATIII → TAT
HCII

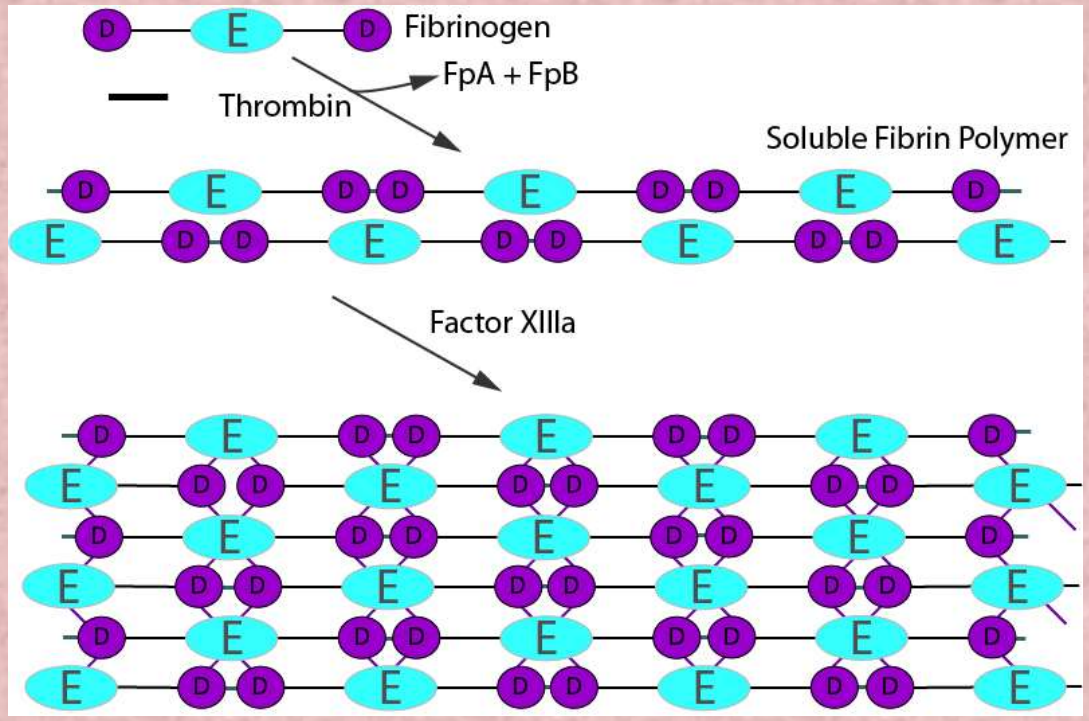
Legend:

TAFI – thrombin activatable fibrinolysis inhibitor
TM – thrombomodulin
PC – protein C
APC – activated PC
ATIII – antithrombin III
TAT – complex thrombin-ATIII
HCII – heparin cofactor II

Thrombin activates factors XI, VIII and V (Coagulation is thus maintained even when there is no more TF – propagation phase)

Fibrin

- Thrombin enables a polymerization of circulating fibrinogen into fibrin fibres by cleaving off the terminal polypeptides A and B (In the presence of Ca^{2+} the fibrin monomers spontaneously polymerize into a fibre)
- Transversal bridges between the fibres are formed by factor XIII (thrombin-activated)

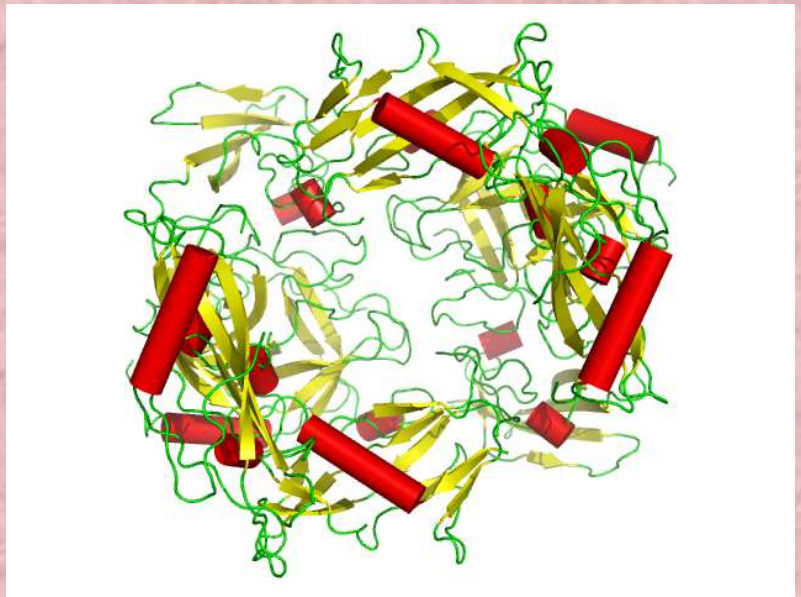


Coagulation inhibition

1) TFPI
   - produced by endothelium
   - forms a complex with factor Xa that is inhibited
   - the complex reacts with a complex TF-VIIa, which stops the formation of factors IXa and Xa through this pathway (feedback loop via factor XI continues)
2) Protein C with a cofactor protein S
   - it binds to factors Va and VIIIa that are cleaved and deactivated
   - protein C is activated by thrombin and thrombomodulin (TM, produced by endothelium
   - protein S is either free or bound to the transporter protein C4B
   - ratio between the free and bound protein S (normally approx. 40%) is a sensitive regulator of coagulation
3) Antithrombin III
   - inactivates all serine proteases of coagulation cascade
   - its effect is much strengthened by polysaccharides heparan and heparin (endogenous or exogenous)
   - heparin and related molecules are inhibited by platelet factor 4 (PF4) released from activated thrombocytes

Fibrinolysis

- Enabled by plasmin
  - serine protease
  - in the circulation, plasmin is present as inactive plasminogen
  - when converted into plasmin, it cleaves fibrin into fibrin degradation products (FDP)
  - out of FDP, D-dimers are important as the markers of fibrinolysis
- Plasminogen is activated by tissue plasminogen activator (tPA) – secreted by the endothelium, or by urokinase (UK, uPA) – secreted by the epithelium
- Plasmin is present in the blood clot bound to fibrin (fibrin also acts as a cofactor for tPA)

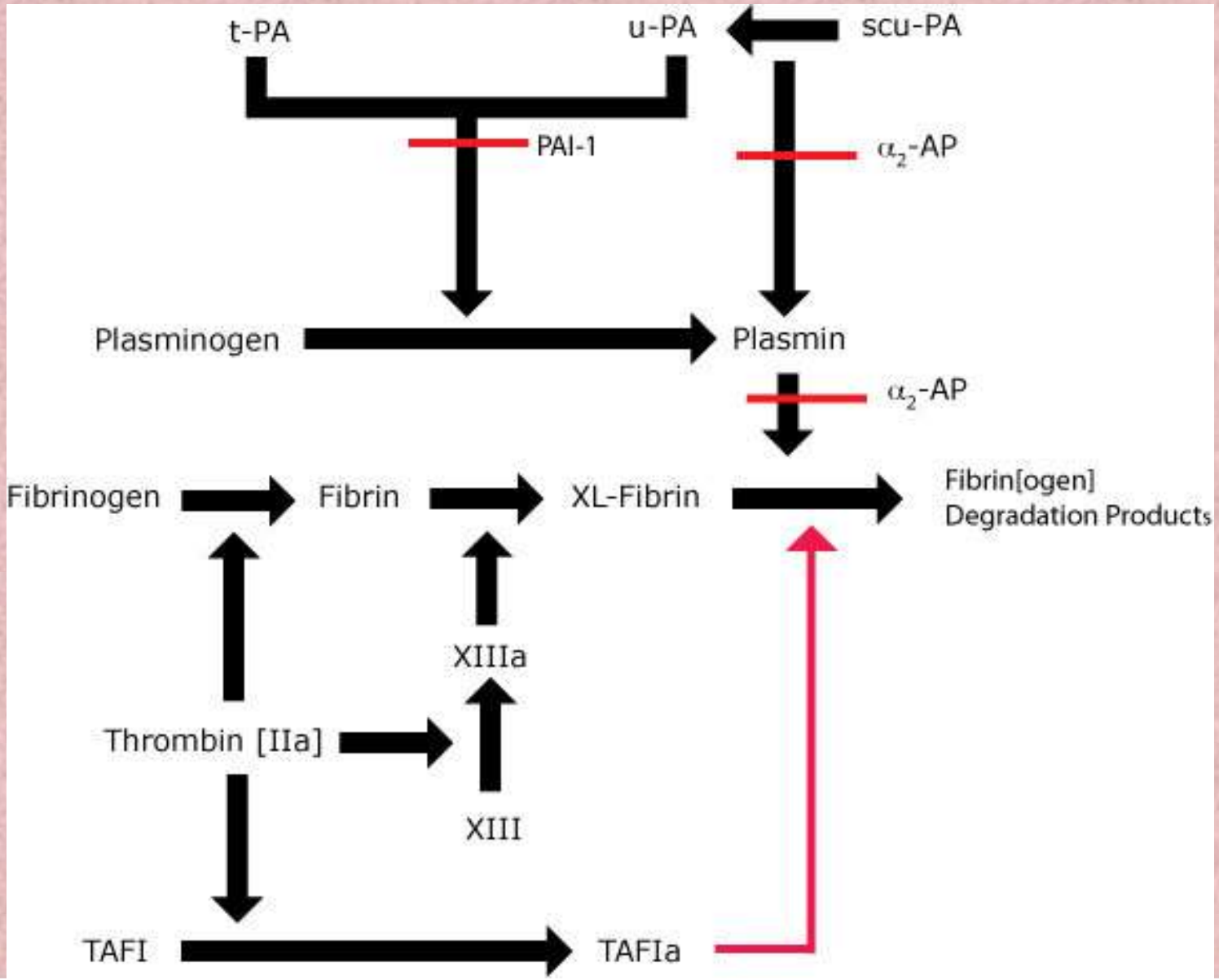


Plasmin molecule

Inhibition of fibrinolysis

- Plasminogen activator inhibitor 1 (PAI-1) – inhibits tPA
- α_2-antiplasmin – cleaves and deactivates free plasmin
  - But not the plasmin bound to fibrin
- Fibrinolysis is ensured by plasmin that is bound in blood clot and tPA from nearby endothelial cells
- In a case of plasmin or tPA leak into the circulation they are readily inactivated by PAI-1 and α_2-antiplasmin
  - Fibrinolysis stays restricted to the blood clot

Fibrinolysis - overview



Tests of coagulation cascade

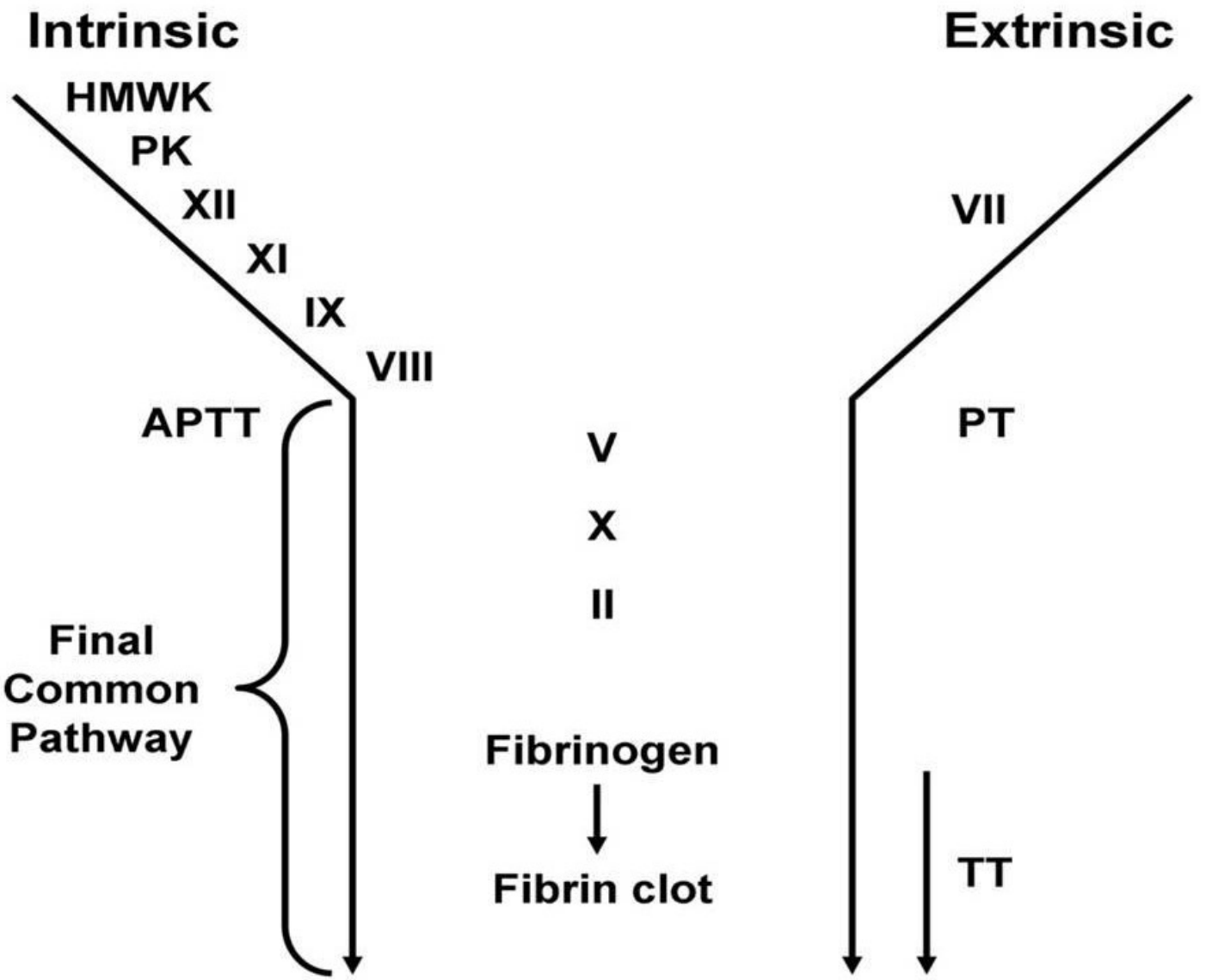
- PT – prothrombin time [s]*
  - TF (factor III) and Ca are added into decalcified blood plasm
  - PT measures the function of extrinsic pathway (it is often used for the monitoring of warfarin effect)
  - it is also known as Quick‘s test

- aPTT – activated partial thromboplastin time [s]
  - by adding Ca, kaolin (clay) and cephalin (phospholipid), factor XII is activated
  - aPTT measures the function of intrinsic pathway (it is often used for the monitoring of heparin effect)

- TT – thrombin time [s]
  - by adding thrombin and Ca, fibrinogen is activated
  - measure the conversion of fibrinogen into fibrin

*PT is often expressed as international normalized ratio (INR), dimensionless quantity

aPTT, PT, TT


Intrinsic
HMWK
PK
XII
XI
IX
VIII
APTT
Final
Common
Pathway
V
X
II
Fibrinogen
Fibrin clot
Extrinsic
VII
PT
TT

Practical

anesthesia

+ heparin 4U/kg

1) preparation of v. jugularis
– application of 2ml of hypotonic solution
2) laparotomy – v. cava caud. ligation
3) thoracotomy – puncture of heart chambers – collection of blood
4) excision of ligated segment

1) Weight of thrombus
2) aPTT

1) preparation of v. jugularis
– application of 2ml of hypotonic solution
2) laparotomy – v. cava caud. ligation
3) thoracotomy – puncture of heart chambers – collection of blood
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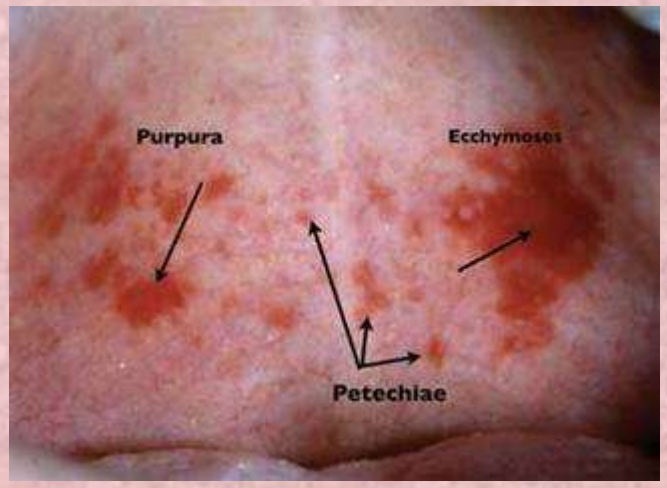
1) Weight of thrombus
2) aPTT

Disorders of hemostasis

- Hypocoagulation
  - Disorders of primary hemostasis
  - Disorders of secondary hemostasis (coagulopathies)
  - Combined disorders
- Hypercoagulation (thrombophilia)
- Combined hypo- and hypercoagulation (TTP, DIC)

Disorders of primary hemostasis

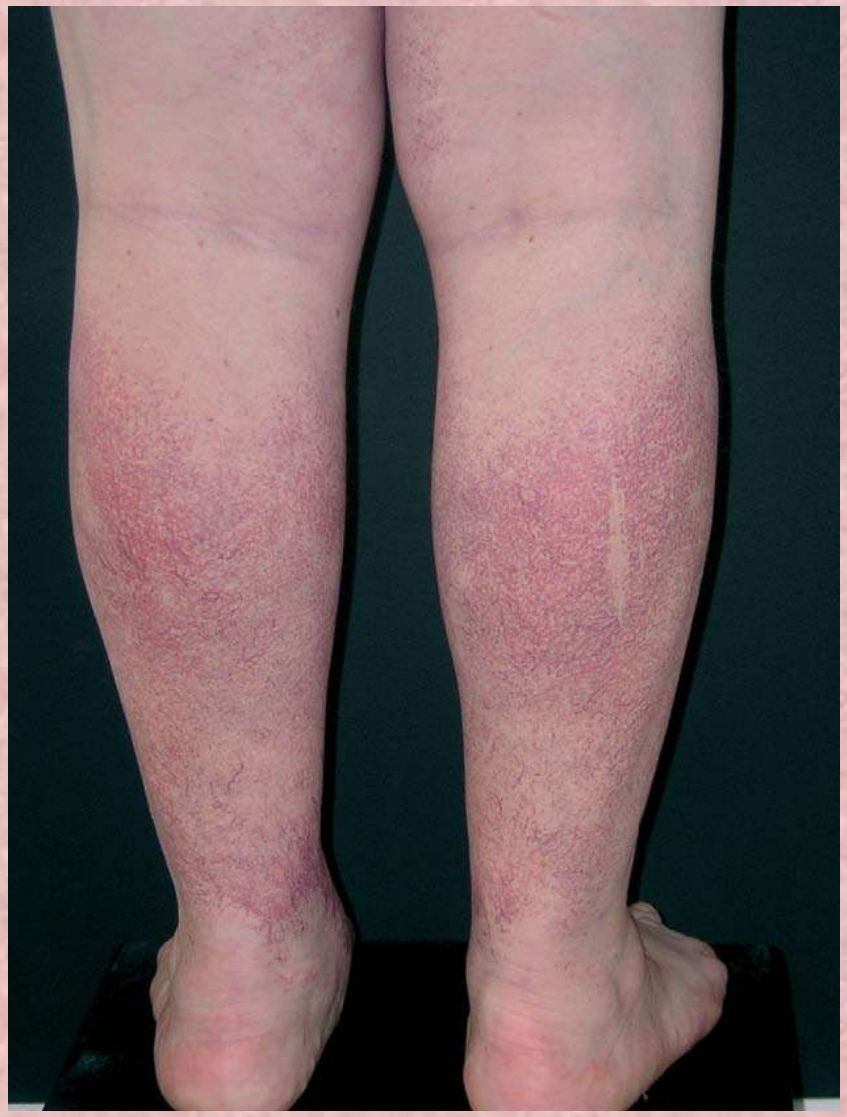
- Problems of either vessel wall (vasculopathies) or platelets (thrombocytopathies/thrombocytopenias)
- Clinically, they usually manifest by petechiae



- Prolonged bleeding time (but this is nonspecific)
- Often epistaxis, hematuria, menorrhagia, gingival bleeding or bleeding into GIT

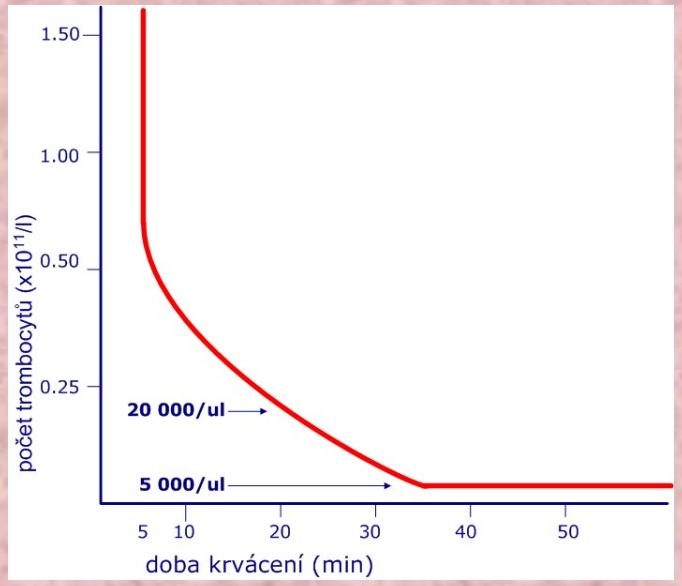
Vasculopathies - examples

- inborn
  - telengiectasia hereditaria (m. Rendu-Osler)
    - AD, attenuation of vessel wall segments → teleangiectasias (skin, mucosa, lungs, urogenital tract)
  - Ehlers-Danlos and Marfan syndrome
    - defect of connective tissue (colagen)
  - m. Kasabach-Merrit
    - Vascular malformations with blood stasis → DIC
- acquired
  - purpura senilis (fragile vessels)
  - bacterial toxins (scarlet fever, morbilli)
  - lack of vit. C (scorbut)
  - imunocomplexes (Henoch-Schönlein purpura)



Thrombocytopenia

- Normal concentration of the platelets is approx. 150 000-450 000/µl
- The thrombocytopenia is clinically manifest when the number of platelets is < 50 000/µl
- Below 20 000/µl spontaneus bleeding may occur
- Causes:
  1) Impaired production (aplasia, myelodysplasia → myelofibrosis)
  2) Increased consumption (TTP/HUS, DIC)
  3) Destruction by the immune system (ITP, systemic autoimmune diseases, drug-induced thrombocytopenia)
  4) Impaired distribution (hypersplenism)

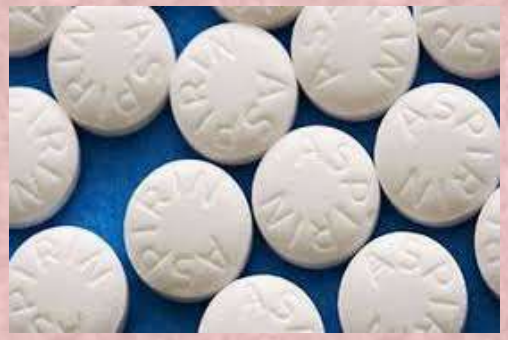


Thrombotic thrombocytopenic purpura (TTP)

- An example of thrombotic microangiopathy (together with hemolytic uremic syndrome, HELLP syndrome in pregnancy and drug-related HUS)
- Thrombotic occlusion of small arteries with following thrombocytopenia and hemolytic anemia is a common feature of thrombotic microangiopathies
- Often unclear pathogenesis
- In classical TTP, big vWF multimers play the key role – they can induce the aggregation without previous binding to collagen
- The vWF multimers are normally cleaved by ADAMTS13 protease
- Patients with classical TTP have antibodies against ADAMTS13

Thrombocytopathies

- Inborn:
- adhesion and aggregation disorders:
  - Bernard-Soulier syndrome (loss of function of GP Ib receptor)
  - Glanzmann thrombastenia (loss of function of GP IIb-IIIa receptor)
- Degranulation disorders
  - Heřmanský-Pudlák syndrome
  - Chédiak-Higashi syndrome
- Acquired:
- paraproteinemia (Ig inhibit fibrinogen binding to the thrombocytes)
- renal failure (guaidin succinate and phenol accumulation)
- dysfunction in myeloproliferative syndromes
- drug-induced thrombocytopathy (often this is actually the goal of the treatment)

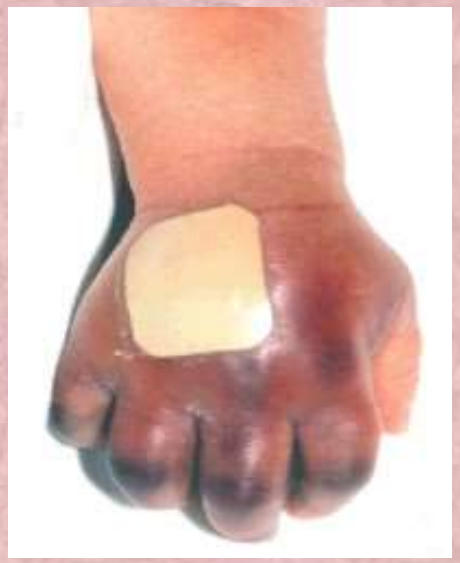


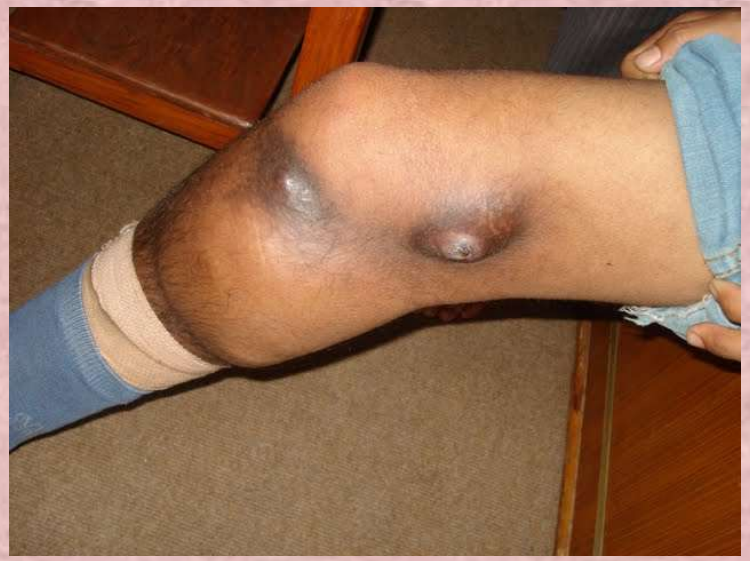
von Willebrand disease

- Either lack or dysfunction of plasmatic vWF
- Both primary and secondary hemostasis is affected
- Several types:
  - Type 1 – low circulating vWF
  - Type 2 – loss of function of vWF
    - several subtypes
    - In type 2N, vWF lacks the binding site for fVIII –same manifestation as hemophilia A
  - Type 3 – lack of vWF and factor VIII
  - Pseudo-vW disease – dysfunction (gain of function) of GP Ib → accelerated removal of circulated vWF

Disorders of secondary hemostasis

- Coagulation cascade dysfunction
- Clinically, they usually manifest by bleeding into body cavities, organs, retroperitoneum, joints, muscles





- Symptoms: joint deformities, nerve comperssion by a hematoma

Abundance and redundancy of coagulation factors (needed for normal values of coagulation tests)

Table 6.2. Rare factor deficiencies

Factor	Plasma Concentration	Level Needed for Hemostasis	Half-Life (hours)	Therapy
I	200-400 mg/dl	100 mg/dl	120	Cryoprecipitate
II	10 mg/dl	25%	50-80	Plasma
V	1 mg/dl	20-25%	24	Plasma, platelets
VII	0.05 mg/dl	15%	6	Plasma, rVIIA
VIII	0.01 mg/dl	100%	12	Concentrate, desmopressin
IX	0.3 mg/dl	100%	24	Concentrate
X	1 mg/dl	10-20%	25-60	Plasma, estrogens
XI	0.5 mg/dl	40-60%	40-80	Plasma
XIII	1-2 mg/dl	1-3%	150	Plasma
$Alpha_2$ antiplasmin	5-7 mg/dl	30% (?)	48	Antifibrinolytic agents
Plasminogen activator 1	0.005 mg/dl			Antifibrinolytic agents

DeLoughery et al., 2004

Hereditary coagulopathies

- hemophilia A (Xq-chromosome linked) – defective fVIII
  - fVIII is a cofactor in the activation of fX in a reaction catalyzed by fIXa
  - lowering the fVIII concentration down to >25% of normal level does not cause coagulopathy, lowering town to 25-1% - mild coagulopathy, <1% severe coagulopathy
  - >150 single nucleotide mutations in the fVIII gene – variable phenotype!!!
  - prevalence in the male population 1:5000 to 1:10000
- hemophilia B (Xq-chromosome linked) – defective fIX
  - ~10 times lower prevalence than hemophilia A
  - >300 mutations in the fIX gene (85% single nucleotide, 3% short deletions and 12% long deletions)
- defects of other factors
  - rare, usually autosomal recessive inheritance, clinically relevant in severe deficiency
    - hemophilia C (defective fXI) – frequent in Ashkenazy and Iraqi Jews, autosomal recessive, 2 causal mutations
      - » Unlike in hemophilia A and B, there is no clear correlation between the severity and levels of circulating fXI
    - dysfibrinogenemia (defective fI)
    - defective α_2-antiplasmin
    - etc…

Hémophilie A : **historique**

Prince Albert de Saxe-Koburg

Reine Victoria d'Angleterre (1819-1901)

Empereur d'Allemagne Frédéric III

Victoria d'Angleterre

Edouard VII

Louis IV Grand-duc de Hessen

Alice

Alfred

Arthur

Léopold d'Albanie (mort à 31 ans)

Hélène de Waldeck

Béatrice

Henri de Battenberg

Empereur Guillaume II

Prince Henri de Prusse

Irène de Hessen

Frédéric (mort à 23 ans)

Alexandra de Hessen

Tsar Nicolas II

Alice

Alexandre de Teck Athlone

Léopold (mort à 23 ans)

Moritz (mort à 23 ans par accident)

Victoire Eugénie de Battenberg

Alphonse XIII d'Espagne

Waldemer (mort à 46 ans)

Sigismund

Henri (mort à 4 ans)

? ? ? ? Tsarévitch Alexei (assassiné à l'âge de 13 ans)

? Rupprecht (mort à 21 ans suite à un accident de la route) ?

Alphonse Jaime (mort à 31 ans après un accident de voiture)

? ? Juan

Gonzales (mort à 20 ans après un accident de voiture)

Juan Carlos d'Espagne

Fille

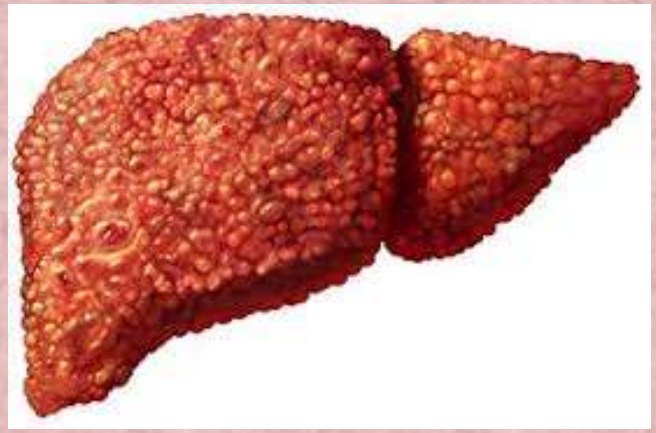
Garçon

Conductrice

Hémophile

Acquired coagulopathies

- Most often accompany the liver failure (coag. factors are synthesized in the liver – moreover the thrombocytopenia can occur as a result of thrombopoetin deficiency)



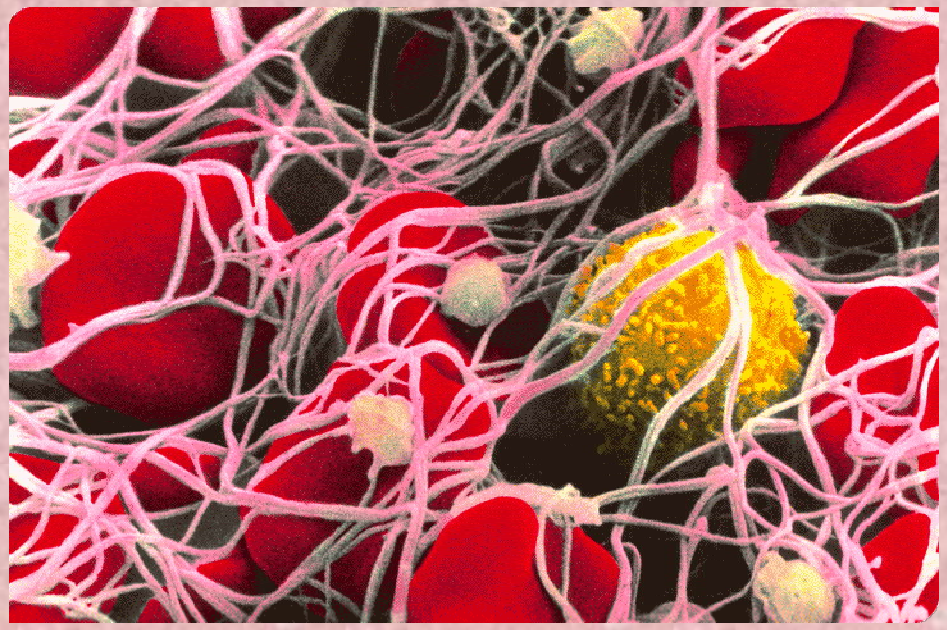
- Vitamin K malabsorption
- DIC
- Anticoagulant therapy (the overdose is particularly frequent in warfarin – inhibits the vitamin K reduction)

Disseminated intravascular coagulopathy (DIC)

- Combination of excessive and insufficient coagulation
- DIC is a consequence of excessive thrombin formation
- The process is usually started by the systemic exposition to TF
- 2 phases:
  1) Formation of microtrombi (with local ischemia)
  2) Bleeding as a result of consummation of coagulation factors

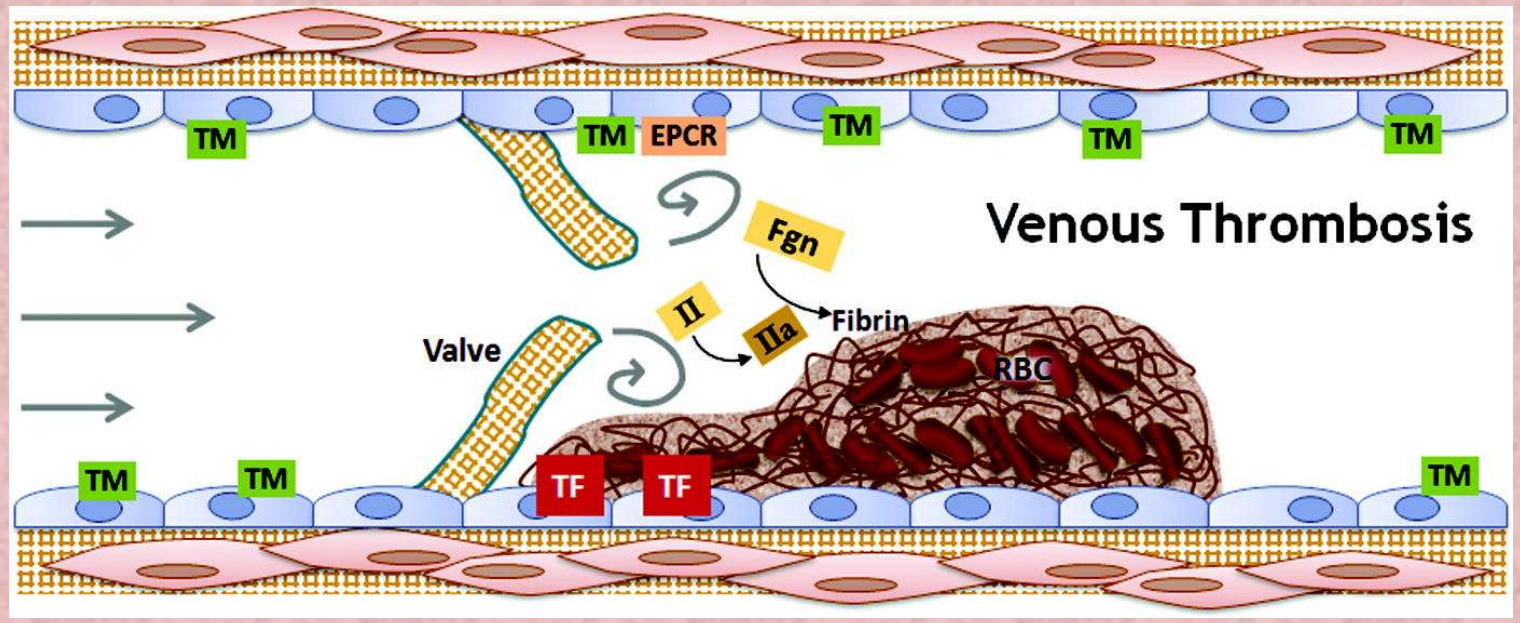
Causes of DIC

- Leucemia
- Solid tumours
- Infections, sepsis
- Shock
- Complication of pregnancy (embolisation of the amniotic fluid)
- Severe traumas
- Hemolysis
- Autoimmune diseases
- TTP/HUS
- M. Kasabach-Merrit



Hypercoagulation and thrombosis

- Pathological activation of hemostasis in vascular lumen or in heart chambers (X hemostatic plug)
- It can lead into the vascular occlusion locally and/or into the embolization and occlusion on distant sites
- When the thrombus occur in the venous system, it embolizes into the lungs



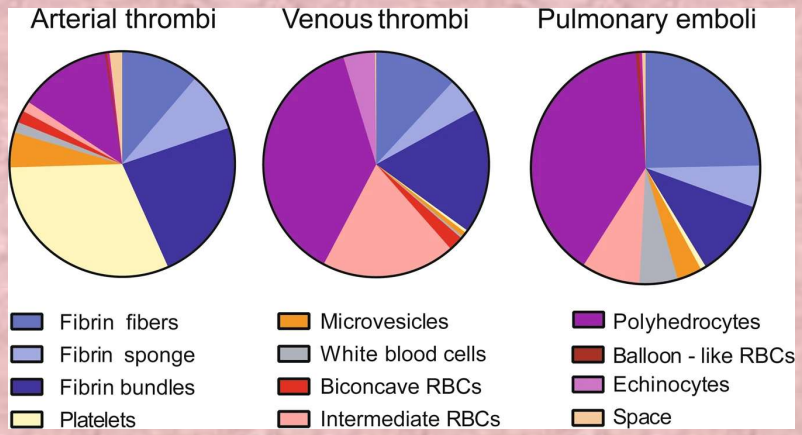
EPCR – endothelial protein C receptor

Red vs. white thrombus

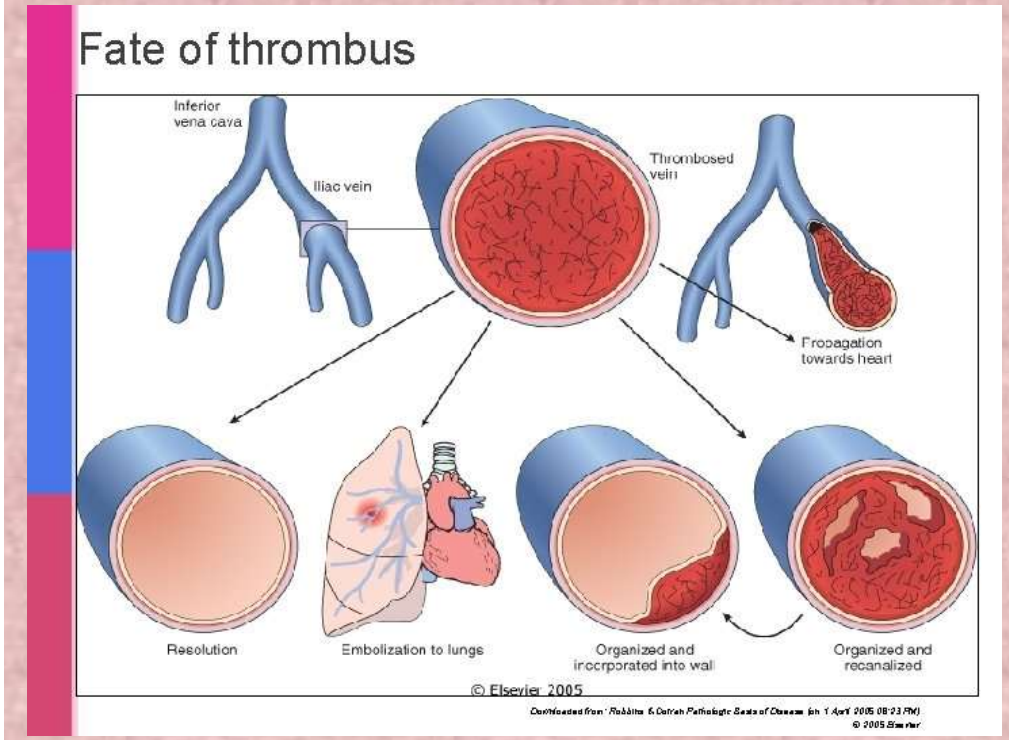
- Red
- dominance of secondary hemostasis
- rich for fibrin and erythrocytes
- blood stasis – veins, heart chambers, emboli
- prevention: mainly anticoagulants

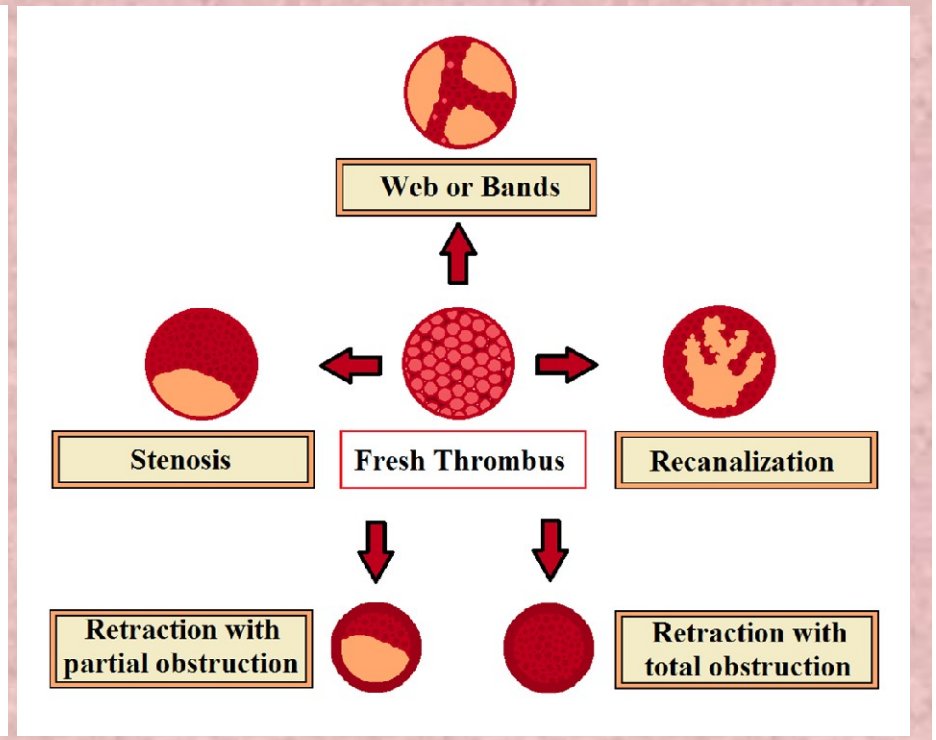
- White
- dominance of primary hemostasis
- Rich for platelets (but fibrin is relatively abundant, too)
- Arterial thrombi
- prevention: mainly antiplatelet drugs

- Mixed
- Arterial aneurysms



Fate of the thrombus





Virchow‘s triad

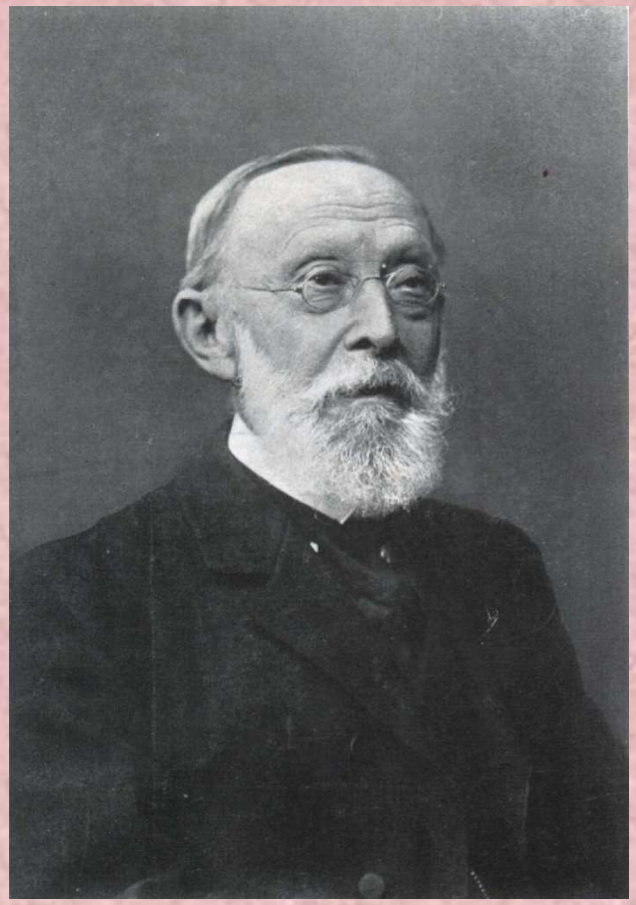
- Three main factors predisposing to thrombosis
  1) slowing of the blood flow

     - e.g. stasis during the immobilization, atrial fibrillation, heart failure

  2) Damage of the vessel wall

     - e.g. ruptured atherosclerotic plaque, artificial surfaces, endothelial damage - ↓trombomodulin

  3) Thrombophilic states



Rudolf Virchow (1821-1902), German pathologist and politician

Thrombophilic states

Inborn

- Protein C dysfunction (paradoxical hypercoagulation at the start of warfarin treatment – K-dependent !)
- Protein S dysfunction
- Resistance of factor V to protein C (Leiden mutation – most frequent hereditary thrombophilia)
- Antithrombin III dysfunction
- Dysfibrinogenemia
- Hyperhomocysteinemia (?)
- Antiphospholipid syndrome

Acquired

- Malignancies
- Post-operational states
- Hyperoestrogenous states (gravidity, peroral contraceptives)
- Heart failure
- Hyperviscosity (e.g. In polycytemia vera)
- Locally everything that leads into blood flow stasis or vessel wall damage

Hyperestrogenous states

Mechanisms

- ↑ endothelial production of vWF
- ↓ protein S
- ↑ coagulation factors
- Concommitant ↑tPA and TAFI (unknown effect)
- In combined treatment, the character of changes depends also on progesterone component

Effects

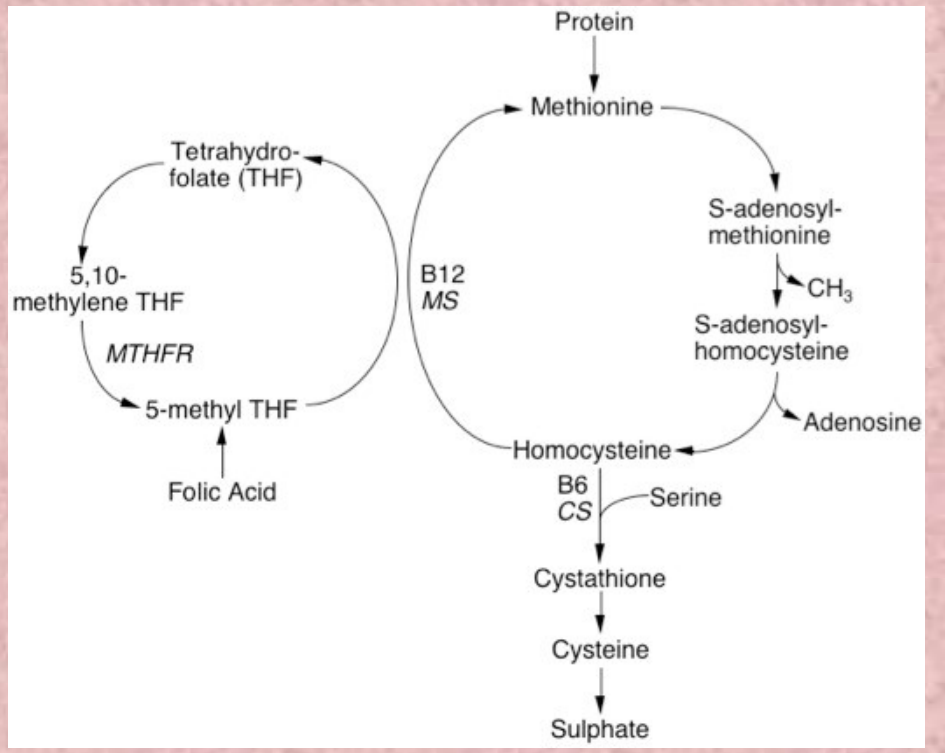
- ↑ risk of venous thrombosis
- Supraaditive risk in Leiden mutation (probably because of protein S – protein C – factor V interaction)
- Low impact on arterial thrombosis

Antiphospholipid syndrome

- increased risk of thrombosis associated with prolonged aPTT
- presence of antiphospholipid antibodies
- frequent abortions
- unclear pathogenesis
- possible mechanisms:
  - induction of TF expression in monocytes
  - platelet activation via Gp Ib
  - endothelial dysfunction (↓ TFPI, ↑ PAI-I)
  - decrease of protein C activity
  - β_2Gp I loss of function (inhibits fXIa generation by thrombin)

Hyperhomocysteinemia

- homocysteine is an intermediary product of methionine transformation in methionine cycle
  - homocysteine is either metabolized to cysteine
  - or it is remethylated back to methionine (in folate cycle)
- Hhcy can be induced by genetic and/or nutritional factors
  - mutations in enzyme-coding genes
  - low supply of vitamin B6, B12 and folic acid (B9)
- HHcy is an independent risk factor of atherosclerosis and thromboembolism, fertility disorders and some developmental and neurological disorders (vertebral clefts)
- But: those are probably mediated by endothelial dysfunction, not hypercoagulation
- Lowering homocysteine does not lead into lowering the thrombosis risk



Forms:

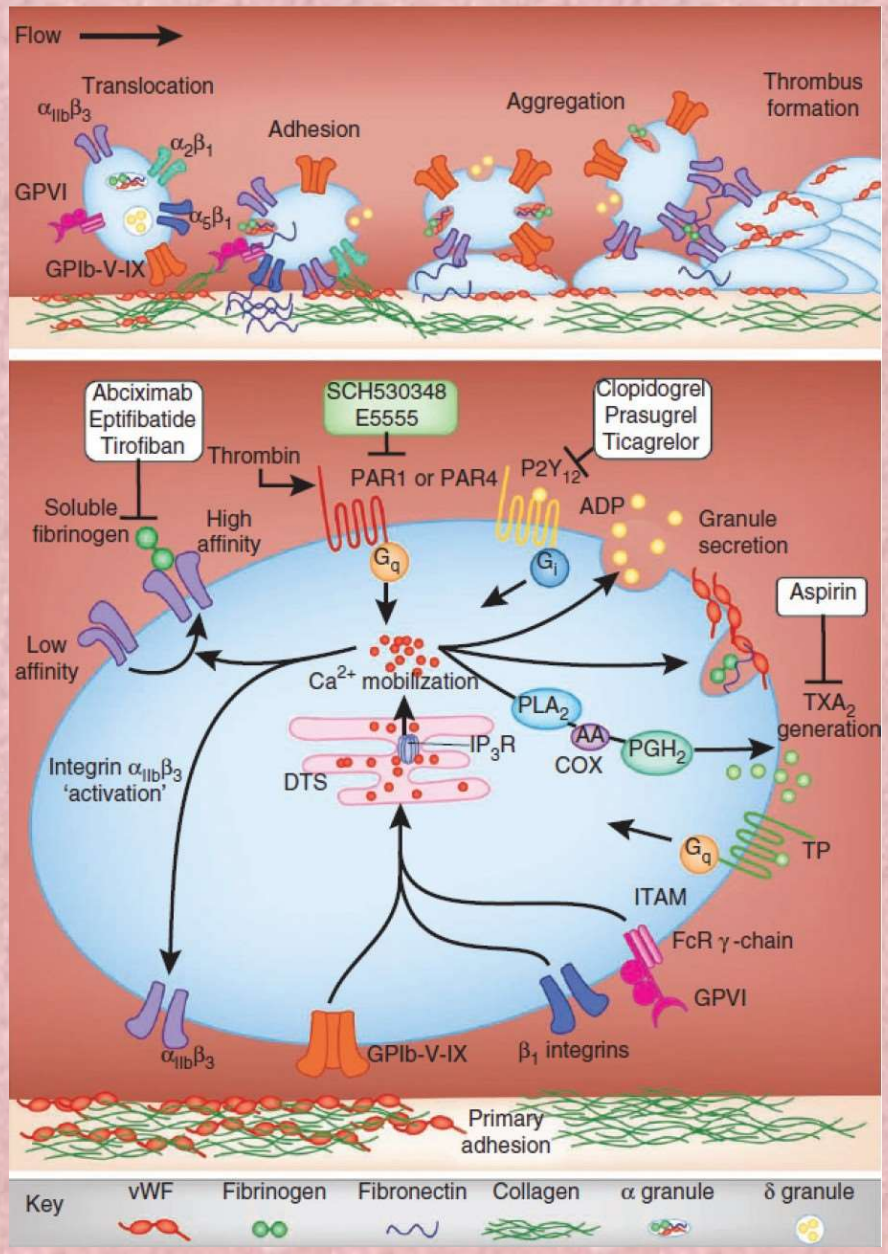
(A) monogenic homocystinuria
Deficiency of cystathionine-β-synthase leads into the marked increase of homocysteine levels in homozygotes, rare disease

(B) Mild hyperhomocysteinemia
Common polymorphism in methylene tetrahydrofolate reductase (MTHFR) gene

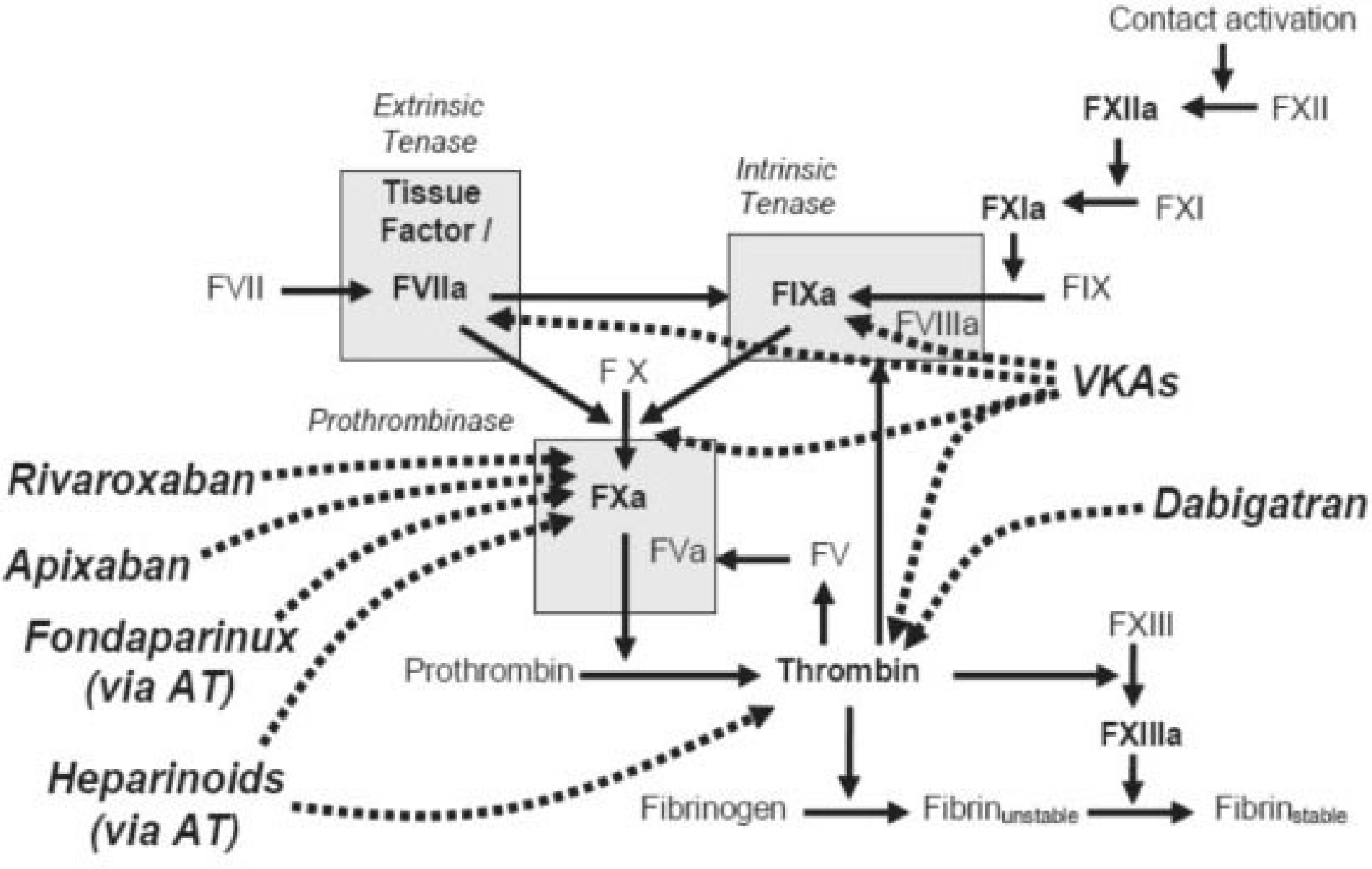
Treatment of insufficient hemostasis

- thrombocytes
- etamsylate (stimulates platelet activation)
- terlipressin (ADH derivative – vasoconstriction)
- frozen plasma
- coagulation factors
- vitamin K
- antifibrinolytics

Strategies of antiplatelet treatment



Strategies of anticoagulant treatment



Fibrinolytics

- Urokinase, streptokinase (act in all circulation)
- tPA (restricted to thrombus)
- reteplase (modified tPA)