

Catalysis: Blood Clotting



Blood clotting

Clotting is a process in which liquid blood is converted into a gelatinous substance that eventually hardens. The aim is to stop the flow of blood from a vessel.

The formation of a clot is the result of a series of enzymatic reactions that are triggered upon injury. The process involves 1) a step of activation (wounding) followed by 2) a cellular response (aggregation of blood platelets) and 3) a molecular response (polymerization of the protein called fibrin to create a

meshwork that hardens). Factors released in the cellular response help activate the molecular response. The process is highly conserved across species.

YouTube Lectures
by Kevin
[HERE](#) & [HERE](#)

Cellular response

Injury to the epithelial lining of a blood vessel begins the process of coagulation almost instantly. The cellular response has an initial action followed by an amplification step. In the cellular response ([Figure 4.68](#)), the platelets bind directly to collagen using Ia/IIa collagen-

binding surface receptors and glycoprotein VI to form a plug. The signal to the platelets to take this action is exposure of the underlying collagen, something that would not happen in the absence of a wound. Upon injury, platelet integrins get activated and bind tightly to the extracellular matrix to anchor them to the site of the wound.

The von Willebrand factor (see below also) assists by forming additional links between the platelets' glycoprotein Ib/IX/V and the fibrils of the collagen.

Amplification

In the amplification part of the cellular response, the activated platelets release a large number of factors, including platelet factor 4 (a cytokine stimulating inflammation and moderating action of the heparin anticoagulant) and thromboxane A₂. The latter has the effect of increasing the "stickiness" of platelets, favoring their aggregation. In addition, a G_q-protein linked receptor cascade is activated, resulting in release of calcium from intracellular stores. This will play a role in the molecular response.

Molecular response

The molecular response results in the creation of a web comprised of polymers of fibrin protein. Like the cellular pathway, the molecular pathway begins with an initiation phase and continues with an amplification phase. Polymerization of fibrin results from convergence of two cascading catalytic pathways. They are the intrinsic pathway (also called the contact activation pathway) and the extrinsic pathway (also referred to as the tissue factor pathway). Of the two pathways, the tissue factor pathway has recently been shown to be the more important.

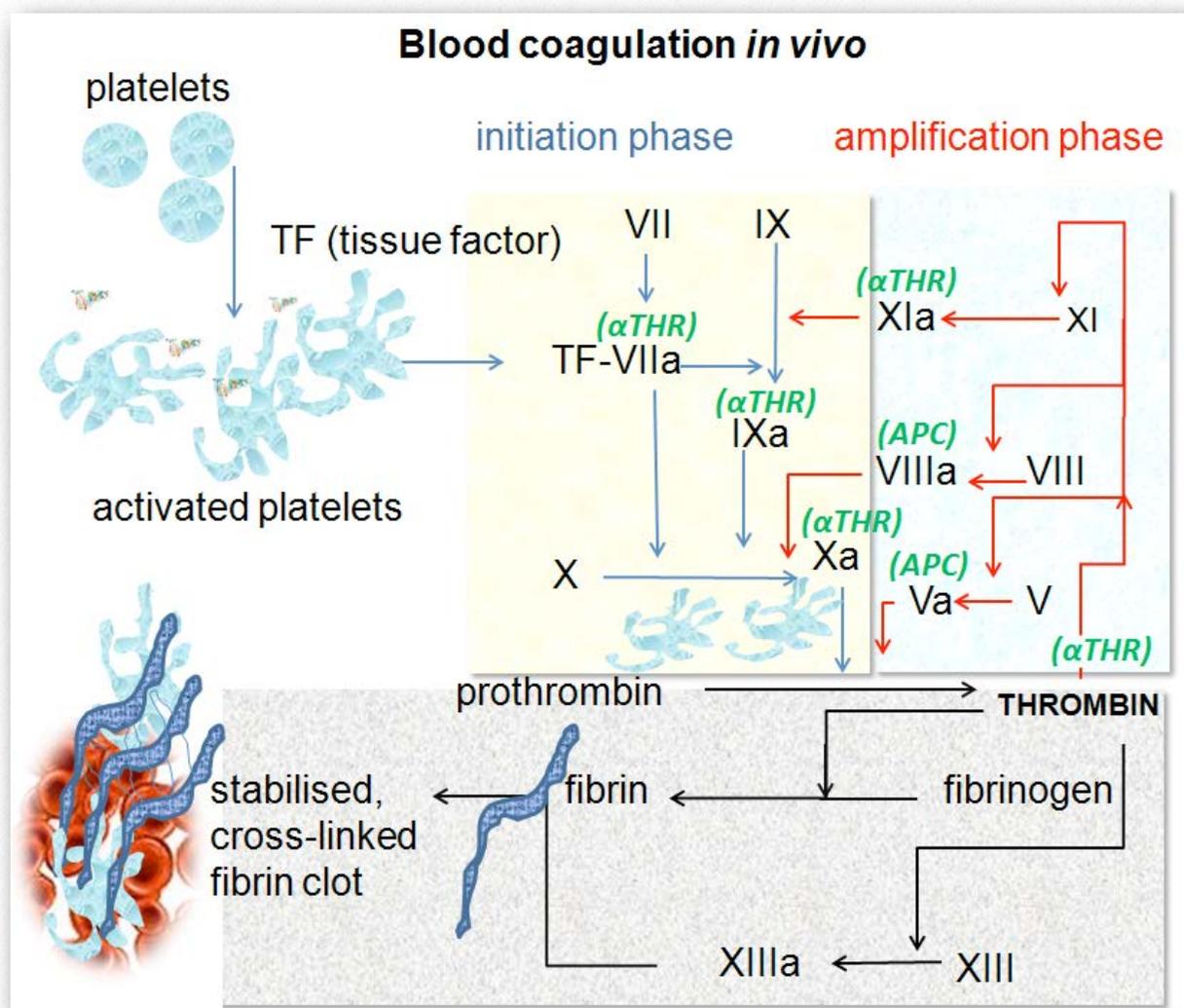


Figure 4.68 - Blood coagulation scheme. The cellular response is shown in white on upper left. Everything else is part of the molecular response

Wikipedia

Serine protease cascade

In both pathways, a series of zymogens of serine proteases are sequentially activated in rapid succession. The advantage of such a cascading system is tremendous amplification of a small signal. At each step of the cascade, activation of a zymogen causes the production of a considerable amount of an active serine protease, which is then able to activate the next zymogen which, in turn, activates an even larger amount of the next zymogen in the system. This results in the ultimate activation of a tremendous amount more fibrin than could be achieved if there were only a sin-

gle step where an enzyme activated fibrinogen to fibrin.

Nomenclature

A note about nomenclature - the zymogen factors in the molecular response are generally labeled with Roman numerals. A lower-case, subscripted 'a' is used to designate an activated form.

The tissue factor pathway functions to create a thrombin burst, a process in which thrombin is activated very quickly. This is the initiation phase. It is fairly straightforward because it has one focus - activation of throm-

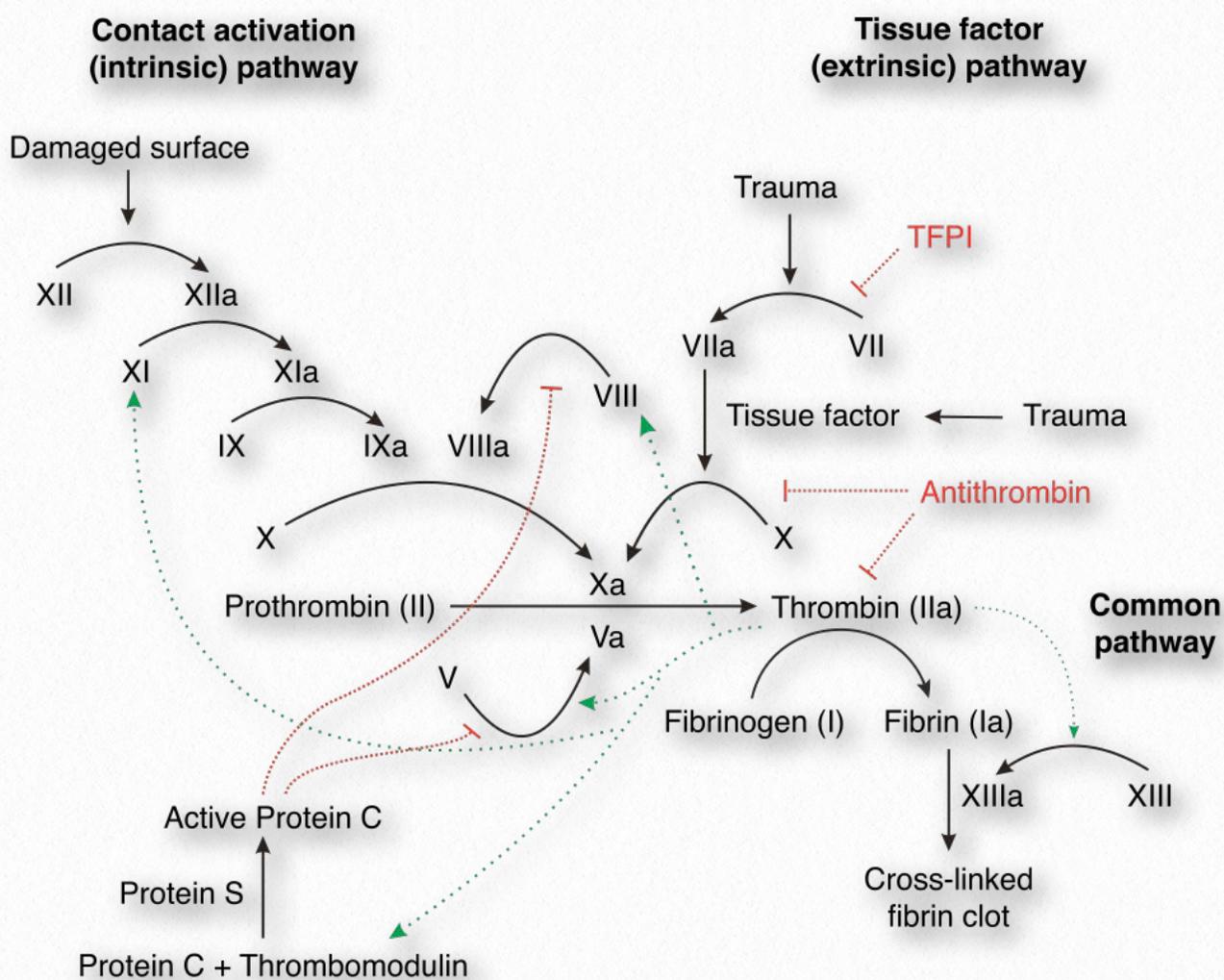


Figure 4.69 - Intrinsic and extrinsic pathways of blood coagulation. The aim is making a fibrin clot (lower right)

bin. Thrombin, which converts fibrinogen into the fibrin of the clot, is central also to the amplification phase, because it activates some of the factors that activate it, creating an enormous increase in signal and making a lot of thrombin active at once.

Initiation phase

The initiation phase of the molecular response begins when Factor VII (the letter 'F' before the Roman numeral is often used as an abbreviation for 'factor') gets activated to FVIIa after damage to the blood vessel (Figure 4.69 & 4.70). This happens as a result of its interac-

tion with Tissue Factor (TF, also called coagulation Factor III) to make a TF-FVIIa complex. The combined efforts of TF-FVIIa, FIXa, and calcium (from the cellular response) inefficiently convert FX to FXa. FXa, FV, and calcium inefficiently convert

prothrombin (zymogen) to thrombin (active). A tiny amount of thrombin has been activated at the end of the initiation phase.



Amplification phase

To make sufficient thrombin to convert enough fibrinogen to fibrin to make a clot,

thrombin activates other factors (FV, FXI, FVIII) that help to make more thrombin. This is the amplification phase of the molecular process and is shown in the light blue portion in the upper right part of Figure 4.68. The amplification phase includes factors in both the intrinsic and extrinsic path-

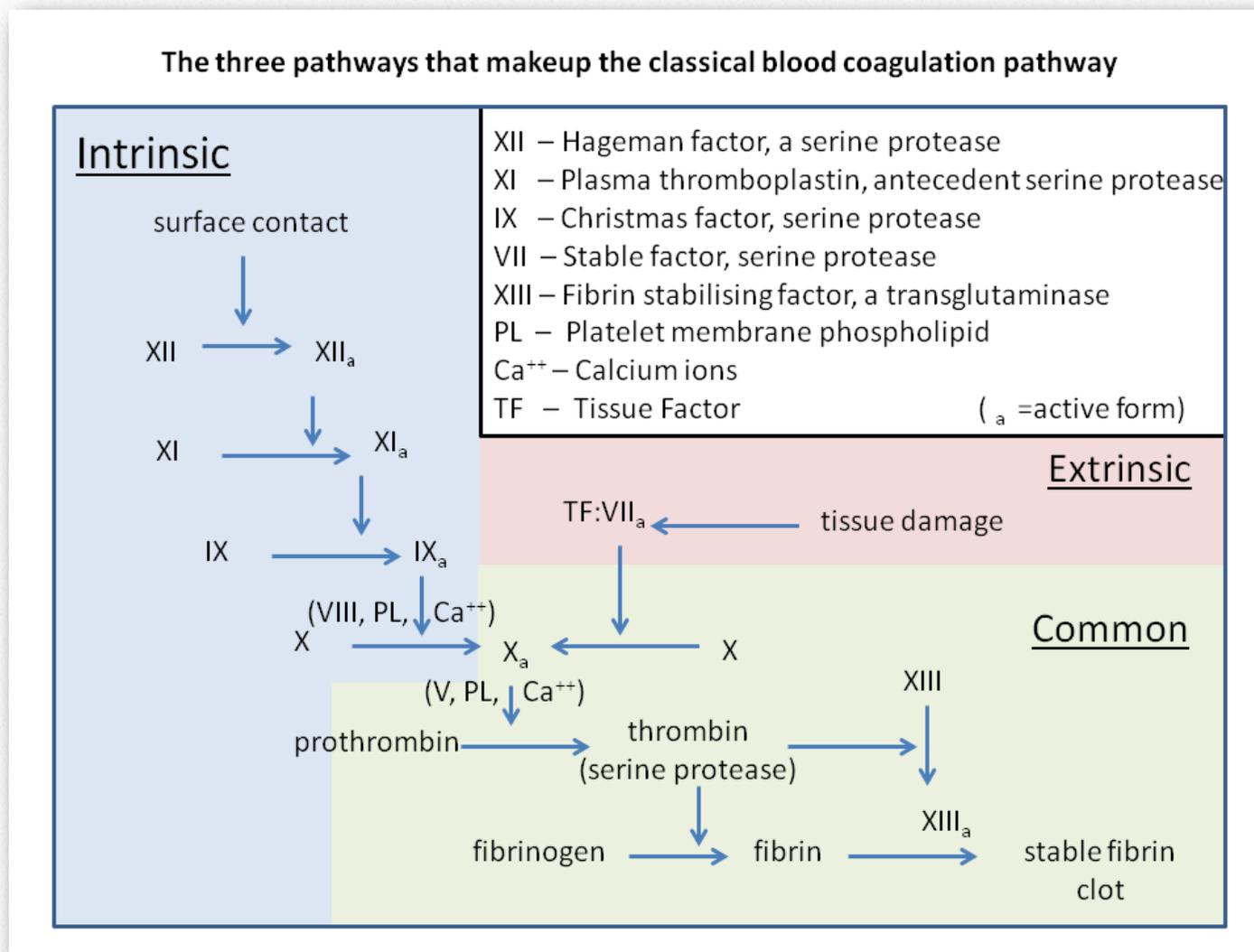


Figure 4.70 - Another view of the molecular response of the blood clotting pathway

Wikipedia

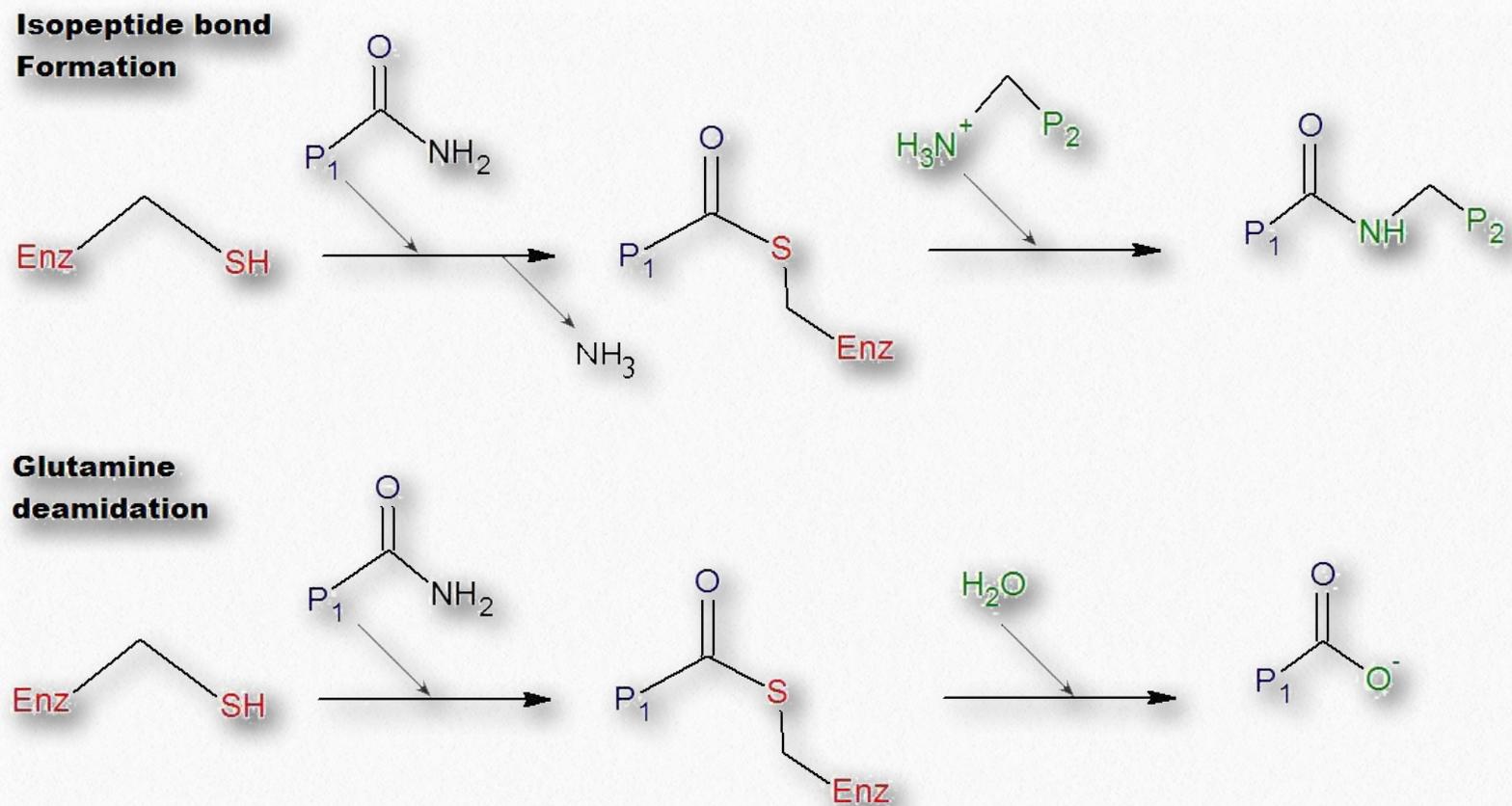


Figure 4.71 - Catalytic action of transglutaminase (top) and breaking of transglutamide bonds by hydrolysis (bottom)

ways. FVIII is normally bound in a complex with the von Willebrand factor and is inactive until it is released by action of thrombin. Activation of FXI to FXIa helps favor production of more FIXa. FIXa plus FVIIIa stimulate production of a considerable amount of FXa. FVa joins FXa and calcium to make a much larger amount of thrombin. Factors FVa and FVIIIa are critical to the amplification process. FVIIIa stimulates FIXa's production of FXa by 3-4 orders of magnitude. FVa helps to stimulate FXa's production of thrombin by about the same magnitude. Thus, thrombin stimulates activation of factors that, in turn, stimulate activation of more thrombin.

Transglutaminase

In addition to helping to amplify product of itself and conversion of fibrinogen to fibrin, thrombin catalyzes the activation of FXIII to FXIIIa. FXIIIa is a transglutami-

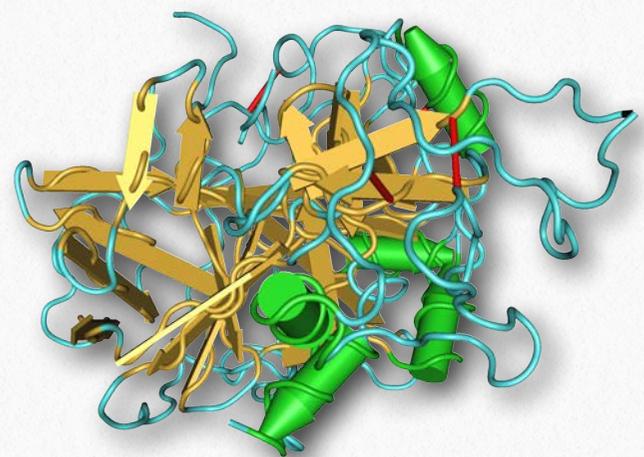


Figure 4.72 - α -thrombin

Wikipedia

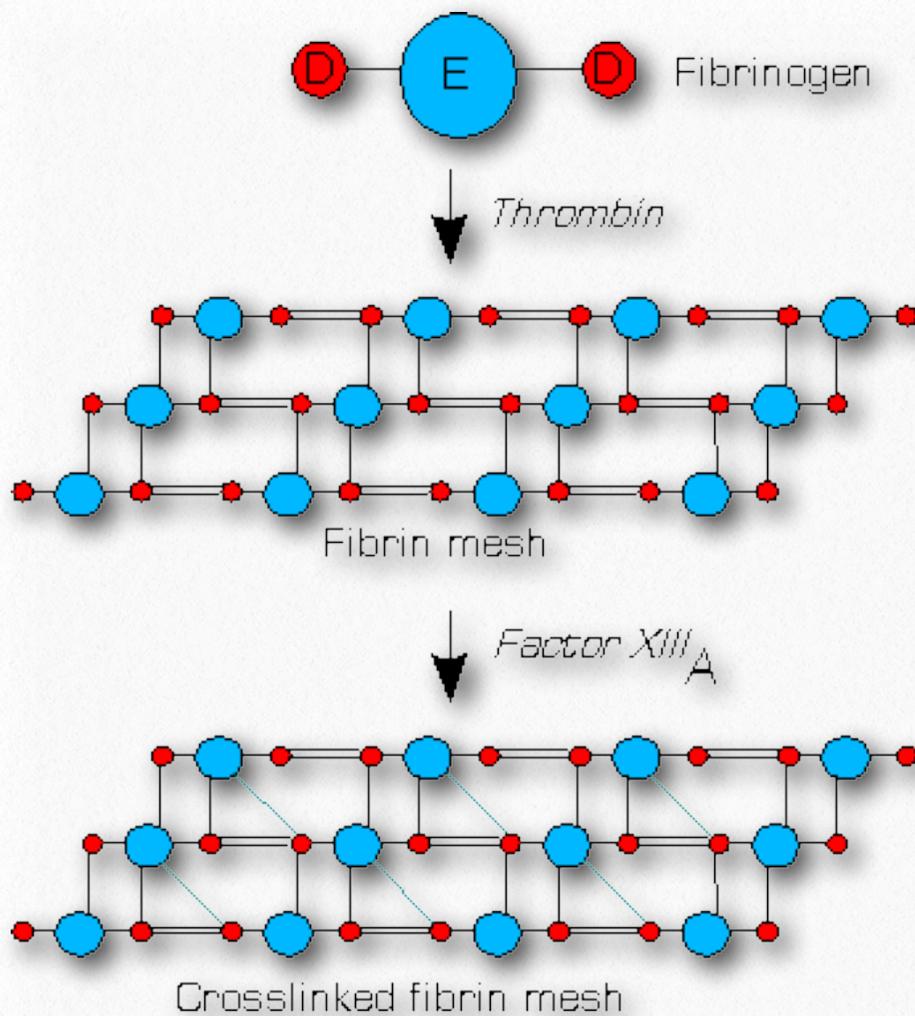


Figure 4.73 - Product of transglutaminase action - cross links

Wikipedia

nase that helps to “harden” the clot (Figure 4.71 & 4.73). It accomplishes this by catalyzing formation of a covalent bond between adjacent glutamine and lysine side chains in the fibrin polymers.

Not all of the factors involved in the clotting process are activated by the pathway, nor are all factors serine proteases. This includes FVIII and FV which are glycoproteins,

and FXIII, which is the transglutaminase described above.

The blood clotting process must be tightly regulated. Formation of clots in places where no damage has occurred can lead to internal clots (thrombosis) cutting off the flow of blood to critical regions of the body, such as heart or brain. Conversely, lack of clotting can lead to internal bleeding or, in severe cases, death due to unregulated external bleeding. Such is a danger for people suffering from hemophilia.

Hemophilia

Hemophilia is a hereditary genetic disorder affecting the blood clotting process in afflicted individuals. The disease is X-linked and thus occurs much more commonly in males. Deficiency of FVIII leads to Hemophilia A (about 1 in 5000 to 10,000 male births) and deficiency of FIX produces Hemophilia B (about 1 in 20,000 to 35,000 male births).

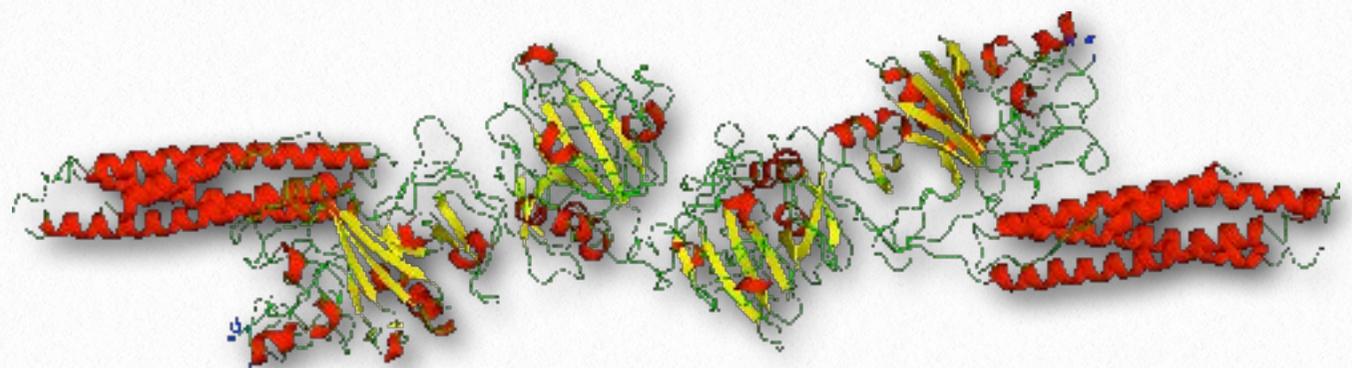


Figure 4.74 - Fibrin dimer - basic unit of a fibrin clot

Wikipedia

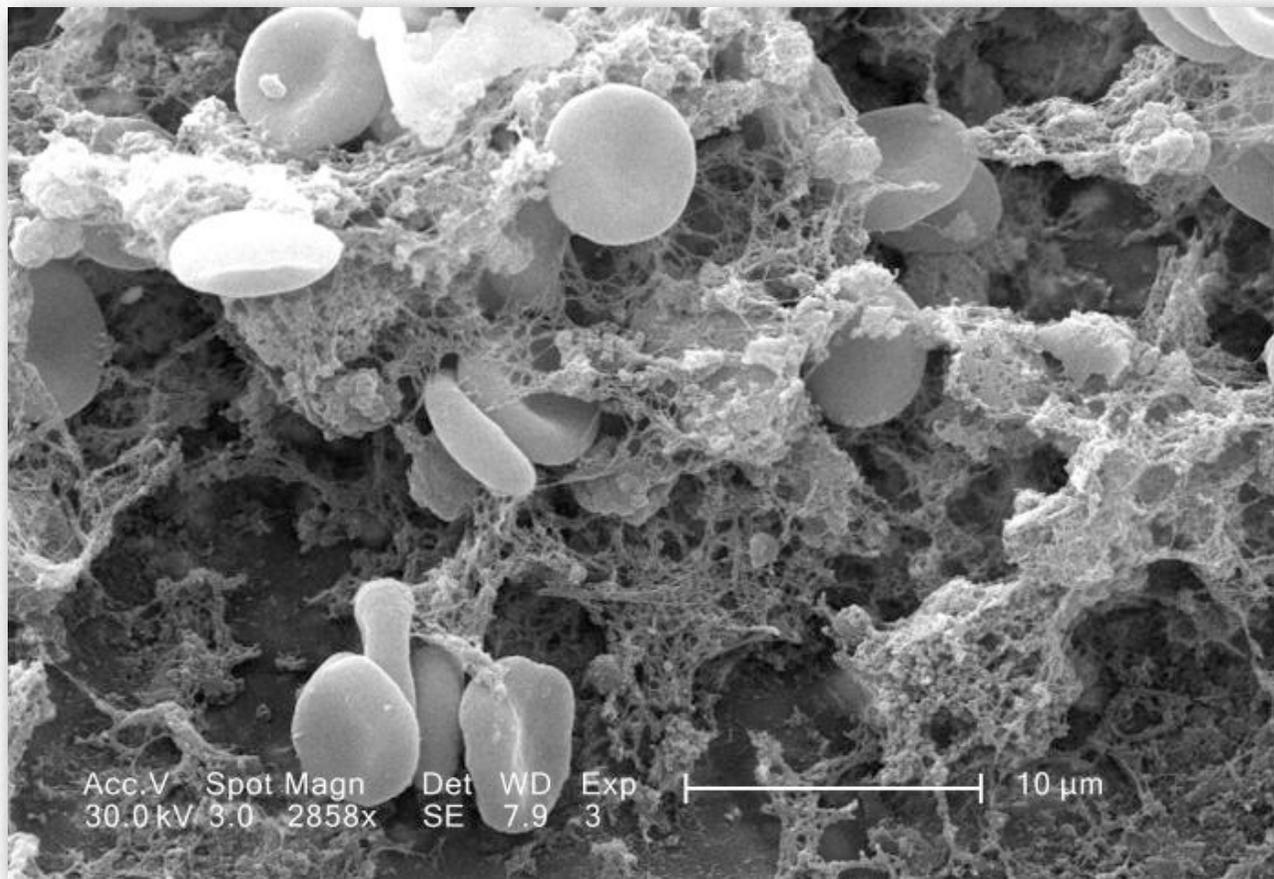


Figure 4.75 - Blood cells enmeshed in a fibrin clot

Hemophilia B spread through the royal families of Europe, beginning with Queen Victoria's son, Leopold. Three of the queen's grandsons and six of her great-grandsons suffered from the disease. Hemophilia is treated by exogenous provision of missing clotting factors and has improved life expectancy dramatically. In 1960, the life expectancy of a hemophiliac was about 11 years. Today, it is over 60.



Figure 4.76 - Queen Victoria, whose descendants suffered from hemophilia B

von Willebrand's disease

A related disease to hemophilia that is also genetically linked is von Willebrand's Disease. The von Willebrand factor plays a role in both the cellular and the molecular responses in blood clotting. First, the factor is a large multimeric glycoprotein present in blood plasma and also is produced in the endothelium lining blood vessels.

The von Willebrand factor helps to anchor platelets near the site of the wound in the cellular response. It binds to several things. First, it binds to platelets' Ib glycoprotein. Second, it binds to heparin and helps moderate its action. Third, it binds to collagen and fourth, the factor binds to FVIII in the molecular response, playing a protective role for it. In the absence of the von Wille-

brand factor, FVIII is destroyed. Fifth, the von Willebrand factor binds to integrin of platelets, helping them to adhere together and form a plug. Defects of the von Willebrand factor lead to various various bleeding disorders.

Blood “thinners”

The clotting of blood is essential for surviving wounds that cause blood loss. However, some people have conditions that predispose them to the formation of clots that can lead to stroke, heart attack, or other problems, like pulmonary embolism. For these people, anti-clotting agents (commonly called blood thinners) are used to reduce the likelihood of undesired clotting.

The first, and more common of these is aspirin. Aspirin is an inhibitor of the production of prostaglandins. Prostaglandins are molecules with 20 carbons derived from arachi-

donic acid that have numerous physiological effects. Metabolically, the prostaglandins are precursors of a class of molecules called the thromboxanes. Thromboxanes play roles in helping platelets to stick together in the cellular response to clotting. By inhibiting the production of prostaglandins, aspirin reduces the production of thromboxanes and reduces platelet stickiness and the likelihood of clotting.

Vitamin K action

Another approach to preventing blood clotting is one that interferes with an important molecular action of Vitamin K. A pro-clotting factor found in the blood, vitamin K is necessary for an important modification to prothrombin and other blood clotting proteins. Vitamin K serves as an enzyme cofactor that helps to catalyze addition of an extra carboxyl group onto the side chain of gluta-

mic acid residues of several clotting enzymes (see [HERE](#)). This modification gives them the ability to bind to calcium ([Figure 4.77](#)), which is important for activating the serine protease cascade. During the reaction that adds carboxyl groups to glutamate, the reduced form of vitamin K becomes oxidized. In

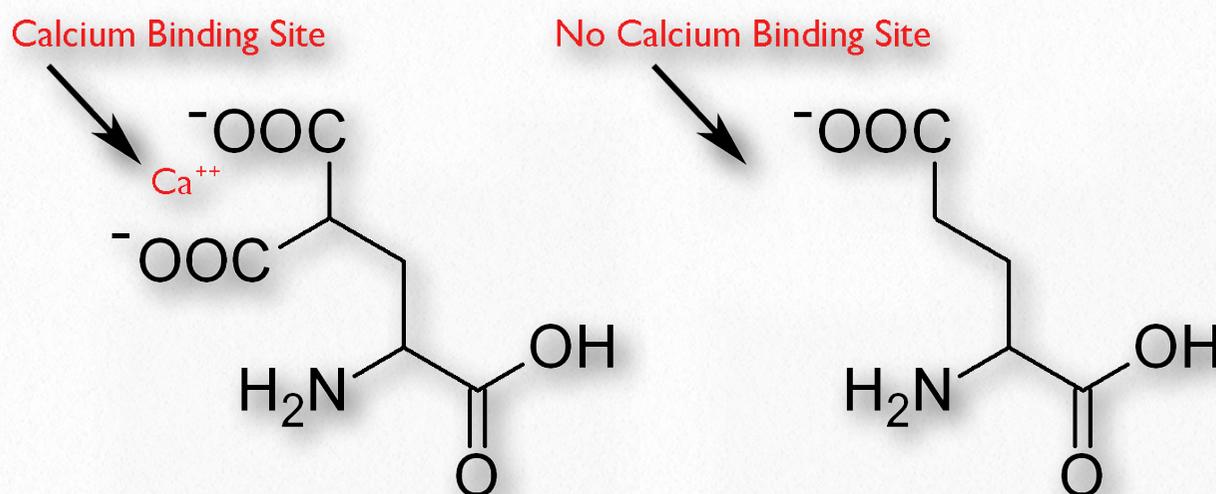


Figure 4.77 - γ -carboxylglutamic acid (left) has a calcium binding Site. Unmodified glutamic acid (right) does not.

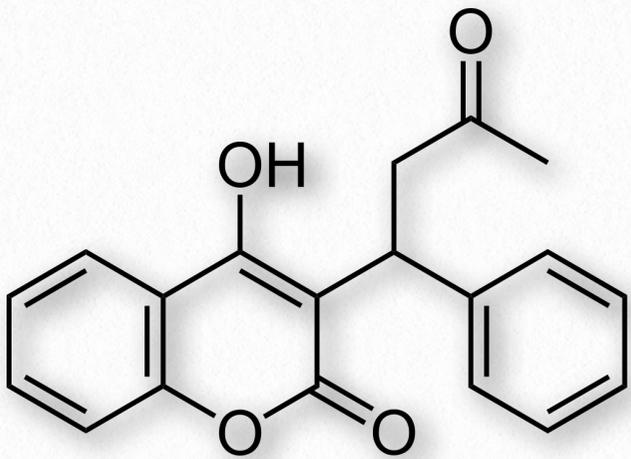


Figure 4.78 - Warfarin

order for vitamin K to stimulate additional carboxylation reactions to occur, the oxidized form of vitamin K must be reduced by the enzyme vitamin K epoxide reductase.

Warfarin blocks reduction

The compound known as warfarin (brand name = coumadin - [Figure 4.78](#)) interferes with the action of vitamin K epoxide reductase and thus, blocks recycling of vitamin K. As a consequence, fewer prothrombins (and other blood clotting proteins) get carboxylated, and less clotting occurs.

Vitamin K-mediated carboxylation of glutamate occurs on the γ carbon of the amino acid's side chain, for 16 different proteins, 7 of which are involved in blood clotting, including prothrombin. When the carboxyl group is added as described, the side chain is able to efficiently bind to calcium ions. In the absence of the carboxyl group, the side chain will not bind to calcium. Calcium released

near the site of the wound in the cellular response to clotting helps to stimulate activation of proteins in the serine protease cascade of the molecular response.

Vitamin K comes in several forms. It is best described chemically as a group of 2-methyl-1,4-naphthoquinone derivatives. There are five different forms recognized as vitamin Ks (K_1 , K_2 , K_3 , K_4 , and K_5). Of these, vitamins K_1 and K_2 come from natural sources and the others are synthetic. Vitamin K_2 , which is made from vitamin K_1 by gut microorganisms, has several forms, with differing lengths of iso-

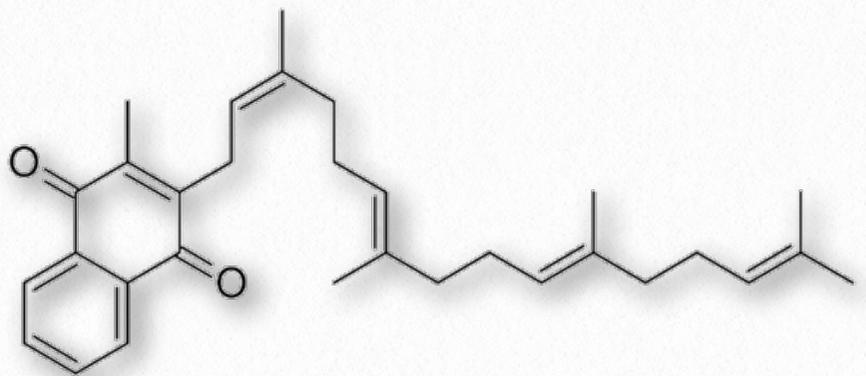


Figure 4.79 - MK-4 (menatetrenone)

prenoid side-chains. The various forms are commonly named as MK-X, where X is a number, and MK stands for menaquinone, which is the name given to this form of vitamin K. [Figure 4.79](#) shows a common form known as MK-4 (menatetrenone).

Hemorrhaging danger

It is very critical that the proper amount of warfarin be given to patients. Too much can result in hemorrhaging. Patients must have

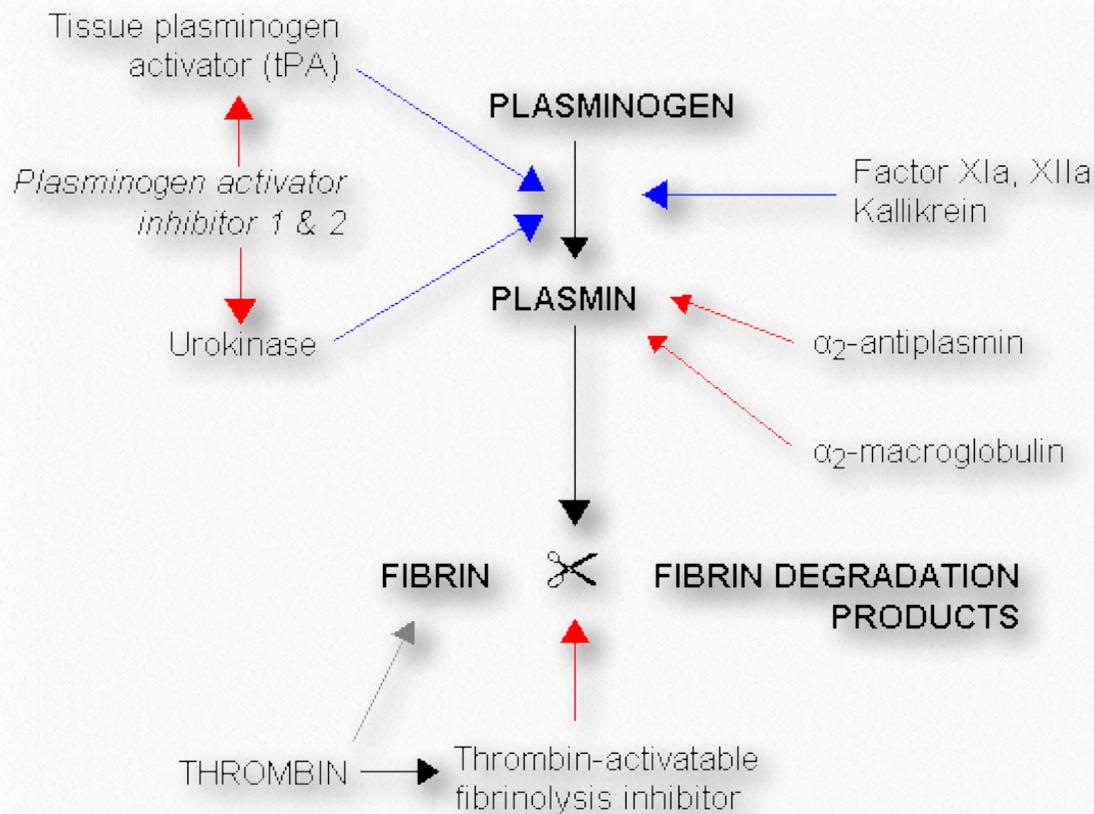


Figure 4.80 - Regulation of fibrin breakdown. Activators in blue. Inhibitors in red.

their clotting times checked regularly to ensure that they are taking the right dose of anti-coagulant medication. Diet and the metabolism of Vitamin K in the body can affect the amount of warfarin needed. Vitamin K is synthesized in plants and plays a role in photosynthesis. It can be found in the highest quantities in vegetables that are green and leafy. Patients whose diet is high in these vegetables may require a different dose than those who rarely eat greens. Dietary vitamin K is also, as mentioned earlier, metabolized by bacteria in the large intestine, where they convert vitamin K₁ into vitamin K₂.

Plasmin

Clots, once made in the body, do not remain there forever. Instead, a tightly regulated en-

zyme known as plasmin is activated, when appropriate, to break down the fibrin-entangled clot. Like many of the enzymes in the blood clotting cascade, plasmin is a serine protease. It is capable of cleaving a wide range of proteins. They include polymerized fibrin clots, fibronectin, thrombospondin, laminin, and the von Willebrand factor.

Plasmin plays a role in activating collagenases and in the process of ovulation by weakening the wall of the Graafian follicle in the ovary. Plasmin is made in the

liver as the zymogen known as plasminogen. Several different enzymes can activate it.

Wikipedia

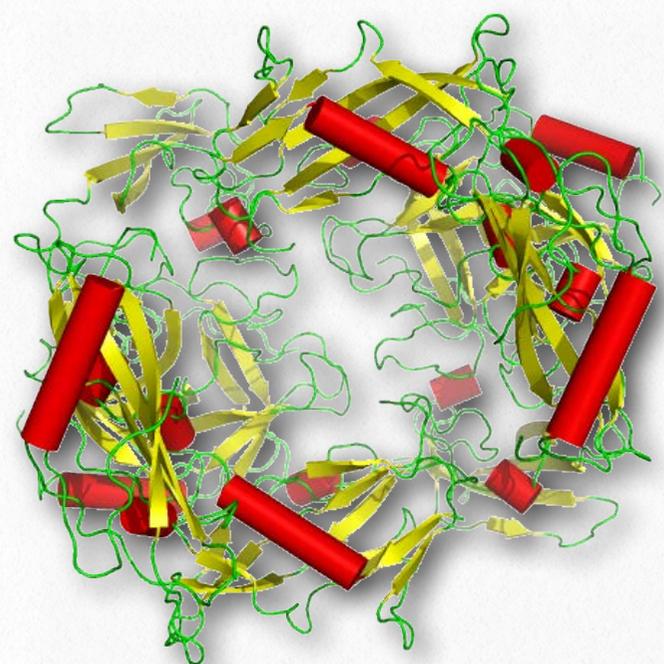


Figure 4.81 - Plasmin

Tissue plasminogen activator (tPA), using fibrin as a co-factor, is one. Others include urokinase plasminogen activator (using urokinase plasminogen activator receptor as a co-factor), kallikrein (plasma serine protease with many forms and blood functions), and FXIa and FXIIa from the clotting cascade.

Plasmin inhibition

Plasmin's activity can also be inhibited. Plasminogen activator inhibitor, for example, can inactivate tPA and urokinase. After plasmin has been activated, it can also be inhibited by α_2 -antiplasmin and α_2 -macroglobulin (Figure 4.80).

Thrombin also plays a role in plasmin's inactivation, stimulating activity of thrombin activatable fibrinolysis inhibitor. Angiostatin is a sub-domain of plasmin produced by auto-proteolytic cleavage. It blocks the growth of new blood vessels and is being investigated for its anti-cancer properties.

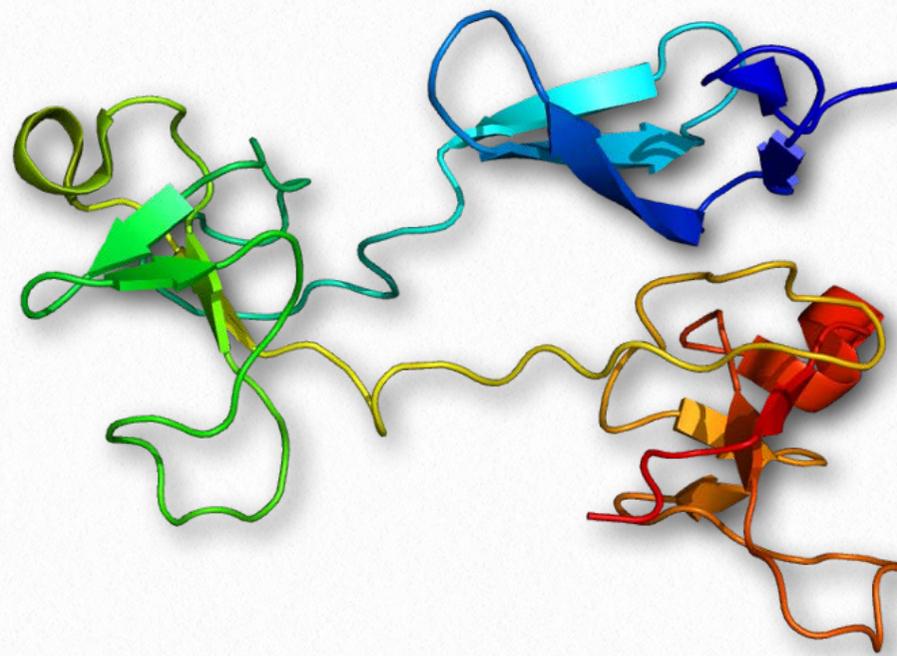


Figure 4.82 - Fibronectin 1

Wikipedia

Fibronectin

Fibronectin is a large (440 kDa) glycoprotein found in the extracellular matrix that binds to integral cellular proteins called integrins and to extracellular proteins, including collagen, fibrin, and heparan sul-

fate. It comes in two forms. The soluble form is found in blood plasma and is made by the liver. It is found in high concentration in the blood stream (300 $\mu\text{g}/\text{ml}$). The insoluble form is found abundantly in the extracellular matrix.

The protein is assembled in the extracellular matrix and plays roles in cellular growth, adhesion, migration, and differentiation. It is very important in wound healing.

YouTube Lectures
by Kevin
[HERE](#) & [HERE](#)

Assists in blood clot formation

Fibronectin from the blood plasma is localized to the site of the wound, assisting in formation of the blood clot to stop bleeding. In the initial stages of wound healing, plasma fibronectin interacts with fibrin in clot formation. It also protects tissue sur-

rounding the wound. Later in the repair process, remodeling of the damaged area begins with the action of fibroblasts and endothelial cells at the wound site. Their task is to degrade proteins of the blood clot matrix, replacing them with a new matrix like the undamaged, surrounding tissue.

Fibroblasts act on the temporary fibronectin-fibrin matrix, remodeling it to replace the plasma fibronectin with cellular fibronectin. This may cause the phenomenon of wound contraction, one of the steps in wound healing. Secretion of cellular fibronectin by fibroblasts is followed by fibronectin assembly and integration with the extracellular matrix.

Embryogenesis

Fibronectin is essential for embryogenesis. Deleting the gene in mice causes lethality before birth. This is likely due to its role in migration and guiding the attachment of cells as the embryo develops. Fibronectin also has a role in the mouth. It is found in saliva and is thought to inhibit colonization of the mouth by pathogenic bacteria.

Platelet activating factor

Platelet Activating Factor (PAF) is a compound (Figure 4.82) produced primarily in

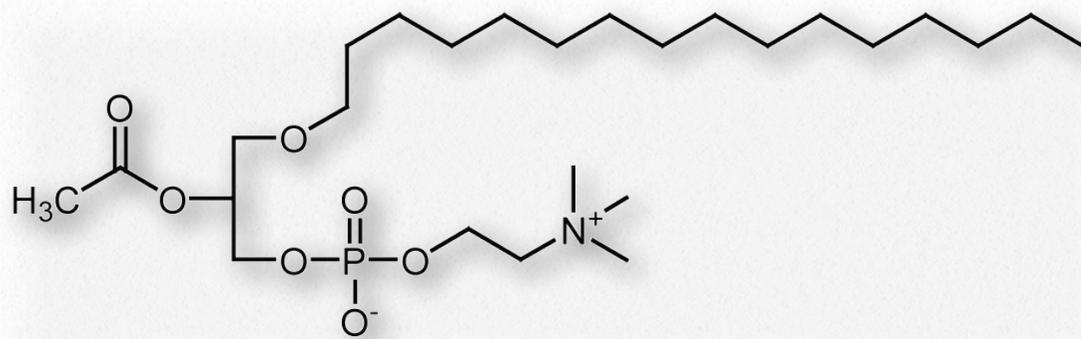


Figure 4.83 - Platelet Activating Factor

Wikipedia

cells involved in host defense. These include platelets, macrophages, neutrophils, and monocytes, among others. It is produced in greater quantities in inflammatory cells upon proper stimulation. The compound acts like a hormone and mediates platelet aggregation/degranulation, inflammation, and anaphylaxis. It can transmit signals between cells to trigger and amplify inflammatory and clotting cascades.

When unregulated, signaling by PAF can cause severe inflammation resulting in sepsis and injury. Inflammation in allergic reactions arises partly as a result of PAF and is an important factor in bronchoconstriction in asthma. In fact, at a concentration of only 10 picomolar, PAF can cause asthmatic inflammation of the airways that is life threatening.

Graphic images in this book were products of the work of several talented students.
Links to their Web pages are below

Click [HERE](#) for
Aleia Kim's
Web Page

Click [HERE](#) for
Pehr Jacobson's
Web Page

Click [HERE](#) for
Penelope Irving's
Web Page

Click [HERE](#) for
Martha Baker's
Web Page

Problem set related to this section [HERE](#)

Point by Point summary of this section [HERE](#)

To get a certificate for mastering this section of the book, click [HERE](#)

Kevin Ahern's free iTunes U Courses - [Basic](#) / [Med School](#) / [Advanced](#)

Biochemistry Free & Easy (our other book) [HERE](#) / [Facebook Page](#)

Kevin and Indira's Guide to Getting into Medical School - [iTunes U Course](#) / [Book](#)

To see Kevin Ahern's OSU ecampus courses - [BB 350](#) / [BB 450](#) / [BB 451](#)

To register for Kevin Ahern's OSU ecampus courses - [BB 350](#) / [BB 450](#) / [BB 451](#)

Biochemistry Free For All [Facebook Page](#) (please like us)

Kevin Ahern's [Web Page](#) / [Facebook Page](#) / Taralyn Tan's [Web Page](#)

Kevin Ahern's free downloads [HERE](#)

OSU's Biochemistry/Biophysics program [HERE](#)

OSU's College of Science [HERE](#)

Oregon State University [HERE](#)

Email [Kevin Ahern](#) / [Indira Rajagopal](#) / [Taralyn Tan](#)

Thank Goodness My Blood is Clotting

To the tune of "Don't Sleep in the Subway Darling"

Metabolic Melodies Website [HERE](#)

I'm feeling so sad
'Cuz I cut . . . myself bad
Now I'm all worried 'bout . . . consequences

It's starting to bleed
There's some clo . . . sure I need
So the body kicks . . . in its defenses

It's happened all so many times before
The blood flows out and then it shuts the door

Thank goodness my blood is clotting
Enmeshing the fibrin chains
Thank goodness my blood is clotting
The zymogens
Are activating and all is well
So I'll stop bleeding again

The vitamin K's
Help to . . . bind to cee-ays
Adding C-O- . . . O-H to amend things
Um-m-um-um-um-um

It hardens and stays
When a glu. . . taminase
Creates co. . . valent bonds . . . for cementing

In just a moment, things are good to go
The clot's in place and it has stopped the flow

But what about clot dissolving?
Untangling fibrin chains?
This calls for some problem solving
There is a way
Just activate up some t-PA
Get plasmin active in veins

Oh, oh, oh.
And thanks to the dis-enclotting'
As part of repairin' veins
It's part of my body's plotting
The wound is gone
I'm back where I started and
Nothing's wrong
My blood flow is normal again.

*Recording by Liz Bacon and David Simmons
Lyrics by Kevin Ahern*