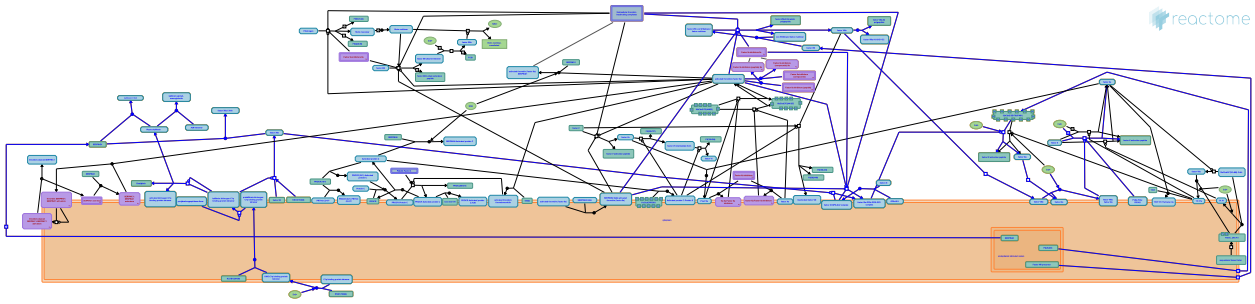
Intrinsic Pathway of Fibrin Clot Formation



D'Eustachio, P., Gómez-Outes, A., Jassal, B., Jupe, S., Mumford, D., Rush, MG., Shamovsky, V., Zhang, B.

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This is just an excerpt of a full-length report for this pathway. To access the complete report, please download it at the Reactome Textbook.

11/05/2024

Introduction

Reactome is open-source, open access, manually curated and peer-reviewed pathway database. Pathway annotations are authored by expert biologists, in collaboration with Reactome editorial staff and cross-referenced to many bioinformatics databases. A system of evidence tracking ensures that all assertions are backed up by the primary literature. Reactome is used by clinicians, geneticists, genomics researchers, and molecular biologists to interpret the results of high-throughput experimental studies, by bioinformaticians seeking to develop novel algorithms for mining knowledge from genomic studies, and by systems biologists building predictive models of normal and disease variant pathways.

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Literature references

Fabregat, A., Sidiropoulos, K., Viteri, G., Forner, O., Marin-Garcia, P., Arnau, V. et al. (2017). Reactome pathway analysis: a high-performance in-memory approach. *BMC bioinformatics, 18*, 142. ↗

Sidiropoulos, K., Viteri, G., Sevilla, C., Jupe, S., Webber, M., Orlic-Milacic, M. et al. (2017). Reactome enhanced pathway visualization. *Bioinformatics, 33*, 3461-3467. ↗

Fabregat, A., Jupe, S., Matthews, L., Sidiropoulos, K., Gillespie, M., Garapati, P. et al. (2018). The Reactome Pathway Knowledgebase. *Nucleic Acids Res, 46*, D649-D655. ↗

Fabregat, A., Korninger, F., Viteri, G., Sidiropoulos, K., Marin-Garcia, P., Ping, P. et al. (2018). Reactome graph database: Efficient access to complex pathway data. *PLoS computational biology, 14*, e1005968. ↗

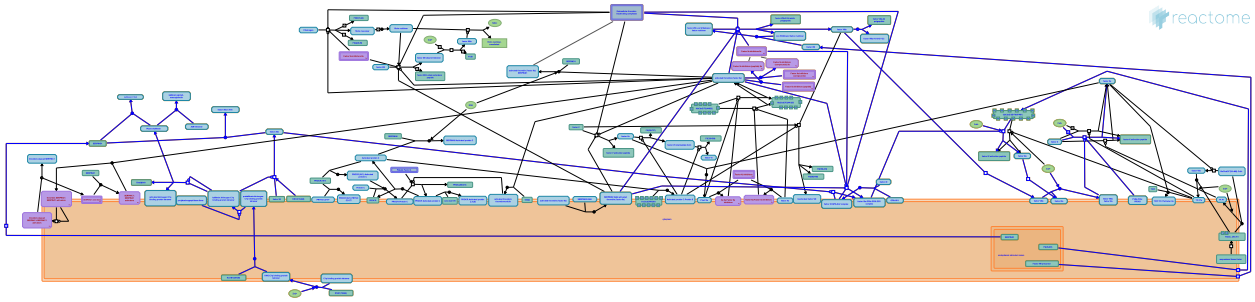
Reactome database release: 88

This document contains 1 pathway and 24 reactions (see Table of Contents)

Intrinsic Pathway of Fibrin Clot Formation ↗

Stable identifier: R-HSA-140837

Compartments: extracellular region



The intrinsic pathway of blood clotting connects interactions among kininogen (high molecular weight kininogen, HK), prekallikrein (PK), and factor XII to the activation of clotting factor X by a series of reactions that is independent of the extrinsic pathway and that is not subject to inhibition by TFPI. It is thus essential for the prolongation of the clotting cascade: while the reactions of the extrinsic pathway appear to be sufficient to initiate clot formation, those of the intrinsic pathway are required to maintain it (Broze 1995; Davie et al. 1991; Monroe et al. 2002). The intrinsic pathway can be divided into three parts: 1) reactions involving interactions of kininogen, prekallikrein, and factor XII, leading to the activation of factor XII, 2) reactions involving factor XI, factor IX, factor VIII, and von Willebrand factor (vWF) leading to the activation of factors VIII and IX, and 3) reactions that inactivate factor XIIa and kallikrein.

Kininogen, prekallikrein, and factor XII were first identified as proteins needed for the rapid formation of clots when whole blood is exposed to negatively charged surfaces in vitro. Early studies in vitro identified several possible sets of interactions, in which small quantities of one or more of these proteins 'autoactivate' and then catalyze the formation of larger quantities of activated factors. Subsequent work, however, suggests that these factors form complexes on endothelial cell surfaces mediated by C1q binding protein (C1q bp), that the first activation event is the cleavage of prekallikrein by prolylcarboxypeptidase, and that the resulting kallikrein catalyzes the activation of factor XII (Schmaier 2004).

The second group of events, occurs in vivo on the surfaces of activated platelets (although most biochemical characterization of the reactions was originally done with purified proteins in solution). Factor XI binds to the platelet glycoprotein (GP) Ib:IX:V complex, where it can be activated by cleavage either by thrombin (generated by reactions of the common pathway) or by activated factor XII (generated in the first part of the intrinsic pathway). Activated factor XI in turn catalyzes the activation of factor IX. Simultaneously, factor VIII, complexed with vWF, is cleaved by thrombin, activating it and causing its release from vWF. Activated factors VIII and IX form a complex on the platelet surface that very efficiently converts factor X to activated factor X. (Activated factors X and V then form a complex that efficiently activates thrombin.)

While these two groups of events can be viewed as forming a single functional pathway (e.g., Davie et al. 1991), human clinical genetic data cast doubt on this view. Individuals deficient in kininogen, prekallikrein, or factor XII proteins exhibit normal blood clot formation in vivo. In contrast, deficiencies of factor XI can be associated with failure of blood clotting under some conditions, and deficiencies of vWF, factor VIII, or factor IX cause severe abnormalities - von Willebrand disease, hemophilia A, and hemophilia B, respectively. These data suggest that while the second group of events is essential for normal clot formation in vivo, the first group has a different function (e.g., Schmaier 2004).

Finally, reactions neutralize proteins activated in the first part of the intrinsic pathway. Kallikrein forms stable complexes with either C1 inhibitor (C1Inh) or with alpha2-macroglobulin, and factor XIIa forms stable complexes with C1Inh. The relevance of these neutralization events to the regulation of blood clotting is unclear, however. The physiological abnormalities observed in individuals who lack C1Inh appear to be due entirely to abnormalities of complement activation; blood clotting appears to proceed normally. This observation is consistent with the hypothesis, above, that factor XIIa plays a limited role in normal blood clotting under physiological conditions.

Literature references

Kisiel, W., Fujikawa, K., Davie, EW. (1991). The coagulation cascade: initiation, maintenance, and regulation. *Biochemistry, 30*, 10363-70. ↗

Monroe, DM., Roberts, HR., Hoffman, M. (2002). Platelets and thrombin generation. *Arterioscler Thromb Vasc Biol, 22*, 1381-9. ↗

Broze, GJ Jr. (1995). Tissue factor pathway inhibitor and the revised theory of coagulation. *Annu Rev Med, 46*, 103-12. ↗

Schmaier, AH. (2004). The physiologic basis of assembly and activation of the plasma kallikrein/kinin system. *Thromb Haemost, 91*, 1-3. ↗

Editions

2004-08-24	Authored	D'Eustachio, P.
2008-01-11	Reviewed	Rush, MG.
2024-03-06	Edited	D'Eustachio, P.

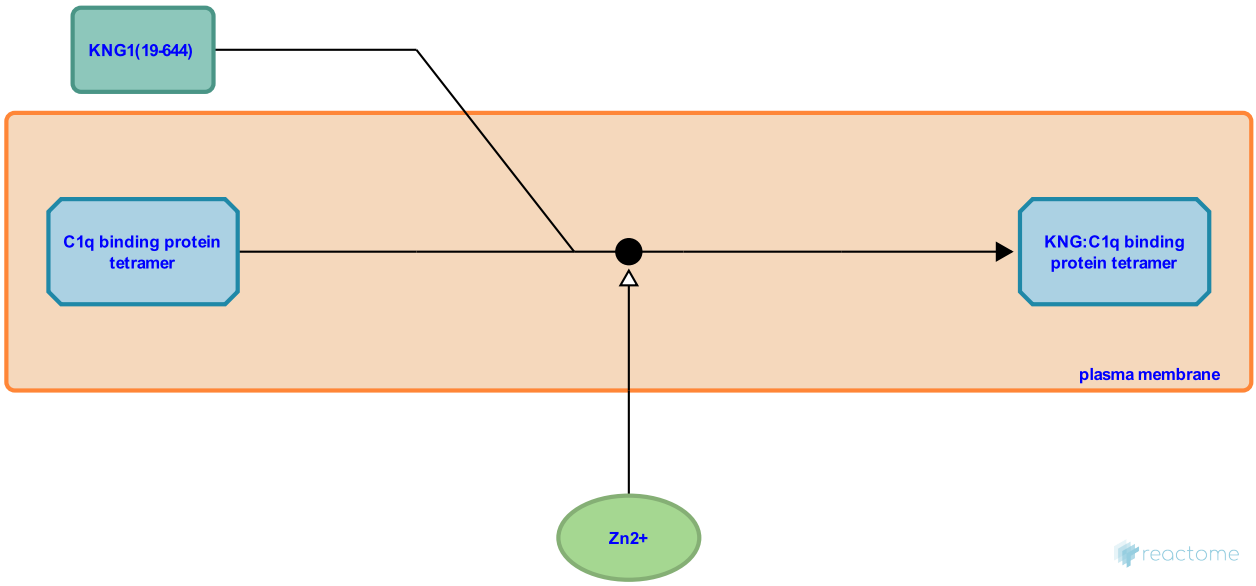
kininogen + C1q binding protein tetramer -> kininogen:C1q binding protein tetramer ↗

Location: Intrinsic Pathway of Fibrin Clot Formation

Stable identifier: R-HSA-158354

Type: binding

Compartments: plasma membrane, extracellular region



Kininogen (high molecular weight kininogen; HK) associates with C1q binding protein on the cell surface in a reaction dependent on Zn++ (Joseph et al. 1996). In the body, the Zn++ needed to drive this reaction may be provided locally by Zn++ release from activated platelets (Mahdi et al. 2002). The C1q binding protein is inferred to form tetramers based on the properties of purified recombinant protein in vitro (Ghebrehiwet et al. 1994); the stoichiometry of the cell surface complex has not been determined directly.

Followed by: prekallikrein + kininogen:C1q binding protein tetramer -> prekallikrein:kininogen:C1q binding protein tetramer

Literature references

Kerbiriou, DM., Griffin, JH. (1979). Human high molecular weight kininogen. Studies of structure-function relationships and of proteolysis of the molecule occurring during contact activation of plasma. *J Biol Chem, 254*, 12020-7. ↗

Lim, BL., Ghebrehiwet, B., Peerschke, EI., Willis, AC., Reid, KB. (1994). Isolation, cDNA cloning, and overexpression of a 33-kD cell surface glycoprotein that binds to the globular "heads" of C1q. *J Exp Med, 179*, 1809-21. ↗

Schmaier, AH., Shariat-Madar, Z., Figueroa, CD., Mahdi, F. (2002). Factor XII interacts with the multiprotein assembly of urokinase plasminogen activator receptor, gC1qR, and cytokeratin 1 on endothelial cell membranes. *Blood, 99*, 3585-96. ↗

Ghebrehiwet, B., Peerschke, EI., Joseph, K., Kaplan, AP., Reid, KB. (1996). Identification of the zinc-dependent endothelial cell binding protein for high molecular weight kininogen and factor XII: identity with the receptor that binds to the globular "heads" of C1q (gC1q-R). *Proc Natl Acad Sci U S A, 93*, 8552-7. ↗

Editions

2005-01-20	Authored	D'Eustachio, P.
2024-03-06	Edited	D'Eustachio, P.

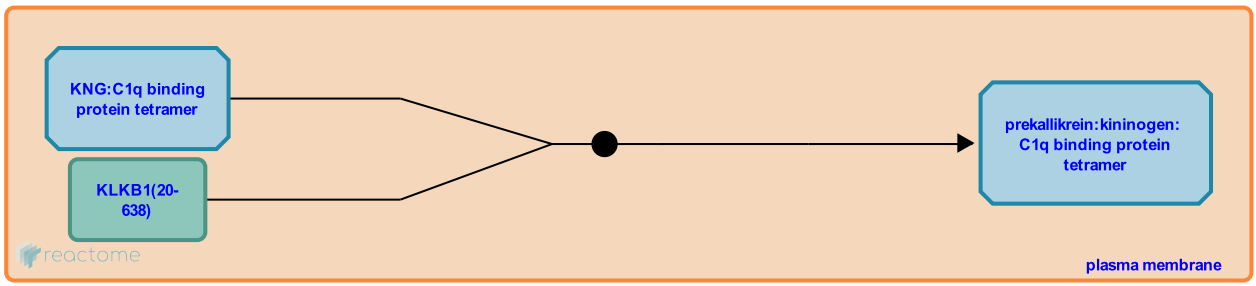
prekallikrein + kininogen:C1q binding protein tetramer -> prekallikrein:kininogen:C1q binding protein tetramer ↗

Location: Intrinsic Pathway of Fibrin Clot Formation

Stable identifier: R-HSA-158218

Type: binding

Compartments: plasma membrane



Prekallikrein (PK) associates specifically with kininogen (HK) on cell surfaces. In vivo, this reaction may occur primarily on the surfaces of endothelial cells in response to platelet activation (Lin et al. 1997; Motta et al. 1998; Mahdi et al. 2003).

Preceded by: kininogen + C1q binding protein tetramer -> kininogen:C1q binding protein tetramer

Followed by: prekallikrein:kininogen:C1q binding protein tetramer -> kallikrein:kininogen:C1q binding protein tetramer

Literature references

Yan, W., Zhang, H., McCrae, KR., Colman, RW., Harris, RB., Lin, Y. (1997). High molecular weight kininogen peptides inhibit the formation of kallikrein on endothelial cell surfaces and subsequent urokinase-dependent plasmin formation. *Blood, 90*, 690-7. ↗

Fujikawa, K., McMullen, BA., Chung, DW., Davie, EW. (1986). Human plasma prekallikrein, a zymogen to a serine protease that contains four tandem repeats. *Biochemistry, 25*, 2410-7. ↗

Schmaier, AH., Shariat-Madar, Z., Mahdi, F. (2003). The relative priority of prekallikrein and factors XI/XIa assembly on cultured endothelial cells. *J Biol Chem, 278*, 43983-90. ↗

Schmaier, AH., Cines, DB., Rojkjaer, R., Hasan, AA., Motta, G. (1998). High molecular weight kininogen regulates prekallikrein assembly and activation on endothelial cells: a novel mechanism for contact activation. *Blood, 91*, 516-28. ↗

Editions

2005-01-20	Authored	D'Eustachio, P.

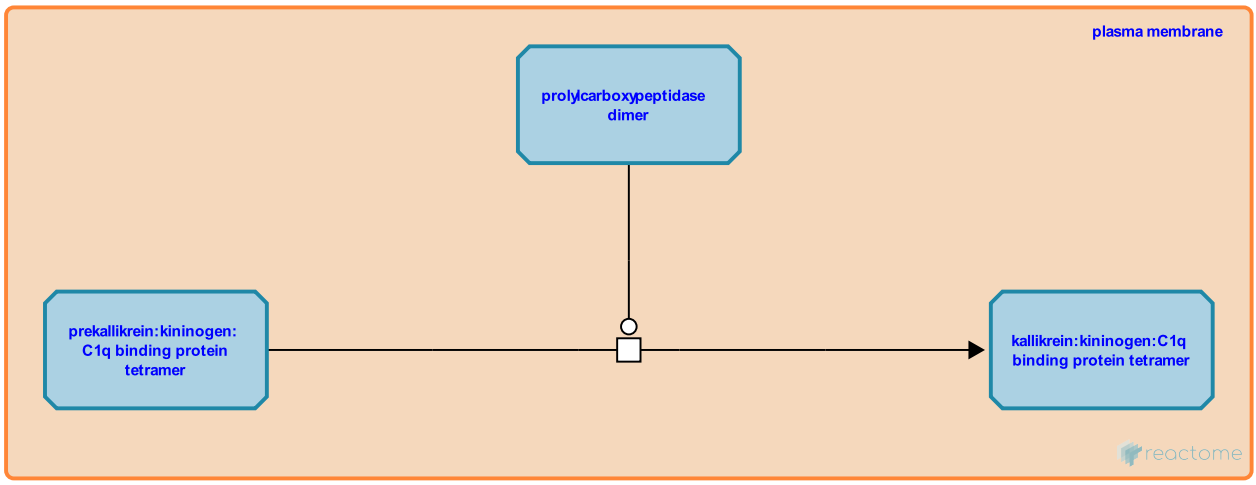
prekallikrein:kininogen:C1q binding protein tetramer -> kallikrein:kininogen:C1q binding protein tetramer ↗

Location: Intrinsic Pathway of Fibrin Clot Formation

Stable identifier: R-HSA-158251

Type: transition

Compartments: plasma membrane



Prekallikrein in a complex with kininogen and C1q binding protein on the plasma membrane is cleaved to generate active kallikrein, which remains bound to the complex. In the body, this reaction appears to occur on the surfaces of endothelial cells and may require the presence of activated platelets. Recent work indicates that the protease that cleaves prekallikrein under these conditions is prolylcarboxypeptidase. Although this enzyme was originally isolated from lysosomes (Odya et al. 1978; Tan et al. 1993), it is associated with plasma membranes of cultured human endothelial cells in vitro (Moreira et al. 2002; Shariat-Madar et al. 2002), and the purified recombinant enzyme efficiently cleaves prekallikrein (Shariat-Madar et al. 2004). In contrast factor XII, despite its activity on prekallikrein in vitro, appears not to be responsible for prekallikrein activation on the cell surface (Rojkjaer et al. 1998).

Preceded by: prekallikrein + kininogen:C1q binding protein tetramer -> prekallikrein:kininogen:C1q binding protein tetramer

Followed by: kallikrein:kininogen:C1q binding protein tetramer -> kallikrein + activated kininogen:C1q binding protein tetramer + bradykinin

Literature references

Schmaier, AH., Shariat-Madar, Z., Mahdi, F. (2002). Identification and characterization of prolylcarboxypeptidase as an endothelial cell prekallikrein activator. *J Biol Chem, 277*, 17962-9. ↗

Skidgel, RA., Erdos, EG., Morris, PW., Tan, F. (1993). Sequencing and cloning of human prolylcarboxypeptidase (angiotensinase C). Similarity to both serine carboxypeptidase and prolylendopeptidase families. *J Biol Chem, 268*, 16631-8. ↗

Marinkovic, DV., Odya, CE., Erdos, EG., Hammon, KJ., Stewart, TA. (1978). Purification and properties of prolylcarboxypeptidase (angiotensinase C) from human kidney. *J Biol Chem, 253*, 5927-31. ↗

Schmaier, AH., Shariat-Madar, Z., Moreira, CR., Nader, HB., Motta, G., Mahdi, F. (2002). Identification of prolylcarboxypeptidase as the cell matrix-associated prekallikrein activator. *FEBS Lett, 523*, 167-70. ↗

Schmaier, AH., Shariat-Madar, Z., Mahdi, F. (2004). Recombinant prolylcarboxypeptidase activates plasma prekallikrein. *Blood, 103*, 4554-61. ↗

Editions

2005-01-20	Authored	D'Eustachio, P.
2024-03-06	Edited	D'Eustachio, P.

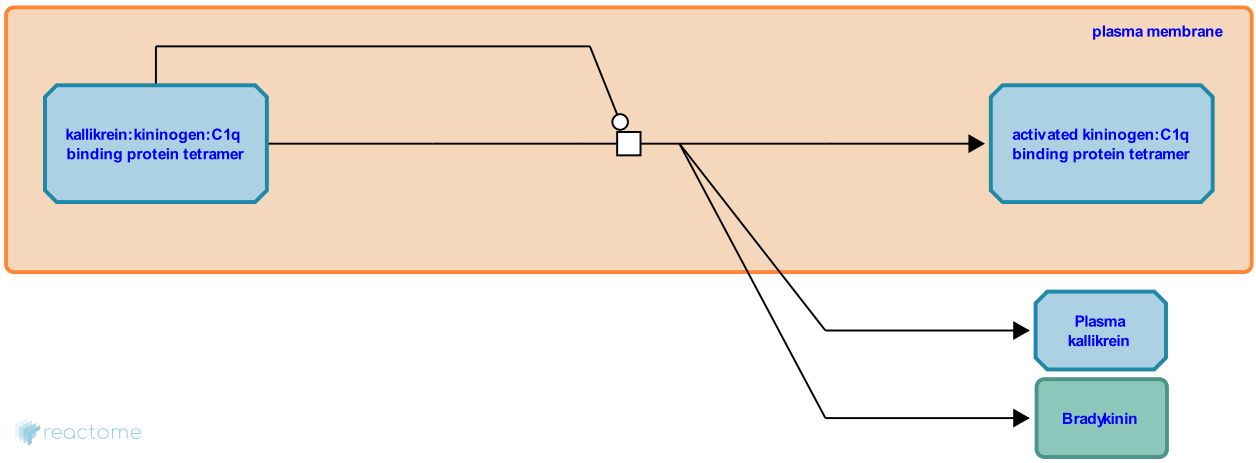
kallikrein:kininogen:C1q binding protein tetramer -> kallikrein + activated kininogen:C1q binding protein tetramer + bradykinin ↗

Location: Intrinsic Pathway of Fibrin Clot Formation

Stable identifier: R-HSA-158311

Type: transition

Compartments: plasma membrane, extracellular region



The cleavage of kininogen (HK, high molecular weight kininogen) yields activated kininogen and the vasoactive peptide bradykinin (Kerbirou and Griffin 1979; Lottspeich et al. 1985; Kellerman et al. 1986). In vivo, this reaction is catalyzed by activated kallikrein, takes places within the kallikrein:kininogen:C1q binding protein tetramer complex on the endothelial cell surface, and results in the release of kallikrein and bradykinin (Motta et al. 1998).

Preceded by: prekallikrein:kininogen:C1q binding protein tetramer -> kallikrein:kininogen:C1q binding protein tetramer

Followed by: kallikrein binds SERPING1, kallikrein + alpha2-macroglobulin -> kallikrein:alpha2-macrogloulin, factor XII -> factor XIIa

Literature references

Muller-Esterl, W., Lottspeich, F., Henschen, A., Kellermann, J. (1986). Completion of the primary structure of human high-molecular-mass kininogen. The amino acid sequence of the entire heavy chain and evidence for its evolution by gene triplication. *Eur J Biochem, 154*, 471-8. ↗

Kerbiriou, DM., Griffin, JH. (1979). Human high molecular weight kininogen. Studies of structure-function relationships and of proteolysis of the molecule occurring during contact activation of plasma. *J Biol Chem, 254*, 12020-7. ↗

Foertsch, B., Muller-Esterl, W., Lottspeich, F., Henschen, A., Kellermann, J. (1985). The amino acid sequence of the light chain of human high-molecular-mass kininogen. *Eur J Biochem, 152*, 307-14. ↗

Schmaier, AH., Cines, DB., Rojkjaer, R., Hasan, AA., Motta, G. (1998). High molecular weight kininogen regulates prekallikrein assembly and activation on endothelial cells: a novel mechanism for contact activation. *Blood, 91*, 516-28. ↗

Editions

2005-01-20	Authored	D'Eustachio, P.
2024-03-06	Edited	D'Eustachio, P.

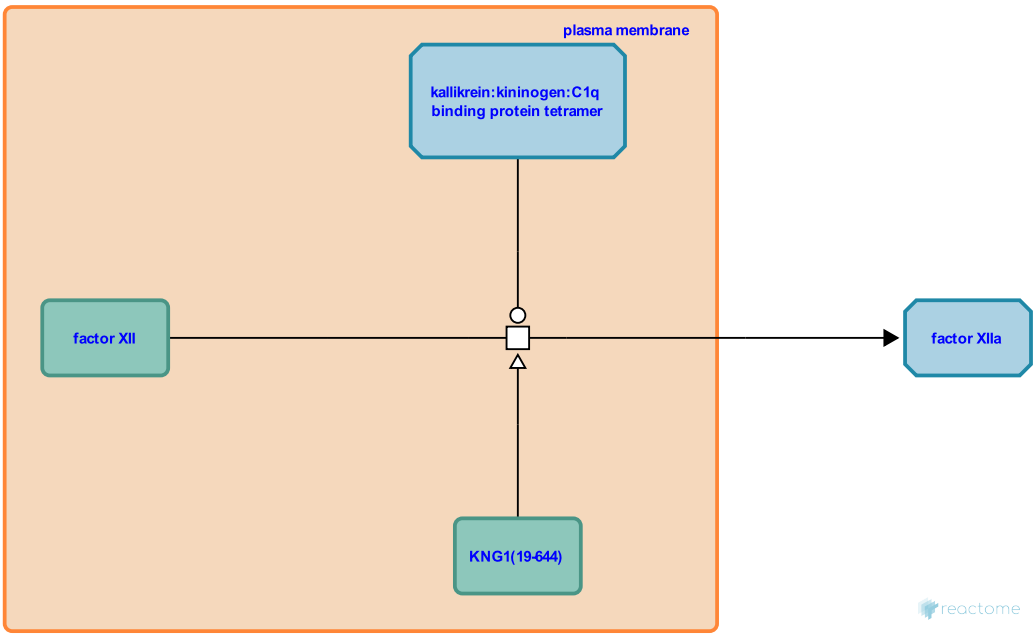
factor XII -> factor XIIa ↗

Location: Intrinsic Pathway of Fibrin Clot Formation

Stable identifier: R-HSA-158313

Type: transition

Compartments: plasma membrane, extracellular region



Cleavage of a single peptide bond converts factor XII to activated factor XII (factor XIIa) (Fujikawa and McMullen 1983; McMullen and Fujikawa 1985). Identification of the catalytic activity or activities responsible for this cleavage has not been straightforward. Studies in vitro have demonstrated the autoactivation of factor XII as well as activation by kallikrein. Both reactions require the presence of negatively charged surfaces and are accelerated in the presence of kininogen (high molecular weight kininogen, HK) (Griffin and Cochrane 1976; Meier et al. 1977; Silverberg et al. 1980). Recent work suggests that factor XII activation in vivo may occur primarily on endothelial cell surfaces and that, as in vitro, association with kininogen may accelerate the reaction (Mahdi et al. 2002; Schmaier 2004), although alternative pathways and alternative mechanisms for associating factor XII with the cell surface have not been excluded (Joseph et al. 2001).

Preceded by: kallikrein:kininogen:C1q binding protein tetramer -> kallikrein + activated kininogen:C1q binding protein tetramer + bradykinin

Followed by: factor XIIa binds SERPING1, factor XI:platelet glycoprotein (GP) Ib:IX:V complex -> factor XIa:platelet glycoprotein (GP) Ib:IX:V complex (XIIa catalyst)

Literature references

Ghebrehiwet, B., Joseph, K., Kaplan, AP., Shibayama, Y. (2001). Factor XII-dependent contact activation on endothelial cells and binding proteins gC1qR and cytokeratin 1. *Thromb Haemost, 85*, 119-24. ↗

Schmaier, AH. (2004). The physiologic basis of assembly and activation of the plasma kallikrein/kinin system. *Thromb Haemost, 91*, 1-3. ↗

Meier, HL., Pierce, JV., Kaplan, AP., Colman, RW. (1977). Activation and function of human Hageman factor. The role of high molecular weight kininogen and prekallikrein. *J Clin Invest, 60*, 18-31. ↗

Cochrane, CG., Griffin, JH. (1976). Mechanisms for the involvement of high molecular weight kininogen in surface-dependent reactions of Hageman factor. *Proc Natl Acad Sci U S A, 73*, 2554-8. ↗

Fujikawa, K., McMullen, BA. (1983). Amino acid sequence of human beta-factor XIIa. *J Biol Chem, 258*, 10924-33. ↗

Editions

2005-01-20	Authored	D'Eustachio, P.
2024-03-06	Edited	D'Eustachio, P.

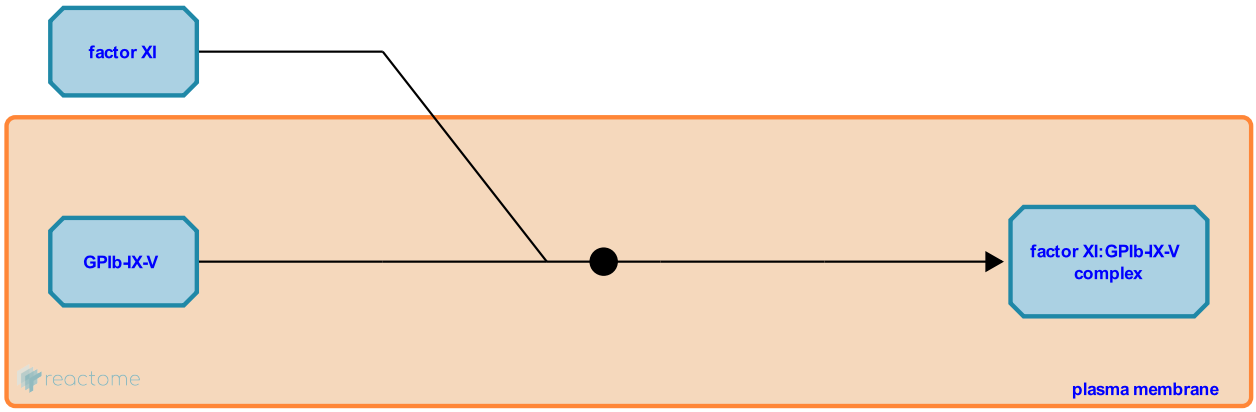
factor XI + platelet glycoprotein (GP) Ib:IX:V complex -> factor XI:platelet glycoprotein (GP) Ib:IX:V complex ↗

Location: Intrinsic Pathway of Fibrin Clot Formation

Stable identifier: R-HSA-158145

Type: binding

Compartments: plasma membrane, extracellular region



Plasma factor XI binds to the platelet glycoprotein Ib:IX:V complex (Baglia et al. 2004; Greengard et al. 1986). In the body, this reaction occurs specifically on the surfaces of activated platelets, but not on endothelial cells (Baird and Walsh 2002). The stoichiometry of the platelet glycoprotein Ib:IX:V complex has not been established directly, but is inferred from the relative abundances of its components in platelet membranes (Modderman et al. 1992; Shrimpton et al. 2002).

Followed by: factor XI:platelet glycoprotein (GP) Ib:IX:V complex -> factor XIa:platelet glycoprotein (GP) Ib:IX:V complex (XIIa catalyst), factor XI:platelet glycoprotein (GP) Ib:IX:V complex -> factor XIa:platelet glycoprotein (GP) Ib:IX:V complex (thrombin catalyst)

Literature references

McCarty, OJT., Tucker, EI., Pang, J., Puy, C., Lorentz, CU., Reitsma, SE. et al. (2021). Role of platelets in regulating activated coagulation factor XI activity. *Am J Physiol Cell Physiol, 320*, C365-C374. ↗

Lopez, JA., Borthakur, G., Shrimpton, CN., Cruz, MA., Dong, JF., Larrucea, S. (2002). Localization of the adhesion receptor glycoprotein Ib-IX-V complex to lipid rafts is required for platelet adhesion and activation. *J Exp Med, 196*, 1057-66. ↗

Heeb, MJ., Griffin, JH., Ersdal, E., Greengard, JS., Walsh, PN. (1986). Binding of coagulation factor XI to washed human platelets. *Biochemistry, 25*, 3884-90. ↗

Admiraal, LG., von dem Borne, AE., Modderman, PW., Sonnenberg, A. (1992). Glycoproteins V and Ib-IX form a noncovalent complex in the platelet membrane. *J Biol Chem, 267*, 364-9. ↗

Lopez, JA., Walsh, PN., Gailani, D., Baglia, FA. (2004). Identification of a binding site for glycoprotein Ibalpha in the Apple 3 domain of factor XI. *J Biol Chem, 279*, 45470-6. ↗

Editions

2005-01-20	Authored	D'Eustachio, P.
2024-03-06	Edited	D'Eustachio, P.

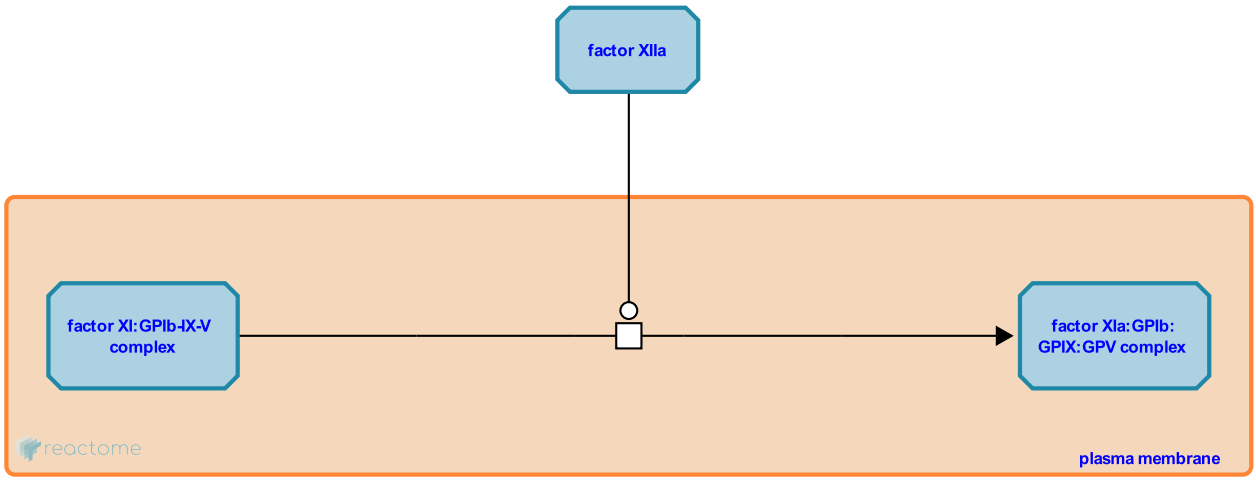
factor XI:platelet glycoprotein (GP) Ib:IX:V complex -> factor XIa:platelet glycoprotein (GP) Ib:IX:V complex (XIIa catalyst) ↗

Location: Intrinsic Pathway of Fibrin Clot Formation

Stable identifier: R-HSA-158300

Type: transition

Compartments: plasma membrane, extracellular region



Factor XI, bound to the cell surface, is converted to activated factor XI (factor XIa). Chemically, this reaction involves the cleavage of a single peptide bond in each subunit of the factor XI homodimer; intra- and inter-chain disulfide bonds hold the resulting four polypeptides together (Bouma and Griffin 1977; Kurachi and Davie 1977; McMullen et al. 1991). In the body, this reaction occurs on the surfaces of activated platelets (Greengard et al. 1986; Baird and Walsh 2002; Reitsma et al. 2021); when this reaction occurs as a step in the contact pathway of blood coagulation, it is catalyzed by activated factor XIIa (Kurachi and Davie 1977) which in turn is generated through the interactions of factor XII, kallikrein, and kininogen on endothelial cell surfaces (Schmaier 2004).

Preceded by: factor XII -> factor XIIa, factor XI + platelet glycoprotein (GP) Ib:IX:V complex -> factor XI:platelet glycoprotein (GP) Ib:IX:V complex

Followed by: factor IX -> factor IXa + factor IX activation peptide (factor XIa catalyst)

Literature references

Griffin, JH., Bouma, BN. (1977). Human blood coagulation factor XI. Purification, properties, and mechanism of activation by activated factor XII. *J Biol Chem, 252*, 6432-7. ↗

Fujikawa, K., McMullen, BA., Davie, EW. (1991). Location of the disulfide bonds in human coagulation factor XI: the presence of tandem apple domains. *Biochemistry, 30*, 2056-60. ↗

Kurachi, K., Davie, EW. (1977). Activation of human factor XI (plasma thromboplastin antecedent) by factor XIIa (activated Hageman factor). *Biochemistry, 16*, 5831-9. ↗

Editions

2005-01-20	Authored	D'Eustachio, P.
2024-03-06	Edited	D'Eustachio, P.

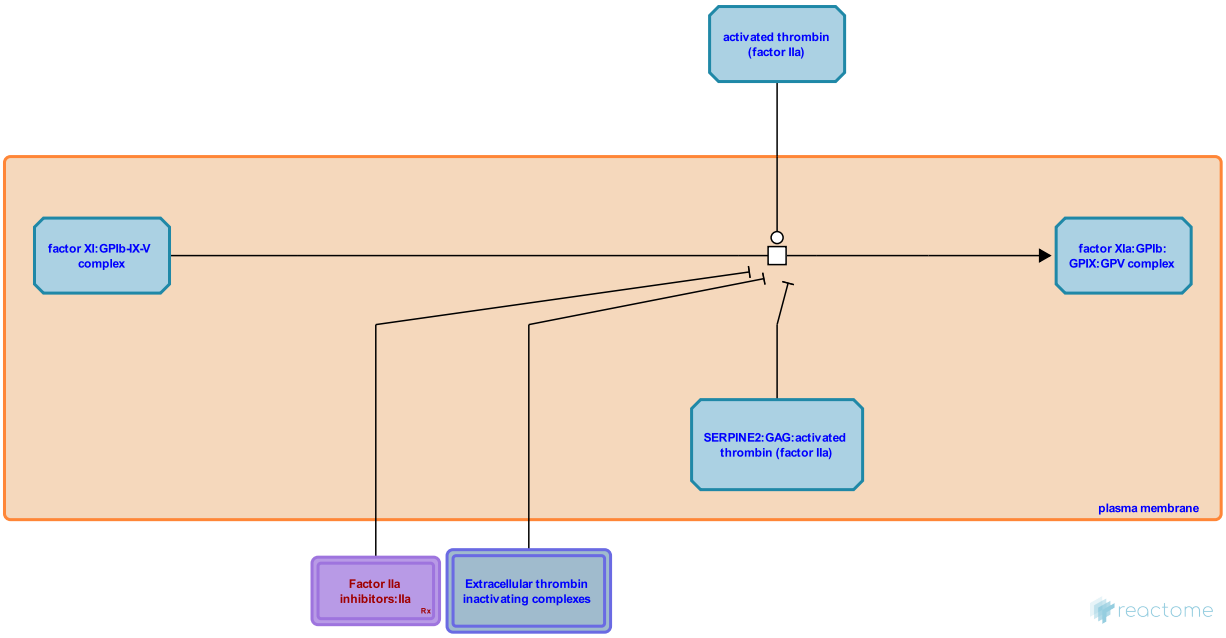
factor XI:platelet glycoprotein (GP) Ib:IX:V complex -> factor XIa:platelet glycoprotein (GP) Ib:IX:V complex (thrombin catalyst) ↗

Location: Intrinsic Pathway of Fibrin Clot Formation

Stable identifier: R-HSA-158419

Type: transition

Compartments: plasma membrane, extracellular region



Factor XI, bound to the cell surface, is converted to activated factor XI (factor XIa). In the body, this reaction occurs on the surfaces of activated platelets (Baglia et al. 2004; White-Adams et al. 2009; Reitsma et al. 2021). Small quantities of factor XI can be activated in a reaction catalyzed by factor XIIa, to initiate formation of a fibrin clot. However, the efficient activation of larger quantities of factor XI, needed to propagate the blood clotting process, appears to be mediated by thrombin (Gailani and Broze 1993; Naito and Fujikawa 1991; Oliver et al. 1999; Monroe et al. 2002).
Some direct oral anticoagulant (DOAC) drugs are potent, competitive direct thrombin inhibitors (DTIs). They reversibly and specifically binds both clot-bound and free thrombin (unlike warfarin or heparin), as well as inhibiting thrombin-induced platelet aggregation. These drugs can be synthetic organic compounds (dabigatran, argatroban) or recombinant peptides (lepirudin, bivalirudin, desirudin). Dabigatran (brand name Pradexa) is formulated as a lipophilic prodrug, dabigatran etexilate, to promote gastrointestinal absorption before it is metabolised to the active drug. The kidneys excrete the majority (80%) of unchanged drug (Stangier et al. 2007). Argatroban is a synthetic inhibitor of thrombin derived from L-arginine, which has a relatively short period of binding only to thrombin's active site (Hursting et al. 1997). It is given intravenously and is metabolised in the liver. Because of its hepatic metabolism, it may be used in patients with renal dysfunction. Lepirudin (brand name Refludan) is a recombinant hirudin derived from yeast cells (Weitz et al. 1990). Hirudin is a naturally occurring anticoagulant produced by the salivary glands of medicinal leeches. Bivalirudin (brand name Angiomax, Angiox) is a synthetic analog of hirudin, with a shorter period of binding to thrombin (Gladwell 2002). Desirudin (brand name Iprivask) is another recombinant hirudin derivative that directly inhibits free and fibrin-bound thrombin (Graetz et al. 2011). Melagatran is the active drug formed from the prodrug ximelagatran and is a competitive and rapid inhibitor of thrombin (Gustafsson et al. 1998). DuP 714 is a potent and specific thrombin inhibitor (Chiu et al. 1991).

Preceded by: factor XI + platelet glycoprotein (GP) Ib:IX:V complex -> factor XI:platelet glycoprotein (GP) Ib:IX:V complex

Followed by: factor IX -> factor IXa + factor IX activation peptide (factor XIa catalyst)

Literature references

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Broze, GJ Jr., Gailani, D. (1993). Factor XII-independent activation of factor XI in plasma: effects of sulfatides on tissue factor-induced coagulation. *Blood, 82*, 813-9. ↗

Editions

2005-01-20	Authored	D'Eustachio, P.
2024-03-06	Edited	D'Eustachio, P.

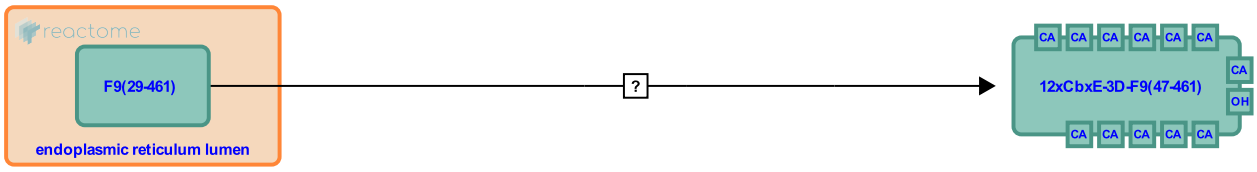
FIX is secreted ↗

Location: Intrinsic Pathway of Fibrin Clot Formation

Stable identifier: R-HSA-9670673

Type: uncertain

Compartments: extracellular region, endoplasmic reticulum lumen



Coagulation Factor IX (FIX) is expressed by hepatocytes (Yoshitake et al. 1985; Kurachi K & Kurachi S 1995). The newly synthesised FIX protein molecule comprising a pre- and pro-sequence (28 and 18 amino acids, respectively) and a mature peptide of 415 amino acids (total length, 461 amino acids) (Yoshitake et al. 1985; Kurachi K & Kurachi S 1995; Andersson LO et al. 1975; Anson DS et al. 1984). The pre-sequence (or signal sequence) directs FIX for secretion and the pro-sequence provides a binding domain for a vitamin K dependent (VKD) gamma (γ)-glutamyl carboxylase (GGCX) (Fryklund L et al. 1976; Galeffi P & Brownlee GG 1987; Lingenfelter SE & Berkner K 1996; Stanley TB et al. 1998). GGCX, an integral membrane protein located in the endoplasmic reticulum (ER) of hepatocytes, carboxylates certain glutamic acid residues in the adjacent GLA domain of FIX (Presnell and Stafford, 2002; Fryklund L et al. 1976; Galeffi P & Brownlee GG 1987). During the γ-carboxylation process, vitamin K hydroquinone is oxidized to vitamin K 2,3 epoxide and a carboxyl group is added to a glutamic acid residue (Wallin R eet al. 2002). In its native form, FIX contains 12 glutamic acid residues in the Gla domain; the first 10 residues are conserved in all VKD proteins, whereas the last two are unique to FIX (Gillis et al. 2008). FIX undergoes several other post-translational modifications before its secretion, including N- and O-linked glycosylation, sulfation, phosphorylation and hydroxylation (Agarwala KL et al. 1994; Bharadwaj D et al. 1995; Kaufman RJ 1998; Bond M et al. 1998; Enjolras N et al. 2004). These post-translational modifications occur within the ER and Golgi apparatus. In the ER, maturation and processing of secreted proteins are orchestrated by a group of molecules which facilitate protein folding and ensure that only correctly folded, assembled and modified proteins are transported along the secretory pathway. The proteins involved in the folding system are lectins such as calreticulin (CRT) or calnexin (CNX). A cellular unfolded protein response induces the ER-resident molecular chaperones such as glucose-regulated protein GRP78/BiP to prevent the aggregation of proteins in the ER. FIX was shown to co-immunoprecipitate with GRP78/BiP and CRT In cell lysates of transiently transfected human hepatocellular carcinoma (HepG2) cells expressing FIX (Enjolras N et al. 2004). After transportation of the carboxylated pro-FIX into the Golgi apparatus, the propeptide (29-46) is removed by the paired basic amino acid cleaving enzyme (PACE) (Wasley LC et al. 1993). The removal of the propeptide by PACE influences the formation of Ca2+-induced secondary and tertiary structures of the Gla domain, thus it is required for normal function of FIX (Pipe, 2008). The mature FIX is secreted and circulates in the plasma as an inactive 57kDa zymogen form (47-461). Domains within the zymogen are identified according to structure or function as follows: the GLA domain is crucial for the interaction with phospholipid surfaces; two epidermal growth factor (EGF)-like domains are critical for the interactions between factor IX and factor VIIIa; the activation peptide is released after proteolytic activation and the catalytic serine protease domain is required for normal function of FIX (Pipe 2008; Yoshitake S et al. 1985; Di Scipio RG et al. 1977; Rees DJ et al. 1988; Freedman SJ et al. 1995). Activation of factor IX involves cleavage of two peptide bonds, one on the C-terminal side of arginine 191 (the α-cleavage) the other on the C-terminal side of arginine 226 (the β-cleavage) (Di Scipio RG et al. 1978; Zögg T & Brandstetter H 2009). Activated factor IX comprising an N-terminal light chain and a C-terminal heavy chain held together by a disulphide bridge between cysteine resides 178 and 335 (Di Scipio RG et al. 1978; Zögg T & Brandstetter H 2009).

Followed by: factor IX -> factor IXa + factor IX activation peptide (factor XIa catalyst)

Literature references

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Kaufman, RJ., Furie, BC., Shoemaker, CB., Furie, B., Wasley, LC. (1986). Expression, purification, and characterization of recombinant gamma-carboxylated factor IX synthesized in Chinese hamster ovary cells. *J. Biol. Chem., 261* , 9622-8. ↗

Editions

2019-12-12	Authored	Shamovsky, V.
2020-01-09	Reviewed	D'Eustachio, P.
2020-04-02	Reviewed	Zhang, B.
2020-05-26	Edited	Shamovsky, V.

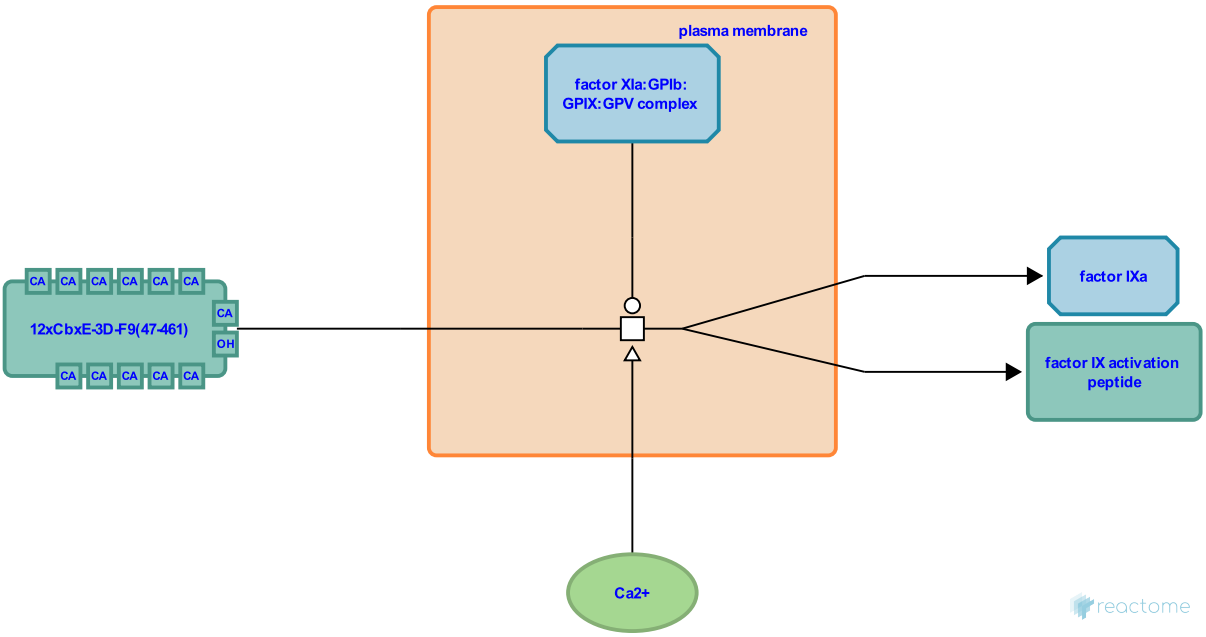
factor IX -> factor IXa + factor IX activation peptide (factor XIa catalyst) ↗

Location: Intrinsic Pathway of Fibrin Clot Formation

Stable identifier: R-HSA-158333

Type: transition

Compartments: plasma membrane, extracellular region



The mature factor IX (FIX) is secreted and circulates in the plasma as an inactive 57kDa zymogen form F9(47-461). Activation of FIX involves cleavage of two peptide bonds, at arginine 191 (R191-A192, the α-cleavage) and at arginine 226 (R226-V227, the β-cleavage), releasing an activation peptide (A192-R226) (Di Scipio RG et al. 1978; Zögg T & Brandstetter H 2009). The activation peptide has no known function. This calcium-dependent reaction is catalyzed by factor XIa (FXIa), bound to platelet glycoprotein (GP) Ib:IX:V on the platelet cell surface (Osterud B et al. 1978; Gailani D et al. 2001; Geng Y et al. 2012). Binding studies showed that FIX does not bind to FXIa in the absence of calcium (Geng Y et al. 2012). Structural studies suggest that both activation of factor XI and binding it to FIX induced conformational changes at the interface between the catalytic and the apple domains of the activated FXIa. The conformational changes of FXIa increased the accessibility to the apple 3 (A3) domain to enable FIX binding (Geng Y et al. 2012; Bar Barroeta A et al. 2019). FIX activation is ordered. FIX first binds to the FXIa A3 domain followed by engagement at the protease active site and cleavage of the R191-Al192 bond (Geng Y et al. 2012, 2013; Gailani D et al. 2014). The cleavage after R191 facilitates cleavage of the R226-V227 bond, forming the activated FIXa (also known as factor IXaβ). Catalytic efficiency for the second cleavage by FXIa is 7-fold greater than for the first cleavage, explaining the low accumulation of the α-cleavage product of FIX (Wolberg AS et al. 1997; Smith SB et al. 2008; Geng Y et al. 2012; Mohammed BM et al. 2018). Activated FIXa comprises an N-terminal light chain and a C-terminal heavy chain held together by a disulphide bridge between cysteine resides 178 and 335 (Di Scipio RG et al. 1978; Zögg T & Brandstetter H 2009). X-ray structure of the FIXa EGF2/protease domain at 1.37 A revealed that a Na+-binding site in association with Ca2+-binding site contributed to stabilization of the FIXa protease domain (Vadivel K et al. 2019).

Preceded by: factor XI:platelet glycoprotein (GP) Ib:IX:V complex -> factor XIa:platelet glycoprotein (GP) Ib:IX:V complex (XIIa catalyst), FIX is secreted, factor XI:platelet glycoprotein (GP) Ib:IX:V complex -> factor XIa:platelet glycoprotein (GP) Ib:IX:V complex (thrombin catalyst)

Followed by: factor VIIIa + factor IXa -> factor VIIIa:factor IXa

Literature references

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Cheng, Q., Ho, D., Sun, MF., Walsh, PN., Gailani, D. (2001). Model for a factor IX activation complex on blood platelets: dimeric conformation of factor XIa is essential. *Blood, 97*, 3117-22. ↗

Bajaj, SP., Verhamme, IM., Messer, A., Smith, SB., Sun, MF., Geng, Y. et al. (2012). A sequential mechanism for exosite-mediated factor IX activation by factor XIa. *J. Biol. Chem., 287*, 38200-9. ↗

Editions

2005-01-20	Authored	D'Eustachio, P.
2024-03-06	Edited	D'Eustachio, P.

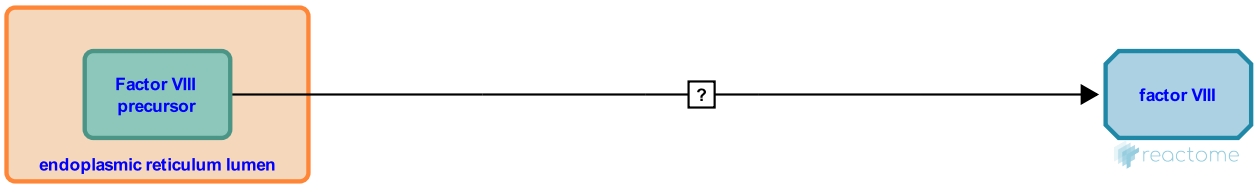
FVIII is secreted ↗

Location: Intrinsic Pathway of Fibrin Clot Formation

Stable identifier: R-HSA-9661625

Type: uncertain

Compartments: extracellular region, endoplasmic reticulum lumen



Coagulation factor VIII (FVIII) is a large glycoprotein of 2351 aminoacids with a discrete domain structure: A1-A2-B-A3-C1-C2 (Wood WI et al. 1984; Vehar GA et al. 1984; Toole JJ et al. 1984). FVIII is synthesized by various tissues, including liver, kidney, and spleen, as an inactive single-chain protein of approximately 293 kDa (Wion KL et al. 1985; Levinson B et al. 1992). Primary human liver sinusoidal endothelial cells (LSECs), blood outgrowth endothelial cells (BOEC), glomerular microvascular endothelial cells (GMVECs) and umbilical vein endothelial cells (HUVECs) were found to produce the FVIII protein, store it in Weibel-Palade bodies (WPB), and secrete in response to EC stimulation (van den Biggelaar M et al. 2009; Shahani T et al. 2014; Turner NA & Moake JL 2015). These findings are in agreement with the reports on the FVIII synthesis in human cultured ECs and in mice suggesting that ECs are the predominant source of plasma FVIII (Jacquemin M et al. 2006; Shahani T et al. 2010; Fahs SA et al. 2014). Evidence on the post-translational processing and secretion of FVIII has been generated from expression of the FVIII complementary DNA (cDNA) in transfected mammalian cells, such as Chinese hamster ovary (CHO), African green monkey kidney (COS-1), HeLa and the human hepatic SK-HEP1cell lines (Pipe SW et al. 1998; Herlitschka SE et al. 1998). Upon synthesis, FVIII is translocated into the lumen of the endoplasmic reticulum (ER), where it undergoes extensive processing including cleavage of a signal peptide and N-linked glycosylation at asparagine residues (Kaufman RJ et al. 1988, 1997; Kaufman RJ 1998). In the ER lumen of mammalian cells FVIII interacts with the protein chaperones calnexin (CNX), calreticulin (CRT), and immunoglobulin-binding protein (BiP or GRP78) that facilitate proper folding of proteins prior to trafficking to the Golgi compartment (Marquette KA et al. 1995; Swaroop M et al. 1997; Pipe SW et al. 1998; Kaufman RJ et al. 1997; Kaufman RJ 1998). Trafficking from the ER to the Golgi compartment is facilitated by LMAN1 and multiple combined factor deficiency 2 (MCFD2) cargo receptor complex (Zhang B et al. 2005; Zheng, C et al. 2010, 2013). Within the Golgi apparatus, FVIII is subject to further processing, including modification of the N-linked oligosaccharides to complex-type structures, O-linked glycosylation, and sulfation of specific Tyr-residues (Michnick DA et al. 1994; Kaufman RJ 1998). In addition, factor VIII is among the many proteins that undergoes intracellular proteolysis. Upon secretion from the cell, FVIII is cleaved at two sites in the B-domain to form a heterodimer consisting of the heavy chain containing the A1-A2-B domains in a metal ion-dependent complex with the light chain consisting of the A3-C1-C2 domains (Kaufman RJ et al. 1997; Kaufman RJ 1998). In the plasma, FVIII is stabilized through interaction with von Willebrand factor (Weiss HJ et al. 1977; Kaufman RJ et al. 1988; Chiu PL et al. 2015). Upon damage to blood vessel walls, thrombin cleaves FVIII and releases the B-domain to form an active FVIII heterotrimer (A1:A2:A3-C1-C2) that binds activated coagulation factor IX on the surface of platelet phospholipid to form the active factor Xase complex (Ahmad SS et al. 2003; Panteleev MA et al. 2006). This complex efficiently cleaves factor X to its active form, which activates prothrombin and leads to the formation of a stable fibrin clot. After conversion into its active conformation, and participation in the factor X activating complex, activated factor VIII rapidly looses its activity (Kaufman RJ et al. 1988; Lenting PJ et al. 1998). This process is governed by both enzymatic degradation and subunit dissociation. At the cellular level the FVIII expression is limited. Inefficient secretion of FVIII is caused by repression at the level of transcription (Lynch CM et al. 1993; Hoeben RC et al. 1995). In addition, a significant portion of the primary translation product is misfolded and ultimately degraded and FVIII is retained within ER through interaction with various ER chaperones including BiP (Marquette KA et al. 1995; Tagliavacca L et al. 2000). Mutations in the F8 gene often result in diminished or inactive plasma factor VIII protein and are the molecular genetic cause of the monogenic, X-linked, bleeding disorder hemophilia A (Al-Allaf FA et al. 2017; Castaman G & Matino D 2019).

Followed by: factor VIII + von Willebrand factor multimer -> factor VIII:von Willibrand factor multimer

Literature references

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Editions

2019-09-18	Authored	Shamovsky, V.
2020-01-09	Reviewed	D'Eustachio, P.
2020-04-02	Reviewed	Zhang, B.
2020-05-26	Edited	Shamovsky, V.

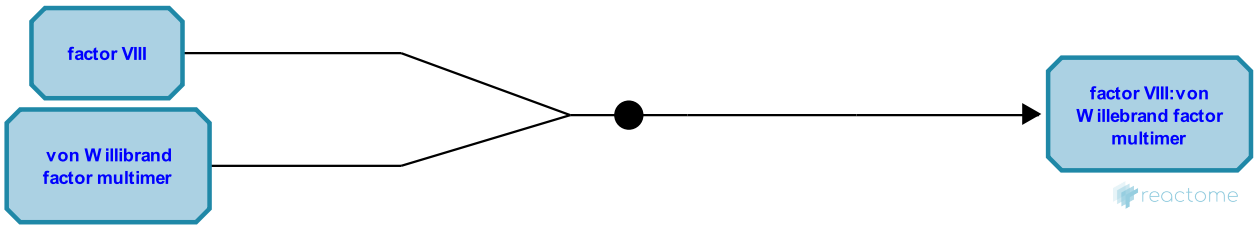
factor VIII + von Willebrand factor multimer -> factor VIII:von Willibrand factor multimer ↗

Location: Intrinsic Pathway of Fibrin Clot Formation

Stable identifier: R-HSA-158118

Type: binding

Compartments: extracellular region



Factor VIII (FVIII) binds to von Willebrand factor (vWF) to form a complex (Lollal P et al. 1988; Leyte A et al. 1989; Vlot et al. 1995). Antibody inhibition data, site-directed deletion and mutagenesis studies suggest that the acidic subdomain a3, C1 & C2 domains of the FVIII light chain together control high affinity binding to vWF (Foster PA et al. 1988; Leyte A et al. 1989, 1991; Shima M et al. 1993; Saenko EL et al. 1994; Saenko EL & Scandella D 1997; Jacquemin M et al. 2000). Structural studies using negative stain electron microscopy (EM) and hydrogen-deuterium exchange mass spectrometry (HDX-MS) have revealed that the TIL' domain of vWF interacts with the C1 domain of FVIII, the E' domain of vWF bridges the TIL' and D3 domains of vWF, whereas the D3 domain of vWF interacts with the C1 and C2 domains of FVIII (Yee A et al. 2015; Chiu PL et al. 2015). In addition, HDX-MS experiments showed that the FVIII a3 subdomain residues V1689-D1697 are directly involved in the interaction (Chiu PL et al. 2015). A combination of NMR spectroscopy and isothermal titration calorimetry (ITC) confirmed direct interaction between the a3 region of FVIII and the TIL' domain of VWF mapping it to the residues in the two β-sheet regions on the VWF TIL' domain (Dagil L et al. 2019). Further, tyrosine sulfation at residue 1699 is required for the interaction of FVIII with vWF (Leyte A et al. 1991). In the absence of sulfation at Y1699 in FVIII, the affinity for vWF was reduced by 5-fold (Leyte A et al. 1991). The nuclear magnetic resonance (NMR) spectrum studies of the complex between FVIII and vWF showed significantly larger residue-specific chemical shift changes when Y1699 was sulfated further highlighting the importance of FVIII sulfation at Y1699 for the binding affinity to vWF (Dagil L et al. 2019). The significance of the sulfation of FVIII at Y1699 in vivo is made evident by the presence of a Y1699F mutation that causes a moderate hemophilia A, likely due to reduced interaction with vWF and decreased plasma half-life (van den Biggelaar M et al. 2011). The vWF stabilizes FVIII, which otherwise has a very short half-life in the blood stream (Kaufman RJ et al. 1988). The interaction of FVIII with vWF allows thrombin to activate the bound FVIII and impedes cleavage of the molecules of nonactivated FVIII by the proteases FXa and activated protein C (APC) (Hamer RJ et al. 1987; Hill-Eubanks DC & Lollar P 1990; Koedam JA et al. 1990; Nogami K et al. 2002). Furthermore, vWF prevents the nonspecific binding of FVIII to the membranes of activated human platelets (Nesheim M et al. 1991; Li X & Gabriel DA 1997).

Factor VIII is a heterodimer containing a heavy and a light polypeptide chain, generated by the proteolytic cleavage of a single large precursor polypeptide (Vehar et al. 1984). Several forms of the heavy chain are found in vivo, all functionally the same but differing in the amount of the B domain removed by proteolysis. The single form annotated here is the shortest one (Eaton et al. 1986; Hill-Eubanks et al. 1989).

It has been demonstrated in in vitro experiments that vWF facilitates the association of FVIII chains and the retention of procoagulant activity in the conditioned medium of cells producing FVIII (Kaufman RJ et al. 1988; Wise RJ et al. 1991). Similar data have been obtained for re-association of FVIII chains in solution (Fay PJ 1988). In vitro, von Willebrand factor (Titani et al. 1986) can form complexes with factor VIII with a 1:1 stoichiometry. The complexes that form in vivo, however, involve large multimers of von Willebrand factor and varied, but always low, proportions of factor VIII (Vlot et al. 1995). A stoichiometry of one molecule of factor VIII associated with 50 of von Willebrand factor is typical in vivo, and is used here to annotate the factor VIII:von Willebrand factor complex.

Preceded by: FVIII is secreted

Followed by: factor VIII:von Willibrand factor multimer -> factor VIIIa + factor VIIIa B A3 acidic polypeptide + von Willibrand factor multimer

Literature references

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Editions

2005-01-20	Authored	D'Eustachio, P.
2024-03-06	Edited	D'Eustachio, P.

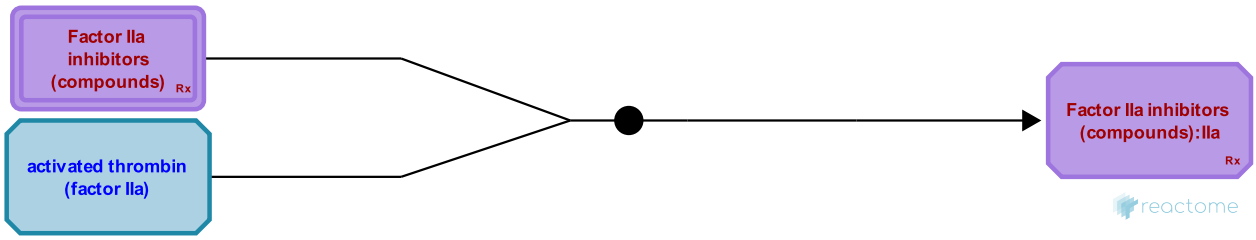
Factor IIa inhibitors (compounds) binds IIa ↗

Location: Intrinsic Pathway of Fibrin Clot Formation

Stable identifier: R-HSA-9015379

Type: binding

Compartments: extracellular region



In the blood coagulation process, prothrombin is proteolytically cleaved to form thrombin (factor IIa) which in turn, acts as a serine protease that converts soluble fibrinogen into insoluble strands of fibrin. Specifically, thrombin converts factor XI to XIa, factor VIII to VIIIa, factor V to Va, fibrinogen to fibrin, and factor XIII to XIIIa. The direct oral anticoagulant (DOAC) synthetic organic drugs dabigatran (brand name Pradaxa), argatroban (brand name Acova, Novastan; Exembol in the UK) and melagatran are potent, competitive direct thrombin inhibitors (DTIs). They reversibly and specifically bind both clot-bound and free thrombin (unlike warfarin or heparin), as well as inhibiting thrombin-induced platelet aggregation (Wienen et al. 2007, Stangier et al. 2007).

Commercially, dabigatran is formulated as a lipophilic prodrug, dabigatran etexilate, to promote gastrointestinal absorption before it is metabolised to the active drug. The kidneys excrete the majority (80%) of unchanged drug (Stangier et al. 2007). Argatroban is a synthetic inhibitor of thrombin derived from L-arginine, which has a relatively short period of binding only to thrombin's active site (Hursting et al. 1997). It is given intravenously and is metabolised in the liver. Because of its hepatic metabolism, it may be used in patients with renal dysfunction. Melagatran is the active drug formed from the prodrug ximelagatran and is a competitive and rapid inhibitor of thrombin (Gustafsson et al. 1998). DuP 714 is a potent and specific thrombin inhibitor (Chiu et al. 1991).

A major downside of DOACs is that they don't have reversing antidotes if internal bleeding arises from their use. However, in the case of severe bleeding of patients on dabigatran, the antibody fragment idarucizumab reversed the anticoagulation effects of dabigatran within minutes (Pollack et al. 2015). This represents a novel anticoagulation reversing mechanism for a DOAC.

Literature references

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Ries, UJ., Hauel, N., Priepke, H., Wienen, W., Stassen, JM. (2007). In-vitro profile and ex-vivo anticoagulant activity of the direct thrombin inhibitor dabigatran and its orally active prodrug, dabigatran etexilate. *Thromb. Haemost., 98*, 155-62. ↗

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Editions

2017-08-10	Authored, Edited	Jassal, B.
2018-03-21	Reviewed	Gómez-Outes, A.

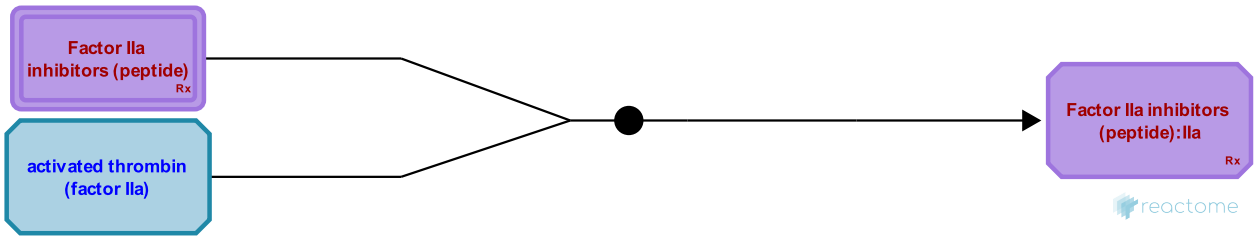
Factor IIa inhibitors (peptide) binds IIa ↗

Location: Intrinsic Pathway of Fibrin Clot Formation

Stable identifier: R-HSA-9603302

Type: binding

Compartments: extracellular region



In the blood coagulation process, prothrombin is proteolytically cleaved to form thrombin (factor IIa) which in turn, acts as a serine protease that converts soluble fibrinogen into insoluble strands of fibrin. Specifically, thrombin converts factor XI to XIa, factor VIII to VIIIa, factor V to Va, fibrinogen to fibrin, and factor XIII to XIIIa. Direct thrombin inhibitors (DTIs) represent a new class of promising anticoagulation agents. DTIs are increasingly being used instead of heparin to provide initial, rapid anticoagulation. Unlike heparin, which requires a mediator (antithrombin) to potentiate anticoagulation, Peptide DTIs can inhibit free and bound thrombin directly. Lepirudin (brand name Refludan) is a recombinant hirudin derived from yeast cells (Weitz et al. 1990). Hirudin is a naturally occurring anticoagulant produced by the salivary glands of medicinal leeches. Bivalirudin (brand name Angiomax, Angiox) is a synthetic analog of hirudin, with a shorter period of binding to thrombin (Gladwell 2002). Desirudin (brand name Iprivask) is another recombinant hirudin derivative that directly inhibits free and fibrin-bound thrombin (Graetz et al. 2011).

Literature references

Gladwell, TD. (2002). Bivalirudin: a direct thrombin inhibitor. *Clin Ther, 24*, 38-58. ↗

Avidan, MS., Smith, JR., Tellor, BR., Graetz, TJ. (2011). Desirudin: a review of the pharmacology and clinical application for the prevention of deep vein thrombosis. *Expert Rev Cardiovasc Ther, 9*, 1101-9. ↗

Massel, D., Hirsh, J., Maraganore, J., Hudoba, M., Weitz, JI. (1990). Clot-bound thrombin is protected from inhibition by heparin-antithrombin III but is susceptible to inactivation by antithrombin III-independent inhibitors. *J. Clin. Invest., 86*, 385-91. ↗

Editions

2018-03-19	Authored, Edited	Jassal, B.
2018-03-21	Reviewed	Gómez-Outes, A.

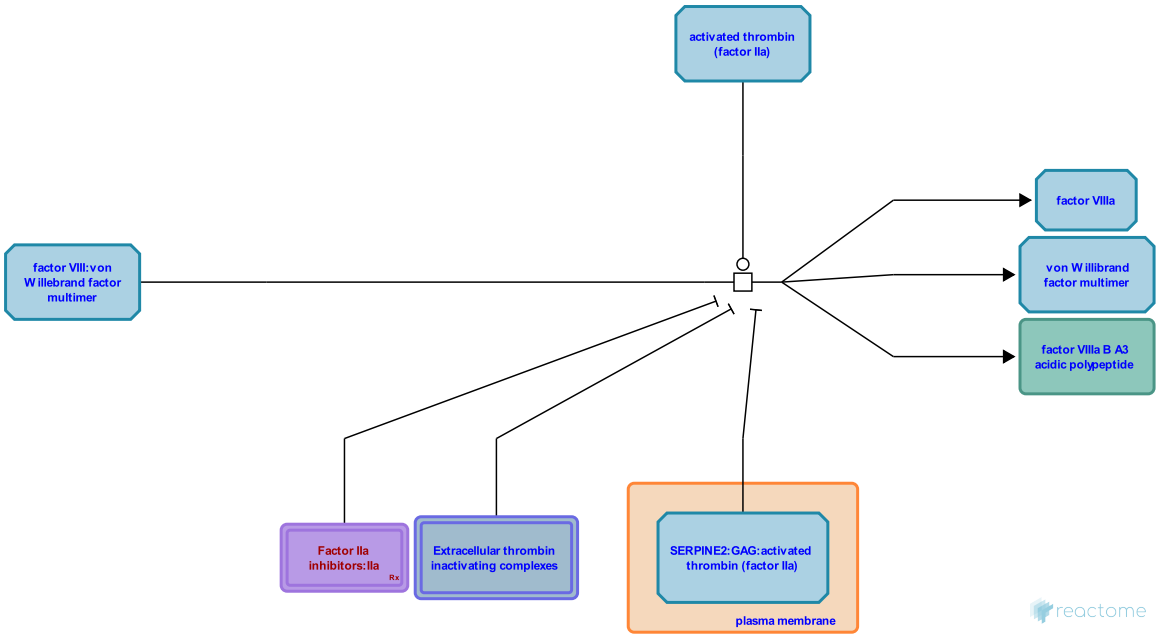
factor VIII:von Willibrand factor multimer -> factor VIIIa + factor VIIIa B A3 acidic polypeptide + von Willibrand factor multimer ↗

Location: Intrinsic Pathway of Fibrin Clot Formation

Stable identifier: R-HSA-158137

Type: transition

Compartments: extracellular region



Factor VIII complexed to von Willibrand factor in the blood is cleaved into several smaller polypeptides that remain associated. The acidic polypeptide on the aminoterminal side of the A3 domain of the light chain is released, however, and as this polypeptide mediates the association of factor VIII with von Willibrand factor, the activated factor VIII is released. While several proteases are capable of catalyzing these cleavages in vitro, only thrombin is active on factor VIII:von Willibrand factor complexes under physiological conditions (Eaton et al. 1986; Hill-Eubanks et al. 1989; Lollar et al. 1988; Pieters et al. 1989).
Some direct oral anticoagulant (DOAC) drugs are potent, competitive direct thrombin inhibitors (DTIs). They reversibly and specifically binds both clot-bound and free thrombin (unlike warfarin or heparin), as well as inhibiting thrombin-induced platelet aggregation. These drugs can be synthetic organic compounds (dabigatran, argatroban) or recombinant peptides (lepirudin, bivalirudin, desirudin). Dabigatran (brand name Pradexa) is formulated as a lipophilic prodrug, dabigatran etexilate, to promote gastrointestinal absorption before it is metabolised to the active drug. The kidneys excrete the majority (80%) of unchanged drug (Stangier et al. 2007). Argatroban is a synthetic inhibitor of thrombin derived from L-arginine, which has a relatively short period of binding only to thrombin's active site (Hursting et al. 1997). It is given intravenously and is metabolised in the liver. Because of its hepatic metabolism, it may be used in patients with renal dysfunction. Lepirudin (brand name Refludan) is a recombinant hirudin derived from yeast cells (Weitz et al. 1990). Hirudin is a naturally occurring anticoagulant produced by the salivary glands of medicinal leeches. Bivalirudin (brand name Angiomax, Angiox) is a synthetic analog of hirudin, with a shorter period of binding to thrombin (Gladwell 2002). Desirudin (brand name Iprivask) is another recombinant hirudin derivative that directly inhibits free and fibrin-bound thrombin (Graetz et al. 2011). Melagatran is the active drug formed from the prodrug ximelagatran and is a competitive and rapid inhibitor of thrombin (Gustafsson et al. 1998). DuP 714 is a potent and specific thrombin inhibitor (Chiu et al. 1991).

Preceded by: factor VIII + von Willebrand factor multimer -> factor VIII:von Willibrand factor multimer

Followed by: Factor VIIIa dissociates , factor VIIIa + factor IXa -> factor VIIIa:factor IXa

Literature references

Lindhout, T., Pieters, J., Hemker, HC. (1989). In situ-generated thrombin is the only enzyme that effectively activates factor VIII and factor V in thromboplastin-activated plasma. *Blood, 74*, 1021-4. ↗

Lollar, P., Hill-Eubanks, DC., Parker, CG. (1989). Differential proteolytic activation of factor VIII-von Willebrand factor complex by thrombin. *Proc Natl Acad Sci U S A, 86*, 6508-12. ↗

Rodriguez, H., Eaton, D., Vehar, GA. (1986). Proteolytic processing of human factor VIII. Correlation of specific cleavages by thrombin, factor Xa, and activated protein C with activation and inactivation of factor VIII coagulant activity. *Biochemistry, 25*, 505-12. ↗

Lollar, P., Hill-Eubanks, DC., Parker, CG. (1988). Association of the factor VIII light chain with von Willebrand factor . *J Biol Chem, 263*, 10451-5. ↗

Editions

2005-01-20	Authored	D'Eustachio, P.
2024-03-06	Edited	D'Eustachio, P.

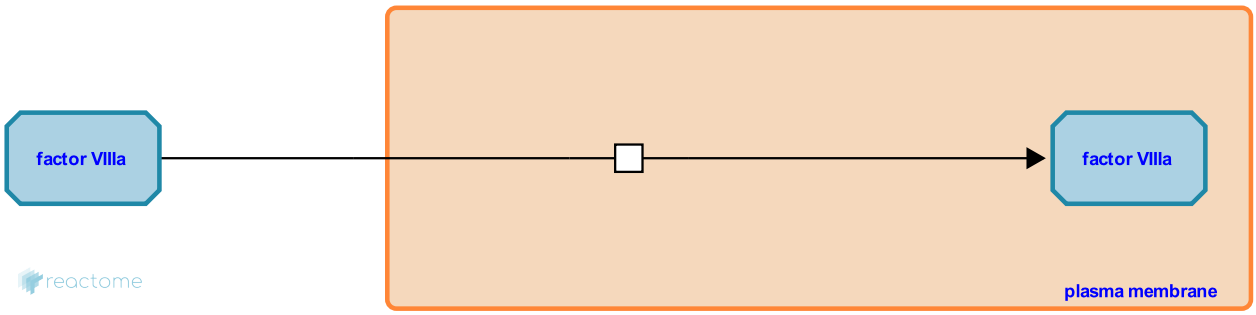
factor VIIIa associates with cell membrane ↗

Location: Intrinsic Pathway of Fibrin Clot Formation

Stable identifier: R-HSA-5607043

Type: transition

Compartments: plasma membrane, extracellular region



Cleavage of factor VIII light chain promotes a change in the conformation of the C2 domain that facilitates dissociation from VWF and enhances the affinity of factor VIIIa for anionic phospholipid surfaces (Saenko et al. 1998). Membrane-bound thrombin-activated factor VIII (FVIIIa) functions as a cofactor for factor IXa in the factor Xase complex. Factors VIIIa and IXa associate with anionic phospholipid surfaces with high affinity (Gilbert et al. 1990, Mertens & Bertina 1984; Panteleev et al 2004). Kd values ranging from 0.01 to 4.8 nM have been reported for FVIII binding to phospholipids (Gilbert et al. 1990,1992; Spaargaren et al. 1995; Raut et al. 1999; Ahmad et al. 2000). Studies using physiologic surfaces provide evidence for coordinated binding interactions of the enzyme, cofactor and substrate to discrete surface structures. For example, the presence of both (active site-modified) factor IXa and factor X increased both the number and the affinity of binding sites on activated platelets for factor VIIIa (Ahmad et al. 2000). However classical receptors for the constituents of factor Xase have not been identified (Fay 2004).

Literature references

Greco, NJ., Saenko, EL., Yakhyaev, AV., Scandella, D. (1998). Activation of factor VIII by thrombin increases its affinity for binding to synthetic phospholipid membranes and activated platelets. *J. Biol. Chem., 273*, 27918-26. ↗

Furie, BC., Gilbert, GE., Furie, B. (1990). Binding of human factor VIII to phospholipid vesicles. *J. Biol. Chem., 265*, 815-22. ↗

Editions

2014-07-14	Authored	Jupe, S.
2014-07-28	Edited	Jupe, S.
2014-09-11	Reviewed	Mumford, D.

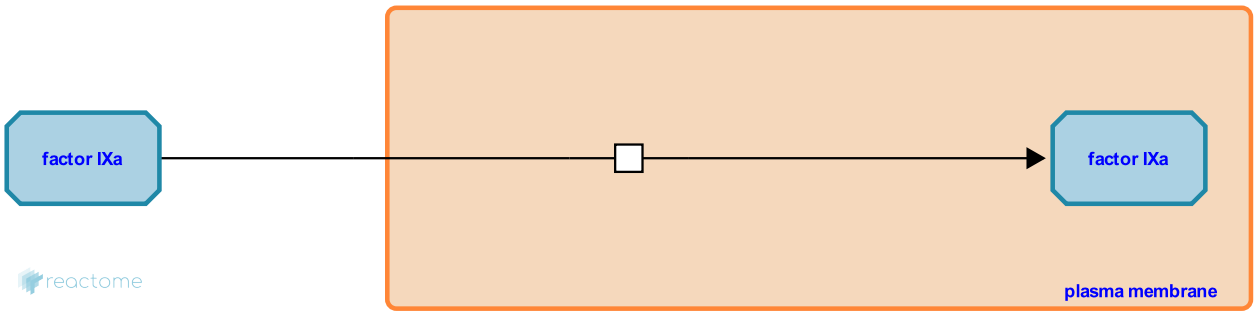
factor IXa associates with cell membrane ↗

Location: Intrinsic Pathway of Fibrin Clot Formation

Stable identifier: R-HSA-5607023

Type: transition

Compartments: plasma membrane, extracellular region



Membrane-bound thrombin-activated factor VIII (fVIIIa) functions as a cofactor for factor IXa in the factor Xase complex. Factors VIIIa and IXa associate with anionic phospholipid surfaces with high affinity (Gilbert et al. 1990, Mertens & Bertina 1984, Mertens et al. 1984; Greengard et al. 1986). Studies using physiologic surfaces provide evidence for coordinated binding interactions of the enzyme, cofactor and substrate to discrete surface structures. For example, the presence of both (active site-modified) factor IXa and factor X increased both the number and the affinity of binding sites on activated platelets for factor VIIIa (Ahmad et al. 2000). However classical receptors for the constituents of factor Xase have not been identified (Fay 2004).

Literature references

Bertina, RM., Cupers, R., Van Wijngaarden, A., Mertens, K. (1984). Binding of human blood-coagulation Factors IXa and X to phospholipid membranes. *Biochem. J., 223*, 599-605. ↗

Heeb, MJ., Griffin, JH., Ersdal, E., Greengard, JS., Walsh, PN. (1986). Binding of coagulation factor XI to washed human platelets. *Biochemistry, 25*, 3884-90. ↗

Editions

2014-07-14	Authored	Jupe, S.
2014-07-28	Edited	Jupe, S.
2014-09-11	Reviewed	Mumford, D.

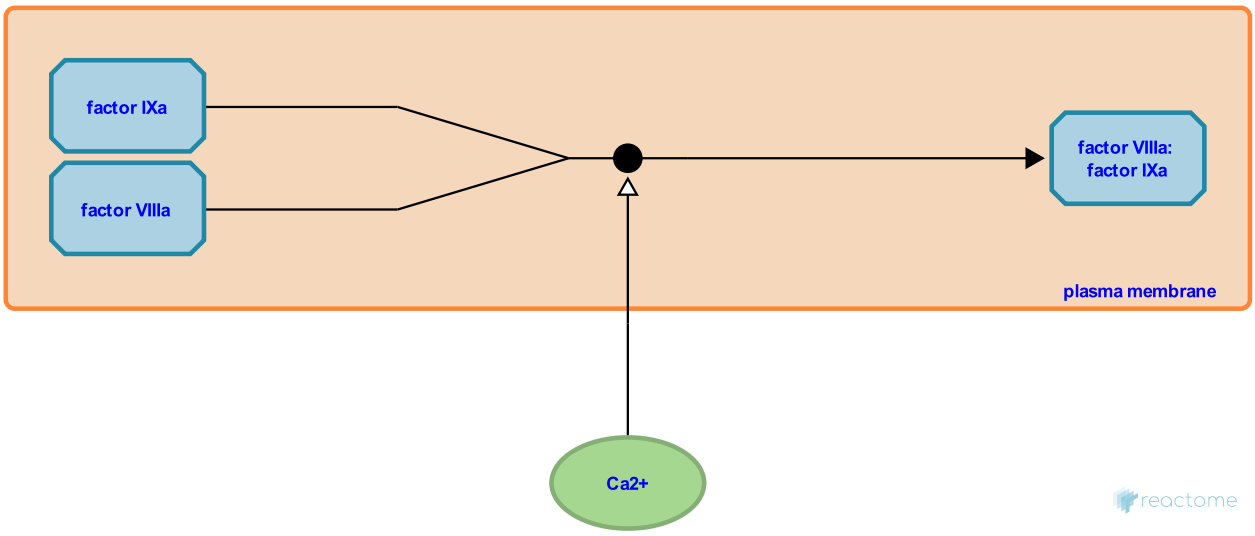
factor VIIIa + factor IXa -> factor VIIIa:factor IXa ↗

Location: Intrinsic Pathway of Fibrin Clot Formation

Stable identifier: R-HSA-158278

Type: binding

Compartments: plasma membrane, extracellular region



Factors VIIIa and IXa associate on cell surfaces to form a complex that very efficiently catalyzes the activation of factor X, the so-called "intrinsic tenase complex". In vitro, negatively charged phospholipids can provide an appropriate surface. In the body, the surface is provided by the plasma membranes of activated platelets (Gilbert and Arena 1996).

Preceded by: factor IX -> factor IXa + factor IX activation peptide (factor XIa catalyst), factor VIII:von Willibrand factor multimer -> factor VIIIa + factor VIIIa B A3 acidic polypeptide + von Willibrand factor multimer

Followed by: factor X -> factor Xa + factor X activation peptide (VIIIa:IXa catalyst)

Literature references

Gilbert, GE., Arena, AA. (1996). Activation of the factor VIIIa-factor IXa enzyme complex of blood coagulation by membranes containing phosphatidyl-L-serine. *J Biol Chem, 271*, 11120-5. ↗

Editions

2005-01-20	Authored	D'Eustachio, P.
2024-03-06	Edited	D'Eustachio, P.

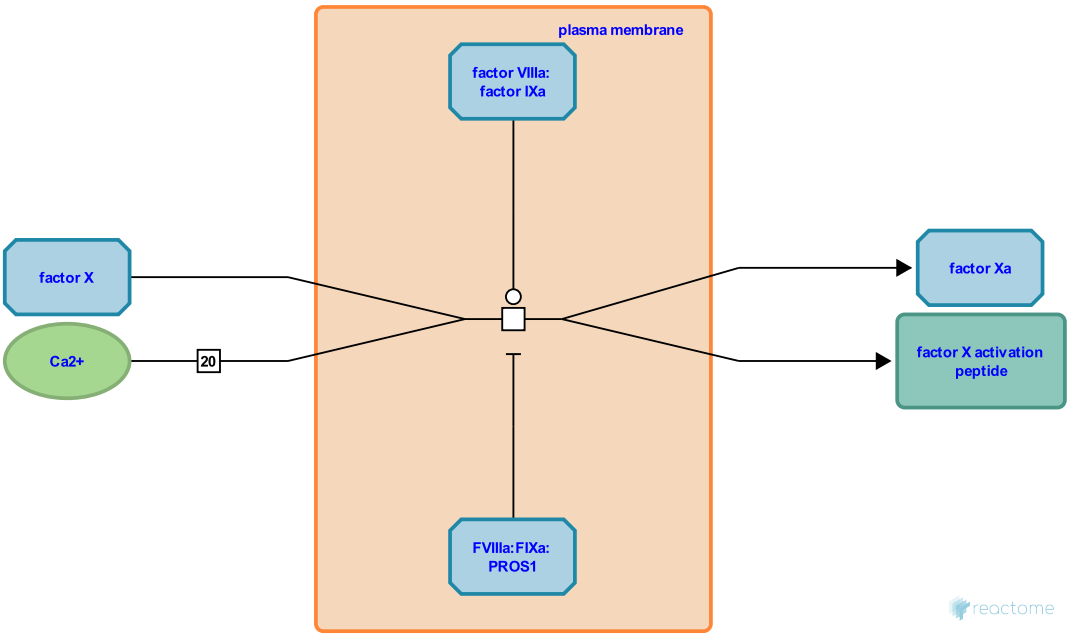
factor X -> factor Xa + factor X activation peptide (VIIIa:IXa catalyst) ↗

Location: Intrinsic Pathway of Fibrin Clot Formation

Stable identifier: R-HSA-158164

Type: transition

Compartments: plasma membrane, extracellular region



Factor IXa, in a complex with factor VIIIa on the surfaces of activated platelets (the "intrinsic tenase complex"), catalyzes the formation of activated factor X with high efficiency. The amino terminal part of the heavy chain of factor X, the factor X activation peptide, is released. (This peptide has no known function.)

Preceded by: factor VIIIa + factor IXa -> factor VIIIa:factor IXa

Literature references

Ahmad, SS., Ashby, B., Walsh, PN., Rawala-Sheikh, R. (1990). Kinetics of coagulation factor X activation by platelet-bound factor IXa. *Biochemistry, 29*, 2606-11. ↗

Editions

2005-01-20	Authored	D'Eustachio, P.
2024-03-06	Edited	D'Eustachio, P.

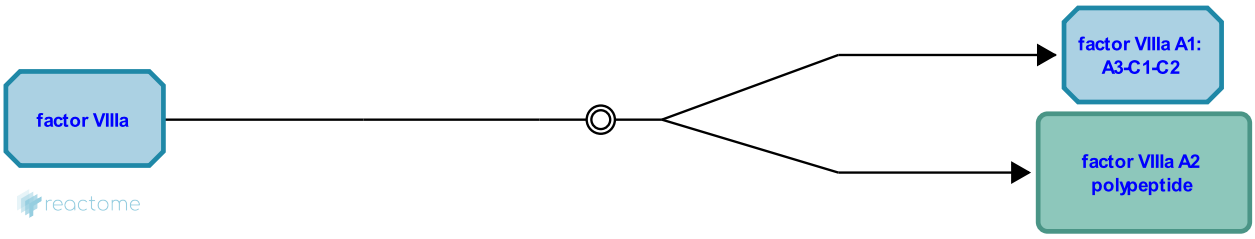
Factor VIIIa dissociates ↗

Location: Intrinsic Pathway of Fibrin Clot Formation

Stable identifier: R-HSA-9670014

Type: dissociation

Compartments: extracellular region



Factor VIII (FVIII) circulates in plasma as a heterodimer (domain structure A1-A2-B:A3-C1-C2) that requires thrombin cleavage to elicit procoagulant activity (Kaufman RJ et al. 1997). Upon activation by thrombin FVIII is converted to the labile FVIIIa, a heterotrimer of A1, A2 and A3C1C2 subunits, which serves as a cofactor for FIXa (Fay PJ 2006). At physiological concentrations, FVIIIa decays as a result of A2 subunit dissociation, which is weakly associated with the A1:A3-C1-C2 dimer by primarily electrostatic interactions (Fay PJet al. 1991; Fay PJ & Smudzin TM 1992; Parker ET et al 2006). Site-directed mutagenesis, functional and structural studies suggest that multiple residues at the A1-A2 and A2-A3 domain interfaces contribute to non-covalent interactions in stabilizing the protein (Parker ET & Lollar P 2007; Wakabayashi H & Fay PJ 2008, 2013; Wakabayashi H et al. 2008; Monaghan M et al. 2016). Retention of A2 polypeptide is required for normal stability of FVIIIa and dissociation of A2 correlates with FVIIIa inactivation and consequent loss of FXase activity.

Preceded by: factor VIII:von Willibrand factor multimer -> factor VIIIa + factor VIIIa B A3 acidic polypeptide + von Willibrand factor multimer

Literature references

Smudzin, TM., Fay, PJ. (1992). Characterization of the interaction between the A2 subunit and A1/A3-C1-C2 dimer in human factor VIIIa. *J. Biol. Chem., 267*, 13246-50. ↗

Haidaris, PJ., Smudzin, TM., Fay, PJ. (1991). Human factor VIIIa subunit structure. Reconstruction of factor VIIIa from the isolated A1/A3-C1-C2 dimer and A2 subunit. *J. Biol. Chem., 266*, 8957-62. ↗

Editions

2019-12-04	Authored	Shamovsky, V.
2020-01-09	Reviewed	D'Eustachio, P.
2020-04-02	Reviewed	Zhang, B.
2020-05-26	Edited	Shamovsky, V.

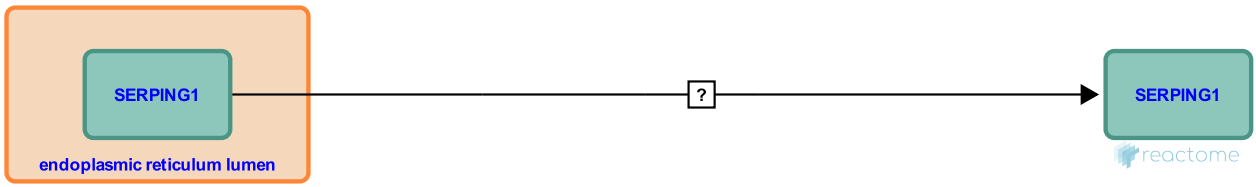
SERPING1 is secreted ↗

Location: Intrinsic Pathway of Fibrin Clot Formation

Stable identifier: R-HSA-9650473

Type: uncertain

Compartments: extracellular region, endoplasmic reticulum lumen



The plasma protease C1-inhibitor (C1-INH, SERPING1)) like all extracellular serine proteinase inhibitors (serpins) is secreted via the endoplasmic reticulum (ER)-Golgi pathway (Pan S et al. 2011). SERPING1 (C1-INH) is produced mainly in hepatocytes, reaching in healthy individuals a plasma concentration of 0.21–0.39 g/l (Prandini MH et al. 1986; Wouters D et al. 2008). SERPING1 can be produced and secreted from other cell types like peripheral blood monocytes, fibroblasts, and endothelial cells (Katz Y & Strunk RC 1989; Schmaier AH et al. 1989; Prada AE et al. 1998). SERPING1 is highly glycosylated plasma protein, bearing both N- and O-glycans (Stavenhagen K et al. 2018). SERPING1 belongs to the serine protease inhibitor (serpin) superfamily of structurally similar but functionally diverse proteins that use a conformational change to inhibit target enzymes (Silverman GA et al. 2001; Gettins PG 2002; Law RH et al. 2006). Serpins are globular proteins with a conserved structure of 7- 9 α-helices and 3 β-pleated sheets and a protruding reactive center loop (RCL) (Silverman GA et al. 2001; Gettins PG 2002; Law RH et al. 2006; Sanrattana W et al. 2019). In native serpins, the RCL, located outside the tertiary core of the serpin, forms a flexible stretch of approximately 20 amino acids, which provides structural flexibility in a solvent-exposed environment. They act on their target proteases by means of a suicide-substrate mechanism involving the cleavage of the RCL and its insertion into β-sheet A (Gettins PG 2002; Pan S et al. 2011; Khan MS et al. 2011). As a result, conformational changes take place in the serpins that ultimately trap and inactivate the targeted protease (Gettins PG 2002; Pan S et al. 2011; Khan MS et al. 2011; Sanrattana W et al. 2019). Serpins are conformationally labile and many of the disease-linked mutations of serpins result in misfolding or in formation of inactive, pathogenic polymers (Law RH et al. 2006). Under normal physiological conditions, SERPING1 (C1-INH) inhibits the activated forms of the serine proteases involved in the complement pathway (C1r and C1s), the contact system (FXIIa, FXIa, and kallikrein) as well as fibrinolytic proteases such as plasmin, tPA, and uPA (Sim et al. 1979; Arlaud et al. 1979; Kaplan AP & Ghebrehiwet B 2010).

Followed by: kallikrein binds SERPING1, factor XIIa binds SERPING1

Literature references

Iannotti, MJ., Sifers, RN., Pan, S. (2011). Analysis of serpin secretion, misfolding, and surveillance in the endoplasmic reticulum. *Meth. Enzymol., 499*, 1-16. ↗

Prandini, MH., Reboul, A., Colomb, MG. (1986). Biosynthesis of complement C1 inhibitor by Hep G2 cells. Reactivity of different glycosylated forms of the inhibitor with C1s. *Biochem. J., 237*, 93-8. ↗

Editions

2019-06-21	Authored	Shamovsky, V.
2020-01-09	Reviewed	D'Eustachio, P.
2020-04-02	Reviewed	Zhang, B.
2020-05-26	Edited	Shamovsky, V.

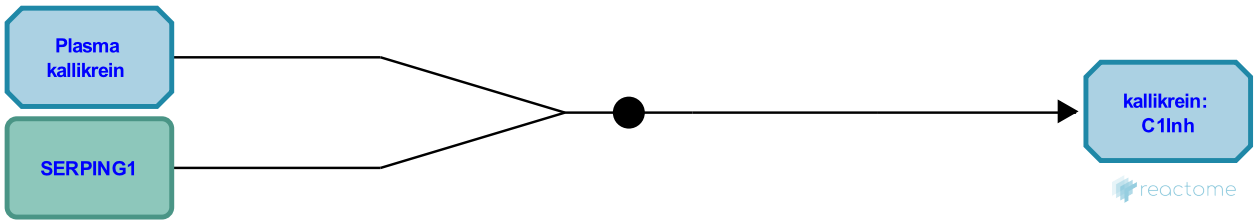
kallikrein binds SERPING1 ↗

Location: Intrinsic Pathway of Fibrin Clot Formation

Stable identifier: R-HSA-158399

Type: binding

Compartments: extracellular region



Activated kallikrein binds to C1Inh (plasma protease C1 inhibitor, SERPING1) (Bock et al. 1986), forming a stable and enzymatically inactive complex. This reaction appears to be the major means by which kallikrein is inactivated (kallikrein can also be inactivated by binding to alpha2-macroglobulin) (Harpel et al. 1985; Ratnoff et al. 1969).

Preceded by: SERPING1 is secreted, kallikrein:kininogen:C1q binding protein tetramer -> kallikrein + activated kininogen:C1q binding protein tetramer + bradykinin

Literature references

Nielsen, E., Marrinan, J., Donaldson, VH., Thogersen, HC., Skriver, K., Eddy, RL. et al. (1986). Human C1 inhibitor: primary structure, cDNA cloning, and chromosomal localization. *Biochemistry, 25*, 4292-301. ↗

Harpel, PC., Lewin, MF., Kaplan, AP. (1985). Distribution of plasma kallikrein between C-1 inactivator and alpha 2-macroglobulin in plasma utilizing a new assay for alpha 2-macroglobulin-kallikrein complexes. *J Biol Chem, 260*, 4257-63. ↗

Naff, GB., Ogston, D., Pensky, J., Ratnoff, OD. (1969). The inhibition of plasmin, plasma kallikrein, plasma permeability factor, and the C'1r subcomponent of the first component of complement by serum C'1 esterase inhibitor. *J Exp Med, 129*, 315-31. ↗

Editions

2005-01-20	Authored	D'Eustachio, P.
2024-03-06	Edited	D'Eustachio, P.

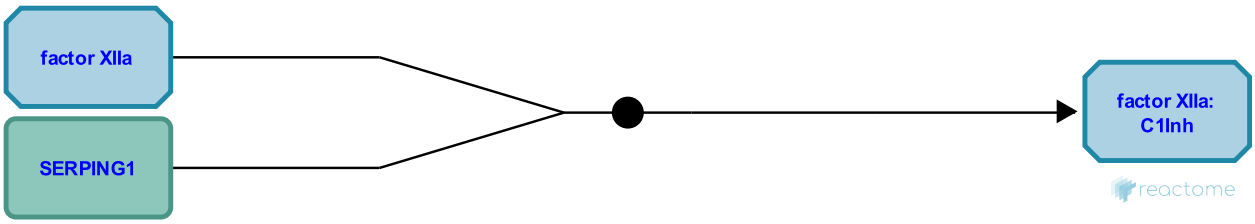
factor XIIa binds SERPING1 ↗

Location: Intrinsic Pathway of Fibrin Clot Formation

Stable identifier: R-HSA-158357

Type: binding

Compartments: extracellular region



Activated factor XII (factor XIIa) binds to C1Inh (C1 inhibitor - Bock et al. 1986) to form a stable, inactive complex (Schneider et al. 1973). While several protease inhibitors can form stable complexes with XIIa in vitro, only C1Inh does so to a significant extent under normal conditions in vivo (Pixley et al. 1985).

Preceded by: factor XII -> factor XIIa, SERPING1 is secreted

Literature references

Nielsen, E., Marrinan, J., Donaldson, VH., Thogersen, HC., Skriver, K., Eddy, RL. et al. (1986). Human C1 inhibitor: primary structure, cDNA cloning, and chromosomal localization. *Biochemistry, 25*, 4292-301. ↗

Schapira, M., Pixley, RA., Colman, RW. (1985). The regulation of human factor XIIa by plasma proteinase inhibitors. *J Biol Chem, 260*, 1723-9. ↗

Kaplan, AP., Austen, KF., Schreiber, AD. (1973). Inhibition by C1INH of Hagemann factor fragment activation of coagulation, fibrinolysis, and kinin generation. *J Clin Invest, 52*, 1402-9. ↗

Editions

2005-01-20	Authored	D'Eustachio, P.
2024-03-06	Edited	D'Eustachio, P.

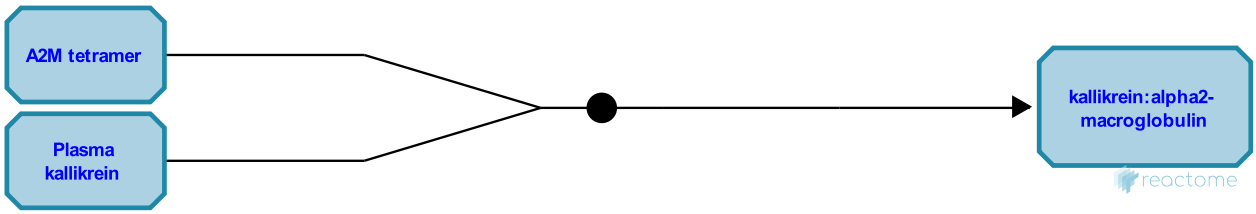
kallikrein + alpha2-macroglobulin -> kallikrein:alpha2-macrogloulin ↗

Location: Intrinsic Pathway of Fibrin Clot Formation

Stable identifier: R-HSA-158340

Type: binding

Compartments: extracellular region



Activated kallikrein binds to alpha2-macroglobulin (Sottrup-Jensen et al. 1984), forming a stable and enzymatically inactive complex. Under normal conditions in vivo, this reaction appears to be responsible for the inactivation of about 1/6 of activated kallikrein (with C1Inh responsible for the inactivation of about 5/6) (Harpel et al. 1985).

Preceded by: kallikrein:kininogen:C1q binding protein tetramer -> kallikrein + activated kininogen:C1q binding protein tetramer + bradykinin

Literature references

Harpel, PC., Lewin, MF., Kaplan, AP. (1985). Distribution of plasma kallikrein between C-1 inactivator and alpha 2-macroglobulin in plasma utilizing a new assay for alpha 2-macroglobulin-kallikrein complexes. *J Biol Chem, 260*, 4257-63. ↗

Sottrup-Jensen, L., Wierzbicki, DM., Lonblad, PB., Petersen, TE., Magnusson, S., Kristensen, T. et al. (1984). Primary structure of human alpha 2-macroglobulin. V. The complete structure. *J Biol Chem, 259*, 8318-27. ↗

Editions

2005-01-20	Authored	D'Eustachio, P.

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