Regulation of human protein S gene (PROS1) transcription

Wolf, Cornelia de

Chapter 1

General introduction

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General Introduction

1.1 Haemostasis and Venous Thrombosis

Haemostasis refers to a physiologic process whereby bleeding is halted (reviewed in (1-3)). When a blood vessel is damaged, several processes occur to staunch the flow of blood. Firstly, vasoconstriction narrows the blood vessel, reducing vessel diameter and slowing bleeding. Then, during primary haemostasis blood platelets bind to collagen in the exposed sub-endothelium to form a haemostatic plug within seconds after an injury. This is followed by secondary haemostasis or coagulation, which involves the activation of a complex cascade of coagulation factors, ultimately resulting in the conversion of fibrinogen into polymerized fibrin, making a clot. Finally, the clot attracts and stimulates the growth of fibroblasts and smooth muscle cells within the vessel wall, and initiates the repair process, which ultimately results in the dissolution of the clot through fibrinolysis (tertiary haemostasis). Disorders of haemostasis can be roughly divided into platelet disorders, such as Glanzmann thrombasthenia and Bernard-Soulier syndrome, and disorders of coagulation, such as haemophilia or thrombosis.

Most coagulation factors circulate as the zymogen of a serine protease. The coagulation cascade is a series of reactions in which the zymogens and their glycoprotein cofactors are activated and then catalyze the next reaction in the cascade. Coagulation is initiated mainly in response to the interaction between factor VII (FVII) and exposed tissue factor (TF) from the vascular sub-endothelium. The TF-activated FVII (FVIIa) complex activates coagulation factors IX (FIX) and X (FX) (4), thereby activating the coagulation cascade (Figure 1). The final product of the cascade, thrombin, increases its own production by activating other components of the coagulation cascade, amongst which factors V (FV), VIII (FVIII), and XI (FXI) and so the cycle continues. The primary role of thrombin is the conversion of fibrinogen into fibrin fibres, the main component of the blood clot together with the platelets.

Activated clotting factors are the driving force of the coagulation cascade. To prevent excessive clotting, activated coagulation factors are inactivated by the circulating blood protease inhibitors; antithrombin (5) and heparin cofactor II (6), by tissue factor pathway inhibitor (TFPI) (7) and by Activated protein C (APC), the end product of the protein C anticoagulant pathway (8). Whereas blood protease inhibitors and TFPI bind to the activated

clotting factors and/or clotting factor complexes thereby rendering them inactive, APC inhibits the coagulation cascade through the proteolytic degradation of the two key coagulation factors activated FV (FVa) and activated FVIII (FVIIIa).

The coagulation cascade is tightly regulated since excessive production of fibrin would lead to the occlusion of blood vessels and thrombosis, whereas too little fibrin would cause (excessive) bleeding and impaired wound healing. When clots are formed in the venous system we refer to venous thrombo-embolism. These clots most often occlude veins in the extremities (e.g. legs; deep vein thrombosis), and may form emboli, which travel through the blood to the narrow pulmonary arteries, where they may cause life-threatening obstructions (pulmonary embolism). The annual incidence of venous thrombosis is 1-3 per 1000 individuals (9;10). A predisposition towards this disease may be genetic or acquired. Acquired risk factors include a.o. aging, immobilisation, trauma, pregnancy, and use of female hormones (11). Genetic risk factors include loss of function mutations in the genes coding for antithrombin, protein C, and protein S (PS) and gain of function mutations in the FV and prothrombin gene (12). The most common genetic risk factor is a mutation in the gene encoding FV, causing an amino acid substitution, R506Q, which renders this coagulation factor resistant to inactivation by APC (13). FV-R506Q is referred to as FV_{Leiden} and occurs in almost 50% of the patients with a family history of venous thrombosis (14).

1.2 The Protein C Anticoagulant Pathway

The protein C anticoagulant pathway is initiated by the activation of protein C to APC by a complex of thrombin and the transmembrane glycoprotein thrombomodulin. Thrombomodulin present in the membranes of vascular endothelial cells amplifies protein C activation more than 1000 fold (15), and when protein C is bound to the Endothelial Protein C Receptor (EPCR), this process is stimulated another 20-fold (16). Upon its generation, APC inactivates F Va and FVIIIa (Figure 1) thus inactivating the prothrombinase and tenase complexes (17;18). Both proteolytic reactions are greatly enhanced by the negatively charged phospholipids present on activated platelets and vascular endothelium, and by PS, the non-enzymatic cofactor to APC (19).

PS forms a complex with APC on the phospholipid surface and increases the affinity of APC for negatively charged phospholipids (17;20;21). Moreover, PS relocates the APC active site closer to the membrane surface, which contains the activated coagulation factors (22). In the inactivation of FVIIIa, PS and FV act as synergistic cofactors to APC (23). PS also has direct anticoagulant properties independent from APC. It was shown to directly inhibit the activity of the tenase and prothrombinase complexes, presumably by binding to factors VIIIa (24), Va and Xa (25-27). The physiological implications of these findings are the subject of active research (28-31).

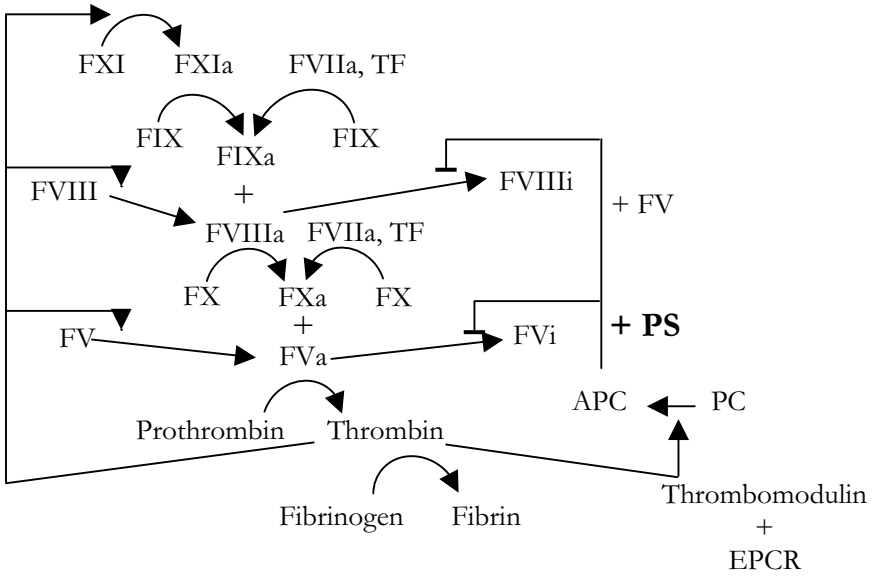

Figure 1 Schematic representation of the coagulation cascade and the protein C anticoagulant pathway. Blunted arrows represent inhibitory reactions.

Next to inhibiting the formation of thrombin, APC also has profibrinolytic properties. During fibrinolysis cross-linked fibrin, the main component of a blood clot, is solubilized by plasmin. Plasmin is generated from its precursor, plasminogen, by tissue-type plasminogen activator (t-PA). t-PA however, is strongly inhibited by plasminogen activator inhibitor-1 (PAI-1). APC is thought to stimulate fibrinolysis through binding and inhibition of PAI-1 (Figure 2) (32-34). The APC-PAI-1 interaction is greatly enhanced upon binding of the extracellular matrix protein vitronectin to PAI-1 (35).

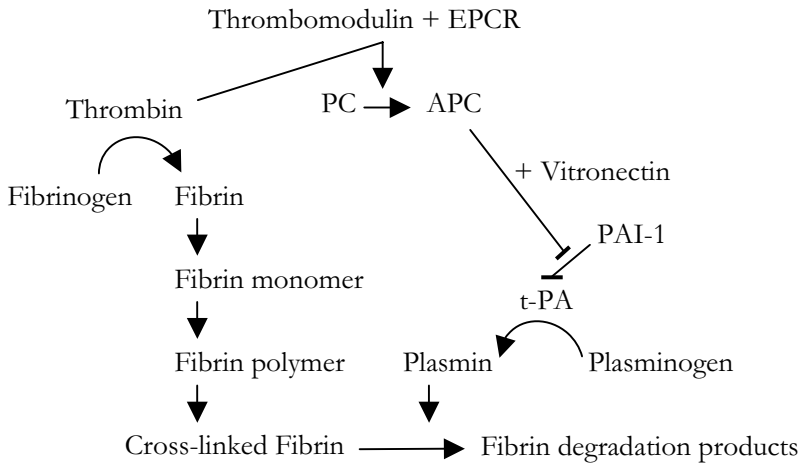

Figure 2 Schematic representation of the proposed role for APC in fibrinolysis. Blunted arrows represent inhibitory reactions.

In addition to their anticoagulant role, both APC and PS have been implicated in other major physiological processes. APC has been shown to have both anti-inflammatory and anti-apoptotic effects (36-38), and successful clinical trials have been conducted for development of its use in the treatment of sepsis (39;40). Similar functions have been allocated to PS (41). The emphasis of the research into a role beyond coagulation for this protein has focused mainly on its mitogenic properties (42;43) and its potential role in the regulation of cell survival (44-47).

Just as the coagulation cascade is under strict regulation by several inhibitory pathways, so are the main components of the protein C anticoagulant pathway. APC anticoagulant activity is inhibited after binding to protein C inhibitor (PCI) and α_1-antitrypsin, both members of the serpin family of blood protease inhibitors (48-50). Moreover, PCI prevents the formation of APC by inhibiting the thrombin-thrombomodulin complex (51). The PAI-1-vitronectin complex was also suggested to be important in limiting APC anticoagulant activity on the platelet and endothelial surface (35). The regulation of PS will be discussed in the following section.

1.3 Protein S

1.3.1 Protein

DiScipio and Davie in Seattle first isolated and named protein S(eattle) in 1977 (52). Not much later Walker described PS as the non-enzymatic cofactor to APC (17;19). PS (Figure 3) is a vitamin K-dependent glycoprotein with a molecular weight of 75 kilo Dalton that is present in plasma at a concentration of ~ 0.35 μM (53-55). The structure and function of its individual domains are well-documented and reviewed elsewhere (56-59) and will therefore not be covered here. Regretfully, a crystal structure of PS has not yet been successfully generated.

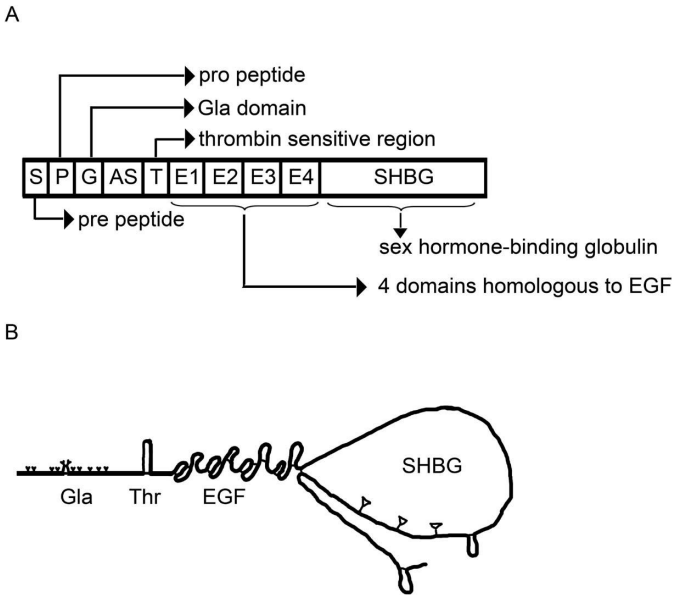

Figure 3 Schematic representation of human Protein S. (**A**) *Immature PS*. AS: aromatic stack, EGF: epidermal growth factor-like domain (**B**) *Mature post-translationally modified PS*. ϒ: carboxylated carboxyglutamic (Gla) residues, ⍱ glycosylation sites, illustration (**B**) taken from (60).

PS is produced and secreted mainly by hepatocytes (61) in the liver but also at low levels by various other cell types, such as megakaryocytes (62), endothelial cells (63;64), Leydig cells (65), osteoblasts (66) and cells of the nervous system (67). In addition, a small portion

(~2.5%) is stored in the α-granules of blood platelets (68). That extra-hepatically produced PS is an important source of plasma PS, is illustrated by the fact that in patients with liver disease PS levels are reduced but not to the same low levels as other vitamin K-dependent coagulation factors (69;70). The anticoagulant reactions in which PS participates (Figure 1), take place on the surface of endothelial cells and activated platelets, cell types that both produce and/or can release PS.

PS can bind to the surface of (endothelial) cells not only through its interaction with negatively charged phospholipids (71), but also by binding to a specific family of membrane receptor tyrosine kinases (Tyro/Axl) (67;72-74). This interaction was first shown for growth arrest-specific 6 (GAS6) (75), a protein structurally related to PS (76). The relevance of this finding is questionable though since in contrast to GAS6, which binds to and stimulates its receptor, PS seems to only bind, not activate, the receptor at physiological relevant concentrations (77).

Functional PS levels in plasma are mainly regulated in two different ways. Firstly, PS can be inactivated by proteolytic cleavage in the thrombin-sensitive region. *In vitro* PS is cleaved by thrombin after Arg 49 and Arg 70 in the thrombin-sensitive region (78). *In vivo* PS is protected against thrombin-mediated cleavage by the binding of calcium ions to the Gla-domain (79). Nevertheless, increased cleaved PS levels were found in patients with disseminated intravascular coagulation (DIC) (80). Long *et al.* demonstrated that *in vitro* PS is cleaved after Arg 60 by FXa (81). This cleavage site was later shown to be the actual cleavage site *in vivo* (82;83). It is therefore assumed that it is FXa that inactivates PS by proteolytic cleavage *in vivo*. Secondly, PS circulates in plasma in two forms; a free form (40%) and in complex with the complement inhibitor, C4b-binding protein (C4BP) (53-55). Multiple binding sites for C4BP are located in the SHBG domain of PS (84-87). The C4BP protein contains 6 or 7 identical α-chains and a single β-chain, although 17% of the C4BP molecules lack the β-chain (55). PS binds with high affinity to C4BP via the β-chain (Figure 4a) (88). Unbound PS circulating in plasma represents the molar excess of PS over C4BPβ (53;55).

Whereas only free PS functions as a cofactor to APC, the APC-independent anticoagulant properties of PS do not seem to be negatively influenced by complexation to C4BP (24;27;31).

Moreover, the newly proposed role for PS in the phagocytosis (44;45) and/or rescue (43;47) of early apoptotic cells is thought to be mediated at least in part by directing C4BP to the surface of apoptotic cells (Figure 4b).

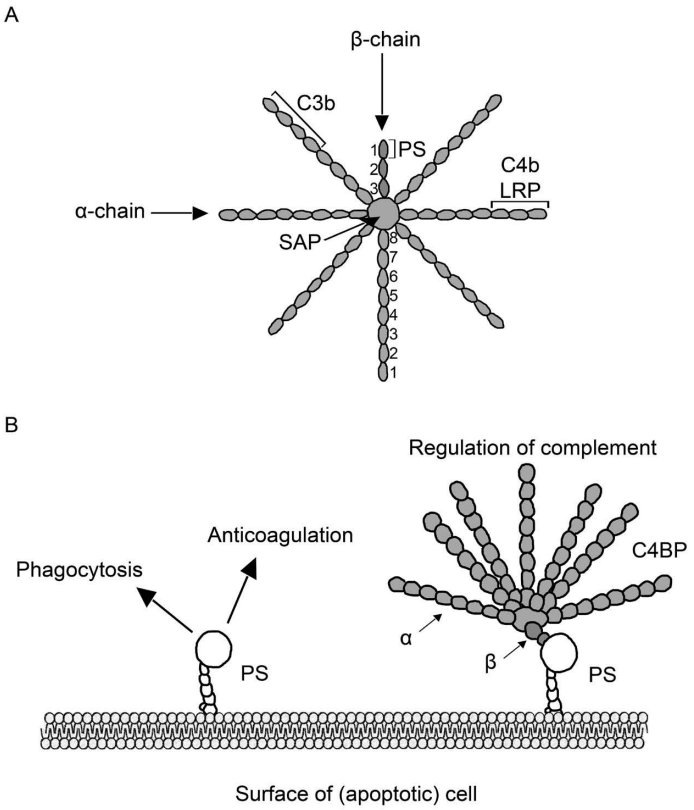

Figure 4 Interaction of PS with C4BP. (**A**) *Schematic representation of binding sites on C4BPβ*$^{+}$. PS binds to the β-chain of C4BP. The binding of other proteins at their specific binding sites does not affect PS binding to C4BP (89;90). C3b: activated complement factor 3, C4b: activated complement factor 4, LRP: low-density lipoprotein receptor-related protein, SAP: Serum Amyloid P component. Illustration adapted from (59). (**B**) *Properties of PS on the cell surface*. Illustration adapted from (91).

1.3.2 Gene

Two copies of the gene for PS, *PROS1* and *PROS2*, are located near the centromeric region of chromosome 3 (3p11.1-3q11.2) (92;93). *PROS* mRNA (94-96) is produced only from the *PROS1* gene, which spans a length of 80 kb and contains 15 exons and 14 introns (97-99). This is because *PROS2* is a pseudogene that lacks the promoter and the first exon. It

contains several frame-shift deletions leading to premature stopcodons that render this gene inactive. *PROS2* most likely originated from the functional gene, *PROS1*, through partial duplication (98). As with promoter regions from other genes coding for vitamin K-dependent coagulation proteins (100-106), a distinct TATA-box is absent from the *PROS1* promoter region. The finding of various transcription start sites reported for both published (94-96) and unpublished (NCBI-dbEST database entries (107)) *PROS1* cDNA sequences, suggests that transcription is initiated through multiple transcription start sites. In addition, the presence of an alternative promoter and first exon was postulated by Ploos van Amstel *et al* (98) upon their finding of two distinct start sites by primer extension analysis and the identification of two putative splice acceptor sites in the promoter region. This last hypothesis was, however, never borne out by experimental data.

In contrast to the coding region of the *PROS1* gene, which has been thoroughly investigated (reviewed in (108;109)), the promoter region has been poorly investigated. A promising abstract on the regulation of the *PROS1* promoter by various transcription factors, which was presented at a meeting of the International Society of Thrombosis and Haemostasis (ISTH) in 1995, was never followed by a paper in a peer reviewed journal (110). Since then, only a single report on the regulation of the promoter region has been published (111). In this last report, transcription directed from *PROS1* promoter-reporter gene constructs was stimulated in hepatoma HepG2 cells *in vitro* by binding of the ubiquitous transcription factor, Sp1. The liver-specific transcription factor, forkhead box A2 (FOXA2, HNF3β), also bound to the *PROS1* promoter, but *trans*-activation studies were not conducted with this transcription factor.

On a completely different level, Hooper and coworkers (112-114) measured PS levels after stimulation of cultured human hepatoma cell line HepG2, primary Human Umbilical Vein Endothelial Cells (HUVEC), and the human microvascular endothelial cell line, HMEC-1, with interleukin 6 (IL6), a mediator of the acute phase response during inflammation (115). IL6 stimulated PS production by all cell types and this could be suppressed by the addition of tumor necrosis factor α (TNFα). Although these authors did not directly investigate transcriptional regulation of *PROS1*, the results allude to the possible binding of the nuclear factors, which are induced by IL6 such as the signal transducer and activator of transcription 3 (STAT3) (116) and the CCAAT/enhancer-binding protein β (C/EBPβ) (117). In Figure 5 the

data from the various studies are combined and putative binding sites for STAT3 and C/EBPβ are included.

All in all, the components that determine (normal) variation in PS levels at the transcriptional level are still largely unknown.

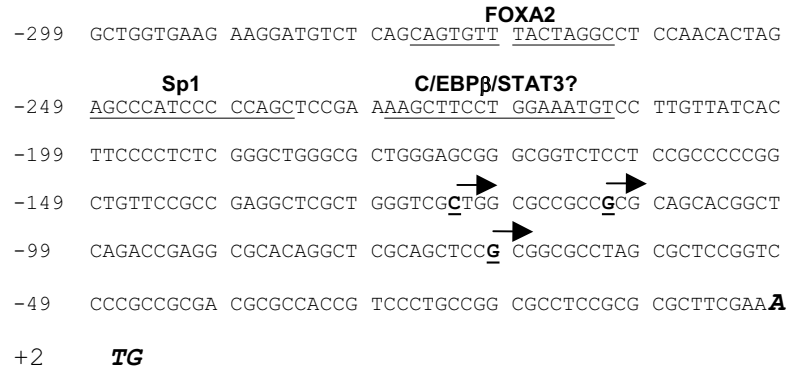

Figure 5 Partial *PROS1*5' sequence. FOXA2 and Sp1 bind the *PROS1* promoter at the underlined regions (111). A putative binding site for C/EBPβ and STAT3 binding is depicted. The arrows indicate published transcription start sites derived from cDNA libraries (65;95;96). +1 is the first nucleotide of the translational startcodon, ATG.

1.4 Protein S Deficiencies

1.4.1 Hereditary PS deficiency

Partial PS deficiency was first reported to be associated with venous thrombotic disease in 1984 (118). In subsequent years it was established that in thrombophilic families heterozygous PS deficiency was associated with an increased risk of venous thrombosis. Homozygous or compound heterozygous PS deficiency is extremely rare and associated with severe purpura fulminans in the neonatal period. Hereditary PS deficiency is classified in three types (119). Type I deficiency corresponds to low levels of both free and complexed PS, type II PS deficiency is characterized by normal total and free PS levels but reduced PS activity, and type

III is defined by normal total PS levels but low free PS levels and activity. The disorder inherits as an autosomal dominant trait with incomplete penetrance (120) and is present in about 2% to 8% of families with hereditary thrombophilia (121;122). Many abnormalities in *PROS1* underlying hereditary PS deficiency have been described (reviewed in (108;109)). More recently Johansson *et al* reported a high incidence of large PS gene deletions in a group of Swedish PS deficient families (123). Not all familial PS deficiencies are explained by an abnormality in *PROS1* though (124). In this respect it must be noted that intronic sequences and 5', and 3' sequences are not routinely included in the investigation of familial PS deficiency and that possible functional mutations and polymorphisms in these regions are therefore not found. Of course, the remainder of unexplained inherited PS deficiencies may also be caused by variations in other genes. The Spanish Genetic Analysis of Idiopathic Thrombophilia (GAIT) project described the genetic linkage between free PS levels and the 1q32 genetic locus, which contains both genes for C4BPα and C4BPβ (125). This was not a surprising finding since PS binds to the C4BPβ-chain present in most C4BP molecules. This binding is of a 1:1 stoichiometric nature, which means that all C4BP-β^+ molecules are bound by one PS molecule. A drop or rise in C4BP-β^+ protein levels thus directly influences free PS levels either positively or negatively, respectively.

An example of a genetic abnormality/polymorphism is the relatively rare Ser 460 to Pro change in $PS_{Heerlen}$ (126) which, in some families, is associated with a type III PS deficiency (127;128). The anticoagulant properties of PS are not negatively affected by this amino acid change (29;31). A recent publication shows that instead, free $PS_{Heerlen}$ is cleared more rapidly than wild type PS from the circulation in mice (129).

1.4.2 Acquired PS deficiency

Whereas hereditary PS deficiency is relatively rare, many circumstances can lead to acquired deficiency, in which case it may be transient. Several non-genetic and environmental factors such as liver disease (130), DIC (80;130), and hormonal status (gender, contraceptive use, pregnancy) (131-133) influence PS levels. In liver disease PS levels as well as C4BP levels are decreased. Patients with DIC have decreased PS activity most likely due to increased cleavage of PS (80). Total PS levels remain similar as in controls and reports on the levels of

free PS in this hypercoagulable state are inconclusive (69;130). PS levels were shown to decrease with age (134), but when these data are corrected for gender the effect appears specific for females (135). It is evident from several studies that PS levels (total and free) are downregulated by female sex hormones, but the mechanism of this effect has not been clarified and would form an interesting area of research.

A more controversial issue is the regulation of PS levels during inflammation. C4BP is an established acute phase reactant with 2-3 fold elevated plasma levels during inflammation (136-139). Although PS levels are upregulated by the acute phase cytokine, IL6, *in vitro* (112), this finding was not confirmed by *in vivo* data. Several studies have demonstrated similar or only slightly increased total plasma PS levels (140-144). A controversy surrounds the levels of free PS during inflammation, with some studies showing reduced free PS levels in patient plasma (140-142), whilst others report stable free PS levels (143;144). A difference in the regulation of C4BP α- and β-chains in patient populations during the acute phase may explain the observed discrepancy (145).

1.5 Aim of this thesis

Overall the components that determine variations in PS levels at the transcriptional level are still largely unknown and the promoter region of *PROS1* has been poorly investigated. Knowledge of the *PROS1* promoter structure and the proteins regulating its transcriptional activity may help in acquiring a greater understanding of PS levels and PS function since;

a. Transcription factors that regulate *PROS1* transcription may be tissue-specific (e.g. liver-specific transcription factors), thereby explaining PS production in certain cell types,
b. Mutations or polymorphisms in the *PROS1* 5' sequence may lead to deficiencies if they are located in important binding sites for transcription factors or for the basal transcriptional machinery,
c. Deficiencies (qualitative or quantitative) in the regulatory factors of *PROS1* transcription may explain idiopathic hereditary PS deficiencies,
d. Transcription factors that upregulate *PROS1* transcription may themselves be triggered by a specific physiological process, thereby linking PS to this process.

The aim of this thesis was to identify the factors regulating PROS1 transcription, to determine whether PROS1 transcriptional regulation has a tissue/cell-specific component and to elucidate the underlying mechanisms.

In **Chapter 2** the transcriptional initiation of endogenous human *PROS1* is examined in liver and the relevant cell types that are exposed to the blood stream. Since PS is produced primarily by hepatocytes and to a lesser extent also by endothelial cells and megakaryocytes, we hypothesized that PS transcription might be regulated in a cell type-dependent manner. Cell-specific transcription may be regulated through a difference in the location of transcription start sites. Four major endogenous start sites were identified, the usage of which differed slightly between cell types. Furthermore, a minimal promoter with optimal transcriptional activity was identified in basal expression studies with *PROS1* promoter-reporter constructs in all cell types. It is this promoter construct that, in **Chapter 3**, was used in a pilot study in which the effect of a range of transcription factors on *PROS1* promoter activity was tested. Phylogenetic footprinting further provided a solid basis on which to select certain conserved regions within the human *PROS1* promoter for further research. **Chapter 4** expands the data presented in chapter 3 with a large scale investigation into the possible binding of nuclear proteins to the sequence contained in the *PROS1* promoter. Sp1 is identified as a transcription factor with multiple binding sites within the *PROS1* promoter. From further functional studies it became apparent that Sp1, and possibly also the related transcription factor Sp3, is almost solely responsible for the basal transcriptional activity of the *PROS1* promoter.

Previously published reports suggest that IL6 has a direct effect on *PROS1* transcription. In **Chapter 5** the IL6 responsive element within the *PROS1* promoter is identified. STAT3 binding to this element was essential for induction of *PROS1* transcription, which is illustrated by the absence of STAT3 binding and IL6 induction of *PROS1* transcriptional activity when this region is mutated. Major stimulatory effects on *PROS1* transcription were also observed upon cotransfection of another mediator of IL6 signalling, C/EBPβ. However, the role for C/EBPβ in *PROS1* promoter regulation was not fully elucidated during the course of this project.

Chapter 6 describes a classic case of serendipity. During the development of a real-time rtPCR analysis (QPCR) for the accurate measurement of *PROS1* transcipt levels, an additional unexpected PCR product was identified. The product was sequenced and resulted in the identification of a relatively abundant alternatively spliced *PROS1* mRNA. The alternative PS product from this alternative mRNA is compared to that of normal recombinant *PROS1* in COS1 cells *in vitro.*

In **Chapter 7** the results described in this thesis are discussed and related to insights from the literature.

References

1. Davie EW. Biochemical and molecular aspects of the coagulation cascade. Thromb.Haemost. 1995;74:1-6.
2. Mann KG. Biochemistry and physiology of blood coagulation. Thromb.Haemost. 1999;82:165-74.
3. Dahlbäck B. Blood coagulation. Lancet 2000;355:1627-32.
4. Österud B, Rapaport SI. Activation of factor IX by the reaction product of tissue factor and factor VII: additional pathway for initiating blood coagulation. Proc.Natl.Acad.Sci.U.S.A. 1977;74:5260-4.
5. Lane DA, Caso R. Antithrombin: structure, genomic organization, function and inherited deficiency. Baillieres Clin.Haematol. 1989;2:961-98.
6. Parker KA, Tollefsen DM. The protease specificity of heparin cofactor II. Inhibition of thrombin generated during coagulation. J.Biol.Chem. 1985;260:3501-5.
7. Broze GJ, Jr. The role of tissue factor pathway inhibitor in a revised coagulation cascade. Semin.Hematol. 1992;29:159-69.
8. Dahlbäck B, Villoutreix BO. The anticoagulant protein C pathway. FEBS Lett.2005;579:3310-6.
9. Anderson FA, Jr., Wheeler HB, Goldberg RJ, Hosmer DW, Patwardhan NA, Jovanovic B *et al.* A population-based perspective of the hospital incidence and case-fatality rates of deep vein thrombosis and pulmonary embolism. The Worcester DVT Study. Arch.Intern.Med. 1991;151:933-8.
10. Nordstrom M, Lindblad B, Bergqvist D, Kjellstrom T. A prospective study of the incidence of deep-vein thrombosis within a defined urban population. J.Intern.Med. 1992;232:155-60.
11. Rosendaal FR. Risk factors for venous thrombotic disease. Thromb.Haemost. 1999;82:610-9.
12. Bauer KA. The thrombophilias: well-defined risk factors with uncertain therapeutic implications. Ann.Intern.Med. 2001;135:367-73.
13. Bertina RM, Koeleman BP, Koster T, Rosendaal FR, Dirven RJ, de Ronde H *et al.* Mutation in blood coagulation factor V associated with resistance to activated protein C. Nature 1994;369:64-7.
14. Bertina RM. Genetic approach to thrombophilia. Thromb.Haemost. 2001;86:92-103.

15. Esmon CT, Esmon NL, Harris KW. Complex formation between thrombin and thrombomodulin inhibits both thrombin-catalyzed fibrin formation and factor V activation. J.Biol.Chem. 1982;257:7944-7.
16. Stearns-Kurosawa DJ, Kurosawa S, Mollica JS, Ferrell GL, Esmon CT. The endothelial cell protein C receptor augments protein C activation by the thrombin-thrombomodulin complex. Proc.Natl.Acad.Sci.U.S.A. 1996;93:10212-6.
17. Walker FJ. Regulation of activated protein C by protein S. The role of phospholipid in factor Va inactivation. J.Biol.Chem. 1981;256:11128-31.
18. Walker FJ, Chavin SI, Fay PJ. Inactivation of factor VIII by activated protein C and protein S. Arch.Biochem.Biophys. 1987;252:322-8.
19. Walker FJ. Regulation of activated protein C by a new protein. A possible function for bovine protein S. J.Biol.Chem. 1980;255:5521-4.
20. Harris KW, Esmon CT. Protein S is required for bovine platelets to support activated protein C binding and activity. J.Biol.Chem. 1985;260:2007-10.
21. Hackeng TM, Hessing M, van 't Veer C, Meijer-Huizinga F, Meijers JC, de Groot PG *et al*. Protein S binding to human endothelial cells is required for expression of cofactor activity for activated protein C. J.Biol.Chem. 1993;268:3993-4000.
22. Yegneswaran S, Wood GM, Esmon CT, Johnson AE. Protein S alters the active site location of activated protein C above the membrane surface. A fluorescence resonance energy transfer study of topography. J.Biol.Chem. 1997;272:25013-21.
23. Shen L, Dahlbäck B. Factor V and protein S as synergistic cofactors to activated protein C in degradation of factor VIIIa. J.Biol.Chem. 1994;269:18735-8.
24. Koppelman SJ, Hackeng TM, Sixma JJ, Bouma BN. Inhibition of the intrinsic factor X activating complex by protein S: evidence for a specific binding of protein S to factor VIII. Blood 1995;86:1062-71.
25. Heeb MJ, Mesters RM, Tans G, Rosing J, Griffin JH. Binding of protein S to factor Va associated with inhibition of prothrombinase that is independent of activated protein C. J.Biol.Chem. 1993;268:2872-7.
26. Heeb MJ, Rosing J, Bakker HM, Fernandez JA, Tans G, Griffin JH. Protein S binds to and inhibits factor Xa. Proc.Natl.Acad.Sci.U.S.A 1994;91:2728-32.
27. Hackeng TM, van 't Veer C, Meijers JC, Bouma BN. Human protein S inhibits prothrombinase complex activity on endothelial cells and platelets via direct interactions with factors Va and Xa. J.Biol.Chem. 1994;269:21051-8.
28. Koenen RR, Tans G, van Oerle R, Hamulyak K, Rosing J, Hackeng TM. The APC-independent anticoagulant activity of protein S in plasma is decreased by elevated prothrombin levels due to the prothrombin G20210A mutation. Blood. 2003;102:1686-92.
29. Koenen RR, Gomes L, Tans G, Rosing J, Hackeng TM. The Ser460Pro mutation in recombinant protein S Heerlen does not affect its APC-cofactor and APC-independent anticoagulant activities. Thromb.Haemost. 2004;91:1105-14.
30. Sere KM, Rosing J, Hackeng TM. Inhibition of thrombin generation by protein S at low procoagulant stimuli: implications for maintenance of the hemostatic balance. Blood 2004;104:3624-30.

31. Heeb MJ, Koenen RR, Fernandez JA, Hackeng TM. Direct anticoagulant activity of protein S-C4b binding protein complex in Heerlen heterozygotes and normals. J.Thromb.Haemost. 2004;2:1766-73.
32. Sakata Y, Loskutoff DJ, Gladson CL, Hekman CM, Griffin JH. Mechanism of protein C-dependent clot lysis: role of plasminogen activator inhibitor. Blood. 1986;68:1218-23.
33. de Fouw NJ, Haverkate F, Bertina RM, Koopman J, van Wijngaarden A, van Hinsbergh VW. The cofactor role of protein S in the acceleration of whole blood clot lysis by activated protein C *in vitro*. Blood. 1986;67:1189-92.
34. de Fouw NJ, de Jong YF, Haverkate F, Bertina RM. Activated protein C increases fibrin clot lysis by neutralization of plasminogen activator inhibitor--no evidence for a cofactor role of protein S. Thromb.Haemost. 1988;60:328-33.
35. Rezaie AR. Vitronectin functions as a cofactor for rapid inhibition of activated protein C by plasminogen activator inhibitor-1. Implications for the mechanism of profibrinolytic action of activated protein C. J.Biol.Chem. 2001;276:15567-70.
36. Hancock WW, Grey ST, Hau L, Akalin E, Orthner C, Sayegh MH *et al.* Binding of activated protein C to a specific receptor on human mononuclear phagocytes inhibits intracellular calcium signalling and monocyte-dependent proliferative responses. Transplantation. 1995;60:1525-32.
37. Joyce DE, Gelbert L, Ciaccia A, DeHoff B, Grinnell BW. Gene expression profile of antithrombotic protein c defines new mechanisms modulating inflammation and apoptosis. J.Biol.Chem. 2001;276:11199-203.
38. Cheng T, Liu D, Griffin JH, Fernandez JA, Castellino F, Rosen ED *et al.* Activated protein C blocks p53-mediated apoptosis in ischemic human brain endothelium and is neuroprotective. Nat.Med. 2003;9:338-42.
39. Bernard GR, Vincent JL, Laterre PF, LaRosa SP, Dhainaut JF, Lopez-Rodriguez A *et al.* Efficacy and safety of recombinant human activated protein C for severe sepsis. N.Engl.J.Med. 2001;344:699-709.
40. Bernard GR, Margolis BD, Shanies HM, Ely EW, Wheeler AP, Levy H *et al.* Extended evaluation of recombinant human activated protein C United States Trial (ENHANCE US): a single-arm, phase 3B, multicenter study of drotrecogin alfa (activated) in severe sepsis. Chest. 2004;125:2206-16.
41. Hancock WW, Tsuchida A, Hau H, Thomson NM, Salem HH. The anticoagulants protein C and protein S display potent antiinflammatory and immunosuppressive effects relevant to transplant biology and therapy. Transplant.Proc. 1992;24:2302-3.
42. Kanthou C, Benzakour O. Cellular effects and signalling pathways activated by the anti-coagulant factor, protein S, in vascular cells protein S cellular effects. Adv.Exp.Med.Biol. 2000;476:155-66.
43. Liu D, Guo H, Griffin JH, Fernandez JA, Zlokovic BV. Protein S confers neuronal protection during ischemic/hypoxic injury in mice. Circulation 2003;107:1791-6.
44. Webb JH, Blom AM, Dahlbäck B. Vitamin K-dependent protein S localizing complement regulator C4b-binding protein to the surface of apoptotic cells. J.Immunol. 2002;169:2580-6.
45. Anderson HA, Maylock CA, Williams JA, Paweletz CP, Shu H, Shacter E. Serum-derived protein S binds to phosphatidylserine and stimulates the phagocytosis of apoptotic cells. Nat.Immunol. 2003;4:87-91.
46. Webb JH, Blom AM, Dahlbäck B. The binding of protein S and the protein S-C4BP complex to neutrophils is apoptosis dependent. Blood Coagul.Fibrinolysis 2003;14:355-9.

47. Kask L, Trouw LA, Dahlbäck B, Blom AM. The C4b-binding protein-protein S complex inhibits the phagocytosis of apoptotic cells. J.Biol.Chem. 2004;279:23869-73.
48. Heeb MJ, Espana F, Griffin JH. Inhibition and complexation of activated protein C by two major inhibitors in plasma. Blood. 1989;73:446-54.
49. Suzuki K, Nishioka J, Kusumoto H, Hashimoto S. Mechanism of inhibition of activated protein C by protein C inhibitor. J.Biochem.(Tokyo) 1984;95:187-95.
50. van der Meer FJ, van Tilburg NH, van Wijngaarden A, van dL, I, Briët E, Bertina RM. A second plasma inhibitor of activated protein C: alpha 1-antitrypsin. Thromb.Haemost. 1989;62:756-62.
51. Rezaie AR, Cooper ST, Church FC, Esmon CT. Protein C inhibitor is a potent inhibitor of the thrombin-thrombomodulin complex. J.Biol.Chem. 1995;270:25336-9.
52. Di Scipio RG, Hermodson MA, Yates SG, Davie EW. A comparison of human prothrombin, factor IX (Christmas factor), factor X (Stuart factor), and protein S. Biochemistry. 1977;16:698-706.
53. Dahlbäck B, Stenflo J. High molecular weight complex in human plasma between vitamin K-dependent protein S and complement component C4b-binding protein. Proc.Natl.Acad.Sci.U.S.A 1981;78:2512-6.
54. Dahlbäck B. Inhibition of protein Ca cofactor function of human and bovine protein S by C4b-binding protein. J.Biol.Chem. 1986;261:12022-7.
55. Griffin JH, Gruber A, Fernandez JA. Reevaluation of total, free, and bound protein S and C4b-binding protein levels in plasma anticoagulated with citrate or hirudin. Blood 1992;79:3203-11.
56. Strickland DK, Kessler CM. Biochemical and functional properties of protein C and protein S. Clin.Chim.Acta. 1987;170:1-23.
57. Heeb, M. J. and Griffin, J. H. The biochemistry of protein S. In: Protein C and related proteins. Bertina RM, ed. 55-70. 1988. Edinburgh, Scotland, Churchill Livingstone.
58. van de Poel RH, Meijers JC, Bouma BN. The interaction between anticoagulant protein S and complement regulatory C4b-binding protein (C4BP). Trends Cardiovasc.Med. 2000;10:71-6.
59. Rezende SM, Simmonds RE, Lane DA. Coagulation, inflammation, and apoptosis: different roles for protein S and the protein S-C4b binding protein complex. Blood. 2004;103:1192-201.
60. Dahlbäck B. Factor V and protein S as cofactors to activated protein C. Haematologica 1997;82:91-5.
61. Fair DS, Marlar RA. Biosynthesis and secretion of factor VII, protein C, protein S, and the Protein C inhibitor from a human hepatoma cell line. Blood 1986;67:64-70.
62. Ogura M, Tanabe N, Nishioka J, Suzuki K, Saito H. Biosynthesis and secretion of functional protein S by a human megakaryoblastic cell line (MEG-01). Blood 1987;70:301-6.
63. Fair DS, Marlar RA, Levin EG. Human endothelial cells synthesize protein S. Blood 1986;67:1168-71.
64. Stern D, Brett J, Harris K, Nawroth P. Participation of endothelial cells in the protein C-protein S anticoagulant pathway: the synthesis and release of protein S. J.Cell Biol. 1986;102:1971-8.
65. Malm J, He XH, Bjartell A, Shen L, Abrahamsson PA, Dahlbäck B. Vitamin K-dependent protein S in Leydig cells of human testis. Biochem.J. 1994;302(Pt 3):845-50.
66. Maillard C, Berruyer M, Serre CM, Dechavanne M, Delmas PD. Protein S, a vitamin K-dependent protein, is a bone matrix component synthesized and secreted by osteoblasts. Endocrinology 1992;130:1599-604.

67. Stitt TN, Conn G, Gore M, Lai C, Bruno J, Radziejewski C *et al.* The anticoagulation factor protein S and its relative, GAS6, are ligands for the Tyro 3/Axl family of receptor tyrosine kinases. Cell 1995;80:661-70.
68. Schwarz HP, Heeb MJ, Wencel-Drake JD, Griffin JH. Identification and quantitation of protein S in human platelets. Blood 1985;66:1452-5.
69. Bertina RM, van Wijngaarden A, Reinalda-Poot J, Poort SR, Bom VJ. Determination of plasma protein S--the protein cofactor of activated protein C. Thromb.Haemost. 1985;53:268-72.
70. Takahashi H, Tatewaki W, Wada K, Shibata A. Plasma protein S in disseminated intravascular coagulation, liver disease, collagen disease, diabetes mellitus, and under oral anticoagulant therapy. Clin.Chim.Acta. 1989;182:195-208.
71. Schwalbe R, Dahlbäck B, Hillarp A, Nelsestuen G. Assembly of protein S and C4b-binding protein on membranes. J.Biol.Chem. 1990;265:16074-81.
72. Crosier PS, Freeman SA, Orlic D, Bodine DM, Crosier KE. The Dtk receptor tyrosine kinase, which binds protein S, is expressed during hematopoiesis. Exp.Hematol. 1996;24:318-23.
73. Nyberg P, He X, Hardig Y, Dahlbäck B, Garcia dF. Stimulation of Sky tyrosine phosphorylation by bovine protein S--domains involved in the receptor-ligand interaction. Eur.J.Biochem. 1997;246:147-54.
74. Wimmel A, Rohner I, Ramaswamy A, Heidtmann HH, Seitz R, Kraus M *et al.* Synthesis and secretion of the anticoagulant protein S and coexpression of the Tyro3 receptor in human lung carcinoma cells. Cancer 1999;86:43-9.
75. Varnum BC, Young C, Elliott G, Garcia A, Bartley TD, Fridell YW *et al.* Axl receptor tyrosine kinase stimulated by the vitamin K-dependent protein encoded by growth-arrest-specific gene 6. Nature. 1995;373:623-6.
76. Manfioletti G, Brancolini C, Avanzi G, Schneider C. The protein encoded by a growth arrest-specific gene (GAS6) is a new member of the vitamin K-dependent proteins related to protein S, a negative coregulator in the blood coagulation cascade. Mol.Cell Biol. 1993;13:4976-85.
77. Evenas P, Dahlbäck B, Garcia dF. The first laminin G-type domain in the SHBG-like region of protein S contains residues essential for activation of the receptor tyrosine kinase sky. Biol.Chem. 2000;381:199-209.
78. Chang GT, Aaldering L, Hackeng TM, Reitsma PH, Bertina RM, Bouma BN. Construction and characterization of thrombin-resistant variants of recombinant human protein S. Thromb.Haemost. 1994;72:693-7.
79. Mitchell CA, Hau L, Salem HH. Control of thrombin mediated cleavage of protein S. Thromb.Haemost. 1986;56:151-4.
80. Heeb MJ, Mosher D, Griffin JH. Activation and complexation of protein C and cleavage and decrease of protein S in plasma of patients with intravascular coagulation. Blood 1989;73:455-61.
81. Long GL, Lu D, Xie RL, Kalafatis M. Human protein S cleavage and inactivation by coagulation factor Xa. J.Biol.Chem. 1998;273:11521-6.
82. Morboeuf O, Borgel D, Gaussem P, Vincenot A, Pittet JL, Aiach M *et al.* Characterization of cleaved plasma protein S with a monoclonal antibody- based assay. Thromb.Haemost. 2000;84:604-10.
83. Borgel D, Reny JL, Fischelis D, Gandrille S, Emmerich J, Fiessinger JN *et al.* Cleaved protein S (PS), total PS, free PS, and activated protein C cofactor activity as risk factors for venous thromboembolism. Clin.Chem. 2003;49:575-80.

84. Nelson RM, Long GL. Binding of protein S to C4b-binding protein. Mutagenesis of protein S. J.Biol.Chem. 1992;267:8140-5.
85. Fernandez JA, Heeb MJ, Griffin JH. Identification of residues 413-433 of plasma protein S as essential for binding to C4b-binding protein. J.Biol.Chem. 1993;268:16788-94.
86. Chang GT, Maas BH, Ploos van Amstel HK, Reitsma PH, Bertina RM, Bouma BN. Studies of the interaction between human protein S and human C4b-binding protein using deletion variants of recombinant human protein S. Thromb.Haemost. 1994;71:461-7.
87. Giri TK, Linse S, Garcia dF, Yamazaki T, Villoutreix BO, Dahlbäck B. Structural requirements of anticoagulant protein S for its binding to the complement regulator C4b-binding protein. J.Biol.Chem. 2002;277:15099-106.
88. Fernandez JA, Griffin JH. A protein S binding site on C4b-binding protein involves beta chain residues 31-45. J.Biol.Chem. 1994;269:2535-40.
89. Dahlbäck B, Hildebrand B. Degradation of human complement component C4b in the presence of the C4b-binding protein-protein S complex. Biochem.J. 1983;209:857-63.
90. Schwalbe RA, Dahlbäck B, Nelsestuen GL. Independent association of serum amyloid P component, protein S, and complement C4b with complement C4b-binding protein and subsequent association of the complex with membranes. J.Biol.Chem. 1990;265:21749-57.
91. Dahlbäck B, Villoutreix BO. Regulation of blood coagulation by the protein C anticoagulant pathway: novel insights into structure-function relationships and molecular recognition. Arterioscler.Thromb.Vasc.Biol. 2005;25:1311-20.
92. Ploos van Amstel JK, van der Zanden AL, Bakker E, Reitsma PH, Bertina RM. Two genes homologous with human protein S cDNA are located on chromosome 3. Thromb.Haemost. 1987;58:982-7.
93. Watkins PC, Eddy R, Fukushima Y, Byers MG, Cohen EH, Dackowski WR *et al*. The gene for protein S maps near the centromere of human chromosome 3. Blood 1988; 71:238-41.
94. Lundwall A, Dackowski W, Cohen E, Shaffer M, Mahr A, Dahlbäck B *et al*. Isolation and sequence of the cDNA for human protein S, a regulator of blood coagulation. Proc.Natl.Acad.Sci.U.S.A 1986;83:6716-20.
95. Hoskins J, Norman DK, Beckmann RJ, Long GL. Cloning and characterization of human liver cDNA encoding a protein S precursor. Proc.Natl.Acad.Sci.U.S.A 1987;84:349-53.
96. Ploos van Amstel HK, van der Zanden AL, Reitsma PH, Bertina RM. Human protein S cDNA encodes Phe-16 and Tyr 222 in consensus sequences for the post-translational processing. FEBS Lett. 1987;222:186-90.
97. Schmidel DK, Tatro AV, Phelps LG, Tomczak JA, Long GL. Organization of the human protein S genes. Biochemistry 1990;29:7845-52.
98. Ploos van Amstel HK, Reitsma PH, van der Logt CP, Bertina RM. Intron-exon organization of the active human protein S gene PS alpha and its pseudogene PS beta: duplication and silencing during primate evolution. Biochemistry 1990;29:7853-61.
99. Edenbrandt CM, Lundwall A, Wydro R, Stenflo J. Molecular analysis of the gene for vitamin K dependent protein S and its pseudogene. Cloning and partial gene organization. Biochemistry 1990;29:7861-8.
100. Yoshitake S, Schach BG, Foster DC, Davie EW, Kurachi K. Nucleotide sequence of the gene for human factor IX (antihemophilic factor B). Biochemistry 1985;24:3736-50.

101. Foster DC, Yoshitake S, Davie EW. The nucleotide sequence of the gene for human protein C. Proc.Natl.Acad.Sci.U.S.A. 1985;82:4673-7.
102. Degen SJ, Davie EW. Nucleotide sequence of the gene for human prothrombin. Biochemistry. 1987;26:6165-77.
103. O'Hara PJ, Grant FJ, Haldeman BA, Gray CL, Insley MY, Hagen FS *et al.* Nucleotide sequence of the gene coding for human factor VII, a vitamin K-dependent protein participating in blood coagulation. Proc.Natl.Acad.Sci.U.S.A 1987;84:5158-62.
104. Jagadeeswaran P, Reddy SV, Rao KJ, Hamsabhushanam K, Lyman G. Cloning and characterization of the 5' end (exon 1) of the gene encoding human factor X. Gene. 1989;84:517-9.
105. Huang MN, Hung HL, Stanfield-Oakley SA, High KA. Characterization of the human blood coagulation factor X promoter. J.Biol.Chem. 1992;267:15440-6.
106. Pollak ES, Hung HL, Godin W, Overton GC, High KA. Functional characterization of the human factor VII 5'-flanking region. J.Biol.Chem. 1996;271:1738-47.
107. Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, Miller W *et al.* Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. 1997;25:3389-402.
108. Gandrille S, Borgel D, Ireland H, Lane DA, Simmonds R, Reitsma PH *et al.* Protein S deficiency: a database of mutations. For the Plasma Coagulation Inhibitors Subcommittee of the Scientific and Standardization Committee of the International Society on Thrombosis and Haemostasis. Thromb.Haemost. 1997;77:1201-14.
109. Gandrille S, Borgel D, Sala N, Espinosa-Parrilla Y, Simmonds R, Rezende S *et al.* Protein S deficiency: a database of mutations--summary of the first update. Thromb.Haemost. 2000;84:918.
110. Hall, A. J., Peake, I. R., and Winship, P. R. Identification of multiple elements regulating transcription of the protein S gene. Thromb.Haemost. 1995;73:1257 (abstract).
111. Tatewaki H, Tsuda H, Kanaji T, Yokoyama K, Hamasaki N. Characterization of the human protein S gene promoter: a possible role of transcription factors Sp1 and HNF3 in liver. Thromb.Haemost. 2003;90:1029-39.
112. Hooper WC, Phillips DJ, Ribeiro M, Benson J, Evatt BL. IL6 upregulates protein S expression in the HepG-2 hepatoma cells. Thromb.Haemost. 1995;73:819-24.
113. Hooper WC, Phillips DJ, Evatt BL. TNF-alpha suppresses IL6 upregulation of protein S in HepG-2 hepatoma cells. Thromb.Res. 1996;81:315-26.
114. Hooper WC, Phillips DJ, Evatt BL. Endothelial cell protein S synthesis is upregulated by the complex of IL6 and soluble IL6 receptor. Thromb.Haemost. 1997;77:1014-9.
115. Ramadori G, Christ B. Cytokines and the hepatic acute-phase response. Semin.Liver Dis. 1999;19:141-55.
116. Heinrich PC, Behrmann I, Haan S, Hermanns HM, Muller-Newen G, Schaper F. Principles of interleukin (IL)-6-type cytokine signalling and its regulation. Biochem.J. 2003;374(Pt 1):1-20.
117. Schrem H, Klempnauer J, Borlak J. Liver-enriched transcription factors in liver function and development. Part II: the C/EBPs and D site-binding protein in cell cycle control, carcinogenesis, circadian gene regulation, liver regeneration, apoptosis, and liver-specific gene regulation. Pharmacol.Rev. 2004;56:291-330.
118. Comp PC, Esmon CT. Recurrent venous thromboembolism in patients with a partial deficiency of protein S. N.Engl.J.Med. 1984;311:1525-8.

119. Bertina, R. M. Nomenclature proposal for protein S deficiency. XXXVI Annual meeting of the Scientific and Standardization Committee of the ISTH. 1990.
120. Bertina RM. Hereditary protein S deficiency. Haemostasis 1985;15:241-6.
121. Lane DA, Mannucci PM, Bauer KA, Bertina RM, Bochkov NP, Boulyjenkov V *et al.* Inherited thrombophilia: Part 1. Thromb.Haemost. 1996;76:651-62.
122. Melissari E, Monte G, Lindo VS, Pemberton KD, Wilson NV, Edmondson R *et al.* Congenital thrombophilia among patients with venous thromboembolism. Blood Coagul.Fibrinolysis. 1992;3:749-58.
123. Johansson AM, Hillarp A, Sall T, Zoller B, Dahlbäck B, Hallden C. Large deletions of the *PROS1* gene in a large fraction of mutation-negative patients with protein S deficiency. Thromb.Haemost. 2005;94:951-7.
124. Koeleman BP, Reitsma PH, Bertina RM. Familial thrombophilia: a complex genetic disorder. Semin.Hematol. 1997;34:256-64.
125. Almasy L, Soria JM, Souto JC, Coll I, Bacq D, Faure A *et al.* A quantitative trait locus influencing free plasma protein S levels on human chromosome 1q: results from the Genetic Analysis of Idiopathic Thrombophilia (GAIT) project. Arterioscler.Thromb.Vasc.Biol. 2003;23:508-11.
126. Bertina RM, Ploos van Amstel HK, van Wijngaarden A, Coenen J, Leemhuis MP, Deutz-Terlouw PP *et al.* Heerlen polymorphism of protein S, an immunologic polymorphism due to dimorphism of residue 460. Blood 1990;76:538-48.
127. Sala, N., Morell, M., Tirado, I., Espinosa, Y., Llobet, D., Fontcuberta, J., Soria, J. M., Volpini, V., and Estivill, X. Linkage disequilibrium between the protein S Heerlen allele and protein S deficiency in Spanish families. Thromb.Haemost. 1995;73:1259 (abstract).
128. Espinosa-Parrilla Y, Morell M, Souto JC, Borrell M, Heine-Suner D, Tirado I *et al.* Absence of linkage between type III protein S deficiency and the *PROS1* and C4BP genes in families carrying the protein S Heerlen allele. Blood 1997;89:2799-806.
129. Denis CV, Roberts SJ, Hackeng TM, Lenting PJ. *In vivo* clearance of human protein S in a mouse model: influence of C4b-binding protein and the Heerlen polymorphism. Arterioscler.Thromb.Vasc.Biol. 2005;25:2209-15.
130. D'Angelo A, Vigano-D'Angelo S, Esmon CT, Comp PC. Acquired deficiencies of protein S. Protein S activity during oral anticoagulation, in liver disease, and in disseminated intravascular coagulation. J.Clin.Invest 1988;81:1445-54.
131. Comp PC, Thurnau GR, Welsh J, Esmon CT. Functional and immunologic protein S levels are decreased during pregnancy. Blood 1986;68:881-5.
132. Boerger LM, Morris PC, Thurnau GR, Esmon CT, Comp PC. Oral contraceptives and gender affect protein S status. Blood 1987;69:692-4.
133. Tans G, Curvers J, Middeldorp S, Thomassen MC, Meijers JC, Prins MH *et al.* A randomized cross-over study on the effects of levonorges. Thromb.Haemost. 2000;84:15-21.
134. Simmonds RE, Zoller B, Ireland H, Thompson E, de Frutos PG, Dahlbäck B *et al.* Genetic and phenotypic analysis of a large (122-member) protein S-deficient kindred provides an explanation for the familial coexistence of type I and type III plasma phenotypes. Blood 1997;89:4364-70.
135. Liberti G, Bertina RM, Rosendaal FR. Hormonal state rather than age influences cut-off values of protein S: reevaluation of the thrombotic risk associated with protein S deficiency. Thromb.Haemost. 1999;82:1093-6.

136. Saeki T, Hirose S, Nukatsuka M, Kusunoki Y, Nagasawa S. Evidence that C4b-binding protein is an acute phase protein. Biochem.Biophys.Res.Commun. 1989;164:1446-51.

137. Barnum SR, Dahlbäck B. C4b-binding protein, a regulatory component of the classical pathway of complement, is an acute-phase protein and is elevated in systemic lupus erythematosus. Complement Inflamm. 1990;7:71-7.

138. Moffat GJ, Tack BF. Regulation of C4b-binding protein gene expression by the acute-phase mediators tumor necrosis factor-alpha, interleukin-6, and interleukin-1. Biochemistry 1992;31:12376-84.

139. Garcia dF, Alim RI, Hardig Y, Zoller B, Dahlbäck B. Differential regulation of alpha and beta chains of C4b-binding protein during acute-phase response resulting in stable plasma levels of free anticoagulant protein S. Blood 1994;84:815-22.

140. D'Angelo A, Gerosa S, D'Angelo SV, Mailhac A, Colombo A, Agazzi A *et al*. Protein S and protein C anticoagulant activity in acute and chronic cardiac ischemic syndromes. Relationship to inflammation, complement activation and *in vivo* thrombin activity. Thromb.Res. 1994;75:133-42.

141. Vila N, Reverter JC, Yague J, Chamorro A. Interaction between interleukin-6 and the natural anticoagulant system in acute stroke. J.Interferon Cytokine Res. 2000;20:325-9.

142. Kaba NK, Francis CW, Hall WJ, Falsey AR, Smith BH. Protein S declines during winter respiratory infections. J.Thromb.Haemost. 2003;1:729-34.

143. Garcia dF, Alim RI, Hardig Y, Zoller B, Dahlbäck B. Differential regulation of alpha and beta chains of C4b-binding protein during acute-phase response resulting in stable plasma levels of free anticoagulant protein S. Blood 1994;84:815-22.

144. Criado-Garcia O, Gonzalez-Rubio C, Lopez-Trascasa M, Pascual-Salcedo D, Munuera L, Rodriguez dC. Modulation of C4b-binding protein isoforms during the acute phase response caused by orthopedic surgery. Haemostasis 1997;27:25-34.

145. Criado GO, Sanchez-Corral P, Rodriguez dC. Isoforms of human C4b-binding protein. II. Differential modulation of the C4BPA and C4BPB genes by acute phase cytokines. J.Immunol. 1995;155:4037-43.