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# CLONING OF THE 1.4-kb mRNA SPECIES OF HUMAN COMPLEMENT FACTOR H REVEALS A NOVEL MEMBER OF THE SHORT CONSENSUS REPEAT FAMILY RELATED TO THE CARBOXY TERMINAL OF THE CLASSICAL 150-kDa MOLECULE[1]

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**Three factor H mRNA species of 4.3 kb, 1.8 kb, and 1.4 kb are constitutively expressed in human liver. Having previously characterized full-length cDNA clones derived from the 4.3-kb and 1.8-kb factor mRNA, we report here the isolation and eucaryotic expression of full-length cDNA clones coding for the 1.4-kb mRNA species. The 1266-bp cDNA codes for a polypeptide of 330 amino acids and contains two polyadenylation signals and a short poly(A)⁺tail. The protein is composed of a leader peptide followed by five short consensus repeat domains. It shows a hybrid structure with the last three domains being almost identical to the carboxy-terminal of the classical 150-kDa factor H molecule and the two first domains representing unique short consensus repeat structures. Eucaryotic expression in COS7 cells revealed two polypeptides derived from one cDNA clone that are also found in human serum. Differences between the cDNA clones within the last three domains indicate two distinct, possibly allelic sequences that, in addition, differ from the authentic 150-kDa factor H sequence. Southern blot results support the notion that the 4.3-kb factor H and the 1.4-kb factor H-related mRNA are transcribed from two separate but highly homologous genes.**

Factor H, a glycoprotein of 150,000 $M_r$, is a regulatory protein of the complement system that plays a key role in the regulation of the alternative pathway. Factor H accelerates the dissociation of Bb from the C3 convertase C3b,Bb (1) and functions as a cofactor for factor I, a serine protease cleaving C3b into inactive iC3b that can no longer form the C3 convertase by binding factor B (2). The C3b binding site of factor H has been localized to the N-terminal of the molecule (3). Previous studies have suggested that factor H has physiologic functions other than the regulation of alternative pathway C3 convertase. Factor H is capable of triggering the respiratory burst in human peripheral monocytes (4) and inhibits the differentiation of peripheral B lymphocytes (5). It furthermore promotes the release of factor I from B lymphocytes (6). It has been shown that factor H binds to lymphocytes, granulocytes, and monocytes by means of a receptor (7). A possible candidate for the factor H receptor has been localized on B lymphoblastoid cell lines (8), and more recently, another protein was isolated that may represent the same receptor (9). The main production of factor H occurs in liver. Synthesis of factor H mRNA has also been described for human peripheral blood monocytes (10), the promonocyte cell line U937 (11), and more recently for human platelet granules (12). In HUVEC[4] and in human skin fibroblasts, the synthesis of factor H could be enhanced by stimulation with IFN-$\gamma$ and TNF-$\alpha$ (13–15).

The protein structure of classical factor $H_{gp150}$ has been established by the isolation of overlapping cDNA clones and is composed of 20 SCR of approximately 60 aa. Each SCR shows a characteristic framework of highly conserved residues (16–18). The SCR feature is a characteristic of a structural protein superfamily made up of several C-like proteins such as C-receptor type 1, C4-binding protein, and decay accelerating factor, and of non-C proteins like factor XIII, IL-2R, and $\beta_2$-glycoprotein I (19). Northern blots of human liver RNA probed with factor H cDNA fragments show three different constitutively expressed mRNA species of 4.3 kb, 1.8 kb, and 1.4 kb (20). We have previously shown by eucaryotic transfection studies that the 4.3-kb mRNA codes for the classical factor $H_{gp150}$ and the 1.8-kb mRNA codes for a truncated form of 43 kDa present in serum (21). In this paper we report the isolation and characterization of factor H-related cDNA clones transcribed from the 1.4-kb mRNA detected in human liver. We show that this transcript is in its C-terminal part (three SCR) almost identical to the sequence of the factor $H_{gp150}$ molecule characterized from the same individual, but codes for a unique N-terminal consisting of two novel domains. Upon transfection into COS7 cells, two polypeptides are recognized in culture supernatants by the mAb 3D11.7, directed to the C-terminal part of factor H (22), which are also found in human serum. Thus, the four factor H-like proteins de-

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[4] Abbreviations used in this paper: HUVEC, human umbilical vein endothelial cells; factor $H_{gp150}$, 150-kDa factor H; cpm, counts per minute; aa, amino acid; fHR, factor H receptor; SCR, short consensus repeat; Bb, catalytic Bb peptide of the alternative pathway of complement activation.

tected in human serum by polyclonal antifactor H antisera are derived from the three mRNA species in liver. Preliminary evidence suggests that they are transcribed from two homologous genes.

## MATERIALS AND METHODS

*RNA preparation and cDNA synthesis.* Total RNA was extracted from a piece of normal, noninfiltrated liver tissue, derived from one patient with liver carcinoma, according to the guanidinium isothiocyanate/CsCl procedure (23). poly(A)$^+$RNA was isolated with an oligo (dT) cellulose spin column obtained from Pharmacia LKB, Freiburg, FRG; 15-$\mu$g poly(A)$^+$RNA was employed for cDNA synthesis using the kit supplied by BRL, Karlsruhe, FRG.

*Construction and screening of a cDNA library in the eucaryotic expression vector CDM8.* The cDNA was blunt-ended with T4-polymerase and ligated to *Bst*XI adaptors. The ligated cDNA was size selected (>1 kb) through a gradient of 5 to 20% potassium acetate and cloned into the gradient purified *Bst*XI cut vector CDM8 following the protocol of Seed, (24, 25). The resultant library of 300,000 colonies was screened with a $\alpha$-[$^{32}$P]dATP-labeled DNA probe of 300 bp derived from the 3′ end of the cDNA clone H46, hybridizing to the 4.3-kb and the 1.4-kb mRNA (20).

*Sequence analysis.* DNA sequencing was performed by the dideoxy chain termination method (26) using the T7 polymerase sequencing kit supplied by Pharmacia/LKB. dscDNA was directly sequenced in both directions using different oligonucleotides: two located 5′ and 3′ of the *Bst*XI cloning site within the CDM8 vector (oCDM8-1/5′: CTGGCTAACTAGAGAAC and oCDM8-2/3′: GATCCTCTAGAGTCGC), and two additional oligonucleotide primers present in 5′ and 3′ orientation within the cDNA insert (underlined in Fig. 3).

*Northern blot analysis.* One microgram of poly(A)$^+$RNA of human liver tissue was electrophoresed in a 1.2% agarose gel containing formaldehyde and transferred to nylon membrane Hybond (Amersham, Braunschweig, FRG) by standard procedures (27). The blots were hybridized with the 4.3-kb insert of the factor H full-length cDNA clone pFH4.3, the 1.8-kb insert of the full length cDNA 5′ clone pFH1.8 (Fig. 1), derived from the 1.8-kb factor H transcript, and two cDNA probes of pFH1.4 containing either SCR3-5 or SCR1-2.

*Southern blot analysis.* Fifteen micrograms of restricted DNA of two human liver DNA preparations (one sample was isolated from the same liver tissue used for cDNA library construction) were separated on a 0.7% agarose gel and transferred to nylon membrane Hybond (28). Blots were hybridized with the 3′*Hin*dIII/*Pst*I fragment present in pFH4.3 and pFH1.4.

*Labeling of DNA probes and hybridization.* Radioactive probes were obtained by labeling the fragments with $\alpha$-[$^{32}$P]dATP according to the random priming procedure (29). Hybridization was performed at a concentration of $5 \times 10^6$ cpm-labeled fragment/ml of hybridization solution, and washing of the blots by the method of Church and Gilbert (30).

*COS cell transfection.* Transfection was carried out by electroporation using a Bio-Rad Laboratories (Richmond, CA) gene pulser and following the recommendations of the supplier. Actively growing COS7 cells were harvested with PBS/2 mM EDTA, washed twice in cold PBS, and resuspended at a concentration of $1 \times 10^7$ cells per ml in ice-cold phosphate-buffered sucrose (272 mM sucrose, 7 mM sodium phosphate, pH 7.4, 1 mM $MgCl_2$); 0.8 ml of cell suspension were mixed with 50 $\mu$g of DNA in a prechilled electroporation cuvette and stored on ice for 10 min. Cells were pulsed with 0.35 kV at 25 $\mu$F and were then allowed to recover on ice for 10 min. Cells were gently resuspended in 10 ml of DMEM supplemented with 10% FCS and seeded into 94 16-mm tissue culture petri dishes. At 24 h, the medium was changed to DMEM containing 1 × Nutridoma-HU (Boehringer, Mannheim, FRG). The supernatants were harvested 72 h after the pulse, concentrated 20-fold, and assayed for the expression of factor H-related proteins by Western blot analysis.

*Deglycosylation.* Five microliters of human serum were incubated with 5 $\mu$l of endoglycosidase F/*N*-glycosidase F (EC3.2.1.96/EC3.2.2.18, Boehringer Mannheim) at 37°C for 24 h. The extent of deglycosylation was tested by loading 5 $\mu$l of the reaction on SDS/PAGE under nonreducing conditions followed by Western blotting.

*SDS-PAGE and Western blotting.* Eighty microliters of concentrated supernatant from COS cell transfectants and 3 $\mu$l of human serum were subjected to SDS-PAGE under nonreducing conditions (31). Proteins were blotted to nitrocellulose (Schleicher and Schuell, Dassel, FRG) (32). Blots were quenched with 1% (w/v) dried milk in PBS and washed with PBS. All antisera were diluted in 2% dried milk/PBS. Staining was carried out with the mAb 3D11.7 (22) and peroxidase-conjugated anti-mouse IgG (Dakopatts, Copenhagen, Denmark) as second antibody, following standard protocol (32).

## RESULTS

A human liver cDNA library was constructed from a liver specimen of a single individual, resulting in 300,000 primary colonies that were screened with the 300-bp *Eco*RV/*Pst*I fragment of clone pFH4.3, a cDNA clone derived from the same cDNA library encoding classical factor $H_{gp150}$ (21) (Fig. 1). The screen yielded six cDNA clones with inserts approximately 1.2 kb in size possibly coding for the 1.4-kb mRNA. Analysis of the clones by restriction with the enzymes *Hin*dIII and *Eco*RV (Fig. 1) demonstrated that the three cDNA clones pFH1.4a,b,c were in the correct orientation for eucaryotic expression in COS7 cells, whereas the clones pFH1.4d,e,f were in the inverted orientation. Sequences of the 5′ and 3′ ends of all six cDNA clones were determined using oligomers within the CDM8 linker. All cDNA sequences coded for the same 5′ untranslated sequence, varying only in length. The cDNA pFH1.4c carried an extended 3′ untranslated region of additional 340 bp that did not hybridize to the 1.4-kb factor H mRNA in human liver (data not shown), thus having been added artificially during the cloning procedure. Two clones, pFH1.4a and pFH1.4b, were chosen for complete DNA sequence determination (Fig. 2). The nucleotide and deduced aa sequence of clones pFH1.4a and b in comparison with classical H cDNA is shown in Figure 3. pFH4.3 is a full-length factor $H_{gp150}$ cDNA clone isolated from the same liver library, and its sequence determined for the 5′ and the 3′ ends (21) (Fig. 3) is identical to the factor H sequence determined by Ripoche et al. (17). The two pFH1.4 cDNA clones were found to vary in seven bases, resulting in three aa substitutions (Fig. 3). Sequence determination of the additional four full-length pFH1.4 cDNA clones in

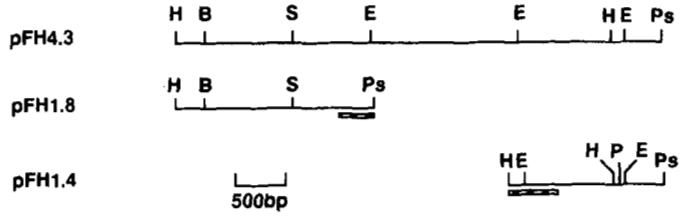


*Figure 1.* Comparison of the restriction map of the full-length cDNA clones derived from the classical 4.3-kb factor H mRNA (pFH4.3), the 1.8-kb mRNA (pFH1.8), and the 1.4-kb factor H mRNA (pFH1.4). The *Hin*dIII site at the 5′ end and the *Pst*I site at the 3′ end belong to the linker sequence of the vector CDM8. The extent of the unique sequences at the 3′ end of pFH1.8 and at the 5′ end of pFH1.4 is indicated below the restriction map. The sites for the following enzymes are shown: *H*, *Hin*dIII; *B*, *Bg*lII; *S*, *Sma*I; *E*, *Eco*RV; *P*, *Pvu*II; *Ps*, *Pst*I.

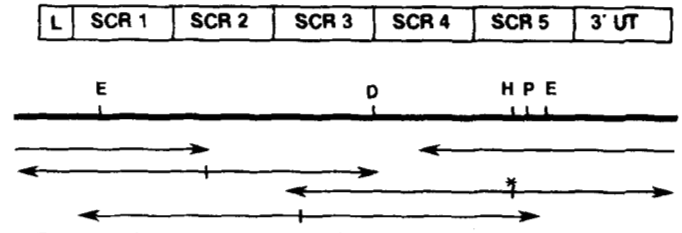


*Figure 2.* Sequencing strategy of cDNA clone pFH1.4a. The extent of the signal sequence, of the SCR domain structures, and of the 3′ untranslated region (3′UT) is indicated above the restriction map with the following enzymes: D, *Dra*I; E, *Eco*RV; H, *Hin*dIII; P, *Pvu*II. *Arrows* mark the extent and direction of the DNA sequence determination (with • showing the sequences obtained from M13 subclones).

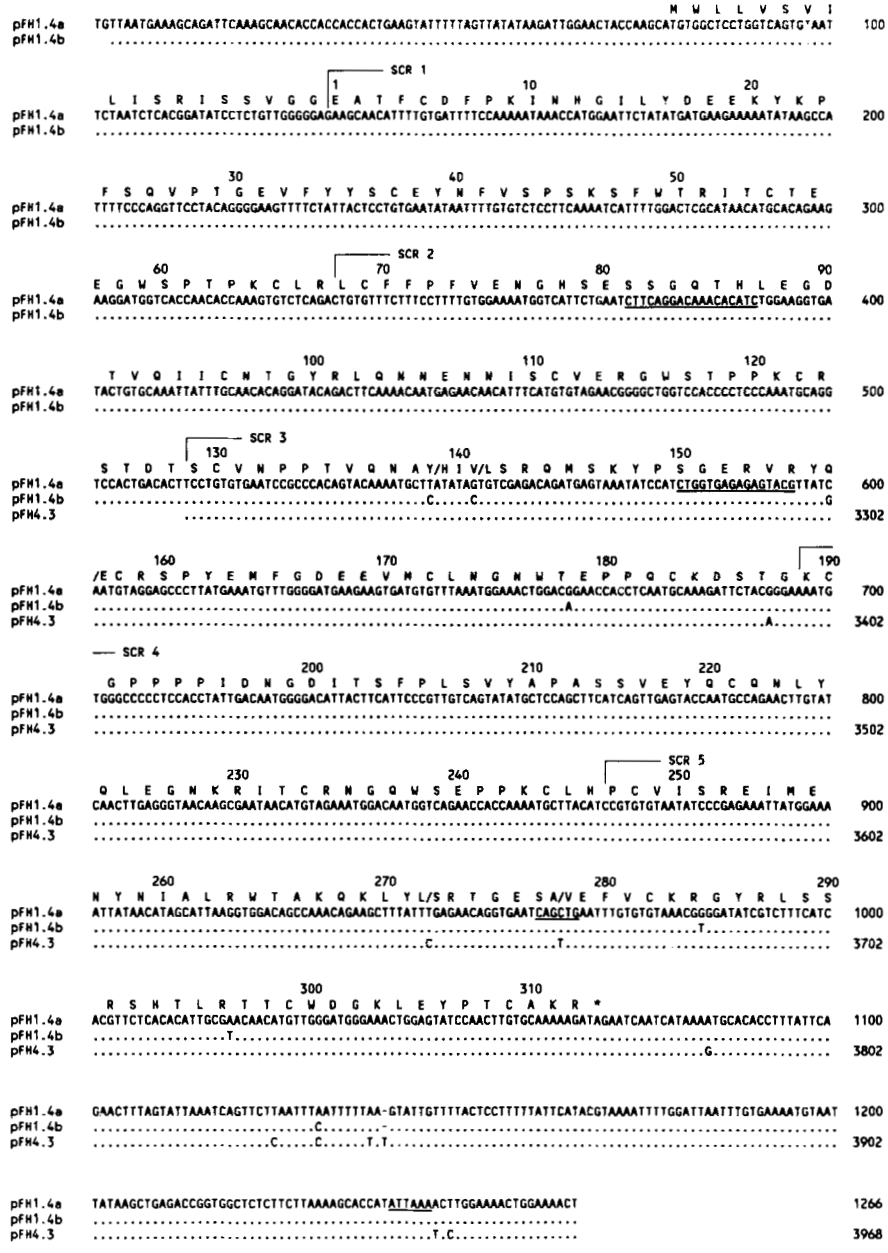

*Figure 3.* Nucleotide and deduced aa sequence of cDNA clones pFH1.4a and b in comparison with classical H cDNA pFH4.3. The oligonucleotides used for sequence analysis, the *Pvu*II restriction site within SCR 5, and the second polyadenylation signal used in the RNA templates are underlined. The beginning of each individual SCR domain is marked. In cases where the base exchanges result in aa substitutions, both amino acids are shown, with the second residue corresponding to the variant position in either pFH1.4b or pFH4.3.

the divergent segments demonstrated that two of them (pFH1.4e and f) are identical to pFH1.4a and the remaining two (pFH1.4c and d) correspond to pFH1.4b. These results indicate that the cDNA clones are derived from two distinct 1.4-kb factor H-like mRNA species representing either allelic or isotypic sequences.

As expected from Northern blot (Fig. 4) and colony screening results, the pFH1.4 sequences are virtually identical to the 3′ end of the classical 4.3-kb factor H transcripts in 754 nucleotides. Ten nucleotide exchanges are found when comparing with the pFH1.4a cDNA leading to two aa substitutions. No homology to known factor H sequences was detected for the 512 bp of the 5′ half of the pFH1.4 cDNA.

The cDNA of pFH1.4a is 1266 bp in length and has one open reading frame of 994 bp encoding a polypeptide of 330 aa. The open reading frame is followed by 189 bp of a 3′ untranslated region that contains two polyadenylation signals at nucleotide positions 1111 and 1241 and a short poly(A) tail. The second polyadenylation signal was used in all our cDNA sequences (1.4 or 4.3 kb). The coding frame starts after 76 bp of 5′ untranslated sequence and is followed by a short hydrophobic stretch of 18 aa of the signal sequence. According to von Heinje (33), the mature protein starts with glutamic acid (E).

The polypeptide encoded by pFH1.4 is, like the other factor H proteins gp150 and p43, a member of the structural SCR superfamily. The protein molecule consists of five SCR domains with domains 1 and 2 representing new sequences. In a Northern blot analysis the corresponding unique 5′ sequence hybridizes exclusively to the small 1.4-kb band (Fig. 4, *lane 4*). No additional transcripts were detected in human liver mRNA. Every SCR domain contains four cysteines at conserved sites such that each domain is determined by two intrachain disulfide bonds. The predicted polypeptide mass of $M_r$ 35,800 contains two possible *N*-glycosylation sites at aa positions 109 and 178 (Fig. 3). The two pFH1.4-encoded variants differ by three aa within domain 3. SCR 3 and 4 of pFH1.4a are identical to the SCR domains 18 and

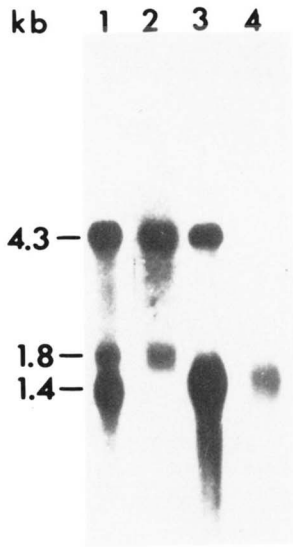


*Figure 4.* Northern blot of poly(A)$^+$RNA from human liver; 1 μg of poly(A)$^+$RNA was separated on an agarose/formaldehyde gel, transferred to nylon membrane, and hybridized with the full-length pFH4.3 cDNA insert (*lane 1*), the full-length pFH1.8 cDNA insert (*lane 2*), a pFH1.4 cDNA probe containing SCR 3–5 (*lane 3*), and a pFH1.4 cDNA probe containing SCR 1–2 (*lane 4*), which is specific for the 1.4-kb factor H mRNA species.

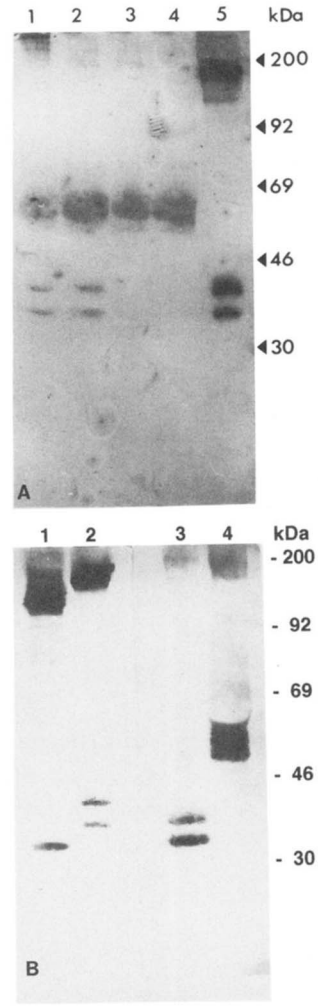


*Figure 5.* Western blot analysis of factor H expression. *A*) COS cell transfectants; 3 μl of normal human serum and 80 μl of 20-fold concentrated supernatants of the individual transfection assays were subjected to SDS/PAGE under nonreducing conditions and then analyzed by Western blotting with the monoclonal antifactor H antibody 3D11.7. *Lane 1*: COS cells transfected with pFH1.4a; *lane 2*: COS cells transfected with pFH1.4b; *lanes 3* and *4*: COS cells transfected with the vector CDM8; *lane 5*: human serum. The mAb 3D11.7 also detects a 55-kDa material in COS cell culture supernatants probably derived from the Nutridoma medium. *B*) Glycosylation of factor H proteins; 5 μl of endoglycosidase F/*N*-glycosidase F-treated human serum (*lanes 1* and *2*) and 2.5 μl of untreated human serum (*lanes 2* and *4*) were analyzed. *Lanes 1* and *2* were stained with the mAb 3D11.7. *Lanes 3* and *4* were stained with a polyclonal antibody to β2-glycoprotein I as a positive control for the deglycosylation procedure (34).

19, respectively, of factor $H_{gp150}$, while SCR 5 corresponds to SCR 20 of factor $H_{gp150}$ with the exception of two aa. Interestingly, the homology of pFH1.4 to pFH4.3 sequences starts exactly with SCR domain 3.

pFH1.4 codes for two factor H-related proteins present in human sera. The plasmid CDM8 used for constructing the cDNA library is a high efficiency eucaryotic expression vector (25). We directly introduced DNA of two cDNA clones pFH1.4a and pFH1.4b into COS7 cells by electroporation. The supernatants were then tested for the encoded proteins by Western blot analysis using an antibody directed against the C-terminal of factor $H_{gp150}$ (22). Interestingly, pFH1.4a and b both code for two proteins of 36,000 and 43,000 $M_r$ (Fig. 5*a*, *lanes 1* and *2*). The same two bands are also detected in human serum (Fig. 5*a*, *lane 5*). These results demonstrate that the mAb 3D11.7 is directed against an epitope present in SCR 18–20.

The finding that one cDNA clone encodes two polypeptides could be explained by different glycosylation forms due to the presence of two *N*-glycosylation sites within the pFH1.4 protein sequence. Treatment with endoglycosidase F/*N*-glycosidase F (Fig. 5*b*) and *N*-glycosidase F alone (data not shown) decreased the molecular mass of both, the 43-kDa and the 36-kDa peptides leading to one molecule of 33 kDa. The 43-kDa peptide was reduced gradually first to 36 kDa and finally to 33 kDa (data not shown). These results indicate that the 43- and 36-kDa molecules are glycoproteins with different degrees of glycosylation probably corresponding to the two glycosylation sites present in each molecule.

The two N-terminal SCR domains specific for the translation product of pFH1.4 show low homology to known human SCR-composed proteins (Fig. 6). Interestingly, these new SCR domains display the highest degree of homology to SCR 6 and 7 of classical factor $H_{gp150}$ (40%) (17), followed by the domains 1 and 2 of $\beta_2$-glycoprotein I (32%) (34), the SCR 15 and 16 of the human complement C3b/C4b receptor CR1 (30%) (35–37) and repeats 1 and 2 of factor XIII (25%) (38). The second SCR of the pFH1.4 protein has a significant homology of 23% to the SCR-like structure of human thyroid peroxidase (39).

Because the cDNA sequences derived from the 4.3-kb and the 1.4-kb mRNA species differ within the shared 3′ region in a *Pvu*II recognition sequence, human genomic DNA was restricted with *Pvu*II and hybridized with the shared 3′ fragment (*Hin*dIII/*Pst*I). All individuals analyzed show two fragments (Fig. 7, *lanes 1–6*). Otherwise, in several additional restriction digests tested (*Bam*HI, *Taq*I, *Bgl*II, *Eco*RV, and *Hin*dIII), this probe detects only one band (Fig. 7, *lanes 7* and *8* for *Hin*dIII), indicating high sequence similarity being also reflected at the gene organization.

## DISCUSSION

We have isolated cDNA clones derived from the small 1.4-kb mRNA of the three factor H-like mRNA species

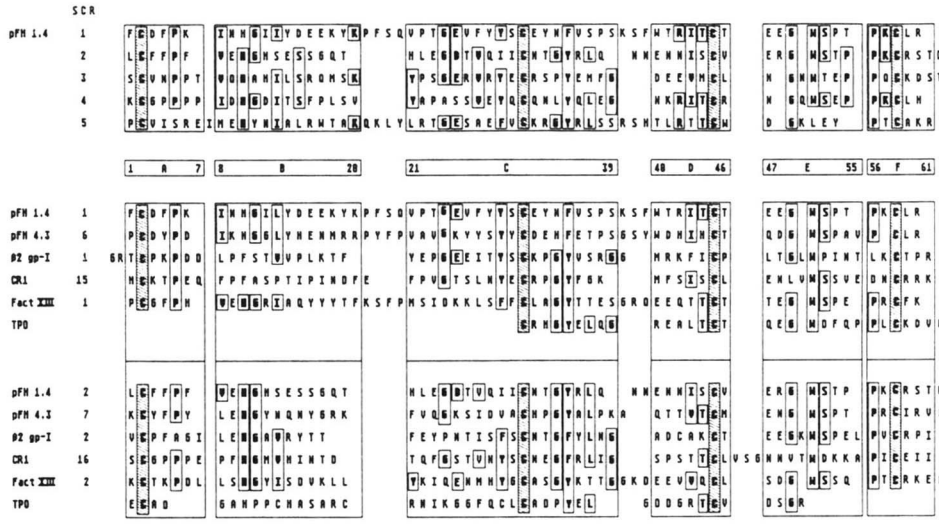

*Figure 6.* The SCR domain structure of the factor H-related polypeptide. The sequences of the SCR repeats are aligned using the pattern of Perkins et al. (18). Conserved residues are boxed. The *upper panel* shows the intramolecular homology in the SCR domains of the factor H-related polypeptide encoded by the 1.4-kb mRNA species. The homology search was performed using the Martinsried Institute Protein Sequences data bank. Only sequences with homology > 23% are shown in this comparison.

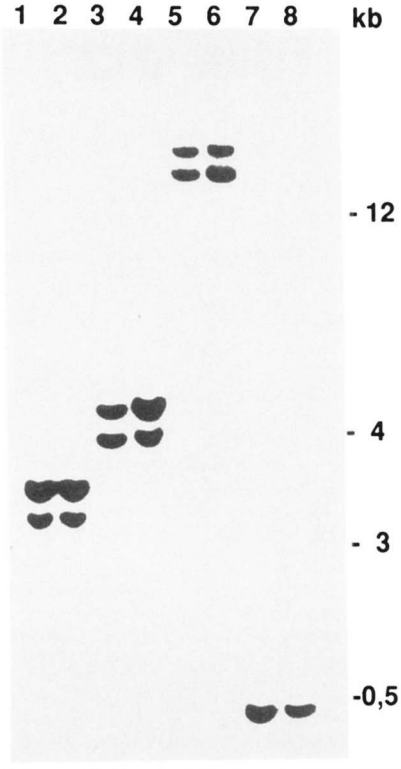


*Figure 7.* Southern blot analysis indicates that highly similar genes code for the factor H 4.3-kb and 1.4-kb mRNA species; 15 μg of restricted human DNA of two individuals was separated on a 0.7% agarose gel and transferred to nylon membrane. Blots were hybridized with the 3′ *Hin*dIII/*Pst*I fragment present in pFH4.3 and pFH1.4. Restriction enzymes were used as follows: *lanes 1* and *2*: *Pvu*II/*Bam*HI; *lanes 3* and *4*: *Pvu*II/*Bgl*II; *lanes 5* and *6*: *Pvu*II; *lanes 7* and *8*: *Hin*dIII.

TABLE I

*Comparison of the human short factor H-related transcript (pFH1.4) with the full-length classical murine factor H sequence (4.3 kb) and the cDNA 13G1, derived from a short factor H-related transcript in the mouse*[a]

| pFH1.4 | Homology to Mouse Factor H cDNA (%) | | | | |
|---|---|---|---|---|---|
| | 13G1 | | 4.3 kb | | |
| | aa | bp | aa | bp | |
| SCR 1 | 81.8 | 75.8 | | | |
| SCR 2 | 77.5 | 70.7 | | | |
| SCR 3 | 51.6 | 40.0 | 72.8 | 60.7 | SCR 18 |
| SCR 4 | 80.4 | 76.8 | 80.7 | 75.4 | SCR 19 |
| SCR 5 | 62.1 | 48.4 | 60.5 | 46.8 | SCR 20 |

[a] On the right the SCR domains of the clone pFH1.4 are numbered, and the SCR 18–20 indicate the C-terminal SCR encoded by the 4.3-kb mouse transcript. The comparison was done for both the aa and the nucleotide (bp) sequences.

present in human liver that encode a new factor H-related protein. The protein is related to the C-terminal of the classical factor $H_{gp150}$ molecule because it shares the last three SCR domains with factor $H_{gp150}$ and contains two SCR domains that appear to be unique for the small protein. The translation product is provisionally named factor $H_{re36}$. The hybrid nature of the 1.4-kb factor H-like mRNA-encoded polypeptide is supported by the isolation of six independent, functional full-length cDNA clones containing these domains. Moreover, it is interesting to observe that the aa sequence of the new domains is joined in frame at a SCR boundary of the following three classical C-terminal domains. Northern blot data, obtained with subfragments, confirm that the sequence of pFH1.4 SCR1 and 2 are linked to SCR 18–20 in the 1.4-kb mRNA species.

The two N-terminal factor $H_{re36}$-specific SCR domains show low homology to two consecutive units in known human SCR-composed proteins (Fig. 6). This finding might indicate that the two SCR structures form a functional domain or may be explained by the duplication process the genes have undergone. The structure of factor H gene of man (40) and mouse (41) shows a grouping of SCR/exons in pairs, with SCR 6 and 7 of the 4.3-kb mRNA being one pair.

Transient expression of pFH1.4a and pFH1.4b shows that the cDNA clones represent viable transcripts of the 1.4-kb factor H-like mRNA of human liver. We were able to demonstrate that both small factor H-like polypeptides detected in human sera with either polyclonal factor H antisera or with the mAb 3D11.7 are encoded by one mRNA. We could demonstrate that both serum proteins are glycosylation products of a 33-kDa polypeptide. Similar results have been described with isolated 43-kDa and 36-kDa polypeptides (42). The calculated size of $M_r$ 36,000 for the pFH1.4-encoded protein is in agreement with the estimated m.w. of 38,000 for the 33-kDa unglycosylated polypeptide when run under reducing conditions (42). The finding that this novel factor H-related molecule is also arranged in SCR units might point to a role in complement regulation. Although to date the functional significance of the repeating units is not known, it

has been shown that many of the complement proteins, built up of SCR domains, interact in some way with either C3b or C4b. The binding site of factor H to C3b is located on the N-terminal (SCR 1–7) of factor $H_{gp150}$. This region is shared by the truncated form of factor $H_{p43}$ encoded by the 1.8-kb mRNA (17, 21). The highest degree of homology of the two novel SCR domains of the pFH1.4 protein to the SCR domains 6 and 7 of factor $H_{gp150}$ and $H_{p43}$ might be significant for the function of this new factor H-related protein, moreover, inasmuch as it is coupled to a C-terminal identical to the one of factor $H_{gp150}$. The pFH1.4-derived factor H-related proteins isolated from human sera have been shown not to exert complement-regulatory functions known to be associated with classical factor H when tested for activity in the dissociation of C3bBb complexes or as cofactor for the cleavage of C3b by factor I (42). They might share some unknown function with the classical factor $H_{gp150}$ carried by the common SCR 18–20 but possibly modified by the presence of the novel N-terminal structure. It is conceivable that the pFH1.4-encoded polypeptides might also bind to the factor H receptor and compete with factor $H_{gp150}$ as only factor H of a certain conformation, $\phi_2$, was able to bind to the factor H receptor of Raji cells (43). Alternatively, the two N-terminal SCR of factor $H_{re36}$ might have an additional new function not fulfilled by factor $H_{gp150}$. A third hypothesis would propose that the combination of the two pFH1.4 specific SCR with the known three-factor H SCR 18–20 would result in a protein with novel functional activities not overlapping with the bioactivity of factor $H_{gp150}$.

With regard to the functional importance of factor $H_{re36}$, it was of interest to learn that a similar molecule evolved among the mouse factor H-like proteins (44) (Table I). Several factor H-related transcripts have been characterized in mouse liver that have not been detected in human liver mRNA. Nucleotide and aa sequence comparison of our factor H-related clone pFH1.4b with the mouse clone 13G1 and classical mouse factor H (4.3 kb) shows a high homology between the two factor H-related sequences of both species, with the polypeptide sequence being more conserved than the nucleotide sequence. The cDNA 13G1, like pFH1.4, codes for two unique N-terminal SCR domains coupled to three domains homologous to the carboxy terminal of murine factor $H_{gp150}$ (SCR 19, 19, 20) also encoded by a 4.3-kb mRNA species. Increased divergence is observed for SCR 3 where the murine polypeptide contains a second SCR 19 homologous domain instead of SCR 18, and in the last SCR 5. Interestingly, whereas human factor $H_{re36}$ shares the carboxy terminal three SCR sequences with factor $H_{gp150}$, the mouse 13G1-encoded protein has a carboxy terminal that is homologous with but quite divergent from the murine factor $H_{gp150}$.

The characterization of the three factor H-related transcripts detected in human liver by cDNA cloning and eucaryotic expression leads to the question whether these mRNA are derived from one gene representing alternatively spliced H gene transcripts. Previous studies have provided convincing evidence that both the 4.3-kb and the 1.8-kb factor H mRNA are transcribed from the same factor H gene (17, 21). The results reported here for the 1.4-kb factor H-related transcript, however, indicate that they are transcribed from another gene(s). First, pFH1.4 differs from the 4.3-kb and 1.8-kb transcripts in the 5′ untranslated region, which points to separate transcription start sites. The notion that 1.4-kb and 4.3/1.8-kb mRNA synthesis is controlled independently is supported by the finding that the 1.4-kb factor H-related transcript is only detected in human liver mRNA, whereas the 4.3-kb and 1.8-kb mRNA species are concomitantly expressed in other tissues (13, 45, 46). Second, pFH1.4 sequences overlapping with the 4.3-kb transcript are not identical to this sequence as determined from the same liver library. The restriction enzyme *Pvu*II, which cleaves only in the 3′ sequence of the pFH1.4a/b (SCR 5, Fig. 3), is absent in 4.3-kb derived clones. Moreover, the isolation of two distinct cDNA encoded by the 1.4-kb mRNA species both differing from the 4.3-kb transcript exclude an explanation of allelic differences.

Southern blot results obtained from human genomic DNA restricted with *Pvu*II and hybridized with the shared 3′ fragment (*Hin*dIII/*Pst*I) show two fragments (Fig. 7, *lanes 1–6*). The results point to at least two genes in the human genome, one coding for the 4.3-kb and 1.8-kb factor H transcripts, and a second one coding for the 1.4-kb factor H-related mRNA.

In the future, the isolation of the gene(s) coding for the 1.4-kb transcript will resolve the question whether the two kinds of pFH1.4 cDNA clones are derived from two discrete loci or whether they represent allelic sequences.

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