A Novel Short Consensus Repeat-containing Molecule Is Related to Human Complement Factor H*

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We have identified a novel factor H-related cDNA, which was isolated from a human liver cDNA library. The DOWN16 clone is 1269 base pairs in size and hybridized to a mRNA of 1.4 kilobases. Similar to the previously described factor H-related proteins, the predicted translation product of 351 amino acids contains a hydrophobic signal sequence followed by a stretch of five short consensus repeats (SCRs). These five SCRs display homology to SCRs of factor H: SCR1-3 (DOWN16) are homologous to SCRs6-8 of factor H, while SCRs4 and -5 are related to SCRs19 and -20. In vitro translation demonstrated that the DOWN16 cDNA encodes a primary translation product of an apparent molecular mass of 37,500 Da which is directed to the secretory pathway and is glycosylated. Thus, we propose that the protein will be present in human serum. The relatedness of structural elements between this novel gene and factor H may suggest common functions of these proteins not yet determined.

The complement glycoprotein factor H plays a regulatory role in the alternative pathway of complement activation, and the corresponding cDNAs have been isolated from human liver cDNA libraries (1-3). The mRNA encoding factor H is 4.4 kb in size, and structural analysis indicates the organization of factor H protein in 20 tandem repeating units of approximately 60 amino acids, termed short consensus repeat (SCR). In addition to factor H, these SCRs are present in several C3- and C4-binding proteins of the complement system; they have also been found in noncomplement proteins such as the b chain of the clotting factor XIII, the lymphocyte homing receptor, the IL-2 receptor, and a vaccina virus-cofactor protein, C3b/C4b receptor (CR1), and C3d/Epstein-Barr virus receptor (CR2).

Several human factor H-related molecules have been isolated at the cDNA and at the protein level. So far, three factor H-related cDNAs have been isolated from human liver cDNA libraries. The 1.5-kb mRNA is supposedly derived from the human factor H gene by alternative splicing (9, 10). Two distinct cDNAs, H36-1 and DDESK59 (11-13), both correspond to the 1.4-kb mRNA and seem transcribed from loci distinct from that of factor H. Two human serum proteins of 37,000 and 42,000 Da (h37, h42), encoded by the H36 cDNA, are both organized in five SCRs (11, 14). The distinct molecular mass of these proteins shows that they represent differently glycosylated forms. Similarly, the human factor H-related serum proteins of 24,000 and 29,000 Da (h24, h29), derived from the DDESK59 cDNA also differ in their degree of glycosylation. These two proteins are organized in four SCRs (11, 13).

The use of cDNA fragments specific for factor H and for the factor H-related molecules as probes for Northern blot analyses indicated the existence of additional factor H-related transcripts in human liver (11). We describe the isolation of a novel factor H-related cDNA DOWN16. The protein encoded by this cDNA displays a signal peptide and is organized in five SCRs. These individual SCRs display homology to SCR6, -7, -8, -19, and -20 of factor H.

MATERIALS AND METHODS

Labeling of Oligonucleotide Probes and Screening—A human liver oligo(dT)-primed cDNA library in λ zap was screened with a cDNA fragment of factor H, representing 1050 bp of the 3' end of the 1.8-kb cDNA (kindly provided by T. Kristensen and B. Tack).

Sequence Analysis of cDNA Clones—The purified cDNA inserts were sequenced by the dideoxy chain termination method (15) using [α-32P]dATP and Sequenase II (U. S. Biochemical Corp.). Various oligonucleotides were synthesized and used as primers to sequence the cDNA in both orientations.

RNA Isolation and Northern Blot Analysis—Total cellular RNA was extracted with guanidinium thiocyanate and isolated by centrifugation over CsCl (16). The RNA concentration was determined spectrophotometrically, and 8 μg of total cellular RNA was separated by electrophoresis in a formaldehyde agarose gel and subsequently transferred to a nylon membrane (PALL).

Southern Blot Analysis—Human DNA (10 μg) isolated from U937 cells was digested to completion with EcoRI or BamHI, separated in a 1.0% agarose gel, and transferred to a nylon membrane (PALL) (17).

Labeling and Hybridization—For library screening, a fragment representing 1050 bp of the 3' end of the factor H 1.8 cDNA was used. A specific TaqI/Xhol fragment (731-1264) of DOWN16 representing mainly SCRS and the 3'-untranslated region was used as a probe for Northern blotting. The cDNA inserts were excised, purified on low melt agarose gels, and, after labeling with 32P by random priming (Amersham), used for hybridization at 42 °C (5 × Denhardt's, 5 × SSC, 0.1% SDS, 250 μg/ml denatured salmon sperm DNA, and 50% formamide). Following hybridization for 14-18 h, the filters were
washed at a final stringency of 0.1% SSC at 55 °C. The filters were exposed at -70 °C using intensifying screens (Quanta III, Du Pont-New England Nuclear).

In Vitro Transcription and Translation—For cell-free expression, the DOWN16 cDNA clone was linearized with HindIII at the 3' end of the coding region, and in vitro transcription was carried out from the T3 promoter. The transcripts were translated in wheat germ lysate in the presence or absence of canine pancreatic microsomes as described previously (18). For the inhibition of high mannos-type N-glycosylation, an NH2-terminal blocked tripeptide (benzyol-Asn-Leu-Thr-N-methylamide) was included in the translation reaction, at a final concentration of 50 μM (19). Proteins were labeled by incorporation of [35S]methionine (Amersham), separated on 15% SDS-polyacrylamide gels, and visualized by autoradiography.

RESULTS

Isolation of DOWN16 cDNA Clones—In order to identify additional factor H-related genes, we screened an oligo(dT)-primed human liver cDNA library with DNA fragments of factor H. The nucleotide sequence of one clone indicated that it was distinct from factor H. Consequently, this clone was used to isolate additional cDNA clones. The sequence of the longest clone (DOWN16) is shown in Fig. 1. This clone is 1269 nucleotides long, including the poly(A) tail. The motif TCA TAC ATG shows a good match (6 out of 9, including the ATG) with the consensus sequence of initiation sites GCC ACC ATG (20). There is a poly(A) signal sequence "AAATAAA" at position 1231–1236.

Deduced Protein Sequence—The nucleotide sequence of clone DOWN16 displays an open reading frame of 331 amino acids encoding a protein of 37,500 Da. Within the predicted amino acid sequence, four potential N-linked glycosylation sites of the type Asn-X-Ser/Thr were found at positions 108–110 (Asn-Ser-Thr), 186–188 (Asn-Ser-Ser), 206–208 (Asn-Ser-Ser), and 310–312 (Asn-Thr-Ser). The NH2-terminal amino acid residues are indicative of a signal peptide which indicates that the product of the DOWN16 gene may be directed to the secretory pathway (21). According to the criteria common for signal peptide cleavage sites, we suggest that the leader sequence is cleaved at position 19 (21). The molecular mass of the secreted, nonglycosylated product was calculated to 35,500 Da.

Structural Analysis and Homology—Structural alignment of the protein encoded by the DOWN16 cDNA indicated a protein composed of an NH2-terminal signal sequence which directs the primary translation product was converted to three major products of larger sizes (Fig. 6A, lane 2). These products were inside microsomal vesicles because they were protected from digestion with post-translationally added protease K (Fig. 6A, lane 3), but they were susceptible to protease if the microsomes were lysed with Triton X-100 (Fig. 6A, lane 4). N-Linked glycosylation occurs in the lumen of the endoplasmic reticulum and results in an increase in molecular mass. In order to assess if the increase in molecular mass was due to N-linked glycosylation, translation and translocation experiments were carried out in the presence of an acceptor peptide which competitively inhibits N-linked glycosylation of newly synthesized polypeptides (Fig. 6B, lanes 2–4). In the presence of microsomal membranes, the primary translation product was converted to a slightly smaller form of approximately 35,500 Da, which in the absence of Triton X-100 was protected from protease K, but not in its presence (Fig. 6B, lanes 1–4). The conversion of the primary translation product to a smaller form (Fig. 6B, lane 2) is consistent with the cleavage of an NH2-terminal signal sequence upon translocation into microsomes (23). These results confirm that the larger products generated in the absence of acceptor peptide have arisen from N-linked glycosylation (Fig. 6A, lanes 2 and 3). Their different masses correspond to variant degrees of glycosylation.

DISCUSSION

We describe the isolation and characterization of a novel factor H-related cDNA clone, DOWN16. The predicted amino acid sequence displays a hydrophobic signal peptide. Similar to factor H and to the other factor H-related proteins, the DOWN16 protein is organized in SCRs. SCRs1 and -2 of DOWN16 display a high degree of homology to SCR6 and -7 of factor H (Fig. 3 and Table I), while the homology of SCRs3–5 to SCRs8, -19, and -20 of human factor H is less pronounced (Table I). In vitro translation experiments demonstrate that the DOWN16 cDNA encodes a primary translation product of an apparent molecular mass of 37,500 Da which is directed to the secretary pathway.

Similar to factor H and to the other factor H-related molecules, the processed DOWN16 product consists exclusively of SCRs. Comparing the mobility of the DOWN16 mRNA to the 18 S ribosomal RNA indicates a size of about 1.4 kb. This size indicates that the DOWN16 cDNA is nearly full-length. The full-length cDNA clones of the other two factor H-related cDNAs (H36 and DDESK59) which are 1266 bp and 1051 bp in length, respectively, are also represented by 1.4-kb mRNAs (11, 13).

The DOWN16 cDNA encodes a protein of 37,500 Da including an NH2-terminal signal sequence which directs the
translation product to the secretory pathway. This indicates that, similar to factor H and the two other factor H-related proteins (h24/h29 and h37/h42), the DOWN16 protein is secreted into human serum, indicating the existence of a family of factor H-related transcripts (11). So far, four human factor H-related cDNA clones have been isolated. One cDNA represents the alternatively spliced 1.8-kb mRNA (24) and the other three distinct factor H-related cDNA clones (DOWN16, DDBDSK59, and H36-1) are all encoded by mRNAs of three distinct factor H-related cDNA clones (DOWN16, -6, and -7). The nucleotide numbers are displayed on the left, and the numbers referring to the amino acid sequence are shown on the right. The polyadenylation signal is underlined. The putative N-linked glycosylation sites are shown in bold characters.

**FIG. 1.** Nucleotide and derived protein sequence of DOWN16 cDNA. The nucleotide numbers are displayed on the left, and the numbers referring to the amino acid sequence are shown on the right. The polyadenylation signal is underlined. The putative N-linked glycosylation sites are shown in bold characters.

**FIG. 2.** Amino acid alignment of the SCRs predicted from the nucleotide sequence of the DOWN16 cDNA clone. The sequences were aligned based on their conserved amino acids according to the SCR structure. Residues conserved in all SCRs are boxed, and the N-linked glycosylation sites are underlined.

The presence of a functional cleaved signal peptide and the similarity of the DOWN16 protein to the human serum protein factor H and to the factor H-related proteins h57/h42 and h27/h29 suggest that the native protein must be secreted into human serum.

Additional factor H-related mRNAs have previously been described in human liver, indicating the existence of a family of factor H-related transcripts (11). So far, four human factor H-related cDNA clones have been isolated. One cDNA represents the alternatively spliced 1.8-kb mRNA (24) and the three distinct factor H-related cDNA clones (DOWN16, DDBDSK59, and H36-1) are all encoded by mRNAs of 1.4 kb (11-13). Despite this large number of factor H-related cDNAs, Southern blot analysis with a specific fragment in-
A

Factor H-related Molecules

Fig. 3. Homology comparison of DOWN16, factor H, and the two factor H-related cDNA clones DDESK59 and H36-1. A, schematic comparison. SCRs of each molecule are numbered consecutively starting with number 1. Related SCRs are indicated by their vertical alignment, and, therefore, gaps indicated by thin lines were introduced for DOWN16 and DDESK59. B, amino acid comparison of the homologous SCRs predicted from the cDNAs of DOWN16, factor H, DDESK59, and H36 using a single-letter code. Identical amino acids are shown by dots. The individual lines represent the signal sequence and SCRs 1-5 of the DOWN16 product. The SCRs of the individual proteins which are used for alignment are indicated on the left.

![Diagram](image)

Table I

| Homology comparison | Amino acid homology of individual SCR elements of the DOWN16 protein to factor H and to the factor H-related proteins h37/h42 and h24/h29, respectively. |
|---------------------|-------------------------------------------------------------------------------------------------------------------------------------|
| DOWN16 H | DDESK59 | H36-1 |
| Leader | 50.0% L | 50.0% L | 50.0% L |
| SCR1 | 93.5% SCR6 | 40.9% SCR1 | 42.4% SCR1 |
| SCR2 | 86.4% SCR7 | 36.2% SCR2 | 36.2% SCR2 |
| SCR3 | 62.9% SCR8 | 32.3% SCR3 | 36.2% SCR3 |
| SCR4 | 62.3% SCR19 | 66.7% SCR4 | 62.3% SCR4 |
| SCR5 | 36.0% SCR20 | 50.5% SCR5 | 39.0% SCR5 |

B

![Diagram](image)

Fig. 4. Northern blot analysis with human liver RNA. Total cellular RNA was extracted, and 8 μg of RNA was separated on denaturing agarose gel electrophoresis and blotted onto nylon membranes. A 32p-labeled cDNA probe specific for DOWN16 was used.
related SCRs of DOWN16 and of factor H exclude these functionally characterized SCRs, the function of the DOWN16 protein seems distinct from that known for factor H. A detailed functional characterization of the protein encoded by the DOWN16 cDNA requires the isolation of the native protein. Except for the alternatively spliced human 1.8-kb mRNA, factor H and all human and murine factor H-related cDNAs (H36, DDES^K59, and DOWN16; 3A4, 23L1, 9C4, and 13G1) isolated so far have at their COOH-terminal end a SCR which is homologous to SCR19 of factor H (11–13, 25, 26). The presence of SCR19 in that large number of molecules suggests a relevant and highly conserved function.

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