

The SPFH domain: implicated in regulating targeted protein turnover in stomatins and other membrane-associated proteins

Stomatin, also known as band 7.2b protein, is one of the major integral membrane proteins of human erythrocytes. This protein is apparently absent in the red cell membrane of patients suffering from overhydrated hereditary stomatocytosis, a form of autosomal dominant hemolytic anemia¹. Although the protein is missing, no mutations have been found within the stomatin gene in patients with this condition. However, an aberrant splice mutation associated with multi-system pathology early in life suggests that the protein is crucial for development (A. Argent, J. Delaunay and G. Stewart, pers. commun.). The genome of the nematode *Caenorhabditis elegans* encodes nine stomatin-related genes, three of which have been genetically characterized.

MEC-2 is a protein expressed predominantly in six touch-receptor neurons and plays an essential role in mechanotransduction^{2,3}, whereas UNC-24 is required for normal locomotion⁴. UNC-1 also affects locomotion and is required for normal responsiveness to volatile anesthetics⁵.

Although proteins of the stomatin family appear to be involved in important biological processes, very little is known about their function *in vivo*. To identify other proteins similar to members of this family, PSI-BLAST searches⁶ were performed on the non-redundant protein sequence database, using stomatin sequences. A domain within the central region of the stomatin family was identified that is strikingly similar to the prohibitin family of mitochondrial

proteins⁷, the caveolae-associated flotillins⁸, the bacterial plasma-membrane proteins HflK and HflC (Ref. 9), and a number of archaeal and bacterial hypothetical proteins (Fig. 1). This conserved region was named the SPFH domain, after the initials of the related protein families: stomatins, prohibitins, flotillins and HflK/C. The core motif of this domain is partially described in the Pfam database (Pfam entry: Band_7, accession number: PF01145).

A dendrogram illustrating evolutionary relationships among family members is shown in Fig. 2. Interestingly, one subfamily of mainly archaeal proteins is highly homologous to the eukaryotic stomatin family. We suggest that these are the prokaryotic members of the stomatin family (p-stomatin). p-Stomatin occurs in four of the five completely sequenced archaeal genomes (it is absent from *Methanococcus jannaschii*) but is present in only one of the 15 completely sequenced bacterial genomes (*Aquifex aeolicus*) and is also present in the partial

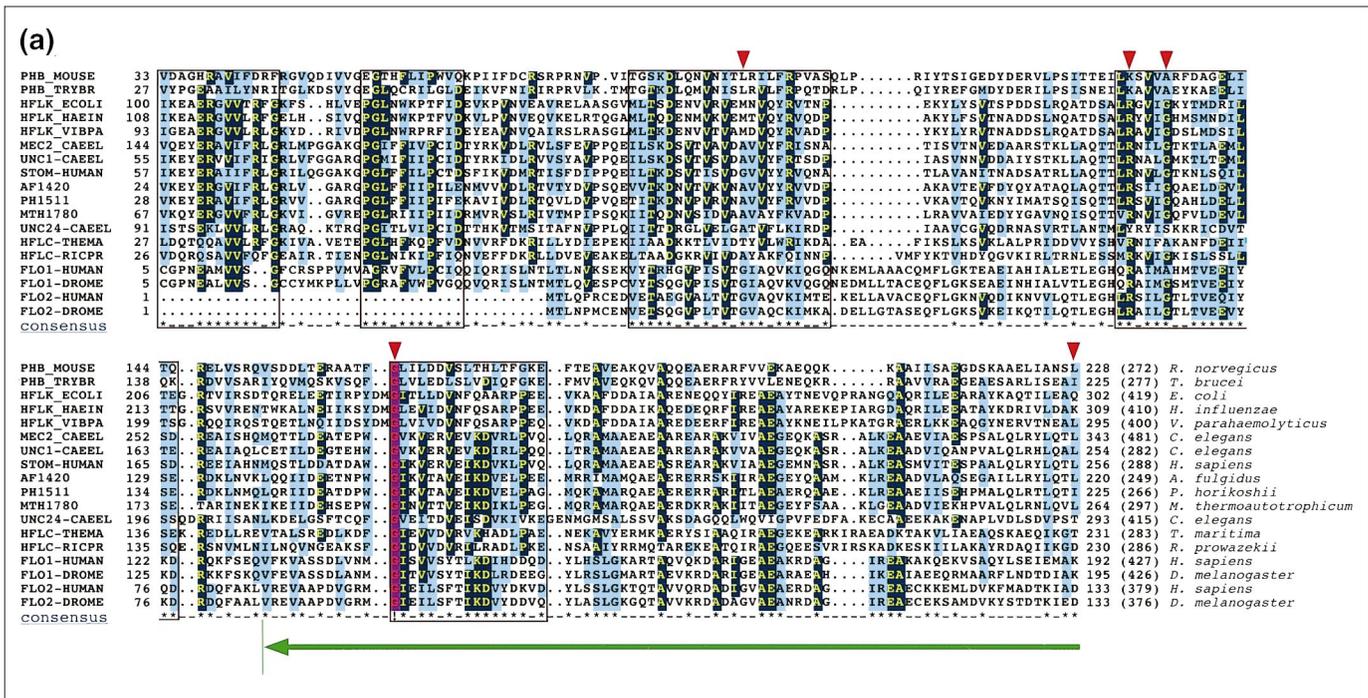


Figure 1

Multiple sequence alignment generated with the ClustalW program¹⁸ and visualized with Boxshade (ISREC, http://www.isrec.isb-sib.ch/8080/software/BOX_form.html). Boxes mark the most conserved blocks of the domain, as identified with HMM searches¹⁹. When sequence conservation is greater than 50%, identical residue positions are highlighted in dark blue and conservative substitutions in light blue. Red arrowheads denote mutations that disrupt the function of MEC-2 or UNC-1 in *Caenorhabditis elegans*. The green arrowbar denotes the region that has been shown to be important for oligomerization of vertebrate stomatin. Amino acid positions are indicated in the margins of the alignment. Numbers in parentheses at the end of the alignment denote the total length of the corresponding protein. Source organisms are also shown. Protein names (and accession numbers, when different) are: PHB_MOUSE, *Rattus norvegicus* prohibitin; PHB_TRYBR, *Trypanosoma brucei* prohibitin; HFLK_ECOLI, *Escherichia coli* hflK protein; HFLK_HAEIN, *Haemophilus influenzae* hflK protein; HFLK_VIBPA, *Vibrio parahaemolyticus* hflK protein; MEC2_CAEEL, *Caenorhabditis elegans* MEC-2 protein; UNC1_CAEEL, *C. elegans* UNC-1 protein (YMM5_CAEEL); STOM-HUMAN, *Homo sapiens* stomatin (gil181184); AF1420, *Archaeoglobus fulgidus* membrane protein (gil2649154); PH1511, *Pyrococcus horikoshii* hypothetical erythrocyte band 7 integral membrane protein (g3257936); MTH1780, *Methanobacterium thermoautotrophicum* stomatin-like protein (gil2622911); UNC24_CAEEL, *C. elegans* UNC-24 protein (gil1353669); HFLC-THEMA, *Thermotoga maritima* hflC protein (gil4982405); HFLC-RICPR, *Rickettsia prowazekii* hflC protein (g3860691); FLO1-HUMAN, *H. sapiens* flotillin-1 (gil3599573); FLO1-DROME, *Drosophila melanogaster* flotillin-1 (gil3115385); FLO2-HUMAN, *H. sapiens* flotillin-2 (gil627431); FLO2-DROME, *D. melanogaster* flotillin-2 (gil3115387). A multiple sequence alignment (alignment number ds39774) has been deposited with the European Bioinformatics Institute (<ftp://ftp.ebi.ac.uk/pub/databases/embl/align/ds39774.dat>).

genome of *Streptomyces coelicolor*. This phylogenetic distribution suggests that the stomatin family is relatively ancient – possibly occurring in the common ancestor of Archaea and Eukarya – and that horizontal gene transfer towards specific bacterial lineages has occurred. A second, distinct, prokaryotic protein family forms a separate branch that is equally distant from the other four main families (Fig. 2). This subfamily consists mainly of bacterial proteins but without members of the completely sequenced bacterial genomes from the archaea *Archaeoglobus fulgidus* and *Aeropyrum pernix*.

Most characterized SPFH family members are integral membrane proteins with the SPFH domain situated in the cytoplasm close to the membrane-associated region (see <http://nekt.rutgers.edu/stomatin/topology.gif>). In most eukaryotic family members, this places the SPFH on the cytoplasmic side of the plasma membrane but prohibition protrudes from the inner mitochondrial membrane into the intermembrane space. In the bacterial HflK/C family, the SPFH domain is on the periplasmic side of the plasma membrane¹⁰.

Biochemical activities of members of two subfamilies, *Escherichia coli* HflK/C and eukaryotic prohibitins, have been studied in some detail and indicate a remarkable conservation of function. HflK and HflC regulate the lysogenic decision during bacteriophage λ infection by modulation of the FtsH protease¹¹, an integral membrane ATP- and Zn²⁺-dependent metalloprotease that is a member of the AAA family of ATPases. The association of FtsH protease with the periplasmic domain of the HflK/C complex is thought to modify its proteolytic activities against specific substrates, such as the heat-shock sigma factor (σ 32), the phage λ CII transcription factor and the SecY subunit of the protein translocase.

Prohibitins are members of a highly conserved eukaryotic protein family with potential roles in senescence and tumor suppression^{12,13}. They are anchored to the mitochondrial inner membrane, and form complexes with and regulate the m-AAA protease, which mediates targeted proteolysis of non-assembled inner membrane proteins. These commonalities suggest that other as yet uncharacterized members of this superfamily might also play a role in the regulation of membrane-associated protein degradation. For example, the flotillins are a family of caveolae-associated integral membrane proteins expressed primarily within the developing nervous systems⁸. Caveolae are plasmalemmal microdomains that are involved in vesicular trafficking and signal transduction, processes for which protein turnover is essential¹⁴. The *reggie-1* and *reggie-2* flotillin-related genes identified in

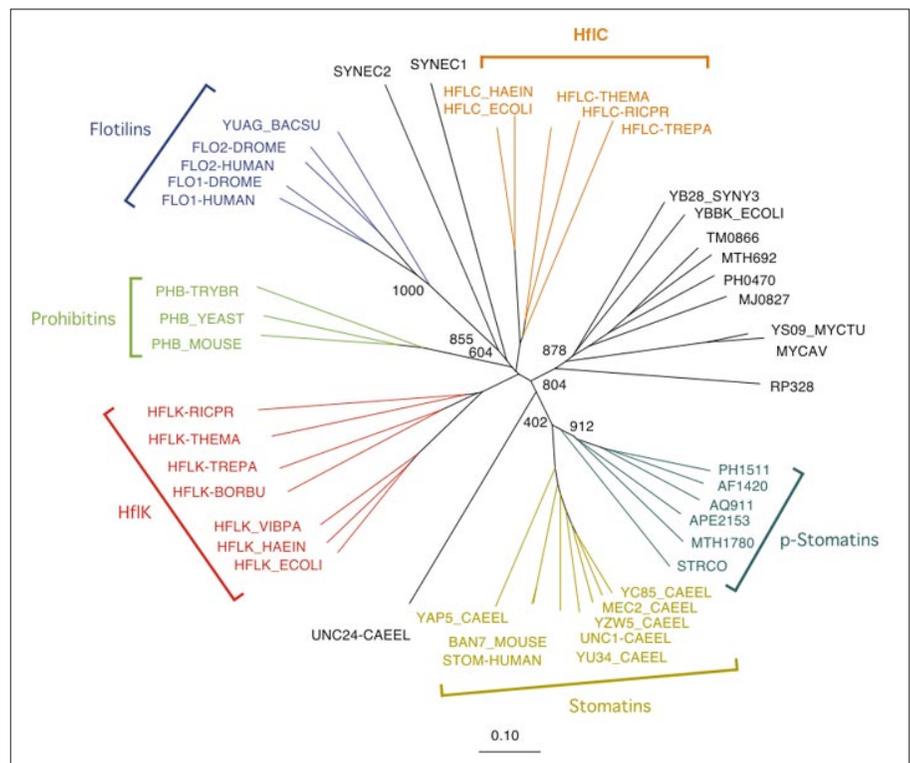


Figure 2

A dendrogram showing distance relationships between most of the stomatin protein superfamily members (the complete ClustalW-generated alignment on which the dendrogram was based is available at <http://nekt.rutgers.edu/stomatin/alignment.gif>). The dendrogram was constructed with the neighbor-joining method²⁰, based on pairwise distance estimates of the expected number of amino acid replacements per site (0.10 in the scale bar) and visualized by TreeTool (<http://geta.life.uiuc.edu>). Bootstrap values out of a thousand tests for some important branching patterns are shown. Protein subfamilies are shown in different colors. Sequence names and their references that are not reported in Fig. 1 are: APE2153, *Aeropyrum pernix* hypothetical erythrocyte band 7 integral membrane protein (g5105852); AQ911, *Aquifex aeolicus* erythrocyte band 7 homolog (gil2983432); BAN7_MOUSE, *Mus musculus* erythrocyte band 7 integral membrane protein; HFLC_ECOLI, *Escherichia coli* hflK protein; HFLC_HAEIN *Haemophilus influenzae* hflC protein; HFLC-TREPA *Treponema pallidum* hflC protein (gil3322377); HFLK-BORBU *Borrelia burgdorferi* hflK protein (gil2688090); HFLK-RICPR *Rickettsia prowazekii* hflK protein (gil3860690); HFLK-THEMA *Thermotoga maritima* hflK protein (gil4982404); HFLK-TREPA *Treponema pallidum* hflK protein (gil3322375); MJ0827, *Methanococcus jannaschii* hypothetical ORF Y827_METJA; MTH692, *Methanobacterium thermoautotrophicum* stomatin-like protein (gil2621777); MYCAV, *Mycobacterium avium* hypothetical protein (gil2183273); PHO470, *Pyrococcus horikoshii* hypothetical membrane protein (gil3256875); PHB_YEAST, *Saccharomyces cerevisiae* prohibitin; RP328, *R. prowazekii* hypothetical protein (gil3860888); STRCO, *Streptomyces coelicolor* hypothetical protein (gil3130017); SYNEC1, *S. PCC7942* hypothetical protein (gil1054892); SYNEC2, *Synechococcus* PCC7942 hypothetical protein (gil1054893); TM0866, *T. maritima* hypothetical protein (g4981401); YAP5_CAEEL, *Caenorhabditis elegans* hypothetical protein; YB28_SYNY3, *Synechocystis* sp. hypothetical protein; YBBK_ECOLI, *E. coli* hypothetical protein; YC85_CAEEL, *C. elegans* hypothetical protein; YS09_MYCTU, *Mycobacterium tuberculosis* hypothetical protein; YU34_CAEEL, *C. elegans* hypothetical protein; YUAG_BACSU, *Bacillus subtilis* hypothetical protein; YZW5_CAEEL, *C. elegans* stomatin-like protein.

database searches via their SPFH domains are highly expressed by retinal ganglion cells upon axon regeneration¹⁵. It was hypothesized that flotillins and *reggie* proteins participate in the localized turnover of specific proteins. Likewise, *C. elegans* and vertebrate stomatins might anchor complexes that regulate local proteolysis (possibly of ion channels) that is crucial for mechanical signaling, locomotion, anesthetic sensitivity and osmotic integrity.

Most mutations that disrupt or alter the activities of *C. elegans* MEC-2, UNC-1 and UNC-24 affect conserved amino acids in the SPFH domain^{2,4,5} (Fig. 1), which suggest that this domain is crucial for function. This region appears to be important in homo-oligomerization of vertebrate stomatin – deletion of the C-terminal region disrupts self-association¹⁶ (Fig. 1). Genetic data in *C. elegans* also support the notion that

homo-oligomerization is a feature of these worm proteins¹⁷. Work with the AAA protease regulators suggests that the region that includes the SPFH domain is essential for complex formation with membrane-associated proteases, although residues that mediate such interactions remain to be precisely defined^{11,13}.

Future definition of the amino acids that mediate protease association and identification of protease partners of various stomatin family members will test the hypothesis that these proteins are core components of membrane-associated proteolytic complexes and extend the understanding of structure–function relationships within the stomatin superfamily.

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NEKTARIOS TAVERNARAKIS AND MONICA DRISCOLL

Department of Molecular Biology and Biochemistry Rutgers, The State University of New Jersey, New Jersey, USA.
Email: tavernarakis@mbcl.rutgers.edu

NIKOS C. KYRPIDES

Integrated Genomics Inc., 2201 W. Campbell Park Drive, Chicago, IL 60612, USA.

Erratum

In the October 1999 issue, we published an article 'Novel oligosaccharide ligands and ligand-processing pathways for the selectins' (*TIBS* 24, 369–372). Unfortunately, in the production process, the positions of different types of arrows in Fig. 3 were altered. The corrected figure is shown here.

Figure 3

Schematic representation of leukocytes (1–3) and endothelial cells (I–VI). Receptor–ligand pairings predicted to favour very strong (black arrows), medium (dark blue arrows) or relatively weak (light blue arrows) interactions of the leukocyte membrane-associated L-selectin with endothelial cells or with other leukocytes; others are predicted to be unfavourable for interaction. On leukocyte 2, the L-selectin is available for interaction with oligosaccharide ligands on endothelium, or on other leukocytes, whereas on leukocyte 1, the carbohydrate-binding activity of the L-selectin is quenched by putative *cis*-interactions³² with oligosaccharide ligands on the same cell surface. The L-selectin on leukocyte 3 is shed from the cell surface. Endothelial cells II, III and V are able to support L-selectin-mediated leukocyte adhesion on account of their having at their surface counter-receptors that bear the 6-sulpho-sialyl-Le^x forms with *N*-acetylated (II and V) sialic acid, among which III is most strongly bound by L-selectin. Endothelial cells I, IV and VI are ligand-negative for L-selectin. In endothelial cell I, the carrier protein does not contain oligosaccharide ligands for L-selectin. In endothelial cell IV, the sialic acid carboxyl group in the 6-sulpho-sialyl-Le^x has become 'cyclized' and in VI the sialyl-Le^x with an unmodified sialic acid is 6'-sulphated at galactose. Symbols for monosaccharides or substituents on oligosaccharides (depicted here as O-glycans) are given in the figure; the sialic acids are *N*-acetyl, de-*N*-acetyl and de-*N*-acetyl with a 'cyclized' carboxyl group, respectively, as listed in the key; 6-O-sulphate on the *N*-acetylglucosamine is commonly referred to as 6-sulphate, and as 6'-sulphate on the galactose.

