

Chapter 2

Prominin-2 and Other Relatives of CD133

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Abstract Several molecules related to prominin-1/CD133, which was first characterized as a marker of mouse neuroepithelial stem cells and human hematopoietic stem cells, have been identified in various species. In mammals, a second *prominin* gene, *prominin-2*, has been identified and characterized, whereas in nonmammalian species, up to three *prominin* genes are potentially expressed. The structural similarities between prominin-1 and prominin-2 are, to some extent, reflected by their biochemical properties; both proteins are selectively concentrated in specific plasma membrane subdomains that protrude into the extracellular space and are released in small extracellular membrane vesicles. In contrast to the apically confined prominin-1, prominin-2 is distributed in a nonpolarized apico-basolateral fashion in polarized epithelial cells and appears to be expressed in separate epithelial cells. Their distinctive localization in plasma membrane protrusions is a hallmark of prominins, validating the naming of the family after its first identified member. Insights into the distinctive and/or complementary roles of the two prominins may be obtained by analyzing the evolutionary history of these proteins and the characteristics of orthologs and paralogs in more distantly related species. In addition, the characterization of prominins may shed light on the still elusive function of CD133.

Keywords CD133 • Cholesterol • Epithelial cell • Microvillus • Prominin • Vesicle

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2.1 Introduction

In spite of the large body of studies on prominin-1/CD133 as a stem cell marker, its function in physiological processes as well as in cancer still remains elusive. I propose to possibly gain insights from a different perspective by presenting the prominin family.

Prominin-1 was first identified 15 years ago as a novel antigenic marker that is present at the apical surface of mouse neuroepithelial cells [1], while its human counterpart, the AC133 antigenic marker, is expressed in a subset of hematopoietic stem and progenitor cells [2, 3]. As described in the first chapter of this book, the intrinsic preference of the protein for plasma membrane protrusions motivated the choice of “prominin” (from the Latin word *prominere*) as the name for the first characterized member of this pentaspan membrane glycoprotein family, which occurs throughout the animal kingdom [4]. Prominin-1-like sequences were soon identified in other vertebrates such as fish and chicken [5, 6] and in invertebrates including worms and flies [1, 5, 7, 8]. Furthermore, the *PROMININ-1* (*PROM1*) gene was found to be the host of mutations that cause retinal degeneration [8] (see Chap. 4).

A second pentaspan membrane glycoprotein structurally related to prominin-1 but encoded by a distinct gene was characterized a few years later in human and rodent through molecular cloning [5]. The tissue distribution profile of this second prominin molecule, named prominin-2, largely overlapped that of prominin-1, with the notable exception of the eye, in which prominin-2 is absent [5]. The existence of prominin-2 suggested that a potential functional redundancy may explain the lack of pathological signs other than retinal phenotype observed in individuals carrying mutations in the *PROM1* gene [8], in spite of the widespread expression of prominin-1 throughout the organism as revealed by Northern blot analysis [1, 3, 5–7, 9]. Further characterization of prominin-2 has since revealed that prominin-1 and prominin-2 harbor similar and distinct cellular specificities.

2.2 Protein and Gene Structure of Prominin-2

Prominin-2 was originally cloned from human, mouse, and rat kidneys [5]. In spite of its low amino acid sequence identity with prominin-1 (26–30% overall identity depending on the species), this 112-kDa glycoprotein has the same pentaspan membrane topology as prominin-1, with an N-terminal domain that is exposed to the extracellular milieu (EC1) and is followed by four alternating, short cytoplasmic (IC1 and IC2) and large glycosylated extracellular loops (EC2 and EC3) and a cytoplasmic C-terminal domain (IC3) (see Fig. 1.1 in Chap. 1, Fig. 2.1). The two extracellular loops contain approximately 250 residues each, and both display potential N-glycosylation sites [5]. Prominin-2 shows no obvious sequence homology to other known proteins except prominin-1.

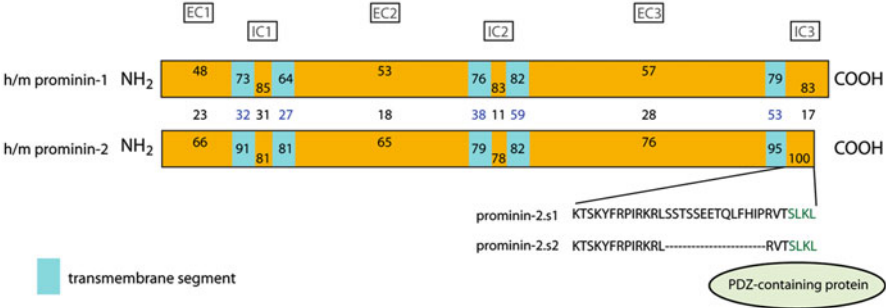


Fig. 2.1 Comparative structure of prominin-1 and prominin-2. Human (*h*) and mouse (*m*) prominin-1 (*top box*) and prominin-2 (*bottom box*) are each comprised of three extracellular domains (EC) and three short intracellular domains (IC) (*orange*) separated by five transmembrane domains (*blue*). Numbers in domains indicate the percentage of amino acid identity between human and mouse orthologs. Numbers between the different domains of prominin-1 and prominin-2 indicate the percentage of identity between the two human paralogs. The sequence of the C-terminal domain is indicated for the two splice variants, prominin-2.s1 and prominin-2.s2, with the putative PDZ-binding site in green (This figure is adapted from Ref. [6])

Prominin-2 shows a higher degree of interspecies conservation than prominin-1 (e.g., 73% and 60%, respectively, between human and mouse) [5]. Strikingly, the cytoplasmic C-terminal tails of human, mouse, and rat prominin-2 are perfectly conserved (100% amino acid identity over 34 residues) and quite distinct from the corresponding domain in prominin-1 (e.g., 17% amino acid identity compared to 26% overall identity between human prominin-1 and prominin-2; see Fig. 2.1) [6, 10]. Moreover this domain is encoded by only two exons, whereas up to four may be involved in the case of prominin-1. A search of predicted genes in the databases at NCBI (National Center for Biotechnology Information) revealed that the last 20 amino acid residues of prominin-2, which are encoded by a single exon (815–834 of human prominin-2), are perfectly conserved among mammals including therians (placentals and marsupials) and monotremes (*Ornithorhynchus anatinus*), with some exceptions (e.g., cat, *Felis catus*; baboon, *Papio anubis*; elephant, *Loxodonta africana*) in which only a single amino acid substitution is present (C.A.F., unpublished data). The conservation rate is approximately 80% in other amniotes (e.g., *Meleagris gallopavo*, *Gallus gallus breed*, *Anolis carolinensis*, *Chrysemys picta*) (not shown) and more divergent in the amphibian *Xenopus laevis* [11]. This sequence conservation suggests that the cytoplasmic tail of prominin-2 is likely important for the function of the molecule.

Interestingly, the C-terminus of mammalian prominin-2 (S-L-K-L) conforms to the pattern of class II PSD95/Dlg1/ZO-1 (PDZ)-binding domains (X-Φ/ψ-X-Φ; X, unspecified amino acids; Φ, hydrophobic residue; ψ, aromatic residue) [12, 13], and this sequence was shown to function as a PDZ-binding site *in vitro*. By means of a yeast two-hybrid screen using the last 31 residues of the C-terminal domain of prominin-2 as bait, Kathrin Opherke, in the research group of Denis Corbeil (Dresden),

has identified a novel splice variant of the glutamate receptor-interacting protein (GRIP1), a multi-PDZ-containing protein involved in anchoring AMPA receptors at the synapse [14], as an interacting partner of prominin-2 [15]. Named GRIP1 κ (GenBank accession numbers, AY294283, AY255674), this 4-PDZ-domain splice variant is similar (only differing in its 11 C-terminal residues) to GRIP1 τ , a testis-specific isoform with a distinct nuclear distribution in addition to the usual cytoplasmic location of GRIP1 [15, 16]. Further studies are needed to determine the physiological relevance of this putative interaction in the function and intracellular trafficking of prominin-2.

The *PROMININ-2* (*PROM2*) gene is located on chromosome 2 in humans (locus p16.2-p12) and mice (2F1), immediately upstream of the *calsenilin/DREAM/KChIP3* (*KCNIP3*) gene, which overlaps with the 3' end of *PROM2*. The *PROM2* gene extends over 16 kb with nearly identical genomic structure, i.e., exon/intron boundaries, among mammals [5]. Remarkably, although the amino acid identity between prominin-1 and prominin-2 is very low (see above), their exon/intron organization is highly similar. Most introns are concordant in position and phase, suggesting an early gene duplication event. Nevertheless, this genomic architecture is not correlated with the structural domains of the protein because the coding region spans at least 23 exons [5].

The development of high-throughput sequencing has revealed that most human gene undergo alternative splicing [17]. Prominin-2 is no exception. Like prominin-1, it may be affected by alternative splicing in the N-terminal domain, the first and second extracellular loops or the cytoplasmic C-terminal domain, suggesting the existence of distinct cytoplasmic protein-interacting partners [5]. The prominin-2 splice variants occur through intron retention, exon skipping, or the use of an internal 3' acceptor site. In particular, exon 23, which encodes the final 20 C-terminal residues, harbors an alternative acceptor site that generates in mouse salivary glands the isoform prominin-2.s2 [4], which has a 14-amino-acid deletion that spares the last 7 residues of the C-terminal domain and thus the potential PDZ-binding site, but removes putative casein kinase II and cAMP- or cGMP-dependent protein kinase phosphorylation sites (Fig. 2.1). Yet, these putative phosphorylation sites occur with high frequency and may therefore be not significant. Several human *PROMININ-2* mRNA variants lacking all or part of exons 5, 6, or 7 (encoding part of EC2) have been amplified by polymerase chain reaction (PCR) (C.A.F., unpublished data). Evidence of the differential splicing of exon 2 (EC1), exons 3 plus 4 (TM1/IC1/TM2), and exon 15 (EC3) and intron retention after exon 16 is present in the databases as expressed sequence tags (EST; GenBank accession number BB617607, CB994746, DA494079, and DA441226). The consequences of these splicing events for the structure of the molecule would vary from short internal deletion to the generation of short proteins with only two transmembrane domains or with an unrelated C-terminus (C.A.F., unpublished data). In human *PROM2*, alternative splicing also affects the 3'-untranslated region (UTR), generating two main mRNA species of 5.0 and 4.2 kb, which are detectable by Northern blot in most tissues expressing prominin-2 [5].

2.3 Cell Biology of Prominin-2

To some extent, the cellular and biochemical properties of prominin-2 reflect its structural similarities with prominin-1.

2.3.1 *Subcellular Localization*

The subcellular localization and cellular trafficking of prominin-2 were investigated by means of a green fluorescent protein (GFP) fusion protein ectopically expressed in Chinese hamster ovary (CHO) and, as a model of polarized epithelial cells, Madin-Darby canine kidney (MDCK) cells [5, 18]. Upon co-transfection with prominin-1, both prominin molecules were found to co-localize in plasma membrane protrusions of CHO cells, indicating that this intrinsic characteristic of prominin-1 is shared with prominin-2 [5]. Like prominin-1 [19, 20], prominin-2 was found in plasma membrane protrusions emerging from the apical domain of MDCK cells [18]. In these protrusions, prominin-2 was observed at the membrane of microvilli and the primary cilium, often concentrated toward their tips, and absent from the neighboring planar areas [18]. GFP-prominin-2 also labeled primary cilia emerging from nearby cells, which engage in long-lasting contacts [18, 21]. Unexpectedly, prominin-2 was also detected at the basal and lateral domains of MDCK cells, in contrast to the exclusive localization of prominin-1 at the apical domain [19]. As revealed by electron microscopy, prominin-2 was concentrated in interdigitated processes growing out from the lateral membrane between adjacent cells and in a few membrane protrusions present at the basal domain. This nonpolarized distribution in polarized epithelial cells may reflect either the absence of apical targeting signal or the presence of two competing signals for each domain [18]. Interestingly, the N-glycan moieties of prominin-2 appear insufficient to mediate an exclusively apical sorting, in contrast to what was observed for other glycoproteins [22, 23].

2.3.2 *Cholesterol Binding and Membrane Microdomain*

In a similar experimental setting that allowed the demonstration of the specific interaction of prominin-1 with membrane cholesterol and membrane microdomains (lipid raft) [24] (for technical details, see Ref. [25]), prominin-2 was shown to interact directly with plasma membrane cholesterol and associate with a cholesterol-dependent membrane microdomain [18]. These biochemical properties may determine the observed preference of prominin-2, like that of prominin-1, for membrane protrusions, irrespective of their subcellular localization.

Table 2.1 Distinctive traits and common features of prominins

Prominin-1	Prominin-2
Strictly apically localized in polarized cells	Apico-basolaterally distributed in polarized cells
Expressed in non-epithelial cells	Singly expressed in the esophagus and the meibomian glands
Singly expressed in the retina	
Used as a stem cell marker	
<i>Common features</i>	
Gene architecture	
Protein structure	
Membrane topology	
Biochemical characteristics	
• Associates with membrane microdomains/interacts with membrane cholesterol • Localizes in plasma membrane protrusions • Associates with extracellular membrane microvesicles	
Expression in epithelial cells	

2.3.3 *Membrane Vesicles*

Prominin-2 is associated with small membrane vesicles, as is prominin-1 [26] (see Chap. 3). Upon transfection in MDCK cells, prominin-1 and prominin-2 (as a GFP fusion protein) could be detected in the same extracellular membrane vesicles released into the apical medium, suggesting common intracellular trafficking [18]. Interestingly, in line with its unpolarized (apico-basolateral) distribution and in contrast to prominin-1, prominin-2-containing membrane vesicles were also released into the basolateral medium [18]. In vivo prominin-2-containing membrane vesicles can be isolated from both mouse and human urine [18, 27] and human saliva [28] by high-speed centrifugation.

Collectively, these investigations have demonstrated that prominin-2, like prominin-1, is (i) selectively concentrated in plasma membrane protrusions, (ii) associated with cholesterol-dependent membrane microdomains through direct interaction with membrane cholesterol, and (iii) released into the extracellular milieu in association with small membrane vesicles. Moreover, as a distinctive feature, prominin-2 is distributed in a nonpolarized apico-basolateral manner at the membrane and in extracellular vesicles (Table 2.1). Therefore, prominin-2 could potentially function as an organizer of plasma membrane protrusions similar to prominin-1 (see Chaps. 1 and 4), particularly in the basolateral compartment, in which prominin-1 is absent.

2.4 Prominin-2 Expression

2.4.1 Tissue Distribution

Although prominin-1 and prominin-2 may assume common functions owing to their shared molecular properties, they seem to have their own specificity, as reflected by their differential tissue distribution (Table 2.1). Whereas prominin-1 is expressed in both epithelial and non-epithelial cells, prominin-2 expression seems rather restricted to epithelial cells [5, 27–29]. Northern blot analyses indicate that the highest human *prominin-2* mRNA levels occur in the adult kidney (like prominin-1), while detectable levels occur in the placenta, mammary gland, prostate, trachea, thyroid gland, salivary gland, and all of the tissues of the digestive tract [5]. Notably, *prominin-2* transcripts are strongly and uniquely expressed in the acini of the meibomian gland, the root sheath of the eyelash/cilium [28], and in the esophagus [5]. Conversely, a major site for prominin-1 expression, the retina, is devoid of prominin-2 [5]. Assuming that, under some conditions, the presence of both prominins may be redundant, this latter observation would at least partially explain why the effect of deleterious mutations in *PROM1* is specific to the eye despite the more widespread distribution of prominin-1 expression [8].

Prominin-2 has been detected in different segments of the male genitourinary tract, including the epididymis, the urothelium of the urinary bladder, and the glandular epithelium of the seminal vesicle, where it is more strongly and uniformly expressed than prominin-1 [29]. Prominin-2 is strongly expressed in the rodent and human prostate [5, 29, 30], and in the latter species, it specifically marks basal epithelial cells [29], which are proposed to give rise to secretory luminal cells [31] (see Chap. 11). In organs in which the expression of both prominins appears to overlap based on dot and Northern blots, refinement of their respective expression profiles through immunohistochemistry and in situ hybridization has shown that they mostly occupy distinct tissue compartments [27–29]. Thus, in the human nephron, *prominin-2* transcripts and protein were confined to the basolateral plasma membrane of the epithelial cells of the distal tubules, including the distal convoluted tubule, the connecting duct, and the collecting duct system. In the thick ascending limb of Henle's loop, prominin-2 was also found at the apical domain, whereas prominin-1 expression featured an exclusive apical localization in epithelial cells of the proximal nephron tubules [1, 32] (see Chap. 8). Interestingly, this differential expression may partly explain the apparent quantitatively weaker expression of prominin-2 in urine vesicles compared to prominin-1, although these prominin-containing membrane vesicles may be derived from other epithelia along the urinary tract. Nevertheless, these findings are of note in the context of a recent report on the proteomic analysis of urinary exosomes from normal individuals and IgA nephropathy and thin basement membrane nephropathy (TBMN) patients. Both prominin-1 and prominin-2 were upregulated in pathological samples, and

prominin-2 was uniquely associated with TBMN, which is characterized by a general thinning of the glomerular basement membrane [33].

A similar situation prevails in murine salivary glands, where both prominins are expressed following distinctive patterns: prominin-1 is preferentially contained in one segment of the duct system, whereas prominin-2 is localized in acinar cells or duct cells or both compartments, depending on the particular gland [28].

Extraocular neuromuscular junctions constitute a particular type of neuromuscular junction with specific physiological properties and subcellular organization, which were recently shown to be reflected by a unique transcriptome and proteome among skeletal muscles [34]. In this study, rat prominin-2 was preferentially expressed in the subsynaptic compartment over the non-synaptic regions of the extraocular muscle, potentially contributing to its differential postsynaptic morphology.

2.4.2 Regulation of Prominin-2 Expression

The factors that regulate the expression of prominin-2 remain largely unknown. An *in silico* comparative analysis of the promoter of the human and mouse *Prom2* genes with ConSite [35] has revealed two regions that contain conserved predicted transcription factor binding sites: one within 750 bp upstream from the first initiation codon (ATG) and another in the 5'-flanking region between nucleotides -1750 and -2850 (Denis Corbeil and C.A.F., unpublished data). These binding sites include Snail, Spz1, HFH-2 (HNF-3/Forkhead Homolog), and E74A [36, 37], which is consistent with the tissue-specific and epithelial expression of prominin-2 and would support a relation with the maintenance of the proper architecture of epithelia [38].

Recently, *prom2* was reported as a nonclassical heat-shock gene [39]. Mouse HSF1 (heat-shock factor 1) is required for the expression of classical heat-shock genes in mouse embryonic fibroblasts (MEFs) [40]. After 6 h of recovery from heat shock, *prominin-2* mRNA was upregulated in MEFs, but this upregulation was not observed in HSF1-null MEF cells, which are deficient in the induced expression of the classical heat-shock genes. However, overexpression of a newly characterized HSF relative, mouse HSF3 (or chicken HSF1, its homolog in this species) by transfection restored the inducibility of *prominin-2* mRNA without affecting the expression of the classical genes that mediate heat-shock responses [39]. It would be interesting to investigate this issue further at the protein level.

Rat prominin-2 was first reported to be a testosterone-regulated gene based on its decreased expression in the rat ventral prostate after castration and restored expression upon hormonal replacement [30]. The confinement of prominin-2 expression to the basal compartment of the human prostate epithelium [29] suggests that this hormonal regulation may be indirect, given the dissociated distribution of *prom-2* and the androgen receptor (AR) within the prostatic epithelium [41]. Therefore, the hormonal regulation of prominin-2 clearly deserves further

investigation. In the same study, Zhang and colleagues attributed pro-apoptotic activity to rat prominin-2 in the prostate based on the expression of a GFP fusion protein construct [30]. However, the evidence provided was questionable, given that this construct would not permit the expression of a prominin-2 fusion protein because of the presence of a stop codon and a single nucleotide insertion in the cDNA, which would introduce a frame shift and the expression of a different amino acid sequence at the C-terminus. These changes resulted in an unusual intracellular localization of the fluorescent signal. Moreover, it is difficult to reconcile this activity with the downregulation of *prominin-2* steady-state mRNA levels upon castration and the concomitant involution of the prostate and upregulation upon androgen replacement that was observed in the same study. In view of the data, the possibility that the transfected constructs had a toxic effect cannot be formally excluded. Such a pro-apoptotic effect has not been observed in other heterologous systems overexpressing mouse or rat prominin-2 [5, 18], although it was not specifically addressed in these studies, and should therefore be considered with caution until confirmation.

2.4.3 *Prominin-2 and Cancer*

Data regarding the expression of prominin-2 in cancer are scarce and preliminary. Expressed sequence tag clones deposited in the GenBank database indicate that prominin-2 may be expressed in tumors of the breast, lung, tongue, and nervous system [5] (data not shown). A microarray gene profiling and quantitative PCR study suggested *PROM2* as a candidate gene marker for segregating chromophobe renal cell carcinoma from benign oncocytoma [42].

Using a pangenomic oligonucleotide microarray, Winnepenninckx and colleagues observed that the expression of *PROMININ-2* was higher in melanomas from patients that did not metastasize to distant sites within 4 years after diagnosis than in those that did, suggesting an anti-metastatic role for this gene [43].

In a recent publication, Duhagon and colleagues proposed that prominin-2 could be a new marker for prostate cancer based on genomic profiling of tumor-initiating prostatospheres (PS) derived from the cancer cell line LNCaP as well as primary prostate cancer stem cells isolated from recurrent prostate tumors through the expression of CD133 [44]. Because prominin-2 is expressed in the basal compartment of the prostate, the authors specifically addressed the expression of prominin-2 and observed its upregulation in LNCaP-derived PS relative to the parental cells. Similar results were obtained with primary tumor-derived PS and confirmed by quantitative PCR in one case of three. Further studies will be needed with a larger panel of samples to determine the potential of prominin-2 as a biomarker for prostate cancer status.

No mutational or pathological relevance that could indicate a physiological function for prominin-2 has been reported, and no phenotypic features of a prominin-2 knockout model have been described.

2.5 Evolution of the Prominin Gene Family

The first phylogenetic analysis of the prominin family performed after the characterization of human and rodent prominin-2 led to the definition of two orthologous groups of mammalian prominin genes [5]. However, none of the complete or partial prominin-related sequences identified at that time in other vertebrate (*Gallus gallus*, *Danio rerio*) or invertebrate (*Drosophila melanogaster*, *Caenorhabditis elegans*) phylogenetic lineages were clearly related to one or the other group, in particular, *G. gallus* prominin-like (GenBank accession number AF406812) [5]. Advances in whole-genome sequencing revealed that in *G. gallus*, prominin-1 and predicted prominin-2 orthologous genes are located on chromosomes 4 and 22, respectively, while the prominin-like gene is on chromosome 6. Therefore, birds may harbor a third prominin that is more similar to one of the three prominin paralogs identified in zebra fish (*Danio rerio*). In 2008, Wotton and colleagues demonstrated that two of the prominin paralogs identifiable in teleosts clearly belong to the *prom1* group, while the third prominin paralog segregates from the tetrapod *prom2* genes to form the *prom3* orthologous group. They proposed that the *prom3* genes may have evolved from the same ancestral locus as *prom2*, which was generated during the first round of vertebrate genome duplication [45]. Thus, the *Danio rerio* paralogs located on chromosomes 14, 1, and 13 are now referred to as prominin-1a, prominin-1b, and prominin-3, respectively [46, 47]. The recent identification of prominin-1, prominin-2, and prominin-3 in *Xenopus laevis* has confirmed the presence of the three orthologous groups in nonmammalian vertebrates [11]. Moreover, the vast array of whole-genome sequencing projects has enabled the identification or prediction of prominin sequences in many species. The elephant shark (*Callorhinchus milii*) genome, which often exhibits higher homology to human sequences than teleosts, includes a significant number of orthologs to human (mammals) genes that are absent from teleosts [48]. This seems to be the case for *prom2*; partial genomic sequences of homologs of each of the three vertebrate family members can be retrieved through TBLASTN searches against mouse or human prominin-2 sequences from the whole-genome shotgun database (WGS). One genomic contig, AAVX01036519, contains a fragment of a *prominin* gene that is more highly related to human prominin-2 than to tetrapod or teleost prominin-1 or prominin-3 sequences. In addition, a spiny dog shark EST sequence (EE886567) was retrieved from the database based on its high sequence homology with mammalian prominin-1 by a TBLASTN search. This sequence encodes the 219 C-terminal residues of a prominin-1 ortholog (Table 2.2). These sequence relationships confirm that the three distinct orthologous groups emerged very early in the vertebrate lineage. Thus, while prominin-2 appears to have been lost in the fish lineage after the radiation of ray-finned fishes and the concomitant whole-genome duplication [49] that resulted in duplicated *prominin-1* genes, prominin-3 vanishes in the mammalian lineage. However, in the platypus (*O. anatinus*) genome, where *prom1* and *prom2* have been predicted, fragments of a gene with similarity to *prom3* can be identified on chromosome 17 by a TBLASTN search. Whether these sequences mark an intermediate stage in

Table 2.2 Existence of three prominin orthologous groups in cartilaginous fishes – comparison of predicted sequences with bony vertebrate prominin members

Bony Vertebrate Prominin Members (Gene ID)	Spiny dogfish shark*	Elephant shark [§]		
		A	B	C
Human prominin-1 (8842 PROM1)	50/93 (54)	48/93 (52)	34/86 (39)	41/92 (45)
Chicken prominin-1 (422825 PROM1)	53/93 (57)	59/93 (63)	29/82 (35)	43/92 (47)
Clawed Frog prominin-1 (100036621 prom1)	52/93 (56)	54/93 (58)	29/86 (34)	42/92 (46)
Zebrafish prominin-1a (322857 prom1a)	55/92 (60)	57/92 (62)	33/81 (41)	42/91 (46)
Zebrafish prominin-1b (378834 prom1b)	54/92 (59)	55/92 (60)	31/85 (36)	41/91 (45)
Human prominin-2 (150696 PROM2)	34/79 (43)	35/81 (43)	54/84 (64)	37/78 (47)
Chicken prominin-2 (428200 PROM2)	38/89 (43)	36/90 (40)	46/82 (56)	37/82 (45)
Clawed Frog prominin-2 (100485371 prom2)	27/78 (35)	27/78 (35)	44/84 (52)	28/81 (35)
Chicken prominin-3 (423849 LOC423849)	47/90 (52)	45/90 (50)	37/81 (46)	50/90 (56)
Clawed Frog prominin-3 (100488690 LOC100488690)	45/91 (49)	42/87 (48)	35/85 (41)	47/90 (52)
Zebrafish prominin-3 (556596 prom2)	40/91(44)	42/91 (46)	32/84 (38)	53/91 (58)

*EST sequence EE886567, 657 bp in length, from the spiny dogfish shark (*Squalus acanthias*), encoding the last 216 C-terminal amino acids of the predicted protein and corresponding to a C-type prominin-1 C-terminus [47] (see Chap. 1)

[§]The three fragments (A, B, and C) of elephant shark (*Callorhynchus milii*) prominin sequences corresponding to exons 20 to 22 of human PROMININ-2 were determined by assembling exonic regions from contig AAVX01091526, the overlapping contigs AAVX01230395 and AAVX01596446, and contig AAVX01036519, respectively, which were previously identified by TBLASTN searches of the GenBank WGS database against human and zebra fish prominin sequences. Introns conform in position and phase to the structure of other prominin genes, and splice sites follow the GT-AG rule. Sequences identity is indicated as the fractional number of exact matches in pairwise alignments with BLAST, with corresponding percentages in parentheses and following the code: red: ≥60, pink: 50–59, green: 40–49, blue: ≤40

mammalian evolution or indicate that monotremes have maintained a functional prominin-3 like many other ancestral characteristics remains to be determined [50]. The presence of three different paralogs in other tetrapods such as birds, reptiles, and amphibians further supports the denomination prominin-3 for the fish paralog that currently appears again as *prom2* gene in the database, after being properly annotated *prom3*.

It is interesting to note that the first orthologous group *prom1*, which is present throughout the different jawed vertebrate lineages, has evolved with longer intronic sequences. These longer intronic sequences differentiate *Prom1* genes from other *Prom* genes by generating an approximately sevenfold lower degree of compactness, depending on the species. This reduction in compactness may be related to a tighter regulation of the expression of the first group [51], in agreement with the commonly observed complex regulation of *prominin-1* gene expression (see Chap. 5).

Table 2.3 Phylogenetic profile of prominins

Human	Platypus	Chicken	Anole	Frog	Zebrafish	Sharks	Drosophila	C. elegans
CDI33^a (8842 PROM1, chr 4)	<i>Predicted prom1</i> (10084810 PROM1, chr 4)	Prominin-1^b (422825 PROM1, chr 4)	<i>AAWZ02006824</i> (chr 4)	Prominin-1^b (100035621 prom1)	Prominin-1a^b (322857 prom1a, chr 14) Prominin-1b^b (378834 prom1b, chr 1)	EST 886567 <i>AAVX01091526^c</i>	Prominin^b (246493, chr 28)	F08B12.1 (181330, chr X)
Prominin-2 (150696 PROM2, chr2)	<i>Predicted prom2</i> (100078032 LOC100078032 unknown)	<i>Predicted prominin-2</i> (428200 PROM2, chr22)	Prominin-2 (100564795 LOC100564795, LGa)	Prominin-2 (100483571 prom2)	Prominin-2 (100483571 prom2)	<i>AAVX01036519^d</i>	Prominin-like (38372, chr 3L)	M28.8 (174641, chr II)
	<i>NC_009110</i> (chr 17)	Prominin-3 (423849 LOC423849, chr 6)	<i>AAWZ02021368</i> (unknown)	Prominin-3 (100488690 LOC100488690)	Prominin-3 (556596 prom2, chr 13)	<i>AAVX01230395,</i> <i>AAVX01596446^e</i>		M28.9 (174642, chr II)
PLACENTALS								
MONOTREMES								
MAMMALS								
BIRDS								
LIZARDS								
AMPHIBIANS								
TETRAPODS								
BONY VERTEBRATES								
RAY-FINNED FISHES								
CARTILAGINOUS FISHES								
ARTHROPODS								
NEMATODES								

Prominin members are indicated for each species; the GenBank gene ID and chromosomal location are given in parentheses. Bold, as characterized/expressed protein. Regular, as EST or mRNA. Italic, genomic identification. Green, orange, and yellow shadings indicate membership in orthologous groups 1, 2, and 3, respectively. Phylogeny is indicated at the bottom of the table.

^a See Chap. 1

^b See Chap. 4

^c AAVX01091526, a 1432-nucleotide (nt) WGS contig from *Callorhinchus milii* displaying four exons of the putative *prom1* gene.

^d AAVX01036519, a 3032-nt contig from *Callorhinchus milii* displaying three exons of the putative *prom2* gene.

^e AAVX01230395, a 1892-nt WGS contig from *Callorhinchus milii* displaying two exons of the putative *prom3* gene and AAVX01596446, an overlapping 799-nt WGS contig displaying two exons (see Table 2.2). Inferred from previous publications [5, 45, 46, 55] and per-sonal unpublished results.

Beyond jawed vertebrates, the evolutionary relationship of prominins is more difficult to establish. Evidence in the EST database supports the existence of at least two different prominins in the sea lamprey, both of which are more similar to prominin-1 and prominin-3 than to prominin-2. As for invertebrates, EST and partial genomic sequences demonstrate that more than one prominin gene is present in echinoderms and mollusks. In addition to F08B12.1, the nematode *C. elegans* harbors two prominin-related molecules (M28.8 and M28.9) that are more distantly related sequences [1, 7], whereas two *prominin* genes have been identified in *D. melanogaster* at distinct locations (2R and 3L) and predicted in other insects [8, 52, 53]. Consequently, the evolutionary and functional relationships of these genes are still unclear. Table 2.3 summarizes the phylogenic profile of the prominin family members.

Nevertheless, the propensity to be concentrated in plasma membrane protrusions that is characteristic of mammalian prominin-1 [9, 19, 24] and prominin-2 [5, 18] and that gave its name to these glycoproteins extends to other members of the family across evolution. F08B12.1 was proposed to be a potential ciliary gene through serial analysis of gene expression (SAGE) performed with ciliated and nonciliated *C. elegans* neuronal cells [54]. Prominin may have evolutionarily ancient functions in vision because *Drosophila* prominin is localized to the stalk membrane and the tips of the microvillus in rhabdomeres (see Chap. 4) [52].

2.6 Conclusions

Members of the prominin family are spread throughout the animal kingdom and are still poorly characterized with the exception of human and rodent prominins, for which common features and distinctive traits have been documented. The prominin family has been enlarged by the definition of an additional orthologous group, but comparative studies of these prominin relatives and their thorough biological characterization are needed. Combined evolutionary perspectives may yield interesting insights for future research aimed at deciphering the different functions of this intriguing family of proteins and that of its most well-known member: CD133.

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