

Protein family review

## The caveolin proteins

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### Summary

The caveolin gene family has three members in vertebrates: caveolin-1, caveolin-2, and caveolin-3. So far, most caveolin-related research has been conducted in mammals, but the proteins have also been found in other animals, including *Xenopus laevis*, *Fugu rubripes*, and *Caenorhabditis elegans*. Caveolins can serve as protein markers of caveolae ('little caves'), invaginations in the plasma membrane 50-100 nanometers in diameter. Caveolins are found predominantly at the plasma membrane but also in the Golgi, the endoplasmic reticulum, in vesicles, and at cytosolic locations. They are expressed ubiquitously in mammals, but their expression levels vary considerably between tissues. The highest levels of caveolin-1 (also called caveolin, Cav-1 and VIP21) are found in terminally-differentiated cell types, such as adipocytes, endothelia, smooth muscle cells, and type I pneumocytes. Caveolin-2 (Cav-2) is colocalized and coexpressed with Cav-1 and requires Cav-1 for proper membrane targeting; the *Cav-2* gene also maps to the same chromosomal region as *Cav-1* (7q31.1 in humans). Caveolin-3 (Cav-3) has greater protein-sequence similarity to Cav-1 than to Cav-2, but it is expressed mainly in muscle cells, including smooth, skeletal, and cardiac myocytes. Caveolins participate in many important cellular processes, including vesicular transport, cholesterol homeostasis, signal transduction, and tumor suppression.

### Gene organization and evolutionary history

Research into caveolae began with their morphological identification in 1953. By transmission electron microscopy, they appear as structures resembling 'little caves', which are vesicular invaginations of the plasma membrane of 50-100 nanometers (nm) in size [1]. Caveolin-1 (also known as caveolin, Cav-1 or VIP21) was the first member of the caveolin family to be identified, and it was shown to be a structural component of caveolae and of transport vesicles derived from the trans-Golgi network [2,3]. It was isolated as one of several proteins that became phosphorylated on tyrosine residues in chicken embryo fibroblasts transformed with the *v-Src* oncogene [4]. Monoclonal antibodies directed against Cav-1 decorated the cytoplasmic protein coat of caveolae, making Cav-1 the first true protein marker of caveolae [2]. Subsequent cloning of the *Cav-1* cDNA revealed that it was identical to another protein,

VIP21, which had been cloned almost simultaneously [3,5]. Interestingly, VIP21 was isolated as an integral membrane protein component of transport vesicles derived from the trans-Golgi network in Madin-Darby canine kidney (MDCK) cells, suggesting that Cav-1/VIP21 may have a role in molecular trafficking as well as oncogenesis.

Caveolin-2 (Cav-2) and caveolin-3 (Cav-3) were identified in 1996 using different experimental methods. Cav-2 was discovered by the microsequencing of a 20 kDa protein that co-purified with adipocyte-derived caveolar membranes [6]. Further characterization revealed that Cav-2 colocalizes with Cav-1 in caveolae, forms hetero-oligomers with Cav-1, is co-expressed in many of the same cells and tissues, and requires Cav-1 for proper membrane localization [7,8]. Cav-3 (also known as M-caveolin) was identified through

database searches and traditional cDNA library screening in an attempt to find Cav-1 homologs [9,10].

Caveolin sequences have been obtained from a range of vertebrates, including human, cow, mouse, *Xenopus*, and *Fugu rubripes*. A caveolin gene family has also been found in *Caenorhabditis elegans* [11,12]. The three mammalian genes encoding members of the caveolin family are similar in sequence (Table 1). A phylogenetic tree of all known caveolins shows that the *C. elegans* Cav-1 sequence is only distantly related to all the others (Figure 1). Interestingly, *Cav-1* and *Cav-2* are in very close proximity (about 19 kilobases (kb) apart) on human chromosome 7q31.1, while *Cav-3* is located on a different chromosome (3p25) [13,14]. Although the evolutionary history of the caveolin genes has not been clearly defined, there are clues within their sequences and genomic organization to suggest possible mechanisms for their origin. For instance, although *C. elegans Cav-1* has two exons, the region that is homologous to mammalian caveolins is encoded by only a single exon, suggesting that mammalian caveolins are derived from this particular exon [11]. Also, two observations derived from the human genomic sequence suggest that some family members may have arisen through gene duplication events: firstly, the exon-intron boundaries in the last exons of *Cav-1*, *Cav-2*, and *Cav-3* are in analogous positions; and secondly, exon 2 of *Cav-2* is divided into two parts (2a and 2b) by an intron, whereas the two homologous portions in the *Cav-1* and *Cav-3* sequences are fused together to form the final exon [13,14]. This second point may suggest that *Cav-2* served as the genomic precursor of *Cav-1* and *Cav-3*.

### Characteristic structural features

Currently, the structural features of this family of proteins are poorly defined, but information gleaned from the protein sequence has enabled some predictions of structure and

motifs within the proteins. For instance, all three caveolins have an invariant 'FEDVIAEP' stretch (in the single-letter amino-acid code) within their hydrophilic amino-terminal domains that has come to be termed the 'caveolin signature motif' [6,9]. The functional importance of this sequence or motif has yet to be determined, however.

Two Cav-1 isoforms ( $\alpha$  and  $\beta$ ) have been identified; the  $\beta$  isoform arises from an internal translational start site that gives a shorter amino terminus than that of the  $\alpha$  form and is truncated by 31 residues [15]. The predicted domains span almost the same number of residues in all three proteins: the amino-terminal domain comprises the first 101 residues in Cav-1 $\alpha$  and the first 70-86 residues in Cav-1 $\beta$ , Cav-2, and Cav-3, with the putative transmembrane domain occupying 33 amino acids and the carboxy-terminal domain containing 43-44 amino acids (Figure 2).

Using a variety of experimental methods, it has been determined that the major sub-cellular location of Cav-1 is at the plasma membrane. From the primary sequence (hydrophilicity plots) and mutational analysis, Cav-1 is predicted to have a membrane-spanning hairpin-like structure, with both amino and carboxyl termini directed towards the cytoplasm (Figure 2). This atypical membrane-spanning model is supported by findings that antibodies directed against the Cav-1 amino or carboxyl terminus require cells to be permeabilized in order to bind Cav-1, that cell-surface biotinylation does not label Cav-1, and that there are known palmitoylation and tyrosine phosphorylation sites within both the amino- and the carboxy-terminal domains of the protein [16-19]; palmitoylation and tyrosine phosphorylation are both cytoplasmically generated post-translational modifications.

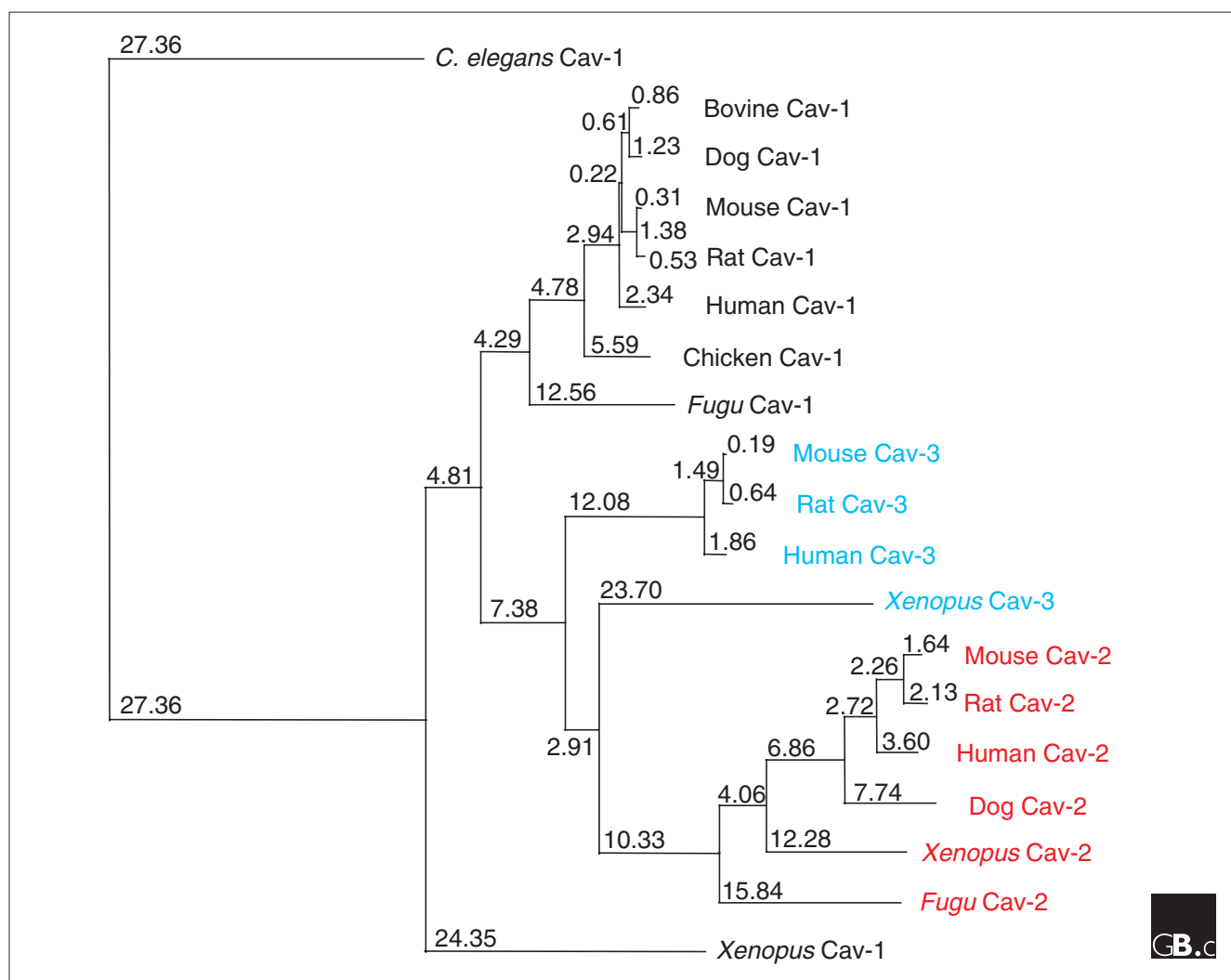
Generally speaking, caveolins are small proteins (18-24 kDa). Structurally, however, perhaps one of the most interesting and significant findings about Cav-1 is that it forms an

**Table 1**

**Genomic organization of the human caveolin genes and properties of their protein products**

Human gene	Chromosomal location	Exon	Exon size (bp)	Intron	Intron size (kb)	Residues encoded by exon	Length of protein (amino acids)	Percentage similarity (identity) to human Cav-1	Percentage similarity (identity) to human Cav-2	Expression patterns
Cav-1	7q31.1	1	30	1	1.47	1-10	178	-	59 (40)	Ubiquitous; highest levels in adipocytes, endothelia, smooth muscle cells, and Type I pneumocytes
		2	165	2	31.8	11-65				
		3	342			66-178				
Cav-2	7q31.1	1	150	1	0.33	1-50	162	58 (38)	-	Co-expressed with Cav-1
		2a	188	2	5.76	51-112				
		2b	151			113-162				
Cav-3	3p25	1	114	1	?	1-38	151	85 (65)	60 (39)	Muscle-specific; primarily in skeletal and cardiac myocytes
		2	342			39-151				

Abbreviations: bp, base pairs; kb, kilobases; ?, unknown. Modified from Razani *et al.* [42].

**Figure 1**

A phylogenetic tree depicting the evolutionary relationships of all known caveolin protein sequences. Note that *C. elegans* Cav-2 was not included in this analysis because of its low similarity to the mammalian caveolins. GenBank-derived protein sequences were entered into the ClustalW program to generate a phylogenetic tree using the neighbor-joining method. Numbers indicate horizontal branch lengths, which correspond to the estimated evolutionary distances between the protein sequences.

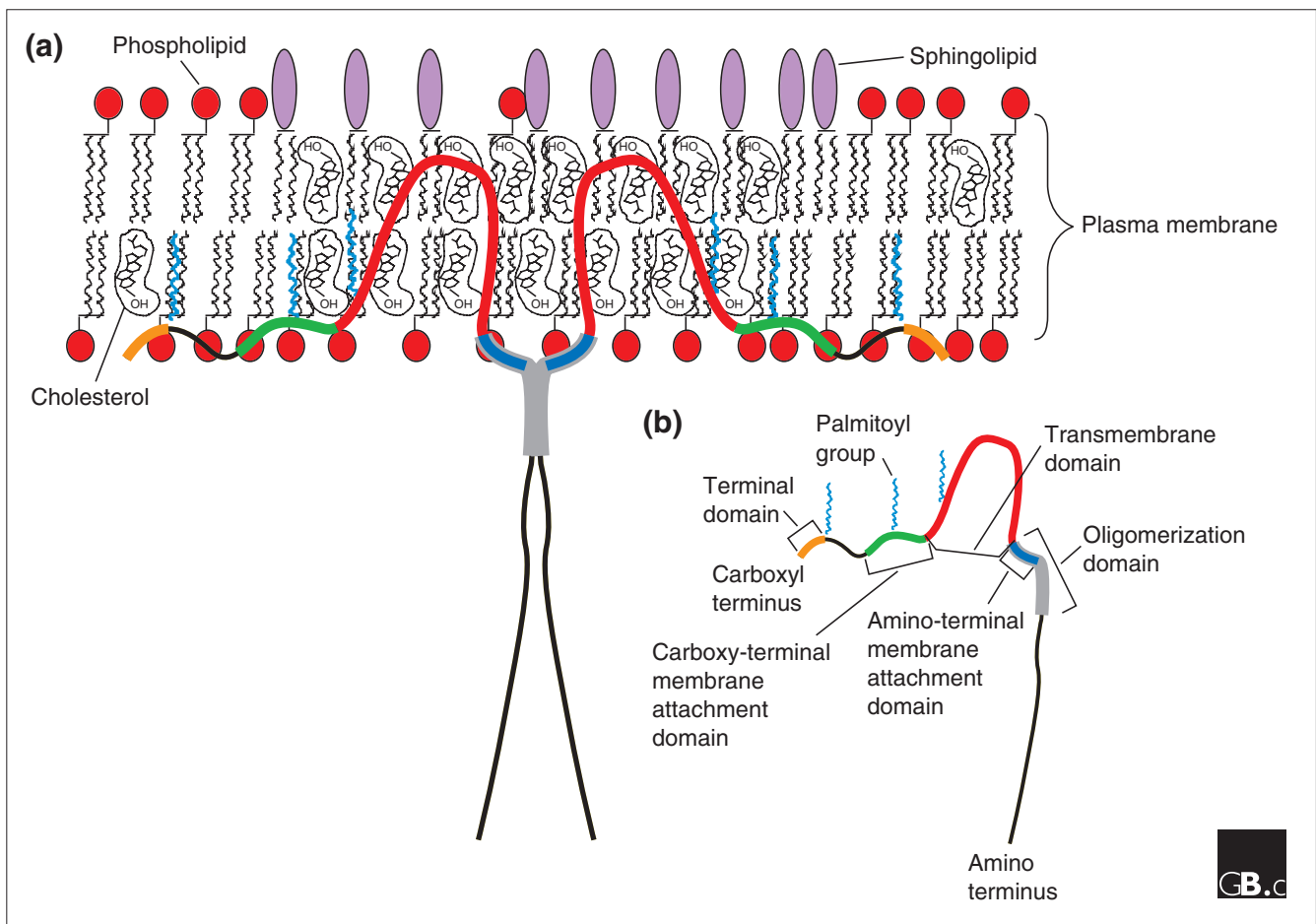
oligomeric complex comprised of approximately 14-16 monomers, as discovered through velocity gradient ultracentrifugation. In this assay system, Cav-1 was found to migrate as 200-400 kDa complexes [16,20]. Experiments with Cav-1 deletion mutants mapped the oligomerization domain to residues 61-101 [16] (Figure 2). Interestingly, Cav-3 also forms large oligomeric complexes of approximately 350-400 kDa *in vivo*, whereas Cav-2 requires Cav-1 to participate in the formation of these high-molecular-mass complexes [7-9].

### Localization and function

Caveolae are considered by many to be a subset of lipid rafts, which are highly-ordered microdomains residing within the plasma membrane that are enriched in certain

lipids [21-23]; this may not be completely accurate, however, as some proteins are known to localize selectively to either lipid rafts or caveolae but not both [24]. Caveolae-enriched membrane fractions can be purified efficiently on the basis of their buoyancy and resistance to solubilization by mild non-ionic detergents at 4°C [25-30], but other types of membrane microdomains may also be enriched by this kind of purification.

Cav-1 localizes to plasma-membrane caveolae and also to the Golgi apparatus and trans-Golgi-derived transport vesicles [3,5,31]. Cav-1 may have a soluble cytoplasmic form, as well as a secreted form, depending on the cell type [32], and the first 31 amino acids may be important in selectively targeting isoforms of Cav-1 to different cellular compartments [33].

**Figure 2**

Primary structure and topology of Cav-1. **(a)** The predicted membrane topology of Cav-1. Two caveolin-1 monomers are shown forming a dimer for simplicity, but about 14-16 monomers normally self-associate to form a single caveolin homo-oligomer (the caveolar assembly unit, akin to the clathrin triskelion). Note that both the amino- and carboxy-terminal domains are oriented towards the cytosolic face of the plasma membrane, with a hairpin loop structure inserted within the membrane bilayer. Modified from Razani *et al.* [42]. **(b)** The domains present in Cav-1. Note that the amino-terminal membrane-attachment domain is also called the caveolin scaffolding domain (CSD).

Cav-1 is expressed ubiquitously, although at different levels in different tissues, with the highest levels in adipocytes, endothelial cells, fibroblasts, smooth-muscle cells, and a variety of epithelial cells. Cav-2 is tightly co-expressed with Cav-1, whereas Cav-3 is expressed predominantly in striated muscle cells [34]. Interestingly, Cav-1 is required for the proper membrane localization of Cav-2.

Mice deficient in *Cav-1*, *Cav-2*, or *Cav-3* are viable and fertile, but each has abnormal cellular and tissue-specific phenotypes peculiar to the specific ablated gene [35-43]. A mutant form found in up to 16% of human breast cancers, Cav-1 (P132L), does not localize properly to the plasma membrane and behaves in a dominant-negative manner, causing the mislocalization and intracellular retention of wild-type Cav-1 [44,45]. An analogous P-to-L mutation in Cav-3 (P104L) has been detected in patients with autosomal dominant limb-girdle muscular dystrophy type-1C, and this mutation also behaves

in a dominant-negative fashion [46-49]. Wild-type Cav-3 localizes to caveolae and the plasma membrane and also associates with the T tubules that form from invaginations of the muscle membrane [50,51].

Caveolin-related research has shown that caveolae function in vesicle trafficking [52], cholesterol homeostasis, signal transduction and tumor suppression. Endothelial caveolae may be involved in transcytosis; they have the molecular components used by other transport vesicles during vesicle formation, docking, and fusion [53], and the motor protein dynamin, which is important for vesicle fission, also localizes to caveolae [54,55]. Regarding endocytosis, it appears that certain ligands and extracellular molecules, such as cholera and tetanus toxins, are transported across the plasma membrane through caveolae, rather than via clathrin-dependent mechanisms [56,57]. Pathogens appear to have evolved mechanisms to gain entry into eukaryotic cells through

caveolae, including simian virus 40 and certain strains of *Escherichia coli* [58].

Caveolae are enriched with cholesterol and Cav-1 is one of the few proteins that binds cholesterol tightly and specifically [59,60]; free cholesterol is required for the proper formation of caveolae [61-63] and regulates the *Cav-1* promoter [64]. Intracellular cholesterol balance may be affected by caveolins [65], as a dominant-negative *Cav-1* mutant causes intracellular retention of free cholesterol as well as a decrease in cholesterol synthesis and its efflux from the cell [66]. Also, caveolae have been linked to the process of reverse cholesterol transport, during which excess free cholesterol is released into the blood plasma via uptake by high-density lipoprotein (HDL) particles [67-71], and caveolae appear to be involved in the uptake of cholesterol esters from the plasma.

Caveolae appear to serve as signaling platforms by compartmentalizing and concentrating signaling molecules (this is referred to as the 'caveolae signaling hypothesis') [27]. Various classes of signaling molecules, including G-protein subunits, receptor and non-receptor tyrosine kinases, endothelial nitric oxide synthase (eNOS), and small GTPases [27,42], bind Cav-1 through its 'caveolin-scaffolding domain' (CSD) (Figure 2). Cav-1 also appears to inhibit the downstream activation and signaling of many proteins, including c-Src, H-Ras, mitogen-activated protein (MAP) kinases, and eNOS [72-78]. The evidence that Cav-2 is a signaling modulator is less clear, partly perhaps because its CSD sequence is divergent from that of Cav-1. The Cav-3 CSD is very similar to the Cav-1 CSD, however, and Cav-3-generated caveolae have been shown to compartmentalize and modulate a number of signaling proteins, including eNOS,  $\beta$ -adrenergic receptors, protein kinase C isoforms, G proteins, Src-family kinases, and multiple components of the dystrophin-glycoprotein complex [34,76-79].

Several lines of evidence have implicated Cav-1 in tumor suppression [18,19,80-84], and there is also accumulating evidence that Cav-1 has an anti-proliferative function. Furthermore, the *Cav-1* and *Cav-2* genes are close to the microsatellite marker D7S522 on human chromosome 7q31.1 [13,14], a region that is commonly deleted and implicated in the pathogenesis of many human epithelial-based cancers, including breast, colorectal, prostate, ovarian, and renal-cell carcinomas. Recent results [85-90] strongly argue that Cav-1 functions either as a negative regulator of cell proliferation or as a tumor suppressor, in both cultured cells and whole animals.

## Frontiers

The ubiquitous nature and diverse tissue expression of caveolin family members in mammals suggest that caveolins are indeed important for normal cellular and tissue physiology

in highly evolved organisms. The discovery of a caveolin gene family in the invertebrate *C. elegans* [11] raises the questions of when caveolins joined the cellular repertoire and whether they are present in more primitive animals, plants or fungi. Another equally important area of research is deciphering the structure of caveolins, as such knowledge would greatly contribute to our understanding of how caveolins function. Recently, gene knockout and transgenic technology has facilitated the study of caveolins in mice, from a whole-organism point of view, allowing the generation of caveolin-deficient or caveolin-overexpressing transgenic mice. The molecular-genetic analysis of these caveolin-deficient mouse models, and cell lines derived from these animals, will greatly facilitate the progress of caveolae-related research into the next decade.

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