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TOPICAL REVIEW

Crowded Little Caves: Structure and Function of Caveolae

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ABSTRACT. Caveolae are small vesicular invaginations of the cell membrane. It is within this organelle that cells perform transcytosis, potocytosis and signal transduction. These "little caves" are composed of a mixture of lipids and proteins unlike those found in the plasma membrane proper. The chief structural proteins of caveolae are caveolins. To date, three caveolins (Cav-1, -2 and -3) with unique tissue distributions have been identified. Caveolins form a scaffold onto which many signalling molecules can assemble, to generate *pre-assembled signalling complexes*. In addition to concentrating these signal transducers within a distinct region of the plasma membrane, caveolin binding may functionally regulate the activation state of caveolae-associated signalling molecules. CELL SIGNAL 10;7:457–463, 1998. © 1998 Elsevier Science Inc.

KEY WORDS. Caveolae, Caveolin proteins, Signal transduction, Oncogenes, Cell transformation, Tumor suppression

BACKGROUND AND OVERVIEW

As far as organelles are concerned, caveolae are a work in progress. Since their discovery more than 40 years ago [1, 2] these 50- to 100-nm vesicles (Fig. 1) have been assigned several important functions. These jobs include potocytosis, transcytosis (in endothelial cells) and signal transduction [3, 4]. The molecular details associated with each of these tasks are being teased from the cells that bear caveolae—namely, endothelial cells; adipocytes; cardiac, smooth and striated myocytes; epithelial cells; and type I pneumocytes.

Although their original definition was morphological, caveolae are now defined as cell-membrane invaginations that contain the marker protein caveolin (a.k.a., VIP21) [5–7] and are enriched in glycosylphosphatidylinositol-anchored proteins, cholesterol and glycosphingolipids. This specialised lipid composition has allowed investigators to purify caveolae by using detergents, inasmuch as caveolae behave as Triton-insoluble complexes. Caveolin was discovered as a phos-phorylation target of the kinase encoded by the Rous sarcoma virus; that is, v-Src [8]. The implications for signal transduction are obvious in hindsight. The discovery of two other caveolins, caveolin-2 [9] and caveolin-3 [10–13], and their differential expression in various tissues suggests other regulatory functions for this gene family (Fig. 1). Caveolin has been re-named caveolin-1 [9, 10].

Because the responsibilities assigned to caveolins and caveolae continue to increase, this review will focus on two main areas of caveolae-related research: (1) caveolin structure and function and (2) caveolae-associated signal transduction. The latter discussion will highlight the involvement of caveolins/caveolae in the Ras-MAP kinase pathway and in phosphatidylinositol (PI) metabolism, as well as their interactions with G proteins, epidermal growth factor receptor (EGF receptor), platelet-derived growth factor receptor (PDGF receptor) and endothelial nitric oxide synthase (eNOS). Recent studies linking caveolae to human disease [cancer, muscular dystrophy (MD), Alzheimer's disease, transmissible spongiform encephalopathy (TSE, scrapie)] also will be considered.

CAVEOLIN STRUCTURE

The Caveolin Gene Family

Caveolin (unless specified, "caveolin" refers to caveolin-1) was identified as a principal protein component of caveolae [6]. Caveolins are a family of ~22,000 M_r integral membrane proteins that assume an unusual hairpin-like structure within the membrane, with both N and C termini facing the cytoplasm (Fig. 2). Human caveolin-1 is 38% identical with and 58% similar to human caveolin-2 [9]. Caveolin-3 is 65% identical with and 85% similar to caveolin-1 [10].

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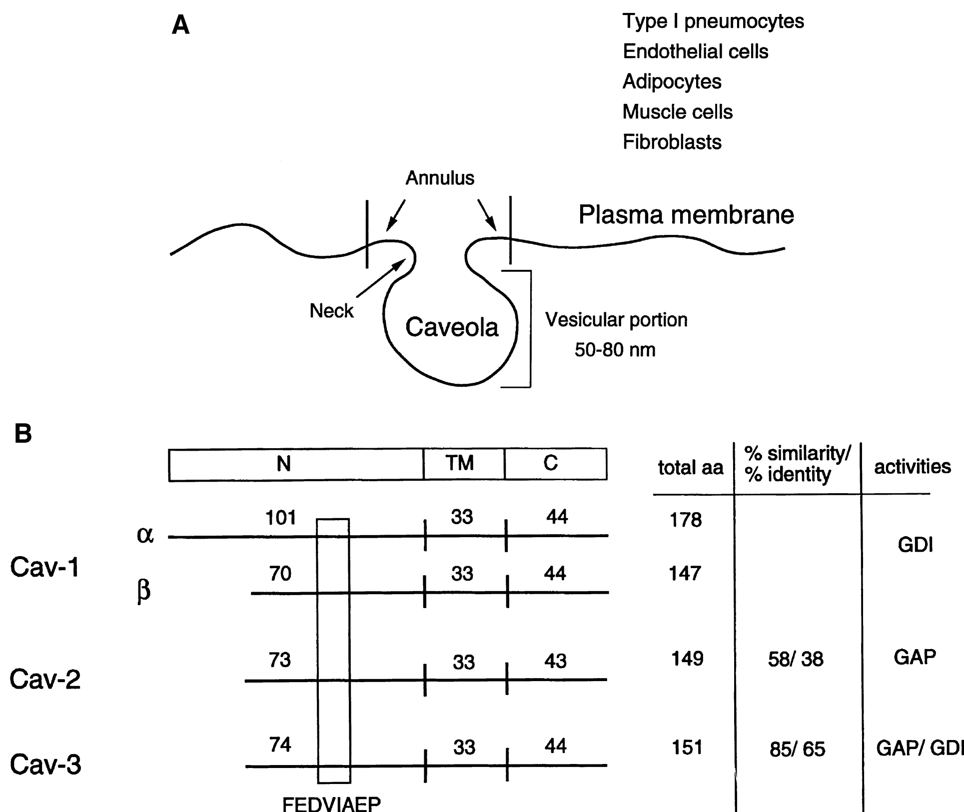

FIGURE 1. Caveolae and caveolins. (A) Caveolae are 50–80-nm invaginations of the plasma membrane and are most abundant in type I pneumocytes, endothelial cells, adipocytes, muscle cells and fibroblasts. Caveolae organelles are separated from the plasma membrane proper by a short caveolar neck or annulus. (B) Caveolins are a gene family of proteins that are the principal structural components of caveolae membranes. To date, three caveolin genes (*cav-1, -2* and *-3*) have been identified in vertebrates. Two isoforms of caveolin-1 (α and β) are known to exist, and they differ in their sites of translation initiation (methionine 1 *vs.* methionine 32). Caveolins 1 and 2 have a similar tissue distribution, whereas the expression of caveolin-3 is muscle specific. Caveolin proteins can all be divided into three distinct regions: (1) a cytoplasmic N-terminal (N) domain, (2) an unusual 33 amino acid (aa) hydrophobic membrane-spanning (TM) segment and (3) a cytoplasmic C-terminal (C) domain. These caveolin gene products differ in their functional interactions with hetero-trimeric G proteins: caveolin-1 possesses GDI activity, caveolin-2 demonstrates GAP activity and caveolin-3 manifests both activities. Abbreviations: GDI, GDP-dissociation inhibitor; GAP, GTPase activating protein.

The caveolin-1 gene appears to be the progenitor of the caveolin-2 and -3 genes [14].

Oligomerization and Caveolar Assembly

Caveolin-1 appears to be an essential component of caveolae [15]. For example, caveolin-1 protein expression directly parallels caveolae formation during adipocyte differentiation [9, 16, 17] (Fig. 3A). Conversely, caveolin-1 mRNA and protein expression are lost or reduced during cell transformation and caveolae are absent from these cell lines [20] (Fig. 3B). Recombinant over-expression of caveolin-1 in caveolin-deficient cell lines results in: (1) the correct biochemical targeting of caveolin-1 to caveolae-enriched membrane fractions [21] and (2) the formation of recombinant caveolae vesicles in lymphocytes [18], Sf21 insect cells [22] and transformed NIH 3T3 cells [23]. These results provide direct evidence that caveolin family members participate in caveolae formation.

However, it remains unknown how caveolin expression induces caveolae formation. This may be related to the self-assembly properties of caveolin-1. Caveolin-1 undergoes two stages of oligomerisation (Fig. 2). First, in the endoplasmic reticulum (ER), caveolin-1 monomers assemble into discrete multi-valent homo-oligomers, containing ~14–16 monomers per oligomer [24, 25]. Subsequently, these individual caveolin-1 homo-oligomers (4–6-nm spherical particles) can interact with one another to form clusters of particles that are ~25–50 nm in diameter [25]. Additionally, caveolin-1 homo-oligomers interact specifically with glycosphingolipids [26] and cholesterol and require a high cholesterol content to insert into model lipid membranes [15, 27]. Thus, we envisage that, through the interaction of caveolin-1 with itself and the caveolin-mediated selection of endogenous lipid components, a caveolae-sized vesicle is generated [15].

The specialized lipid composition of caveolae is thought to convey resistance of this membrane domain to detergent solubilisation by Triton X-100 (at low temperatures) [28–33]. This property appears to be unique to caveolae membranes. For example, when intact cells were fixed in *para*-formaldehyde, extracted with Triton X-100 and then exam-

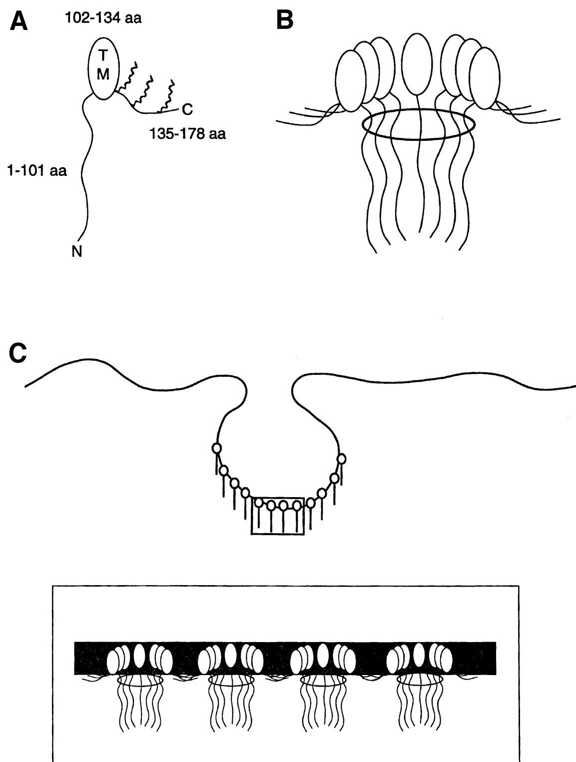

FIGURE 2. **Caveolin oligomers and caveolae assembly. (A) The domain organization of a caveolin-1 monomer. Caveolin-1 contains an N-terminal domain of 101 amino acids, a transmembrane domain of 33 amino acids, and a C-terminal domain of 44 amino acids. Note that the C-terminal domain contains three cysteine residues that are modified by *S*-acylation (palmitoylation). (B) Caveolin-1 monomers assemble into discrete homo-oligomers containing ∼14–16 individual caveolin molecules. Homo-oligomerisation is mediated by residues 61–101 of the membrane-proximal region of the N-terminal domain. (C) Caveolin-1 oligomers are thought to pack side by side within caveolae membranes. Each caveolin-1 homo-oligomer is attached to its neighbours through multiple C-terminal– C-terminal interactions. A section of caveolae membrane containing four such homo-oligomers is enclosed by a rectangle and is shown in more detail.**

ined by electron microscopy, the insoluble membranes that remained were found to be caveolae [34]. However, it is not known whether caveolin-1 contributes to the detergent insolubility of caveolae membranes.

SIGNAL TRANSDUCTION

Src-Family Tyrosine Kinases and the Caveolin Scaffolding Domain

Caveolin was first identified as the 22,000 M_r target of v-Src [8]. Li *et al.* [35] showed that caveolin binds cytosolic c-Src tyrosine kinase and sequesters it in an inactive form. With the same approach used to map caveolin homo-oligomerisation, residues 82–101 (overlapping with the homo-oligomerisation domain) have been observed to stably interact with c-Src but not with the constitutively active v-Src. This domain also binds Fyn, a related Src family tyrosine kinase. Furthermore, a peptide encoding residues 82–101 suppresses auto-activation of purified c-Src and Fyn. Lastly, when caveolin and c-Src are co-expressed in 293T cells, c-Src tyrosine kinase activity is heavily suppressed. Because this region of caveolin (residues 82–101) is required for homo-oligomerisation and interacts directly with many substrates including Gα subunits and H-Ras, it has been termed the caveolin scaffolding domain (CSD).

Using a phage display library encoding random peptide sequences, Couet *et al.* [36] isolated peptide ligands that specifically recognise the CSD. Two related caveolin-binding motifs were identified among the peptides that interact with the CSD: ΦXΦXXXXΦ and ΦXXXXΦXXΦ, where Φ is an aromatic amino acid (Trp, Tyr or Phe). Because these caveolin-binding motifs are present in many caveolae-associated proteins [36] [including G proteins, Src-like kinases, eNOS, protein kinase C (PKC), and receptor tyrosine kinases—e.g., EGF-R and PDGF-R], our studies could provide a general mechanism for caveolin-mediated sequestration and inactivation of a diverse group of signalling molecules within caveolae membranes—for regulated activation by receptor ligands [36]. Thus, the CSD may function like other modular protein domains (SH-2, SH-3, PH, WW and others) to generate membrane-bound oligomeric complexes that contain signalling molecules and cytoskeletal elements [36]. *In essence, caveolin may act as molecular "velcro" to nucleate the formation of signal transduction complexes, holding these molecules in the* off *state.*

Ras and EGF-R Signalling

Epidermal growth factor initiates activation of the Ras-MAP kinase cascade. After EGF binds to its receptor, activated Ras recruits Raf-1 to the membrane. This recruitment allows Raf-1 to phosphorylate MAP kinase kinase (MAPKK), the next messenger in the cascade. Mineo *et al.* [37] found in Rat-1 cells that EGF receptor and Ras are greatly enriched within caveolae membranes. Thirty seconds after the addition of EGF, Raf-1 appears in caveolae but not in other membrane domains. Furthermore, caveolar Raf-1 can phosphorylate MAPKK, but cytosolic Raf-1 fails to phosphorylate MAPKK. In addition, expression of constitutively active H-Ras (Gly 12→Val, H-Ras^{G12V}) causes Raf-1 membrane recruitment to take place independently of EGF stimulation.

Song *et al.* [38] showed that inactive H-Ras preferentially interacts with caveolin both *in vitro* and *in vivo*; in contrast, mutationally activated H-Ras^{G12V} failed to form a stable complex with caveolin. Furthermore, interaction of H-Ras with caveolin is through the CSD [36], suggesting that one of caveolin's main functions is the sequestration of inactive signalling molecules until they are activated to relay a message.

Hetero-Trimeric G-Proteins

In Madin Darby canine kidney (MDCK) cells, ⩾98% of wt $G_{s\alpha}$ is membrane bound, [39], whereas only ∼16% of constitutively active (Gln 227→Leu) $G_{s\alpha}$ is bound to the membrane. Because G_α subunits localise to caveolae, Li *et al.* [39] determined whether this protein is bound in an active or inac-

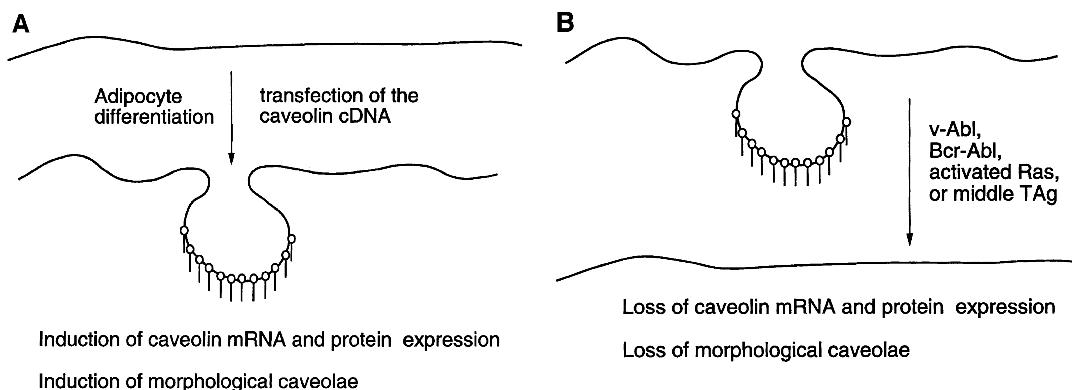

FIGURE 3. Caveolin expression drives the formation of caveolae membranes. (A) During adipocyte differentiation, both caveolin-1 mRNA and protein levels are induced ~20–25-fold. Similarly, the number of caveolae increases by ~10-fold. This suggests that increases in caveolin-1 expression can drive the morphological formation of caveolae membranes [9, 16, 17]. This notion has recently been confirmed by transfecting the caveolin-1 cDNA into caveolae negative cells. Recombinant expression of caveolin-1 is sufficient to induce the formation of caveolae membranes [15, 18]. (B) Modification or inactivation of caveolin-1 expression appears to be a common feature of the transformed phenotype. Historically, caveolin was first identified as a major v-Src substrate in Rous-sarcoma-virus-transformed cells [19]. On the basis of this observation, Glenney and co-workers proposed that caveolin may be a critical target during cell transformation [8]. In direct support of this notion, caveolin-1 mRNA and protein expression are reduced or absent in NIH 3T3 cells transformed by a variety of activated oncogenes [v-*abl*, *bcr-abl*, H-*ras* (G12V)] caveolae organelles also are missing from these transformed cells [20].

tive form. They found that 15–20% of wt $G_{s\alpha}$ co-purifies with caveolin from MDCK cells, whereas the constitutively active mutant is quantitatively excluded from caveolae-rich fractions. This indicates that inactive $G_{s\alpha}$ is specifically enriched ~20–40-fold within caveolae membranes relative to the plasma membrane.

Synthetic peptides encoding the scaffolding domains of caveolin-1 and caveolin-2 interact with G_α subunits [36]. However, these peptides have different effects on the GTPase activity of G_{i2} and G_o. Whereas the caveolin-2 peptide activates GTP hydrolysis, the caveolin-1 peptide inhibits GTP hydrolysis. Because caveolin-1 peptide also suppresses basal G_o binding of GTPγS by 70% and caveolin-2 peptides show no effect on GTPγS binding, these workers conclude that caveolin-2 has GTPase activator protein-like (GAP) activity and caveolin-1 has GDP dissociation inhibitor-like (GDI) activity. Li *et al.* [39] suggest that these seemingly opposing modalities can co-exist in the same caveolae because they work in concert, "holding" (GDI) or "actively placing" (GAP) G_α subunits in the inactive GDP-bound state.

Phosphoinositol Metabolism

Many stimuli cause the liberation of 1,4,5-triphosphoinositol (IP_3) and diacylglycerol. These two signalling molecules are the phospholipase C catabolites of phosphatidylinositol 4,5-bisphosphate (PtdIns 4,5-P_2). When IP_3 binds its receptor, a cascade is initiated [40]. Fujimoto *et al.* [41] found that an isoform of the IP_3 receptor is localised in plasmalemmal caveolae.

Prompted by the finding that the IP_3 receptor targets to caveolae and their own observation that PtdIns 4,5-P_2 is enriched in detergent-insoluble lipid domains containing caveolin, Pike and Casey [42] determined whether caveolar PtdIns 4,5-P_2 is degraded after treatment with growth hormones.

In A431 cells, at least half of the PtdIns 4,5-P_2 is present in caveolae. Treatment with EGF or bradykinin reduces this pool of phospholipids in a time-dependent manner [42]. This result underscores how caveolae function as a focal point for much of the cell's signalling machinery, inasmuch as both a second-messenger progenitor (i.e., PtdIns 4,5-P_2) and the second messenger's receptor (i.e., IP_3 the receptor) are housed within these cell membrane invaginations. Similarly, the target of diacylglycerol (i.e., PKCα) has been localised to caveolae membranes [43, 44].

Platelet-Derived Growth Factor

PDGF receptor transmits its signal after hormone binding by dimerising, autophosphorylating tyrosine and phosphorylating several effectors including PtdIns 3-kinase, non-receptor tyrosine phosphatases and adaptor molecules [45]. Liu *et al.* [46] found, through biochemical and microscopic examination, that PDGF receptor localises to and undergoes PDGF-induced phosphorylation in normal human fibroblast caveolae. Binding of PDGF to the receptor stimulates phosphorylation of several caveolar targets, including the tyrosine phosphatase Syp, the adapter molecule Shc 66 and MAP kinase. In unstimulated cells, these molecules and Src, Nck and the noncatalytic subunits of Ptdlns 3-kinase are enriched in the caveolar fraction. Additionally, a 190,000 M_r phospho-protein of unknown function appears within the caveolar fraction subsequent to PDGF stimulation. This tyrosine phosphoprotein co-immunoprecipitates with caveolin. However, this protein does not appear when cells are treated with acidic fibroblast growth factor, insulin or EGF.

Nitric Oxide Synthase

eNOS is the NOS isoform found in endothelial cells. It catalyses the conversion of L-arginine and O_2 to L-citruline and NO. In the context of blood vessels, NO is a vasodilator and an inhibitor of platelet aggregation. Deficiencies in this enzyme are associated with cardiovascular disease [47].

Hecker *et al.* [48] found that e-NOS is mainly associated with the plasma membrane. Because the activity of eNOS is modulated by many caveolae residents (e.g., the muscarinic acetylcholine receptor, a plasma-membrane Ca^{2+} channel and PKC), Shaul *et al.* [49] examined caveolae from foetal lamb pulmonary artery endothelial cells for the presence of eNOS. The overwhelming majority of eNOS was found within caveolar membranes: the protein was ~9.4-fold enriched within caveolar membranes *vs.* whole plasma membranes. These workers also observed that transfection of eNOS cDNA into COS7 cells, which bear caveolae but do not express eNOS, resulted in caveolar localisation of eNOS. Perhaps most interestingly, these authors showed that both *N*-myristoylation and thiopalmitoylation are necessary to target eNOS to caveolae. They estimate that each acylation process enhances targeting by ~10-fold. These values were calculated by comparing the activities of eNOS and its mutant derivatives (lacking both acylations or missing only thiopalmitoylation) in transfected COS7 cells [49]. These findings were recently corroborated [50].

García-Cardeña *et al.* [51] observed similar acylation-dependent localisation in bovine lung microvasculature endothelial cells. Transfection of NIH 3T3 cells with wt eNOS cDNA leads to acylation-dependent co-localisation with caveolin, whereas transfection of these cells with the palmitoylation-deficient (pal−) eNOS cDNA does not. This same group showed that palmitoylation of eNOS is required for maximal NO release [52]. On stimulation with the calcium ionophore ionomycin, however, membrane association of pal− eNOS was comparable to that of wt eNOS. The authors propose that other hydrophobic factors mediate eNOS's membrane association.

Shortly after these results were reported, Feron *et al.* [53] showed that eNOS interacts with caveolin-1 in bovine aortic endothelial cells (BAECs) and with caveolin-3 in heart muscle (i.e., in adult rat ventricular myocytes). In endothelial cells, eNOS is quantitatively immunoprecipitated by antibodies against caveolin-1. In cardiac myocyte lysates, essentially all the eNOS can be immunoprecipitated by antibodies directed against caveolin-3. Conversely, eNOS anti-serum immunoprecipitates caveolin-3. The association with different caveolins in endothelial cells and cardiac myocytes suggests a mechanism for regulation of eNOS, either by different caveolins directly or by other signalling molecules found in caveolae.

García-Cardeña *et al.* [54] demonstrated that the latter hypothesis may be correct: eNOS is phosphorylated in BAECs. When these cells are treated with hydrogen peroxide and the protein tyrosine phosphatase inhibitor sodium orthovanadate, increased eNOS phosphorylation takes place. This modification causes enzyme activity to drop by 50%. In addition, phosphorylated eNOS interacts with caveolin-1 by immunoprecipitation. Hence, caveolin-1 is the first eNOS-associated protein.

HUMAN DISEASE

Cancer

Caveolae are the launching pads of many signalling cascades. A hallmark of cellular transformation is the shedding of growth- factor requirements [55]. These two observations led Koleske and co-workers [20] to examine caveolin-1 expression in transformed and non-transformed NIH 3T3 cells. They found that cells transformed by expression of *v-abl*, *bcr-abl*, H-*ras*^{G12V}, polyoma virus mTAg or *crk1* oncogenes express substantially less caveolin-1 than do non-transformed cells. Caveolin mRNA levels were similarly affected. Furthermore, the levels of expression correlated inversely with colony size in transformed cells grown in agar; only transformed cells can grow in soft agar.

Engelman *et al.* [23] expressed caveolin-1 in oncogenically transformed cells under the control of an inducible-expression system. Regulated induction of caveolin-1 expression was monitored by Western blot analysis and immunofluorescence microscopy. Caveolin-1 protein was expressed well with the use of this system and correctly localised to the plasma membrane. Induction of caveolin-1 expression in v-*abl*-transformed and H-*ras*^{G12V}-transformed NIH 3T3 cells abrogated the anchorage-independent growth of these cells in soft agar and resulted in the *de novo* formation of caveolae as seen by transmission electron microscopy. Consistent with its antagonism of Ras-mediated cell transformation, caveolin-1 expression dramatically inhibited both Ras/MAPK-mediated and basal transcriptional activation of a mitogen-sensitive promoter. With the use of an established system for detecting apoptotic cell death, it appears that the effects of caveolin-1 may, in part, be attributed to its ability to initiate apoptosis in rapidly dividing cells. In addition, Engleman and colleagues [23] showed that caveolin-1 expression levels were reversibly down-regulated by two distinct oncogenic stimuli. Taken together, these results indicate that down-regulation of caveolin-1 expression and caveolae organelles may be critical to maintaining the transformed phenotype in certain cell populations.

These findings may also have relevance to human cancers. Using differential display and subtractive hybridisation techniques, Sager and co-workers [56] identified a number of "candidate tumour suppressor genes"; these are genes whose mRNAs are down-regulated in human mammary carcinomas. In this screening approach, caveolin-1 was independently identified as 1 of 26 gene products down-regulated during mammary tumorigenesis. In addition, caveolin-1 expression was absent in several transformed cell lines derived from human mammary carcinomas including MT-1, MCF-7, ZR-75-1, T47D, MDA-MB-361 and MDA-MB-474 [56]. In contrast, caveolin-1 mRNA was abundantly expressed in normal mammary epithelium.

Muscular Dystrophy

As stated earlier, caveolin-3 expression is limited to striated (skeletal/cardiac) and smooth muscle cells. This 21,000–24,000 M_r integral membrane protein is a component of the sarcolemma and co-immunoprecipitates with dystrophin [11]. This biochemical finding was corroborated with immunofluorescence staining of mouse skeletal muscle: caveolin-3 and dystrophin co-localise at the sarcolemma. Dystrophin is a cytoskeletal protein that is mutated in both Duchenne's and Becker's muscular dystrophy (MD) [57]. This protein and its associated proteins (dystrophin-associated proteins) form a high-molecular complex. The complex includes dystroglycans α and β (156,000 and 43,000 M_r, respectively), sarcoglycans α, β and γ (50,000, 43,000 and 35,000 M_r, respectively) and an unidentified integral membrane protein of ~20,000–25,000 M_r. The dystrophin-associated proteins are believed to anchor dystrophin to the cytoplasmic face of myocyte plasmalamellae. Song *et al.* [11] hypothesised that caveolin-3 is the missing ~20,000–25,000 M_r component of the dystrophin complex. This is consistent with earlier morphological observations demonstrating that skeletal muscle cell caveolae appear abnormal in size, distribution and number in patients with Duchenne's MD (see [11]).

Transmissible Spongiform Encephalophathy and Alzheimer's Disease

In Alzheimer's disease and in tranmissible spengiform encephalophathy (TSE), molecules responsible for or associated with the pathologic state localise to caveolae-like domains. In TSE, the conversion of the cellular prion protein (PrP^c) into the virulent scrapie form PrP^{Sc} [58] takes place within caveolae-like domains [59]. Bouillot *et al.* [60] found that the Alzheimer's marker axonal amyloid protein (Ax-APP), which localises to axons, and F3 (a GPI-linked glycoprotein) are present within membranous microdomains that bear biophysical properties similar to those of caveolae. This finding is significant because the βA4 peptide found in extracellular amyloid plaques characteristic of Alzheimer's disease is a fragment of the transmembrane precursor APP.

FUTURE DIRECTIONS

Molecular Biology and Genetic Systems

Methods have been recently developed for the generation and purification of "recombinant caveolae" through the over-expression of caveolin in insect cells [22]. When Sf21 insect cells are infected with a recombinant baculo-virus encoding the caveolin-1 protein, they accumulate hundreds of 50- to 120-nm (diameter) cytoplasmic vesicles. These cytoplasmic vesicles are the same size as caveolae and plasmalemmal vesicles seen in mammalian cells. The abundance of these cytoplasmic vesicles and their ease of purification make for an attractive method of generating large amounts of recombinant caveolae. Furthermore, co-expression of caveolin-1 and other proteins is possible with this system.

Motivated by similar desires to ease caveolae study, Kübler *et al.* [61] purified low-density detergent-insoluble (LDTI) membranes from the yeast *Saccharomyces cerevisiae*. These LDTI membranes bear the same proteins (e.g., G proteins) as those from mammalian cells. It appears, therefore, that yeast organise their signal-transduction machinery much as higher organisms do. Although caveolin-like proteins in yeast have not been identified yet, the power of yeast genetics can now be applied to this problem.

The recent identification, cloning and expression of a caveolin from *Caenorhabditis elegans* opens a new avenue of research [14]. This protein, named Cav^{ce}, has the same membrane topology as that of mammalian caveolins. It can self-associate to form high-molecular-weight complexes just as mammalian caveolins can, and it interacts with G proteins in a manner similar to that of caveolin-1. On a practical level, *C. elegans* is a tractable invertebrate model that can now be used to study the function of caveolin *in vivo*. This finding also allows us to place caveolin in evolutionary context. The *cav*^{ce} gene has two exons (1–276 bp and 277–705 bp) linked by a 125-bp intron. The protein's sequence is similar to all three mammalian caveolins, with its second exon bearing strongest resemblance to caveolins found in mammals. This suggests that mammalian caveolins may have evolved from this second exon.

A New Family of Caveolae-Associated Integral Membrane Proteins

Bickel *et al.* [62] identified and cloned the cDNA for an approximately 45,000 M_r protein that co-purifies with caveolin-1. This novel protein, termed flotillin, is one of ten predominant polypeptides detected by Ponceau S staining (Fig. 1B,) [21]. Flotillin is homologous to epidermal surface antigen (ESA), an integral membrane protein found in keratinocytes. Thus, flotillin and its homologue ESA define a new gene family of caveolae-associated integral membrane proteins. Flotillin's function is unknown, but its discovery adds even more depth to the caveolae story. Identification and characterisation of other novel caveolae components is a challenge that is central to the future of caveolae-related research.

These "little caves" are crowded, indeed.

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