Pharmaceutical Research, Vol. 17, No. 9, 2000

Review Article

Caveolae: An Alternative Membrane Transport Compartment

Mark Gumbleton,[1,2] Abedel-nasser G. Abulrob,[1] and Lee Campbell[1]

Received February 23, 2000; accepted May 20, 2000

Caveolae are omega-shaped invaginations of the plasma membrane with a diameter of 50–100 nm. Caveolae invaginations can detach from the plasma membrane to form discrete functional caveolae vesicles within the cell cytoplasm. Caveolae are most prominent in adipocytes, fibroblasts, muscle cells (skeletal, smooth and cardiac), capillary endothelium and type I pneumocytes, although other cell types also display these structures but at a lower numerical density. The key structural and functional protein for caveolae is caveolin. At the plasma membrane caveolae serve to compartmentalise and integrate a wide range of signal transduction processes. Caveolae also serve transport functions including that of the vesicular internalisation of small molecules by the process of potocytosis, and the endocytic and transcytotic movements of macromolecules. Opportunities exist for basic and applied investigators working within the pharmaceutical sciences to exploit caveolae membrane interactions with the aim to develop novel cellular or transcellular drug delivery strategies.

KEY WORDS: caveolae; caveolin; plasmalemmal vesicles; transport; endocytosis; transcytosis; potocytosis; drug delivery; drug targeting.

INTRODUCTION

An understanding of the fundamental mechanisms by which cells internalise solutes from their external environment via vesicle mediated endocytosis with the potential for subsequent transcytosis has traditionally centered around clathrin-coated pits. Recent years have witnessed a growing interest in similar processes that are facilitated by other vesicular populations. Caveolae are a subpopulation of cellular plasma membrane invaginations that comprise a wider spectrum of structures variously termed 'non-coated' or 'smooth-coated' plasmalemmal invaginations due to the lack, at the electron-microscopic level, of an electron-dense cytoplasmic coat characteristic of clathrin-coated pits. The exact function and interactions of caveolae within the cell are far from fully elucidated, however, functional involvement in a wide range of cellular processes has been reported including the regulation of signal transduction and cellular growth control (see reviews 1–4). Further, at least certain populations of caveolae invaginations can detach from the plasma membrane to form discrete caveolae vesicles within the cell cytoplasm (5), affording the potential to mediate the vesicular transport of both low molecular weight solutes and macromolecules. Studies have also demonstrated involvement of the caveolae system in the uptake of, or association with, infectious agents such as viruses (6).

This review aims to provide the reader with a thorough survey of the literature relating to the function of caveolae in transport processes. Appropriate analysis is given to afford appreciation of the potential role of caveolae in pharmaceutical drug transport, and for pharmaceutical scientists to consider targeting this vesicular system for the enhanced delivery of therapeutic agents. Beyond this, the co-ordinated interaction between the processes of signal transduction and transport establish caveolae as unique subcellular tubulo-vesicular structures of interest to pharmaceutical scientists in the broadest definition of the term. As a necessary prelude to the above, a concise overview of the cell biology of caveolae will be provided, however, more detailed reviews on this particular aspect are available (1–4).

CELL BIOLOGY OF CAVEOLAE

Caveolae are most frequently observed at the electron-microscopic level as omega-shaped invaginations (diameter of 50–100 nm at the widest point) connected to the plasma membrane or plasmalemma by a neck-like structure which affords spatial continuity with the extracellular environment (Figure 1). Caveolae are recognised as prominent morphological features in differentiated cell types, notably adipocytes, muscle cells (skeletal, cardiac and smooth), fibroblasts, capillary endothelium and type I pneumocytes, although to varying extents many other cell types display caveolae structures. A principal component and critical structural and functional element of caveolae is the cytoplasmically orientated integral membrane protein, caveolin (7). As a biochemical marker caveolin has provided for an additional broader definition for caveolae, i.e. as a flattened (as opposed to invaginated) caveolin-rich membrane microdomain morphologically indistinguishable from the plasmalemma proper (Figure 5.). Caveolae membranes are enriched not only in caveolin, but also in

[1] Pharmaceutical Cell Biology, Welsh School of Pharmacy, Cardiff University, King Edward VII Avenue, Cardiff CF10 3XF, UK.

[2] To whom correspondence should be addressed. (email: gumbleton@cardiff.ac.uk)

0724-8741/00/0900-1035$18.00/0

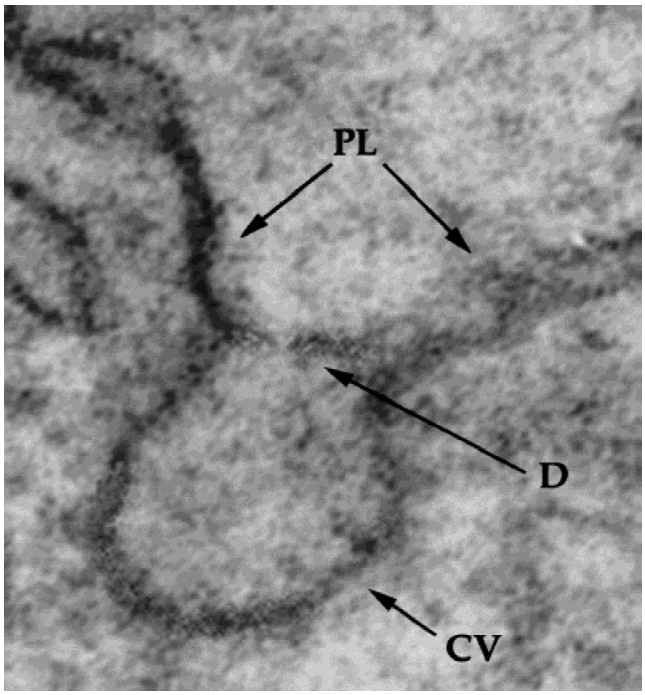

Fig. 1. Electron micrograph of a 'flask-shaped' caveolae invagination (CV) of diameter approximately 80 nm located within the apical plasmalemma (PL). The invagination lacks an electron dense cytoplasmic coat characteristic of a clathrin coated pit. At the neck of the caveolae can be seen a membranous diaphragm (D) restricting the caveolae opening to a 20–40 nm.

cholesterol, sphingomyelin and glycosphingolipids (8). The dynamic clustering of these lipids within the membrane giving rise to detergent-resistant membrane domains or rafts which serve as platforms for membrane-linked functions (8).

Caveolin comprises a family of at least four proteins: [a] caveolin-1 isoforms -1α (24 *kDa* 178 amino acids) and -1β (21 *kDa,* 147 amino acids) (9) derived from the same mRNA transcript but where protein translation is initiated at different start sites; [b] caveolin–2 (20 *kDa,* 149 amino acids) (10), and [c] caveolin-3 (17.2*kDa,* 151 amino acids), a muscle specific form (11,14). Caveolins possess a conserved hydrophobic transmembrane region (33 amino acids in length) which allows for both the carboxyl- and amino–termini domains of the protein to be cytoplasmically located and providing a 'hairpin-like' structure to the monomer unit as it resides within the membrane (Figure 2a) (12). Caveolin proteins also possess a 'scaffolding' domain (19–21 amino acids in length), which for caveolins –1 and –3 is central to many of the reported signal regulation, and indeed probably transport, functions ascribed to caveolae (1–4).

Caveolin-1 is the most widely investigated of the caveolin proteins, and in cell types other than cardiac and skeletal muscle, caveolin-1 is identified as the main caveolin protein family member influencing the structural and functional properties of caveolae. Both caveolin-1 isoforms, 1α and 1β, target to caveolae membranes, with considerable overlapping subcellular co-localisation of the isoforms noted (9) in cultured rat thyroid epithelial (FRT) cells recombinantly engineered to express caveolin-1. At the level of the organised tissue relatively strong expression for caveolin-1 protein (13,14) is recognised in lung and fat, with moderate signals evident in pancreas, heart and skeletal muscle; liver tissue is notably devoid of caveolin-1. While caveolin-1 is also expressed in smooth muscle cells, it's expression in skeletal and cardiac muscle tissue may well reflect that associated with the endothelium within these tissues and not the muscle fibres themselves. At the level of the cell, caveolin-1 expression is most conspicuous in adipocytes, fibroblasts, endothelial cells and type I pneumocytes. Among other cell types reported to express caveolin-1 are kidney epithelial cells (15), brain endothelial cells (16), primary cultures of brain astrocytes (17) and dorsal root ganglion neurons (18). Hemopoeitic cells including lymphocytes and neutrophils are generally recognised as caveolin-1 negative (19), although some leukemia cell lines have been shown to express caveolin-1 (20). Data from our own laboratory (unpublished) has shown primary human monocyte and macrophage cells to be devoid of caveolin-1 and -2, although other workers (21) have reported caveolin-1 expression and the functional presence of caveolae in rat peritoneal macrophages activated *in-vivo*, following administration of Freund's adjuvant.

Caveolin-2 displays a tissue and cellular distribution closely resembling that of caveolin-1, with expression noted in fat and lung tissue, and in adipocyte, fibroblast, smooth muscle and endothelial cell types (10,22). Caveolins -1 and -2 have been shown (22) to form stable hetero-oligomeric complexes, and to be co-localized in caveolae plasma membrane domains and other internal cellular membranes. The regulation of caveolin -1 and -2 gene expression has, however, been shown to be independently controlled (22). Work from recent papers (23) has documented the requirement for caveolin-1 to facilitate the transport of caveolin-2 from the Golgi complex to the plasma membrane, i.e. in cells caveolin-1 deficient, caveolin-2 displays a restricted distribution to the Golgi complex, but following recombinant caveolin-1 expression, caveolin-2 can redistribute to the plasma membrane as part of hetero-oligomeric caveolin -1/-2 complexes. The other caveolin family member, caveolin-3, shows a muscle specific distribution (11,14) with expression in cardiac and skeletal muscle fibres, where it serves as the principal caveolin protein family member promoting the assembly of caveolae. Caveolin-3 is also expressed together with the other family members (-1 and -2) in smooth muscle cell fibres.

The structural unit for caveolin protein within the plasma membrane is considered to be in the form of high molecular weight caveolin homo-oligomers (12,24,25) (Figure 2b). The interaction of caveolin with membrane lipids, particularly cholesterol, is critical for caveolin membrane incorporation and oligomer stabilisation (26,27). While caveolin-1 and caveolin-3 are able to undergo homo-oligomerisation, i.e. oligomers comprising only caveolin-1 or caveolin-3 monomers, respectively, caveolin-2 exists alone only in monomeric or homo-dimeric forms, not participating in high molecular weight caveolin-2 homo-oligomeric structures (28). Nevertheless, as noted earlier, caveolin-1 is able to recruit caveolin -2 to form high molecular weight caveolin-1/-2 hetero-oligomeric complexes.

Evidence that caveolin-1 is a critical, but not necessarily the sole, determining factor in caveolae biogenesis includes correlative data between caveolae formation and caveolin-1 expression levels in differentiating 3T3- L1 adipocytes (29), in transdifferentiating alveolar epithelial type II to type I cells (30) and in oncogenically transformed cells (31). The rela-

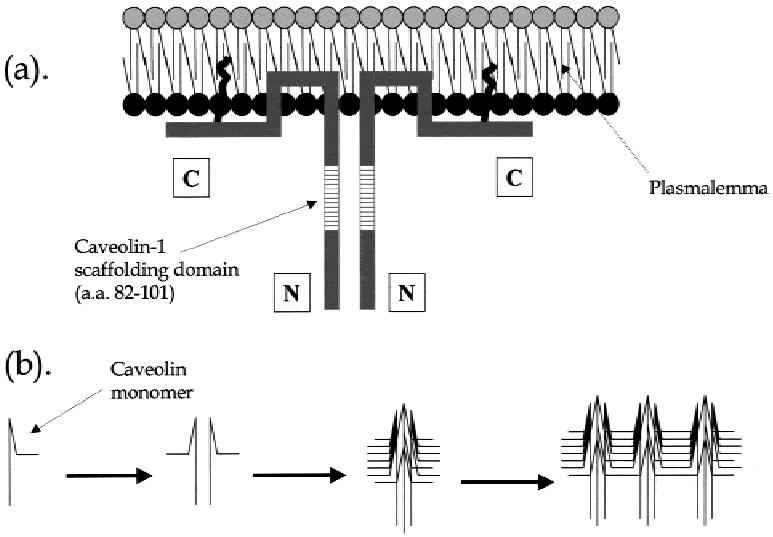

Fig. 2. (a) Schematic of caveolin membrane topology highlighting the membrane incorporation of caveolin transmembrane domain, the cytoplasmic orientation of both carboxyl- and amino- termini, and the caveolin-1 scaffolding domain (a.a. 82–101). Encompassing the caveolin scaffolding domain is the region responsible for homo-oligomerisation (a.a. 61–101); (b) Schematic of caveolin oligomerisation with self-assembly of high molecular mass oligomers of approximately 400 kDa (estimated to comprise 14–16 caveolin monomer units) through protein-protein interactions mediated through caveolin's oligomerisation domain. Later, within caveolae membranes, caveolin-oligomers interact with each other through carboxy-terminal domains to form a caveolin-rich lattice network.

tionship between caveolin-1 expression and caveolae biogenesis appears to require a threshold level of caveolin expression for the formation of caveolae (30,32), such that caveolin expression alone does not necessarily imply the presence of caveolae within a cell. More direct evidence for the structural role of caveolin-1 includes the recombinant expression of caveolin-1 driving the *de novo* formation of caveolae in previously caveolin negative cells (32,33), including the recombinant expression in wild-type caveolin-negative CaCo-2 cells (34). Studies by Li et al. (33) using baculovirus-based caveolin expression vectors have demonstrated that each of the caveolin-1 isoforms (-1α and -1β) can independently generate caveolae, the identity of which was confirmed by caveolin-1 immunolabelling of the vesicle structures formed. Further, using the same model system these workers subsequently showed the requirement for oligomerisation of caveolin-1 or -3 protein for vesicle formation, and the lack of vesicle formation when caveolin-2 was expressed alone (28).

CAVEOLAE MEDIATED TRANSPORT

Inhibitors of Caveolae Trafficking

Recent years have witnessed a succession of studies that have largely unravelled the molecular composition of caveolae and progressed the understanding of how caveolin-1 protein, and probably caveolae vesicles themselves, are transported from one subcellular compartment to another. Through these studies a broad range of pharmacological reagents have been identified that may be employed to probe molecule internalisation by plasma membrane caveolae and/or intracellular trafficking by caveolin-1 associated vesicular complexes.

In early studies it was discovered that the enzyme cholesterol oxidase (which catalyzes the conversion of plasma membrane cholesterol to cholestenone) could induce in fibroblasts the translocation of caveolin-1 protein from the cell surface to the Golgi apparatus (35). This initial study resulted in a series of reports that led to the elucidation of an intracellular trafficking route for caveolin-1 recently designated the CERGA (Caveolae to Endoplasmic Reticulum to Golgi Apparatus) pathway (as described in detail in reference 36). In this pathway caveolin-1 is shown to constituitively cycle between a plasmalemma location (where it is either associated with a morphologically identifiable caveolae invagination, or indeed as a flattened 'caveolin-rich' domain, directly to the lumen of the endoplasmic recticulum and then routed to the Golgi complex from where it is eventually returned to the cell surface in the form of a caveolae vesicle. Surprisingly, however, despite induction of the translocation of caveolin-1 protein from the cell surface to the Golgi apparatus, treatment with cholesterol oxidase, at least in fibroblasts, does not lead to a significant reduction in the number of caveolar invaginations at the plasma membrane (35). Additionally, in human intestinal cells, it is shown that cholesterol oxidase does not appear to perturb caveolae dynamics with respect to cholesterol trafficking, thereby making it an unlikely choice of reagent for the general inhibition of caveolae mediated internalisation pathways (37).

Various reagents known to disrupt caveolae structure and function have been identified and have been reliably used to decipher caveolae mediated trafficking pathways in a number of epithelial and endothelial cell types. Filipin, a macrolide antibiotic, selectively binds cholesterol and has been shown to reversibly decrease the number of invaginated plasmalemmal caveolae and as a corollary reduce the endocytic

and transcytotic capacities of caveolae in endothelial cells, without any apparent effect on clathrin pathways (38). Similarly, nystatin has been shown to inhibit the dynamics of caveolae by this same mechanism (39). A study examining the role of caveolae in the intracellular trafficking of newly synthesised cholesterol (40) highlighted progesterone as a suitable agent for the disruption of caveolae mediated trafficking. The mode of action of this particular inhibitor is again reliant upon the fact that caveolae structure is dependant upon cholesterol, and progesterone is shown to prevent the passage of cholesterol from its site of synthesis in the endoplasmic recticulum to the plasma membrane.

Several other pharmacological molecules have been identified (39) to inhibit caveolae mediated transport, and in particularly the transport process termed potocytosis (see later), these molecules include phorbol myristate acetate, indomethacin, and okadaic acid, the latter two like filipin are able to mediate a reduction in the number of caveolae invaginations evident at the plasma membrane cell surface. Indomethacin and the phosphatase inhibitor, okadaic acid, appear to inhibit both facets of caveolae trafficking, i.e. internalisation and vesicle recycling back to the plasmalemma. Phorbol myristate acetate reduces caveolae internalisation without affecting the return of plasmalemmal vesicles to the cell surface, and elicits its effects by activation of protein kinase C-α, a signal transduction molecule that appears critical in regulating the invagination of caveolae (71). Caveolae inhibition with indomethacin appears to involve indomethacin's recognised action to elevate arachidonate. The thioalkylating agent *N*-ethylmaleimide (NEM) has been reported to inhibit transcytosis and endocytosis by caveolae in endothelial cells (41,42). Although electron microscopical evidence obtained from these *in-vitro* and *in-situ* studies clearly display a reduction in the transendothelial movement of tracers by caveolae, NEM cannot be considered specific. The cellular effects of NEM are well documented and include inhibition of vesicular trafficking proteins, e.g. NEM-sensitive Factor (NSF), responsible for the budding and fusion of a wide range of vesicle populations, including clathrin coated vesicles.

Collectively, studies exploiting inhibitors of caveolae have demonstrated a transport function for caveolae, or at the very least of caveolin associated complexes. However, it is likely that the precise cytoplasmic routing of caveolae / caveolin proteins will vary dependent upon cell type. For example, it is recognised that the various members of the caveolin protein family display multiple interactions with each other, with each type of interaction potentially leading to altered subcellular trafficking. Indeed the caveolin proteins have been shown to possess several distinct domains influencing their subcellular trafficking between intracellular compartments: caveolin-1 possesses a domain (amino acid residues 66 and 70) shown to regulate its departure from the endoplasmic reticulum. Two additional domains within the caveolin-1 molecule (amino acid residues 91–100 and 134–154) appear to control the transport of the oligomerised form of caveolin-1 from the Golgi apparatus to the plasma membrane (43). Another study (44) has identified a cis-Golgi targeting domain within the C-terminus of the caveolin-3 molecule. A better understanding of the movement of caveolin and/or caveolae between the various intracellular compartments will provide invaluable information for drug delivery scientists wishing to specifically target certain organelles.

Potocytosis

Potocytosis is a term used to describe a specialised cyclic vesicle internalisation process in which cells sequester, concentrate and internalise small molecules and ions through a receptor-mediated mechanism (45,46), where such molecules may represent either nutrients or molecules mediating receipt and transmission of extracellular signals to within the host cell. Potocytosis is distinct from the receptor-mediated endocytosis of macromolecules which involves extensive membrane trafficking, in that potocytosis is perceived as providing a particular form of spatial and temporal control over small molecule delivery to the cell cytoplasm. Potocytosis has been shown to be largely dependent upon functional caveolae, with insights into the sequential mechanistic events of the potocytotic process gained largely through the study of 5-methyltetrahydrofolate internalisation (47–49). With this ligand as an example, the potocytosis cycle (Figure 3) is initiated following the binding of 5-methyltetrahydrofolate to its receptor, a GPI-anchored membrane protein that is extracellularly orientated. This binding leads to the translocation of ligand-receptor complexes to a caveolae domain that becomes progressively invaginated, finally resulting in the formation of a discrete closed vesicle no longer contiguous with the plasma membrane but remaining at or near the cell surface (45). In response to a reduction in vesicle pH, the folate dissociates from its receptor, and the relatively high concentrations of folate within the closed caveolae vesicle then favours the its passive facilitated diffusion into the cytoplasm via an organic anion carrier (50). The folate receptor is subsequently recycled back to the plasma membrane for re-utilisation. Acidification of caveolae has been shown to be mediated by a vacuolar-type proton ATPase in a manner similar to endosomes derived from other vesicular populations (51). Studies undertaken *in-vitro* suggest that the potocytotic cycle can be regulated by hormones, e.g. histamine which appears to elicit its effect by promoting the uncoupling of PKC-α from caveolar membranes and thereby resulting in reduced phosphorylation of key membrane substrates necessary for the invagination of caveolae to proceed (52).

From a pharmaceutical perspective the targeting of caveolae and the potocytosis pathway warrants further investi-

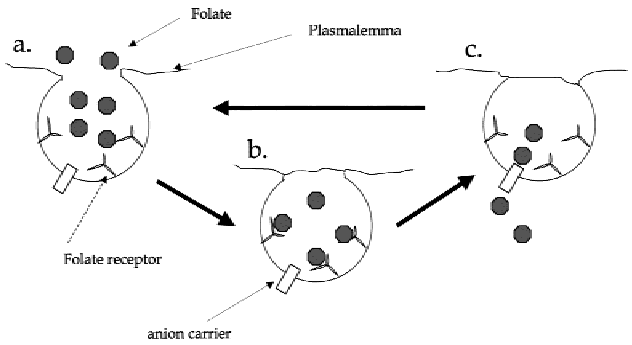

Fig. 3. Potocytosis of folate (adapted from reference 45). (a) Caveolae open at the plasmalemma and bind folate molecules via specific folate receptors; (b) Caveolae closes and possibly detaches from plasmalemma; (c) A proton gradient is generated that causes folate to dissociate from its receptor. The high concentration of folate within the caveolae creates a gradient that favours movement of folate across the membrane into the cytoplasm via an anionic carrier.

gation, especially with recent interest shown in the use of folate as a targetting ligand (53). However, while caveolae are clearly involved in folate potocytosis the endocytic internalisation of folate, and as a corollary folate targetted delivery systems, may also exploit trafficking via an alternative vesicle pathway.

Cholesterol Trafficking

The maintenance of cholesterol homeostasis within cells, particularly that of unesterified cholesterol (free cholesterol), is critical for the viability of a cell. Recent research (40,54) into the intracellular processing of lipoprotein derived cholesterol, and in particular free cholesterol, has consolidated the intimate relationship that exists between caveolae, caveolin and cholesterol transport.

It is recognised that greater than 80% of a cell's free cholesterol is contained within the plasma membrane, and several reports indicate that at least in certain cell types caveolae situated at the plasma membrane participate in cholesterol homeostasis, specifically representing a regulatory efflux pathway facilitating the removal of excess free cholesterol to the cell exterior (55). Insights into the mechanistics of cholesterol trafficking were contributed by a series of closely related studies that utilised quiescent fibroblast cultures, and demonstrated that free cholesterol derived from low density lipoproteins (LDL) accesses the cell by receptor mediated events initially occurring within clathrin coated pits (54) (Figure 4). Subcellular fractionation and inhibitor studies (54,56) determined that following internalisation the cholesterol is transported to intracellular sites, primarily the trans-Golgi network, where upon any excess free cholesterol is eventually exocytosed at the plasma membrane through caveolae for release to high density lipoproteins (HDL) (Figure 4). Corroborating evidence of a role for caveolae in the homeostasis of free cholesterol was further provided by pulse-chase experiments (40) showing that the 'rapid' (10–20 min) transit of newly synthesised radiolabelled cholesterol occurs from the endoplasmic reticulum to plasma membrane caveolae in cultured human fibroblasts. Caveolae mediated cholesterol efflux has also been reported in other cell types including arterial smooth muscle cells (both *in-vivo* and *in-vitro*) (57) and may represent the predominant pathway by which most cell types rich in caveolae discharge excess cholesterol and thereby contribute to cholesterol homeostasis within the cell. Interestingly, a recent study (36) has reported the secretion of caveolin in a soluble form from serous exocrine cells of the pancreas upon stimulation with various secretagogues (secretin and dexamethasone). The exact significance of this is not known but the association of soluble caveolin with lipoproteins led the authors to allude to the possibility that caveolin may serve to regulate the extracellular trafficking of lipids in exocrine compartments of the body such as the gastrointestinal tract.

While the above studies support a role for caveolae in regulating cholesterol flux at the level of the plasma membrane, little evidence exists for caveolae serving as discrete vesicular organelles for the trafficking of cholesterol between intracellular compartments. Intriguingly Uittenbogaard and co-workers (58) report upon data collected within human fibroblasts indicating that caveolin may mediate the movement of cholesterol through the cell independent of membrane vesicles. This direct transport of cholesterol to the plasma membrane from the endoplasmic reticulum involving a cyto-

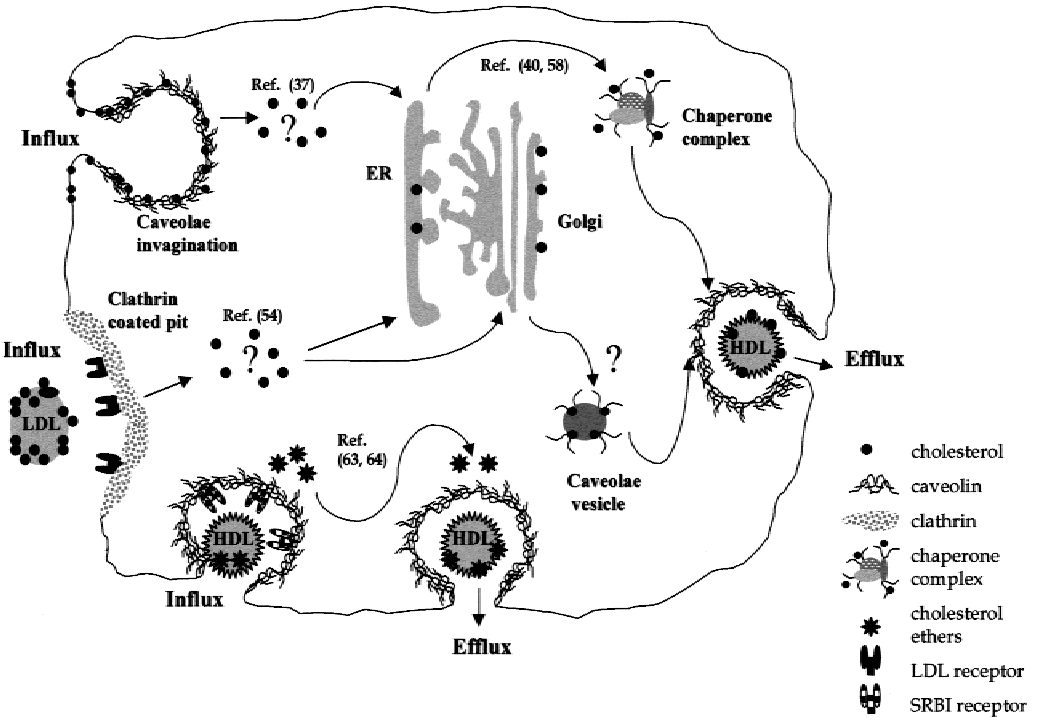

Fig. 4. A simplified model highlighting cholesterol efflux and influx based upon published data (see references in Figure and text) concerning the role of caveolae and/or caveolin chaperone complexes in the intracellular trafficking of cholesterol and lipid homeostasis within cells.

solic caveolin-chaperone complex comprising caveolin-1 together with the cytosolic molecular chaperones, cyclophilin A, cyclophilin 40 and heat shock protein 56 (Figure 4). This intracellular trafficking pathway for cholesterol could be blocked by treatment of the cells with cyclosporin or rapamycin; agents that disrupt cyclophilin containing chaperone complexes and which until that time were not implicated in modulating a caveolin-1 linked transport pathway.

Levels of caveolin-1 have been shown to be inversely regulated with respect to the cholesterol status of the cell. Increases of 200–300% in the levels of caveolin-1 mRNA have been reported following exposure of human fibroblast monolayers to LDL particles enriched with free cholesterol (59), and also following increases in intracellular cholesterol levels in MDCK cells (60). The mechanism by which caveolin-1 is up-regulated in response to increases in free cholesterol has been elucidated (61) and appears to involve the inhibition by free cholesterol of the cleavage of a sterol regulatory element binding protein (SREBP), whose cleavage fragment, SREBP-1, acts as a transcriptional inhibitor for caveolin-1, i.e. free cholesterol represses the production of a caveolin-1 inhibitor. Consistent with this Fielding and co-workers (62) have shown in synchronised dividing skin fibroblasts, i.e. cells with a high cholesterol requirement, a down-regulation of caveolin-1 mRNA which is associated with a parallel decrease in the cellular efflux of cholesterol.

Recent studies using HDL particles loaded with cholesterol ethers have provided some evidence to support the participation of caveolae in the selective uptake of conjugated cholesterol species (Figure 4). Specifically, uptake of HDL particles via the scavenger receptor, SR-BI (generally accepted as the physiological receptor for HDL particles), in a macrophage cell line (THP-1) induced to express caveolin-1 (63), and in a SR-BI transfected Chinese hamster ovary cell line where HDL mediated uptake of cholesterol was shown to be highly compartmentalised to plasma membrane caveolae (64), and within which the recombinantly expressed SR-BI receptors were found to be highly enriched; a proportion (50%) of the internalised cholesterol ethers shown to be reversibly effluxed (Figure 4). These findings consolidate the relationship between caveolae and the uptake of cholesterol species derived from HDL particles, leading investigators (64) to hypothesise that caveolae and SR-BI could be instrumental in the potentiation or abrogation of foam cell formation and subsequent development or aversion of atheroma, respectively.

Caveolae mediated influx of cholesterol from the plasma membrane to the endoplasmic reticulum has been reported in the human colonic adenocarcinoma cell line, CaCo-2, following the equilibriation of the cells with radiolabelled cholesterol (37) (Figure 4). The movement of cholesterol from the plasmalemma to the ER could be inhibited by upto 70% with filipin treatment. In contrast to the studies by Field and co-workers (37) in CaCo-2 and also the demonstration by these workers of caveolin-1 expression in primary human intestinal epithelial cells, the existence of caveolin and caveolae in wild-type CaCo-2 cells is refuted by a number of laboratories (34,65). Such an apparent contradiction may be explained by CaCo-2 cell line comprising a number of clones that display considerable phenotypic variability. This should be of significance to drug delivery scientists using the widely exploited CaCo-2 cell line as an *in-vitro* model to predict the transport and permeability of pharmaceutical drugs across the gastrointestinal tract.

It is clear that the extensive research that has been undertaken regarding the cellular trafficking of cholesterol has given investigators an invaluable insight into the intracellular trafficking pathways utilised by caveolin and possibly caveolae vesicles themselves. As the concept of exploiting caveolae for the enhanced delivery of pharmaceutically relevant compounds gains acceptance, improved knowledge of such mechanisms will prove essential to the refinement of drug delivery strategies to cell types that are rich in caveolae.

Caveolae and Potential Role in Transport-Mediated Multidrug Resistance

A major problem associated with cancer chemotherapy is the development by tumor cells of resistance to the cytotoxic effects of anti-cancer drugs. The most characterised feature of multidrug resistance (MDR) relates to the overexpression of P-glycoprotein (P-gp), a plasma membrane ATP-dependent drug efflux pump. However, the acquisition of MDR in cancer cells is a multifactorial process with significant membrane alterations shown to occur in MDR cells, including the up-regulation of membrane glucosylceramide, sphingomyelin, and cholesterol (66). Recently, an increase in the expression of caveolin-1 together with concomitant caveolae biogenesis has been observed during the development of an MDR phenotype in the breast cancer cell line MCF7/ADR (67). The precise relationship between caveolae and MDR, however, is currently not established. Nevertheless, the induction of caveolin-1 expression and the formation of caveolae in the resistant phenotype can be a distinct phenomenon independent of P-gp expression, for example induction of low level Taxol resistance (9-fold induction) in P-gp negative A549 cells (68) is associated with a 3 to 4-fold increase in caveolin-1 expression. Further, the effects of cell exposure to Taxol are acute, with increases in caveolin-1 expression evident within 48 hr of drug exposure. Preliminary data from our laboratory has found a similar upregulation of caveolin-1 in A549 cells treated with very low concentrations of the cytotoxics vinblastine or doxorubicin (unpublished data). Lavie and co-workers (66) have hypothesised that caveolae may serve a similar role in MDR as that undertaken in maintaining homeostasis of free cholesterol, i.e. in the resistant phenotype caveolae may facilitate the export of lipophilic cytotoxic drugs. However, such a mechanism would initially appear to lack consistency with the prevailing view that caveolin and caveolae together with cholesterol efflux are down regulated in actively dividing cells, at least in fibroblasts (62). Clearly the role of caveolae and caveolin in the MDR phenotype is an intriguing area for further research and may expand to address interaction between caveolae / caveolin and constituitively expressed membrane transporters.

Endothelial Transport

Plasma membrane invaginations conforming to the morphological definition of caveolae have long been recognised in electron micrographs of capillary endothelium and subsequently defined biochemically as caveolae. Based upon morphometric analysis of pulmonary capillary endothelium (69,70) the numerical density of what are now recognised as

caveolae has been estimated at 150–400 per μm^3 cell volume which would approximate to 0.2×10^6 per cell, and with a significant proportion (~70%) of the endothelial cell plasmalemma located within caveolae membrane invaginations (Figure 7). However, it must be noted that endothelial cells display phenotypic heterogeneity and the full characterisation of caveolae within the endothelium of different organs is required before efforts are made to exploit these structures for improved drug targeting and delivery.

A long considered function of caveolae within endothelial cells has been the transport of plasma macromolecules (70). Until comparatively recently, however, this was much debated due in part to the lack of recognised inhibitors specific for caveolae mediated pathways, but also to a paucity in knowledge of the underlying mechanisms modulating membrane vesicle dynamics. An approach widely used to investigate and characterise the permeability of intact endothelial barriers involves the modelling of theoretical pore populations defining the differential transendothelial transport of a series of hydrophillic solutes of varying molecular size. For intact continuous vascular endothelium theoretical pore models generally predict the presence of a small pore population (diameter 10 nm; ~ 18 units / μm^2) and a large pore population (diameter $\leq$ 50 nm) of much lower numerical density. Pioneering work by Predescu and Palade during the 1990s (41,72,73) has identified caveolae to serve as the structural equivalents of both small and large pores in continuous microvascular endothelium. Using electron microscopical techniques the above series of *in-situ* studies directly demonstrated the exclusive transcytosis of labelled albumin and orosomucoid (tracers that qualify as large and small pore probes respectively) via endothelial caveolae. The authors hypothesised that caveolae act as large pores when fully opened and as small pores when the neck of the plasma membrane invagination is constricted to less than 10 nm diameter (Figure 5). Conversely, the small and large pores may represent distinct subpopulations of caveolae that are postulated to exist in microvascular endothelial cells (38). The transport via caveolae may not be mediated solely by vesicle trafficking through the cytoplasm from luminal to abluminal surfaces, i.e. transcytosis, but may also involve the fusion of vesicles to form transient transcellular channels (Figure 5.) allowing for the convective flow of solutes, a feature that has been noted in particular in the attentuated (150–300 nm thickness) regions of endothelial microvasculature cells at sites distal from the nuclear cell body (70,74).

Based upon well-defined *in-vitro* and *in-situ* models, functional studies conducted with pulmonary microvascular endothelial cells (38,42) have demonstrated an unequivocal role for caveolae in the receptor mediated internalisation and transcytosis of native albumin, and the endocytosis of modified albumins. In cultured microvascular lung endothelial cells Schnitzer and coworkers (38) showed the ability of sterol binding agents, in particular filipin, to diassemble caveolae leading to a reduction in the surface density (to less than 15% of control) of plasmalemmal caveolae invaginations, but without effect on the structural integrity of coated pits. Further, these workers showed filipin treatment to give rise to concentration-dependent inhibition (up to approximately 50%) in the transendothelial transport of native albumin across both *in-vitro* cultured endothelial monolayers and *in-situ* rat lung capillaries. They further showed that fillipin treatment exhibited no affect upon paracellular transport pathways or indeed upon the transport of α_2-macroglobulin (whose internalisation pathway via clathrin-coated pits is well established). The caveolae localisation of albumin receptors, gp18 and gp30, is shown to mediate the delivery of modified albumins to lysosomes for eventual degradation and represents a scavenging function for endothelial caveolae (38). This pathway maybe responsible for the selective removal of damaged tissue products in response to pathological processes or normal ageing. The caveolae-mediated vectorial transendothelial transport of native albumin via the gp60 receptor involving the shuttling of individual caveolae between apical and basolateral surfaces, provides a direct route for the transfer of intact albumin between blood and tissue. Therefore the endocytosis of modified albumin, and transcytosis of native albumin suggests the existence of functionally distinct subpopulations of caveolae

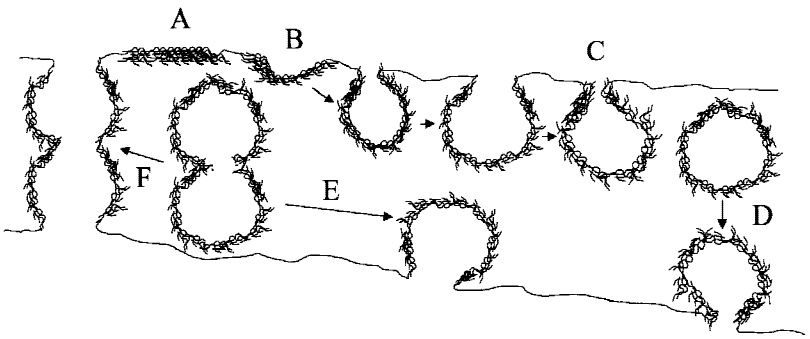

Fig. 5. A schematic representation of putative caveolae dynamics within endothelial cells. Flattened caveolin-rich domains (A) may exist in the plasma membrane that steadily increase in curvature (B $\Rightarrow$ C) following appropriate physiological stimuli such as ligand-receptor interactions. The resultant invagination may remain attached to the plasma membrane (C) or completely close and detach to form a discrete intracellular vesicle that can traffic between luminal and abluminal endothelial surfaces (D). Additionally a detached caveolae may connect with an open invagination on the opposing membrane surface (E) where the transfer of vesicular cargo occurs. Conversely, several caveolae in close proximity could merge to form transient endothelial channels (F), allowing the direct transport of solutes from the blood to subendothelial compartments.

that differ in their subcellular trafficking pathways. There is evidence to suggest a relationship between the receptor mediated endocytosis of albumin and fluid phase uptake by caveolae. Tiruppathi and co-workers (75) examined the binding of native albumin to the surface of aortic endothelial cells and reported that the binding invoked phosphorylation of the gp60 albumin receptor and an increase in the caveolae-mediated uptake of fluid phase markers.

When the potential exists for a solute to participate in dual transport pathways the relative quantitative significance of a caveolae–mediated route is clearly an issue that warrents address. In the *in-vitro* study of Roberts and Sandra (76), comparison was made of the relative contribution of a clathrin-coated pit pathway and a non-coated vesicle (morphologically identifiable as caveolae) pathway in the transcytosis of insulin across cultured bovine pulmonary artery endothelial cells. Using semi-quantitative immunocytochemical analyses they reported that both vesicular populations were associated with insulin, although a greater (approximately 70% of the total) amount of the gold-labelled insulin probe was associated with caveolae structures. However, morphometric evaluation determined the surface density of clathrin coated pits in their cultured cell type to approximate only 5% of that for caveolae, leading the authors to interpret that, when normalised for vesicle density, insulin shows a preferential interaction with clathrin-coated pits.

The molecular machinery involved in the accurate trafficking and fusion of caveolae vesicles to their target membranes is currently under study. In particular the role of a conglomerate of small proteins collectively termed the SNARE complex (77) (Figure 6). Certain components of the SNARE machinery, namely SNAP-25 and syntaxin, reside on the target membrane whilst other components reside in the membrane of the free cytoplasmic vesicles (vesicle associated membrane protein-VAMP). Other components, N-ethylmaleimide-sensitive factor (NSF) and soluble NSF attachment protein (α-SNAP) represent two soluble cytosolic proteins that mediate the docking of free vesicles to target membranes. It is proposed that the SNARE protein complex functions in the budding, directional transport and targetted docking of caveolae within microvascular endothelium (78). Schnitzer and co-workers (78) undertook an extensive examination of caveolae isolated from pulmonary microvascular endothelium, and revealed the co-localisation of caveolin and several key components of the SNARE complex, namely VAMPS, NSF and SNAPs. Additionally, members of the annexin family (II and IV) and heterotrimeric GTP-binding proteins, which are both believed to influence plasma membrane dynamics, were also co-localised to endothelial caveolae. An interesting recent finding (79) further supports the direct involvement of certain SNARE components in the intracellular trafficking of caveolae. Specifically, the demonstration of a selective inhibition in the endothelial internalisation of cholera toxin subunit B (a putative caveolar transport marker) following the cleavage of VAMP-2 by a VAMP-specific botulinium toxin. The same workers (80) recently co-localised dynamin, a member of a multigene family of large GTPases, to the neck of endothelial caveolae and shown its functional involvement in severing the caveolae invagination from the plasma membrane to form transport vesicles. Dynamin has also been implicated in severing clathrin coated pits from the plasmalemma during receptor mediated endocytosis. Indeed caveolae and clathrin-dependent internalisation pathways show considerable overlap including the involvement of the SNARE machinery (81). An important question to address is to what extent the components of the SNARE machinery contribute to caveolar dynamics in other non-endothelial cell types, particularly in epithelial barriers known to display high numerical densities of caveolae, such as the lung alveolar epithelium.

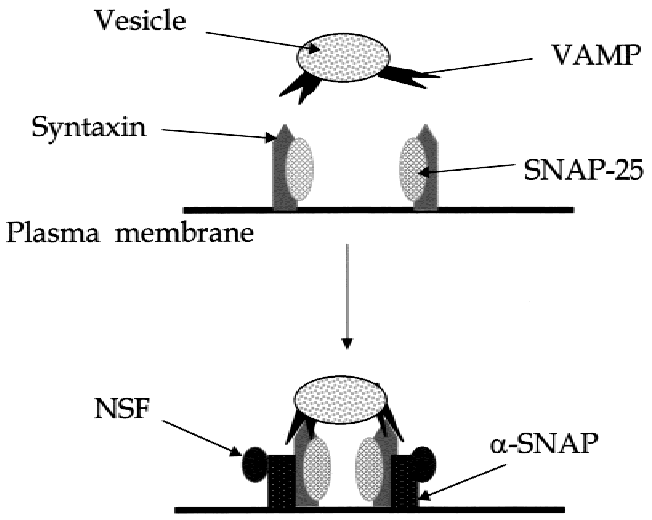

Fig. 6. Schematic of the functioning of the SNARE vesicle trafficking machinery. SNAP-25 and syntaxin reside on the target membrane whilst other components (VAMP) reside in the membrane of the free cytoplasmic vesicle. Soluble cytosolic proteins, the ATPase N-ethylmaleimide-sensitive factor (NSF) and NSF attachment protein (α-SNAP), bind with syntaxin and SNAP-25 to mediate the docking of free vesicles to target membranes.

Brain capillary endothelial cells comprise the cellular basis for the blood brain barrier (BBB), and are specialised to limit the passive brain permeation of hydrophillic or macromolecular solutes. This is achieved to a greater extent by the lack within blood brain endothelium of fenestrations, and the presence of restrictive inter-cellular tight junctional complexes. It is generally considered that capillary endothelial cells in the brain possess relatively fewer transport vesicles than present within peripheral capillary endothelial cells. Nevertheless, the vesicular delivery of essential nutrients across the BBB to the central nervous system must be maintained, and selective transcytotic pathways are clearly functional within the BBB, although evidence that caveolae fulfil such a role remains limited. An ultrastructural study in the mid-70's (82) provided a morphometric analysis of caveolae within cerebral cortex endothelial cells of the rat, and with tracer techniques observed internalisation within these caveolae structures of horseradish peroxidase that had been administered intravenously. Using *in-vitro* cultured bovine brain capillary endothelial cells Dehouck and coworkers (83) demonstrated a receptor-mediated transcytotic pathway for low density lipoprotein (LDL) that was distinct from that of LDL internalisation and receptor recycling mediated via clathrin-coated pits. These investigators noted the presence of caveolae structures in their cultured endothelial cells and that the transcytosis of LDL could be inhibited by filipin, a cholesterol binding agent disassembling caveolae and shown by the investigators to be without effects upon clathrin pathways.

One of the most important transporters within the BBB is P-glycoprotein (P-gp), present at high concentrations within brain capillaries and mediating the efflux of a range of lipophillic molecules out from the brain capillary endothelial cell back into blood; in fulfilling this task P-gp serves as a functional element of the barrier. Intriguingly, a very recent report has stated that P-gp contains a caveolin-1 binding motif (84) raising the possibility that caveolin-1 and P-gp are involved in a functional interaction. With further work to substantiate an actual enrichment of P-gp within purified caveolae membrane from the BBB then a basis for investigating the role of caveolin in regulating this aspect of BBB function will have been provided.

Alveolar Epithelial Transport

The potential for exploitation of the pulmonary route for the systemic delivery of macromolecule therapeutics, particularly recombinant proteins and polypeptides, is increasingly realised (85). Anatomical and biochemical determinants indicate that for significant systemic absorption, particularly for macromolecules, the alveolar epithelium would appear the most appropriate absorption surface to target. Indeed, further substantiating the role of the lung periphery in systemic absorption is experimental data showing the extent of protein absorption to positively correlate with the depth of solute deposition within the lung (86,87). As a corollary the mechanisms of macromolecule transfer across alveolar epithelium are the subject of intense investigation.

Alveolar epithelium is predominantly comprised of two cell types, the squamous alveolar epithelial type I cell which constitutes approximately 93% of alveolar epithelial surface area (88), and the surfactant producing cuboidal alveolar epithelial type II cell. The alveolar epithelial type I cell contains numerous morphologically characteristic omega-shaped plasmalemmal invaginations (69) (Figure 7). We have recently (13) confirmed biochemically that these alveolar epithelial type I cell invaginations immunolabel with caveolin-1 and therefore satisfy a biochemical definition of caveolae. The numerical densities of what are now confirmed to be caveolae within the alveolar epithelial type I cells have been variously determined at 145–260 per μm^3 cell volume through to 150–250 per μm^2 cell surface (89,90). Given the dimensions of the human alveolar epithelial type I cell (88) the above determinations would estimate there to be $> 0.4 \times 10^6$ caveolae per alveolar type I cell.

Speculation has focussed upon alveolar epithelial type I cell caveolae as a transcytotic pathway for the absorption of therapeutic proteins (91), especially given the numerical densities of caveolae within this cell type and the very thin nature of the alveolar epithelial type I cell itself. However, due to anatomic complexity only limited experimental data in organised lung tissue is available. Investigators have attempted to examine caveolae-mediated trafficking within intact lung tissue using electron microscopic techniques with gold-labelled macromolecule probes (see review 91), but there is little substantive evidence showing a direct association of probe within caveolae. Atwal and co-workers (92) demonstrated an association of cationic ferritin with plasmalemmal vesicles of the luminal membrane of alveolar epithelial type I cells following instillation into the lung of a goat via a bronchoscope. Tracer was also evident on the abluminal front of the alveolar epithelial type I cells, suggestive of vesicular transcytotic activity. However, the interpretation of such electron micrographs can be imprecise, especially with the realisation that dynamic membrane events such as vesicle internalisation and trafficking will continue for some time (up to 9 seconds) after the initiation of tissue fixation (41). Nevertheless, given the nature of the alveolar lining fluid and what is known about caveolae transport in other tissues and cell types, it would be surprising if caveolae in alveolar epithelial type I cells did not fulfill a trans-alveolar macromolecule trafficking function. As mentioned in the previous section, the SNARE protein, VAMP-2, has been functionally localised to caveolar mem-

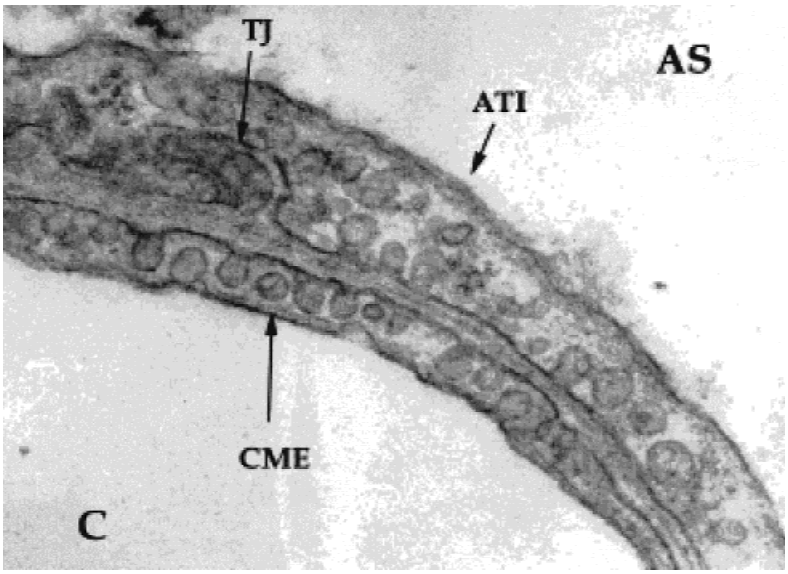

Fig. 7. Electron micrograph of the alveolar –capillary barrier in the rat lung, showing 'flask-shaped' caveolae invaginations and free cytosolic vesicles within both capillary microvascular endothelial (CME) cells and alveolar type I epithelial (ATI) cells. AS = alveolar airspace, C = capillary lumen and TJ = Tight Junction.

branes of rat lung microvascular endothelium (79). The same paper has reported on the level of VAMP-2 specific colloidal gold label associated with the alveolar type I epithelial cells within intact lung tissue. Compared to pulmonary microvascular endothelium the immunostaining in the alveolar type I epithelial cell was significantly reduced even when differences in caveolae density between the two cell types are taken into consideration. While this data would not exclude the caveolae of the type I epithelial cell from being dynamic entities, able to detach from a plasma membrane location it raises the need to characterise the molecular transport machinary within particular cell types, for example, caveolae (or subpopulations thereof) within alveolar type I epithelial cells may have a reduced requirement for VAMP-2, or functionally utilise other distinct VAMP related molecules.

Since an established role of caveolae in the transport of potential inhaled therapeutic macromolecules across the alveolar epithelium remains to be fully determined, clues to this function may be gained from the study of caveolae in cell types of non-alveolar lineage. Insulin is perhaps the most studied protein therapeutic in regard to pulmonary absorption, the insulin receptor has been localised to caveolae in endothelial (76) and adipocyte (93) cell types, although the insulin receptor can also undergo dynamic transfer between different membrane domains, including clathrin coated pits. Schnitzer and colleagues (38) have shown receptor-mediated endothelial transcytosis of insulin to be undertaken, at least in part, by caveolae. Using immunocytochemistry we have recently shown the presence of insulin receptor on the apical surface of the alveolar type I cells *in-vivo* (unpublished). Recombinant human growth hormone (rhGH) is another protein molecule whose delivery via the lung has been investigated. In Chinese hamster ovary cells bearing recombinant rhGH-receptor, a component in the internalisation of rhGH has been shown to be mediated via caveolae (94). The kinetics of rhGH internalisation displayed a bi-phasic response which consisted of a relatively more rapid initial period of internalisation (5–15min), followed by a slower uptake phase. The inhibitor for caveolae formation, the sterol binding agent fillipin, reduced internalisation during the slower component of uptake only. The mechanisms by which certain chemokines penetrate the alveolar barrier to stimulate systemic granulocytes is of interest to both pharmaceutical and biomedical science disciplines. Recombinant human granulocyte colony stimulating factor (G-CSF) is a 18.8 kDa polypeptide haemopoietic growth factor known to regulate the systemic production of polymorphonuclear leukocytes. Niven and co-workers (95) have examined the pharmacological response and pharmacokinetics of G-CSF in hamsters following intratracheal administration. The study concluded that the absorption of G-CSF is both rapid and extensive, and led the authors to speculate on the involvement of the caveolae system in its absorption across the pulmonary epithelium. To date this speculation has not been tested, although the G-CSF receptor is expressed in human alveolar epithelial type I cells (unpublished data) but its role in transport has yet to be assessed. However, the chemokine, interleukin-8 (IL-8), has been shown to be internalised and transcytosed by caveolae in dermal endothelial cells (96), and indeed the Duffy antigen, a broad spectrum scavenging receptor for chemokines including IL-8, has been localised to the caveolae within alveolar epithelium (97).

The complex nature of the lung architecture means that the alveolar epithelium is not readily accessible. Therefore the use of cultures of alveolar cells as a reliable *in-vitro* experimental model for the prediction of the extent, rate and mechanism of alveolar absorption of pharmaceuticals has gained acceptance amongst investigators (98). Specifically, the use of primary cultures of isolated alveolar epithelial type II cells which, when grown over a 5–8 day period on semipermeable polycarbonate membranes, generate monolayers with high transepithelial resistance (>1000 $\Omega \cdot cm^2$) resembling that of the *in-vivo* pulmonary barrier. Many lines of evidence indicate that isolated alveolar epithelial type II cells with time in culture transdifferentiate, losing their characteristic alveolar type II phenotype, coupled with the acquisition of certain morphological and biochemical markers distinctive of the *in-vivo* alveolar epithelial type I cell. With further characterisation, the alveolar type I-like monolayers may provide for a suitable *in-vitro* model system to examine the *in-vitro* alveolar transport of potentially therapeutic molecules. In this regard we have recently shown (30) in alveolar epithelial type I-like cell monolayers that the expression of caveolin-1 and the biogenesis of caveolae is evident as a function of the transdifferentiation process, and indeed such expression and caveolae biogenesis is upregulated during the transdifferentiation process by the presence of dexamethasone in the culture media (99). Intriguingly, in an *in-vivo* study it has been shown that the clearance of an isomolar albumin (5%) solution from the alveolar region of adult rat lung is 80% greater in groups that have been pre-treated with dexamethasone for 48 hr prior to instillation (100). Based upon these two observations one could hypothesise that that glucocorticoid treatment for acute lung injury may convey its therapeutic benefit in part through the upregulation of caveolar dynamics within alveolar type I epithelial cells, leading to the removal of oedematous fluid from the alveolar region. Ultimately a better understanding of the dynamics of caveolae in alveolar epithelium should lead to more specific drug targeting approaches for promoting pulmonary delivery.

Caveolae Mediated Delivery of Gene Based Therapeutics

With increasing knowledge about the genetic basis of human disease the employment of DNA-based therapies, including the use of somatic gene transfer or of single stranded oligonucleotide technology, would appear to offer enormous potential. However, efficient intracellular delivery of such DNA - based therapeutics is at present a major limitation in their full exploitation. Surprisingly, few studies have specifically addressed the role that caveolae may fulfill in the internalisation and cellular trafficking of potential DNA therapeutics. This is particularly unexpected with regard to gene delivery to the *in-vivo* endothelial cell, and especially the microvascular endothelial cell, which is generally acknowledged as possessing a high relative numerical density of caveolae and would offer potential in a broad range of therapeutic programmes, including for example, cancer, chronic inflammatory disease or cardiovascular and cerebrovascular disease.

Although no direct study has been reported of endothelial cell uptake of DNA species by caveolae, the work of Thurston and co-workers (101) (primarily showing that angiogenic blood vessels avidly bind cationic liposomes and li-

posome-DNA complexes) does provide some morphological evidence of an association of gold-labelled cationic liposome with caveolae structures. However, the role of caveolae in mediating the intracellular delivery of the gene vectors was not specifically addressed. Similarly, some *in-vivo* morphological data (102) has described an association between plasmid DNA and the caveolae of mammalian (rat) skeletal muscle cells following the intra-muscular injection of uncomplexed plasmid DNA. However, while an association of plasmid DNA to caveolae membrane was observed, and reporter gene expression evident, the role of caveolae in mediating plasmid DNA internalisation could not be confirmed. Recent work (103) undertaken in MA104 cells (possessing high numbers of caveolae) has examined a role for caveolae-mediated transfection of plasmid DNA complexed with either cationic liposome or cationic liposome-protamine vectors. It was reported that co-incubation with the caveolae inhibitor, filipin, during the cell transfection period (duration 6 hr) resulted in significant reductions in the expression of the reporter gene, Green Fluorescent Protein (GFP). The filipin treatment was reported not to affect the physical characteristics (size, zeta potential) of the non-viral gene complexes nor the cell viability. Further, in another cell line, A549 (essentially devoid of caveolae), while GFP expression was significant it was not affected by filipin co-incubation. While this latter research represents only preliminary *in-vitro* data it nevertheless demonstrates that despite the restricted size of the caveolae structure they would appear capable of fulfilling a role in the internalisation of non-viral gene complexes, a role that would clearly be more important in cell types displaying a high numerical density of caveolae compared to other vesicle structures, if indeed caveolae mediate such internalisation.

The use of folate as a targeting ligand for exploiting receptor-mediated internalisation of potential therapeutic agents, including plasmid DNA and oligonucleotides, has been documented (53). However, as stated in an earlier section, while much evidence supports a role for caveolae in mediating folate potocytosis there exists little evidence for the involvement of either caveolae in folate-mediated endocytosis, or for the potocytosis cycle to deliver macromolecules to the cell cytoplasm. For example, while it has been reported (104) that serum albumin-folic acid conjugates are internalised by caveolae structures in KB cells, a distinction between internalisation via a folic acid-mediated, versus an albumin–mediated, endocytic pathway was not clear. Further, it is perceived that the potocytosis process, while largely dependent upon caveolae, provides a regulated pathway for the cytoplasmic delivery of low molecular species and is a process which may not involve caveolae fusion with other internal tubulo-vesicular structures (45). Nevertheless, full characterisation of the receptor populance within caveolae membrane domains of a particular cell type may pinpoint potential targeting strategies. Ultimately, selected peptides derived from the respective known natural ligands may be complexed with exogenous DNA or other pharmaceutical adjuvants to construct carriers that will facilitate enhanced membrane interactions and subsequent internalisation.

Ultrastructural studies have shown that the ganglioside GM1 is concentrated in the caveolae of epithelial cells (105). This ganglioside is considered the physiological receptor for the non-toxic pentameric B subunit of cholera toxin (CTB). The construction and targeting of lipid vesicles incorporating the CTB has recently been described (106). This would represent a strategy to be tested for the enhanced delivery of encapsulated gene constructs or antisense oligonucleotides to certain cells. During the last decade the use of simian virus 40 (SV40) derived vectors to deliver foreign genetic material and sustain transgene expression has proved to be effective. Recent *in-vitro* experiments have implicated caveolae in the exclusive internalisation of SV40 (6,107,108), an internalisation shown to be mediated by molecules of the class I major histocompatibility complex (MHC I). Data obtained from initial functional studies (108) has provided evidence to suggest that caveolae or caveolin containing complexes invoke the translocation of virus to the endoplasmic reticulum, either by a direct or indirect route, and which was modulated by the inhibitor for caveolae formation, nystatin. However, it is noted that such entry is appreciably slower (2–3 hr) than that for other viral strains that commandeer the classic clathrin coated pathway for internalisation. One postulated (6) mechanism of SV40 entry via caveolae involves the recruitment of caveolin to MHC I - rich plasma membrane domains following viral binding. Such caveolin recruitment may serve to invoke membrane invagination and further mobilise components of the transport machinery such as VAMPS and NSF in order to initiate vesicular uptake.

Thus there is todate little quantitative evidence showing caveolae to fulfill a role in mediating the uptake of DNA based therapeutics. However, it is clear that further detailed studies are required which will ultimately provide for a better understanding of potential for exploitation of the caveolae pathway in the internalisation and intracellular delivery of gene based medicines.

CONCLUSIONS

There has been an exponential growth in caveolae / caveolin research since the early 1990s. The caveolae membrane system comprises unique lipid and protein domains, and fulfills a role in a wide range of processes. Although the transport function of caveolae has long been considered, it is only through the molecular characterisation of caveolae that investigators have begun to understand the interaction of ligands with caveolae associated receptors, and the subsequent cellular trafficking pathways for caveolae vesicles. Study and exploitation of this pathway by membrane and drug delivery scientists will undoubtedly prove rewarding in the understanding of the transport and delivery optimisation of pharmaceuticals.

REFERENCES

1. A. Schlegel, D. Volonte, J. A. Engelman, F. Galbiati, P. Mehta, X.-L. Zhang, P. E Scherer, and M. P. Lisanti. Crowded little caves: Structure and function of caveolae. *Cell Signal* **10**:457–463 (1998).
2. J. Couet, S. Li, T. Okamoto, P. E. Scherer, and M. P. Lisanti. Molecular and cellular biology of caveolae: Paradoxes and plasticities. *Trends Cardiovasc. Med.* **7**:103–110 (1997).
3. P. W. Shaul and R. G. W. Anderson. Role of plasmalemmal vesicles in signal transduction. *Am. J. Physiol.* **275**:L843–L851 (1998).
4. R. G. W. Anderson. The caveolae membrane system. *Annu. Rev. Biochem.* **67**:199–225 (1998).
5. R. G. Parton, B. Joggerst, and K. Simons. Regulated internalisation of caveolae. *J. Cell Biol.* **127**:1199–1215 (1994).
6. R. G. Parton and M. Lindsey. Exploitation of major histocom-

patibility complex class I molecules and caveolae by simian virus 40. *Immunol. Rev.* **168**:23–31 (1999).
7. K. G. Rothberg, J. E. Heuser, W. C. Donzell, Y. Ying, J. R. Glenny, and R. G. W. Anderson. Caveolin, a protein component of caveolae membrane coats. *Cell* **68**:673–682 (1992).
8. K. Simons and E. Ikonen. Functional rafts in cell membranes. *Nature* **387**:569–572 (1997).
9. P. E. Scherer, Z. L. Tang, M. Chuns, M. Sargiacomo, H. F. Lodish, and M. P. Lisanti. Caveolin isoforms differ in their N-terminal protein sequence and subcellular distribution. *J. Biol. Chem.* **270**:16395–16401 (1995).
10. P. E. Scherer, T. Okamoto, M. Chun, I. Nishimoto, H. F. Lodish, and M. P. Lisanti. Identification, sequence, and expression of caveolin-2 defines a caveolin gene family. *Proc. Natl. Acad. Sci. USA* **93**:131–135 (1996).
11. Z.-L. Tang, P. E. Scherer, T. Okamoto, K. Song, C. Chu, D. S. Kohtz, I. Nishimoto, H. F. Lodish, and M. P. Lisanti. Molecular cloning of caveolin-3, a novel member of the caveolin gene family expressed predominantly in muscle. *J. Biol. Chem.* **271**:2255–2261 (1996).
12. S. Monier, R. G. Parton, F. Vogel, J. Behlke, A. Henske, and T. V. Kurzchalia. VIP21-caveolin, a membrane protein constituent of caveolae coat, oligomerizes *in-vivo* and *in-vitro*. *Mol. Biol. Cell* **6**:911–927 (1995).
13. G. R. Newman, L. Campbell, C. von Ruhland, B. Jasani, and M. Gumbleton. Caveolin and its cellular and subcellular immunolocalisation in lung alveolar epithelium: Implications for alveolar type I cell function. *Cell Tissue Res.* **295**:111–120 (1999).
14. K. S. Song, P. E. Scherer, Z.-L. Tang. T. Okamoto, S. Li, M. Chafel, C. Chu, D. S. Kohtz, and M. P. Lisanti. Expression of caveolin-3 in skeletal, cardiac and smooth muscle cells. *J. Biol. Chem.* **271**:15160–15165 (1996).
15. P. Schieffele, P. Verkade, A. M. Fra, H. Virta, K. Simons, and E. Ikonen. Caveolin-1 and -2 in the exocytic pathway of MDCK cells. *J. Cell Biol.* **140:**795–806 (1998).
16. T. Ikeza, H. Ueda, B. D. Trapp, K. Nishiyama, J. F. Sha, D. Volonte, F. Galbiati, A. L. Byrd, G. Bassell, H. Serizawa, W. S. Lane, M. P. Lisanti, and T. Okamoto. Affinity-purification and characterisation of cavoelins from the brain: Differential expression of caveolin-1, -2, and -3 in brain endothelial and astroglial cell types. *Brain Res.* **804**:177–192 (1998).
17. P. L. Cameron, J. W. Ruffin, R. Bollag, H. Rasmussen, and R. S. Cameron. Identification of caveolin and caveolin-related proteins in the brain. *J. Neurosci.* **17**:9520–9535 (1997).
18. F. Galbiati, D. Volonte, O. Gil, G. Zanazzi, J. L. Salzer, M. Sargiacomo, P. E. Scherer, J. A. Engelman, A. Schlegel, M. Parenti, T. Okamoto, and M. P. Lisanti. Expression of caveolin-1 and -2 in differentiating PC12 cells and dorsal root ganglion neurons: Caveolin-2 is upregulated in response to injury. *Proc. Natl. Acad. Sci. USA* **95:**10257–10262 (1998).
19. H. Sengelov, M. Voldstedlund, J. Vinten, and N. Borregaard. Human neutrophils are devoid of the integral membrane protein caveolin. J. Leukoc. Biol. **63**:563–6 (1998).
20. M. Hatanaka, T. Maeda, T. Ikemoto, H. Mori, T. Seya, and A. Shimizu. Expression of caveolin-1 in human T-cell leukemia cell lines. *Biochem. Biophys. Res. Comm.* **253**:382–387 (1998).
21. A. L. Kiss and H. J. Geuze. Caveolae can be alternative endocytic structures in elicited macrophages. *Eur. J. Cell Biol.* **73**:19–27 (1997).
22. P. E. Scherer, R. Y. Lewis, D. Volonte, J. A. Engelman, F. Galbiati, J. Couet, D. S. Kohtz, E. van Donselaar, and M. P. Lisanti. Cell-type and tissue-specific expression of caveolin-2. Caveolins 1 and 2 co-localize and form a stable heterooligomeric complex *in-vivo*. *J Biol. Chem.* **272**:29337–29346 (1997).
23. R. Mora, V. L. Bonilha, A. Marmorstein, P. E. Scherer, D. Brown, M. P. Lisanti, and E. Rodriguez-Boulan. Caveolin-2 localises to the Golgi complex but redistributes to plamsa membrane, caveolae and rafts when co-expressed with caveolin-1. *J. Biol. Chem.* **274**:25708–25717 (1999).
24. M. Sargiacomo, P. E. Scherer, Z.-L. Tang, E. Kubler, K. S. Song, M. C. Sanders, and M. P. Lisanti. Oligomeric structure of caveolin: Implications for caveolae membrane organisation. *Proc. Natl. Acad. Sci. USA* **92**:9407–9411 (1995).
25. K. S. Song, Z.-L.Tang, S. Li, and M. P. Lisanti. Mutational analysis of the properties of caveolin-1. *J. Biol. Chem.* **272**:4398–4403 (1997).
26. M. Murata, J. Peranen, R. Schreiner, F. Wieland, T. V. Kurzchalia, and K. Simons. VIP21/caveolin is a cholesterol-binding protein. *Proc. Natl. Acad. Sci. USA* **92**:10339–10343 (1995).
27. S. Monier, D. J. Dietzen, W. R. Hastings, D. M. Lublin, and T. V. Kurzchalia. Oligomerisation of VIP21-caveolin *in-vitro* is stabilized by long chain fatty acylation or cholesterol. *FEBS Lett.* **388**:143–149 (1996).
28. S. Li, F. Galbiati, D. Volonte, M. Sargiacomo, J. A. Engelman, K. Das, P. E. Scherer, and M. P. Lisanti. Mutational analysis of caveolin-induced vesicle formation. *FEBS Lett.* **434**:127–134 (1998).
29. P. E.Scherer, M. P. Lisanti, G. Baldini, M. Sargiacomo, C. C. Mastick, and H. F. Lodish. Induction of caveolin during adipogenesis and association of GLUT4 with caveolin-rich vesicles. *J. Cell. Biol.* **127**:1233–1243 (1994).
30. L. Campbell, A. J. Hollins, A. Al-Eid, G. R. Newman, C. von Ruhland, and M. Gumbleton. Caveolin-1 expresion and caveolae biogenesis during cell transdifferentiation in lung alveolar epithelial primary cultures. *Biochem. Biophys. Res. Comm.* **262**:744–751 (1999).
31. A. J. Koleske, D. Baltimore, and M. P. Lisanti. Reduction of caveolin and caveolae in oncogenically transformed cells. *Proc. Natl. Acad. Sci. USA*. **92**:1381–1385 (1995).
32. A. M. Fra, E. Williamson, K. Simons, and R. G. Parton. De novo formation of caveolae in lymphocytes by expression of VIP21-caveolin. *Cell Biol.* **92**:8655–8659 (1995).
33. S. Li, K. S. Song, S. S. Koh, A. Kikuchi, and M. P. Lisanti. Baculovirus-based expression of mammalian caveolin in Sf21 insect cells. *J. Biol. Chem.* **271**:28647–28654 (1996).
34. U. Vogel, K. Sandvig, and B. van Deurs. Expression of caveolin-1 and polarised formation of invaginated caveolae in Caco-2 and MDCK II cells. *J. Cell Sci.* **111**:825–832 (1998).
35. E. J. Smart, Y.-S Ying, P. A. Conrad, and R. G. W. Anderson. Caveolin moves from caveolae to Golgi apparatus in response to cholesterol oxidation. *J. Cell Biol.* **127**:1185–1197 (1994).
36. P. Liu., W.-P. Li, T. Machleidt, and R. G. W. Anderson. Identification of caveolin-1 in lipoprotein particles secreted by exocrine cells. *Nature Cell Biol.* **1**:369–375 (1999).
37. E. J.Field, E. Born, S. Murphy, and S. N. Mather. Caveolin is present in intestinal cells: role in cholesterol trafficking. *J. Lipid Res.* **39**:1938–1950 (1998).
38. J. E. Schnitzer, P. Oh, E. Pinney, and J. Allard. Fillipin-sensitive caveolae-mediated transport in endothelium: Reduced transcytosis, scavenger endocytosis and capillary permeability of select macromolecules. *J. Cell Biol.* **127**:1217–1232 (1994).
39. E. J. Smart, K. Estes, and R. G. W. Anderson. Inhibitors that block both the internalization of caveolae and the return of plasmalemmal vesicles. *Cold Spring Harbor Symp. Quant. Biol.* LX, 243–248 (1995).
40. E. J. Smart, Y.-S. Ying, W. C. Donzell, and R. G. W. Anderson. A role for caveolin in transport of cholesterol from endoplasmic reticulum to plasma membrane. *J. Biol. Chem.* **271**:29427–29435 (1996).
41. D. Predescu, R. Horvat, S. Predescu, and G. Palade. Transcytosis in the continuous endothelium of the myocardial microvasculature is inhibited by N-ethylmaleimide. *Proc. Natl. Acad. Sci. USA* **91**:3014–3018 (1994).
42. J. E. Schnitzer, J. Allard, and P. Oh. NEM inhibits transcytosis, endocytosis, and capillary permeability: implication of caveolae fusion in endothelia. *Am. J. Physiol.* **268**:H48–H55 (1995).
43. T. Machleidt, W.-P. Li, P. Liu, and R. G. W. Anderson. Multiple domains in caveolin-1 control its intracellular traffic. *J. Cell Biol.* **148**:17–28 (2000).
44. R. Luetterforst, E. Stang, N. Zorzi, A. Carozzi, M. Way, and R. G. Parton. Molecular characterisation of caveolin association with the Golgi Complex: Identification of a Cis-Golgi targeting domain in the caveolin molecule. *J. Cell Biol.* **145**:1443–1459 (1999).
45. R. G. W. Anderson, B. A. Kamen, K. G. Rothberg, and S. W. Lacey. Potocytosis: Sequestration and transport of small molecules by caveolae. *Science* **255**:410–411 (1992).
46. R. G. W. Anderson. Caveolae: Where incoming and outgoing

messengers meet. *Proc. Natl. Acad. Sci. USA* **90**:10909–10913 (1993).
47. K. A. Rothberg, Y.-S. Ying, B. A. Kamen, and R. G. W. Anderson. Cholesterol controls the clustering of the glycophospholipid-anchored membrane receptor for 5-methyltetrahydrofolate. *J. Cell Biol.* **111**:2931–2938 (1990).
48. W.-J. Chang, K. G. Rothberg, B. A. Kamen, and R. G. W. Anderson. Lowering the Cholesterol content of MA104 cells inhibits receptor-mediated transport of folate. *J. Cell Biol.* **118**: 63–69 (1992).
49. E. J. Smart, C. Mineo, and R. G. W. Anderson. Clustered folate receptors deliver 5-methyltetrahydrofolate to cytoplasm of MA104 cells. *J. Cell Biol.* **134**:1169–1177 (1996).
50. B. A. Kamen, A. K. Smith, and R. G. W. Anderson. The folate receptor works in tandem with a probenecid-sensitive anion carrier. *J. Clin. Invest.* **84**:1442–1449 (1991).
51. C. Mineo and R. G. W. Anderson. A vacuolar proton ATPase mediates acidification of plasmalemmal vesicles during potocytosis. *Exp. Cell Res.* **224**:237–242 (1996).
52. E. J.Smart, Y. S. Ying, and R. G. Anderson. Hormonal regulation of caveolae internalisation. *J. Cell Biol.* **131**:929–938 (1995).
53. S.Wang and P. S. Low. Folate-mediated targeting of antineoplastic drugs, imaging agents, and nucleic acids to cancer cells. *J. Controlled Rel.* **53**:39–48 (1998).
54. P. E. Fielding and C. J. Fielding. Intracellular transport of low density lipoprotein derived cholesterol begins at clathrin coated pits and terminates at cell surface caveolae. *Biochemistry* **35**: 14932–14938 (1996).
55. C. J. Fielding and P. E. Fielding. Intracellular cholesterol transport. *J. Lipid. Res.* **38**:1503–1521 (1997).
56. P. E. Fielding and C. J. Fielding. Plasma membrane caveolae mediate the efflux of cellular free cholesterol. *Biochemistry* **34**: 14288–14292 (1995).
57. J. Thyberg, F. Calara, P. Dimayuga, J. Nilsson, and J. Regnstrom. Role of caveolae in cholesterol transport in arterial smooth muscle cells exposed to lipoproteins *in-vitro* and *in-vivo*. *Lab. Invest.* **78**:825–837 (1998).
58. A. Uittenbogaard, Y.-S. Ying, and E. J. Smart. Characterization of a cytosolic heat-shock protein-caveolin chaperone complex. *J. Biol. Chem.* **273**:6525–6532 (1998).
59. J. C. Fielding, A. Bist, and P. E. Fielding. Caveolin mRNA levels are up-regulated by free cholesterol and down-regulated by oxysterols in fibroblast monolayers. *Proc. Natl. Acad. Sci. USA.* **94**:3753–3758 (1997).
60. D. Hailstone, L. S. Sleer, R. G. Parton, and K. K. Stanley. Regulation of caveolin and caveolae by cholesterol in MDCK cells. *J. Lipid. Res.* **39**:369–379 (1998).
61. A. Bist, P. E. Fielding, and C. J. Fielding. Two sterol regulatory element-like sequences mediate up-regulation of caveolin gene transcription in response to low density lipoprotein free cholesterol. *Proc. Natl. Acad. Sci. USA.* **94**:10693–10698 (1997).
62. J. C. Fielding, A. Bist, and P. E. Fielding. Intracellular cholsterol transport in synchronised human skin fibroblasts. *Biochemistry* **38**:2506–2513 (1999).
63. S. Matveev, D. R. van der Westhuyzen, and E. J. Smart. Co-expression of scavenger receptor-BI and caveolin-1 is associated with enhanced selective cholesteryl ester uptake in THP-1 macrophages. *J. Lipid Res.* **40**:1647–1654 (1999).
64. G. A. Graf, P. M. Connell, D. R. van der Westhuyzen, and E. J. Smart. The class B, type I scavenger receptor promotes the selective uptake of high density lipoprotein cholesterol ethers into caveolae. *J. Biol. Chem.* **274**:12043–12048 (1999).
65. C. Mirre, L. Monlauzeur, M. Garcia, M. H. Delgrossi, and A. Le Bivic. Detergent-resistant microdomains from Caco-2 cells do not contain caveolin. *Am. J. Physiol.* **271**:C887–C894 (1996).
66. Y. Lavie, H. Cao, S. L. Bursten, A. E. Giuliano, and M. C. Cabot. Accumulation of glucosylceramides in multidrug-resistant cancer cells. J. Biol. Chem. **271**:19530–19536 (1996).
67. Y.Lavie, G. Fiucci, and M. Liscovitch. Up-regulation of caveolar constituents in multidrug-resistant cancer cells. *J. Biol. Chem.* **273**:32380–32383 (1998).
68. C.-P. H.Yang, F. Galbiati, D. Volonte, S. B. Horwitz, and M. P. Lisanti. Upregulation of caveolin-1 and caveolae organelles in Taxol-resistant A549 cells. *FEBS Lett.* **439**:368–372 (1998).
69. J. Gil. Number and distribution of plasmalemma vesicles in the lung. *Fed. Proc.* **42**:2414–2418 (1983).
70. M. Simionescu and N. Simionescu. Endothelial transport of macromolecules: Transcytosis and endocytosis. *Cell Biol. Rev.* **25**:1–80 (1991).
71. E. J. Smart, D. C. Foster, Y.-S. Ying, B. A. Kamen, and R. G. W. Anderson. Protein kinase C activators inhibit receptor-mediated potocytosis by preventing internalisation of caveolae. *J. Cell Biol.* **124**:307–313 (1994).
72. D. Predescu and G. Palade. Plasmalemmal vesicles represent the large pore system of continuous microvascular endothelium. *Am. J. Physiol.* **265**:H725–H733 (1993).
73. S. Predescu, D. Predescu, and G. Palade. Plasmalemmal vesicles function as transcytotic carriers for small proteins in the continuous endothelium. *Am. J. Physiol.* **272**:H937–H949 (1997).
74. C. C. Michel. Transport of macromolecules through microvascular walls. *Cardiovas. Res.* **32**:644–653 (1996).
75. C. Tirruppath, W. Song, M. Bergenfeld, P. Sass, and A.B. Malik. Gp60 activation mediates the albumin transcytosis in endothelial cell by tyrosine kinase-dependent pathway. *J. Biol. Chem.* **272**:25968–25975 (1997).
76. R. L. Roberts and A. Sandra. Receptor-meidated endocytosis of insulin by cultured endothelial cells. *Tissue. Cell* **24**:603–611 (1992).
77. M. Linial. SNARE Proteins—Why so many, Why so few? *J. Neurochem.* **69**:1781–1792 (1997).
78. J.E. Schnitzer, J. Liu, and P. Oh. Endothelial caveolae have the molecular transport machinery for vesicle budding, docking, and fusion including VAMP, NSF, SNAP, annexins, and GTPases. *J. Biol. Chem.* **24**:14399–14404 (1995).
79. D. P. McIntosh and J. E. Schnitzer. Caveolae require intact VAMP for targeted transport in vascular endothelium. *Am. J. Physiol.* **277**:H2222–H2232 (1999).
80. P. Oh, D. P. McIntosh, and J. E. Schnitzer. Dynamin at the neck of caveolae mediates their budding to form transport vesicles by GTP-driven fission from the plasma membrane of endothelium. *J. Cell Biol.* **141**:101–114 (1998).
81. G. J. Steel, M. Tagaya, and P. G. Woodman. Association of the fusion protein NSF with clathrin-coated vesicle membranes. *EMBO J.* **15**:745–752 (1996).
82. H. Reyners, E. G. Reyners, J. M. Jadin, and J. R. Maisin. An ultrastructural quantitative method for the evaluation of the permeability to horseradish peroxidase of cerebral cortex endothelial cells of the rat. *Cell Tiss. Res.* **157**:93–99 (1975).
83. B. Dehouck, L. Fenart, M. P. Dehouck, A. Pierce, G. Torpier, and R. Cecchelli. A new function for the LDL-receptor: Transcytosis of LDL across the blood brain barrier. *J. Biol. Chem.* **138**:877–889 (1997).
84. M. Demeule, J. Jodoin, D. Gingras, and R. Beliveau. P-glycoprotein is localised in caveolae in resistant cells and in brain capillaries. *FEBS Lett.* **466**:219–224 (2000).
85. J. S. Patton. Breathing life into protein drugs. *Nat. Biotechnol.* **16**:141–143 (1998).
86. P. Colthorpe, S. J. Farr, G. Taylor, I. J. Smith, and D. Wyatt. The pharmacokinetics of pulmonary delivered insulin: A comparison of intra-tracheal and aersol administration to the rabbit. *Pharm. Res.* **9**:765–768 (1992).
87. P. Colthorpe, S. J. Farr, I. J. Smith, D. Wyatt, and G. Taylor. The influence of regional deposition on the pharmacokinetics of pulmonary-delivered human growth hormone in rabbits. *Pharm. Res.* **12**:356–359 (1995).
88. J. D. Crapo, B. E. Barry, P. Gehr, M. Bachofen, and E. R. Weibel. Cell number and cell characteristics of normal human lung. *Am. Rev. Respir. Dis.* **125**:332–337 (1982).
89. D. O. Defouw and F. P. Chinard. Numerical densities of cellular vesicles in pulmonary alveolar septa of edematous dog lungs. *Microvasc. Res.* **23**:248 (1982).
90. J. Gil, D. A. Silage, and J. M. McNiff. Distribution of vesicles in cells of the air-blood barrier in the rabbit. *J. Appl. Physiol.* **50**:334–340 (1981).
91. J. S. Patton. Mechanisms of macromolecule absorption by the lungs. *Adv. Drug Deliv. Rev.* **19**:3–36 (1996).
92. O. S. Atwal, L. Viel, and K. J. Minhas. An uptake of cationized ferritin by alveolar type I cells in airway-instilled goat lung:

distribution of anionic sites on the epithelial surface. *J. Submicrosc. Cytol. Pathol.* **22**:425–432 (1990).
93. R. I. Goldberg, R. M. Smith, and L. Jarrett. Insulin and α_2-macroglobulin-methylamine undergo endocytosis by different mechanisms in rat adipocytes: Comparison of cell surface events. *J. Cell. Physiol.* **133**:203–212 (1987).
94. P. E. Lobie, R. Sadir, R. Graichen, H. C. Mertani, and M. Morel. Caveolar internalisation of growth hormone. *Exp. Cell Res.* **246**: 47–55 (1999).
95. R. W. Niven, F. D. Lott, and J. M. Cribbs. Pulmonary absorption of recombinant methionyl human granulocyte colony stimulating factor after intratracheal instillation to hamster. *Pharm. Res.* **10**:1604–1610 (1993).
96. J. Middleton, S. Neil, J. Wintle, I. Clarke-Lewis, H.Moore, C. Lam, M. Auer, E. Hub, and A. Rot. Transcytosis and surface presentation of IL-8 by venular endothelial cells. *Cell* **91**:385–395 (1997).
97. A. Chaudhuri, S. Nielsen, M.-L. Elkjaer, V. Zbrzezna, F. Fang, and A. O. Pogo. Detection of duffy antigen in the plasma membranes and caveolae of vascular endothelial and epithelial cells of nonerythroid organs. *Blood* **89**:701–712 (1997).
98. N. R. Mathias, F. Yamashita, and V. H. L. Lee. Respiratory epithelial cell culture models for evaluation of ion and drug transport. *Adv. Drug Deliv. Rev.* **22**:215–249 (1996)
99. A. J. Hollins, A. El-Aid, L. Campbell, and M. Gumbleton Effect of glucocorticoid upon cell differentiation within an alveolar epithelial cell culture used to model pulmonary absorption *in-vitro. Pharm. Sci.* **1**: (abtsract suppl.) 2629, S-257 (1999).
100. H. G. Folkesson, A. Norlin, Y.Wang, P. Abedinpour, and M. A. Matthay. Dexamethasone and thyroid hormone pretreatment upregulate alveolar fluid clearance in adult rats. *J. Appl. Physiol.* **88**:416–424 (2000).
101. G. Thurston, J. W. McLean, M. Rizen, P.Baluk, A. Haskell, T. J. Murphy, D. Hanahan, and D. M. McDonald. Cationic liposomes target angiogenic endothelial cells on tumors and chronic inflammation in mice. *J. Clin. Invest.* **101**:1401–13 (1998).
102. J. A. Wolff, M. E. Dowty, S. Jiao, G. Repetto, R. K. Berg, J. J. Ludtke, P. Williams, and D. B. Slautterback. Expression of naked plasmids by cultured myotubes and entry of plasmids into T tubules and caveolae of mammalian skeletal muscle. *J. Cell Sci.* **103**:1249–1259 (1992).
103. S. Al-Mousa, P. J. Nicholls, and M. Gumbleton. Evidence for the role of caveolae in gene delivery. *J. Pharm. Pharmacol.* **51**:(abstract suppl.) #178 (1999).
104. J. J. Turek, C. P. Leamon, and P.S. Low. Endocytosis of folate-protein conjugates: Ultrastructural localisation in KB cells. *J. Cell Sci.* **106**:423–430 (1993).
105. R. G. Parton. Ultrastructural localisation of Gangliosides; GM_1 is concentrated in caveolae. *J. Histochem. Cytochem.* **42**:155–166 (1994).
106. T. Lian and R. J. Y. Ho. Cholera toxin B-mediated targeting of lipid vesicles containing ganglioside GM1 to mucosal epithelial cells. *Pharm. Res.* **14**:1309–1315 (1997).
107. E. Stang, J. Kartenbeck, and R. G. Parton Major histocompatibility complex class I molecules mediate association of SV40 with caveolae. *Mol. Biol. Cell* **8**:47–57 (1997).
108. H. A. Anderson, Y. Chen, and L. C. Norkin. Bound simian virus 40 translocates to caveolin-enriched domains, and its entry is inhibited by drugs that selectively disrupt caveolae. *Mol. Biol. Cell* **7**:1825–1834 (1996).