Regulation of caveolin and caveolae by cholesterol in MDCK cells

Deborah Hailstones,* Leanne S. Sleer,* Robert G. Parton,† and Keith K. Stanley‡,*

The Heart Research Institute,* 145 Missenden Road, Camperdown NSW 2050, Sydney, Australia, and Centre for Microscopy and Microanalysis† Department of Physiology and Pharmacology, and Centre for Molecular and Cellular Biology, University of Queensland, QLD 4092, Brisbane, Australia

Abstract  We have examined the expression of caveolin in MDCK cells under conditions that vary cellular cholesterol concentration. Caveolin mRNA levels dropped to one-sixth of control levels after treatment with simvastatin, an inhibitor of cholesterol synthesis, or β-trimethyl cyclodextrin (CD), a cholesterol sequestering drug. Both simvastatin and CD treatment decreased total cellular cholesterol levels to about 50% of control values. The potent activator of the sterol regulatory element, 25-hydroxycholesterol, showed no direct regulation of caveolin mRNA levels. Caveolin protein concentration was also decreased to 50% of control values in cholesterol-depleted cells, giving rise to a severe attenuation of caveolin expression detected by indirect immunofluorescence labeling. Quantitative electron microscopy showed a total loss of morphologically recognizable invaginated caveolae after these cholesterol depletion treatments. When the number of invaginated caveolae per cell was expressed as a function of the cellular cholesterol content, a threshold phenomenon was observed, suggesting that caveolae only form when the steady state cellular cholesterol is above 50% of control values. These findings indicate that caveolins, and caveolae, may play an important part in cellular cholesterol homeostasis.—Hailstones, D., L. S. Sleer, R. G. Parton, and K. K. Stanley. Regulation of caveolin and caveolae by cholesterol in MDCK cells. J. Lipid Res. 1998. 39:369–379.

Supplementary key words  caveolae • caveolin • cyclodextrin • 25-hydroxycholesterol • simvastatin

Caveolae are distinctive, flask-shaped invaginations of the plasma membrane found in many cells. They have a characteristic lipid composition, rich in cholesterol and gangliosides (1–3), and are associated with the presence of a 22 kD protein called caveolin. Caveolin has an unusual membrane topography with both the amino and carboxy terminus exposed to the cytoplasmic face of the caveolar membrane, and a long hydrophobic segment of 33 amino acids (4). In addition, it is palmitoylated on cysteine residues in the C-terminal domain of the protein (5, 6). Four isoforms of caveolin have been described: caveolin-1α and 1β are differentially spliced from a single gene (7); caveolin-2 is the product of a second gene that is often found expressed in the same cells as caveolin-1 (8); and caveolin-3 is encoded by a third gene that is predominantly expressed in muscle tissue (9, 10). Most cells contain one or more types of caveolin, and in those cells where caveolin has not been found it is possible that additional caveolin isoforms exist.

A close and interdependent relationship between cholesterol and caveolin has been observed for the formation of stable caveolae. Thus it has been shown that lymphocytes, which do not express any known isoform of caveolin, do not have morphologically recognizable caveolae (11). However, transfection of caveolin-1 causes de novo formation of caveolae (12). On the other hand, when fibroblasts were treated with the cholesterol-binding drug, filipin, the caveolae collapsed into the plasma membrane, suggesting a requirement for cholesterol to maintain the morphology of caveolae (13). A possible explanation for a need for both caveolin and cholesterol in the formation of invaginated caveolae is that caveolin is itself a cholesterol-binding protein (14). The presence of cholesterol promotes oligomerization of the protein (14–17) and these oligomers are likely to contribute to the striated coat seen around caveolae in electron micrographs (15).

Caveolin is also found in the trans Golgi network (TGN) of fibroblasts by immunofluorescence (4) and has been described as a component of apical and basolateral transport vesicles in Madin-Darby canine kidney (MDCK) cells (18). Furthermore, it has been reported

Abbreviations: CD, β-trimethyl cyclodextrin; HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; MDCK, Madin-Darby canine kidney cells; 25-OHC, 25-hydroxycholesterol; SREBP, sterol regulatory element binding protein.

1To whom correspondence should be addressed.
that the intracellular distribution of caveolin can be experimentally manipulated in some cells by enzymatic oxidation of surface cholesterol (19) or by depolymerization of microtubules (20). These studies suggest that caveolin location is also dynamic within the cell and might have a role in cholesterol transport.

The function of caveolin is not known, although roles in the uptake of small solutes (13), transcytosis of lipoprotein particles across endothelial cells (21), and signal transduction (22, 23) have been proposed (24). In addition, it has been reported that exchange of cholesterol from exogenous low density lipoprotein (LDL) particles to fibroblasts results in selective transfer to caveolae (25). Newly synthesized cholesterol was also selectively removed from a caveolae fraction when [3H] mevalonate-labeled fibroblasts were incubated in plasma (25). Thus, understanding the mechanisms controlling the formation of caveolae might be of considerable importance in the reversal of atherosclerosis. In this paper we describe the control of caveolin expression by the level of endogenous cholesterol and a threshold effect of cellular cholesterol on the number of caveolae found per cell.

MATERIALS AND METHODS

Cell culture

Type II MDCK cells were routinely grown in complete Dulbecco’s modified Eagle’s medium (DMEM) + 10% fetal calf serum supplemented with 1-glutamine, penicillin, and streptomycin) in T75 flasks and were certified free of Mycoplasma contamination. Cells were passaged every 3 to 4 days at confluence, with feeding every 2 days. For cholesterol depletion experiments, confluent monolayers were first changed to appropriate media (complete DMEM (CM)), as above except with 10% human serum or lipoprotein-deficient serum (LPDS)-DMEM containing 10% lipoprotein-depleted human serum (26) with other additions as indicated. In all cases, parallel cultures were tested for cell viability by trypan blue exclusion and shown to be over 95%.

Preparation of RNA

Cells were seeded into 60-mm dishes for experiments where a preparation of total RNA was sufficient. A single dish was used for each condition, and all experiments were performed on at least three separate occasions. Results were the same in all cases. Total RNA was prepared using a modification of the guanidinium isothiocyanate method (27). Briefly, cells were washed twice in PBS and then lysed directly into denaturing solution containing guanidinium thiocyanate. This lysate was acidified with sodium acetate, extracted with acidified phenol and then chloroform and isoamyl alcohol, microfuged, and then total RNA was precipitated from isopropanol. The resulting pellet was resuspended in guanidinium thiocyanate and reproprecipitated from isopropanol. The final pellet contained between 20 and 60 μg of total RNA, depending on the experimental condition.

Northern blotting and hybridization

Northern blots and hybridizations were performed essentially as described (28). Briefly, RNA was electrophoresed on (formaldehyde-free) 1% agarose gels with 1 × MOPS running buffer. Samples were loaded in 1 × MOPS, 50% deionized formamide, 6% formaldehyde, and 5% glycerol. After electrophoresis, the gel was stained with ethidium bromide (presence of 28S, 18S and 5S ribosomal RNA (rRNA) bands indicating intact RNA) and then transferred to Hybond-N+ under vacuum, using alkaline conditions.

Caveolin blots were prehybridized for 4–24 h at 65°C, and then hybridized overnight at 65°C to the probe at 2 × 10^6 cpm per ml of hybridization buffer. Prehybridization and hybridization buffers were the same (4 × SSC (where 1 × SSC is 0.15 m NaCl and 0.015 m sodium citrate), 5 × Denhardt’s solution and 50 mm sodium phosphate, pH 7.0) except that hybridization buffer also contained 10% dextran sulfate. Blots were then washed twice in 0.5 × SSC, 0.1% SDS for 30 min at 65°C and then once in 0.2× SSC, 0.1% SDS for 30 min at 65°C. Washed blots were then exposed to a phosphorimage plate and read on a Fuji/Berthold 1500 phosphorimage analyzer.

Subsequently, total RNA blots were hybridized to a 30-mer oligonucleotide complimentary to the 18S rRNA to correct for loading and transfer errors. These hybridizations were performed in essentially the same manner, except that the hybridization was performed at 55°C and washes were performed in 4 × SSC, 0.1% SDS twice at room temperature and then once at 55°C. In addition, an excess of unlabeled 18S oligonucleotide was always added to the hybridization reaction to ensure that the probe was not limiting.

The probe for caveolin was a 670 bp EcoRI-Hpal fragment of the MDCK caveolin cDNA, originally described as VIP21 (18) but found to be identical to caveolin (29), which was primed with an antisense oligonucleotide corresponding to bp 610–635. It was labeled using the Pharmacia Ready-to-go kit, and was routinely labeled to a specific activity of better than 10^9 dpm per μg DNA.
Measurement of endogenous cholesterol concentration

Cellular cholesterol concentrations were measured by high performance liquid chromatography (HPLC) (30). Cells were seeded in quadruplicate for each experiment (generally performed on at least three separate occasions) and media were supplied as appropriate. At the conclusion of each experiment, cells were washed twice in PBS and then lysed in 200 mm sodium hydroxide. An aliquot of each sample was retained for protein estimation, the remainder was extracted into methanol and hexane (in the presence of the antioxidants: 20 μm butyl hydroxytoluene and 20 mm EDTA). The hexane fraction was then evaporated, resuspended, and the cholesterol was analyzed on a reverse phase C18 column with detection at 210 nm.

Electron microscopy

Cells were rinsed twice in PBS containing 0.1 mm CaCl₂ and 1 mm MgCl₂ at room temperature and then fixed in 2 ml of 2.5% glutaraldehyde in 50 mm cacodylate buffer, pH 7.35, containing 70 mm KCl, 1.2 mm CaCl₂, and 1.2 mm MgCl₂. After 1 h at room temperature, the cells were washed 4 times in 100 mm cacodylate buffer, pH 7.35, and then processed for epon embedding after removing the cells from the dishes using propylene oxide (31). After embedding and curing, 50- to 60-nm sections were prepared and counterstained with uranyl acetate and lead citrate. Sections were viewed in a Jeol 1010 electron microscope. The number of surface caveolae was quantitated in a blind fashion using a code that was only decoded after the completion of the analysis. Caveolae were defined as uncoated 55–65 nm surface invaginations clearly connected to the plasma membrane. Through necessity this definition excluded any caveolae connected to the cell surface by a neck that was not visible in the plane of section. The number of invaginated caveolae was estimated by counting the number of caveolar profiles per cell profile and over 50 cells were analyzed for each experiment. Results were confirmed in two independently processed cell cultures.

Immunofluorescence labeling

Cells were fixed and stained as described by Bos, Wraight, and Stanley (32) except that methanol-acetone 1:1 was used as fixative at −20°C for 10 min and 5% FCS in PBS was used as quench buffer. The caveolin polyclonal antiserum was purchased from Transduction Laboratories.

Western Blot

Cells were washed 3 × in PBS and then solubilized in 1 ml of 2% SDS. Proteins were separated on 12% polyacrylamide gels and then transferred to nitrocellulose paper. The caveolin was detected by Western blot using a 1:1000 dilution of polyclonal primary antibody (Transduction Laboratories) and 125I-labeled protein A. After 60 min incubation in the 125I-labeled protein A (0.1 μCi/ml), the filters were washed 3 × in PBS containing 0.02% Tween 20 and dried milk quenching protein, followed by one wash in PBS. The filters were then dried and quantitated in a phosphorimager. In some gels equal protein (80 μg) was loaded onto each lane. Alternatively, equal volumes were added and caveolin was related to the original protein concentration or cell density. Protein content was measured by the bicinchoninic acid method.

RESULTS

Caveolin-1, originally termed VIP-21, has been cloned from MDCK cells (18) where it has been shown to be present in surface caveolae and associated with transport vesicles leaving the trans Golgi network (4). We therefore used this well-characterized cell system to examine the regulation of caveolin expression by cholesterol.

Type II MDCK cells were grown in normal culture media containing 10% FCS until they reached confluence. The medium was then changed to DMEM containing 10% human serum with various additions, and the cells were grown for a further 3 days before extraction of lipids and cholesterol analysis by HPLC. In control cells, between 10 and 15 μg of cholesterol was detected per mg cell protein, with a mean = 13 ± 0.77 (SE) μg/mg (n = 8). In order to deplete cells of endogenous cholesterol, we incubated them for 3 days in LPDS-DMEM with the addition of either 25 μm simvastatin (an HMG-CoA reductase inhibitor; Fig. 1A, column 3), or overnight in LPDS-DMEM containing 1 mg/ml of β-trimethyl cyclodextrin (CD; Fig. 1A, column 5). Both treatments lowered the cellular cholesterol to between 40 and 65% of control levels, with slight variations between experiments. Treatment for longer periods of time with CD caused the cells to detach from the tissue culture dish; however, after all the treatments in Fig. 1, cell viability measured by trypan blue exclusion remained above 95%.

In a parallel experiment, the caveolin mRNA concentration was quantitated by Northern blot of total RNA using a caveolin 1 cDNA probe (Fig. 1, panel B). A single hybridizing band was found at approximately 2.3 kb, running in between the positions of 18S and 23S rRNA, consistent with the observation of Scherer et al. (8). Loading of RNA was determined by hybridization of the same blot with an 18S rRNA probe after...
waiting for the caveolin signal to decay (Fig. 1, panel C). Both Northern blots were quantitated on a phosphorimage analyzer and the ratio of caveolin:18S rRNA was taken as a measure of the level of expression of caveolin mRNA per cell (Fig. 1, panel D). It can be seen in Fig. 1D that the steady state level of caveolin mRNA decreased significantly as the cellular cholesterol was lowered. In the CD-treated cells (Fig. 1D, column 5), caveolin message was 6-fold decreased relative to cells grown in medium containing 10% human serum (Fig. 1D, column 1). This effect was not due to the CD itself, as a decrease of expression was also observed in LPDS medium containing simvastatin (Fig. 1D, column 3). Furthermore, when the CD was added to complete medium rather than LPDS medium, the cellular cholesterol was reduced to a lesser extent (Fig. 1A, column 4 compared with column 5) and the attenuation of caveolin mRNA was also inhibited (Fig. 1D, columns 4 and 5).

Sterols have previously been demonstrated to regulate the transcription of a number of genes important in cholesterol metabolism via the activation of sterol regulatory element binding proteins (SREBP) (33). We therefore examined the effect of 25-hydroxycholesterol (25-OHC), the most potent of SREBP activators (34), on caveolin mRNA expression. In Fig. 1A, column 2, it can be seen that addition of 25-OHC (0.5 \( \mu \)g/ml) and cholesterol (10 \( \mu \)g/ml) to the complete medium for 3 days caused an attenuation of caveolin mRNA similar to CD (Fig. 1D, column 2). The decrease in mRNA reflects a decrease in the amount of caveolin protein, as shown by Western blot analysis (Fig. 2).

Fig. 1. Caveolin expression in MDCK cells with decreasing cellular cholesterol. MDCK cells were incubated for 3 days in DMEM containing 10% human serum (complete medium, lane 1), complete medium containing 0.5 \( \mu \)g/ml 25-hydroxycholesterol (lane 2), LPDS-DMEM containing 25 \( \mu \)m simvastatin (lane 3), overnight treatment in complete medium containing 1 mg/ml \( \beta \)-trimethyl cyclodextrin (lane 4), or overnight treatment in LPDS-DMEM containing 1 mg/ml \( \beta \)-trimethyl cyclodextrin (lane 5). Panel A shows the mean and standard error of total cell cholesterol measurements from one experiment as percent of control, panel B a Northern blot with a caveolin I probe, panel C the same filter as used in panel B with an 18S rRNA probe, and panel D the quantitated intensity of the caveolin band in panel B divided by the intensity of the 18S band measured in panel C.

Fig. 2. Quantitation of caveolin protein in MDCK cells under cholesterol-lowering conditions by Western blot. Cells were treated as in Fig. 1, washed in PBS, and solubilized in SDS sample buffer. Eighty \( \mu \)g of protein was analyzed by SDS PAGE and Western blot. The mean and standard error of three determinations from one experiment are shown. Similar data were obtained in a second experiment. Control (column 1), cells grown in DMEM + 10% human serum; 25-OHC, cells grown in this medium with addition of 0.5 \( \mu \)g/ml 25-hydroxycholesterol and 10 \( \mu \)g/ml cholesterol for 3 days; simvastatin, cells grown in LPDS-DMEM containing 25 \( \mu \)m simvastatin for 3 days; LDL, cells grown in 10% human serum containing 0.2 mg/ml of purified human LDL. Symbols indicate significance relative to control, *\( P = 0.01 \), †\( P = 0.004 \), ‡\( P = 0.06 \).
days decreased total cellular cholesterol to 76% of control values. Treatment with 25-OHC reduced the caveolin expression to 77% of control values (Fig. 1D, column 2). Thus no effect of 25-OHC, other than via cellular cholesterol levels, on the steady state level of caveolin mRNA was detected.

The effect of cholesterol depletion on caveolin protein expression was determined by Western blot of cell
lysates. MDCK cells incubated under control and cholesterol-lowering conditions were washed in PBS, lysed in SDS PAGE sample buffer, and equal amounts of protein were loaded onto a 12% SDS gel. After Western transfer and immunoblot with a polyclonal antiserum, the 22 kD caveolin band was quantitated by densitometric scanning of the film. In Fig. 2 it can be seen that treatment with simvastatin in LPDS-DMEM medium for 3 days caused a 58% decrease in the expression of caveolin protein (Fig. 2, column 4). Despite the short treatment time, CD also caused a significant decrease in caveolin protein levels, to 51% of control levels after an overnight incubation (Fig. 2, column 3). 25-OHC had no detectable effect on caveolin protein expression in this experiment. Thus, decreasing the cellular cholesterol, using either simvastatin or CD, caused a significant decrease in both caveolin mRNA and protein levels. In order to study the effect of increased cholesterol on caveolin expression, we incubated cells in 10% human serum with the addition of 0.2 mg/ml of purified human LDL (Fig. 2, column 5). The cellular cholesterol increased only 9% under this treatment and gave a small increase in caveolin concentration (23%, $P = 0.06$).

Caveolin has been reported at two intracellular locations, in cholesterol-rich domains found in the Golgi and in caveolae at the cell surface. Indirect immunofluorescence staining of MDCK cells grown in

![Fig. 4]
medium containing 10% human serum showed intensely stained vesicular structures in a perinuclear region and a punctate distribution at the cell surface (Fig. 3A). After overnight treatment in LPDS-DMEM containing 1 mg/ml CD, both these patterns of fluorescence were severely attenuated (Fig. 3b). No staining was observed in the absence of the polyclonal anticyeolins antiserum (data not shown). This confirms the result by Western blot and shows that the decrease in caveolin concentration is caused by the down-regulation of caveolin protein within each cell. It appears that both surface and intracellular pools of caveolin are affected after an overnight treatment in CD medium.

Cells treated under cholesterol-depleting conditions were also examined by electron microscopy. Control cells (Fig. 4a) showed abundant basolateral invaginated caveolae. In contrast, after treatment with CD overnight (Fig. 4b) no invaginated caveolae remained, despite an otherwise normal morphology of the cells. A similar loss of invaginated caveolae was observed after cholesterol depletion using simvastatin in LPDS-DMEM medium. This enabled us to investigate the reappearance of caveolae as cells survive this condition well. After 3 days treatment with 25 µm simvastatin in LPDS-DMEM, a high concentration of mevalonate (2 mm) was added to the medium, bypassing the block in HMG-CoA synthesis, and allowing the cells to synthe-
size cholesterol. At times from 1 to 24 h we analyzed total cellular cholesterol (Fig. 5A), and the number of invaginated caveolae by a quantitative ultrastructural analysis (Fig. 5B). After simvastatin treatment, no invaginated caveolae were visible in the cells, as before. Invaginated caveolae first became visible after 4 h in 2 mm mevalonate, and increased in number until, at 24 h, approximately 22% of the number of caveolae seen in normal cells were observed. These caveolae showed the typical morphology of caveolae in control cells. In a separate experiment we measured the expression of caveolin as cells recovered from cholesterol depletion (Fig. 6). In this experiment the cholesterol levels recovered with kinetics similar to Fig. 5. Caveolin expression lagged slightly behind the rise in cholesterol concentration, reaching 60% of normal values at the 24 h time point.

The lack of morphologically identifiable caveolae in cells expressing 40-50% of normal caveolin levels suggested a threshold effect of cholesterol levels on caveolae number. To further investigate this, we devised an experiment in which cells grown in normal medium were transferred to LPDS-DMEM containing 25 μm simvastatin and different amounts (0, 20, 200, or 2000 μm) of mevalonate for 3 days. This resulted in steady state levels of total cellular cholesterol ranging from 54% of control values (no mevalonate added) to 70% of control values (2000 μm mevalonate added). This resulted in steady state levels of total cellular cholesterol ranging from 54% of control values (no mevalonate added) to 70% of control values (2000 μm mevalonate added). As previously determined, the number of caveolae in cholesterol-depleted cells with no mevalonate added was low (10% of control in this experiment) and increased to control values after the cellular cholesterol reached 66% of control values (Fig. 7A). When data from other experiments, where normal or depleted cholesterol was at steady state, were plotted on this graph, the same

Fig. 5. Caveolae formation in response to cholesterol synthesis in cholesterol-depleted MDCK cells. MDCK cells were treated for 3 days in LPDS-DMEM containing 25 μm simvastatin. Mevalonate (2 mm) was then added for the times shown and cells were analyzed for cholesterol (panel A) and the number of caveolae by quantitative electron microscopy (panel B). The cellular cholesterol in control cells (100%) was 11.8 μg/mg protein in this experiment.

Fig. 6. Caveolin expression in response to cholesterol synthesis in cholesterol-depleted MDCK cells. MDCK cells were depleted of cholesterol for 3 days as in Fig. 5. Mevalonate (2 mm) was then added for the times shown and cells were analyzed for cholesterol (panel A) and the expression of caveolin protein (panel B). The cellular cholesterol in control cells (100%) was 9.9 μg/mg protein in this experiment.
threshold effect was observed. Each data point is the result of counting over 50 cell profiles, carried out without knowledge of the sample history. In a separate experiment we measured the amount of caveolin expressed under similar conditions (Fig 7B). Unlike the number of caveolae, the expression of caveolin showed an approximately linear relationship with the cellular cholesterol concentration.

**DISCUSSION**

Caveolin is a cholesterol-binding protein found associated with cholesterol-rich domains of the cell surface and Golgi complex (35). Recently it has been shown that sterol flux, both out of cells after synthesis in the endoplasmic reticulum and into cells after exchange with exogenously added lipoproteins, occurs with concomitant accumulation in surface caveolae (25, 36). Thus caveolin and caveolae might have an important role in maintaining cellular cholesterol homeostasis. At the time this manuscript was submitted for publication another report of caveolin mRNA regulation using fibroblasts was published (37). In this report Fielding, Bist, and Fielding (37) demonstrated that caveolin mRNA was regulated in parallel with free cholesterol efflux, and that efflux of cholesterol was proportional to the amount of oxidizable cholesterol in intact cells, which was taken as a measure of free cholesterol in caveolae. We have independently tested the relationship between caveolin expression, cellular cholesterol concentration, and the number of caveolae.

In agreement with Fielding et al. (37) we find that caveolin mRNA levels can be regulated under conditions where the cellular cholesterol level is varied. Unlike Fielding et al. (37) however, we chose conditions where no extracellular lipoproteins were present in the medium and cholesterol concentrations were modulated by the addition of inhibitors of cholesterol synthesis, cholesterol chelating drugs, and mevalonate. This allowed a greater variation in cellular cholesterol concentration to be achieved. Our data show that caveolin protein concentration can be regulated over a 2- to 3-fold range in the same direction as the cellular cholesterol concentration, giving rise to an approximately linear relationship. Small changes were observed with the addition of excess LDL to 10% human serum, suggesting that caveolin concentrations are likely to be regulated by cholesterol in vivo. It should be noted that caveolin is regulated by cholesterol in the opposite direction to that of the HMG-CoA reductase, cholesterol synthetase, and the LDL receptor (33), which are all up-regulated after cholesterol depletion. Indeed, 25-hydroxycholesterol, the most potent activator of the sterol regulatory element binding protein (34), had only a small effect on caveolin mRNA levels and no detectable effect on caveolin protein expression. The fact that caveolin protein is regulated in the absence of lipoproteins in the medium suggests that the mechanism of regulation is more likely to be triggered by cellular cholesterol than by the rate of cholesterol influx, as suggested by Fielding et al. (37).

Although caveolin mRNA and protein levels fell rapidly after cholesterol depletion, the recovery after addition of 2 mm mevalonate to the medium was slower, giving a 22% increase in the numbers of caveolae over 24 h. In parallel experiments, cholesterol and caveolin concentrations rose to approximately 80% and 60%,
respectively, in this time. Thus caveolae formation is likely to be dependent on sufficient concentrations of both cholesterol and caveolin protein in the cell as previously reported (12, 13).

As caveolin is a cholesterol-binding protein, one possible function of increasing caveolin expression under conditions of high cholesterol in the cell would be to provide a ‘sink’ for excess cholesterol in the membrane. Excess plasma membrane cholesterol could be sequestered in caveolae by caveolin and released from the cell to extracellular carriers (25, 37). Conversely, when the cells are depleted of cholesterol, the lower expression of caveolin decreases the number of caveolae, and would be expected therefore to decrease the efflux of cholesterol from the cell. We have shown that depleting MDCK cells of approximately half of their cholesterol and caveolin protein caused total ablation of morphologically recognizable caveolae without affecting cell viability or general morphology. This suggests that a threshold concentration of caveolin and cholesterol is required for these structures to form, consistent with a function of caveolae as a ‘sink’ for excess cholesterol or as an organelle functioning in the export of excess cholesterol. A similar phenomenon was observed by Fra et al. (12) when caveolin cDNA was expressed in lymphocytes, cells that normally do not express caveolin. In lymphocytes expressing high levels of caveolin, clusters of small uniformly sized vesicles were observed beneath the plasma membrane that were labeled by immuno-gold labeling with an antibody against caveolin. In contrast, in cells expressing low levels of caveolin, labeling was dispersed over the plasma membrane without the formation of caveolae-like profiles (12). As caveolin polymers have been suggested to form the striated coat around caveolae (15), it is plausible that the formation of invaginated caveolae would be highly dependent on caveolin concentration.

Another consequence of increased numbers of caveolae might be an increase in caveolae-mediated uptake of ligands into the cell. In endothelial cells, where caveolae have been shown to mediate lipoprotein movement into the arterial intima (21), elevated levels of circulating lipoprotein have been shown to increase the transcellular transport of LDL (38). In this case cholesterol exchanging into the endothelial cell from the circulating lipoproteins might stimulate transcytosis via increased cellular caveolin concentration and increased numbers of caveolae. Thus the atherogenic risk associated with increased transport of lipoproteins across endothelial cells into the arterial intima (39) might, in part, be due to the effects of cholesterol on caveolin expression.

In conclusion, our results, in conjunction with those recently published by Fielding et al. (25, 37), suggest that caveolin expression regulated by cellular cholesterol level might regulate the number of caveolae per cell and hence cholesterol export. As such it could represent an alternative method for cholesterol homeostasis in cells.

We thank the National Heart Foundation of Australia for support to L. S. and the NH&MRC of Australia for support to R. G. P. We would also like to thank Natasha Zorzi for excellent technical assistance.

Manuscript received 18 April 1997, in revised form 20 August 1997, and in revised form 20 October 1997.

REFERENCES


