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review

Cellular spelunking: exploring adipocyte caveolae

Paul F. Pilch,[1,*] Ricardo P. Souto,[2,*] Libin Liu,[*] Mark P. Jedrychowski,[*,†] Eric A. Berg,[*,§] Catherine E. Costello,[*,§] and Steven P. Gygi[†]

Department of Biochemistry* and Mass Spectrometry Resource,[§] Boston University School of Medicine, Boston, MA 02118; and Department of Cell Biology,[†] Harvard Medical School, Boston, MA 02115

Abstract It has been known for decades that the adipocyte cell surface is particularly rich in small invaginations we now know to be caveolae. These structures are common to many cell types but are not ubiquitous. They have generated considerable curiosity, as manifested by the numerous publications on the topic that describe various, sometimes contradictory, caveolae functions. Here, we review the field from an "adipocentric" point of view and suggest that caveolae may have a function of particular use for the fat cell, namely the modulation of fatty acid flux across the plasma membrane. Other functions for adipocyte caveolae that have been postulated include participation in signal transduction and membrane trafficking pathways, and it will require further experimental scrutiny to resolve controversies surrounding these possible activities.—Pilch, P. F., R. P. Souto, L. Liu, M. P. Jedrychowski, E. A. Berg, C. E. Costello, and S. P. Gygi. **Cellular spelunking: exploring adipocyte caveolae.** ***J. Lipid Res.*** **2007.** 48: **2103–2111.**

Supplementary key words caveolin • cavin • fatty acid • uptake • proteomics

Adipocytes have long been understood to be the major energy storage depot for numerous organisms of many phyla. Triglycerides are the energy-rich storage fuels in these cells that are generally obtained from the diet as fatty acids (FAs), but they can also be synthesized from carbohydrate precursors. Fatty acids (and glycerol) are released from adipocytes into the circulation by lipolysis when they are needed as fuel in other tissues, principally skeletal and cardiac muscle in mammals. It is now appreciated that adipocytes have an important dynamic role in metabolic regulation at the organismal level by virtue of their ability to sense metabolic states and produce hormones, called adipokines, that in turn affect nutrient intake, metabolism, and energy expenditure at numerous levels (1, 2). Nevertheless, FA flux across the plasma membrane is the most frequent and quantitatively robust activity of the adipocyte, which raises the mechanistic question of how this flux is mediated. Interestingly, the plasma membrane of adipocytes is particularly rich in small invaginations, as was determined quite some time ago in several independent morphological studies (3–5). We now realize that these structures are caveolae, based on their specific labeling (6) by antibodies directed to caveolin, a structural protein necessary for the formation of these structures (7, 8). It is our hypothesis that the abundance of caveolae in the adipocyte plasma membrane is related to FA flux across this barrier, as will be elaborated below, although additional roles for caveolae in adipocyte biology are virtually certain.

Caveolae were first identified by electron microscopy as small (60–100 nm) invaginations of endothelial cells by Palade in 1953 (9), and soon thereafter, they were named as such based on their resemblance to small caves (10). The characterization of caveolae was restricted to morphological techniques until caveolin, now caveolin-1, was determined to be a protein marker for these structures (7, 8). There are three caveolin isoforms, caveolin-3 being muscle-specific and caveolin-2 requiring caveolin-1 for robust expression. Ectopic expression of caveolin-1 or caveolin-3 will result in the formation of morphologically identifiable caveolae with their characteristic curvature (**Fig. 1**) (for review, see Ref. 11). With marker proteins and various cDNA constructs available, numerous investigators began to enrich or perturb this membrane domain and characterize its contents and potential function. The various isolation techniques that have been used in this regard are critical to the interpretation of the resultant data and will be discussed in detail below. The exceptional scientific and medically related interest in caveolae has resulted in numerous reviews of the topic, and for more background, we steer the reader to some examples that are relatively general in scope (12–15). Hereafter, we focus primarily on caveolae in adipocytes, whereas in the broader context discussed in Ref. 14, there is considerable controversy concerning their composition, associations, and potential functions. The key question is this: why do adipocytes have so many caveolae?

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[1] To whom correspondence should be addressed.
e-mail: ppilch@bu.edu
[2] Current address of R. P. Souto: Faculdade de Medicina do ABC, Santo Andre, SP, Brazil, and Departamento de Bioquimica, Instituto de Quimica, Universidade de Sao Paulo, Sao Paulo, SP, Brazil.

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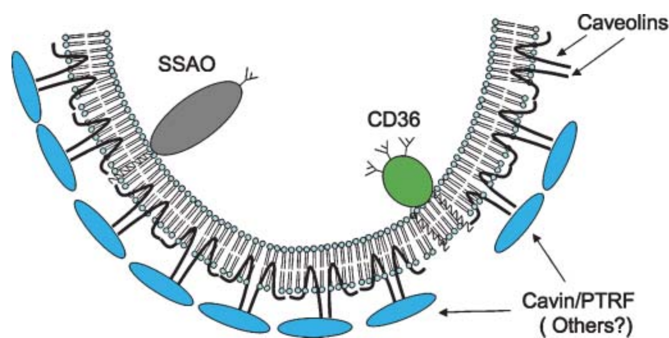

Fig. 1. Representation of the protein contents of rat adipocyte caveolae. Caveolins are integral membrane proteins with an unusual "hairpin" topography (14). We identified just two transmembrane proteins, CD36 and semicarbazide-sensitive amine oxidase (SSAO) (one copy of each for illustration purposes), as caveolae components and cavin/polymerase I and transcript release factor (PTRF) as a peripheral membrane protein of the cytoplasmic face. See text for details about these proteins.

CAVEOLAE ISOLATION, PROTOCOLS, AND PROBLEMS

A large part of the controversies concerning caveolae contents almost certainly derives from the methodology used for their isolation and characterization. Caveolae are contiguous with the plasma membrane; therefore, their isolation requires methodology that will allow their separation from the bulk cell surface membrane. The first procedures developed in this regard relied on the relative insolubility of caveolae when briefly exposed to cold Triton X-100 for both cultured (16) and primary (17) cells. When Triton-resistant protein complexes were subjected to a sucrose gradient, caveolin was greatly enriched in a fraction of light buoyant density that was deemed to be "caveolae" by biochemical and morphological criteria. However, Triton resistance is also a property of the cellular "skeletal framework" described by Penman and colleagues (18), and consistent with this, isolated caveolin/caveolae colocalize with cytoskeletal elements (17), possibly because caveolae may be positionally stabilized or anchored at the cell surface by the cortical cytoskeleton (19). In addition, detergent-resistant "lipid rafts" lacking caveolin, such as those marked by flotillin in brain, a tissue that lacks caveolin (20), are likely present in most or all cells and will be enriched by typical detergent resistance protocols designed to isolate caveolae. In fact, flotillin is present in detergent-resistant domains from caveolin-1 null fibroblasts (21), clearly showing that it is not necessarily a caveolae-associated protein, as was suggested originally (20).

Caveolae have been considered a type of lipid raft, but the distinction between lipid rafts and caveolae is blurred in many publications. Caveolae in situ are clearly definable, relatively stable morphological structures as noted above, whereas lipid rafts in cells are highly dynamic in the plane of the membrane and are heterogeneous in size, with no consensus regarding what their absolute composition is and what their existence means for physiological processes (22). Moreover, lipid rafts isolated in vitro on the basis of detergent resistance are not as likely to be the equivalent of those existing in situ (23). The nature of the nonionic detergent used and the temperature are parameters regarding detergent resistance that will have an effect on the physical state of membrane lipids and, consequently, will affect the experimental outcomes and interpretations of raft/caveolae enrichment by this method (24). Despite these caveats, many published studies use detergent resistance as a major criterion for the characterization of caveolae and lipid rafts, and this protocol can be useful if its limitations are understood and the data are interpreted with circumspection. For example, with regard to adipocytes, the SNARE proteins involved in membrane trafficking and fusion (25) are enriched in detergent-resistant domains (26), although what this means under physiological conditions regarding Glut4 trafficking (see below) remains uncertain. Caveats also apply to the use of detergents in immunoprecipitation/immunoisolation experiments designed to determine the identity of caveolin-associated proteins. The detergent-insoluble fraction from such procedures will consist of a complex mixture that may or may not bear any relationship to the protein-protein interactions in the native membrane, and anti-caveolin antibodies can and will pull down the entire detergent-insoluble "pellet."

Consequently, protocols have been developed that do not use detergent and involve sonication to release caveolae from the plasma membrane. One such procedure for cultured cells uses an ultracentrifugation step to obtain an enriched plasma membrane preparation, which is then sonicated to pinch off caveolae. A fraction of unique buoyant density, enriched in caveolin, is obtained after two successive Optiprep gradients (27). This approach may be preferable to the detergent resistance method, but the ultimate purity of the resultant caveolae is still not completely clear, as a proteomics study from such preparations identified numerous endoplasmic reticulum and mitochondrial proteins as possibly being caveolae-associated (28), a result at odds with the great majority of morphological studies localizing caveolin. A second protocol involves the sonication of cell lysates at alkaline pH followed by an overnight sucrose gradient spin (29). However, this process yields the same protein profile in the "caveolae" fraction from cells 90% depleted in caveolin-1 as it does for the caveolin-positive cells, save for caveolin-1 of course (30). Another procedure for caveolae isolation that has been applied to endothelial cells in situ is to perfuse them with colloidal silica particles too large to enter caveolae. After isolation, the cells are sonicated to release caveolae, which are much lighter than the bulk of the silica-coated plasma membrane (31). A variation of the silica coating technique for endothelial cells involves sonication, centrifugation, and immunoisolation (32). However, simple sonication and immunoisolation of endothelial cell caveolae seem to work just as well as the silica-based technique (33), and this procedure is similar to the one we use for adipocytes, as illustrated in **Fig. 2**.

Thus, primary adipocytes are isolated, homogenized, and fractionated, and then caveolae are isolated from the

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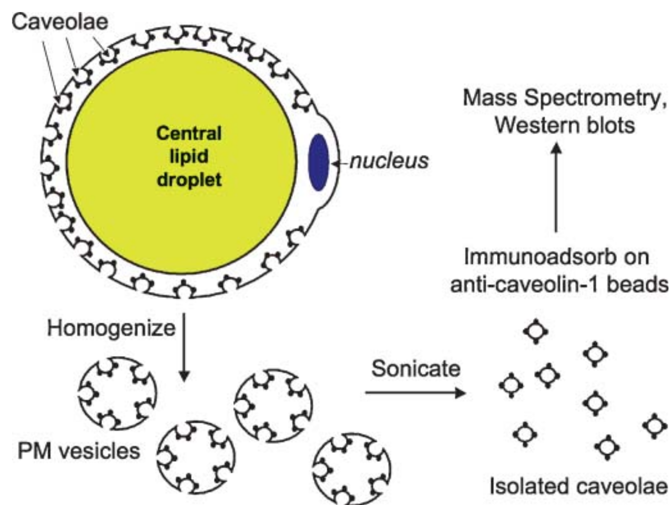

Fig. 2. Schematic representation of caveolae isolation from rat adipocytes. See text for details about this procedure.

light microsomal fraction with anti-caveolin-1-immoblized beads in relatively small yield (34). However, we have now obtained caveolae in much higher yield (~30%) from sonicated plasma membranes, and we have compared them with those naturally pinched off during homogenization. As shown in **Fig. 3**, we see exactly the same

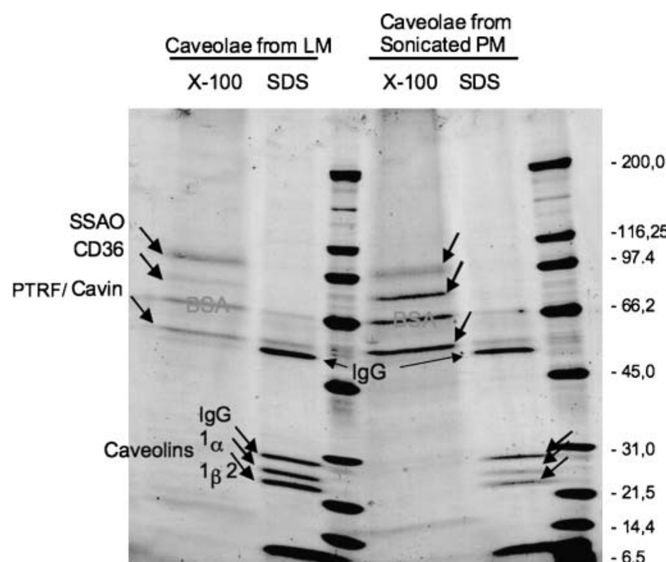

Fig. 3. Protein contents of caveolae immunoisolated from adipocyte membranes. Adipocyte light microsomes (LM; 6 mg of protein) were obtained as described (34). In parallel, 1 mg of plasma membrane (PM) was processed as outlined in Fig. 2. Both samples were immunoadsorbed with 300 μl of beads coupled to anti-caveolin-1 antibody. Protein was recovered from beads by successive elution with 1% Triton X-100, then SDS, and all of it was loaded onto a 6–15% SDS-PAGE gel and stained with colloidal Coomassie blue as shown. Protein bands were excised, digested with trypsin, and identified as the indicated proteins (arrows) by analysis of their mass spectra.

protein-staining pattern, albeit with different intensities for individual bands. The relative staining intensity of each band varies from preparation to preparation, but the overall pattern is always the same (data not shown). Because of the unique composition of the adipocyte, a large cell surface, and a unilocular fat droplet that occupies 90% of the cell, the plasma membrane fraction is obtainable in exceptional purity, and along with other adipocyte membrane fractions, it has been carefully characterized (35). Consequently, caveolae immunoisolation from sonicated plasma membrane, as outlined in Fig. 2, is rapid and requires a single ultracentrifugation step to isolate the plasma membrane on a sucrose cushion (35). Moreover, the immunoadsorption step ensures that we obtain only caveolae and not lipid raft domains that lack caveolin.

To obtain further information about their identity, we subjected the proteins stained in Fig. 2 to mass spectrometry, and in addition to what we previously identified by sequencing or by Western blotting, we found cavin [also known as polymerase I and transcript release factor (PTRF) (36)] to be a major constituent, in confirmation of the work of Vinten and colleagues (37, 38) in rat adipocytes and that of Aboulaich et al. (39, 40), who used human fat cells. The role of cavin in caveolae structure/function remains to be determined, but its relative abundance, its tissue distribution that mirrors that of caveolin-1 and -3 (37, 41), and its apparent lack of enzymatic activity (36) suggest a possible structural role, although a functional role in adipocytes has been suggested (40) (see lipid trafficking section below). As in our previous Western blotting experiments (34), we see no mass spectrometry-based evidence for the caveolae localization of adipocyte proteins involved in insulin's actions, namely the insulin receptor and one of its downstream targets, Glut4. Indeed, the protein composition of adipocyte caveolae obtained rapidly and in reasonable yield is quite simple and in agreement with a previous freeze-fracture study of cultured adipocytes that concluded that there were relatively few proteins in them (42). However, we cannot rule out the possibility that some caveolae-localized proteins escaped our scrutiny and/or that the caveolae population is heterogeneous, as has been suggested (43). In fact, we only isolated the major Coomassie blue-staining bands for analysis by mass spectrometry; therefore, we could have missed minor, potentially important caveolae constituents. The possibility of caveolae heterogeneity raises mechanistic questions relative to protein targeting (namely, how the same coat protein, caveolin, in a given cell could recruit different populations of partners), which is not consistent with the accepted notion that caveolin composition is relatively uniform in a given cell type (14).

POSSIBLE FUNCTIONS OF ADIPOCYTE CAVEOLAE

Insulin action

Caveolae have been implicated to be the locus of an insulin signaling pathway in adipocytes that leads to the translocation of Glut4 but is independent of insulin re-

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ceptor substrate (IRS) proteins and phosphatidylinositol 3-kinase (44). The canonical insulin signaling pathway involves the ligand-dependent activation of the insulin receptor's intrinsic tyrosine kinase activity, tyrosine phosphorylation of IRSs, IRS-1 and IRS-2 being the major metabolically relevant species (45). Binding of phosphatidylinositol 3-kinase to a tyrosine-phosphorylated IRS activates its lipid kinase activity, leading to the activation of the serine/threonine kinase PDK-1, which phosphorylates another serine/threonine kinase, Akt; it is this protein whose action diverges onto various metabolic processes (46), including the glucose transport activation via Glut4 translocation being considered below. Alternatively, it has been postulated that caveolae-localized insulin receptor phosphorylates the adaptor/substrate proteins c-cbl and APS, which leads to the activation of the small GTPase TC10, which, in turn, signals to the cytoskeleton in such a way that Glut4 is recruited to the cell surface (44).

This hypothesis has been called into question based on the fact that ablation of c-cbl in vivo (47) and down-regulation of c-cbl in vitro (48) result in enhanced insulin sensitivity or no change in this parameter, respectively. Still, consistent with the cbl/TC10 hypothesis, there are a number of reports that show the association of insulin receptors with caveolin and/or their localization in caveolae (49–52). Moreover, insulin-dependent tyrosine phosphorylation of caveolin-1 occurs in adipocytes (Fig. 3), but Fyn is the tyrosine kinase implicated in this process (53). On the other hand, we (34) and others (53) fail to find insulin receptors associated with caveolae. A possible explanation for these discrepancies may have been revealed in a very recent study in which it was shown by electron microscopy that insulin receptors associate with the necks of caveolae and not the "bulb" region (54). Thus, depending on the nuances of the various protocols used in the studies cited above (34, 49–53), the insulin receptor could be seen to associate with caveolin/caveolae or not. However, it remains unclear how and if the caveolae neck localization affects insulin receptor function regarding glucose transport, because fat cells are fully responsive to insulin when $<3\%$ of its receptors are occupied (55); thus, a very small subset of receptors could represent those critical for transport activation, and these could reside anywhere.

Glucose transport stimulation downstream of insulin signaling in adipocytes results from the translocation of the muscle/fat-expressed glucose transporter isoform, Glut4, from an intracellular locus where $>95\%$ of it resides to the cell surface where it can function. This process is the intersection of a signaling pathway with a complex membrane trafficking pathway, and it has been reviewed extensively (56–60). Briefly, intracellular Glut4 is distributed in the endosomal compartments characteristic of a protein that traffics to and from the cell surface. However, unlike most proteins that traffic in this way, about half of Glut4 is sequestered into a unique compartment called Glut4 storage vesicles. Insulin signaling to Akt converges on Glut4 storage vesicles at one or more steps of its movement to the cell surface, resulting in a dramatic increase in cell surface Glut4 and continual cycling. As with the insulin receptor, there are publications from several groups that support a role for caveolae in Glut4 trafficking based on their colocalization under certain conditions. (61–65). On the other hand, several independent studies do not see Glut4 in caveolae (6, 66–68).

In trying to reconcile these contradictions, considerations concerning caveolae and Glut4 trafficking are quite different from those relevant for the insulin receptor localization in caveolae. Whereas a very small percentage of insulin receptors are needed for maximal signaling, all Glut4 molecules are involved in trafficking and Glut4 attains a steady-state distribution, cell surface versus internal, ~10 min after insulin exposure, when exocytic and endocytic rates are equal (69–71). In this time frame, we see no evidence that any insulin-dependent changes occur in cell surface caveolae composition (34), consistent with the finding that bulk caveolae are not very dynamic structures (72, 73). At steady state, there is ample evidence that Glut4 endocytosis involves clathrin-coated pits (74–77), which requires that the endocytic vesicles lose their clathrin coat (78). In contrast, caveolae do not lose their caveolin coat even in the small population that can undergo endocytosis (73, 79). Thus, any transit of Glut4 through caveolae would involve a mechanism whereby the integral membrane protein caveolin is retained in caveolae, whereas Glut4 is able to move in and out of these structures while they maintain their integrity. Such a mechanism has no precedent and seems inconsistent with our current knowledge of the cell biology of membrane trafficking, but the data are the data. Therefore, the devil is most likely in the details of the experimental considerations and caveats noted in the previous section.

Lipid traffic

Caveolae are detergent-resistant domains, and FAs are mild detergents. FAs released by lipolysis in adipocytes can attain concentrations potentially deleterious to cells; therefore, we postulate that caveolin/caveolae might serve to modulate or buffer FA flux across the plasma membrane. This would serve to protect the cell from the detergent effects of high FA concentrations. In fact, caveolin-1 has been shown by photolabeling to be a FA binding protein (80). Adipocytes take up and metabolize FA very rapidly, making it difficult to study individual steps of FA movement (81). Therefore, we created a model cell system for the purpose of studying the role of caveolin in transmembrane FA flux in which FA movement and metabolism could be distinguished: namely, HEK293 cells stably expressing caveolin-1. The parental cells express no detectable caveolin, and we created four lines with increasing expression levels. Interestingly, we determined that at a certain threshold of expression, caveolin-1 alters transmembrane FA flux such that the flip from the outer to the inner bilayer of the membrane is enhanced by 2-fold (82), and we interpret this to mean that caveolin-rich domains or rafts can in fact sequester higher levels of FAs than nonraft domains. The effect is independent of fatty acid type, with *cis*- and *trans*-unsaturated and saturated FAs

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all exhibiting this phenomenon. We have now obtained additional data supporting the role of caveolae/caveolin in modulating transmembrane FA flux by showing that transfection of caveolin-3 has an effect identical to that of caveolin-1 in modulating FA flux in HEK cells (data not shown). Because these isoforms have the same functions, albeit in different cell types (14), they would be expected to function similarly in HEK cells, and they do. Because caveolin expression results in increased levels of cholesterol (83) and sphingolipids (84), we are in the process of assessing the relative roles of the various caveolae proteins and membrane lipids involved in modulating transmembrane FA movement.

What about the possibility of an FA transport protein equivalent to a sugar transporter for transmembrane FA movement? From the properties of these two types of molecules, a transporter is clearly required to mediate the transmembrane movement of highly water-soluble sugars, as they cannot cross this barrier by diffusion with any kinetics compatible with their rate of metabolism. On the other hand, because of their hydrophobic properties, FAs are poorly water-soluble but readily partition into biological membranes and can diffuse across them by a flip-flop of their nonprotonated form (85). The argument is made that cellular FA uptake needs to be regulated and diffusion is not a regulated process, but the very robust glucose utilization in the liver is not regulated at its entry into the cell; rather, it is regulated by glucokinase-dependent phosphorylation and by other downstream enzymes (86). The analogous mechanism is that cellular FA entry is regulated at the level of its conversion to CoA derivatives, as has been suggested (87) and as would readily occur in fat cells. Nonetheless, this begs the question of why the adipocyte makes relatively elaborate structures like caveolae in such large amounts, which would at face value seem to be overkill just to modulate FA flux. Therefore, this effect may be a side benefit to some as yet unspecified cellular function for caveolae.

Consistent with the observation noted above that caveolin expression and cholesterol levels parallel one another, caveolin-1 has been determined to be a cholesterol binding protein (88). In addition, caveolae have been suggested to be a major site of cellular cholesterol efflux (89), and recently, cholesterol exposure of adipocytes was shown to drive caveolin-1 from the cell surface to lipid droplets where triglycerides are stored (90). Indeed, there is considerable evidence for caveolin's association with lipid droplets (91–95), structures in adipocytes and steroidogenic cells that are increasingly recognized as a dynamic organelle with a relatively complex protein coat (96), which is necessary for the regulation of lipid uptake and release (97, 98). The physiological role of caveolin on lipid droplets and its trafficking there to and from the cell surface remain to be clarified, although it has been suggested that triglyceride synthesis may be initiated in a subtype of caveolae (99), one that also expresses perilipin, the characteristic lipid droplet marker protein (98). Again, this notion raises questions and contradictions about the presumed biogenesis of lipid droplets from the endoplasmic reticulum, but it is certainly consistent with the theme that almost everything about caveolae is contentious.

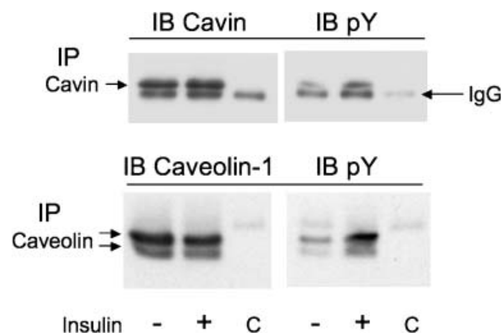

Fig. 4. Caveolin-1 and cavin are tyrosine phosphorylated in an insulin-dependent manner in rat adipocytes. Rat adipocyte plasma membrane (300 μg of protein) from insulin-treated (100 nM, 15 min) (+) and untreated (−) adipocytes were solubilized and immunoprecipitated with anti-cavin or anti-caveolin-1 monoclonal antibody or nonspecific IgG (C). The immunoprecipitates (IP) were subjected to SDS-PAGE and analyzed by Western blot with the same antibodies (left panels) or a mix of anti-phosphotyrosine antibodies (pY, 4G10 + PY20; right panels). Final detection was by chemiluminescence. IB, immunoblot.

FUTURE DIRECTIONS

Considering the many important cellular processes attributed to caveolae, it is surprising that caveolin-1 knockout mice are viable, although they are hardly normal and exhibit major vascular, pulmonary, and cardiac problems (100–102). Importantly, aged caveolin-1 null animals are lean, develop dyslipidemia, and have abnormal-appearing adipocytes (103). This phenotype is completely consistent with the role we propose for caveolin/caveolae in modulating lipid flux across the adipocyte plasma membrane (82), a process that was discussed above. Inability to nor-

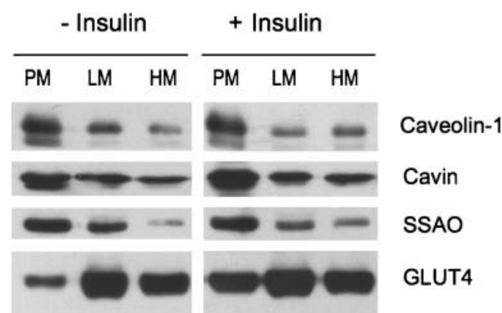

Fig. 5. Caveolae proteins caveolin-1, cavin/PTRF, and SSAO have no insulin-dependent dynamic behavior. Membrane fractions from rat adipocytes [plasma membrane (PM), heavy microsomes (HM), and light microsomes (LM)] from resting and insulin-treated (100 nM, 15 min) adipocytes were obtained, and equal proportions of each fraction were subjected to SDS-PAGE and Western blotted with the antibodies indicated before detection by chemiluminescence.

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mally modulate FA flux in adipocytes would lead to their unusual appearance according to this hypothesis. And although it will not be experimentally convenient to study primary adipocytes from these animals, cultured adipocytes derived from caveolin-1 null fibroblasts have potential utility in addressing the role of caveolae/caveolin in insulin action and lipid trafficking. Thus, we are in the process of creating a permanent adipocyte cell line from caveolin-1 null fibroblasts for use in this regard. We might expect, for example, that these cells would have altered transmembrane FA flux compared with normal, caveolin-1-positive cells.

The major proteins we and others have identified in adipocyte caveolae to date are caveolin-1 and -2, the transmembrane proteins CD36 and semicarbazide-sensitive amine oxidase (SSAO) (34) (Figs. 1, 3), and cavin/PTRF, a protein of its cytoplasmic face (38, 39). CD36 is a lipoprotein receptor (104) that also has been postulated to be a fatty acid transport protein (105). The transport function of CD36 is controversial (106) (see above), and its sequence is unlike that of any known transport protein. However, its absence in knockout animals diminishes FA uptake and metabolism in adipocytes in situ (107), clearly indicating a role in overall lipid homeostasis. Recently, it was suggested that caveolin-1 is required for cell surface CD36 expression, as it is absent from the plasma membrane in caveolin-1 null fibroblasts (108). Thus, there appears to be a strong connection between FA, CD36, and caveolae, but a clear CD36 function there remains elusive. This is likewise the case for SSAO, a highly abundant adipocyte protein with eponymous activity (109). It is localized to caveolae in rat and human cells (34, 39), but its physiological function there remains unknown.

In addition to the limited studies noted above, cavin/PTRF has been shown to undergo insulin-dependent tyrosine phosphorylation (110, 111) in cultured adipocytes, as does caveolin-1 (53), and we confirm this result for primary rat adipocytes, as shown in **Fig. 4**. It was recently suggested that in human adipocytes, cavin/PTRF undergoes insulin-dependent movement from the plasma membrane to the cytosol and nucleus and can interact with hormone-sensitive lipase in the cytosol (40). We have performed similar experiments in rat adipocytes, as shown in **Fig. 5**, and we fail to see any substantial loss of cavin/PTRF from the plasma membrane in the same time frame and insulin concentration that was used for the human cells. This result does not preclude the possibility that a very small portion of cavin/PTRF could move into the cytosol under these conditions. Glut4 translocation from light microsomes to the plasma membrane is shown as a control for insulin responsiveness, and we fail to see any dynamic behavior for caveolin-1 and SSAO, as we reported previously (34).

Whether or not these apparently discrepant results are attributable to a species difference remains to be determined, but regardless, the data suggest that cavin/PTRF is a target of a tyrosine kinase, and as a peripheral membrane protein, it has the possibility of undergoing relatively rapid dynamic behavior. Therefore, the identification of the kinase responsible for the observed phosphorylation is likely to be informative, and it will also be interesting to determine what parameters can affect cavin/PTRF dynamics, the cytoskeleton in particular. Moreover, because the caveolins and cavin/PTRF show very similar tissue distribution, efforts are underway to understand their relationship. Preliminary data suggest that small interfering RNA-mediated cavin knockdown in adipocytes results in a roughly comparable loss of caveolin-1 (data not shown), suggesting that their expression and possible function are linked. Indeed, caveolin-1 expression is markedly enhanced during adipocyte differentiation (61, 66), yet very little is known about what regulates its expression in these cells. In conclusion, adipocytes are a rich source of information about the biology of caveolins, caveolae, and their associated protein contents, and they promise to be so in the future.

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