Caveolin-associated Filamentous Actin (Cav-actin) Defines a Novel F-actin Structure in Adipocytes*

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Dynamic actin remodeling has been implicated in the translocation of the insulin-responsive glucose transporter 4 (GLUT4) to the plasma membrane in adipocytes. Here we show that fully differentiated 3T3L1 adipocytes have unique cortical filamentous actin structure, designated Cav-actin (caveola-associated F-actin). During 3T3L1 adipocyte differentiation, rhodamine-phalloidin staining demonstrated the formation of a cortical actin cytoskeleton that is composed of small dot-like F-actin spikes lining the inside of the plasma membrane. Double labeling with a caveolin antibody indicated that these F-actin spikes emanate from organized rosette-like clusters of caveolae/lipid raft microdomains. In contrast, there was no obvious relationship between F-actin and caveolin localization and/or organization in 3T3L1 preadipocytes (fibroblasts). Treatments of differentiated adipocytes with latrunculin B, Clostridium difficile toxin B or a dominant-interfering TC10 mutant (TC10/T31N) disrupted the Cav-actin structure without significantly affecting the organization of clustered caveolae. Similarly, disruption of the clustered caveolae with methyl-β-cyclodextrin also dispersed the Cav-actin structure. These data demonstrate that this novel Cav-actin structure is organized through clustered caveolae but that the formation of caveolae-rosettes are not dependent upon F-actin.

Insulin stimulation of glucose uptake in striated muscle and adipose tissue is achieved through the translocation of intracellular localized GLUT4 protein to the cell surface membrane (1–5). This primarily results from an increase in the rate of exocytosis such that ~50% of the GLUT4 protein is redistributed to the plasma membrane (6–9). This highly complex and dynamic membrane trafficking process requires a phosphatidylinositol (PI) 3-kinase pathway leading to the activation of protein kinase B/Akt and/or the atypical protein kinase C, PKCγ/PKCδ (10–14). In addition, a parallel PI 3-kinase-independent pathway leading to the activation of small GTP-binding protein TC10 appears to function in concert with the PI 3-kinase pathway (15–17). Although the specific sites of action and/or the molecular targets of these insulin signaling cascades that lead to GLUT4 translocation have remained unclear, both PI 3-kinase and TC10 have been reported to play an important role in regulating actin cytoskeleton in various cell types (18–20). For example, TC10 is a member of Rho family GTPases that has been reported to be a potent actin regulator in various cell types including adipocytes (18–21).

The actin cytoskeleton is a dynamic filament network that is essential for multiple cellular functions including cell movement, morphogenesis, polarity, and cell division (22–24). In particular, treatment of adipocytes with actin-depolymerizing agents cytochalasin D and latrunculin A or B and the actin-stabilizing agent jasplakinolide all inhibit insulin-stimulated GLUT4 translocation (20, 25–28). Furthermore, insulin stimulates dynamic actin remodeling at both the inner surface of the plasma membrane and in the perinuclear region that is sensitive to Clostridium difficile toxin B, a Rho family-specific toxin (20, 29). Taken together these data suggest that dynamic actin remodeling is essential in the GLUT4 translocation process.

Recent evidence has also demonstrated that cholesterol-enriched lipid raft microdomains serve as critical compartmentalized membrane regions that generate specific insulin signals (15–17, 30). For example, TC10 is mainly localized to the lipid raft compartment enriched in caveolin, the major structural protein of a subset of lipid raft microdomains that forms characteristic Ω-shaped invaginations of the plasma membrane termed caveolae (31, 32). In adipocytes, these caveolin-containing lipid raft microdomains are necessary for the activation of TC10 through a CAP-Cbl signaling pathway (15, 16). Based upon these data, we hypothesized that lipid microdomains might function as pivotal signaling platforms regulating and/or assembly-specific cellular machinery necessary for insulin action. In this article we demonstrate that adipocytes have a uniquely organized F-actin structure emanating from the caveolin-enriched clustered lipid raft microdomains at the plasma membrane.

EXPERIMENTAL PROCEDURES

Materials—C. difficile toxin B was obtained from Techlab Inc. (Blacksburg, VA). Latrunculin B was purchased from Calbiochem. Wortmannin, methyl-β-cyclodextrin (MβCD), and rhodamine-phalloidin were purchased from Sigma. pKH3-TC10/T31N and TC10/Q75L were prepared as described previously (15). pEGFP-actin cDNA was purchased from CLONTECH (Palo Alto, CA). The caveolin 1 and caveolin 2 antibodies were purchased from Transduction Laboratories (Lexington, KY). The hemagglutinin and Myc epitope tag antibodies were purchased from Upstate Biotechnology. Fluorescent secondary antibodies were purchased from Jackson ImmunoResearch Laboratories (West Grove, PA) and Molecular Probes. Horseradish peroxidase-conjugated secondary antibodies were from Pierce.

Cell Culture, Transfection, and Fluorescent Analysis of 3T3L1 Adipocytes—Murine 3T3L1 preadipocytes were purchased from the American Type Tissue Culture repository and differentiated as described by Min et al. (33). Single cell microinjection, isolation of plasma membrane sheets, and image analysis of 3T3L1 adipocytes were performed as described previously (34, 35).

Cholesterol Extraction—Methyl-β-cyclodextrin was added directly to serum-free Dulbecco’s modified Eagle’s medium at a final concentration of 10 mM, and the cells were incubated at 37 °C for 30 min.

RESULTS AND DISCUSSION

Recent studies have demonstrated that insulin signaling induces dynamic actin rearrangements and that prevention of
these events by various types of actin-disrupting reagents, toxins, and inhibitory proteins result in a significant inhibition of insulin-stimulated GLUT4 translocation to the plasma membrane (20, 25–28). We have also reported that the organization of the actin cytoskeleton is dramatically changed during the differentiation of adipocytes from a typical stress fiber actin structure in fibroblasts to a relatively thick cortical actin lining the inner surface of the plasma membrane in adipocytes (20). These findings suggest that the reorganization of stress fiber actin to that of cortical actin is an important process of adipocyte insulin responsiveness.

To further investigate this differentiation-dependent reorganization of the actin cytoskeleton, we initially compared the intracellular distribution of F-actin with caveolin (Fig. 1). As previously observed, predifferentiated 3T3L1 fibroblasts displayed long organized F-actin stress fibers as detected by rhodamine-phalloidin labeling (Fig. 1, panel c). Caveolin was dispersed throughout the cells with no apparent relationship to the localization pattern of caveolin 1 (Fig. 1a, panels a, c, and i). Two days after initiation of adipocyte differentiation, the amount of F-actin stress fibers visualized at the cell bottom were markedly reduced, being more diffuse and decreasing in both thickness and length (Fig. 1a, panel f). At this time, caveolin began to develop a more organized pattern in some of the cells (Fig. 1a, panels b, f, and j). However, after 6 and 10 days of adipocyte differentiation the stress fiber F-actin became small patches of punctate actin that were co-localized with the caveolin-positive clusters that are characteristic of lipid raft microdomains in adipocytes (Fig. 1a, panels c, d, g, h, k, and l).

We and others have found that individual caveolae in differentiated 3T3L1 adipocytes are often clustered into ring-like arrays (caveolae-rosettes) that can be visualized by fluorescent microscopy (16, 36, 37). Consistent with these data, these ring-like caveola organized structures were observed in the plasma membrane of differentiated 3T3L1 adipocytes (Fig. 1b, panel a). A similar pattern was also observed in the labeling of F-actin with rhodamine-phalloidin (Fig. 1b, panels b and c). At higher magnification the caveola-rosette structures are readily apparent and are co-localized with rhodamine-phalloidin labeling of F-actin (Fig. 1b, panels d–f). In many cases, the F-actin appears to localize along the inner circumference of the large caveolae-rosettes (Fig. 1b, panels d–f, arrowheads) and sometimes fills the center regions of the smaller caveolae-rosettes.

FIG. 3. Depolymerization of F-actin does not affect caveola-rossete organization. Fully differentiated 3T3L1 cells were either left untreated (panels a, c, and d) or incubated with 20 μM latrunculin B (LatB) for 120 min (panels b, f, and j), 0.5 μg/ml toxin B for 120 min (panels c, g, and k), or 10 mM MβCD for 30 min (panels d, h, and l) as described under “Experimental Procedures.” Plasma membrane sheets were then prepared and subjected to confocal fluorescent microscopy using a caveolin 1 antibody (panels a–d) and rhodamine-phalloidin (panels e–h). The merged images are shown in panels i–l. These are representative images from experiments independently performed three times.
T31N) expression on Cav-actin (Fig. 2). The expressed TC10/T31N protein displayed a plasma membrane localization similar to that of caveolin and importantly did not disrupt the caveolae-rosette structures (Fig. 2, panels a and b). However, rhodamine-phalloidin staining demonstrated a near complete loss of Cav-actin structure (Fig. 2, panels c and d). The disruption of Cav-actin is consistent with total loss of cortical actin that also occurs in adipocytes expressing TC10/T31N (20).

The fact that expression of TC10/T31N disrupts the Cav-actin structure without significantly affecting caveolae-rosettes indicates that the clustered organization of caveolae are not dependent upon F-actin. To test this prediction, we next examined the effect of latrunculin B, a red sea sponge toxin that binds to monomeric actin and prevents its polymerization to F-actin (Fig. 3). As previously observed, differentiated adipocytes displayed the typical organization of caveolae-rosettes with the co-localization of F-actin (Fig. 3, panels a, c, e, and i). Latrunculin B treatment of differentiated adipocytes for 2 h resulted in a marked loss of the Cav-actin structure at the bottom of the cell with little effect on the caveolae-rosette organization (Fig. 3, panels b, f, j, and k). As previously reported (20), under these conditions there was also a complete disruption of cortical actin lining the inner surface of the plasma membrane (data not shown). In addition, following removal of latrunculin B the Cav-actin structure reassembled, consistent with these structures undergoing continuous remodeling (data not shown). Similarly the Cav-actin structures were disrupted by toxin B treatment without significant changes in the organization of the caveolae-rosettes (Fig. 3, panels c, g, and i). This also occurred concomitant with the disappearance of the F-actin staining at the center of the cells (data not shown). In contrast, cholesterol depletion with MβCD effectively disrupted the localization of F-actin and, in parallel, dispersed the caveolae-rosette organization (Fig. 3, panels d, h, and l). Together these data indicate that the caveolae-rosettes are responsible for organization of F-actin into the Cav-actin structure.

At present, the functional role of this unusual adipocyte Cav-actin structure has not yet been defined. Nevertheless several lines of evidence suggest that Cav-actin may play an important role in the insulin regulation of GLUT4 translocation. For example, depolymerization or stabilization of F-actin inhibits insulin-stimulated GLUT4 translocation (20, 25, 26, 27). TC10 inhibition of GLUT4 translocation only occurs when TC10 is targeted to adipocyte caveolae-rosettes in parallel with disruption of cortical actin (16). Furthermore, cholesterol depletion or interference with caveolae-rosette assembly also prevents insulin-stimulated GLUT4 translocation (16, 38). These findings coupled with the apparent necessary role of plasma membrane lipid raft microdomains in the assembly of the C2b-Cbl-TC10 signaling cascade (15, 17) provide compelling evidence for this pathway in the control of Cav-actin function.

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