Caveolin-1 Functions as a Novel Cdc42 Guanine Nucleotide Dissociation Inhibitor in Pancreatic β-Cells*

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The cycling of the small Rho family GTPase Cdc42 is required for insulin granule exocytosis, although the regulatory proteins involved in Cdc42 cycling in pancreatic β-cells are unknown. Here we demonstrate that the caveolar protein caveolin-1 (Cav-1) is a Cdc42-binding protein in β-cells. Cav-1 associated with Cdc42-VAMP2-bound granules present near the plasma membrane under basal conditions. However, stimulation with glucose induced the dissociation of Cav-1 from Cdc42-VAMP2 complexes, coordinate with the timing of Cdc42 activation. Analyses of the Cav-1 scaffolding domain revealed a motif conserved in guanine nucleotide dissociation inhibitors (GDIs), which suggested a novel role for Cav-1 as a Cdc42 GDI in β-cells. The novel role was further supported by: 1) in vitro binding analyses that demonstrated a direct interaction between Cav-1 and Cdc42; 2) GST-Cdc42 interaction assays showing preferential Cav-1 binding to GDP-Cdc42 over that of GTP-Cdc42; 3) Cav-1 depletion studies resulting in an inappropriate 40% induction of activated Cdc42 in the absence of stimuli and also a 40% increase in basal insulin release from both MIN6 cells and islets. Expression of wild-type Cav-1 in Cav-1-depleted cells restored basal level secretion to normal, whereas expression of a scaffolding domain mutant of Cav-1 failed to normalize secretion. Taken together, these data suggest that Cav-1 functions as a Cdc42 GDI in β-cells, maintaining Cdc42 in an inactive state and regulating basal secretion in the absence of stimuli. Through its interaction with the Cdc42-VAMP2-bound insulin granule complex, Cav-1 may contribute to the specific targeting of granules to “active sites” of exocytosis organized by caveolae.

Regulated insulin granule exocytosis is elicited by the fusion of a primed and readily releasable pool of plasma membrane-localized granules and through the mobilization and trafficking of intracellularly localized insulin granules (1, 2). Granule fusion is initiated through the entry of glucose into the pancreatic β-cells via the GLUT2 glucose transporter, leading to elevation of the intracellular ATP/ADP ratio, which in turn closes the ATP-sensitive K+ ($K_{ATP}$) channels (3, 4). This triggers the opening of the voltage-dependent calcium channels and increased intracellular cytoplasmic calcium concentration (5), which culminates in fusion of insulin secretory granules from the multiple intracellular pools (6, 7). Granules fuse with the plasma membrane through the pairing of their vesicular soluble NSF2 attachment protein receptor (v-SNARE) VAMP2 with the plasma membrane target membrane SNARE (t-SNARE) proteins Syntaxin 1A and SNAP-25 (8–12). Although this provides a mechanism for fusion of granules/vesicles with the plasma membrane, the upstream events required for targeting the granules/vesicles specifically to “active fusion sites” on the plasma membrane where their cognate t-SNAREs reside remains unclear. In addition, the mechanism that restricts fusion of granules near the plasma membrane in the absence of stimulus is not known.

The small Rho family GTPase Cdc42 has been demonstrated to co-localize with VAMP2-bound insulin secretory granules in pancreatic β-cells (13, 14) and may function in targeting insulin-secretory granules to Syntaxin 1A fusion sites at the plasma membrane. Cdc42 is found bound directly to the NH2 terminus of VAMP2 under basal conditions. Stimulation by glucose triggers the activation of Cdc42, and activated Cdc42 bound to VAMP2 localizes insulin granules at the plasma membrane. Cdc42 binds indirectly to Syntaxin 1A (15), and the interaction is bridged by VAMP2 to form a heteromeric complex (16). The interactions among Cdc42 and the SNARE proteins are functionally important for SNARE-mediated insulin exocytosis (16), as is the cycling of Cdc42 between its GDP- and GTP-bound conformations. However, although it is clear that glucose activates Cdc42 at a step proximal to or at $K_{ATP}$ channel closure (17), the precise regulatory proteins involved in this Cdc42 cycling pathway are unknown. Putative regulatory proteins to investigate would probably fall into three categories: 1) guanine nucleotide exchange factors, which catalyze nucleotide exchange and mediate activation; 2) GTPase-activating proteins, which stimulate GTP hydrolysis leading to inactivation; 3) proteins, which stimulate GTP hydrolysis leading to inactivation;
and 3) guanine nucleotide dissociation inhibitors (GDIs), which prevent interaction with the plasma membrane, exchange factors, and downstream effector proteins (for reviews, see Refs. 18 and 19).

Cdc42 has been reported to localize to caveolar domains at the plasma membrane (20). Similarly, Syntaxin 1A and SNAP-25 have been found “clustered” in highly active fusion centers by caveolae in neurosecretory PC12 cells (21, 22). Caveolae are microdomains that are enriched in cholesterol and sphingolipids (23) located in the plasma membrane as well as in membranous vesicles residing close to the plasma membrane (24). These plasmalemmal organelles have been implicated in a wide variety of cellular functions, including vesicle transport and cell signaling (25). Furthermore, caveolar clustering of SNARE proteins into “fusion centers” has been suggested to increase the efficiency of neurotransmitter secretion (21).

Recently, studies have shown electron micrographs of islet β-cell membranes revealing caveolar structures that contain the protein caveolin-1 (Cav-1), suggesting that caveolae may also function in insulin exocytosis (26, 27).

However, there is also a significant proportion of Syntaxin 1A proteins outside of these caveolar domains (21), suggesting that the preferential targeting of neurotransmitter-containing vesicles to caveolar-clustered “fusion centers” probably requires additional cues. Cav-1 appears to have potential as one of the “additional cues” to preferentially direct granules to Syntaxin 1A fusion sites within the caveolar clusters. A key protein of caveolae and plasmalemmal organelles, Cav-1 is a transmembrane protein present on caveolae that forms a hairpin-like structure with both the NH₂ and COOH termini facing the cytoplasm (28). Cav-1 directly binds cholesterol (29) and forms homo- and heterooligomers (28, 30, 31). Cav-1 contains an oligomerization domain, juxtaposed to a scaffolding domain, and it is the latter of these domains that participates in signal transduction events (32, 33). Importantly, Cav-1 has been reported to associate with Cdc42 in other cell types, although the functional role of this interaction has not been evaluated.

In this report, we present evidence to support a novel role for Cav-1 as a Cdc42 GDI and suggest that the Cav-1-Cdc42 association may provide a link between caveolar clustering of SNARE proteins in the plasma membrane and efficient targeting of VAMP2-bound insulin granules to plasma membrane Syntaxin 1A-based fusion centers clustered by caveolae. In support of a functional role for Cav-1 as a Cdc42 GDI, we show that 1) Cav-1 interacted preferentially with GDP-bound Cdc42 and that the interaction was direct; 2) Cav-1 bound to Cdc42 via a conserved GDI motif located within the Cav-1 scaffolding domain; 3) Cav-1 was required to maintain low levels of secretion and inactivation of Cdc42 in the absence of stimuli. Taken together, these data suggest a mechanism whereby Cav-1 binds Cdc42-GDP under basal conditions and glucose stimulates the dissociation of the complex via GTP-loading of Cdc42 to facilitate regulated granule targeting to active fusion sites, ensuring that the appropriate interactions occur in an efficient spatial and temporal manner.

**EXPERIMENTAL PROCEDURES**

**Materials**—Radioimmunoassay grade bovine serum albumin and D-glucose were obtained from Sigma. The rabbit polyclonal anti-caveolin-1, mouse monoclonal anti-Myc (9E10), and actin antibodies were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Mouse monoclonal anti-VAMP2 antibody and rabbit polyclonal anti-VAMP2 were purchased from Synaptic Systems (Gottingen, Germany) and Chemicon (Temecula, CA), respectively. The Syntaxin antibody was obtained from Upstate Biotechnology, Inc. (Lake Placid, NY). Monoclonal anti-Cdc42, caveolin-1, and SNAP-25 antibodies were purchased from BD Biosciences. Rabbit anti-GST antibody was obtained from Affinity BioReagents (Golden, CO). The MIN6 cells and rabbit polyclonal phogrin antibody were a gift from Dr. John Hutton (University of Colorado Health Sciences Center). The n-octyl glucoside and GGTL-2147 were purchased from Research Products International Corp. (Mt. Prospect, IL) and Calbiochem, respectively. Recombinant Cdc42-His proteins were purchased from Cytoskeleton Inc. (Denver, CO). The ECL kit and Hyperfilm-MP were obtained from Amersham Biosciences. Tfx-50 lipofection reagent was purchased from Promega (Madison, WI). Vectashield was obtained from Vector Laboratories (Burlingame, CA). Human C-peptide and rat insulin RIA kits were purchased from Linco Research (St. Charles, MO).

**Plasmids**—The pCI52-Cav-1 wild-type and pCI52-Cav-1 SD (F92A/V94A) mutant constructs have been described previously (34) and were a gift from Dr. Michael Quon (National Institutes of Health). The pGEX-Cdc42 construct was a gift from Dr. Lawrence Quilliam (Indiana University School of Medicine). The pSilencer-Cav-1 construct was generated by insertion of annealed complementary double-stranded oligonucleotides encoding 19 nt, GCCCAACAACAAGGCCATG, of canine caveolin-1, followed by a loop region (TTCAAGAG) and then the antisense of the 19 nt. The pSilencer-control construct was generated in an identical manner, using the following 19-nt sequence, which fails to match any known mammalian protein using BLAST (NCBI): GCGCGCTTTGTAGGATTCG into the AflII site and GCCCAACAACAAGGCCATG into the 5'- and 3'-ends for insertion into the pSilencer1.0 vector (Ambion, Inc., Austin, TX). Nucleotide sequences encoding Cav-1 full-length (FL) amino acids and hydrophilic domain amino acids (residues 1–101) were amplified by PCR using oligonucleotides designed to contain EcoRI and XhoI restriction sites at the 5' and 3' ends, respectively. PCR products were subcloned into the EcoRI and XhoI of the pGEX4T-1 vector. The siCav-1-Ad adenoviral shuttle vector was generated by insertion of the Cav-1 siRNA sequence (5’-GCCCAACAAACAGGCCATG) into the 5’ AflII site and the 3’ Spel site of the pMighty vector (Viraquest, North Liberty, IA). The construct was linearized by restriction digestion with NdeI for recombination and virus-packaged with EGFP to enable visualization of infection efficiency. The siCon-Ad shuttle vector was generated by insertion of the Control siRNA sequence (5’-GCGCGCTTTGTAGGATTCG) into the AflII site and the 3’ Spel site of the pMighty vector and adenovirus made as described above. Cesium chloride-purified adenoviral
particles were used at an MOI of 100, and the efficiency of transduction was gauged visually by EGFP fluorescence.

**Cell Culture and Transient Transfection**—CHO-K1 cells were purchased from the American Type Culture Collection (Manassas, VA) and cultured in Ham’s F-12 medium supplemented with 10% fetal bovine serum, 100 units/ml penicillin, 100 μg/ml streptomycin, and 292 μg/ml l-glutamine. At 80–90% confluence, cells were electroporated with 40 μg of DNA as described previously (36). Under these conditions, ~70–80% of cells were transfected. After a 48-h incubation in media cells were harvested in Nonidet P-40 lysis buffer (25 mM Hepes, pH 7.4, 1% Nonidet P-40, 10% glycerol, 50 μM sodium fluoride, 10 mM sodium pyrophosphate, 137 mM sodium chloride, 1 mM sodium vanadate, 1 mM phenylmethylsulfonyl fluoride, 10 μg/ml aprotinin, 1 μg/ml pepstatin, and 5 μg/ml leupeptin), and lysates were cleared by microcentrifugation for 10 min at 4 °C for subsequent use in co-immunoprecipitation experiments. MIN6 cells were cultured in Dulbecco’s modified Eagle’s medium (with 25 mM glucose) supplemented with 15% fetal bovine serum, 100 units/ml penicillin, 100 μg/ml streptomycin, 292 μg/ml l-glutamine, and 50 μM β-mercaptoethanol, as described (17, 37, 38). MIN6 cells plated in 10-cm tissue culture dishes at 40–60% confluence were electroporated with 300 μg of plasmid DNA per cuvette (one 10-cm dish/cuvette) to obtain ~50% transfection efficiency using a procedure previously described (39). After 48 h of incubation, cells were washed twice with and incubated for 2 h in 1 ml of modified Krebs-Ringer bicarbonate buffer (MKRBB) (17, 37) and stimulated with glucose (20 mM) or KCl (50 mM). For studies with the geranylgeranylation inhibitor, GGTI-2147, MIN6 cells were cultured overnight in medium supplemented with vehicle (Me₂SO) or 20 μM GGTI-2147 followed by incubation in MKRBB with vehicle or GGTI-2147 for 2 h. Insulin secreted into the MKRBB was quantitated by radioimmunoassay. Cells were subsequently lysed in Nonidet P-40 lysis buffer to generate cleared cell detergent homogenates for quantitation of insulin content by insulin RIA and for co-immunoprecipitation assays. For measurement of human C-peptide release, MIN6 cells were transiently co-transfected with the human proinsulin expression vector (pCB6/INS), a kind gift from Dr. Chris Newgard (Duke University) using Tfx-50 with 2.5 μg of plasmid DNA per cuvette (one 10-cm dish/cuvette) to yield ~70–80% of cells were transfected. After a 48-h incubation in media cells were harvested in Nonidet P-40 lysis buffer (25 mM Hepes, pH 7.4, 1% Nonidet P-40, 10% glycerol, 50 μM sodium fluoride, 10 mM sodium pyrophosphate, 137 mM sodium chloride, 1 mM sodium vanadate, 1 mM phenylmethylsulfonyl fluoride, 10 μg/ml aprotinin, 1 μg/ml pepstatin, and 5 μg/ml leupeptin) for 2 h at 4 °C. Following three washes with Nonidet P-40 lysis buffer, proteins were eluted from the Sepharose beads and subjected to 12% SDS-PAGE followed by transfer to PVDF membrane for immunoblotting.

**Co-immunoprecipitation and Immunoblotting**—MIN6 cell lysates (3 mg) cleared in a lysis buffer containing 0.25% Triton X-100 and 60 mM n-octyl glucoside, a nonionic detergent for solubilizing membrane-bound proteins, were combined with 3 μg of rabbit anti-Cdc42, mouse anti-Cav-1, or mouse anti-VAMP2 antibody for 2 h at 4 °C, followed by a second incubation with protein G Plus-agarose for 2 h. The resultant immunoprecipitates were subjected to electrophoresis on 12% SDS-PAGE followed by transfer to PVDF membranes for immunoblotting. Primary antibodies were used at 1:250–1000 dilutions, and secondary antibodies conjugated to horseradish peroxidase diluted at 1:5000 for visualization by ECL.

**Subcellular Fractionation**—Subcellular fractions were isolated as previously described (42). Briefly, MIN6 cells at 70–80% confluence were washed with cold PBS and harvested into 1 ml of homogenization buffer (20 mM Tris–HCl, pH 7.4, 0.5 mM EDTA, 0.5 mM EGTA, 250 mM sucrose, and 1 mM dithiothreitol containing the following protease inhibitors: leupeptin (10 μg/ml), aprotinin (4 μg/ml), pepstatin (2 μg/ml), and phenylmethylsulfonyl fluoride (100 μM). Cells were disrupted by 10 strokes through a 27-gauge needle, and homogenates were centrifuged at 900 × g for 10 min. Postnuclear supernatants were centrifuged at 5500 × g for 15 min, and the subsequent supernatant was centrifuged at 25,000 × g for 20 min to obtain the secretory granule fraction in the pellet. The supernatant was further centrifuged at 100,000 × g for 1 h to obtain the cytosolic fraction. All steps were performed at 4 °C. Highly purified plasma membrane fractions were obtained using a protocol by Hubbard et al. (43). Briefly, the postnuclear pellet from the initial 900 × g centrifugation was mixed with 1 ml of Buffer A (0.25 M sucrose, 1 mM MgCl₂, and 10 mM Tris–HCl, pH 7.4) and 2 volumes of Buffer B (2 M sucrose, 1 mM MgCl₂, and 10 mM Tris–HCl, pH 7.4). The mixture was overlaid with Buffer A and centrifuged at 113,000 × g for 1 h to obtain an interface containing the plasma membrane. The interface was collected and diluted to 2 ml with homogenization buffer for centrifugation at 3000 × g for 10 min, and the resulting pellet was collected as the
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plasma membrane fraction. Fractions were assayed for soluble protein content as described (44).

**Immunofluorescence and Confocal Microscopy**—MIN6 cells at 40% confluence plated onto glass coverslips were incubated in MKRBB for 2 h, followed by stimulation with 20 mM glucose, and then fixed and permeabilized in 4% paraformaldehyde and 0.1% Triton X-100 for 10 min at 4 °C. Fixed cells were blocked in 1% bovine serum albumin and 5% donkey serum for 1 h at room temperature, followed by incubation with primary antibody (1:100) for 1 h. Cells were then washed with PBS and incubated with Texas Red secondary antibody (1:100) for 1 h. Cells were washed again in PBS and overlaid with Vectashield mounting medium, and coverslips were mounted onto slides for confocal fluorescence microscopy using a Zeiss 510 confocal microscope. Images presented within a figure were captured using identical settings unless otherwise specified.

**Cdc42 Activation Assay and Immunoblotting**—A glutathione S-transferase (GST) fusion protein, corresponding to the p21-binding domain of p21-activated kinase (Pak1) was used to specifically detect and interact with the GTP form of Cdc42 in MIN6 cell plasma membrane fractions using the EZ-Detect Cdc42 activation kit from Pierce. Briefly, plasma membrane fractions were prepared from cells incubated in MKRBB buffer for 2 h at 37 °C and stimulated with glucose for 3 min. Freshly made fractions (100 µg) were combined with 20 µg of PKA p21-binding domain-agarose for 1 h at 4 °C. After three washes with lysis buffer, proteins were eluted from the agarose beads and subjected to electrophoresis on 12% SDS-PAGE, followed by transfer to PVDF membrane. Membranes were immunoblotted with mouse anti-Cdc42 or rabbit anti-GST antibodies, and proteins were visualized by ECL.

**Adenoviral Transduction of Isolated Mouse Islets**—Pancreatic mouse islets were isolated as previously described (35), as modified from Lacy and Kostianovsky (45). Briefly, pancreata from 8–10-week-old male C57B16J mice were digested with collagenase and purified using a ficoll gradient. After isolation, islets were immediately transduced at an MOI of 100 with either siCon-Ad or siCav-1-Ad CsCl-purified particles for 1 h at 37 °C. Islets were washed twice and incubated for 48 h in RPMI 1640 at 37 °C and 5% CO₂. Transduction efficiency was determined by EGFP fluorescence. EGFP fluorescent islets were hand-picked for static culture insulin secretion analysis.

**Static Culture**—Fresh islets from wild-type mice were hand-picked into groups of 10, preincubated in KRBB (10 mM Hepes (pH 7.4), 134 mM NaCl, 5 mM NaCO₃, 4.8 mM KCl, 1 mM CaCl₂, 1.2 mM MgSO₄, 1.2 mM KHPO₄) containing 2.8 mM glucose and 0.1% bovine serum albumin for 4 h. Media were collected to measure insulin secretion, and islets were solubilized in Nonidet P-40 lysis buffer as described above to determine cellular insulin content by RIA.

**Statistical Analysis**—All data are expressed as mean ± S.E. Data were evaluated for statistical significance using Student’s t test.

**RESULTS**

**Cav-1 Interacts with Cdc42 and SNARE Proteins**—The presence of Cav-1 protein has been reported in multiple β-cell lines such as HIT-T15 and INS-1 (27). To first verify that Cav-1 was also present in the MIN6 β-cell line, cells were lysed in a buffer containing 0.25% Triton X-100 and 60 mM n-octyl glucoside, a nonionic detergent for solubilizing membrane-bound proteins (32), and lysates were subsequently used for Cav-1 immunoprecipitation. As seen in Fig. 1A, Cav-1 protein was immunoprecipitated by anti-Cav-1 antibody, demonstrating its presence in MIN6 cells. Moreover, VAMP2 and Cdc42 were co-immunoprecipitated with Cav-1, suggesting association among these proteins. IgG control antibody failed to immunoprecipitate any of the three proteins. Reciprocal immunoprecipitation with anti-VAMP2 and anti-Cdc42 antibodies co-precipitated Cav-1, confirming the finding that Cav-1 associated with Cdc42 and VAMP2 (Fig. 1B). This association was specific for the Cav-1 isoform of caveolin, since Cav-2 protein was undetectable in MIN6 cell lysate and was not co-precipitated by Cdc42 or VAMP2 antibodies (data not shown).

VAMP2 and Cdc42 localize together at the plasma membrane and on insulin secretory granules in β-cells (14, 16, 46). The plasma membrane compartment contains several pools of granules, namely to their proximity to the plasma membrane (i.e. immediate releasable, readily releasable, or intracellular storage). To investigate the pool of granules to which Cav-1 was localized, we performed subcellular fractionation experiments. As described previously, MIN6 cells can be fractionated by differential centrifugation into plasma membrane (PM), cytosolic, and insulin granule storage (Gran) fractions (16, 42). The fractionation procedure was validated in two ways: 1) demonstration of highest insulin content in the insulin...
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granule fraction, with less in the PM and little if any in the cytosolic fraction (data not shown; see Refs. 16, 47, and 48); 2) presence of marker proteins Syntaxin 1A in the PM, VAMP2 and phogrin in the Gran, and Cdc42 in all three fractions (Fig. 2A). Although Cdc42 is a soluble protein, it also localizes to membrane fractions via associations with other membrane-bound proteins (16) and post-translational modifications (19). Among these fractions, Cav-1 was found localized to the plasma membrane and insulin granule fractions, consistent with other reports localizing Cav-1 to both the plasma membrane and to plasmalemmal vesicles and/or granules (26–28).

We next questioned which pool of granules Cav-1 was associated with and whether its association with the Cdc42-VAMP2 bound granules occurred in a glucose-sensitive manner. PM or Gran fractions prepared from MIN6 cells left unstimulated or stimulated with glucose for 3 min were used for co-immunoprecipitation with anti-VAMP2 (Fig. 2B). To specifically assess the ability of Cav-1 to associate with Cdc42 localized to granules and not free Cdc42, anti-VAMP2 antibody was used for co-immunoprecipitation. VAMP2 co-immunoprecipitated both Cdc42 and Cav-1 from both PM and Gran fractions under basal and glucose-stimulated conditions. However, co-precipitation of Cav-1 was significantly reduced in the PM fraction in response to glucose stimulation (Fig. 2B, lanes 1 and 2). Quantitation by optical density scanning revealed the decrease in Cav-1 co-precipitation with VAMP2 from the PM fraction to be 60 ± 6% (p < 0.05) (Fig. 2C). This decrease failed to occur in cells stimulated with KCl, indicating that the dissociation of Cav-1 from the VAMP2-Cdc42 complex required glucose. The response was also determined to be specific to D-glucose, since L-glucose failed to elicit dissociation of Cav-1 from Cdc42 and thus was not a default response to osmotic changes (data not shown). These data indicated that Cav-1 associated with Cdc42-VAMP2-bound granules in a pool near the plasma membrane and in a glucose-sensitive manner.

Cav-1 Associates with GDP-bound Cdc42—Cav-1 dissociation from the Cdc42-VAMP2 granules may have resulted from glucose-induced modifications to Cav-1, Cdc42, or VAMP2. Since we have previously shown that glucose induces the activation of Cdc42 within 3 min (17), we first questioned whether Cav-1 might preferentially interact with GDP-bound Cdc42. GST-Cdc42 protein was purified from E. coli and complexed to Sepharose beads loaded with either GDP or GTPyS, a nonhydrolyzable analogue of GTP, and immediately incubated beads with cleared MIN6 lysates prepared from cells stimulated with glucose for 3 min (Fig. 3A). Precipitation of GST-Cdc42-GDP beads resulted in markedly increased amounts of Cav-1 compared with GST-Cdc42-GTPyS beads (Fig. 3A, lanes 2 and 3). In addition, GST alone failed to associate with Cav-1, indicating that the interaction between Cdc42-GDP and Cav-1 was specific (Fig. 3A, lane 4). In multiple experiments, the GST-Cdc42-GDP also bound Cav-1 from lysates prepared from unstimulated MIN6 cells (data not shown), although the band intensity of Cav-1 was consistently stronger in reactions using the glucose-stimulated lysates. One explanation for this may be the glucose-stimulated dissociation of endogenous Cav-1-Cdc42 complexes freed Cav-1, increasing the amount of Cav-1 available for interaction with the exogenous GST-Cdc42-GDP.

To investigate if the interaction of Cav-1 with Cdc42 required an islet cell-specific factor, Cav-1 was expressed in CHO-K1 cells, and cleared cell lysates were prepared for incubation with GST-Cdc42 beads loaded with GDP or GTPyS (Fig. 3B). Like the binding observed in the MIN6 cell lysates, Cav-1 preferentially associated with the GDP-loaded Cdc42 compared with GTPyS-Cdc42 (Fig. 3B, lanes 2 and 3) and failed to bind to the GST-beads alone (Fig. 3B, lane 4). These data indicated that Cav-1 preferentially interacted with GDP-bound Cdc42 and that the interaction was not restricted to β-cells.

Cdc42 Interacts with a Conserved Motif Present in Cav-1 and Other GDI Proteins—Since Cav-1 preferentially bound to the GDP-form of Cdc42, we assessed its putative role as a GTPase...
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A) MIN6 cells

B) CHO cells

FIGURE 3. Caveolin-1 interacts preferentially with GDP-bound Cdc42. GST-Cdc42 linked to Sepharose beads was preloaded with GDP or GTP\(^\gamma\)S and incubated with MIN6 cell lysates prepared from cells stimulated with glucose for 3 min (A) or with CHO-K1 detergent cell lysates prepared from cells expressing recombinant Cav-Wt protein (B). GST protein alone linked to Sepharose beads was used as a control for specificity of binding. Beads were washed, and proteins were eluted for resolution on 12% SDS-PAGE and transferred to PVDF for immunoblotting (IB) with Cav-1 and GST antibodies. Data shown are representative of 3–5 independent experiments.

FIGURE 4. Amino acid sequence comparison between Cav-1 and various mammalian forms of GDI proteins. GenBank\(^\text{TM}\) accession numbers for Cav-1 (NM_001003296); mouse skeletal muscle GDI-2 (Rab mGDI-2, AAB16908); Rab GDI-β (L36314) and GDI-2 (NM_001001643) are indicated. Amino acid residue numbers are listed to the right of each sequence, and the conserved residues of the binding motif are shaded. Φ, basic amino acid Arg or Lys.

GDI. Cav-1 contains a Ras-binding region within the scaffolding domain (amino acids 82–101), and alignment of this region of Cav-1 with known mammalian Rho and Rab GDIs revealed a 10-amino acid region of high conservation (Fig. 4). Of these 10 residues, all were conserved among Cav-1 sequences in mice, rats, canines, chickens, and humans. More than 45% of the residues shared identity with a similar region in sequence alignments with murine skeletal muscle isoform of RabGDI-2 (mGDI-2), murine Rab GDI-β, and porcine Rab GDI-2 (shaded). In the other 5–6 residues of this region, there remained ~27% sequence similarity (conservative substitution). A core sequence of 6 residues is overall highly conserved and was defined by the consensus motif FT-VTΦY, where Φ represents an arginine or lysine residue.

To determine if the binding interaction between Cdc42-GDP and Cav-1 required these conserved residues of Cav-1, GST-Cdc42 loaded with GDP or GTP\(^\gamma\)S was combined with lysates prepared from CHO-K1 cells overexpressing Myc-tagged wild-type (Cav-Wt) or mutant (Cav-Mut, point mutations F92A and V94A) forms of Cav-1 (34). The Myc-Cav-Wt protein bound preferentially with the GDP-bound GST-Cdc42 (Fig. 5, lanes 1 and 5). In contrast, Myc-Cav-Mut failed to bind to GST-Cdc42-GDP or -GTP\(^\gamma\)S (Fig. 5, lanes 2 and 6). Neither Cav-Wt nor Cav-Mut bound to GST beads alone (Fig. 5, lanes 3 and 4). The lack of Cav-Mut binding to GST-Cdc42-GDP was not due to reduced protein expression, since Cav-Wt and Cav-Mut proteins were equivalently expressed in lysates (Fig. 5, lanes 7 and 8). These data suggested that the interaction between Cdc42-GDP and Cav-1 was mediated by the consensus motif present in the Ras-binding/scaffolding domain of Cav-1.

In addition to binding to GDP-bound forms of GTPases, GDIs form high affinity complexes with the geranylgeranyl membrane-targeting moiety present at the COOH terminus of the GTPase (for reviews, see Refs. 18, 49, and 50). In MIN6 cells, the geranylgeranyltransferase inhibitor GGTI-2147 was found to decrease the association between Cav-1 and Cdc42 by 22% (p < 0.05). This decrease is consistent with that of other reports showing similar functional effects of this inhibitor (51, 52). These data suggested that the interaction between Cdc42-GDP and Cav-1 was mediated by the consensus motif present in the Ras-binding/scaffolding domain of Cav-1.

Cav-1 Directly Interacts with Cdc42—GDI proteins interact directly with their GTPases. To determine whether the interaction between Cav-1 and Cdc42 was direct or indirect, recombi-
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Cav-1 Depletion Leads to Increased Basal Secretion—Functionally, GDIs regulate access of GTPases to exchange factors and effectors present at sites of activation in specific membrane compartments. To evaluate the functional requirement for Cav-1 in regulating glucose-stimulated insulin secretion, we generated two siRNAs directed against different regions of the canine Cav-1 cDNA in a plasmid delivery system (pSilencer1.0; Ambion). Initially, CHO-K1 cells were electroporated with Cav-Wt and Cav-Mut expression levels, since both were found localized to both intracellular membranes and plasma membrane in an identical manner (Fig. 7C, bars 3 and 4). Moreover, the functional difference was not attributed to differences in Cav-Wt and Cav-Mut expression levels, since both were found to be expressed in equivalent abundances under control conditions and both showed similar depletion by Cav-1 siRNA (Fig. 7E). Therefore, these data suggest that the interaction of Cdc42 with the conserved FT-VTΦ motif of Cav-1 mediates basal secretion.

To determine whether the depletion of Cav-1 from primary islets would similarly result in increased basal secretion, freshly isolated wild-type mouse islets were transduced with Cav-1 (siCav-1-Ad) or control (siCon-Ad) siRNA-expressing adenoviral particles (packaged with enhanced green fluorescent protein for positive identification of transduced islets). 48 h following transduction, the green fluorescent protein-expressing islets were identified and hand-picked into batches of 10 for analysis of insulin secretion under static culture conditions.

Cav-1 siRNA showed no increase in secretion beyond basal secretion level release. Cellular insulin content was unaffected in cells depleted of Cav-1 by siRNA, under either unstimulated or glucose-stimulated conditions (data not shown). Thus, these data demonstrated that siRNA-mediated depletion of Cav-1 resulted in significant elevation in basal insulin secretion with no significant responsiveness to glucose within 10 min, suggesting that Cav-1 plays a role in maintenance of basal secretion and may be required for glucose-induced initiation of secretion.

To determine if the requirement for Cav-1 in the maintenance of basal secretion was related to its ability to interact with Cdc42 through the conserved FT-VTΦ motif, Cav-1 levels were restored in Cav-1 siRNA-depleted cells by transfection of either Cav-Wt or Cav-Mut DNA. MIN6 cells were simultaneously co-transfected with the human proinsulin cDNA, since it is immunologically distinct from the mouse C-peptide secreted from the MIN6 cells and is used to detect secretion specifically from transfected cells (53, 54). In the human C-peptide assay system, basal release was significantly elevated in Cav-1 siRNA-transfected cells relative to control cells (Fig. 7C, bars 1 and 2). Although the release was only 10% higher than control in this system, rather than the 40% observed in the electroporated cells, the elevation of basal level with the Cav-1 siRNA was statistically significant and observed in all experiments. This discrepancy may have resulted from dilution of the effect coming from some cells in the population not having taken up all three types of DNA during the transfection. Consistent with a requirement for Cav-1 in maintenance of basal insulin release, replenishment of Cav-Wt expression in Cav-1-depleted cells normalized basal secretion down to the level exhibited by control cells (Fig. 7C, bars 3 and 4). However, Cav-Mut expression in Cav-1-depleted cells failed to normalize basal secretion in Cav-1-depleted cells (Fig. 7C, bars 5 and 6). This functional difference was not the result of differential cellular localization of Cav-Wt versus Cav-Mut, since both were found localized to both intracellular membranes and plasma membrane in an identical manner (Fig. 7D). Therefore, these data suggest that the interaction of Cdc42 with the conserved FT-VTΦ motif of Cav-1 mediates basal secretion.

increase in basal secretion is very high. Control siRNA-expressing cells exhibited a 2-fold increase in insulin release within 10 min of glucose stimulation, consistent with other reports using the MIN6 cell line (35). However, cells expressing the Cav-1 siRNA showed no increase in secretion beyond basal secretion level release. Cellular insulin content was unaffected in cells depleted of Cav-1 by siRNA, under either unstimulated or glucose-stimulated conditions (data not shown). Thus, these data demonstrated that siRNA-mediated depletion of Cav-1 resulted in significant elevation in basal insulin secretion with no significant responsiveness to glucose within 10 min, suggesting that Cav-1 plays a role in maintenance of basal secretion and may be required for glucose-induced initiation of secretion.

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To determine whether the depletion of Cav-1 from primary islets would similarly result in increased basal secretion, freshly isolated wild-type mouse islets were transduced with Cav-1 (siCav-1-Ad) or control (siCon-Ad) siRNA-expressing adenoviral particles (packaged with enhanced green fluorescent protein for positive identification of transduced islets). 48 h following transduction, the green fluorescent protein-expressing islets were identified and hand-picked into batches of 10 for analysis of insulin secretion under static culture conditions.
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FIGURE 7. Cav-1 depletion by siRNA-mediated knockdown results in increased basal secretion. MIN6 cells were electroporated with pSilencer 1.0-control or Cav-1 siRNA plasmid DNAs, and after a 48-h incubation, cells were incubated in glucose-free MKRBB for 2 h. Cells were subsequently left unstimulated or were stimulated with 20 mM glucose for 10 min, after which buffer was collected and detergent lysates were prepared. A, immunoblot (IB) analysis of Cav-1 protein abundance (SNAP-25 was assayed as a control for siRNA specificity). B, insulin released into the buffer was assayed by radioimmunoassay, and data were normalized to insulin content for each experiment. Data represent 4–6 independent sets of cells; p < 0.05 versus control siRNA. C, restoration of basal secretion by expression of Cav-Wt but not Cav-Mut in Cav-1-depleted MIN6 cells. MIN6 cells were transiently co-transfected with either control or Cav-1 siRNA plus either Cav-Wt, Cav-Mut, or vector DNA. All cells were also co-transfected with human proinsulin DNA as a reporter of granule secretion specifically from transfectable cells. Human C-peptide released into the MKRBB after a 2-h incubation was measured by RIA. Data represent the mean ± S.E. from four independent experiments (normalized to control siRNA = 100 for each DNA construct in each experiment) *, p < 0.05 versus control siRNA. D, protein localization of wild-type or mutant forms of recombinant caveolin-1 in MIN6 cells. MIN6 cells were electroporated with Cav-Wt or Cav-Mut and incubated for 48 h, followed by fixation and permeabilization for immunostaining with anti-Myc antibody. Confocal immunofluorescent images shown were taken using a Zeiss 510 with a ×100 objective and a ×2 zoom. E, protein expression of wild type or mutant forms of recombinant Cav-1 in control or Cav-1-depleted CHO-K1 cells. CHO-K1 cells were co-electroporated with control or Cav-1 siRNA plus either Cav-Wt or Cav-Mut. Detergent cell lysates were prepared, and protein was resolved on 12% SDS-PAGE for immunodetection of Myc-tagged recombinant (upper band) and endogenous (lower band) caveolin-1 proteins. Data are representative of three independent electroporation experiments.

Cav-1 Maintains Cdc42 in the Inactivated State—Since GDIs preferentially interact with the GDP-bound form of the GTPase and maintain the GTPase in the inactive state, we next questioned whether the depletion of Cav-1 would result in the inappropriate activation of Cdc42 under basal conditions. MIN6 cells were transduced with the siCon-Ad and siCav-1-Ad adenoviral particles (MOI = 100), stimulated with glucose for 3 min, or left unstimulated and then fractionated to examine Cdc42 activation in the PM compartment. Cdc42 activation assays were performed using the various PM fractions (Fig. 9A). PM fractions prepared from cells transduced with siCon-Ad showed low basal level Cdc42 activation, which increased upon stimulation with glucose for 3 min (Fig. 9B). In contrast, cells transduced with siCav-1-Ad showed 40% higher levels of basal Cdc42 activation, and glucose failed to elicit an increase in Cdc42 activation beyond basal levels. These findings are consistent with the functional data showing a 40% increase in basal levels of insulin secretion from siCav-depleted MIN6 cells (Fig. 7B). Taken together, these data show that Cav-1 is required to maintain Cdc42 in its inactive state under basal conditions and provide further support for the characterization of Cav-1 as a GDI for Cdc42.

DISCUSSION

In this report, we have presented data that suggest a novel role for Cav-1 as a link between Cdc42, SNARE proteins, and caveolae in insulin granule exocytosis. Mechanistically, under basal conditions, Cav-1 functions as a GDI by binding to the inactive GDP-bound Cdc42 present on VAMP2-bound insulin granules localized near the plasma membrane in close proximity to Syntaxin 1A fusion centers, restricting fusion of granules. Upon glucose stimulation, Cdc42 becomes activated and dissociates from Cav-1, allowing access of Cdc42-VAMP2-bound granules to interact with caveolae-localized Syntaxin 1A fusion centers. Importantly, although it has been previously reported that Cav-1 can associate with Cdc42 (55), our report is the first to

Islets transduced with siCav-1-Ad showed a 1.9-fold increase in basal secretion compared with siCon-Ad islets, whereas insulin content remained similar in siCon- and siCav-1-expressing islets (Fig. 8). These data are supportive of a potential physiological role for Cav-1 in the islet to maintain low levels of secretion in the absence of appropriate stimuli (basal conditions).
document that Cav-1 plays a novel role as a GDI for Cdc42 and participates directly in the regulation of insulin exocytosis.

Ras-related proteins are usually found in the inactive GDP-bound state in resting cells, although GTP is in a higher concentration in the cytosolic compartment. In pancreatic β-cells, a mechanism must exist to keep Cdc42 inactive until activation is required, and we propose that this mechanism is mediated through the GDI activities of Cav-1. Cav-1 shows sequence homology with several GDIs. Some of the defining features of GDIs are to 1) maintain Rho/Rab GTPases in the inactive conformation, 2) prevent the biological response of activation, and 3) bind in a direct manner to the GTPase. We showed that Cav-1 fits these criteria for a GDI: 1) Cav-1 preferentially interacted with the GDP-bound form of GST-Cdc42; 2) siRNA-mediated depletion of Cav-1 resulted in dysregulated insulin release from isolated mouse islets and clonal β-cells; and 3) Cav-1 bound directly to Cdc42. Whereas most GDI-GTPase complexes are found in the cytosol (for a review, see Ref. 56), Cav-1 is membrane-localized. However, RhoGDI-3 is also non-cytosolic and found to be in the detergent-resistant subcellular membrane fraction (57). Another Cdc42 GDI has been shown to exist in β-cells but is cytosolic (58). Thus, Cav-1 might function specifically with the plasmalemmal pool of Cdc42 on granules, whereas the other GDI mediates Cdc42 activities in the cytosol. Other activities held by GDIs are to prevent activation and modulate cycling between compartments. We showed that depletion of Cav-1 resulted in the inappropriate activation of Cdc42 in the plasma membrane compartment under basal conditions, suggesting that Cav-1 is needed to prevent inappropriate Cdc42 activation in the absence of relevant stimuli.

The discovery that glucose-stimulated activation of Cdc42 correlates with its dissociation from Cav-1 raises the issue of whether Cav-1 is an upstream signal for Cdc42 activation. One mechanism by which Cav-1 might function upstream of Cdc42 would be that Cav-1 undergoes tyrosine phosphorylation in response to glucose stimulation, which leads to Cdc42 activation. For example, DerMardirossian et al. (59) showed that phosphorylation of RhoGDI by Pak-1 resulted in selective release of Rac1 from the GDI complex, leading to interaction with guanine nucleotide exchange factors and activation. We have recently shown that Pak-1 is in MIN6 cells and interacts with Cdc42 (16) and therefore could potentially mediate the interaction between Cav-1 and Cdc42. However, Rho and RabGDIs have been shown to be regulated by both phosphorylation and dephosphorylation such that phosphorylation or dephosphorylation of the GDI modulates its interaction with GTPases. For example, it has been shown that RhoGDI is constitutively phosphorylated in resting neutrophils and that dephosphorylation of RhoGDI results in decreased affinity for RhoA (60). Conversely, phosphorylation at Tyr249 of Rab GD1-2 is required for membrane release of the GTPase Rab in 3T3-L1 adipocytes (61). Tyrosine-phosphorylated Cav-1 is reported to cluster/dimerize more than nonphosphorylated Cav-1 (62). However, it will be important to determine if the phosphorylation of Cav-1 is induced by glucose and if it is required for Cdc42 activation to place it in this pathway upstream of Cdc42. Alternatively, conversion of Cdc42 to its GTP-bound state may be the trigger for dissociation of Cav-1, and Cav-1 phosphorylation may occur as a downstream event. It has also been reported that Cdc42 can be phosphorylated in response to stimuli (63), and thus Cdc42 phosphorylation may be part of the dissociation mechanism from Cav-1. A mechanism involving glucose-induced modification of Cdc42 rather that modification of Cav-1 would fit well with our data that showed that Cav-1 present in lysates prepared from glucose-

| Glucose (min): | siCon-Ad | siCav-1-Ad |
|---------------|---------|------------|
| 0             |         |            |
| 3             |         |            |
| 0             |         |            |
| 3             |         |            |

**FIGURE 8. Cav-1 depletion from isolated islets results in increased basal insulin secretion.** Ilets isolated from wild-type C57BL/6j mice were immediately transduced at an MOI of 100 with adenoviral particles packed with EGFP encoding siControl (siCon-Ad) or siCav-1 (siCav-1-Ad) for 1 h. Islets were cultured for 48 h followed by insulin secretion analysis. Ilets showing green fluorescent protein fluorescence were hand-picked into groups of 10 and incubated in KRBH buffer containing 2.8 mM glucose for 4 h. Insulin released into KRBH buffer was measured by radioimmunoassay. Data represent the mean ± S.E. from three independent experiments (normalized to basal = 1 for each experiment). *, p < 0.05 versus siCon-Ad.

**FIGURE 9. Cav-1 depletion results in inappropriate Cdc42 activation under basal conditions.** A: MIN6 cells were transduced at an MOI of 100 with adenoviral particles packed with EGFP encoding siControl (siCon-Ad) or siCav-1 (siCav-1-Ad) for 1 h and cultured for 48 h. MIN6 cells were left unstimulated or stimulated with glucose (20 mM) for 3 min followed by subcellular fractionation. PM fractions were subjected to Cdc42 activation assays, and proteins were separated on 12% SDS-PAGE and transferred to PVDF for immunoblotting (IB). B: optical density scanning quantitation of three independent sets of PM fractions immunoblotted for Cav-1 and GST. Data were normalized to unstimulated = 1 for each fraction per experiment and are shown as means ± S.E. *, p < 0.05 versus unstimulated using unpaired Student’s t test. Data are representative of three independent sets of fractions.
stimulated MIN6 cells was capable of binding well to the exogenous GST-Cdc42-GDP. Investigations exploring the mechanism of Cdc42 dissociation from Cav-1 and association with guanine nucleotide exchange factors in islet β-cells are under way in our laboratory.

Cav-1 depletion mediated by siRNA expression revealed that Cav-1 functionally restricts secretion in the absence of appropriate stimuli in both isolated islets and MIN6 cells. We also showed that glucose failed to increase release of insulin within 10 min in MIN6 cells. However, glucose stimulation for 30 min did elicit a slight increase in secretion (data not shown), suggesting that Cav-1 either functions in early secretory events only or that Cav-1 depletion slowed the overall rate of secretion. Since glucose stimulated a dissociation of Cdc42 from Cav-1 within 3 min, we focused upon the role of Cav-1 during basal and early events in secretion. Cav-1 has also been reported to act as a negative regulator of numerous signaling molecules, including mitogen-activated protein kinase (64), nitric oxide and eNOS (65, 66), G proteins, and growth factor receptors (67–69). In addition, Cav-1 inhibits insulin action in rat adipose cells through a reduction in the recruitment of GLUT4 to the cell surface, a process that is VAMP2-dependent and can be mediated by Cdc42 (34, 70), suggesting that Cav-1 has many different functions. Thus, the novel role for Cav-1 as a GDI may underlie other signaling events where Cav-1 acts in a restrictive capacity.

Whereas Cav-Wt could restore basal secretion to Cav-1-depleted cells, Cav-Mut could not. Since Cav-Wt binds to Cdc42 but Cav-Mut does not, these data suggest that Cav-1 binding to Cdc42 is important for function. More specifically, Cav-1 bound to GDP-Cdc42 through its scaffolding domain, which is consistent with the binding of Cav-1 to the GTPase Ras as reported previously (71). Moreover, we have also identified two critical residues required for this interaction as Phe92 and Val94 within the GDI consensus motif of Cav-1, defined here as FT-VTΦY. It has also been demonstrated that expression of a truncated form of Cav-1 expressing only residues 82–101 functionally affects the basal GTPase activity and GTP binding of purified heterotrimeric G proteins (71). This is consistent with a model whereby Cav-1 binding negatively regulates GTP binding of Cdc42 and serves to hold Cdc42 in an inactive conformation in the absence of secretagogue. This would restrict access of granules juxtaposed to the membrane surface from inappropriate releasing insulin in the absence of stimuli. This concept is consistent with our data showing the interaction between VAMP2-Cdc42 and Cav-1 in the plasma membrane fraction under basal conditions and also with the release of Cav-1 upon glucose stimulation and activation of Cdc42.

There are conflicting data in the literature as to whether Syntaxin 1A and other SNARE proteins are localized into or juxtaposed to caveolar cholesterol-rich lipid microdomains. For example, two studies report the inclusion of Syntaxin 1A, SNAP-25, and VAMP2 in lipid rafts in pancreatic β-cells and neurosecretory PC12 cells (21, 72). Conversely, Lang et al. (22) suggest that in PC12 cells, syntaxin clusters are distinct from cholesterol-dependent membrane rafts, since they are Triton X-100-soluble and do not co-localize with raft markers. This notion is further supported by atomic force microscopy studies of syntaxin in liposomes, where it was determined that syntaxin is excluded from sphingomyelin-enriched domains in a cholesterol-dependent manner (73). In our hands, immunoprecipitation of Syntaxin 1A resulted in the co-immunoprecipitation of Cav-1 from MIN6 lysates (data not shown), suggesting that the proteins are either in close enough proximity to bind or interact indirectly through a common binding partner. Since Cdc42 binds to Syntaxin 1A through direct interaction with VAMP2, and we show here that Cav-1 binds directly to Cdc42, the interaction between Syntaxin 1A and Cav-1 may be dependent upon interaction of each with the same subset of Cdc42 molecules.

Data presented here show that depletion of Cav-1 in islets or MIN6 β-cells resulted in a significant increase in basal level insulin release, which correlated with the inappropriate activation of Cdc42 under basal conditions. However, this finding is in conflict with results obtained from the Cav-1 knock-out mice, which were reported to have normal fasting plasma insulin levels and develop hyperinsulinemia when placed on a high fat diet (74). However, plasma insulin levels are not an exclusive readout of insulin secretion; rather, these levels represent the net result of insulin secretion plus insulin action. Moreover, fasting plasma insulin levels in mice are highly variable, and thus the potential to detect any increase in fasting insulin levels may have been obscured by the variability. Thus, to examine the requirement of Cav-1 in insulin secretion, the islet (specifically, the β-cells of the islet) must be studied. To do this, we isolated islets from Cav-1 knock-out mice (Jackson Laboratories). However, we found that islet size from the knock-out mice was significantly smaller than in control mice and that the amount of insulin released was 10-fold less than that released by the C57BL/6 mouse islets tested in parallel studies. The background strain of the Cav-1 knock-out mice may have impacted the islet morphology and function as well as the level of plasma insulin. The Cav-1 knock-out mice (Jackson Laboratories) are primarily a mix of Sv129 and C57BL/6 with a minor contribution from the SJL background (contributed from the originating ES cell line). However, it has been shown that Sv129T2 mice have higher fasting plasma glucose levels and lower fasting insulin levels compared with other strains, such as C57BL/6, and that Sv129T2 mice were glucose-intolerant and secreted significantly less insulin in response to glucose compared with C57BL/6 mice (75). Another possibility may be that the Cav-1 knock-out mice underwent an adaptive response to the chronic absence of Cav-1, which impacted their islet development and/or function. Thus, for these reasons, we chose to acutely deplete Cav-1 using siRNA-mediated depletion from C57BL/6 islets to investigate the physiological importance of Cav-1 in the process of insulin secretion. However, to assess the physiological impact of Cav-1 depletion upon insulin secretion in vivo, the generation of Cav-1 conditional knock-out mice on the C57BL/6 mouse strain background will be required.

In conclusion, we report a novel interaction between Cdc42 and a GDI consensus motif FT-VTΦY within the scaffolding domain of Cav-1 and show this interaction to be functionally important for the overall maintenance of regulated insulin secretion. These data provide evidence for a novel function for Cav-1 as a Cdc42 GDI and a possible link between caveola-
localized SNARE fusion centers and Cdc42 targeting of granules to the plasma membrane. Last, this interaction was recapitulated in CHO-K1 cells as well, suggesting that the interaction may be part of a general mechanism for vesicle targeting to the SNARE machinery at the plasma membrane and may not be specific to the islet β-cell.

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