JBC RESEARCH ARTICLE

β-Cells retain a pool of insulin-containing secretory vesicles regulated by adherens junctions and the cadherin-binding protein p120 catenin

Received for publication, April 11, 2022, and in revised form, June 16, 2022 Published, Papers in Press, July 6, 2022,
https://doi.org/10.1016/j.jbc.2022.102240

Waruni C. Dissanayake[1,2] **and Peter R. Shepherd**[1,2,*]

From the [1]*Department of Molecular Medicine and Pathology, Faculty of Medical and Health Sciences, and* [2]*Maurice Wilkins Centre for Molecular Biodiscovery, The University of Auckland, Auckland, New Zealand*

Edited by Qi-Qun Tang

The β-cells of the islets of Langerhans are the sole producers of insulin in the human body. In response to rising glucose levels, insulin-containing vesicles inside β-cells fuse with the plasma membrane and release their cargo. However, the mechanisms regulating this process are only partly understood. Previous evidence indicated reductions in α-catenin elevate insulin release, while reductions in β-catenin decrease insulin release. α- and β-catenin contribute to cellular regulation in a range of ways but one is as members of the adherens junction complex. Therefore, we investigated the effects of adherens junctions on insulin release. We show in INS-1E β-cells knockdown of either E- or N-cadherin had only small effects on insulin secretion, but simultaneous knockdown of both cadherins resulted in a significant increase in basal insulin release to the same level as glucose-stimulated release. This double knockdown also significantly attenuated levels of p120 catenin, a cadherin-binding partner involved in regulating cadherin turnover. Conversely, reducing p120 catenin levels with siRNA destabilized both E- and N-cadherin, and this was also associated with an increase in levels of insulin secreted from INS-1E cells. Furthermore, there were also changes in these cells consistent with higher insulin release, namely reductions in levels of F-actin and increased intracellular free Ca^{2+} levels in response to KCl-induced membrane depolarization. Taken together, these data provide evidence that adherens junctions play important roles in retaining a pool of insulin secretory vesicles within the cell and establish a role for p120 catenin in regulating this process.

The β-cells of the islets of Langerhans are the only cell type in the body to produce insulin, and appropriate levels of glucose-stimulated insulin secretion (GSIS) from these cells are crucial for the regulation of glucose metabolism (1). Several features of the mechanisms regulating the secretion in response to glucose are understood including the processes by which glucose triggers calcium influx (2, 3) and the basic outline of how insulin granules traffic to and fuse with the plasma membrane, including the need for rearrangements of the actin cytoskeleton needed to facilitate this (1, 4). The processes regulating insulin secretory granules are partially understood and have some similarities with those regulating the secretion of neurotransmitter containing vesicles at the synapses of neurons (1, 2) and with the GLUT4 glucose transporter containing vesicles in adipocytes and fat tissue (5). However a characteristic feature of all regulated secretory processes is the ability to arrest secretory vesicles at a step immediately proximal to the final stages of fusion with the plasma membrane (2), and there is evidence indicating interactions of the cell with other cells or with extracellular matrices plays an important role in these processes (6). β-cells are in fact polarized cells and contain adherens junctions and integrin-mediated junctions (7–9). Both cadherin-mediated and integrin-mediated cell junctions have been implicated in the regulation of insulin secretion (7, 8).

Adherens junctions are formed by homophilic Ca^{2+}-dependent interactions of the extracellular domains of cadherin proteins (10). The intracellular domains of cadherins bind to a range of catenin proteins including with the four members of the p120 catenin family (including the prototypical member of the family encoded by the CTNND1 gene, hereafter referred to as p120-catenin) (11, 12), with β-catenin, and with plakoglobin (10, 13). These bind at the juxtamembrane and distal intracellular regions of cadherins, respectively. β-Catenin can in turn interact with α-catenin and thus have impacts on the actin cytoskeleton in the region of adherens junctions (10, 14).

We have previously shown in cultured β-cell models and in the 3D context of mouse islets that β-catenin is required for establishing a glucose sensitive pool of insulin secretory granules (15–17). This process can be regulated by changes in extracellular glucose levels, which are associated with changes in phosphorylation status of β-catenin (16, 17). However, we find that β-catenin–dependent gene expression is not required for this but that actin rearrangements are consistent with this being a mechanism for dynamic modulation of β-cell secretory function (15, 16). Loss of α-E-catenin or

* For correspondence: Peter R. Shepherd, peter.shepherd@auckland.ac.nz.

α-N-catenin has the opposite effect as it allows increased levels of insulin release and increased K^+-stimulated Ca^{2+} flux (18). This raises the question of how other components of the adherens junction complex contribute to these effects (10, 14). Previous studies have established that cadherins are important for forming adhesion between β-cells (19, 20). Antibodies to E-cadherin that disrupt adherens junction partially attenuate GSIS in islets (21) and pseudoislets (22) and loss of E-cadherin selectively in β-cells in mice is associated with impairments in control of glucose metabolism (23). Conversely culturing β-cells on plates containing extracellular domains for either N-cadherin or E-cadherin creates artificial adherens junctions and this boosts GSIS (24). Loss of N-cadherin in mouse islets also affects insulin secretion from β-cells (25). Together, this suggests that both these cadherins potentially play roles in regulating the ability of β-cells to properly regulate secretion of insulin in response to glucose, but the relative role of different N- and E-cadherins remains to be established. Here, we sought understand the relative role these cadherins play in establishing and regulating the glucose-sensitive pool of insulin vesicles in the INS-1E β-cell model.

Results

Reducing expression of either Cdh1 (E-Cadherin) or Cdh2 (N-Cadherin) with siRNA caused a nonsignificant increase in GSIS (Fig. 1*A*), although the knockdown of E-cadherin alone significantly decreases the fold change in insulin secretion compared to low glucose level (Fig. 1*B*). However, basal insulin secretion at low glucose was significantly enhanced when both E-cadherin and N-cadherin levels were reduced such that no further effect on insulin secretion was seen with the addition of glucose (Fig. 1*A*). To check whether any of the effects observed on insulin secretion might be secondary to effects on cell viability, we investigated effects of the siRNA treatments on morphology of the cells (data not shown) and on cell viability by performing sulforhodamine B colorimetric (SRB) assays (Fig. 1*C*) and found no effect on either. Cadherins stabilize a pool of β-catenin at the plasma membrane, and in some cell types, loss of cadherins can lead to release of this pool and a corresponding increase in the nuclear pool of β-catenin where it can induce expression of a range of genes including Cyclin-D2 (26). However, Cyclin-D2 levels are not affected by cadherin knockdown despite decrease in β-catenin levels in dual Cdh1/Cdh2 knockdown cells, suggesting effects of the

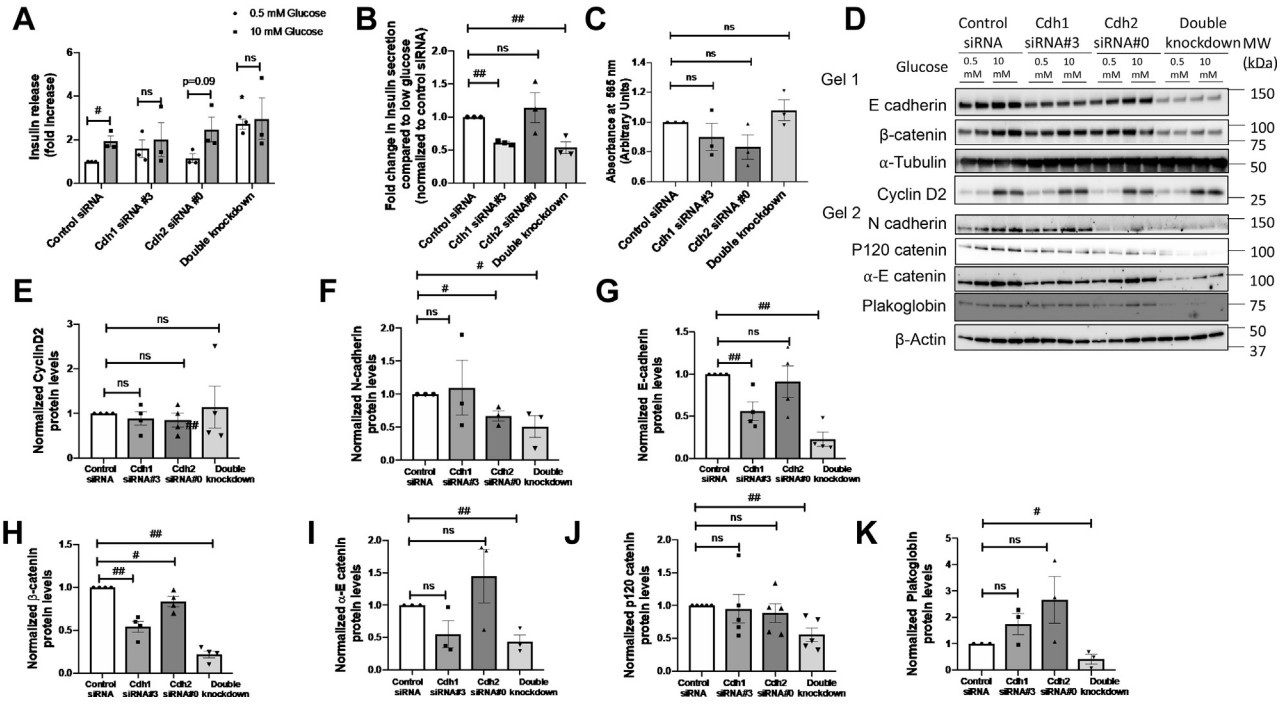

Figure 1. Simultaneous depletion of E-Cadherin and N-cadherin increase the basal insulin secretion. INS-1E cells were transfected with either control siRNA or siRNA targeting E-cadherin (Cdh1), N-cadherin (Cdh2), or both Cdh1 and Cdh2 siRNA. About 48 h after transfection, cells were serum starved for 2 h and then treated either 0.5 mM glucose or 10 mM glucose for further 2 h. *A*, insulin concentrations in the supernatants in siRNA transfected INS-1E cells measured by performing Alpha Liza. *B*, fold changes in insulin secretion compared to low glucose level were analyzed and values were normalized to fold change in control siRNA. Results are mean ± SEM of at least three independent experiments. $^{*}p < 0.05$ and $^{**}p < 0.01$ compared with control siRNA glucose stimulated condition as assessed by two-way ANOVA with LSD post hoc. $^{\#}p < 0.05$ compared with control siRNA as assessed by *t* test. *C*, SRB assays were performed in INS-1E cells 48 h after transfection with the indicated siRNAs targeting cadherins. Results are mean of at least three independent experiments. *D*, Western blot analysis of aforementioned cell lysates using E-cadherin, N-cadherin, β-catenin, Cyclin-D2, p120 catenin, plakoglobin, α-catenin, and α-tubulin antibodies. Representative Western blots of three independent experiments shown here. *E–K*, densitometry analysis of the expression of E-cadherin, N-cadherin, β-catenin, Cyclin-D2, p120 catenin, plakoglobin, and α-E-catenin proteins. Results are mean ± SEM of at least three independent experiments with average for each experiment indicated by symbols. $^{*}p < 0.05$ and $^{**}p < 0.01$ compared with control siRNA glucose stimulated condition as assessed by two-way ANOVA with LSD post hoc. $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$ compared with control siRNA as assessed by *t* test. LSD, least significance difference; SRB, sulforhodamine B colorimetric.

cadherin loss were not being mediated by effects on gene expression (Fig. 1, *D* and *E*). Knockdown of Cdh1/Cdh2 does though affect the levels of other proteins linked to adherens junction complexes (Fig. 1, *F–K*). Notably, the levels of β-catenin, plakoglobin, and p120 catenin were all reduced but only when both cadherins were lost, implying these proteins were stabilized by the presence of cadherins in this cell type.

Given the finding that p120 catenin levels were reduced in cadherin knockdown cells and that p120 is known to regulate levels of cadherins in other cell types (27), we also investigated the impacts of loss of p120 catenin in INS-1E rat β-cell model. Using siRNA, we were able to greatly attenuate expression of p120 catenin (Fig. 2, *A* and *B*). Reduction in p120 catenin expression resulted in an almost a complete loss of E-cadherin (Fig. 2, *A* and *C*) and also a strong reduction in levels of N-cadherin (Fig. 2, *A* and *D*). This is consistent with previous data indicating p120 catenin stabilizes cadherin proteins at the plasma membrane by inhibiting the internalization process (27). Since the stability of the intracellular pool of β-catenin and α-catenin protein is affected by their ability to bind to cadherin, we measured protein levels of β-catenin and α-E-catenin and found both were significantly reduced by p120 siRNA (Fig. 2, *A*, *E* and *F*). Together, this indicates that p120 catenin is required for the stability of the adherens junction complex in INS-1E cells.

We investigated the impact of p120 catenin depletion on GSIS. We find that transfection of INS-1E cells with three different siRNAs targeting p120 catenin (Fig. 3, *A* and *B*) increases the amounts of insulin released in response to glucose and basal insulin release (Fig. 3*C*). This is similar to the effects seen with double knockdown of Cdh1 and Cdh2. This effect of p120 catenin is also similar to α-catenin knockdown effect in INS-1E cells (18), suggesting the potential involvement of both p120 catenin, cadherins, and α-catenin in the mechanisms retaining insulin secretory granules in the cells. To investigate the mechanism by which p120 catenin regulates insulin secretion, we analyze whether changes in levels of p120 catenin affected the total insulin synthesis. However, there were no significant differences in total insulin content between control siRNA transfected and p120 catenin siRNA transfected samples (Fig. 2*D*). Therefore, it is likely that p120-mediated regulation of insulin release is linked to modulation of insulin granule translocation or insulin granule fusion.

Influx of calcium ions through voltage-gated calcium channels in responses to glucose-induced depolarization of β-cell plasma membranes is a key step in promoting insulin granule fusion with the plasma membrane, leading to increase in insulin release (1, 2). However, INS-1E cells are not a good model for studying glucose-induced calcium fluxes as the level of response is low and is pulsatile (28, 29). To be able to

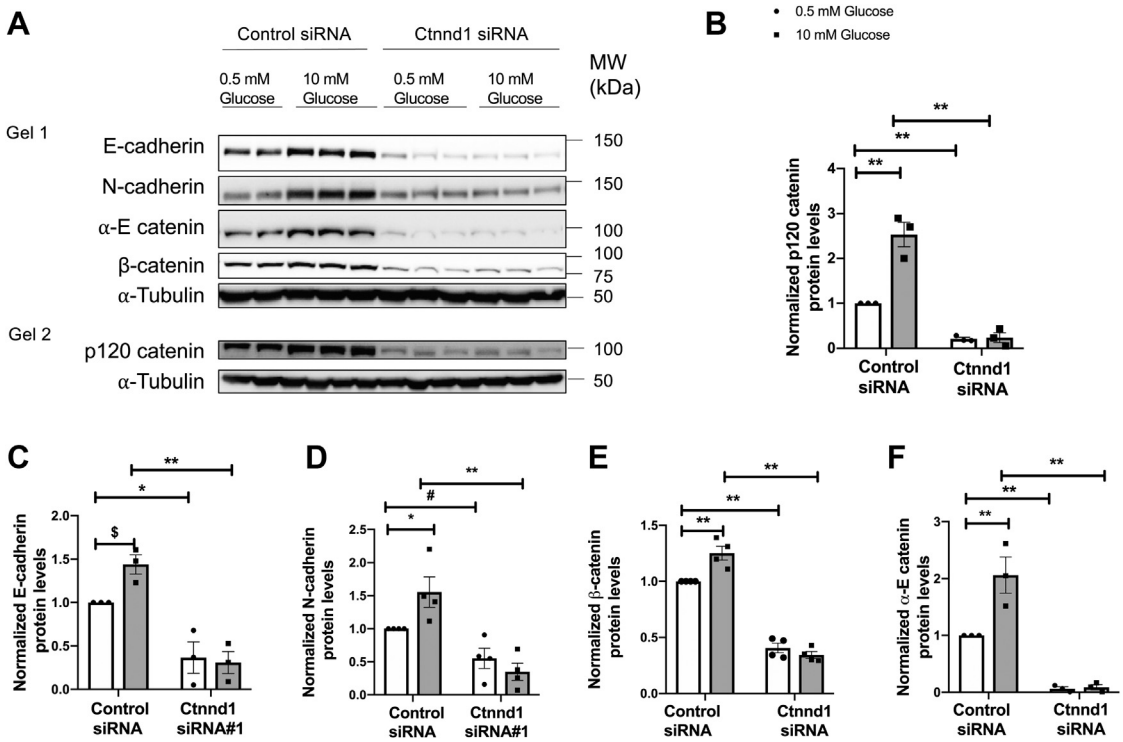

Figure 2. p120 catenin depletion decreases the protein expressions of E-cadherin, N-cadherin, β-catenin, and p-120 catenin. INS-1E cells were transfected with either control siRNA or siRNA targeting p120 catenin (Ctnnd1 siRNA). About 48 h after transfection, cells were serum starved for 2 h and then treated either 0.5 mM glucose or 10 mM glucose. *A*, cell lysates were subjected for Western blot analysis E-cadherin, N-cadherin, β-catenin, p120 catenin, α-E catenin, and α-tubulin antibodies. Densitometry analysis of (*B*) p120 catenin (*C*) E-cadherin (*D*) N-cadherin (*E*) β-catenin (*F*) α-E catenin protein expression after p120 catenin depletion. Results are mean ± SEM of at least three independent experiments. $^{*}p < 0.05$ and $^{**}p < 0.01$ compared with control siRNA as assessed by two-way ANOVA with LSD post hoc analysis. $^{\#\#}p < 0.01$ compared with control siRNA 0.5 mM glucose condition as assessed by *t* test. LSD, least significance difference.

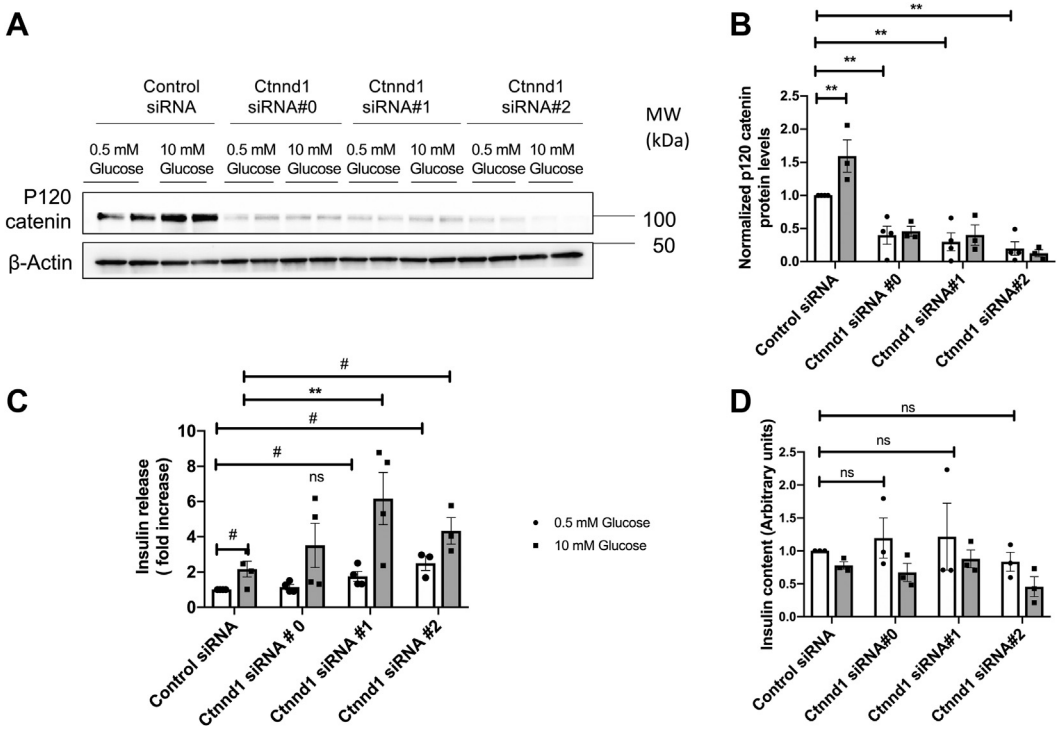

Figure 3. p120 catenin negatively regulate the glucose stimulated insulin secretion. INS-1E cells were transfected with either control siRNA or three different siRNAs targeting Ctnnd1. After serum starvation, cells were treated with either 0.5 mM glucose or 10 mM glucose. *A*, cell lysates were subjected for Western blot analysis using p120 catenin and β-actin antibodies and (*B*) Western blot images were quantified. *C*, secreted insulin concentrations in the supernatants of siRNA transfected cells were measured. *D*, total insulin content in the p120 catenin transfected cells were measured using total cell lysates. Results are mean ± SEM of at least three independent experiments $^{*}p < 0.05$ and $^{**}p < 0.01$ compared with control siRNA as assessed by two-way ANOVA with LSD post hoc analysis. $^{\#}p < 0.05$ compared with control siRNA as assessed by *t* test. LSD, least significance difference.

reproducibly assess the impact that loss of p120 catenin had on calcium flux, we instead induced maximal voltage-dependent calcium influx by depolarizing the plasma membrane with KCl. Consistent with the role of p120 catenin in increasing insulin secretion, here we found that depletion of p120 catenin increases the KCl-stimulated calcium influx (Fig. 4*A*) and that in parallel p120 catenin knockdown also increases the KCl-stimulated insulin secretion in INS-1E cells (Fig. 4, *B* and *C*).

We have previously found that adherens junction components β-catenin and α-catenin are involved in regulating vesicle trafficking to the plasma membrane in β-cell models and that this is associated with changes in the actin cytoskeleton (15, 16, 18). Actin cytoskeleton has dual mode of actions on insulin secretion; under basal condition, actin web formed at cell periphery prevents insulin granule exocytosis while filamentous actin provides transportation tracts for insulin granules (7). Cadherins and p120 catenin are also known to be involved in actin remodeling (30). Therefore, we investigated the effect of siRNA-mediated knockdown of p120 catenin on actin polymerization and found a significant decrease in the F/G actin ratio compared to the scrambled siRNA transfected cells (Fig. 5, *A* and *B*). The p120 catenin depletion effect on actin polymerization is consistent with its impact on insulin secretion given that high glucose levels that increase insulin secretion also decrease the actin polymerization (7). When we treated cells with the Arp2/3 inhibitor-CK666 that blocks actin branching and the p120 catenin knockdown effect on insulin secretion is attenuated (Fig. 5*C*). This observation is similar to what we found in α-E catenin knockdown samples (18), and this would be consistent with a model where reduction of either p120 catenin or α-E catenin results more branched actin in the cells leading to increase in insulin release. However, inhibition of actin branching in these catenin-depleted cells leads to an accumulation of unbranched actin filaments, causing attenuation of insulin release induced by catenin knockdown. Further supporting the role of p120 catenin in actin remodeling, we found that p120 catenin knockdown also modulates the phosphorylation (Ser3) of cofilin protein (Fig. 5, *D* and *E*), which is known to regulate actin polymerization (31), actin severing (32), and insulin secretion (33). Given that Ser3 phosphorylation negatively regulates the cofilin activity (34) and here we found that p120 catenin knockdown increases Ser3 phosphorylation, we concluded that 120 catenin mediated actin remodeling may also occur through the modulation of cofilin activity.

Changes in glucose levels are known to result in modulation of the actin cytoskeleton in β-cells (7), and we have previously shown that exposure of INS1E cells to different levels of glucose modulates levels of α-catenin and β-catenin (15, 18). Therefore, we compared expression levels of cadherin proteins and p120 catenin in INS1E β-cells in the presence of very low ambient glucose (0.5 mM) or a level equivalent to post prandial levels (10 mM) for 2 h. These studies show a significant effect of glucose on p120 catenin levels (Figs. 2, *A* and *B* and 3, *A* and *B*), N-cadherin, and E-cadherin (Fig. 2, *A*, *C* and *D*). This therefore has potential to contribute to longer term

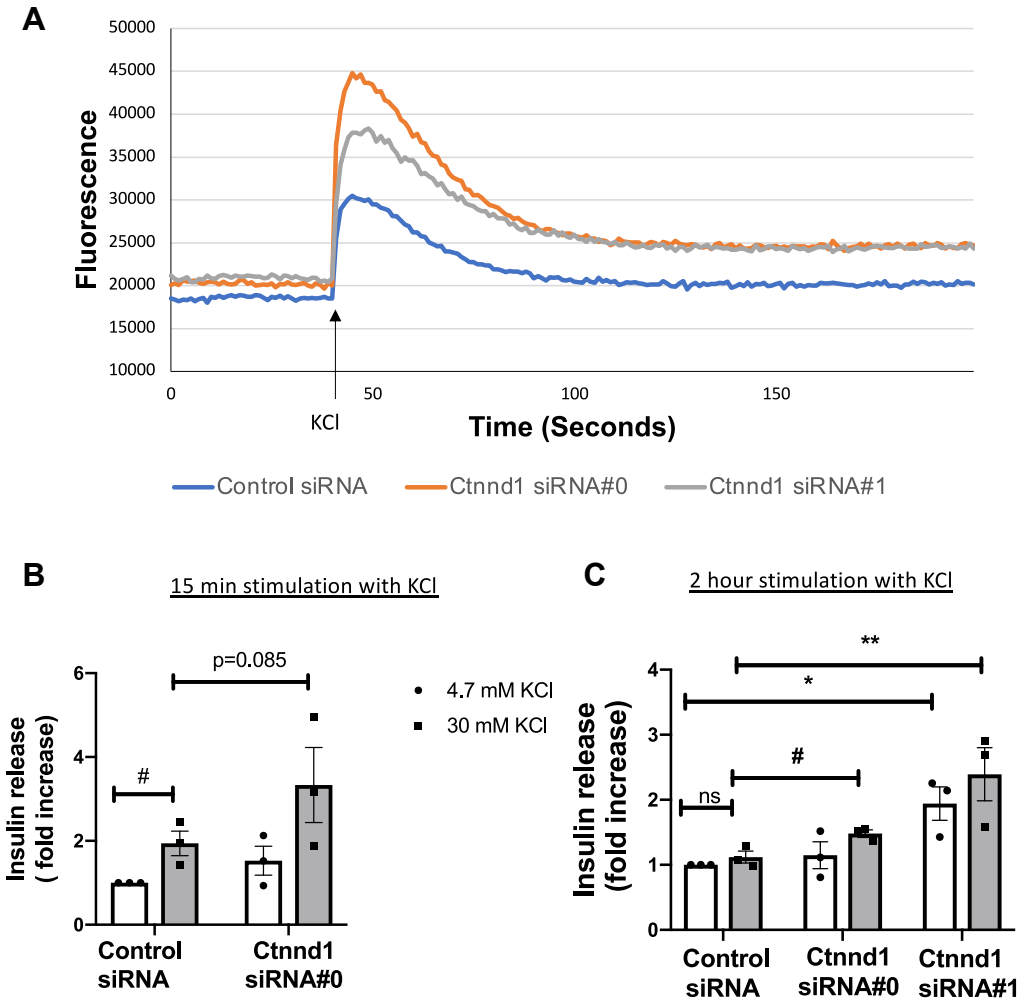

Figure 4. Reduction of p120 catenin protein level is associated with increase in KCl stimulated Ca^{2+} influx and KCl stimulated insulin secretion. *A*, forty-eight hours after siRNA transfection cells were incubated with calcium-binding dye for 1 h at 37 °C. At 40 s, 30 mM KCl (final concentration) was added to each well and fluorescence readings were taken at every 2 s for 200 s using Fluostar optima plate reader. Similar results were observed in three independent experiments with Ctnnd1 siRNA#0 and two independent experiments with Ctnnd1 siRNA#1. *B* and *C*, p120 catenin transfected INS-1E cells were stimulated with 30 mM KCl and secreted insulin levels were measured using Alpha-Liza assays. Results are mean ± SEM of at least three independent experiments $^{*}p < 0.05$ and $^{**}p < 0.01$ compared with control siRNA as assessed by two-way ANOVA with LSD post hoc analysis. $^{\#}p < 0.05$ compared with control siRNA as assessed by *t* test. LSD, least significance difference.

modulation of a β-cell's insulin secretory capability in response to changing blood glucose levels.

Discussion

The current studies define a role of adherens junction proteins in the regulation of insulin secretion. At least in the INS1E β-cell model, N- and E-cadherin appear to be the main cadherins as loss of both of these is sufficient to induce complete loss of β-catenin, plakoglobin, and p120 catenin. While there are many similarities in the way the various cadherins function (10), differences have also been reported in roles played by E- and N-cadherin (35). However, the presence of either N- or E-cadherin is sufficient to partially suppress levels of basal and glucose-stimulated insulin secretion and more significant effects are only seen when both of these are knocked down. Thus N- and E-cadherin play functionally redundant roles in this context. This could explain why *in vivo* KO of either E-cadherin or N-cadherin in mouse β-cells only have partial effects on insulin secretion (23, 25). Overall, this demonstrates the cadherin complexes are important in reducing the basal levels of insulin secretion and thus in establishing conditions that allow regulated secretion to occur.

There is also evidence that adherens junctions are important in other cell types for establishing the conditions required to establish regulated fusion of intracellular vesicles at the plasma membrane. For example, in muscle and adipocytes, β-catenin has been implicated in regulating increases in GLUT4 at the plasma membrane in response to insulin or exercise (36–38), with M-cadherin being also implicated in the case of muscle (37). Also, overexpression of p120 catenin reduces basal and insulin-stimulated trafficking of GLUT4, mannose-6-phosphate receptor, and transferrin receptor in adipocytes (39), which is consistent with our findings here. Adherens junctions also play an important role in regulating the recruitment of synaptic vesicles in neurons (40–42). Together,

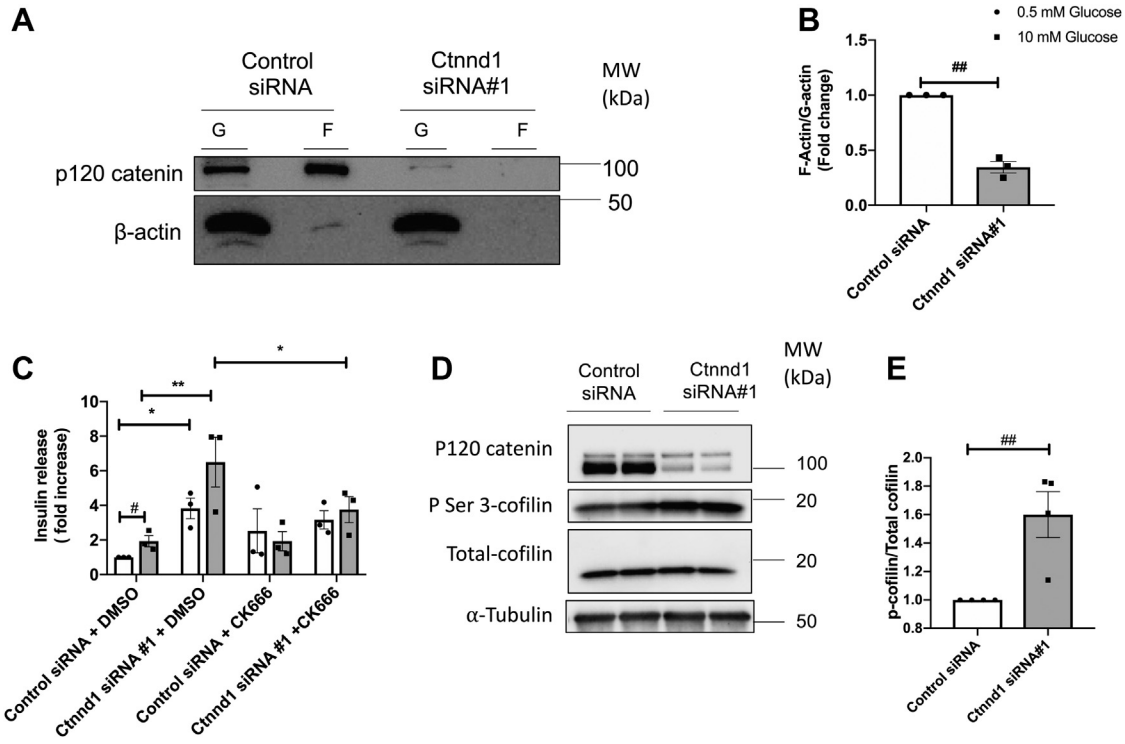

Figure 5. p120 catenin knockdown is associated with actin remodeling. INS1-E cells were transfected with either control siRNA or Ctnnd1 siRNA. *A*, F-actin and G-actin were separated by ultracentrifugation at 100,000*g* for 1 h and expression of F-actin and G-actin were analyzed by performing Western blot analysis using β-actin antibody. *B*, F-actin and G-actin ratio was analyzed by densitometry. *C*, siRNA transfected INS-1E cells were pretreated with either DMSO or 100 μM CK666 for 30 min and stimulated with glucose for 2 h in the presence of DMSO/CK666. Secreted insulin concentrations in the supernatants were measured by performing Alpha Liza assays. *D*, INS1-E cells were transfected with either control siRNA for Ctnnd1 siRNA, and 48 h after transfection, cells were lysed, and cell lysates were subjected to Western blot analysis. *E*, densitometry analysis of p-cofilin/total cofilin. Results are mean ± SEM of at least three independent experiments. $^{*}p < 0.05$ and $^{**}p < 0.01$ compared with control siRNA as assessed by two-way ANOVA with LSD post hoc analysis. $^{\#}p < 0.05$ compared with control siRNA as assessed by *t* test. DMSO, dimethyl sulfoxide; LSD, least significance difference.

this implies that the adherens junctions are controlling mechanisms that these different types of vesicles in different cells all require for proper regulated vesicle trafficking to occur. While there are some differences in the way these all traffic to the plasma membrane, the one thing they all share is similarities in the final stage of this journey in the steps involved in fusing with the plasma membrane.

There are several reasons why regulation of actin remodeling is a strong candidate as a mechanism for being a common mechanism linking these diverse vesicle trafficking processes with cadherin. Actin remodeling is known to be required for regulated trafficking of insulin granules, particularly in second phase insulin secretion (43). It is also required for regulating neurotransmitter release at synapses, and as with insulin secretion, it seems to be more involved with secondary phase of such processes rather than the acute release of predocked vesicles (44). Actin remodeling is also required for regulating the trafficking of GLUT4 containing vesicles in response to either insulin or contraction (38, 45, 46). In support of this, actin can sequester syntaxin-1 and SNAP-25 in a glucose-dependent manner (47) and it is known that actin can mask functional domains of the key SNARE protein syntaxin-4 in β-cell models (48). In this model, actin remodeling after cell stimulation would relieve these inhibitory effects and allow vesicle fusion. This is supported by the fact that treatment of β-cells with the actin depolymerizing agent latrunculin increases insulin release to a similar level as glucose (47).

Research on the role of cell adhesion molecules in the regulation of actin in relation to vesicle trafficking at the cell surface has to date largely focused on the role of integrins (reviewed in (7)). In the case of insulin secretion, there is clearly a role for such processes as the final fusion of vesicles and secretion for insulin occurs at the basolateral face, which interacts with the extracellular matrix *via* integrins (49). However, this does not also preclude a role for adherens junctions in directly regulating insulin vesicle secretion. Indeed, in elegant experiments, directly comparing the effects of integrin engagement and E-cadherin on insulin vesicle release, significant amounts of insulin were found to be secreted in areas of β-cell membranes engaged by E-cadherin (49). Adherens junctions are well placed to act as a major regulatory point for cortical actin as they are highly efficient at recruiting actin fibers (50). For example, experiments show that exogenous labeled actin introduced into keratinocytes does not disperse but instead rapidly locates to sites of adherens junctions (51). Thus, it is possible that adherens junctions play a direct role in regulating the trafficking and fusion of insulin secretory granules. Alternatively, these junctions could also create a sink of actin that can trap molecules required for vesicles fusion in an inactive state and divert these away from

active sites of vesicle fusion at the basolateral surfaces until signals arrive to release this inhibition.

If actin constrains insulin secretory vesicles from fusing with the plasma membrane, then there must be mechanisms to release this inhibition when insulin secretion is required. Cadherin complexes contain molecules that can regulate actin cytoskeleton turnover and thus potentially relieve such inhibitory mechanisms and contribute to increasing insulin release in response to secretagogues such as glucose. One molecule that can contribute to such mechanisms is p120 catenin, which can regulate actin cytoskeleton through modulation of Rho/Rac/cdc42 GTPases (30, 52, 53). In particular, p120 catenin is known to activate Rac1 and Cdc42 while inhibiting RhoA (54). This is consistent with promoting insulin release as Rac1 (55, 56) and Cdc42 (57) are known to promote insulin release while RhoA inhibits insulin secretion. Moreover, p120 catenin is also known to interact and/or modulate several actin-related proteins including Arp3 (58), cortactin (58), N-WASP (59), and cofilin (60) in other cell lines. Additionally, β-catenin is involved in regulating actin cytoskeleton in β-cells (15). This is in part mediated by PAK-1 mediated phosphorylation of β-catenin (16) but also involves interactions with α-catenin (18). Cadherins could also regulate actin cytoskeleton remodeling *via* a complex involving β-catenin and β-Pix, a Rac/Cdc42 GEF (61). Notably, p120 catenin has also been reported to regulate the phosphorylation of β-catenin on Tyr142, thus regulating the interaction with α-catenin and its subsequent effects on the actin cytoskeleton (62). Together, these provide a plausible mechanism by which adherens junctions could regulate fusion at the plasma membrane of multiple different types of vesicles in various different cell types by regulating actin remodeling.

Our studies also establish a role for adherens junctions in regulating voltage-sensitive calcium channels at the plasma membrane in β-cells, and this would also contribute to the effects of these junctions on insulin secretion. This has parallels with neurons where N-cadherin is known to regulate voltage-sensitive calcium channels *via* mechanisms that involve p120 catenin (53, 63). This could be secondary to the effects of p120 catenin and adherens junction proteins on the actin cytoskeleton as there is evidence that the localization of this type of calcium channel at the plasma membrane can be regulated by association with actin cytoskeleton (7, 64). This appears to be mediated by the association with actin of the Ca(v)β protein that regulates the subcellular localization and activity of the Ca(v)α channels (65, 66). In support of this, the effect of K^+ on calcium influx is elevated and F-actin is also reduced in INS1-E cells where α-catenin has been depleted (18). It should be noted that Ca^{2+} in the cell can also regulate actin levels through the calcium-dependent protease gelsolin (43), so it is possible the regulation of the voltage-sensitive calcium channels could in fact be a contributor to the effects of cadherins on the cytoskeleton.

For the cadherin system to play a role in regulating a dynamic process such as insulin secretion, we would expect some mechanism for regulation of this system that was consistent with the changes in the nutrients that trigger insulin release. We do observe rapid changes in β-catenin phosphorylation in response to changes in glucose levels and these correlate with effects on insulin secretion, so providing a potential mechanism for short term regulation of the system (16, 37). Over longer time periods of glucose stimulation, changes in levels of cadherins are observed here but even bigger changes are seen in p120 catenin. This agrees with previous studies showing β-catenin and α-catenin levels change over 1 to 2 h in response in changes in glucose levels in β-cells (15–18, 37). A glucose induced increase in adherens junctions would be predicted to cause an increase in F-actin and this is what is seen in β-cell models (67). Further, the high levels of F-actin in high glucose conditions (glucotoxicity) were resistant to remodeling and this was associated with reduced GSIS (67).

In conclusion, the finding that N- and E-cadherins and also p120 catenin are involved in regulating the mechanisms controlling the release of insulin from INS-1E β-cells establishes a role for the adherens junctions overall in regulating this process. Our results indicate that this involves control of the actin cytoskeleton and could also involve regulation of voltage-sensitive Ca^{2+} influx. While we have only studied one cell type here, the fact that other components of the adherens junctions contribute to insulin release in intact islets and also the regulation of a range of other vesicle trafficking processes in a similar way in a range of cell types suggests that this is an important process shared by all these vesicle trafficking systems. Overall, these findings are consistent with models where pools of filamentous actin regulated by the adherens junctions either plays a direct role in regulating vesicle trafficking or sequesters key molecules required for final steps of vesicle fusion and that these are released upon appropriate stimulation following actin remodeling.

Experimental procedures

Cell culture

A rat pancreatic β-cell model, INS-1E was kindly provided by Professor C. B. Wollheim. INS-1E cells were maintained in RPMI1640 medium with 10% fetal bovine serum, 1% antibiotic–antimycotic (100 units/ml penicillin, 100 μg/ml streptomycin, 0.25 μg/ml gibco amphotericin B), 10 mM Hepes, 2 mM L-glutamine, 1 mM sodium pyruvate (all Gibco, Life Technologies), and 50 μM β-mercaptoethanol.

siRNA transfection

All siRNAs including negative control siRNA (Stealth RNAi siRNA negative control, Med GC) and siRNA specific for E-cadherin (RSS331003), N-cadherin (RSS372940), and p120 catenin (RSS318990, RSS318991, RSS318992) were purchased from Life Technologies and used according to manufacturer's instructions. Thirty naomolar of either negative control siRNA or p120 catenin siRNAs was transfected to 5×10^5 INS-1E cells using Lipofectamine 2000 transfection reagent, and cells were plated in antibiotic-free RMPI medium. Twenty-four hours after transfection, medium was changed to complete RMPI medium, and experiments were performed 48 h after transfection.

Assays for GSIS

Cells were incubated in RPMI (11.1 mM glucose) for 48 h post transfection and then cells were glucose and serum-starved in Krebs-Ringer bicarbonate Hepes buffer (119 mM NaCl, 4.74 mM KCl, 1.19 mM MgSO4, 25 mM NaHCO3, 1.19 mM KH2PO4, 2.54 mM CaCl2, and 50 mM Hepes), pH 7.4, with 0.2% bovine serum albumin (low fatty acid) for 2 h and then stimulated with either 0.5 mM or 10 mM glucose for 2 h. Supernatants were collected, and secreted insulin in the supernatants were measured by using AlphaLISA Insulin Assay kit (PerkinElmer) according to the manufacturer's instructions. Insulin concentrations were normalized to the total protein levels in each sample.

Western blot analysis

Western blot analysis was performed using primary antibodies against p120 catenin (1:1000; Cell Signaling Technologies, #59854), total β-catenin (1:1000; Cell Signaling Tech nologies, #8480), N-cadherin (1:1000; Cell Signaling Technologies, #13116), E-cadherin (1:1000; Cell Signaling Technologies, #3195), plakoglobin (1:1000; Cell Signaling Technologies, #2309), Cyclin D2 (1:1000; Cell Signaling Tech nologies, #D52F9), p-ser 3 cofilin (1:1000; Cell Signaling Technologies, #3313), total-cofilin (1:1000; Cell Signaling Technologies, #5175), α-tubulin (1:20,000; Sigma–Aldrich, T6074), and β-actin (1:20,000; Sigma–Aldrich, A1978). After overnight incubation at 4 °C with aforementioned primary antibodies, membranes were washed and incubated with respective secondary antibodies anti-rabbit IgG horseradish peroxidase (1:10,000 Santa Cruz biotechnology), antimouse IgG horseradish peroxidase (1:20,000; Sigma–Aldrich) for 1 h at room temperature (RT) and developed with Clarity Western ECL substrate (Bio-Rad Laboratories).

Assay for F/G actin

Forty-eight hours after siRNA transfection, cells were washed with PBS and lysed with actin stabilization buffer (50 mM NaCl, 5 mM $MgCl_2$, 50 mM Pipes, 5 mM EGTA, 5% (v/v) glycerol, 0.1% nonidet P-40, 0.1% Triton X-100, 0.1% Tween 20, 0.1% 2-mercaptoethanol, 0.001% antifoam A, 1 mM ATP, and protease inhibitors). Collected cell lysates were homogenized by passing through a 24-gauge needle several times and then incubated at 37 °C for 10 min. Cell lysates were then centrifuged at 2000*g* for 5 min to separate the cell debris. The G-actin was separated from F-actin by centrifuging at 100,000*g* for 1 h at 37 °C. F-Actin pellet was resuspended in actin depolymerization buffer (Milli-Q water containing 10 μM of cytochalasin D) and incubated in ice for 1 h. Samples were run on 8% SDS-PAGE gel and the amount F- and G-actin were analyzed by performing Western blot using β-actin antibody.

Assay for calcium influx

Intracellular calcium levels in INS-1E cells were measured using FLIPR calcium5 assay kit (Molecular Devices, Inc) according to manufacturer's instructions. Briefly INS-1E cells were transfected with relevant siRNAs and plated on 96-well black wall, clear bottom plates. About 7×10^4 cells per well were used for each transfection, and 48 h after transfection, cells were washed with PBS and serum starved in 100 μl Krebs-Ringer bicarbonate Hepes buffer for 1 h. Then medium was replaced by 100 μl of Hank's balanced salt solution buffer plus 20 mM Hepes pH 7.4, and 100 μl of loading dye was added to each well containing Hank's balanced salt solution/Hepes buffer and incubated for 1 h at 37 °C. Ten microliter of H_2O was injected at 10 s and 10 μl of 660 mM KCl (30 mM final KCl concentration) injected to the same well after another 30 s. Readings were collected at every 2 s for 200 s using Fluostar optima plate reader.

SRB assay

SRB assays were performed as described previously (68). Briefly, INS-1E cells (4×10^7 cells per well) were transfected with relevant (30 nM) siRNAs in 96-well plates. Forty-eighy hours after siRNA transfection, cells were fixed by adding 100 μl of 10% cold trichloroacetic acid and incubated for 1 h at 4 °C. Cells were then washed four times with water and then air dried at RT. Hundred microliter of 0.057% SRB solution was added to each well and incubated at RT for 30 min while on the shaker. Plates were quickly rinsed with 1% acetic acid (four times) and air dried at RT. Bound dye was solubilized by adding 200 μl of 10 mM Tris base solution (pH 10.5) to each well, and plates were incubated at RT for 10 min while on the shaker. Absorbance readings were taken at 565 nm and 690 nm, and final absorbance measurements were calculated by subtracting the background absorbance at 690 nm from 565 nm reading.

Statistical analysis

Results are presented as means ± SEM of at least three independent experiments. Statistical differences were determined using unpaired *t* tests or two-way ANOVA with LSD post hoc test as indicated in the figure legends. Statistical significances are displayed as $^*p < 0.05$ or $^{**}p < 0.01$. Statistical analyses were performed using statistical software package GraphPad Prism 6.0 (GraphPad Software Inc).

Data availability

No large datasets associated with this study. All raw data for Western blots and insulin secretion assays are available upon request.

Acknowledgments—This work was supported by the Maurice Wilkins Centre and the Health Research Council of New Zealand.

Author contributions—P. R. S. and W. C. D. conceptualization; W. C. D. methodology; W. C. D. formal analysis; W. C. D. investigation; P. R. S. writing-original draft; P. R. S. and W. C. D. writing-review and editing.

Conflict of interest—The authors declare that they have no conflicts of interest with the contents of this article.

Abbreviations—The abbreviations used are: GSIS, glucose-stimulated insulin secretion; SRB, sulforhodamine B colorimetric.

References

1. Roder, P. V., Wong, X., Hong, W., and Han, W. (2016) Molecular regulation of insulin granule biogenesis and exocytosis. *Biochem. J.* **473**, 2737–2756
2. Gerber, S. H., and Sudhof, T. C. (2002) Molecular determinants of regulated exocytosis. *Diabetes* **51 Suppl 1**, S3–S11
3. Tarasov, A., Dusonchet, J., and Ashcroft, F. (2004) Metabolic regulation of the pancreatic beta-cell ATP-sensitive K+ channel: a pas de deux. *Diabetes* **53 Suppl 3**, S113–S122
4. Thurmond, D. C., and Gaisano, H. Y. (2020) Recent insights into beta-cell exocytosis in type 2 diabetes. *J. Mol. Biol.* **432**, 1310–1325
5. Pessin, J. E., Thurmond, D. C., Elmendorf, J. S., Coker, K. J., and Okada, S. (1999) Molecular basis of insulin-stimulated GLUT4 vesicle trafficking. Location! Location! Location! *J. Biol. Chem.* **274**, 2593–2596
6. Lammert, E., and Thorn, P. (2020) The role of the islet Niche on beta cell structure and function. *J. Mol. Biol.* **432**, 1407–1418
7. Arous, C., and Halban, P. A. (2015) The skeleton in the closet: actin cytoskeletal remodeling in β-cell function. *Am. J. Physiol. Endocrinol. Metab.* **309**, E611–E620
8. Dissanayake, W. C., Sorrenson, B., and Shepherd, P. R. (2018) The role of adherens junction proteins in the regulation of insulin secretion. *Biosci. Rep.* **38**. BSR20170989
9. Gan, W. J., Zavortink, M., Ludick, C., Templin, R., Webb, R., Webb, R., *et al.* (2017) Cell polarity defines three distinct domains in pancreatic β-cells. *J. Cell Sci.* **130**, 143–151
10. Pokutta, S., and Weis, W. I. (2007) Structure and mechanism of cadherins and catenins in cell-cell contacts. *Annu. Rev. Cell Dev. Biol.* **23**, 237–261
11. Anastasiadis, P. Z. (2007) p120-ctn: a nexus for contextual signaling via Rho GTPases. *Biochim. Biophys. Acta* **1773**, 34–46
12. Hatzfeld, M. (2005) The p120 family of cell adhesion molecules. *Eur. J. Cell Biol.* **84**, 205–214
13. Zhurinsky, J., Shtutman, M., and Ben-Ze'ev, A. (2000) Plakoglobin and beta-catenin: protein interactions, regulation and biological roles. *J. Cell Sci.* **113**, 3127–3139
14. Gul, I. S., Hulpiau, P., Saeys, Y., and van Roy, F. (2017) Evolution and diversity of cadherins and catenins. *Exp. Cell Res.* **358**, 3–9
15. Sorrenson, B., Cognard, E., Lee, K. L., Dissanayake, W. C., Fu, Y., Han, W., *et al.* (2016) A critical role for β-catenin in modulating levels of insulin secretion from β-cells by regulating actin cytoskeleton and insulin vesicle localization. *J. Biol. Chem.* **291**, 25888–25900
16. Sorrenson, B., Dissanayake, W. C., Hu, F., Lee, K. L., and Shepherd, P. R. (2021) A role for PAK1 mediated phosphorylation of beta-catenin Ser552 in the regulation of insulin secretion. *Biochem. J.* **478**, 1605–1615
17. Cognard, E., Dargaville, C. G., Hay, D. L., and Shepherd, P. R. (2013) Identification of a pathway by which glucose regulates β-catenin signalling via the cAMP/protein kinase A pathway in β-cell models. *Biochem. J.* **449**, 803–811
18. Dissanayake, W. C., Sorrenson, B., Lee, K. L., Barre, S., and Shepherd, P. R. (2020) α-catenin isoforms are regulated by glucose and involved in regulating insulin secretion in rat clonal β-cell models. *Biochem. J.* **477**, 763–772
19. Rouiller, D. G., Cirulli, V., and Halban, P. A. (1991) Uvomorulin mediates calcium-dependent aggregation of islet cells, whereas calcium-independent cell adhesion molecules distinguish between islet cell types. *Dev. Biol.* **148**, 233–242
20. Hauge-Evans, A. C., Squires, P. E., Persaud, S. J., and Jones, P. M. (1999) Pancreatic beta-cell-to-beta-cell interactions are required for integrated responses to nutrient stimuli: enhanced Ca2+ and insulin secretory responses of MIN6 pseudoislets. *Diabetes* **48**, 1402–1408
21. Yamagata, K., Nammo, T., Moriwaki, M., Ihara, A., Iizuka, K., Yang, Q., *et al.* (2002) Overexpression of dominant-negative mutant hepatocyte nuclear fctor-1 alpha in pancreatic beta-cells causes abnormal islet architecture with decreased expression of E-cadherin, reduced beta-cell proliferation, and diabetes. *Diabetes* **51**, 114–123
22. Rogers, G. J., Hodgkin, M. N., and Squires, P. E. (2007) E-Cadherin and cell adhesion: a role in architecture and function in the pancreatic islet. *Cell. Physiol. Biochem.* **20**, 987–994
23. Wakae-Takada, N., Xuan, S., Watanabe, K., Meda, P., and Leibel, R. L. (2013) Molecular basis for the regulation of islet beta cell mass in mice: the role of E-cadherin. *Diabetologia* **56**, 856–866
24. Parnaud, G., Lavallard, V., Bedat, B., Matthey-Doret, D., Morel, P., Berney, T., *et al.* (2015) Cadherin engagement improves insulin secretion of single human β-cells. *Diabetes* **64**, 887–896
25. Johansson, J. K., Voss, U., Kesavan, G., Kostetskii, I., Wierup, N., Radice, G. L., *et al.* (2010) N-cadherin is dispensable for pancreas development but required for beta-cell granule turnover. *Genesis* **48**, 374–381
26. Rulifson, I. C., Karnik, S. K., Heiser, P. W., ten Berge, D., Chen, H., Gu, X., *et al.* (2007) Wnt signaling regulates pancreatic beta cell proliferation. *Proc. Natl. Acad. Sci. U. S. A.* **104**, 6247–6252
27. Peifer, M., and Yap, A. S. (2003) Traffic control: p120-catenin acts as a gatekeeper to control the fate of classical cadherins in mammalian cells. *J. Cell Biol.* **163**, 437–440
28. Langlhofer, G., Kogel, A., and Schaefer, M. (2021) Glucose-induced [Ca2+]i oscillations in β cells are composed of trains of spikes within a subplasmalemmal microdomain. *Cell Calcium* **99**, 102469
29. Liu, G., Hilliard, N., and Hockerman, G. H. (2004) Cav1.3 is preferentially coupled to glucose-induced [Ca2+]i oscillations in the pancreatic beta cell line INS-1. *Mol. Pharmacol.* **65**, 1269–1277
30. Noren, N. K., Liu, B. P., Burridge, K., and Kreft, B. (2000) p120 catenin regulates the actin cytoskeleton via Rho family GTPases. *J. Cell Biol.* **150**, 567–580
31. Ghosh, M., Song, X., Mouneimne, G., Sidani, M., Lawrence, D. S., and Condeelis, J. S. (2004) Cofilin promotes actin polymerization and defines the direction of cell motility. *Science* **304**, 743–746
32. Pavlov, D., Muhlrad, A., Cooper, J., Wear, M., and Reisler, E. (2007) Actin filament severing by cofilin. *J. Mol. Biol.* **365**, 1350–1358
33. Uenishi, E., Shibasaki, T., Takahashi, H., Seki, C., Hamaguchi, H., Yasuda, T., *et al.* (2013) Actin dynamics regulated by the balance of neuronal Wiskott-Aldrich syndrome protein (N-WASP) and cofilin activities determines the biphasic response of glucose-induced insulin secretion. *J. Biol. Chem.* **288**, 25851–25864
34. Arber, S., Barbayannis, F. A., Hanser, H., Schneider, C., Stanyon, C. A., Bernard, O., *et al.* (1998) Regulation of actin dynamics through phosphorylation of cofilin by LIM-kinase. *Nature* **393**, 805–809
35. Seidel, B., Braeg, S., Adler, G., Wedlich, D., and Menke, A. (2004) E- and N-cadherin differ with respect to their associated p120ctn isoforms and their ability to suppress invasive growth in pancreatic cancer cells. *Oncogene* **23**, 5532–5542
36. Dissanayake, W. C., Sorrenson, B., Cognard, E., Hughes, W. E., and Shepherd, P. R. (2018) β-catenin is important for the development of an insulin responsive pool of GLUT4 glucose transporters in 3T3-L1 adipocytes. *Exp. Cell Res.* **366**, 49–54
37. Masson, S. W. C., Sorrenson, B., Shepherd, P. R., and Merry, T. L. (2020) β-catenin regulates muscle glucose transport via actin remodelling and M-cadherin binding. *Mol. Metab.* **42**, 101091
38. Masson, S. W. C., Woodhead, J. S. T., D'Souza, R. F., Broome, S. C., MacRae, C., Cho, H. C., *et al.* (2021) β-Catenin is required for optimal exercise- and contraction-stimulated skeletal muscle glucose uptake. *J. Physiol.* **599**, 3897–3912
39. Hou, J. C., Shigematsu, S., Crawford, H. C., Anastasiadis, P. Z., and Pessin, J. E. (2006) Dual regulation of Rho and Rac by p120 catenin controls adipocyte plasma membrane trafficking. *J. Biol. Chem.* **281**, 23307–23312
40. Bamji, S. X., Rico, B., Kimes, N., and Reichardt, L. F. (2006) BDNF mobilizes synaptic vesicles and enhances synapse formation by disrupting cadherin-beta-catenin interactions. *J. Cell Biol.* **174**, 289–299
41. Bamji, S. X., Shimazu, K., Kimes, N., Huelsken, J., Birchmeier, W., Lu, B., *et al.* (2003) Role of beta-catenin in synaptic vesicle localization and presynaptic assembly. *Neuron* **40**, 719–731

42. Brigidi, G. S., and Bamji, S. X. (2011) Cadherin-catenin adhesion complexes at the synapse. *Curr. Opin. Neurobiol.* **21**, 208–214
43. Kalwat, M. A., and Thurmond, D. C. (2013) Signaling mechanisms of glucose-induced F-actin remodeling in pancreatic islet β cells. *Exp. Mol. Med.* **45**, e37
44. Lee, J. S., Ho, W. K., and Lee, S. H. (2012) Actin-dependent rapid recruitment of reluctant synaptic vesicles into a fast-releasing vesicle pool. *Proc. Natl. Acad. Sci. U. S. A.* **109**, E765–E774
45. Lopez, J. A., Burchfield, J. G., Blair, D. H., Mele, K., Ng, Y., Vallotton, P., *et al.* (2009) Identification of a distal GLUT4 trafficking event controlled by actin polymerization. *Mol. Biol. Cell* **20**, 3918–3929
46. Kanzaki, M., and Pessin, J. E. (2001) Insulin-stimulated GLUT4 translocation in adipocytes is dependent upon cortical actin remodeling. *J. Biol. Chem.* **276**, 42436–42444
47. Thurmond, D. C., Gonelle-Gispert, C., Furukawa, M., Halban, P. A., and Pessin, J. E. (2003) Glucose-stimulated insulin secretion is coupled to the interaction of actin with the t-SNARE (target membrane soluble N-ethylmaleimide-sensitive factor attachment protein receptor protein) complex. *Mol. Endocrinol.* **17**, 732–742
48. Jewell, J. L., Luo, W., Oh, E., Wang, Z., and Thurmond, D. C. (2008) Filamentous actin regulates insulin exocytosis through direct interaction with Syntaxin 4. *J. Biol. Chem.* **283**, 10716–10726
49. Gan, W. J., Do, O. H., Cottle, L., Ma, W., Kosobrodova, E., Cooper-White, J., *et al.* (2018) Local integrin activation in pancreatic β cells targets insulin secretion to the vasculature. *Cell Rep.* **24**, 2819–2826 e3
50. Yonemura, S. (2011) Cadherin-actin interactions at adherens junctions. *Curr. Opin. Cell Biol.* **23**, 515–522
51. Zhang, J., Betson, M., Erasmus, J., Zeikos, K., Bailly, M., Cramer, L. P., *et al.* (2005) Actin at cell-cell junctions is composed of two dynamic and functional populations. *J. Cell Sci.* **118**, 5549–5562
52. Zebda, N., Tian, Y., Tian, X., Gawlak, G., Higginbotham, K., Reynolds, A. B., *et al.* (2013) Interaction of p190RhoGAP with C-terminal domain of p120-catenin modulates endothelial cytoskeleton and permeability. *J. Biol. Chem.* **288**, 18290–18299
53. Marrs, G. S., Theisen, C. S., and Bruses, J. L. (2009) N-cadherin modulates voltage activated calcium influx via RhoA, p120-catenin, and myosin-actin interaction. *Mol. Cell. Neurosci.* **40**, 390–400
54. Anastasiadis, P. Z., Moon, S. Y., Thoreson, M. A., Mariner, D. J., Crawford, H. C., Zheng, Y., *et al.* (2000) Inhibition of RhoA by p120 catenin. *Nat. Cell Biol.* **2**, 637–644
55. Asahara, S., Shibutani, Y., Teruyama, K., Inoue, H. Y., Kawada, Y., Etoh, H., *et al.* (2013) Ras-related C3 botulinum toxin substrate 1 (RAC1) regulates glucose-stimulated insulin secretion via modulation of F-actin. *Diabetologia* **56**, 1088–1097
56. Li, J., Luo, R., Kowluru, A., and Li, G. (2004) Novel regulation by Rac1 of glucose- and forskolin-induced insulin secretion in INS-1 beta-cells. *Am. J. Physiol. Endocrinol. Metab.* **286**, E818–E827
57. Wang, Z., Oh, E., and Thurmond, D. C. (2007) Glucose-stimulated Cdc42 signaling is essential for the second phase of insulin secretion. *J. Biol. Chem.* **282**, 9536–9546
58. Boguslavsky, S., Grosheva, I., Landau, E., Shtutman, M., Cohen, M., Arnold, K., *et al.* (2007) p120 catenin regulates lamellipodial dynamics and cell adhesion in cooperation with cortactin. *Proc. Natl. Acad. Sci. U. S. A.* **104**, 10882–10887
59. Rajput, C., Kini, V., Smith, M., Yazbeck, P., Chavez, A., Schmidt, T., *et al.* (2013) Neural Wiskott-Aldrich syndrome protein (N-WASP)-mediated p120-catenin interaction with Arp2-Actin complex stabilizes endothelial adherens junctions. *J. Biol. Chem.* **288**, 4241–4250
60. Dohn, M. R., Brown, M. V., and Reynolds, A. B. (2009) An essential role for p120-catenin in Src- and Rac1-mediated anchorage-independent cell growth. *J. Cell Biol.* **184**, 437–450
61. Sun, Y., and Bamji, S. X. (2011) β-Pix modulates actin-mediated recruitment of synaptic vesicles to synapses. *J. Neurosci.* **31**, 17123–17133
62. Piedra, J., Miravet, S., Castaño, J., Pálmer, H. G., Heisterkamp, N., García de Herreros, A., *et al.* (2003) p120 catenin-associated Fer and Fyn tyrosine kinases regulate beta-catenin Tyr-142 phosphorylation and beta-catenin-alpha-catenin Interaction. *Mol. Cell. Biol.* **23**, 2287–2297
63. Piccoli, G., Rutishauser, U., and Bruses, J. L. (2004) N-cadherin juxtamembrane domain modulates voltage-gated Ca2+ current via RhoA GTPase and Rho-associated kinase. *J. Neurosci.* **24**, 10918–10923
64. Gandini, M. A., and Felix, R. (2015) Molecular and functional interplay of voltage-gated Ca^{2+} channels with the cytoskeleton. *Curr. Mol. Pharmacol.* **8**, 69–80
65. Guzman, G. A., Guzman, R. E., Jordan, N., and Hidalgo, P. (2019) A tripartite interaction among the calcium channel α1- and β-subunits and F-actin increases the readily releasable pool of vesicles and its recovery after depletion. *Front. Cell. Neurosci.* **13**, 125
66. Stolting, G., de Oliveira, R. C., Guzman, R. E., Miranda-Laferte, E., Conrad, R., Jordan, N., *et al.* (2015) Direct interaction of CaVβ with actin up-regulates L-type calcium currents in HL-1 cardiomyocytes. *J. Biol. Chem.* **290**, 4561–4572
67. Quinault, A., Gausseres, B., Bailbe, D., Chebbah, N., Portha, B., Movassat, J., *et al.* (2016) Disrupted dynamics of F-actin and insulin granule fusion in INS-1 832/13 beta-cells exposed to glucotoxicity: partial restoration by glucagon-like peptide 1. *Biochim. Biophys. Acta* **1862**, 1401–1411
68. Vichai, V., and Kirtikara, K. (2006) Sulforhodamine B colorimetric assay for cytotoxicity screening. *Nat. Protoc.* **1**, 1112–1116