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Regulation of pancreatic β -cell insulin secretion by actin cytoskeleton remodelling: role of gelsolin and cooperation with the MAPK signalling pathway

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Summary

We have previously isolated two MIN6 β -cell sublines, B1, highly responsive to glucose-stimulated insulin secretion, and C3, markedly refractory (Lilla, V., Webb, G., Rickenbach, K., Maturana, A., Steiner, D. F., Halban, P. A. and Irminger, J. C. (2003) *Endocrinology* 144, 1368-1379). We now demonstrate that C3 cells have substantially increased amounts of F-actin stress fibres whereas B1 cells have shorter cortical F-actin. Consistent with these data, B1 cells display glucose-dependent actin remodelling whereas, in C3 cells, F-actin is refractory to this secretagogue. Furthermore, F-actin depolymerisation with latrunculin B restores glucose-stimulated insulin secretion in C3 cells. In parallel, glucose-stimulated ERK1/2 activation is greater in B1 than in C3 cells, and is potentiated in both sublines following F-actin depolymerisation. Glucose-activated phosphoERK1/2

accumulates at actin filament tips adjacent to the plasma membrane, indicating that these are the main sites of action for this kinase during insulin secretion. In addition, B1 cell expression of the calcium-dependent F-actin severing protein gelsolin is >100-fold higher than that of C3 cells. Knock-down of gelsolin reduced glucose-stimulated insulin secretion, whereas gelsolin over-expression potentiated secretion from B1 cells. Gelsolin localised along depolymerised actin fibres after glucose stimulation. Taken together, these data demonstrate that F-actin reorganization prior to insulin secretion requires gelsolin and plays a role in the glucose-dependent MAPK signal transduction that regulates β -cell insulin secretion.

Key words: Actin dynamics, Latrunculin B, Beta cells, Insulin secretion, Gelsolin, ERK1/2

Introduction

The actin cytoskeleton is a highly dynamic structure that is remodelled in response to a variety of signals. It plays essential roles in the regulation of numerous cellular processes, such as cell migration, wound repair and vesicle exocytosis (Sun et al., 1999; Gasman et al., 2004).

In pancreatic β -cells, the main role of which is to control glucose homeostasis, insulin-containing granules undergo tightly regulated exocytosis as a response to extrinsic stimuli (such as glucose) that lead to an elevation in cytosolic calcium (Easom, 2000; Ashcroft et al., 1994), in a process that shares many similarities with the calcium-regulated release of neurotransmitters and hormones by neurons and neuroendocrine cells (Bader et al., 2004). A number of previous studies support the idea of actin cytoskeleton reorganisation being coupled to calcium-regulated exocytosis, but the precise role of actin has not been elucidated (Aunis and Bader, 1988; Bader et al., 2004; Eitzen, 2003). F-actin, which in pancreatic β -cells is organised as a dense web beneath the plasma membrane (Orci et al., 1972), is transiently depolymerised after glucose stimulation of insulin secretion (Thurmond et al., 2003). This secretion is facilitated by the disruption of the F-actin network with cytochalasin B (Jijakli et al., 2002), latrunculin B (Thurmond et al., 2003) or *Clostridium botulinum* C2 toxin (Li et al., 1994) in insulin-

secreting cell lines, suggesting that F-actin limits the access of insulin granules to the plasma membrane. By contrast, actin filament disruption with C2 toxin inhibited insulin secretion in poorly granulated HIT-T15 cells (Li et al., 1994), indicating an alternative role for F-actin in the recruitment of secretory granules to the releasable pool. Recent reports have underlined a possible role for the Rho GTPases Cdc42 (Nevins and Thurmond, 2003; Kowluru et al., 1997) and Rac (Li et al., 2004; Kowluru et al., 1997) in cortical F-actin reorganisation during regulated insulin secretion, but the exact mechanism of action, as well as the key proteins implicated in this important process, remain unknown.

In this study, two different sublines derived from the parental mouse pancreatic β -cell line MIN6, designated B1 and C3, which have been previously characterised as having similar insulin content but different secretory properties [with B1 but not C3 cells responding to glucose in a concentration-dependent manner (Lilla et al., 2003)], have been employed with a view to identifying novel genes and mechanisms involved in the regulation of β -cell actin organisation and insulin secretion. First, we establish that these two sublines have very different actin cytoskeleton organisation and remodelling properties. We further employ the comparison of B1 and C3 cells to provide the first evidence of the involvement of the Ca^{2+} -dependent actin remodelling protein gelsolin in

glucose-regulated insulin secretion, as well as to unveil a link between F-actin remodelling and glucose-dependent activation and subcellular localisation of the mitogen-activated protein kinase (MAPK) signalling cascade member extracellular signal-regulated kinase 1/2 (ERK1/2), two of the processes implicated in the potentiation of regulated insulin secretion in the β -cell.

Results

Differences in actin cytoskeleton organisation and remodelling between MIN6 sublines B1 and C3

In order to establish the validity of the B1 versus C3 cell model for the study of the mechanisms implicated in β -cell actin reorganisation and its importance in regulated insulin secretion, we determined whether there was any difference in the actin cytoskeleton organisation and remodelling between the well-regulated insulin-secreting B1 subline and the dysregulated glucose-insensitive C3 subline. Phalloidin staining of cell monolayers from these two sublines showed marked differences in their F-actin cytoskeleton properties (Fig. 1). In basal conditions (0 mM glucose), B1 cells display shorter more curved cortical actin filaments compared to C3 cells, the latter being rich in long stress fibres underlying the cell membrane. As shown in Fig. 1A, and as previously reported for MIN6 cells using these same conditions (Thurmond et al., 2003), the actin cytoskeleton of B1 cells partially depolymerises shortly after stimulation with 20 mM glucose, whereas the actin cytoskeleton of C3 cells remains unchanged. Similar results were obtained under more physiologically relevant basal conditions (2.8 mM glucose) followed by stimulation at 16.7 mM glucose (Fig. 1B).

The partial depolymerisation of the F-actin cytoskeleton did not take place when the cAMP phosphodiesterase inhibitor IBMX, which increases intracellular cAMP levels and potentiates insulin secretion (Siegel et al., 1980), was used instead of glucose as the stimulatory agent (Fig. 1C).

Actin depolymerisation by latrunculin B treatment potentiates glucose-regulated insulin secretion in the MIN6 B1 subline and restores regulated insulin secretion in the MIN6 C3 subline

Treatment with the actin depolymerising agent latrunculin B has been previously demonstrated to potentiate glucose-stimulated insulin secretion in MIN6 cells (Thurmond et al., 2003). In view of the abnormal distribution and glucose-dependent actin remodelling properties of the secretion-defective C3 subline, we explored the possible effects of latrunculin B on the glucose-dependent insulin secretion of this as well as of the glucose-responsive B1 subline. The effectiveness of latrunculin B treatment on the actin depolymerisation of B1 and C3 cells was assessed by phalloidin staining of cell samples pre-treated with the drug (or with DMSO as a negative control) for 2 hours at basal conditions followed by 10 minutes stimulation with 20 mM glucose (Fig. 2A). Untreated B1 cells display a partial depolymerisation of cortical actin similar to that shown in Fig. 1A, whereas latrunculin B pre-treated B1 cells had a disrupted actin cytoskeleton, with F-actin extensively fragmented and scattered throughout the cell. In the case of the C3 subline, untreated cells retained a stress fibre-rich actin cytoskeleton after glucose stimulation, but latrunculin B pre-treated cells

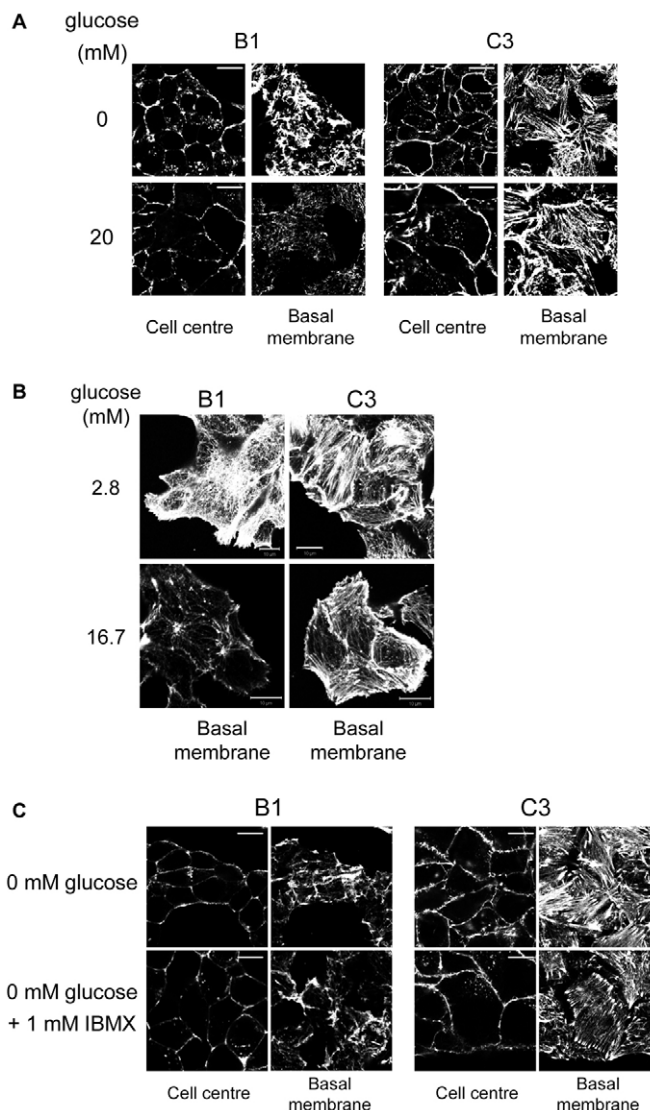


Fig. 1. Confocal microscopy images showing the differences in the organisation and dynamic properties of the actin cytoskeleton in B1 and C3 cells. Response of cells to (A) 10 minutes stimulation with 20 mM glucose after 0 mM glucose; (B) 16.7 mM after 2.8 mM glucose; and (C) 1 mM IBMX without glucose. F-actin was stained with phalloidin. Bars, 10 μ m.

displayed a similar level of actin disruption to latrunculin B-treated B1 cells.

Pre-treatment of B1 cells with latrunculin B resulted in an increase of 3.7-fold (from 15.7 ± 1.9 to 58.1 ± 8.0 stimulated versus basal fold increase) in glucose-stimulated insulin secretion without any significant impact on basal secretion (Fig. 2B, top panels). This increase was sustained throughout the 1 hour stimulatory period (Fig. 2C, left panel). As shown previously (Lilla et al., 2003), the C3 subline did not display any increase in insulin secretion in response to glucose (fold-stimulation ≈ 1). However, there was an increase of 10.9-fold (from 0.92 ± 0.06 to 9.985 ± 0.62 stimulated vs basal fold increase) in the secretory response to glucose after latrunculin B-induced actin depolymerisation in this subline (Fig. 2B, bottom panels, and Fig. 2C, right panel), with a 54.5-fold

increase of stimulated insulin secretion and a lower but significant impact (fivefold increase) on basal secretion. The increase in secretion measured after latrunculin B pre-treatment in both B1 and C3 sublines was not accompanied by significant differences in the total (secreted + intracellular) insulin content of either subline as compared to control DMSO-treated cells (Fig. 2D).

Differential expression of the F-actin remodelling protein gelsolin between MIN6 B1 and C3 cells

B1 and C3 cells have been subjected in the past to a thorough analysis of differentially expressed genes by high density (Affymetrix) oligonucleotide microarrays (Lilla et al., 2003). In this study, the candidate Ca^{2+} -dependent actin remodelling protein gelsolin was identified as being grossly over-expressed in B1 compared to C3 cells. In order to verify the microarray results, the level of gelsolin mRNA was determined in both B1 and C3 cells by real-time RT-PCR. The results obtained were comparable to the microarray analysis and indicated an increase of more than 100-fold in the level of gelsolin mRNA in B1 compared with C3 cells (Table 1). This increase in gelsolin expression was subsequently confirmed at the protein level by western blot analysis of protein samples from both sublines, and also from sorted primary mouse and rat pancreatic β -cells for comparison (Fig. 3A). Gelsolin protein levels were clearly increased in B1 compared to C3 cells, with mouse primary β -cells displaying a similar level of gelsolin to B1 cells, and rat primary β -cells exhibiting an intermediate level of expression, but clearly higher than that of the defective C3 subline. Immunofluorescence analysis of gelsolin subcellular localisation in B1 cells showed that the protein partially co-localises with cortical actin beneath the plasma membrane (Fig. 3B).

RNA interference analysis indicates that gelsolin participates in extracellular Ca^{2+} -dependent glucose-stimulated insulin secretion in MIN6 B1 cells

In view of the difference in the level of expression of gelsolin between the glucose-responsive insulin-secreting B1 and the glucose-insensitive C3 sublines, and the documented role of this protein in dynamic alterations of the actin

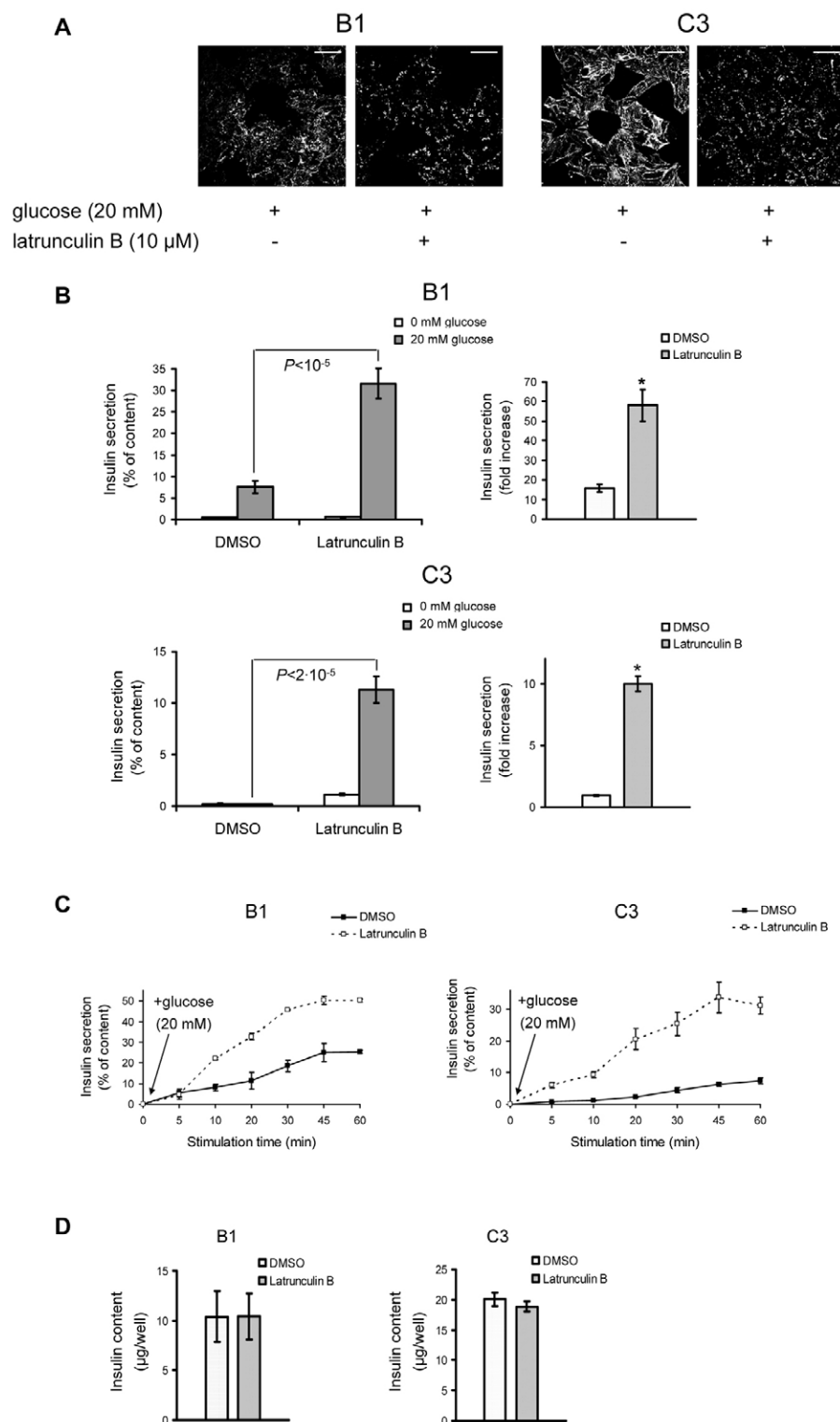


Fig. 2. Effect of actin depolymerisation on the secretory response to glucose in B1 and C3 cells. (A) Latrunculin B-dependent depolymerisation of F-actin in B1 and C3 cells. Bar, 20 μm . (B) Stimulatory effect of actin depolymerisation by 10 μM latrunculin B on glucose-dependent insulin secretion in B1 and C3 cells. Left panels: basal (0 mM glucose for 1 hour) and stimulated (20 mM glucose for 1 hour) insulin secretion as a percentage of cell content; right panels: fold-stimulation ($*P < 10^{-4}$). (C) Time-course of insulin secretion (20 mM glucose) by B1 and C3 cells with or without 10 μM latrunculin B. (D) Insulin content in B1 and C3 sublines with or without 10 μM latrunculin B treatment. Data in all cases are mean $\pm$ s.e.m. of three independent experiments.

Table 1. Differences in gelsolin mRNA levels in MIN6 B1 and C3 cells

	Ratio B1/C3	
	Mean	± s.e.m.
Microarray	145.7	48.9
Real time RT-PCR	115.8	31.7

Data are the mean ± s.e.m. of three independent experiments.

Results for microarray analysis are taken from Lilla et al. (Lilla et al., 2003).

cytoskeleton (Sun et al., 1999; Kwiatkowski, 1999), we decided to analyse the role of gelsolin in regulated insulin secretion. For this purpose, we employed the RNA interference technique to knock-down gelsolin in B1 cells. In order to assess the efficacy of gelsolin down-regulation, B1 cells were transiently transfected with plasmids expressing two different short hairpin RNA (shRNA) sequences specific for mouse gelsolin mRNA and the fluorescent protein GFP as a marker for transfected cells. As a negative control, cells were transfected with the same type of plasmid expressing a non-mammalian unrelated shRNA. Three days after transfection, cells were collected and analysed by fluorescence-activated cell sorting in which only the GFP-positive shRNA-expressing cells were collected. Gelsolin protein levels were later determined in sorted cell samples by western blot analysis, as shown in Fig. 4A for the most effective of the two gelsolin shRNAs analysed. This construct knocked down gelsolin in B1 cells to very low levels when compared to negative control shRNA-expressing cells, and was therefore subsequently used to examine the impact of gelsolin on insulin secretion.

To determine the involvement of gelsolin in glucose-stimulated insulin secretion, B1 cells were co-transfected with the plasmid expressing shRNA for gelsolin and a human growth hormone (hGH)-expressing vector. Human growth hormone was used as a surrogate marker for insulin secretion

from the subpopulation of transfected cells, as this hormone is stored (Schweitzer and Kelly, 1985) and co-secreted with insulin in pancreatic β -cells (Holz et al., 1994; Wang et al., 1997; Iezzi et al., 1999). B1 cells transfected with the control non-specific shRNA plasmid displayed a fivefold increase in growth hormone secretion after raising the level of glucose in the incubation medium from 2.8 mM to 16.7 mM for 1 hour, whereas B1 cells transfected with the gelsolin-specific shRNA-expressing vector reached only a 3.2-fold increase in growth hormone secretion after glucose stimulation (Fig. 4B). Gelsolin-depleted B1 cells therefore lost a statistically significant 36% of their secretory response to glucose compared to control cells.

To characterise further this loss of secretion in gelsolin-depleted cells, growth hormone secretion experiments were repeated using 1 mM IBMX alone (Fig. 4C, top panels) or 1 mM IBMX supplemented with 16.7 mM glucose (Fig. 4C, bottom panels) as secretagogues. We could not measure any significant decrease in growth hormone secretion from gelsolin-depleted cells compared with control cells in any of these instances, indicating a glucose-specific role for gelsolin in insulin secretion.

We next determined whether the stimulatory effect of latrunculin B treatment on glucose-induced secretion in B1 cells was counteracted by gelsolin depletion. For this purpose, similar hGH secretion experiments were performed on control and gelsolin shRNA-transfected B1 cells after 2 hours latrunculin B pre-treatment (Fig. 4D). Under these conditions, and contrary to the results obtained for the secretion of insulin (Fig. 2B), latrunculin B pre-treatment caused an increase in basal growth hormone secretion in both control and gelsolin-depleted cells (compare Fig. 2B and Fig. 4D). We could nevertheless measure a 31% reduction in the level of stimulated growth hormone secretion in gelsolin-depleted cells as compared to the control, indicating that gelsolin depletion can partially counteract the effect of latrunculin B on stimulated growth hormone secretion in B1 cells.

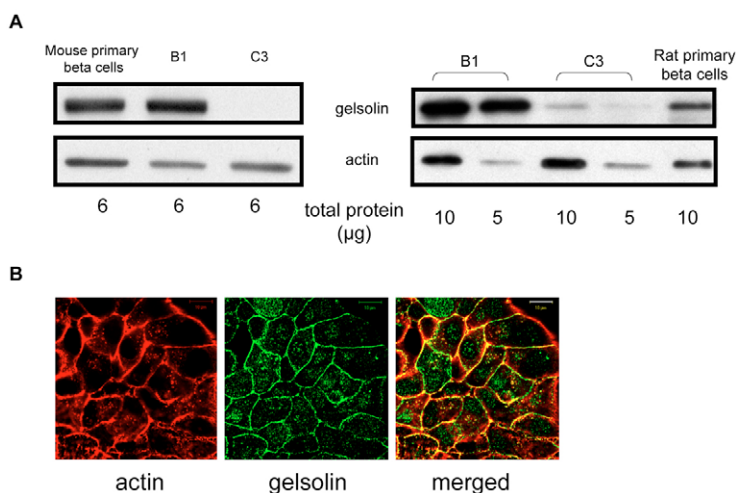


Fig. 3. (A) Western blot analysis of the differences in the level of gelsolin in B1, C3, mouse and rat primary pancreatic β -cells. (B) Subcellular localisation of gelsolin (green) and F-actin (red) in B1 cells. No immunoreactivity was observed using control rabbit IgG (not shown). Bar, 10 μ m.

Effect of over-expression of gelsolin in the glucose-dependent actin remodelling properties and the secretory response of MIN6 B1 and C3 cells

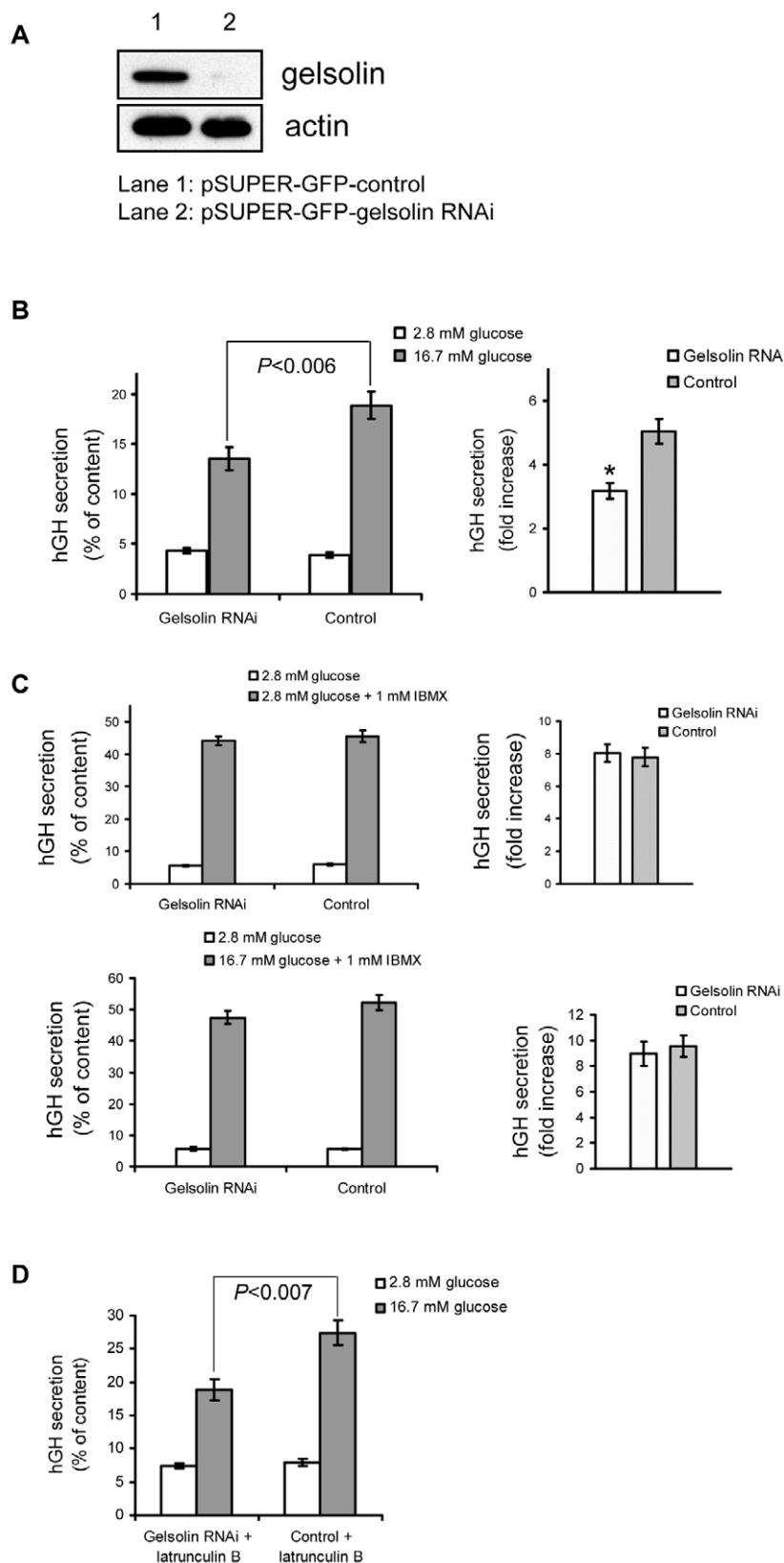
To examine further the role of gelsolin in short-term actin remodelling and its link to insulin secretion, we transfected both B1 and C3 cells with a plasmid expressing mouse gelsolin cDNA fused to an HA tag (pcDNA3.1+hygro-HA-mGsn; a gift from H. Fujita, National Cardiovascular Center Research Institute, Osaka, Japan), or with the same plasmid in which the HA-gelsolin construct had been excised for control purposes (pcDNA3.1+hygro). In Fig. 5A, the level of gelsolin in untransfected (wt) cells is compared to that in cells transfected with the control or the HA-gelsolin expression plasmids for both B1 and C3 sublines. These results show that transfection with the HA-gelsolin expression plasmid results in an over-expression of gelsolin in B1 cells and a restoration of gelsolin expression in C3 cells.

Both B1 and C3 sublines were subsequently transfected with the HA-gelsolin expression plasmid,

Fig. 4. Analysis of the role of gelsolin in insulin secretion by RNAi in B1 cells. (A) Western blot showing the effect of RNAi treatment on gelsolin protein levels in B1 cells. GFP-positive B1 cells transfected with either pSUPER-GFP gelsolin RNAi or pSUPER-GFP control plasmids were selected by FACS and used for immunoblotting. Equal loading was confirmed with an anti-actin antibody. (B,C) B1 cells were co-transfected with pSUPER-gelsolin RNAi or pSUPER-control RNAi plasmids and an hGH-expressing vector, and growth hormone secretion was measured after stimulation with 16.7 mM glucose (B), 1 mM IBMX (C, top panels), or a combination of 16.7 mM glucose and 1 mM IBMX (C, bottom panels). Data are mean $\pm$ s.e.m. of five independent experiments (B, right panel: $*P < 4 \times 10^{-4}$). (D) Growth hormone secretion in B1 cells co-transfected with pSUPER-gelsolin RNAi or pSUPER-control RNAi plasmids and an hGH-expressing vector after a 2 hour pre-incubation with 10 μ M latrunculin B. Data are mean $\pm$ s.e.m. of two independent experiments.

and the subcellular localisation of HA-gelsolin was analysed in conjunction with the F-actin cytoskeleton by confocal immunofluorescence at both basal (0 mM glucose) and stimulated (20 mM glucose) conditions (Fig. 5B). In B1 cells under basal conditions (Fig. 5B, top panels, 0 mM glucose), the level of actin polymerisation in transfected HA-tag-positive cells is comparable to that of untransfected cells on the same dish. Upon glucose stimulation, HA-gelsolin relocates specifically to actin filaments and the extent of glucose-induced actin fibre depolymerisation appears to be increased in transfected cells as compared to untransfected cells on the same dish (Fig. 5B, top panels, 20 mM glucose). By contrast, in C3 cells HA-gelsolin is clearly localised along the entire length of actin filaments even in basal conditions (Fig. 5B, bottom panels, 0 mM glucose), and depolymerisation of these actin filaments is increased in transfected cells in both basal and stimulated conditions as compared to untransfected cells on the same dish (Fig. 5B, bottom panels, 0 and 20 mM glucose).

We then analysed the level of growth hormone secretion in B1 and C3 cells that had been co-transfected with either the HA-gelsolin expression plasmid or the control empty plasmid and an hGH-expressing vector. For B1 cells, we measured an increase of 42% in stimulated growth hormone secretion in gelsolin over-expressing cells as compared to the control without any significant effect on basal secretion (Fig. 5C). However, we could not measure any significant increase of stimulated growth hormone secretion between HA-gelsolin and control C3 transfected cells (data not shown). This seemingly contradictory result might be explained in combination with the immunofluorescence data in Fig. 5B as resulting from dysregulation of gelsolin action



on the defective C3 subline, where gelsolin appears to promote actin depolymerisation even in the absence of glucose.

The actin cytoskeleton depolymerisation state is linked to the glucose-dependent activation of the MAPK signalling cascade during stimulation of insulin secretion in MIN6 cells

In order to deepen our understanding of the role of short-term actin remodelling in glucose-stimulated insulin secretion, and prompted by previous reports showing a link between the actin cytoskeleton and the activation of the MAPK pathway by insulin in insulin-responsive muscle cells (Tsakiridis et al., 1998), we examined whether there was a connection between

the glucose-induced actin remodelling and the activation of the MAPK pathway in β -cells. Specifically, we investigated if there were differences in the glucose-dependent activation of ERK1/2 and its subcellular localisation in B1 compared with C3 cells, and if these possible differences were related to the distinct actin remodelling characteristics identified in the two MIN6 sublines.

To confirm that ERK1/2 played a role in insulin secretion in B1 cells, the selective pharmacological inhibitor PD98059 was used to inhibit MEK1/2, the upstream ERK1/2 kinase (Pang et al., 1995). To verify that PD98059 treatment abolished ERK1/2 activation by glucose, B1 cells were pre-incubated for 2 hours with PD98059 (or DMSO as a negative control), and stimulated with 10 mM glucose. Cells were collected after 5-, 15- and 30-minute incubations and ERK1/2 activation was analysed by western blot using an antibody specific for the phosphorylated active form of the protein (Fig. 6A). Both ERK isoforms 1 and 2 were phosphorylated following incubation at 10 mM glucose (see also Fig. 7A), and this phosphorylation was blocked by pre-treatment with PD98059.

We next analysed the effect of PD98059 pre-treatment on insulin secretion from B1 cells after stimulation with 10 mM glucose for the same three time periods as above (Fig. 6B). There was a 60% decrease in the fold-stimulation of insulin secretion (stimulated versus basal condition) in PD98059-treated cells when compared to control DMSO-treated cells after 5 minutes glucose stimulation (top panel). However, after 15 minutes in glucose, this difference

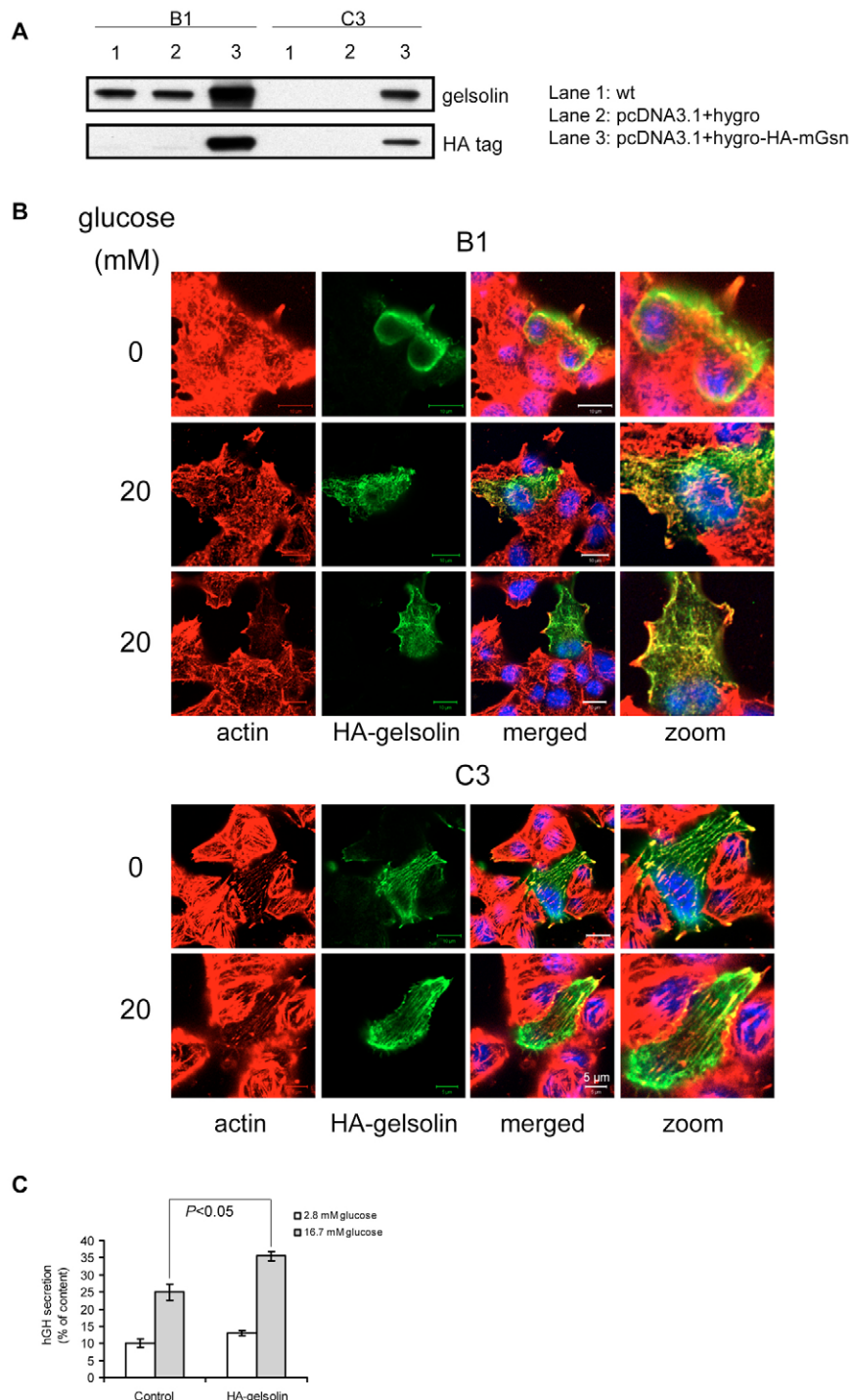


Fig. 5. Analysis of the role of gelsolin in actin depolymerisation and insulin secretion by exogenous expression of an HA-tagged gelsolin cDNA construct in B1 and C3 cells.

(A) Western blot showing the effect of exogenous expression of HA-gelsolin in B1 and C3 cells on the total level of gelsolin. Exogenous HA-gelsolin was specifically detected with an anti-HA-tag antibody (10 μ g total protein/lane). (B) Immunofluorescence analysis of the subcellular localisation of HA-gelsolin (green) and the actin cytoskeleton (red) on B1 (top panels) and C3 cells (bottom panels) with or without 20 mM glucose stimulation. Nuclei (blue) were stained with DRAQ5TM. For glucose-stimulated B1 cells, two examples of HA-gelsolin-expressing cells are given and the actin (red) signal is boosted in the higher magnification image (zoom) to facilitate visualisation of actin/gelsolin co-localisation. Bars, 10 μ m (unless stated otherwise). (C) Growth hormone secretion by B1 cells co-transfected with an HA-gelsolin expression plasmid or with a control empty plasmid. Data are mean $\pm$ s.e.m. of three replicates of a single experiment.

had been reduced to 30% (middle panel), and no significant difference was observed after 30 minutes glucose stimulation. These results show that ERK1/2 activity contributes to the short-term but not to the long-term glucose-induced insulin secretion of B1 cells.

Once it was established that ERK1/2 activation has an important role in the short-term insulin secretion of B1 cells, we compared the levels of activation of ERK1/2 after short-term (5 minutes) glucose exposure in B1 and C3 cells, and we also investigated the effect of actin depolymerisation by latrunculin B pre-treatment on the level of activation of ERK1/2 in these two sublines. For these purposes, B1 and C3 cells were pre-treated with either latrunculin B or DMSO (as a negative control) in the absence of glucose for 2 hours, and stimulated for 5 minutes with 20 mM glucose, and the levels of ERK1/2 phosphorylation were subsequently determined by western blotting (Fig. 7A). Both B1 and C3 cells showed no activation of ERK1/2 in the absence of glucose for either the control or the latrunculin B-treated cell samples, this being an indication of the absence of secondary effects of the latrunculin B pre-treatment per se on ERK1/2 phosphorylation. After glucose stimulation, ERK1/2 was phosphorylated in both B1 and C3 cells, but the level of phosphorylation of the ERK2 isoform was reduced in C3 as compared to B1 cells (2.15-fold increase B1/C3), indicating a partial defect in MAPK activation by glucose in the secretion-impaired C3 cell line. After actin depolymerisation by latrunculin B pre-treatment, the level of glucose-induced ERK1/2 phosphorylation increased in both B1 and C3 cells, this increase being greater for the C3 cell line (7.5-fold increase with or without latrunculin B pre-treatment).

Activated phosphoERK1/2 accumulates at the tips of actin filaments in the vicinity of the plasma membrane upon stimulation with glucose

The short time frame associated with the activity of ERK1/2 on insulin secretion precludes a classic mechanism of action in which this kinase would translocate to the nucleus in order to regulate gene transcription. Instead, a mechanism in which phosphoERK1/2 is involved in the activation of specific cytoplasmic targets that would potentiate insulin secretion is more readily explainable. In order to gain more insight into this question, we analysed the subcellular localisation of phosphoERK1/2 in conjunction with the F-actin cytoskeleton by confocal immunofluorescence at basal (0 mM glucose) and stimulated (20 mM glucose) conditions in both B1 and C3 cells (Fig. 7B). As expected, we did not detect any signal for the phosphorylated form of ERK1/2 in the absence of glucose in either B1 or C3 cells. Upon glucose stimulation, phosphoERK1/2 localised throughout

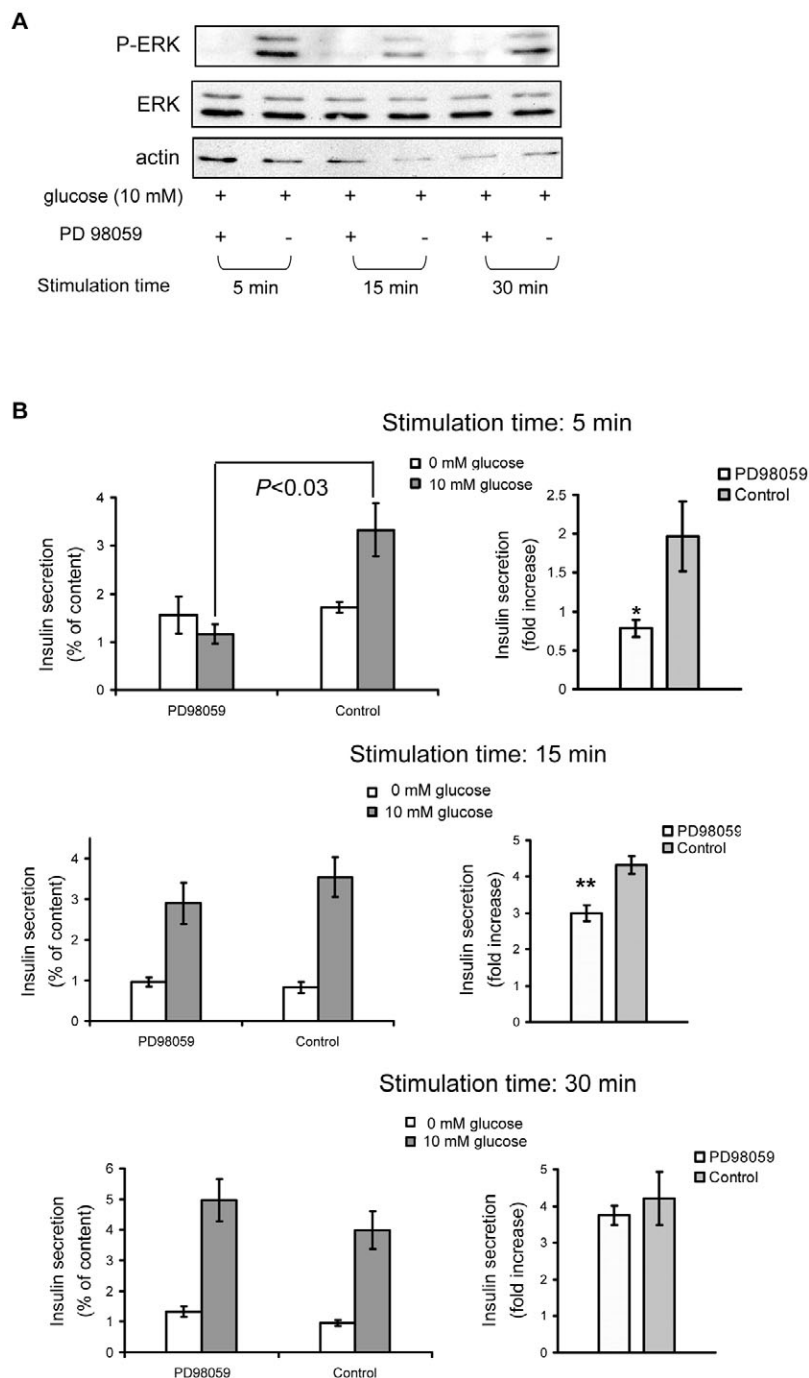


Fig. 6. ERK1/2 activity contributes to the short-term glucose-induced insulin secretion in B1 cells. (A) Western blot analysis of the inhibitory effect of PD98059 on the activation (by phosphorylation) of ERK1/2 after 10 mM glucose stimulation in B1 cells. (B) Insulin secretion by B1 cells treated with PD98059 at three different time points. After 5 minutes glucose stimulation (top), B1 cells display a 60% decrease in their secretory response as compared to control DMSO-treated cells ($*P < 0.05$). However, after 15 minutes stimulation (middle), the decrease in insulin secretion is reduced to 30% ($**P < 0.02$), and is not present after 30 minutes glucose stimulation (bottom). Data in all cases are mean $\pm$ s.e.m. for basal and stimulated % insulin secretion (left panels) and secretion fold-stimulation (right panels) of three replicates of a single experiment. Stimulation conditions of 0 to 10 mM glucose were used to facilitate direct comparison with previously published work showing the effect of glucose-dependent ERK1/2 activation on insulin secretion (Longuet et al., 2005).

the cytoplasm, displaying a punctate pattern in both sublines. Upon closer examination, we detected an accumulation of the phosphoERK1/2-specific signal at the tips of remodelled actin filaments at sites adjacent to the plasma membrane in B1 cells stimulated at both 20 mM glucose and at the more physiological concentration of 16.7 mM glucose (Fig. 7C). This subcellular localisation of phosphoERK1/2 at the tips of

actin fibres was also present but substantially diminished in C3 cells (Fig. 7B, bottom panels).

We next determined whether the localisation of activated phosphoERK1/2 at the tips of actin filaments could be responsible for the process of actin remodelling itself, rather than being a downstream consequence of this remodelling process that would be further involved in the phosphorylation

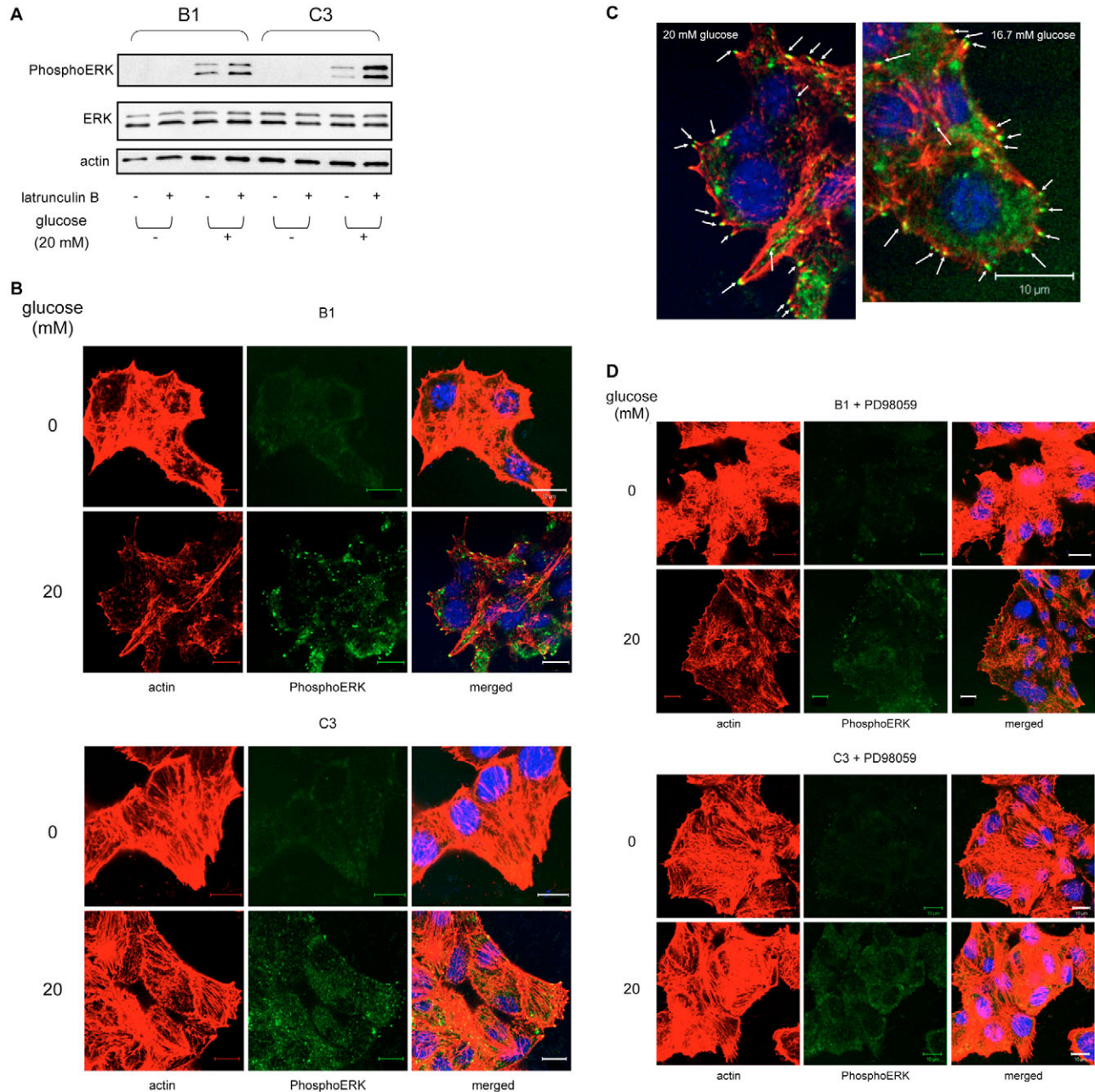


Fig. 7. (A) Western blot analysis showing the differences in short-term glucose-induced ERK1/2 activation between B1 and C3 sublines and the stimulatory effect of latrunculin B pre-treatment on the short-term phosphorylation of ERK1/2. (B) Immunofluorescence analysis of the subcellular localisation of activated phosphoERK1/2 (green) and the actin cytoskeleton (red) on B1 (top panels) and C3 cells (bottom panels) before and after 5 minutes stimulation with 20 mM glucose. Nuclei (blue) were stained with DRAQ5TM. No immunoreactivity was observed using control rabbit IgG (not shown). Bars, 10 μ m. (C) PhosphoERK1/2 accumulates at the tips of actin fibres in the vicinity of the plasma membrane (white arrows) after 20 mM glucose (left) and 16.7 mM glucose (right) stimulation in B1 cells. Bar, 10 μ m. (D) Inhibition of ERK1/2 activation by PD98059 has no effect on the actin cytoskeleton remodelling properties of B1 or C3 cells as shown by confocal analysis of phalloidin-labelled cell samples + PD98059 before and after 5 minutes glucose stimulation. Bars, 10 μ m.

of specific targets implicated in the potentiation of insulin secretion. For this purpose, we pre-incubated both B1 and C3 cells with the ERK1/2 inhibitor PD98059 prior to glucose stimulation and analysed the effects on actin cytoskeleton reorganisation by immunofluorescence (Fig. 7D). Under these experimental conditions, we could not detect any differences in the actin cytoskeleton remodelling properties of these two sublines as compared with untreated cells (Fig. 7D, compare with Fig. 1A,B).

Taken together, these results indicate that glucose-induced actin remodelling contributes towards the initiation of a rapid-response signalling pathway that leads to the short-term activation of ERK1/2, this activation either occurring at the tips of remodelled actin filaments or promoting the rapid relocalisation of phosphoERK1/2 to these actin filament tips, where it could function in the potentiation of the rapid glucose-dependent insulin secretory response in MIN6 β -cells, most probably by phosphorylating specific downstream targets at these precise cell sites in the vicinity of the plasma membrane and in close contact with the actin cytoskeleton.

Discussion

The dynamic control of actin distribution during regulated secretion is a complex process in which a number of regulatory proteins including the small GTPases cdc42 (Nevens and Thurmond, 2003) and Rac1 (Li et al., 2004), structural proteins such as fodrin (Nakano et al., 2001) and motor proteins such as the myosins (Wilson et al., 2001; Rudolf et al., 2003) have already been identified, even if the precise mechanism of action remains to be established.

In the present study we have characterised the differences in actin cytoskeleton organisation and remodelling between two β -cell sublines generated from mouse MIN6 cells with a view to identifying novel genes involved in regulated insulin secretion. Importantly for the present study, differences in secretory pattern between B1 and C3 cells were not attributed to any metabolic defect. Rather, C3 cells, which fail to respond to glucose or most other fuel and non-fuel secretagogues, appeared to have a lesion late in the exocytotic pathway (Lilla et al., 2003).

The actin cytoskeleton of the glucose-responsive B1 subline was found to be a dynamic structure resembling that of the parental MIN6 cell line in its capacity to partially depolymerise in response to glucose (Thurmond et al., 2003; Nevins and Thurmond, 2003), whereas the glucose-insensitive C3 subline had a rigid cytoskeleton, with an abundance of stress fibres in the subplasmalemmal region that were not remodelled after glucose stimulation.

Transient depolymerisation of the actin network in response to stimuli in pancreatic β -cells and other secretory cell types has often been correlated with their capacity for stimulated secretion (Thurmond et al., 2003; Koffer et al., 1990; Rose et al., 2001; Wilson et al., 2001). In agreement with this, and as previously shown for the parental MIN6 cell line (Thurmond et al., 2003), treatment with the actin depolymerising drug latrunculin B potentiated insulin secretion in B1 cells. Moreover, the normally secretory-impaired C3 cells became glucose-responsive under the same conditions of latrunculin B treatment, demonstrating a direct link between the actin polymerisation state and the capacity for regulated insulin secretion in these sublines.

The differences in actin cytoskeleton morphology and remodelling between the B1 and C3 sublines and the restoration of regulated insulin secretion after actin depolymerisation in C3 cells make the comparison between these two subclones an attractive model for the study of the mechanism of glucose-induced actin remodelling and its importance for insulin secretion.

We employed this model to identify the protein gelsolin as a candidate for glucose-induced actin remodelling in MIN6 cells. Gelsolin is well known for its involvement in dynamic changes of the actin cytoskeleton in response to changes in intracellular Ca^{2+} levels (Sun et al., 1999; Kwiatkowski, 1999), and was previously identified by microarray analysis as being over-expressed in B1 compared with C3 cells (Lilla et al., 2003). In confirmation of this earlier observation, both gelsolin mRNA and protein were found to be expressed at much higher levels in B1 than in C3 cells, and the level of expression of gelsolin in primary mouse β -cells was comparable to that found in B1 cells. In B1 cells, in common with other secretory cell types such as chromaffin cells (Aunis and Bader, 1988), gelsolin was found to localise to the subplasmalemmal area of the actin cytoskeleton. The presence of pronounced actin stress fibres in the secretion-impaired C3 cells might be related to the loss of gelsolin expression in this subline, as this same phenotype has been previously reported in fibroblasts from gelsolin null mice (Witke et al., 1995).

When gelsolin was knocked down by RNA interference in B1 cells, there was a significant decrease in glucose-stimulated secretion. Gelsolin knock-down was also able to partially counteract the stimulation of secretion after actin depolymerisation by latrunculin B, showing a close relationship between the actin polymerisation level and the level of secretion in B1 cells. Conversely, gelsolin knock-down had little or no effect on the secretory response induced by elevation of cAMP by IBMX. This observation, which correlates with the absence of actin depolymerisation after IBMX treatment, is most probably explained by the divergent mechanisms of stimulation of insulin secretion between glucose and cAMP (Yajima et al., 1999; Dyachok and Gylfe, 2004). By contrast, gelsolin over-expression resulted in a significant increase in stimulated hGH secretion in transfected B1 cells. This result, together with the loss of secretion after gelsolin knock-down, demonstrates a link between the level of this protein and the extent of regulated secretion in the B1 subline.

The decrease in glucose-dependent hGH secretion observed after gelsolin knock-down in B1 cells was partial, whereas regulated secretion was totally impaired in C3 cells. Several factors may contribute to this outcome, such as the partial efficacy of the co-transfection method in selecting knocked-down cells or the residual level of gelsolin left after the shRNA treatment. In addition, other proteins involved in the process of actin remodelling could be over-expressed or alternative pathways switched on to guarantee partial functionality of the secretion process. In this context, it is worth noting that C3 cells have extensive differences in their mRNA expression profile compared to B1 cells, with more than 200 genes either over- or under-expressed an average of twofold or more between these two sublines (Lilla et al., 2003), indicating that C3 cells suffer from an extensive number of defects, the combination of which most probably leads to the altered

phenotype. The above-mentioned results correlate well with the fact that actin depolymerisation by latrunculin B treatment in B1 cells results in a 58-fold increase in insulin secretion, whereas the same treatment in C3 cells results only in a tenfold increase.

The accumulated defects harboured in C3 cells might explain not only the partial secretory decrease observed in B1 cells after gelsolin knock-down compared with the total loss of secretion in C3 cells, but also the inability of C3 cells transfected with HA-tagged gelsolin to recover their capacity for regulated secretion. However, this latter result might also be the consequence of a dysregulation of calcium homeostasis resulting in the abnormal activation of gelsolin in C3 cells, as suggested by the immunolocalisation of HA-tagged gelsolin along extensively depolymerised actin filaments in basal conditions in these cells (this only occurs after glucose stimulation in B1 cells).

Gelsolin, which has previously been identified as being expressed in a number of hormone-secreting tissues (Nelson and Boyd, III, 1985), has been shown to affect the mobility of chromaffin granules (Miyamoto et al., 1993) and synaptic vesicles (Miyamoto, 1995) by disassembling actin filament networks *in vitro*. These observations are consistent with a role for gelsolin in coupling the Ca^{2+} signal elicited after glucose stimulation (Lang, 1999) and the rearrangement of the actin cytoskeleton at the onset of secretion. Scinderin, the closest homologue to gelsolin, has been previously suggested to play a role in β -cell actin remodelling (Bruun et al., 2000), but the very low to no expression of this protein outside a small number of tissues, such as renal tubular and intestinal epithelial cells, together with the differential pattern of gelsolin and scinderin expression (Lueck et al., 1998) conflict with the proposed model for scinderin function in insulin exocytosis (Kwiatkowski, 1999).

The small GTPase Rac1 has recently been described as playing a role in insulin secretion. Glucose exposure induces Rac1 activation in the rat pancreatic INS cell line and expression of a dominant-negative Rac1 mutant inhibits second phase insulin secretion (Li et al., 2004). Gelsolin is known to act as a downstream effector of Rac (Azuma et al., 1998). Active Rac indirectly dissociates gelsolin-actin complexes (Arcaro, 1998), probably via its association with phosphatidylinositol 5-kinases. This association promotes the translocation of phosphatidylinositol 5-kinases to the plasma membrane, increasing the local concentration of phosphatidylinositol 4,5-bisphosphate (Anderson et al., 1999), an inhibitor of gelsolin severing activity (Sun et al., 1999). In pancreatic β -cells, gelsolin activation by Ca^{2+} could promote actin filament severing resulting in the transient disruption of the actin cytoskeleton during the first phase of insulin secretion. Gelsolin could then be regulated by glucose-activated Rac1, inactivating the actin-severing process, and triggering actin filament uncapping and creation of new actin barbed ends. This would promote rapid actin filament assembly required for membrane ruffling and recruitment of granules to the plasma membrane during the second phase of insulin secretion (Li et al., 1994). Alternatively, gelsolin may affect insulin secretion in a totally different manner, either by modulating stimulus-dependent ion channel function (Furukawa et al., 1997), or by modulating lipid signalling events via its interaction with phosphoinositides (Kwiatkowski, 1999).

In the last part of this study we have explored the possibility of the actin cytoskeleton playing a role in the propagation of signalling events leading to insulin secretion in β -cells. In particular, we have investigated whether the state of actin polymerisation in β -cells has any effect on the glucose-induced activation of the MAPK member ERK1/2. The MAPK pathway has important roles in many cellular events, including proliferation, differentiation, survival and secretion (Hunter, 1995). In β -cells, glucose is known to activate ERK1/2 (Frodin et al., 1995; Briaud et al., 2003), and a recent report has shown that glucose-induced ERK1/2 activity remains in the cytoplasm and participates in the regulation of insulin secretion in MIN6 cells (Longuet et al., 2005).

In this context, it is worth noting that, in contrast to what has been found in the above-mentioned study (Longuet et al., 2005), we could only establish a contribution of ERK1/2 during the first phase but not the second phase of insulin secretion in B1 cells. The role of ERK1/2 in insulin secretion therefore coincides temporally with the transient remodelling of the actin cytoskeleton exerted by glucose in MIN6 (Thurmond et al., 2003) and MIN6 B1 cells. In addition, actin disassembly by latrunculin B pre-treatment was found to enhance the short-term glucose-induced ERK1/2 phosphorylation in both B1 and C3 cells. These results point towards a link between glucose-induced transient actin depolymerisation and the short-term transduction of signals from glucose to the MAPK pathway in MIN6 cells.

Although nuclear translocation of ERK1/2 is required for transcriptional changes, the cellular consequences of cytoplasmic signalling have not yet been defined. The role of ERK1/2 in the regulation of insulin secretion is most probably exerted via the phosphorylation of specific targets, one of which is the protein synapsin I (Longuet et al., 2005), known to be associated with insulin granules (Matsumoto et al., 1999), and proposed to be involved in granule translocation prior to insulin exocytosis (Yamamoto et al., 2003). In adult synapses, synapsin I tethers synaptic vesicles to the actin cytoskeleton in a phosphorylation-dependent manner, regulating the proportion of vesicles available for release (Jovanovic et al., 1996). Our observation that activated phosphoERK1/2 accumulates at the tips of actin filaments adjacent to the plasma membrane after 5 minutes of glucose stimulation indicates that these are the main sites of action of phosphoERK1/2 for the potentiation of insulin secretion. Membrane-proximal ERK1/2 localisation has been previously implicated in specific processes such as the activation of M-calpain downstream of epidermal growth factor receptor signalling (Glading et al., 2001). In view of our own results, we propose a mechanism of action in which ERK1/2 is activated specifically at the tips of actin filaments in close contact with the plasma membrane after glucose-dependent F-actin remodelling. This mechanism does not preclude the previously reported role for a cAMP/PKA-dependent pathway in the glucose-induced activation of ERK1/2 (Briaud et al., 2003), but simply suggests that remodelling of actin in response to glucose favours accessibility of ERK1/2 to its specific activation sites. Activated phosphoERK1/2 would subsequently phosphorylate specific targets at these sites, such as synapsin I present in insulin granules at the proximity of the plasma membrane, facilitating the onset of secretion.

In conclusion, we have established a relationship between

the differing actin cytoskeleton polymerisation levels and secretory properties of the B1 and C3 sublines and we have demonstrated a role for the Ca^{2+} -dependent actin remodelling protein gelsolin in regulated insulin secretion. We have also unveiled a connection between the state of actin polymerisation and the transduction of signals through the MAPK pathway, showing that actin remodelling in pancreatic β -cells can have functional repercussions that go beyond the removal of a physical barrier for insulin granules to access the membrane.

Materials and Methods

Drugs and antibodies

Latrunculin B and the MEK1/2 inhibitor PD98059 were purchased from Calbiochem (La Jolla, CA, USA). Latrunculin B was used at 10 μM and PD98059 at 100 μM final concentrations. Alexa Fluor 546-phalloidin was obtained from Molecular Probes (Eugene, OR, USA). Primary antibodies were, mouse anti-pan actin monoclonal antibody (mAb) from Chemicon International (Temecula, CA, USA), rabbit anti-ERK1/2 polyclonal antibody (pAb) and rabbit anti-phosphoERK1/2 pAb from Cell Signaling (Beverly, MA, USA), mouse anti-HA tag mAb from Roche (Basel, Switzerland) and rabbit anti-gelsolin pAb (a gift from C. Chaponnier, University of Geneva Medical School, Switzerland). Secondary antibodies were: donkey anti-rabbit Alexa Fluor 555, goat anti-mouse Alexa Fluor 488 and donkey anti-rabbit Alexa Fluor 488 from Molecular Probes; donkey anti-rabbit horseradish peroxidase (HRP) and sheep anti-mouse HRP from Amersham Biosciences (Little Chalfont, UK).

Primary β -cell purification

Islets of Langerhans were isolated by collagenase digestion of pancreas from adult male C57BL/6 mice or Wistar rats followed by Ficoll purification based on a method adapted from (Rouiller et al., 1990). Pancreatic β -cells were separated from non- β -cells by autofluorescence-activated sorting using the fluorescence-activated cell sorter FACStar-Plus (BD Biosciences, NJ, USA) and used for western blot analysis of gelsolin levels.

Cell lines and culture conditions

Both B1 and C3 cells were cultured in complete medium as described previously (Lilla et al., 2003). In general, cells were subcultured into 35 mm wells up to passage 25 to ~80% confluency before the experiments. For confocal microscopy, cells were plated, before fixation, onto 35-mm glass bottom microwell dishes (MatTek, Ashland, MA, USA) coated with extracellular matrix from 804G rat bladder carcinoma cells to favour cell attachment (Bosco et al., 2000).

Insulin secretion assays

Cells were washed twice with a modified Krebs-Ringer bicarbonate Hepes buffer [KRBH: 125 mM NaCl, 4.74 mM KCl, 1 mM CaCl_2 , 1.2 mM KH_2PO_4 , 1.2 mM MgSO_4 , 5 mM NaHCO_3 , 25 mM Hepes (pH 7.4), 0.1% BSA] and pre-incubated with this same buffer containing the drug of interest or DMSO (as a control) for 2 hours at 37°C. Cells were then incubated for 1 hour at 37°C in KRBH with drugs or DMSO (basal conditions), followed by 1 hour in KRBH supplemented with 10 or 20 mM glucose (stimulated conditions). For time-course analysis of insulin secretion, cells were pre-incubated as above and incubated in stimulated conditions for the different time points analysed. Basal and stimulated incubation buffers were recovered, and cells were extracted with acid-ethanol. The amount of insulin in the incubation buffers and cell extracts was measured by radioimmunoassay (RIA) as described previously (Lilla et al., 2003; Herbert et al., 1965). Insulin secretion is expressed as a percentage of the total insulin content, which is the sum of insulin contained in basal and stimulated buffers and in cell extracts.

Real-time reverse-transcription (RT)-PCR

Total RNA was extracted with the RNeasy Mini Kit (Qiagen, Germany) following the manufacturer's instructions, and used for cDNA synthesis with Superscript II (Invitrogen, Carlsbad, CA, USA). Primers were designed using the Primer Express software, and real-time RT-PCR was performed using the qPCR core kit for SYBR Green I (Eurogentec, Belgium) in the ABI PRISM 7000 Sequence Detection System (PE Applied Biosystems, CA, USA). Actin was used as a control for data normalisation.

RNA interference (RNAi)-mediated silencing of endogenous gelsolin

Two 64 base pair (bp) sequences encoding two different 21-bp-long small hairpin RNAs (shRNAs) specific for mouse gelsolin were cloned into both pSUPER and pSUPER-GFP plasmids (from OligoEngine, Inc., Seattle, WA, USA). A similar sequence encoding a non-specific shRNA without mammalian homology was also cloned and used as a negative control. Both shRNAs were tested for their capacity to knock-down gelsolin in B1 cells as follows. Cells were transiently transfected

with the pSUPER-GFP plasmid containing either one of the shRNAs against gelsolin or the negative control shRNA. Cells were cultured for 72 hours for RNAi expression before selection of GFP-positive cells by fluorescence-activated cell sorting (FACS). Selected cells were pooled and lysed for western blot analysis of gelsolin levels compared to negative control cells. The shRNA sequence 5'-AAAGGAGGTGTGGCATCTGGA-3' exhibited a greater silencing capacity and was employed in all subsequent experiments.

Transient transfection of cells

Cells were transfected with the different plasmids using Lipofectamine™ 2000 reagent (Invitrogen, Carlsbad, CA, USA) following the manufacturer's instructions. Transfected cells were incubated for 72 hours to allow for RNAi expression.

Growth hormone secretion assays

To study secretion specifically from transfected cells, the cells were co-transfected with a human growth hormone (hGH)-expressing vector and the vector of interest (1:3 DNA ratio), and incubated for 72 hours before analysis of hGH secretion (used as a surrogate marker for insulin secretion from transfected cells).

Co-transfected cells were washed twice in KRBH supplemented with 2.8 mM glucose and pre-incubated with this same buffer for 2 hours at 37°C. Cells were then incubated for 1 hour at 37°C in KRBH with 2.8 mM glucose, followed by 1 hour incubation with KRBH supplemented with 16.7 mM glucose or 16.7 mM glucose + 1 mM of 3-isobutyl-1-methylxanthine (IBMX) (stimulated conditions). Basal and stimulated incubation buffers were recovered, and cells were extracted with acid-ethanol. The amount of growth hormone in the incubation buffers and cell extracts was measured by ELISA using the hGH ELISA kit from Roche Diagnostics (Basel, Switzerland), following the manufacturer's instructions. Growth hormone secretion is expressed as a percentage of the total growth hormone content, which is the sum of growth hormone contained in the basal and stimulated buffers and cell extracts.

Immunofluorescence and confocal microscopy

Cells were washed in PBS, fixed/permeabilised on ice for 30 minutes in fixation solution (2% paraformaldehyde + 0.1% Triton X-100 in PBS), washed four times in PBS and blocked for 15 minutes with 1% BSA. Fixed cells were incubated in a moist chamber with primary antibody in 1% BSA for 1 hour, followed by four washes of 5–10 minutes each in PBS before incubation with the secondary antibody in 1% BSA for a further hour. For actin labelling, cells were incubated with Alexa Fluor 546-phalloidin for 20 minutes after fixation. Nuclei were detected with the DNA-binding dye DRAQ5™ (Biosstatus Ltd., UK). Cell samples were observed under a Zeiss LSM 510 inverted confocal microscope using a 63 $\times$ oil immersion lens. Images were acquired and processed using the Lsm510 software (Carl Zeiss AG, Germany).

SDS-PAGE and western blotting

Cultured cells were washed in PBS and solubilised in lysis buffer (20 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% NP-40) supplemented with Complete Mini protease inhibitor cocktail (Roche, Switzerland). Lysates were pre-cleared at 16,000 g for 5 minutes at 4°C, and total protein levels were quantified with the DC protein assay kit (BioRad, Hercules, CA, USA). Protein samples were re-suspended in SDS-PAGE sample buffer, boiled for 5 minutes, and resolved by SDS-polyacrylamide gel electrophoresis on discontinuous mini-gels. Proteins were then electroblotted for 45 minutes onto a Protran nitrocellulose Transfer Membrane (Schleicher & Schuell GmbH, Germany). Membranes were treated with blocking solution [5% skimmed milk in TBS-Tween (TBS + 0.05% Tween 20)] for 1 hour at room temperature and incubated for 16 hours at 4°C with primary antibody in blocking solution (or TBS-Tween + 5% BSA for anti-phosphoERK1/2 pAb). Following three TBS-Tween washes, membranes were incubated with secondary antibody for 90 minutes, also in blocking solution. After three more TBS-Tween washes, blots were incubated with the ECL Plus Western Blotting detection system (Amersham Biosciences, UK) and exposed to BioMax MR Films (Kodak, Rochester, NY, USA). The films were developed in a Kodak M35 X-OMAT Processor, and scanned. Scan band density was determined with Scion Image for Windows.

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References

- Anderson, R. A., Boronenkov, I. V., Doughman, S. D., Kunz, J. and Loijens, J. C. (1999). Phosphatidylinositol phosphate kinases, a multifaceted family of signaling enzymes. *J. Biol. Chem.* **274**, 9907–9910.

- Arcaro, A. (1998). The small GTP-binding protein Rac promotes the dissociation of gelsolin from actin filaments in neutrophils. *J. Biol. Chem.* **273**, 805-813.
- Ashcroft, F. M., Proks, P., Smith, P. A., Ammala, C., Bokvist, K. and Rorsman, P. (1994). Stimulus-secretion coupling in pancreatic beta cells. *J. Cell Biochem.* **55**, S54-S65.
- Aunis, D. and Bader, M. F. (1988). The cytoskeleton as a barrier to exocytosis in secretory cells. *J. Exp. Biol.* **139**, 253-266.
- Azuma, T., Witke, W., Stossel, T. P., Hartwig, J. H. and Kwiatkowski, D. J. (1998). Gelsolin is a downstream effector of rac for fibroblast motility. *EMBO J.* **17**, 1362-1370.
- Bader, M. F., Doussau, F., Chasserot-Golaz, S., Vitale, N. and Gasman, S. (2004). Coupling actin and membrane dynamics during calcium-regulated exocytosis: a role for Rho and ARF GTPases. *Biochim. Biophys. Acta* **1742**, 37-49.
- Bosco, D., Meda, P., Halban, P. A. and Rouiller, D. G. (2000). Importance of cell-matrix interactions in rat islet beta-cell secretion in vitro: role of alpha6beta1 integrin. *Diabetes* **49**, 233-243.
- Briaud, I., Lingohr, M. K., Dickson, L. M., Wrede, C. E. and Rhodes, C. J. (2003). Differential activation mechanisms of Erk-1/2 and p70(S6K) by glucose in pancreatic beta-cells. *Diabetes* **52**, 974-983.
- Bruun, T. Z., Hoy, M. and Gromada, J. (2000). Scinderin-derived actin-binding peptides inhibit Ca(2+)- and GTPgammaS-dependent exocytosis in mouse pancreatic beta-cells. *Eur. J. Pharmacol.* **403**, 221-224.
- Dyachok, O. and Gylfe, E. (2004). Ca(2+)-induced Ca(2+) release via inositol 1,4,5-trisphosphate receptors is amplified by protein kinase A and triggers exocytosis in pancreatic beta-cells. *J. Biol. Chem.* **279**, 45455-45461.
- Easom, R. A. (2000). Beta-granule transport and exocytosis. *Semin. Cell Dev. Biol.* **11**, 253-266.
- Eitzen, G. (2003). Actin remodeling to facilitate membrane fusion. *Biochim. Biophys. Acta* **1641**, 175-181.
- Frodin, M., Sekine, N., Roche, E., Filloux, C., Prentki, M., Wollheim, C. B. and Van O. E. (1995). Glucose, other secretagogues, and nerve growth factor stimulate mitogen-activated protein kinase in the insulin-secreting beta-cell line, INS-1. *J. Biol. Chem.* **270**, 7882-7889.
- Furukawa, K., Fu, W., Li, Y., Witke, W., Kwiatkowski, D. J. and Mattson, M. P. (1997). The actin-severing protein gelsolin modulates calcium channel and NMDA receptor activities and vulnerability to excitotoxicity in hippocampal neurons. *J. Neurosci.* **17**, 8178-8186.
- Gasman, S., Chasserot-Golaz, S., Malacombe, M., Way, M. and Bader, M. F. (2004). Regulated exocytosis in neuroendocrine cells: a role for subplasmalemmal Cdc42/N-WASP-induced actin filaments. *Mol. Biol. Cell* **15**, 520-531.
- Glading, A., Uberall, F., Keyse, S. M., Lauffenburger, D. A. and Wells, A. (2001). Membrane proximal ERK signaling is required for M-calpain activation downstream of epidermal growth factor receptor signaling. *J. Biol. Chem.* **276**, 23341-23348.
- Herbert, V., Lau, K. S., Gottlieb, C. W. and Bleicher, S. J. (1965). Coated charcoal immunoassay of insulin. *J. Clin. Endocrinol. Metab.* **25**, 1375-1384.
- Holz, R. W., Brondyk, W. H., Senter, R. A., Kuizon, L. and Macara, I. G. (1994). Evidence for the involvement of Rab3A in Ca(2+)-dependent exocytosis from adrenal chromaffin cells. *J. Biol. Chem.* **269**, 10229-10234.
- Hunter, T. (1995). Protein kinases and phosphatases: the yin and yang of protein phosphorylation and signaling. *Cell* **80**, 225-236.
- Iezzi, M., Escher, G., Meda, P., Charollais, A., Baldini, G., Darchen, F., Wollheim, C. B. and Regazzi, R. (1999). Subcellular distribution and function of Rab3A, B, C, and D isoforms in insulin-secreting cells. *Mol. Endocrinol.* **13**, 202-212.
- Jijakli, H., Zhang, H. X., Dura, E., Ramirez, R., Sener, A. and Malaisse, W. J. (2002). Effects of cytochalasin B and D upon insulin release and pancreatic islet cell metabolism. *Int. J. Mol. Med.* **9**, 165-172.
- Jovanovic, J. N., Benfenati, F., Siow, Y. L., Sihra, T. S., Sanghera, J. S., Pelech, S. L., Greengard, P. and Czernik, A. J. (1996). Neurotrophins stimulate phosphorylation of synapsin I by MAP kinase and regulate synapsin I-actin interactions. *Proc. Natl. Acad. Sci. USA* **93**, 3679-3683.
- Koffer, A., Tatham, P. E. and Gomperts, B. D. (1990). Changes in the state of actin during the exocytotic reaction of permeabilized rat mast cells. *J. Cell Biol.* **111**, 919-927.
- Kowluru, A., Li, G., Rabaglia, M. E., Segu, V. B., Hofmann, F., Aktories, K. and Metz, S. A. (1997). Evidence for differential roles of the Rho subfamily of GTP-binding proteins in glucose- and calcium-induced insulin secretion from pancreatic beta cells. *Biochem. Pharmacol.* **54**, 1097-1108.
- Kwiatkowski, D. J. (1999). Functions of gelsolin: motility, signaling, apoptosis, cancer. *Curr. Opin. Cell Biol.* **11**, 103-108.
- Lang, J. (1999). Molecular mechanisms and regulation of insulin exocytosis as a paradigm of endocrine secretion. *Eur. J. Biochem.* **259**, 3-17.
- Li, G., Rungger-Brandle, E., Just, I., Jonas, J. C., Aktories, K. and Wollheim, C. B. (1994). Effect of disruption of actin filaments by Clostridium botulinum C2 toxin on insulin secretion in HIT-T15 cells and pancreatic islets. *Mol. Biol. Cell* **5**, 1199-1213.
- 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.
- Lilla, V., Webb, G., Rickenbach, K., Maturana, A., Steiner, D. F., Halban, P. A. and Irminger, J. C. (2003). Differential gene expression in well-regulated and dysregulated pancreatic beta-cell (MIN6) sublines. *Endocrinology* **144**, 1368-1379.
- Longuet, C., Broca, C., Costes, S., Hani, E. H., Bataille, D. and Dalle, S. (2005). Extracellularly regulated kinases 1/2 (p44/42 mitogen-activated protein kinases) phosphorylate synapsin I and regulate insulin secretion in the MIN6 beta-cell line and islets of Langerhans. *Endocrinology* **146**, 643-654.
- Lueck, A., Brown, D. and Kwiatkowski, D. J. (1998). The actin-binding proteins adseverin and gelsolin are both highly expressed but differentially localized in kidney and intestine. *J. Cell Sci.* **111**, 3633-3643.
- Matsumoto, K., Ebihara, K., Yamamoto, H., Tabuchi, H., Fukunaga, K., Yasunami, M., Ohkubo, H., Shichiri, M. and Miyamoto, E. (1999). Cloning from insulinoma cells of synapsin I associated with insulin secretory granules. *J. Biol. Chem.* **274**, 2053-2059.
- Miyamoto, S. (1995). Changes in mobility of synaptic vesicles with assembly and disassembly of actin network. *Biochim. Biophys. Acta* **1244**, 85-91.
- Miyamoto, S., Funatsu, T., Ishiwata, S. and Fujime, S. (1993). Changes in mobility of chromaffin granules in actin network with its assembly and Ca(2+)-dependent disassembly by gelsolin. *Biophys. J.* **64**, 1139-1149.
- Nakano, M., Nogami, S., Sato, S., Terano, A. and Shirataki, H. (2001). Interaction of syntaxin with alpha-fodrin, a major component of the submembranous cytoskeleton. *Biochem. Biophys. Res. Commun.* **288**, 468-475.
- Nelson, T. Y. and Boyd, A. E., III (1985). Gelsolin, a Ca2+-dependent actin-binding protein in a hamster insulin-secreting cell line. *J. Clin. Invest.* **75**, 1015-1022.
- Nevins, A. K. and Thurmond, D. C. (2003). Glucose regulates the cortical actin network through modulation of Cdc42 cycling to stimulate insulin secretion. *Am. J. Physiol. Cell Physiol.* **285**, C698-C710.
- Orci, L., Gabbay, K. H. and Malaisse, W. J. (1972). Pancreatic beta-cell web: its possible role in insulin secretion. *Science* **175**, 1128-1130.
- Pang, L., Sawada, T., Decker, S. J. and Saltiel, A. R. (1995). Inhibition of MAP kinase blocks the differentiation of PC-12 cells induced by nerve growth factor. *J. Biol. Chem.* **270**, 13585-13588.
- Rose, S. D., Lejen, T., Zhang, L. and Trifaro, J. M. (2001). Chromaffin cell F-actin disassembly and potentiation of catecholamine release in response to protein kinase C activation by phorbol esters is mediated through myristoylated alanine-rich C kinase substrate phosphorylation. *J. Biol. Chem.* **276**, 36757-36763.
- Rouiller, D. G., Cirulli, V. and Halban, P. A. (1990). Differences in aggregation properties and levels of the neural cell adhesion molecule (NCAM) between islet cell types. *Exp. Cell Res.* **191**, 305-312.
- Rudolf, R., Kogel, T., Kuznetsov, S. A., Salm, T., Schlicker, O., Hellwig, A., Hammer, J. A., III and Gerdes, H. H. (2003). Myosin Va facilitates the distribution of secretory granules in the F-actin rich cortex of PC12 cells. *J. Cell Sci.* **116**, 1339-1348.
- Schweitzer, E. S. and Kelly, R. B. (1985). Selective packaging of human growth hormone into synaptic vesicles in a rat neuronal (PC12) cell line. *J. Cell Biol.* **101**, 667-676.
- Siegel, E. G., Wollheim, C. B., Kikuchi, M., Renold, A. E. and Sharp, G. W. (1980). Dependency of cyclic AMP-induced insulin release on intra- and extracellular calcium in rat islets of Langerhans. *J. Clin. Invest.* **65**, 233-241.
- Sun, H. Q., Yamamoto, M., Mejillano, M. and Yin, H. L. (1999). Gelsolin, a multifunctional actin regulatory protein. *J. Biol. Chem.* **274**, 33179-33182.
- 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.
- Tsakiridis, T., Bergman, A., Somwar, R., Taha, C., Aktories, K., Cruz, T. F., Klip, A. and Downey, G. P. (1998). Actin filaments facilitate insulin activation of the src and collagen homologous/mitogen-activated protein kinase pathway leading to DNA synthesis and c-fos expression. *J. Biol. Chem.* **273**, 28322-28331.
- Wang, Y., Okamoto, M., Schmitz, F., Hofmann, K. and Sudhof, T. C. (1997). Rim is a putative Rab3 effector in regulating synaptic-vesicle fusion. *Nature* **388**, 593-598.
- Wilson, J. R., Ludowyke, R. I. and Biden, T. J. (2001). A redistribution of actin and myosin IIA accompanies Ca(2+)-dependent insulin secretion. *FEBS Lett.* **492**, 101-106.
- Witke, W., Sharpe, A. H., Hartwig, J. H., Azuma, T., Stossel, T. P. and Kwiatkowski, D. J. (1995). Hemostatic, inflammatory, and fibroblast responses are blunted in mice lacking gelsolin. *Cell* **81**, 41-51.
- Yajima, H., Komatsu, M., Schermerhorn, T., Aizawa, T., Kaneko, T., Nagai, M., Sharp, G. W. and Hashizume, K. (1999). cAMP enhances insulin secretion by an action on the ATP-sensitive K+ channel-independent pathway of glucose signaling in rat pancreatic islets. *Diabetes* **48**, 1006-1012.
- Yamamoto, H., Matsumoto, K., Araki, E. and Miyamoto, E. (2003). New aspects of neurotransmitter release and exocytosis: involvement of Ca2+/calmodulin-dependent phosphorylation of synapsin I in insulin exocytosis. *J. Pharmacol. Sci.* **93**, 30-34.