Dynamics of Glucose-induced Membrane Recruitment of Protein Kinase C βII in Living Pancreatic Islet β-Cells

The mechanisms by which glucose may affect protein kinase C (PKC) activity in the pancreatic islet β-cell are presently unclear. By developing adenovirally expressed chimeras encoding fusion proteins between green fluorescent protein and conventional (βII), novel (δ), or atypical (ζ) PKCs, we show that glucose selectively alters the subcellular localization of these enzymes dynamically in primary islet and MIN6 β-cells. Examined by laser scanning confocal or total internal reflection fluorescence microscopy, elevated glucose concentrations induced oscillatory translocations of PKCβII to spatially confined regions of the plasma membrane. Suggesting that increases in free cytosolic Ca\textsuperscript{2+} concentrations ([Ca\textsuperscript{2+}]\textsubscript{c}) were primarily responsible, prevention of [Ca\textsuperscript{2+}]\textsubscript{c} increases with EGTA or diazoxide completely eliminated membrane recruitment, whereas elevation of cytosolic [Ca\textsuperscript{2+}]\textsubscript{c} with KCl or tolbutamide was highly effective in redistributing PKCβII both to the plasma membrane and to the surface of dense core secretory vesicles. By contrast, the distribution of PKC\textsubscript{δ}-EGFP, which binds diacylglycerol but not Ca\textsuperscript{2+}, was unaffected by glucose. Measurement of [Ca\textsuperscript{2+}]\textsubscript{c} immediately beneath the plasma membrane with a ratiometric "pericam," fused to synaptic vesicle-associated protein-25, revealed that depolarization induced significantly larger increases in [Ca\textsuperscript{2+}]\textsubscript{c} in this domain. These data demonstrate that nutrient stimulation of β-cells causes spatially and temporally complex changes in the subcellular localization of PKCβII, possibly resulting from the generation of Ca\textsuperscript{2+} microdomains. Localized changes in PKCβII activity may thus have a role in the spatial control of insulin exocytosis.

Ca\textsuperscript{2+} and phospholipid-dependent protein kinases (PKC) are important mediators of intracellular signals (1). PKC isoforms can be divided into three subfamilies. Conventional PKCs are activated via recruitment to membranes, mediated by the Ca\textsuperscript{2+}-dependent binding of a C2 domain to phospholipids, and this effect is further potentiated by the binding of diacylglycerol (DAG) to C1 domains (1). By contrast, novel PKCs bind DAG, but not Ca\textsuperscript{2+} and phospholipids, while atypical PKCs are not affected by any of the above activators (1).

Biochemical studies of the activation of PKC are complicated by the need for cell disruption and isolation of membrane and cytosol fractions (2) or for cell fixation and immunocytochemistry (2–4). Each of these approaches is limited by the difficulty of detecting any changes in subcellular localization, which are spatially or temporally complex. To overcome this limitation, fusion constructs between enhanced green fluorescent protein (EGFP) (5) and PKCγ (6), PKCδ (7), PKCζ (8), and PKCβII (9) have recently been used to monitor the dynamics of membrane translocation of PKCs in a number of non-excitable cell types and appear faithfully to reflect the behavior of the endogenous PK isoforms. However, while PKC may play an important role in agonist stimulation of exocytosis from neurosecretory cells (10), no data are presently available on the dynamics of conventional PKCs in any excitable cell type.

Elevated glucose concentrations stimulate insulin secretion from β-cells via metabolism of the sugar (11, 12) and increases in cytosolic free ATP concentration (13). Closure of ATP-sensitive K\textsuperscript+ channels (14) then leads to depolarization of the plasma membrane, influx of Ca\textsuperscript{2+} through voltage-gated Ca\textsuperscript{2+} channels (15), and secretory vesicle fusion (16). PKC activity is present in both primary pancreatic islets (17) and derived β-cell lines (18, 19). Furthermore, conventional (α, βI, βII; sensitive to Ca\textsuperscript{2+} and DAG), novel (δ; sensitive to DAG but not Ca\textsuperscript{2+}), and atypical (ζ, λ; insensitive to Ca\textsuperscript{2+} and DAG) PKC isoforms (20–23) have all been reported in islet cells. However, the role of PKC in the stimulation of insulin secretion is controversial. Acute activation of conventional and novel PKCs with the phorbol ester 12-O-tetradecanoyl-phorbol-13-acetate strongly stimulates insulin secretion (19, 24) without affecting β-cell electrical activity or cytosolic free Ca\textsuperscript{2+} ([Ca\textsuperscript{2+}]\textsubscript{c}) (25, 26). On the other hand, inhibition of PKC activity with the broad specificity inhibitor staurosporine (27), or an inhibitor specific for classical PKC isoforms (Go6976), slightly enhances the first phase of glucose-stimulated insulin release from rat islets (28).
PKC Translocation in Pancreatic β-Cells

while diminishing the sustained phase. Down-regulation of conventional PKC isoforms with phorbol esters has little effect on glucose-stimulated insulin release (29).

To determine whether active PKCs may play a role in the spatial coordination of exocytosis in individual β-cells without necessarily affecting total insulin release, we have therefore generated fusion constructs between EGFFP and PKCβII, PKCδ, and PKCγ. PKCβII and PKCδ represent the major conventional PKC isoforms in β-cells (20), and PKCβII activity has recently been shown to be important for the regulation of the preproinsulin gene (23). Expression of these constructs has allowed the dynamics of each isoform to be studied in real time in both primary islet and clonal β-cells. Using confocal and total internal reflection fluorescence (TIRF)/evanescent wave (30–34) imaging, we show that elevated glucose concentrations cause complex, oscillatory translocations to the plasma and other membranes of PKCβII in primary β-cells and clonal MIN6 cells. These changes appear to be produced largely by transient depolarizations of the plasma membrane and stimulated Ca2+ influx. The formation of microdomains of [Ca2+]i, immediately beneath the plasma membrane, demonstrated directly by targeting a green fluorescent protein-based Ca2+ probe (“pericam”) (35) exclusively to this domain, may be critical for the generation of complex movements of PKC.

**EXPERIMENTAL PROCEDURES**

**Materials and Methods**

Cell culture reagents were obtained from Invitrogen or Sigma, and molecular biologicals from Roche Molecular Biochemicals.

**Adenoviral Generation**—Adenoviruses were constructed and amplified using the pAdEasy system (36) as previously described (37). The PKCβII/EFP, PKCδ/EFP, and PKCγ/EFP (9) cDNAs were transferred into plasmid pShuttle-CMV as KpnI/XhoI fragments. Adenoviral generation from the recombinant shuttle vectors was performed, and infection of cells and islets was performed as previously described (37).

Recombination with pAdEasy-1, transfection into HEK 293 cells, and viral amplification of the pShuttle-CMV based plasmids encoding each recombinant PKC isoform-GFP fusion proteins was performed essentially as previously described (37). Determination of viral concentration was by comparison of the absorbance at 260 nm with a viral stock of known titer (37). MIN6 cells were infected with a multiplicity of infection of 30–16 h prior to imaging.

**Cell Culture and Adenoviral Infec­tion**—Primary isolated islet β-cells and MIN6 cells (passages nos. 20 to 30) were cultured and infected with adenoviruses as previously described (38). In each case, the concentration of glucose was lowered to 3 mM for 16 h before experiments.

**Confocal Imaging Analysis**—Coverslips (24 mm in diameter) were placed in a thermostatted Leyden chamber, (model TC-202A, Medical Instruments). MIN6 cells were incubated at 37 °C until use. Images were acquired every 2 s. To analyze the data, translocation events were manually selected and the average fluorescence intensity of individual plasma membrane regions was calculated.

**Statistical Analysis**

Data are given as means ± S.E. of at least three individual experiments. Comparisons between means were performed using one-tailed Student’s t test for paired data with Microsoft ExcelTM or OriginTM (OriginLab, Northampton, MA) software.

**RESULTS**

**Responses of PKCβII to Glucose and Other Agonists in Primary β-Cells**—Fig. 1A shows the responses to a stepped increase in glucose concentration from 3 to 25 mM of adenovirally expressed PKCβII/EGFP, imaged by laser-scanning confocal microscopy in primary β-cells. An increase in fluorescence ratio (plasma membrane: cytosol) was observed in 7 of 16 cells examined (Fig. 1A). Imaging [Ca2+]i, increases in these effects of glucose, cell depolarization with 35 mM KCl (Fig. 1B) or stimulation of muscarinic receptors with carbachol, 100 μM (Fig. 1C), also caused a clear increase in the proportion of plasma membrane-bound PKCβII and in each case the appearance of focal points of high fluorescence (arrows).

In contrast to PKCβII/EGFP, neither PKCδ/EGFP nor
while phorbol 12-myristate 13-acetate (PMA) caused translocation of PKCβII in MIN6 cells (Fig. 2A). Retranslocation to the cytosol was clearly evident in almost half (three of seven) of the cells examined. To provide greater temporal and spatial resolution we next employed TIRF microscopy (31–34). This technique involves the generation of a thin (<100 nm) field of fluorescence at the surface of the coverslip and thus at the surface of an attached cell. Hence a fluorophore such as PKCβII-EGFP will only fluoresce as it approaches very close to (within ~50 nm) the plasma membrane while molecules in the cytosol remain in darkness. Since MIN6 cells display a flattened morphology, this technique was anticipated to permit a more precise quantification of plasma membrane-associated PKCβII.

PKCβII-EGFP was translocated to the plasma membrane with a half-time of ~60 s and a peak increase in membrane:cytosolic PKCβII-EGFP of ~1.5 (Fig. 2B). Translocation was not provoked by a non-metabolizable sugar (galactose, not shown) and was completely suppressed by chelation of extracellular Ca^{2+} with EGTA or by cell hyperpolarization with the ATP-sensitive K+ channel opener, diazoxide (Fig. 2B, trace 4). In some cells, “hot spots” and waves of PKC were clearly detectable (see movie “Fig. 2B” at http://www.jbc.org). These effects were not observed in cells expressing a membrane-targeted GFP chimera (not shown) and are thus unlikely to result simply from changes in the shape of the cell. Moreover, the effects of glucose upon translocation were only marginally reduced by inhibition of phospholipase C activity with U73122 (Fig. 2B, trace 5) but at a concentration of (10 μM) that completely inhibited the effects of carbachol on PKCβII translocation (see Fig. 5B, trace 3; see movie “Fig. 5B” at http://www.jbc.org for the effect of carbachol alone or [Ca^{2+}], not shown). Measurement of Global or Localized Ca^{2+} Changes with Recombinant Targeted Pericams—Changes in free Ca^{2+} concentration were measured throughout the cell cytosol after expression of a ratiometric pericam (35) in this compartment. Elevating the glucose concentration from 3 to 30 mM caused a gradual increase in [Ca^{2+}], from ~200 nM to close to 1 μM (resting 0.18 ± 0.1 μM, maximum 1.03 ± 0.34 μM, n = 10 cells in each case; Fig. 2C), with a half-time similar to that for the increases in PKCβII associated with the plasma membrane (Fig. 2A and B). In some cells (4 of 10 examined) the glucose-induced increases were more oscillatory, consisting of spikes on a steadily increasing baseline (Fig. 2D).

We next tested the possibility that highly localized changes in [Ca^{2+}], immediately beneath the plasma membrane may be involved in PKCβII membrane recruitment. The formation of such a Ca^{2+} microdomain would be expected to permit phospholipid-dependent interaction of the PKCβII C2 domain with the membrane inner leaflet (45), independently of the DAG binding domain (C1) (46).

To achieve measurements of [Ca^{2+}], close to the inner surface of plasma membrane (<10 nm), we targeted the Ca^{2+} sensor, ratiometric pericam (35) to this region of the cell. cDNA encoding the pericam was fused in frame with that encoding the soluble N-ethyl maleimide-sensitive factor receptor (t-SNARE), synaptosome-associated protein of 25 kDa (SNAP25), which binds to membranes after palmitoylation (47). The SNAP25-pericam chimera displayed a largely plasma membrane localization with some fluorescence on intracellular structures, possibly corresponding to the Golgi apparatus or mature secretory vesicles (Fig. 2E, monochrome panel) (37). Importantly, the molecular targeting of this construct eliminated the need for spatially selective excitation (i.e. by confocal or TIRF microscopy), permitting ratiometric measurement of...
fluorescence by conventional epifluorescence microscopy. The membrane-targeted pericam displayed a dissociation constant for Ca$^{2+}$ close to that previously reported for the untargeted construct (1.7 μM) (35) in digitonin-permeabilized cells (not shown). Resting Ca$^{2+}$ concentrations reported with this peri-cam were not significantly different (0.21 μM) compared with the untargeted reporter (0.18 μM, Fig. 2, C and D). However, greater heterogeneity was apparent in the Ca$^{2+}$ increases elicited by elevated glucose concentrations (Fig. 2E, trace 1 versus 2) in cells expressing the plasma membrane-targeted pericam, with an average difference in the peak [Ca$^{2+}$]c of 0.22 ± 0.06 μM (n = 8 separate cells). By contrast, no significant differences in peak [Ca$^{2+}$]c in different areas of the cell cytoplasm were detected using the untargeted pericam, either in cells displaying a monotonic response to the sugar (Fig. 2C) or in those in which [Ca$^{2+}$]c oscillations were apparent (Fig. 2D).

**Effect of Stimulated Ca$^{2+}$ Influx on PKCβII Localization**—Stimulation of Ca$^{2+}$ influx with either depolarizing concentrations of KCl (in 29 of 34 cells examined, Fig. 3, A and B; see movie “Fig. 3B” at http://www.jbc.org) or tolbutamide, which closes ATP-sensitive K+ channels (in 16 of 19 cells examined, Fig. 4, A and B) caused clear and rapid translocation of PKCβII to the cell surface. Tolbutamide stimulation was usually also followed by a series of oscillations in both PKCβII translocation (Fig. 4B; see movie “Fig. 4B” at http://www.jbc.org) to the plasma membrane, as well as cytosolic (Fig. 4C) and plasma membrane [Ca$^{2+}$]c (Fig. 4D). Arguing against the possibility that the transient nature of the KCl-induced PKCβII translo-
cation was due to a short-lived increase in DAG generation by phospholipid hydrolysis, glucose-stimulated translocation of PKCβII-EGFP was entirely unaffected by the pharmacological PLC inhibitor, U73122 (Fig. 3B, trace 3).

Imaged by confocal microscopy (Fig. 3C), PKCβII was found also to translocate to intracellular structures in response to KCl. The identity of the majority of these structures was revealed as mature insulin secretory vesicles by simultaneous imaging of a co-expressed dense core vesicle membrane protein, phogrin (39), conjugated to cyan fluorescent protein (47).

Changes in \([\text{Ca}^{2+}]_c\) in the bulk cytosol and beneath the membrane in response to cell depolarization induced by KCl or tolbutamide were explored with targeted pericams. In contrast to untargeted pericam, which reported an increase in intracellular Ca²⁺, targeted pericams revealed a substantially larger increase in \([\text{Ca}^{2+}]_{\text{PM}}\) (D) than \([\text{Ca}^{2+}]_c\) (C) following KCl addition.

**Fig. 3.** Effect of membrane depolarization with KCl on PKCβII distribution (A, B, C) and intracellular Ca²⁺ changes (D, E). Cells expressing either PKCβII (A, B, C), plus phogrin-ECFP (C) or targeted pericams (D, E), were incubated with the indicated concentrations of KCl. In C, images were captured by alternate illumination at 430 and 488 nm as described under “Experimental Procedures”; points of colocalization between PKCβII and phogrin-ECFP-containing dense core vesicles are indicated with arrows. Other details were as Fig. 2. Note the substantially larger increase in \([\text{Ca}^{2+}]_{\text{PM}}\) than \([\text{Ca}^{2+}]_c\) following KCl addition.
lular Ca$^{2+}$ upon cell depolarization to 1.38 ± 0.26 μM (n = 20 cells; Fig. 3D), plasma membrane localized SNAP25-pericam reported an increase to 1.82 ± 0.31 μM (n = 20 cells, p < 0.05 with respect to cytosolically targeted pericam; Fig. 3E).

**Simultaneous Imaging of PKCβII Translocation and Depolarization-induced [Ca$^{2+}$]$_i$ Increases in Single Cells**—We next sought evidence that the larger increase in Ca$^{2+}$ beneath the plasma membrane may be important for the recruitment of PKCβII. If the glucose-induced translocation of PKCβII were due solely to a global increase in intracellular [Ca$^{2+}$]$_i$, it would be predicted that the kinetics of the increases in [Ca$^{2+}$]$_i$ and the membrane content of PKCβII would be very similar. Indeed, glucose induced changes in PKCβII-EGFP distribution, and cytosolic Ca$^{2+}$ displayed grossly similar kinetics (Fig. 2, A and B versus C and D). However, when cells were stimulated with KCl, this prediction only held true during the initial recruitment of the chimera (see Fig. 6). At later time points (>30 s) PKCβII-EGFP dissociated from the membrane while [Ca$^{2+}$]$_i$, remained close to maximal. These data suggest that PKCβII association with the plasma membrane may be controlled by locally high Ca$^{2+}$ concentrations.

**Impact of Intracellular Ca$^{2+}$ Mobilization on PKCβII Localization**—Activation of muscarinic receptors with carbachol and mobilization of intracellular Ca$^{2+}$ caused a rapid, transient translocation to the plasma membrane (in 25 of 29 cells examined, Fig. 5, A and B). This effect was entirely blocked by the presence of the phospholipase C inhibitor U73122 (Fig. 5B, trace 3). In contrast to depolarizing stimuli (Figs. 3 and 4) carbachol caused an essentially identical increase in [Ca$^{2+}$]$_{PM}$ (to 2.62 ± 0.42 μM, n = 10 cells; Fig. 5D) as [Ca$^{2+}$]$_i$ (to 2.48 ± 0.36 μM, n = 10 cells; Fig. 5C). Interestingly, the response to carbachol was significantly accelerated at high glucose concentrations (Fig. 5A, images 3 and 4 and lower graph; solid versus dashed trace), presumably reflecting glucose-induced Ca$^{2+}$ influx and/or DAG production (see “Discussion”).

**Effects of Glucose on the Subcellular Distribution of Novel and Atypical PKC Isoforms**—The ineffectiveness of the phospholipase C inhibitor to prevent glucose or KCl-induced recruitment of PKCβII to the plasma membrane (Fig. 3B) suggested that DAG production and binding to C1 domains may have played a relatively small part in translocation. In line with this view, the distribution of neither the novel isoform PKCδ (no C2 domain) (48) nor PKCζ (lacking both C1 and C2 domains) were affected by glucose (Fig. 7) or depolarizing stimuli (not shown). By contrast, PKCζ was rapidly translocated (half-time ~20 s in each case) to both the nuclear membrane and cell surface in response to addition of the phorbol ester, PMA (Fig. 7, A and B).

**FIG. 4.** Effect of K$_{ATP}$ channel closure on PKCβII distribution (A, B) and intracellular Ca$^{2+}$ changes (C, D). Cells expressing either PKCβII (A, B), or targeted pericams were incubated with the indicated concentrations of tolbutamide. Other details as Fig. 2.
DISCUSSION

Dynamics of PKCβII-EGFP Translocation—We show here, for the first time in single living β-cells, that elevated glucose concentrations cause complex and dynamic changes in the localization of a conventional PKC isoform PKCβII. This behavior was observed in both primary islet β-cells (Fig. 1A) and, more dramatically, in clonal MIN6 β-cells (Fig. 2, A and B). In the latter case, TIRF microscopy revealed the creation by elevated glucose concentrations of hot spots and waves of PKCβII at the plasma membrane (see also movie “Fig. 2B” at http://www.jbc.org). In this respect, the behavior of PKCβII (9) as well as the conventional PKC isoforms PKCγ (6) and PKCα (7) is reminiscent of that previously described in non-excitable cells using GFP chimeras and confocal microscopy. However, by the use of TIRF microscopy, we also reveal the creation by elevated glucose concentrations of hot spots and waves of PKCβII at the plasma membrane, phenomena recently described for PKCα in astrocytes (49). Arguing against the possibility that this behavior reflects a nonspecific coagulation of GFP molecules on the membrane, such hot spots are rarely observed using phospholipid-dependent membrane-targeted EGFP chimeras that incorporate pleckstrin homology domains using either confocal (50) or TIRF microscopy.2 The present data are also consistent with the findings of Yedovitzky et al. (4) and Ganesan et al. (3) who demonstrated the translocation of PKCα to the plasma membrane of β-cells by immunocytochemistry and biochemical analyses, respectively.

2 T. Tsuboi, Q. Qian, and G. A. Rutter, unpublished observations.
Interestingly, we failed to find any change in the localization of either PKCα or PKCζ in response to glucose or other secretagogue stimuli (Fig. 7). These results contrast with reports of an important role of PKCζ in the regulation of the preproinsulin gene by glucose (51), although it should be emphasized that we did not explore the localization of this isoform beyond rel-}

**Mechanisms Involved in PKCβII Translocation, Role of Ca²⁺ Microdomains**—We provide evidence that the changes in PKCβII distribution are likely to result from localized changes in cytosolic Ca²⁺ concentration generated beneath the plasma membrane during the depolarization-induced opening of L-type Ca²⁺ changes (15). Thus, depolarizing concentrations of KCl (Fig. 3, D and E) or tolbutamide (Fig. 4, C and D) increased [Ca²⁺]ᵢ in this domain ([Ca²⁺]₉₀) to concentrations 1.3–1.5-fold higher than those in the bulk cytosol and caused robust translocation of PKCβII-EGFP to the membrane. However, the partial inhibition of glucose-induced PKCβII translocation by blockade of phospholipase C activity (Fig. 2B, trace 5) suggests that the local generation of DAG, caused by phospholipid hydrolysis, may contribute to the recruitment of conventional PKCs to the membrane in response to glucose. In this regard it should be mentioned that total islet DAG content is reported to increase only slightly (52) if at all (53) at elevated glucose concentrations, largely through de novo synthesis of DAG from glucose-derived palmitate (52). Importantly, such changes are not expected to be blocked by inhibitors of phospholipase C (Fig. 2B). However, arguing that glucose-induced increases in DAG content are small in the MIN6 cell system studied here, we failed to observed any translocation of PKCβ to the cell surface in response to elevated glucose concentrations (Fig. 7, A and B). On the other hand, because PKCα activity is regulated by several long chain acyl-CoA esters (54), a possible role for glucose-induced increase in the concentrations of these latter species (55) in the observed recruitment of PKCβII to the plasma membrane cannot be ruled out.

Our observations (Fig. 6) that cytosolic [Ca²⁺] and PKC membrane localization could be dissociated in the same single cell are perhaps most simply explained by the fact that in the absence of generation of DAG a “threshold” concentration of Ca²⁺, probably ≥ 1 μM, is required to ensure the binding of the C2 domain of PKCβII to membrane phospholipids (44) as previously proposed for PKCα (56). Interestingly, the concentra-tions of Ca²⁺ measured here immediately beneath the membrane of stimulated MIN6 β-cells (2–3 μM) are similar to, if somewhat lower than, those previously reported at greater distances from the plasma membrane (0.5–1.0 μM) of β-cells using diffusible dyes (6–10 μM) (57). Thus, the present data, which were obtained using a molecularly targeted probe, would seem to rule out the notion of a generalized large gradient of Ca²⁺ concentration stretching across the whole interior surface of the cell membrane. However, more localized [Ca²⁺] domains (for example at the mouth of individual Ca²⁺ channels) (58, 59) cannot be excluded. In contrast to the impact of stimulated Ca²⁺ influx, the stimulation of intracellular Ca²⁺ release and DAG production with a muscarinic agonist elicited efficient membrane localization of PKCβII (Fig. 5), presumably reflecting a slightly larger increase in plasma membrane [Ca²⁺] as well as the cooperation of C1 and C2 domains in membrane association (44). Interestingly, this effect of carbachol was significantly accelerated by elevated glucose concentrations (see legend to Fig. 5), possibly reflecting the de novo synthesis of DAG from glucose (52).

**Potential Roles of PKCβII Translocation in Regulated Insu-lin Secretion and Gene Expression**—What may be the consequences of the translocation of PKCβII (and other conventional PKCs) to the plasma membrane? Arguing that the enzyme is at least partly activated upon membrane translocation in β-cells, only kinase-active PKCβII, but not an active site (K371R) mu-

---

**Fig. 6.** Cytosolic relocation of PKCβII precedes the decay in [Ca²⁺] following K⁺–induced membrane depolarization. The time course of [Ca²⁺] elevation following stimulation with 20 mM KCl was monitored using Calcium Crimson in a single cell infected with virus PKCβII-EGFP. Elevation of [Ca²⁺] (408), was reported as a relative increase in Calcium Crimson fluorescence with unstimulated conditions. Plasma membrane localization of PKCβII-EGFP was recorded as a relative increase in the ratio of EGFP fluorescence in the vicinity of the plasma membrane to that of the bulk cytosol. Images were acquired every 2 s. The trace is a single cell representative of five cells from three separate experiments.

**Fig. 7.** Effects of glucose and PMA on the subcellular distribution of PKCβ-EGFP (A, B) and PKCζ-EGFP (C) in MIN6 cells. A, cells expressing PKCβ-EGFP were incubated in KRB containing 3 mM glucose and stimulated with 25 mM glucose for 300 s, prior to addition of 10 μM PMA. Images were collected using the confocal microscope at the start of the incubation, 300 s after the addition of 25 mM glucose, and 300 s after the subsequent addition of PMA. B, time course of changes in fluorescence at the plasma membrane and nuclear membrane in the single cell shown in A. C, cells expressing PKCζ-EGFP were imaged during identical manipulations to those described in A.
PKC Translocation in Pancreatic \( \beta \)-Cells

K. J. Mitchell and G. A. Rutter

37710

PKC Translocation in Pancreatic \( \beta \)-Cells

K. J. Mitchell and G. A. Rutter

37710

PKC Translocation in Pancreatic \( \beta \)-Cells

K. J. Mitchell and G. A. Rutter

37710

PKC Translocation in Pancreatic \( \beta \)-Cells

K. J. Mitchell and G. A. Rutter

37710

PKC Translocation in Pancreatic \( \beta \)-Cells

K. J. Mitchell and G. A. Rutter

37710

PKC Translocation in Pancreatic \( \beta \)-Cells

K. J. Mitchell and G. A. Rutter

37710

PKC Translocation in Pancreatic \( \beta \)-Cells

K. J. Mitchell and G. A. Rutter

37710

PKC Translocation in Pancreatic \( \beta \)-Cells

K. J. Mitchell and G. A. Rutter

37710

PKC Translocation in Pancreatic \( \beta \)-Cells

K. J. Mitchell and G. A. Rutter

37710

PKC Translocation in Pancreatic \( \beta \)-Cells

K. J. Mitchell and G. A. Rutter

37710

PKC Translocation in Pancreatic \( \beta \)-Cells

K. J. Mitchell and G. A. Rutter

37710

PKC Translocation in Pancreatic \( \beta \)-Cells

K. J. Mitchell and G. A. Rutter

37710

PKC Translocation in Pancreatic \( \beta \)-Cells

K. J. Mitchell and G. A. Rutter

37710

PKC Translocation in Pancreatic \( \beta \)-Cells

K. J. Mitchell and G. A. Rutter

37710

PKC Translocation in Pancreatic \( \beta \)-Cells

K. J. Mitchell and G. A. Rutter

37710

PKC Translocation in Pancreatic \( \beta \)-Cells

K. J. Mitchell and G. A. Rutter

37710

PKC Translocation in Pancreatic \( \beta \)-Cells

K. J. Mitchell and G. A. Rutter

37710

PKC Translocation in Pancreatic \( \beta \)-Cells

K. J. Mitchell and G. A. Rutter

37710

PKC Translocation in Pancreatic \( \beta \)-Cells

K. J. Mitchell and G. A. Rutter

37710

PKC Translocation in Pancreatic \( \beta \)-Cells

K. J. Mitchell and G. A. Rutter

37710

PKC Translocation in Pancreatic \( \beta \)-Cells

K. J. Mitchell and G. A. Rutter

37710

PKC Translocation in Pancreatic \( \beta \)-Cells

K. J. Mitchell and G. A. Rutter

37710

PKC Translocation in Pancreatic \( \beta \)-Cells

K. J. Mitchell and G. A. Rutter

37710

PKC Translocation in Pancreatic \( \beta \)-Cells

K. J. Mitchell and G. A. Rutter

37710

PKC Translocation in Pancreatic \( \beta \)-Cells

K. J. Mitchell and G. A. Rutter

37710

PKC Translocation in Pancreatic \( \beta \)-Cells

K. J. Mitchell and G. A. Rutter

37710

PKC Translocation in Pancreatic \( \beta \)-Cells

K. J. Mitchell and G. A. Rutter

37710

PKC Translocation in Pancreatic \( \beta \)-Cells

K. J. Mitchell and G. A. Rutter

37710

PKC Translocation in Pancreatic \( \beta \)-Cells

K. J. Mitchell and G. A. Rutter

37710

PKC Translocation in Pancreatic \( \beta \)-Cells

K. J. Mitchell and G. A. Rutter

37710

PKC Translocation in Pancreatic \( \beta \)-Cells

K. J. Mitchell and G. A. Rutter

37710

PKC Translocation in Pancreatic \( \beta \)-Cells

K. J. Mitchell and G. A. Rutter

37710

PKC Translocation in Pancreatic \( \beta \)-Cells

K. J. Mitchell and G. A. Rutter

37710

PKC Translocation in Pancreatic \( \beta \)-Cells

K. J. Mitchell and G. A. Rutter

37710

PKC Translocation in Pancreatic \( \beta \)-Cells

K. J. Mitchell and G. A. Rutter

37710

PKC Translocation in Pancreatic \( \beta \)-Cells

K. J. Mitchell and G. A. Rutter

37710

PKC Translocation in Pancreatic \( \beta \)-Cells

K. J. Mitchell and G. A. Rutter

37710

PKC Translocation in Pancreatic \( \beta \)-Cells

K. J. Mitchell and G. A. Rutter

37710

PKC Translocation in Pancreatic \( \beta \)-Cells

K. J. Mitchell and G. A. Rutter

37710

PKC Translocation in Pancreatic \( \beta \)-Cells

K. J. Mitchell and G. A. Rutter

37710

PKC Translocation in Pancreatic \( \beta \)-Cells

K. J. Mitchell and G. A. Rutter

37710

PKC Translocation in Pancreatic \( \beta \)-Cells

K. J. Mitchell and G. A. Rutter

37710

PKC Translocation in Pancreatic \( \beta \)-Cells

K. J. Mitchell and G. A. Rutter

37710

PKC Translocation in Pancreatic \( \beta \)-Cells

K. J. Mitchell and G. A. Rutter

37710

PKC Translocation in Pancreatic \( \beta \)-Cells

K. J. Mitchell and G. A. Rutter

37710

PKC Translocation in Pancreatic \( \beta \)-Cells

K. J. Mitchell and G. A. Rutter

37710

PKC Translocation in Pancreatic \( \beta \)-Cells

K. J. Mitchell and G. A. Rutter

37710

PKC Translocation in Pancreatic \( \beta \)-Cells

K. J. Mitchell and G. A. Rutter

37710

PKC Translocation in Pancreatic \( \beta \)-Cells

K. J. Mitchell and G. A. Rutter

37710

PKC Translocation in Pancreatic \( \beta \)-Cells

K. J. Mitchell and G. A. Rutter

37710

PKC Translocation in Pancreatic \( \beta \)-Cells

K. J. Mitchell and G. A. Rutter

37710

PKC Translocation in Pancreatic \( \beta \)-Cells

K. J. Mitchell and G. A. Rutter

37710

PKC Translocation in Pancreatic \( \beta \)-Cells

K. J. Mitchell and G. A. Rutter

37710

PKC Translocation in Pancreatic \( \beta \)-Cells

K. J. Mitchell and G. A. Rutter

37710

PKC Translocation in Pancreatic \( \beta \)-Cells

K. J. Mitchell and G. A. Rutter

37710

PKC Translocation in Pancreatic \( \beta \)-Cells

K. J. Mitchell and G. A. Rutter

37710

PKC Translocation in Pancreatic \( \beta \)-Cells

K. J. Mitchell and G. A. Rutter

37710

PKC Translocation in Pancreatic \( \beta \)-Cells

K. J. Mitchell and G. A. Rutter

37710

PKC Translocation in Pancreatic \( \beta \)-Cells

K. J. Mitchell and G. A. Rutter

37710

PKC Translocation in Pancreatic \( \beta \)-Cells

K. J. Mitchell and G. A. Rutter

37710

PKC Translocation in Pancreatic \( \beta \)-Cells

K. J. Mitchell and G. A. Rutter

37710

PKC Translocation in Pancreatic \( \beta \)-Cells