Glucose-dependent Translocation of Insulin Promoter Factor-1 (IPF-1) between the Nuclear Periphery and the Nucleoplasm of Single MIN6 β-Cells*

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Using laser-scanning confocal microscopy, we have monitored glucose-induced changes in the subcellular localization of insulin promoter factor-1 (IPF-1) labeled with a c-myc epitope tag. This construct trans-activated the insulin promoter in single living MIN6-β-cells as assessed by luciferase-based promoter analysis. IPF-1.c-myc expression also enhanced the response of the insulin promoter to elevations in extracellular glucose concentration. In the majority (148/235, 63%) of cells maintained at low (3 mM) extracellular glucose concentration, IPF-1.c-myc immunoreactivity was confined to the nuclear periphery. Incubation of cells at stimulatory (30 mM) glucose concentrations caused a rapid redistribution of the chimera to the nucleoplasm (775/958, 81% of cells). By contrast, the irrelevant transcription factor c-Fos, tagged with either c-myc or as a chimera with luciferase, was localized exclusively to the nucleoplasm irrespective of the glucose concentration. Furthermore, IPF-1 extended with the bulky (27 kDa) enhanced green fluorescent protein (EGFP) group was confined largely to the nucleoplasm at all glucose concentrations tested and did not support trans-activation of the insulin promoter by glucose. Movement of endogenous IPF-1 from the nuclear periphery to the nucleoplasm may therefore increase the trans-activational capacity of this factor in native β-cells exposed to high extracellular glucose concentrations.

Expression of the insulin gene in islet β-cells is increased at elevated blood glucose concentrations at least in part through the activation of transcription (1). Transcriptional activity is regulated by numerous trans-acting factors acting at multiple recognition sites in the immediate 5′ flanking region of the gene (2) (see Fig. 1). Recent evidence has suggested that insulin promoter factor-1 (IPF-1) (3) (also called PDX-1, IDX-1, STF-1, gene (2) (see Fig. 1). Recent evidence has suggested that insulin promoter factor-1 (IPF-1) between the Nuclear Periphery and the nucleoplasm (5). Second, mutations in the human pdx-1/IPF-1 gene are linked to the development of a form of maturity onset (non-insulin-dependent) diabetes mellitus in the young (MODY4) (6). In addition, a naturally occurring mutant of the pdx-1 gene leads to arrested development of the pancreas (agenesis) in man (7). Finally, other β-cell genes, including those encoding the islet/liver glucose transporter, GLUT2 (8, 9), glucokinase (10), and islet amyloid polypeptide (11) are likely targets for transactivation by IPF-1.

The mechanisms involved in the IPF-1-mediated activation of insulin gene expression by glucose are unclear. For a number of other transcription factors, including the steroid-receptor superfamily (12) and cytokine-activated signal transducers and activators of transcription (STAT) (13), a shift from the cytosol to the nucleoplasm is an important means of activation. To determine whether such a mechanism may be involved in the activation of IPF-1 by glucose, we have expressed epitope-tagged IPF-1 in the glucose-responsive β-cell line MIN6. The localization of the protein was then monitored by immunocytochemistry and laser-scanning confocal microscopy. We show that in the majority of single cells incubated at low glucose concentrations, IPF-1.c-myc is associated with the nuclear periphery and the nuclear membrane region. Incubation of cells at elevated glucose concentrations stimulated the transcriptional activity of an insulin promoter reporter construct monitored in single living MIN6 β-cells and caused a concomitant shift in the localization of IPF-1 into the nucleoplasm. These data implicate translocation of this factor as an important element in the activation of the insulin gene by elevations in blood glucose concentration.

EXPERIMENTAL PROCEDURES

Materials

Monoclonal anti-c-myc antibody 9E10 was the kind gift of Dr. Gerard Evan (Imperial Cancer Research Fund, Lincoln’s Inn, London). Monoclonal anti-nucleoporin antibody was from Babco (Richmond, CA). Rabbit polyclonal anti-firefly luciferase antisemur and primers pRL.CMV, encoding Renilla reniformis luciferase under cytomegalovirus immediate-early gene promoter control, was obtained from Promega (Madison, WI). All other reagents were obtained from Promega or Life Technologies, Inc.

Methods

Preparation of Constructs

IPF-1.c-myc—cDNA encoding full-length (852 nucleotides) murine IPF-1 (3) was amplified using reverse transcription PCR (Ingenius, R & D Systems, Abingdon, UK) with poly(A)+ mRNA isolated from MIN6 β-cells using primer 1, 5′-TTT.AAG.CTT.GAC.GAG.ATG.AAC.ATG.GAG.CAG.CA with HindIII site (underlined) and Kozak sequence (italalic), and primer 2, 5′-T.TTT.GGA.TCC.TCA.CTT.CAG.GTC.CTC.CTC.CGA.CAT.CAG.CTT.CTC.CAT.CAG.CAG.CTT.CTC.CAT.CCC.GGG.TTC.CTG.CGG including BamHI site (underlined), 12 amino acid c-myc epitope tag, and IPF-1 sequence (bold). The amplified 888-nucleotide fragment was restricted and ligated into the mammalian expression vector pcDNA3...
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**Fig. 1.** Maps of plasmid pINS(260–60)-Luc (a) and IPF-1-c-myc (b). Scale bar represents 100 base pairs (b). See Ref. 2 for definition of the cis regulatory elements in the insulin promoter; Th, minimal thymidine kinase promoter; Luc+, humanized firefly luciferase.

(Invitrogen) under cytomegalovirus immediate-early gene promoter control.

**IPF-1.EGFP**—Full-length IPF-1 cDNA was amplified using plasmid IPF-1.C (Invitrogen) as template with primer 1 and primer 3: T.TTT.GGA.TCC.C.TTC.GGA.TGG.CGG.GGT.TCC.TGC.GGT.GCG.AGT (BamHI site underlined), restricted and ligated into plasmid pEGFP.N1 (CLONTECH) encoding enhanced green fluorescent protein with S65T, F64L, and codon-usage mutations.

pINS(260–60).Luc—A 200-nucleotide fragment corresponding to the region −260 to −60 nucleotides upstream of the transcriptional start site of the human insulin gene was released from plasmid pBCTKp200 (14) with HinclI and PvuII and cloned into the Smal site of plasmid pGL3.TK. The latter was based on plasmid pGL3 (Promega) encoding firefly luciferase, modified by removal of the immediate C-terminal peroxisomal targeting sequence (15), and including the herpes simplex virus minimal thymidine kinase promoter (16). Plasmid c-Fos.Luc (17) comprised cDNA encoding a chimera between the entire coding region of human c-Fos (18) and 711 nucleotides of the immediate 5′ flanking region (19). Plasmid cFos.c-myc (18) was kindly supplied by Dr. H. Bading (University of Cambridge). All plasmids were purified on a CsCl density gradient (19).

**Generation of Antibodies**

Polyclonal antibody to murine IPF-1 was raised in rabbits using a 17-amino acid peptide, corresponding to the immediate C terminus of IPF-1.C (CSPQPSSIAPLRPQEPRO) (3) and conjugated to keyhole limpet hemocyanin. Purified IgG fraction was obtained by caprylic acid precipitation and affinity purification on an IPF-1-peptide column and quantitated by Western analysis versus IPF-1 expressed in LipofectAMINE-transfected COS-7 cells. A single band migrating with apparent molecular mass = 43 kDa (as previously observed for IPF-1 (6)) represented >90% of the recognized protein.

**Cell Culture and Transfection**

MIN6 cells (20) (passage 15–25) were cultured on poly-L-lysine-coated (Sigma) coverslips in Dulbecco’s modified Eagle’s medium containing 15% (v/v) fetal bovine serum and either 30 or 3 mM glucose as indicated. CHO.T cells were cultured in Ham’s F-12 medium as described (21). Pressure microinjection was performed as described previously (22, 23) using glass borosilicate capillaries and a total plasmid concentration of 0.3–0.5 mg/ml in 2 ml Tris-HCl, 0.2 mM EDTA. Liposome-mediated transfection was performed using Tfx-50™ (Promega) using 2.5 μg of DNA/35-mm dish and a 3:1 charge ratio of Tx-f-50:DNA in serum-free medium. Cells were transfected by incubation for 2 h at 37 °C with the DNA mixture. Transfection efficiency was typically between 1–5%.

**Photon Counting Digital Imaging**

Photon counting digital imaging was performed using a cooled intensified photon camera (Photek, Lewes, UK) and Olympus IX-70 microscope (× 10, 0.4 numerical aperture objective) as described previously (21, 23). Briefly, firefly luciferase activity was measured by imaging 5 min in the presence of 1 mM luciferin, and the sum of firefly and Renilla luciferases were assessed by a further 5-min imaging period in the presence of 5 μM coelenterazine.

**Immunocytochemistry and Confocal Imaging**

Cells were fixed and permeabilized with 4% (v/v) parafomaldehyde plus 0.2% Triton X-100. Primary antibodies were revealed with fluorophore-conjugated secondary antibodies in 0.2% (v/v) bovine serum albumin. Cells were washed with phosphate-buffered saline between incubations and mounted on coverslips with Mowiol before analysis. Confocal images were captured using a Leica TCS 4D/DM IRBE laser-scanning confocal microscope (× 63/1.32 NA PL-Apo oil-immersion lens)
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TABLE I

Effect of IPF-1.c-myc on the response to elevated glucose concentrations of the insulin promoter (−260 to −60) region in single MIN6 β-cells

| Plasmid/experiment | Glucose | Incubation time | Insulin promoter activity as ratio firefly:Renilla luciferase | Fold effect of 30 mM glucose |
|---------------------|---------|----------------|---------------------------------------------------------------|-----------------------------|
| pcDNA3              |         |                |                                                               |                             |
| 1                   | 3       | 24             | 0.134 ± 0.026                                                | 27                          |
| 2                   | 30      |                | 0.22 ± 0.058                                                 | 25                          |
| 3                   | 3       | 5              | 0.30 ± 0.056                                                 | 19                          |
| 4                   | 30      |                | 0.25 ± 0.08                                                 | 11                          |
| IPF-1.c-myc         |         |                |                                                               |                             |
| 3                   | 3       | 24             | 0.006 ± 0.002                                                | 22                          |
| 4                   | 30      |                | 0.027 ± 0.011*                                              | 19                          |
| 4                   | 3       | 5              | 0.078 ± 0.024                                                | 33                          |
| 4                   | 30      |                | 0.17 ± 0.028**                                              | 28                          |

Cells were maintained for 48 h in medium containing 3 mM glucose before microinjection with pINS(260–60)Luc (0.35 mg/ml), pRL.CMV (0.08 mg/ml), and empty pcDNA3 (experiments 1, 0.15 mg/ml; 2, 0.05 mg/ml) or IPF-1.c-myc (concentrations as experiment 1 and 2, respectively). In experiments 1 and 3, cells were transferred directly after microinjection into medium containing the indicated glucose concentration. In experiments 2 and 4, cells were maintained for 24 h (experiment 2) or 40 h (experiment 4) at 3 mM glucose, before transfer to the indicated glucose concentrations for 5 h. Firefly and R. reniformis luciferase activities were determined by photon counting imaging as described under “Methods.” *, p < 0.05; **, p < 0.01; NS, not significant. Data are representative of 3 further experiments.

The expression of IPF-1 was detected in MIN6 cells by reverse-transcription-PCR (data not shown), and was analyzed off-line using Adobe PhotoshopTM.

In Vivo Confocal Imaging

Cells were incubated in Krebs-Ringer medium comprising (in mM) 125 NaCl, 3.5 KCl, 1.5 CaCl2, 0.5 MgSO4, 0.5 KH2PO4, 2.5 NaHCO3, 3 glucose, 10 Hepes-Na*, pH 7.4, equilibrated with O2:CO2 (95:5 %). Cells were then transferred to the thermostatted (37°C) stage of a Leica DM IRBE inverted optics confocal microscope fitted with a × 40/1.0 NA PL Fluotar oil-immersion objective and controlled by TCS-NT4 software (Leica). Optical sections (0.5 μm) were obtained with 8–16 sweeps of the laser.

Statistical Analysis

Data are presented as means ± S.E. for the number of parameters given in parentheses.

RESULTS

Construction and Expression of IPF-1.c-myc—We first explored the possibility of monitoring the localization of endogenous IPF-1 in MIN6 β-cells by direct immunocytochemistry. An anti-peptide antibody was generated to the immediate C terminus of the protein, a region predicted to show high antigenicity. Analyzed by Western blotting and immunocytochemistry, the immunopurified polyclonal antibody reacted strongly and specifically with IPF-1.c-myc overexpressed in either COS-7 (data not shown) or MIN6 β-cells (see Fig. 3, panels g and j). However, the level of endogenous IPF-1 in single MIN6 cells was below that which could be detected with this antibody.

Because IPF-1 mRNA could readily be detected in MIN6 cells by reverse-transcription-PCR (data not shown), we used this technique to construct and overexpress cDNA encoding c-myc epitope-tagged IPF-1. To confirm that the recombinant expressed chimera retained normal trans-activation capacity, we examined the ability of expressed IPF-1.c-myc to enhance the activity of the proximal promoter region of the human insulin gene (Fig. 1). Photon counting digital imaging of single living MIN6 cells (21, 22) was used to compare the activity of firefly luciferase, expressed under insulin promoter control, to that of the distinct R. reniformis luciferase. The latter, distinct photoprotein, was expressed under cytomegalovirus immediate-early gene (CMV) promoter control. This technique allowed correction for marked differences in the activity of the basal transcriptional/translational machinery between individual cells and cell cultures. Co-microinjection of cDNA encoding IPF-1.c-myc increased the ratio of firefly:R. reniformis luciferases 6.5-fold 24 h after injection, from 0.026 ± 0.003 (n = 31 cells co-microinjected with empty pcDNA3) to 0.17 ± 0.061 (n = 9 cells microinjected with plasmid IPF-1.c-myc, p < 0.001) in MIN6 cells maintained at stimulatory (30 mM) glucose concentrations. Similarly, IPF-1.c-myc expression increased the firefly:R. reniformis luciferase ratio from 0.037 ± 0.016 (n = 28 cells) to 0.086 ± 0.024 (n = 21) in CHO.T cells, confirming the transactivational activity of the recombinant factor in the absence of other β-cell proteins.

Overexpression of IPF-1.c-myc increased the response of the insulin promoter to culture of MIN6 cells at high versus low glucose concentrations (Fig. 2, Table I). Thus, the response of the insulin promoter to an increase in glucose concentration from 3 to 30 mM was small or zero in cells microinjected with empty pcDNA3 plasmid (Fig. 2, a–d; Table I). By contrast, in cells co-microinjected with IPF-1.c-myc, elevations of extracellular glucose to 30 mM increased markedly the ratio of firefly: Renilla luciferase (Fig. 2, e–h; Table I), indicating insulin promoter activation. This effect of glucose was rapid, with a similar -fold activation observed after incubation for 5 or 24 h at elevated glucose concentrations (Table I, experiments 3 and 4).

Effect of Glucose Concentration on the Subcellular Localization of IPF-1.c-myc—Overexpressed IPF-1.c-myc could readily be visualized in fixed and permeabilized MIN6 cells, using either anti-c-myc antibodies (Figs. 3 and 4), or IgG derived from polyclonal anti-mouse IPF-1 antiserum (Fig. 3, images g and j). In MIN6 cells previously cultured 24 h at 3 mM glucose, IPF-1.c-myc was localized in the majority of cells to the nuclear periphery (Table II; Fig. 3, a, g, and m). In the remaining cells, the construct was localized to both the nuclear periphery and the nucleoplasm, with exclusion from the nucleosomes (data not shown). No appreciable IPF-1.c-myc immunoreactivity was detectable in the cell cytosol.

In cells maintained at 3 mM glucose, the stained region was shown to correspond to the nuclear periphery by co-labeling of the nucleoplasm with a DNA-binding stain (Fig. 3, a–f), and by colocalization with antibodies to the nuclear pore protein, nucleoporin (Fig. 3, g–l). By contrast, cells incubated for the same period in the presence of 30 mM glucose displayed largely nucleoplasmic localization of IPF-1 (Fig. 3, d, j, and p). However, the subcellular localization of a chimera between c-Fos and firefly luciferase (c-Fos.luc) was unaffected by glucose in cells where clear redistribution of IPF-1.c-myc was apparent (Fig. 3, m–r).

We next analyzed the ability of the IPF-1.c-myc chimera to translocate from the nuclear periphery to the nucleoplasm over
the time-frame of the gene expression experiments. We performed quantitative confocal digital image analysis to determine the extent of the shift of IPF-1.c-myc elicited by a 60-min incubation at high glucose concentrations (Fig. 4). In three cells cultured at 3 mM glucose and analyzed as in Fig. 4, the mean ratio of nucleoplasmic:peripheral fluorescence was $0.32 \pm 0.09$. This ratio was increased in cells maintained in the presence of 30 mM glucose to $1.22 \pm 0.35$ ($n = 4$ cells). The shift from the nuclear membrane to the nucleoplasm was assessed in large populations exposed to elevated glucose for 60 min, 120 min, or 24 h (Table II). The distribution of the chimera was scored as predominantly either peripheral to the nucleus or nucleoplasmic. Incubation at 30 mM glucose caused a dramatic increase in the proportion of cells displaying predominantly nucleoplasmic localization (Table II). This was not associated with any overall increase in the level of expression of IPF-1.c-myc at the higher glucose concentration. No correlation was observed between overall fluorescence intensity and subcellular distribution at either glucose concentration examined (data not shown).

By contrast to the apparent shift of IPF-1.c-myc, no significant change in the localization of c-Fos.c-myc was apparent (Table II) as also observed with a c-Fos.luciferase chimera (Fig. 3). With each of these chimeras, predominantly nucleoplasmic localization was observed at either 3 or 30 mM glucose. These data indicate that the c-myc epitope tag of IPF-1.c-myc was not responsible for the localization of the protein at the nuclear.

**FIG. 3.** Effect of glucose concentration on intracellular distribution of IPF-1.c-myc. MIN6 cells were incubated for 24 h in medium containing 3 mM glucose before transfection with IPF-1.c-myc and other plasmids as indicated (see "Methods"). Cells were then cultured for a further 48 h in the presence of the indicated glucose concentrations (mM). After fixation and permeabilization, cells were probed with antibodies as follows. a–f, IPF-1.c-myc was probed with mouse monoclonal anti-c-myc (9E:10; 1:200) and revealed with fluorescein isothiocyanate (FITC)-conjugated anti-mouse IgG (1:500); images a and d). The nuclear region was revealed by co-staining with a nuclear Hoechst DNA stain (1.5 µg/ml; images b and e). Images a and b, and d and e, are superimposed in panels c and f, respectively. g–l, IPF-1.c-myc was probed with polyclonal anti-IPF-1 IgG (1:50) and revealed with tetramethylrhodamine isothiocyanate-conjugated anti-rabbit IgG (1:500; images g and j). No staining of endogenous IPF-1 was apparent in untransfected cells using this antibody (see "Results"). Nuclear pore complex proteins were probed with mouse monoclonal anti-nucleoporin antibody (1:1,000) and revealed with FITC-conjugated anti-mouse IgG (images h and k). Images g and h, and j and k, are superimposed in panels i and l, respectively. m–r, cells were cotransfected with c-Fos.luc and probed with antibody 9E:10 (revealed with FITC-conjugated secondary antibody; images m and p) and with polyclonal anti-luciferase antibody (revealed with tetramethylrhodamine isothiocyanate-conjugated anti-rabbit IgG; images n and q). Images o and r show the superimposition of images m and n, and images p and q, respectively.
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TABLE II

Effect of glucose on subcellular distribution of IPF-1.c-myc

| Plasmid/incubation time | Number of cells showing predominantly peripheral or nucleoplasmic localization of the c-myc epitope tag at the indicated glucose concentration in % |
|-------------------------|---------------------------------------------------------------------------------------------------------------|
|                         | 3.0                                                                                                           | 30.0                                                                                       |
| IPF-1.c-myc             |                                                                                                               |                                                                                           |
| (a) 48 h                | Nuclear periphery: 148, Peripheral: 87, 63%                                                                 | Nuclear periphery: 183, Peripheral: 775, 19.1%                                              |
|                         | (b) 60 min.                                                                                                   |                                                                                           |
|                         | Nuclear periphery: 55, Peripheral: 195, 20.9%                                                               |                                                                                           |
|                         | (c) 120 min.                                                                                                  |                                                                                           |
|                         | Nuclear periphery: 51, Peripheral: 244, 17.2%                                                               |                                                                                           |
| c-Fos.c-myc             |                                                                                                               |                                                                                           |
| (d) 24 h                | Nuclear periphery: 1, Peripheral: 35, 2.7%                                                                    |                                                                                           |
|                         |                                                                                                               |                                                                                           |

DISCUSSION

We describe here the construction and expression in MIN6 cells of an IPF-1.c-myc chimera. The recombinant factor was transcriptionally active and enhanced the responsiveness of the proximal regulatory region of the human insulin promoter to increases in extracellular glucose concentration. In cells maintained at low glucose concentrations, IPF-1.c-myc was localized to an area of the nucleoplasm immediately beneath the nuclear membrane. Incubation at elevated glucose concentrations caused a rapid redistribution of IPF-1.c-myc to the nucleoplasm. This translocation was not the result of artifacts caused by the overexpression of myc-tagged IPF-1, there was no correlation between the level of IPF-1 expression and the distribution of the chimera between the nuclear periphery and the nucleoplasm. Furthermore, we did not observe any translocation of two other nuclear targeted proteins (c-myc-tagged c-Fos, Table II, and a c-Fos.firefly luciferase chimera, Fig. 2, m–r). The latter observation would appear also to exclude the possibility that fixation/permeabilization artifacts could be responsible for the apparent localization of the chimera at the nuclear periphery.

What may be the physical basis of the distribution of IPF-1.c-myc at the nuclear periphery? IPF-1.c-myc was not localized exclusively to the nuclear membrane nor did the chimera perfectly colocalize in the region presumed to be the membrane, with the nuclear pore complex protein, nucleoporin. Instead, there was a steep gradient of IPF-1.c-myc from the nuclear membrane leading into the center of the nucleoplasm (e.g., see Fig. 3, g and i). Similarly, in a minority of cells expressing IPF-1.EGFP, and maintained at 3 mM (but not 30 mM) glucose, fluorescence was observed at the rim of the nucleus. This fluorescence was largely immobile, as monitored by time-lapse confocal microscopy over > 30 min (data not shown). Together, these data suggest that in cells exposed to 3 mM glucose, endogenous IPF-1 may be tethered to immobile anchors immediately beneath the plasma membrane. The nature of these tetherers is unclear, however, although it might be speculated that needle-like microfilaments/microtubules may be involved. The markedly reduced appearance of EGFP-extended IPF-1 at the nuclear periphery, and the failure of the chimera to respond to elevated glucose concentrations, may indicate that the bulky (27 kDa) EGFP moiety (24) disrupts an interaction between IPF-1 and the usual tethering site near the nuclear membrane.

Recent data have suggested that other transcription factors may also be associated with the nuclear periphery under certain circumstances, via specific interactions with nuclear membrane proteins. For example, the transcription factor Sp1 (25) has been shown by yeast 2-hybrid analysis to interact strongly with the nuclear pore protein p62. Of particular relevance to the current work, Sp1 may be involved in the regulation by extracellular glucose of the fatty acid synthase promoter (26). Furthermore, close inspection of the data of Bonny et al. (27) (Fig. 3E) suggests that the β-cell-specific JIP homologue IB1 may also be localized to the nuclear periphery. Like IPF-1, the latter transcription factor is also implicated in the regulation of the glucose-sensitive GLUT-2 gene in β-cells (27), suggesting that localization to the nuclear periphery may be a general phenomenon for glucose-sensitive transcription factors.

The mechanisms responsible for eliciting the shift in IPF-1 are unknown. However, previous studies (28) have suggested that changes in the DNA binding activity of IPF-1 by glucose may involve changes in the phosphorylation state of the protein. Phosphorylation of IPF-1 on one or more sites would appear a plausible means of altering the interaction between this factor and its putative tethering molecule.

What may be the role for the placement of IPF-1 at the nuclear periphery? In unstimulated cells, incubated at low glucose concentrations, this would be expected to reduce the concentration of the transcription factor in the nucleoplasm where it is able to interact with the promoter regions of insulin (and other gene(s)). Activation of the insulin, GLUT2, and glucokinase promoters might therefore be expected under conditions where there was a shift of IPF-1 to the nucleoplasm. This was indeed observed with an insulin promoter-reporter construct in single living MIN6 cells after exposure to elevated glucose concentrations. In support of the view that the translocation to the nucleoplasm may be important for the activation of the insulin gene by glucose, we failed to observe any stimulation in cells overexpressing an IPF-1-EGFP chimera. In the majority of cells examined, this chimera failed to translocate between the nuclear periphery and the nucleoplasm. Furthermore, movement of IPF-1.c-myc between the nuclear periphery and the nucleoplasm was not observed in another β-cell line, INS-1 (data not shown), where glucose has little or no effect on insulin gene expression (data not shown and Ref. 29). In addition to a role in transcriptional regulation, it might also be speculated that the localization of IPF-1 at the nuclear periphery plays a role in controlling the flow of traffic across the nuclear membrane. For example, this may inhibit the exit from the nucleus of insulin and other mRNAs at low glucose concentrations.
In conclusion, translocation of IPF-1 from the nuclear periphery to the nucleoplasm may be an important mechanism for the activation of the insulin and other promoters in response to elevations in extracellular glucose. Defective translocation of IPF-1 may therefore represent a potential defect in non-insulin-dependent diabetes mellitus.

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**FIG. 4. Quantitation of IPF-1.c-myc localization.** Cells were incubated in 3 mM glucose for 24 h prior to transfection with IPF-1.c-myc. The cell represented in panels a, c, and e was then incubated for 48 h in 3 mM glucose and that shown in panels b, d, and f for 48 h in 3 mM glucose followed by 60 min in the presence of 30 mM glucose. Each cell was immunostained with anti-c-myc antibody 9E:10 revealed with FITC-conjugated antimouse IgG (see legend to Fig. 3) before confocal analysis (see “Methods”). Pseudocolor (blue, low; white, high) and three-dimensional images were generated off-line using Hamamatsu Argus-50 software (bar = 1 μm). e and f, fluorescence intensity corresponding to the single line across the center of the images shown in panels a and b was generated using NIHimage (freeware from ftp://zippy.nih.gov/pub/image/) and imported into Excel (Microsoft) for quantitation. The extent of nucleoplasmic versus peripheral localization was calculated by summing the total intensity in the region at the center of the nucleus (area B) and dividing by the sum of the intensities in the region of the nuclear periphery (areas A + C), as apparent in cells incubated at 3 mM glucose (panel e). Calculated ratios for the cells shown were 0.17 (panel a) and 1.98 (panel b). Mean data for further analyzed cells are given under “Results.” Scale bar, 1 μm.
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