FEBS Letters 456 (1999) 332–338 FEBS 22420

Glucokinase regulatory protein is essential for the proper subcellular localisation of liver glucokinase

Núria de la Iglesia[a], Maria Veiga-da-Cunha[b], Emile Van Schaftingen[b], Joan J. Guinovart[a], Juan C. Ferrer[a,*]

[a] *Departament de Bioquímica i Biologia Molecular, Universitat de Barcelona, Martí i Franquès, 1, E-08028 Barcelona, Spain*
[b] *Laboratory of Physiological Chemistry, Christian de Duve Institute of Cellular Pathology and Université Catholique de Louvain, B-1200 Brussels, Belgium*

Received in revised form 24 June 1999

Abstract Glucokinase (GK), a key enzyme in the glucose homeostatic responses of the liver, changes its intracellular localisation depending on the metabolic status of the cell. Rat liver GK and *Xenopus laevis* GK, fused to the green fluorescent protein (GFP), concentrated in the nucleus of cultured rat hepatocytes at low glucose and translocated to the cytoplasm at high glucose. Three mutant forms of *Xenopus* GK with reduced affinity for GK regulatory protein (GKRP) did not concentrate in the hepatocyte nuclei, even at low glucose. In COS-1 and HeLa cells, a blue fluorescent protein (BFP)-tagged version of rat liver GK was only able to accumulate in the nucleus when it was co-expressed with GKRP-GFP. At low glucose, both proteins concentrated in the nuclear compartment and at high glucose, BFP-GK translocated to the cytosol while GKRP-GFP remained in the nucleus. These findings indicate that the presence of and binding to GKRP are necessary and sufficient for the proper intracellular localisation of GK and directly involve GKRP in the control of the GK subcellular distribution.

Key words: Glucokinase regulatory protein; Protein-protein interaction; Fluorescence microscopy

1. Introduction

Glucokinase (GK), one of the enzymes that catalyse glucose phosphorylation in liver and in pancreatic α- and β-cells, is a key piece of the glucose sensing machinery in mammals [1,2]. It plays a fundamental role in the whole body glucose homeostasis and mutations of the GK gene are associated with MODY 2, a form of maturity-onset diabetes of the young [3].

GK, a monomeric 50 kDa protein, exhibits kinetic properties quite different from those of the other members of the mammalian hexokinase family. It is not inhibited by its product, glucose 6-phosphate, and possesses a sigmoidal saturation curve and a relatively low affinity for glucose (Hill coefficient = 1.5–1.8, $S_{0.5}$ = 2–8 mM, depending on the species), so that the flux through GK is sensitively modulated by fluctuations in the concentration of its substrate in the physiological range [1]. In the liver, the activity of GK is also modulated by a regulatory protein (GKRP) that binds and inhibits GK competitively with respect to glucose. The inhibitory effect of GKRP is enhanced by fructose 6-phosphate and suppressed by fructose 1-phosphate, both of which bind to GKRP and modify its affinity for GK [4]. This 68 kDa protein is only found in the livers of species that express GK and although there is some evidence for its presence in pancreatic tissue [5,6], a direct demonstration is still not available.

In in vitro assays, rat GKRP can inhibit human pancreatic GK, which shows a high degree of identity to the rat liver isoform [7], as well as *Xenopus laevis* liver GK [8], a more distantly related protein. This suggests that the residues involved in the binding to GKRP are conserved among the members of the GK family. Through the analysis of several mutated forms of *Xenopus* GK and human β-cell GK, the domains involved in this interaction have been further delineated [7,8].

Experiments with digitonin-permeabilised hepatocytes first showed that fructose or elevated concentrations of glucose cause GK, but not GKRP, to translocate from a Mg^{2+}-dependent binding site to a site where it is more easily released by the detergent [9,10]. Immunostaining of GK and GKRP in perfused rat livers [11,12] and in rat cultured hepatocytes [13], it revealed a nuclear localisation of both proteins in the presence of low concentrations of glucose. Rat GK was reported to translocate to the cytoplasm upon incubation with fructose [13] or high concentrations of glucose [11,13]. However, there is still controversy about the behaviour of GKRP, since it has been reported to remain in the nucleus [13] or to accompany GK in its movement [12].

In the present study, using the green fluorescent protein (GFP) and a blue variant (BFP) as tags for localisation of GKRP and wild-type and mutated forms of GK, we show that the relative affinity of GK for GKRP is an absolute determinant of the nuclear localisation of GK. Furthermore, by the co-expression of rat liver GK and GKRP, we have been able to engineer a glucose sensing device in two cell lines unrelated to hepatocytes, COS-1 and HeLa, making that the intracellular distribution of GK in these cells is controlled by the levels of glucose.

2. Materials and methods

2.1. Plasmid construction

Standard molecular cloning techniques were used throughout. The full coding sequence of rat GK was amplified from rat liver mRNA by PCR using the Superscript One-Step RT-PCR System (Gibco BRL) and the two oligonucleotides TCTCTACTTCCCCAACGACCCC (sense), corresponding to nucleotides 28–49 of the 5′ untranslated region of the rat liver GK cDNA (GeneBank number J04218), and TTTGTGGTGTGTGGAGTCCCC (antisense), complementary to nucleotides 1501–1521. It was then re-amplified with Pfu DNA polymerase following the supplier's instructions and using the sense primer (Boehringer Mannheim) TACGTACCCCATGGCTATGGATACTA-

*Corresponding author. Fax: (34) (93) 4021219.
E-mail: ferrer@sun.bq.ub.es

Abbreviations: GK, glucokinase; GKRP, glucokinase regulatory protein; GFP, green fluorescent protein; BFP, blue fluorescent protein

PII: S0014-5793(99)00971-0

CAAG, which contains a *Nco*I site (underlined) at the start codon, and the antisense primer TAGCTACGTCGACCTGGATTTCACTGGGCC, which introduces a *Sal*I site (underlined) and is complementary to nucleotides 1456–1475. The fragment obtained was cloned into the blunt-ended and dephosphorylated plasmid pUC18 *Sma*I/BAP using the SureClone Ligation kit (Pharmacia Biotech). The resulting plasmid was digested with *Nco*I and blunt-ended with the Klenow fragment of *Escherichia coli* DNA polymerase I, followed by digestion with *Sal*I. The fragment was ligated into pEGFP-C1 (Clontech) which previously had been digested with *Bgl*II, Klenow-filled and digested with *Sal*I. This ensured the in-frame fusion of rat liver GK at the C-terminus of the GFP coding sequence plus a linker of five amino acids under the control of the constitutive immediate early promoter of the human cytomegalovirus.

The full coding sequence of rat liver GKRP was amplified from the pBluescript/GKRP plasmid [14] by PCR using Pfu DNA polymerase and the oligonucleotides GAAGATCTCGCCACCATGCCAGGCACCAAACGATATCAGC (sense) and GCAGGATCCAATTCAGGGTCCCACAGGCGGG (antisense). The sense oligonucleotide introduces the *Bgl*II restriction site at its 5′ end followed by a Kozak consensus translation initiation site, to further increase the translation efficiency in eukaryotic cells, and nucleotides 22–46 of the rat GKRP cDNA (GeneBank number X68497). The antisense oligonucleotide, which is complementary to nucleotides 1882–1904, was designed to eliminate the GKRP stop codon and to introduce a *Bam*HI restriction site at its 3′ end. The PCR product was digested with *Bam*HI, Klenow-filled, digested with *Bgl*II and then ligated into pEGFP-N1 (Clontech), which had previously been digested with *Sal*I, Klenow-filled and digested with *Bgl*II. This ensured the in-frame fusion of GFP to the C-terminus of the GKRP coding sequence plus a linker of 15 amino acids.

A blue variant of pEGFP-C1/rat GK was obtained by introducing the mutations Y67H and Y146F in the GFP coding region [15], using the oligonucleotides CGTGACCACCCTGACCCATGGCGTGCAGTGCTTCAGC (sense) and GCTGAAGCACTGCACGCCATGGGTCAGGGTGGTCACG (antisense) and GCTGGAGTACAACTTTAACAGCCACAACG (sense) and CGTTGTGGCTGTTAAAGTTGTACTCCAGC (antisense), respectively, and the QuickChange site-directed mutagenesis kit (Stratagene).

The mutants of *Xenopus* liver GK, constructed as previously described, were cloned into the pEGFP-C1 plasmid as follows. The pET-XGK-A, pET-XGK-C and pET-XGK-A-C plasmids [8] were digested with *Nde*I, blunt-ended and digested with *Bam*HI. The mutant *Xenopus* GK cDNAs were ligated into pEGFP-C1, which had previously been digested with *Bgl*II, Klenow-filled and digested with *Bam*HI.

The final plasmids were purified by ion-exchange chromatography (Qiagen) and finally dissolved in 10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0. DNA encoding the fusion proteins was sequenced using the ABI-PRISM DNA sequencing kit and the ABI-PRISM 377 automatic DNA sequencer (Perkin Elmer Applied Biosystems) to rule out PCR errors.

2.2. Cell culture and transfection

COS-1 and HeLa cells were grown in DMEM supplemented with 25 mM glucose, 10% foetal bovine serum (FBS) and penicillin/streptomycin. Liposome-mediated transfection of cells was performed at 70–80% confluence, using 4 μg of Clonfectin (Clontech) and 4 μg of plasmid DNA per 35 mm dish, following the manufacturer's instructions. After transfection (3–4 h) at 37°C in humidified 5% CO_2:95% air, cells were washed in phosphate-buffered saline (PBS) and incubated in DMEM containing 25 mM glucose and 10% FBS. Prior to the experiments, performed 48 h after transfection, cells were preincubated overnight in DMEM containing 20% FBS, 17.5 mM glucose and 1 μg/ml insulin.

Hepatocytes were isolated from 24 h starved male Wistar rats (Interfauna) by collagenase perfusion as described [16]. Cells were resuspended in DMEM supplemented with 10 mM glucose, 10% FBS, 100 nM insulin (Sigma), 100 nM dexamethasone (Sigma) and penicillin/streptomycin and seeded (6×10^4 cells/cm^2) onto gelatine-coated glass coverslips placed on tissue culture plates. After 4 h at 37°C in humidified 5% CO_2:95% air, unattached cells were removed by washing with PBS and transfection was performed as described above. After transfection, cells were washed in PBS and incubated overnight in DMEM containing 20% FBS, 17.5 mM glucose and 1 μg/ml insulin.

On the day of the experiment, cells were washed in PBS and incubated in plain DMEM with 5.5 mM glucose for 2 h (controls), followed by a 2 h incubation with plain DMEM with 30 mM glucose. At the end of the incubations, cells were washed twice with PBS, fixed for 20 min in PBS containing 4% paraformaldehyde and were washed several times with PBS.

2.3. Immunofluorescence analyses

Following fixation, cells were incubated with $NaBH_4$ (1 mg/ml), permeabilised with 0.2% (v/v) Triton X-100 in PBS and blocked in 3% bovine serum albumin-PBS (w/v). The cells were then incubated with antibodies directed against rat liver GK [17] or GKRP [18] for 45 min at room temperature, washed in PBS and treated with a fluorescein isothiocyanate (FITC)-conjugated anti-sheep or anti-rabbit immunoglobulin (Chemicon and Molecular Probes, respectively) for 30 min. Finally, cells were washed in PBS. Controls included examination of cultures for autofluorescence and specificity of antibodies, after incubating the fixed cells with only the labelled secondary antibody.

For nuclei staining in red, coverslips were treated with 1 μg/ml RNAse (DNAse free) in PBS for 30 min and with 0.2 μg/ml propidium iodide for 10 min, followed by washing in PBS. Finally, coverslips were air-dried and mounted on glass microscope slides in Immuno Fluore Mounting Medium (ICN).

2.4. Confocal and epifluorescence microscopy

Confocal fluorescent images were obtained with a Leica TCS 4D (Leica Lasertechnik, Heidelberg, Germany) confocal scanning laser microscope adapted to an inverted Leitz DMIRBE microscope and 63×/1.4 oil Plan-Apo objective. The light source was an argon/krypton laser (75 mW). Green fluorescence (from GFP recombinants or the FITC-conjugated secondary antibodies) and red fluorescence (from Texas red-conjugated secondary antibodies) were excited at 488 and 568 nm, respectively, with the laser. Optical sections (0.5 μm) were obtained.

Epifluorescent images were taken with a cooled CCD Micromax camera, format RTE 782-Y, adapted to a LEICA DMRB fluorescence microscope and a 63×/1.3 oil Plan-Apo objective. Blue fluorescence from BFP recombinants was excited with an UV filter set (excitation filter of 340/80 nm and emission filter of 430 nm), green fluorescence from GFP recombinants and FITC-conjugated secondary antibodies with a blue filter set (excitation filter of 450/90 nm and emission filter of 515/60 nm) and red fluorescence from Texas red-conjugated secondary antibodies with a green filter set (excitation filter of 515/60 nm and emission filter of 580 nm). To analyse the subcellular localisation of the different fusion proteins, a minimum of 50 transfected cells were counted for each construct and each culture condition. The quantitative measurements of fluorescence intensity in a pre-defined region were performed with Metamorph Imaging System software, version 3.5 for Microsoft Windows 95 (Universal Imaging Corporation), and were expressed as a percentage of the total fluorescence of the cell.

3. Results

3.1. GFP tagging does not affect GK or GKRP localisation

To ensure that the fusion proteins behaved like the endogenous unmodified proteins, cultured rat hepatocytes were transiently transfected with the plasmids encoding GFP (pEGFP-C1), GFP- and BFP fusions of wild-type rat liver GK (pEGFP-C1/rat GK and pBFP-C1/rat GK) and GFP fusion of rat GKRP (pEGFP-N1/GKRP). As it has been observed in other cell types, overexpressed GFP uniformly stained both the nucleus and the cytoplasm of transfected cells, although the former was slightly brighter. This pattern was not modified upon changes in the concentration of glucose (data not shown).

Hepatocytes expressing the GFP-GK or BFP-GK fusion proteins showed a clear nuclear concentration of the fluorescence at 5.5 mM glucose (control conditions). When the medium contained 30 mM glucose, there was a diffuse staining of the cytoplasm and nucleus (Fig. 1a). As a control, non-trans-

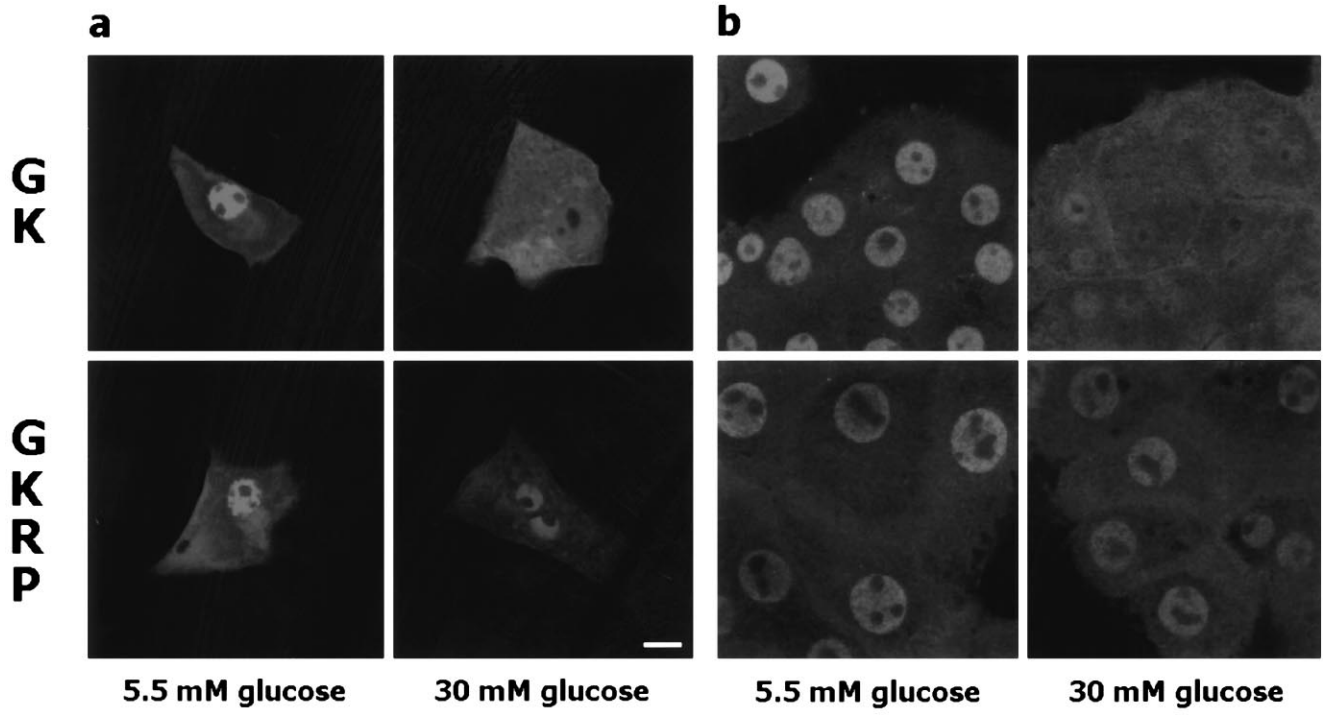

Fig. 1. Confocal images of cultured rat hepatocytes. (a) Intracellular localisation of the GFP-rat liver GK and rat GKRP-GFP fusion proteins at low and high concentrations of glucose. BFP-rat liver GK displayed the same pattern as GFP-rat liver GK at the two concentrations of glucose used (not shown). (b) Immunostaining of endogenous rat liver GK and GKRP at low and high concentrations of glucose. Scale bar, 10 μm.

fected hepatocytes isolated and seeded the same day were immunostained for GK. Endogenous GK responded to a rise in the glucose concentration (Fig. 1b), as it has already been described [13], displaying the same pattern as the overexpressed GFP-GK and BFP-GK (not shown). It must be noted that although GK clearly leaves the nucleus, there is always some residual nuclear staining which results in a diffuse fluorescence of the whole cell.

Although there always was some faint cytosolic staining, the green fluorescence from GKRP-GFP concentrated in the nucleus of transfected hepatocytes, irrespective of the glucose concentration (Fig. 1a). Immunostaining of non-transfected hepatocytes also revealed a nuclear concentration of endogenous liver GKRP, at both low and high glucose concentrations (Fig. 1b). These results agree with the findings of Brown et al. [13], but are at variance with those of Toyoda and coworkers [12] and show that tagging with GFP or BFP does not interfere in GK or GKRP localisation.

3.2. Subcellular localisation of GK mutants with a lowered affinity for GKRP

The residues involved in the catalytic activity and binding to GKRP are assumed to be within the conserved region of different GKs [8], since these functions seem to be shared by all animal GKs [19]. It was then of interest to study the behaviour of *X. laevis* liver GK, which is relatively distant from mammalian GKs. *Xenopus* GK is inhibited by rat GKRP, although the concentration of purified rat GKRP necessary to decrease its activity to the same extent as purified *Xenopus* GKRP is slightly higher (5-fold) [8]. Several *Xenopus* GK mutants have previously been constructed by replacing GK-specific residues with the equivalent ones in the C-terminal half of rat hexokinase I. In an in vitro assay, some of these mutants showed a kinetic $S_{0.5}$ value for glucose similar to that of the wild-type protein, but had a lowered affinity for both *Xenopus* and rat GKRP. Thus, mutant A (E51S, E52K) and mutant C (H141G, K142P, K143R, L144M) displayed respectively a 9- and 40-fold reduction of their affinity for rat GKRP, while the double mutant A/C was completely insensitive to the highest amount of GKRP tested [8].

Cultured rat hepatocytes were transfected with plasmids encoding GFP fused to wild-type *Xenopus* GK and to mutants A, C and A/C. GFP-*Xenopus* GK concentrated in the nucleus when the cells were incubated in the presence of 5.5 mM glucose and translocated to the cytoplasm upon incubation with 30 mM glucose, like the rat enzyme (Fig. 2, upper panels). However, the nuclear concentration of *Xenopus* GK at control conditions was apparent only in ~80% of the cells. Approximately 20% of the transfected hepatocytes displayed a diffuse staining of the nucleus and the cytoplasm, indicating that in some cells, the overexpressed protein was not completely sequestered inside the nucleus. Moreover, the staining pattern at high levels of glucose was also slightly different between the rat and the *Xenopus* wild-type fusion proteins. GFP-*Xenopus* GK displayed a diffuse staining of the cytoplasm and the nucleus in ~70% of the cells, but stained only the cytosol in ~30% of the transfected hepatocytes. As mentioned above, the rat enzyme was never found to leave the nucleus completely at high glucose concentrations. The slightly lower affinity of *Xenopus* GK for rat GKRP and its lower affinity for glucose [8] could probably account for these differences.

GFP-*Xenopus* GK mutants with a decreased affinity for GKRP concentrated much less in the nucleus under control

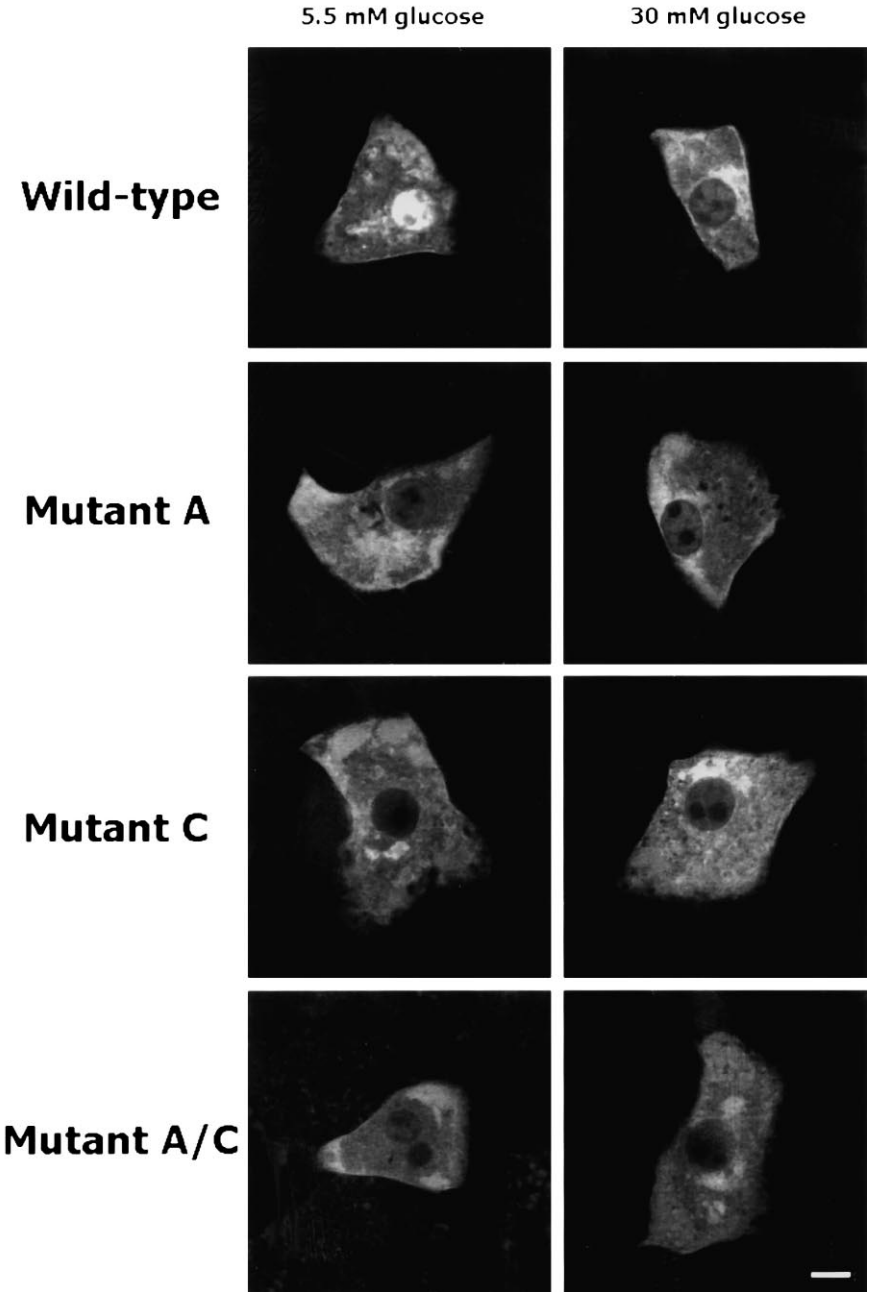

Fig. 2. Confocal images of cultured rat hepatocytes expressing GFP fused to *Xenopus* wild-type GK and to *Xenopus* GK mutants A, C and A/C, with a reduced affinity for rat GKRP, at low and high glucose concentrations. Scale bar, 10 μm.

conditions. The level of mistargeting varied with the mutant: the lower the in vitro affinity for rat GKRP, the larger the percentage of cells showing a cytoplasmic localisation of the GFP-*Xenopus* GK construct. Mutants A, C and A/C did not concentrate in the nucleus in ~65, ~75 and ~90% of the cells, respectively, and their intracellular distribution did not change upon incubation with high glucose concentrations (Fig. 2). These results suggest that the binding of GK to GKRP at low glucose concentrations is responsible for its nuclear localisation.

3.3. *Co-expression of BFP-GK and GKRP-GFP in other cell types*

To further characterise the mechanism of GK translocation, we analysed the behaviour of the two proteins in non-hepatic cells. Two established cell lines that derive from non-hepatic tissues, COS-1 and HeLa cells, were transiently transfected with plasmids encoding BFP-rat GK or GKRP-GFP fusion proteins and were immunostained with antibodies directed against rat liver GK and GKRP. Only cells expressing BFP-GK were immunostained with the anti-GK antibody and positive staining with the anti-GKRP antibody was only found in cells also displaying green fluorescence from the GKRP-GFP fusion protein (data not shown). This indicates that COS-1 or HeLa cells do not endogenously express GK or GKRP.

In a control experiment, GFP and BFP were overexpressed in COS-1 and HeLa cells. After fixation, the cells showed a diffuse labelling of the cytosol and the nucleus, with a slight

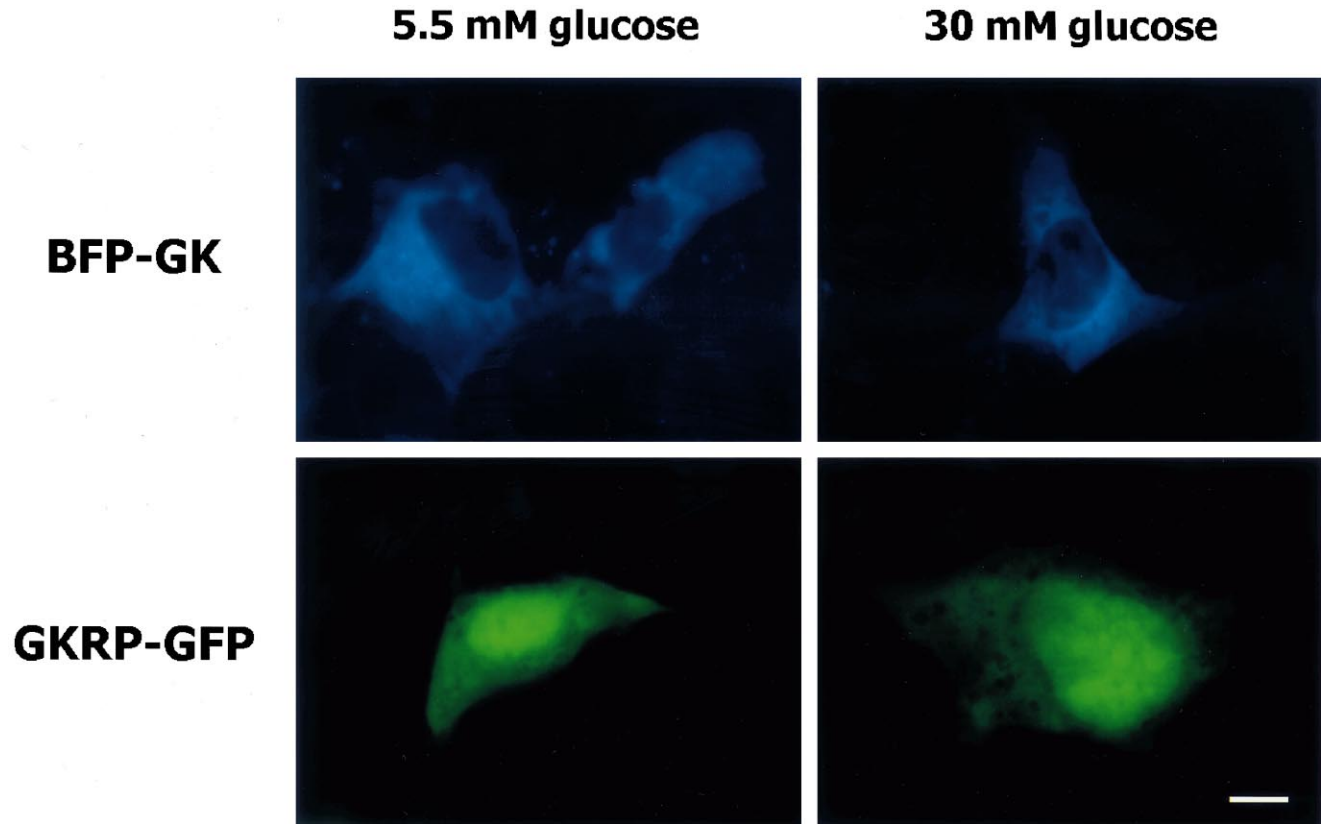

Fig. 3. Epifluorescence images of COS-1 cells expressing BFP-rat liver GK or GFP-rat GKRP, at low and high glucose concentrations. Scale bar, 20 μm.

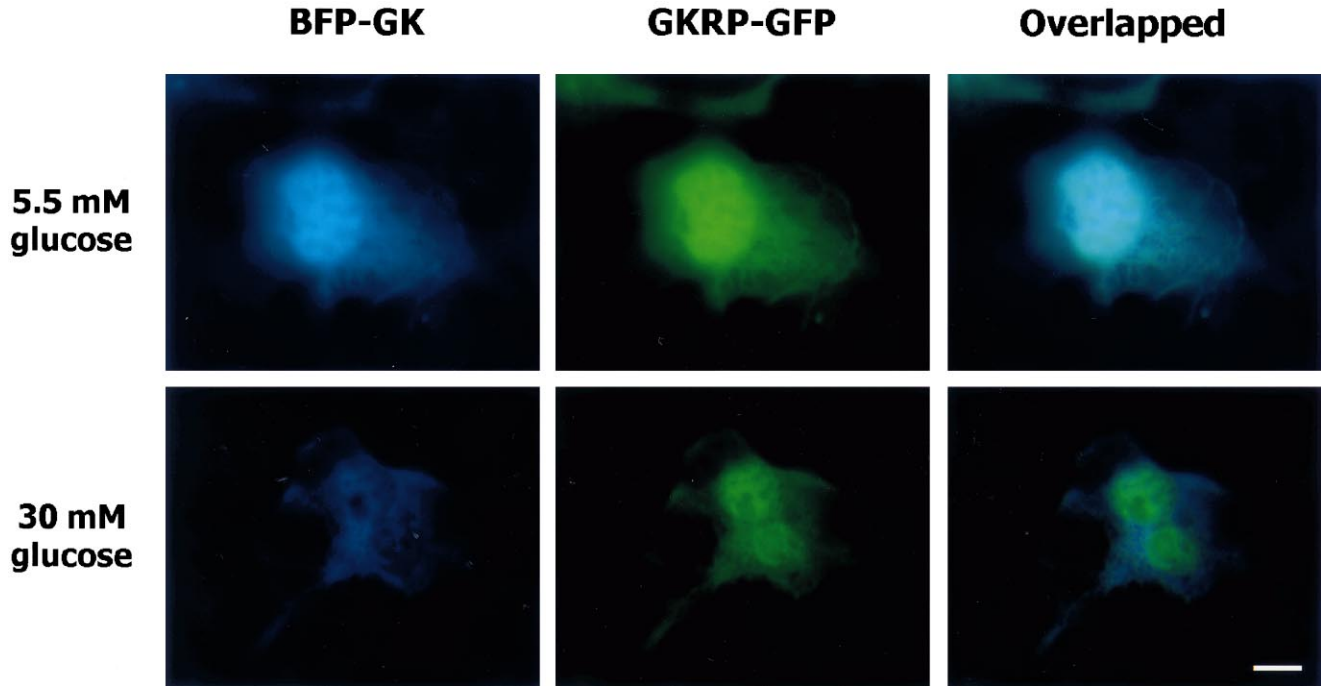

Fig. 4. Epifluorescence images of COS-1 cells co-expressing BFP-rat liver GK and rat GKRP-GFP, at low and high glucose concentrations. The overlapped image was obtained with the Metamorph Imaging System software. Scale bar, 20 μm.

concentration of the fluorescence in this last compartment. This distribution was unaffected by changes in the concentration of glucose. When GKRP-GFP was expressed alone in COS-1 cells, it concentrated in the nuclear compartment (Fig. 3) and the green fluorescence partly overlapped with the propidium iodide staining (not shown) at the two concentrations of glucose tested. On the contrary, in cells expressing BFP-rat GK alone, the enzyme was always found in the cytoplasm and excluded from the nucleus, even at low glucose concentrations (Fig. 3). When COS-1 cells were co-transfected with both plasmids, there was a clear nuclear concentration of the GK and GKRP fusion proteins at 5.5 mM glucose (Fig. 4), which again partly overlapped with the red propidium iodide staining (not shown). An increase in the glucose concentration to 30 mM led to the translocation of GK, but not of GKRP, to the cytoplasm, mimicking their behaviour in the hepatocyte (Fig. 4). Identical results were obtained when HeLa cells were used (not shown). To quantitate the phenomenon we measured, at low and high glucose, the total blue fluorescence in the nucleus and the whole cell, determined as the integrated fluorescence intensity in the selected region. At a low glucose concentration, $55.2 \pm 3.4\%$ (mean ± S.E.M., $n = 6$) of the blue fluorescence of the BFP-GK construct was found in the nucleus and this percentage lowered to $25.8 \pm 2.3\%$ ($n = 11$) upon incubation with 30 mM glucose. These results indicate that the presence of GKRP is absolutely necessary for the correct intracellular localisation of GK and suggest that GKRP is directly involved in the control of the mechanism by which GK shuttles between the nucleus and the cytoplasm, in response to changing metabolic conditions.

4. Discussion

Hepatic GK changes its intracellular localisation depending on the concentration of glucose or precursors of fructose 1-phosphate. In cultured hepatocytes and in perfused rat livers, GK is inside the nucleus under conditions mimicking the fasted state of the whole animal. It translocates to the cytoplasm when the cell is exposed to glucose concentrations similar to that of the portal vein in the fed state or to low concentrations of fructose [11,13]. While there is general agreement over this point, there is controversy about the subcellular localisation of GKRP [12,13]. In our hands, GKRP concentrates in the nucleus and does not leave this compartment when cultured rat hepatocytes are exposed to high concentrations of glucose. It has to be noted that both immunolocalisation of endogenous GKRP and overexpression of GKRP-GFP in cultured hepatocytes always reveal the presence of some protein in the cytosol, although there are no apparent changes in its distribution upon increasing the concentration of glucose.

In the present study, we have shown that the interaction between GKRP and GK plays a crucial role in the proper subcellular localisation of the enzyme. First of all, *Xenopus* GK mutants with a lowered affinity for GKRP are unable to concentrate in the hepatocyte nucleus at low concentrations of glucose. Secondly, GK is excluded form the nuclei of cells that do not express GKRP. Finally and most importantly, we have been able to reconstitute a glucose sensing machinery in cell lines not related to hepatic or pancreatic cells, by the co-expression of both rat GK and GKRP. In these conditions, GK is able to concentrate in the nucleus, when the glucose levels are low, and move to the cytoplasm at higher concentrations of the sugar. This last result also shows that the molecular mechanisms involved in the translocation of GK are not exclusive of hepatic cells but are also present in other cell types.

As it has already been proposed, GKRP can be viewed as a nuclear retention factor of GK [10,20]. At least in cultured hepatocytes, GKRP is always concentrated in the nucleus and, under metabolic conditions in which GK activity must be repressed, exhibits affinity for GK. Nevertheless, this does not explain how GK shuttles between the nuclear and the cytosolic compartments. The molecular mass of GK, below the exclusion limit of the nuclear pore [21], should in principle allow for its passive diffusion through the nuclear membrane. In this scenario, the GK subcellular distribution would only be controlled by its relative affinity for GKRP. However, there are several lines of evidence that indicate that the import and export of GK in and out of the nucleus is not a merely diffusional process, but rather an active transport mechanism. First of all, translocation of GK in both directions is a rapid process [11,13]. Secondly, in the experiments performed in this work with *Xenopus* GK, the addition of glucose to the medium causes in some cells the total translocation of the enzyme to the cytosol, leaving the nucleus empty. This observation is difficult to reconcile with a purely diffusional mechanism of translocation, unless the existence of a GK cytosolic retention factor is postulated. Finally, and most convincingly, the fusion protein GFP-GK, with a molecular mass of 78 kDa, is well above the size of the proteins that can passively diffuse across the nuclear membrane and, nevertheless, is able to reproduce the intracellular movement of GK.

Both rat and *Xenopus* GK contain in their primary structures a conserved stretch of basic residues (KHKKL) that could act as a nuclear localisation signal (NLS) [22]. In mutant C of *Xenopus* GK, which does not concentrate in the nuclear compartment, this sequence has been disrupted to become KGPRM. One may therefore wonder if the effect of mutation C is not due to disruption of this potential NLS. However, mutant A, which has been mutated in a sequence that certainly does not resemble a nuclear targeting signal, displays the same behaviour. Furthermore, GK is not targeted to the nucleus in the absence of GKRP, indicating that the KHKKL sequence is not enough to cause GK nuclear concentration.

Similar to NLS, nuclear export signals (NES) are short sequence motifs which are sufficient to mediate the rapid nuclear export of large proteins [23]. NES-mediated export is inhibited by leptomycin B [24], but in cultured hepatocytes, this drug does not prevent GK translocation from the nucleus to the cytosol (unpublished results). All these observations suggest that the intracellular movement of GK in response to changing metabolic conditions constitutes an example of a novel nuclear import-export mechanism. Further studies are in course to elucidate at the molecular level how the distribution of GK is controlled inside the cell.

Acknowledgements: We thank Susanna Baqué and Mar García-Rocha for helpful discussions and Susanna Castel and Anna Adrover for technical assistance with the confocal and epifluorescence microscopes and with the primary cultures of hepatocytes, respectively. This work was supported by Grant PB96-0992 from DGICYT (Spain), by the ARC programme of the Communauté Française de Belgique, by the Belgian Federal Service for Scientific, Technical and Cultural Affairs and by the Juvenile Diabetes Foundation International. N.I. was

the recipient of a doctoral fellowship from the Generalitat de Catalunya.

References

[1] Iynedjian, P.B. (1993) Biochem. J. 293, 1–13.
[2] Matschinsky, F.M. (1990) Diabetes 39, 647–652.
[3] Bell, G.I., Pilkis, S.J., Weber, I.T. and Polonsky, K.S. (1996) Ann. Rev. Physiol. 58, 171–186.
[4] Van Schaftingen, E., Veiga-da-Cunha, M. and Niculescu, L. (1997) Biochem. Soc. Trans. 25, 136–140.
[5] Malaisse, W.J., Malaisse-Lagae, F., Davies, D.R., Vandercammen, A. and Van Schaftingen, E. (1990) Eur. J. Biochem. 190, 539–545.
[6] Becker, T.C., Noel, R.J., Johnson, J.H., Lynch, R.M., Hirose, H., Tokuyama, Y., Bell, G.I. and Newgard, C.B. (1996) J. Biol. Chem. 271, 390–394.
[7] Veiga-da-Cunha, M., Xu, L.Z., Lee, Y.H., Marotta, D., Pilkis, S.J. and Van Schaftingen, E. (1996) Diabetologia 39, 1173–1179.
[8] Veiga-da-Cunha, M., Courtois, S., Michel, A., Gosselain, E. and Van Schaftingen, E. (1996) J. Biol. Chem. 271, 6292–6297.
[9] Agius, L. (1994) Biochem. J. 303, 841–845.
[10] Agius, L., Peak, M. and Van Schaftingen, E. (1995) Biochem. J. 309, 711–713.
[11] Toyoda, Y., Miwa, I., Kamiya, M., Ogiso, S., Nonogaki, T., Aoki, S. and Okuda, J. (1994) Biochem. Biophys. Res. Commun. 204, 252–256.
[12] Toyoda, Y., Miwa, I., Satake, S., Anai, M. and Oka, Y. (1995) Biochem. Biophys. Res. Commun. 215, 467–473.
[13] Brown, K.S., Kalinowski, S.S., Megill, J.R., Durham, S.K. and Mookhtiar, K.A. (1997) Diabetes 46, 179–186.
[14] Detheux, M., Vandekerckhove, J. and Van Schaftingen, E. (1993) FEBS Lett. 321, 111–115.
[15] Heim, R. and Tsien, R.Y. (1996) Curr. Biol. 6, 178–182.
[16] Massagué, J. and Guinovart, J.J. (1977) FEBS Lett. 82, 317–320.
[17] Quaade, C., Hughes, S.D., Coats, W.S., Sestak, A.L., Iynedjian, P.B. and Newgard, C.B. (1991) FEBS Lett. 280, 47–52.
[18] Vandercammen, A. and Van Schaftingen, E. (1993) Eur. J. Biochem. 294, 551–556.
[19] Vandercammen, A. and Van Schaftingen, E. (1991) Eur. J. Biochem. 200, 545–551.
[20] Niculescu, L. and Van Schaftingen, E. (1998) Diabetologia 41, 947–954.
[21] Nigg, E.A., Baeuerle, P.A. and Lührmann, R. (1991) Cell 66, 15–22.
[22] Nigg, E.A. (1997) Nature 386, 779–787.
[23] Nakielny, S. and Dreyfuss, G. (1997) Curr. Opin. Cell Biol. 9, 420–429.
[24] Wolff, B., Sanglier, J.J. and Wang, Y. (1997) Chem. Biol. 4, 139–147.