Conditional expression of hepatocyte nuclear factor-1 β , the maturity-onset diabetes of the young-5 gene product, influences the viability and functional competence of pancreatic β -cells

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Abstract

Mutations in the gene encoding hepatocyte nuclear factor (HNF)1 β result in maturity-onset diabetes of the young- (MODY)5, by impairing insulin secretory responses and, possibly, by reducing β -cell mass. The functional role of HNF1 β in normal β -cells is poorly understood; therefore, in the present study, wild-type (WT) HNF1 β , or one of two naturally occurring MODY5 mutations (an activating mutation, P328L329del, or a dominant-negative form, A263insGG) were conditionally expressed in the pancreatic β -cell line, insulin-1 (INS-1), and the functional consequences examined. Surprisingly, overexpression of the dominant-negative mutant did not modify any of the functional properties of the cells studied (including insulin secretion, cell growth and viability). By contrast, expression of WT HNF1 β was associated with a time- and dose-dependent inhibition of INS-1 cell proliferation and a marked increase in

apoptosis. Induction of WT HNF1 β also inhibited the insulin secretory response to nutrient stimuli, membrane depolarisation or activation of protein kinases A and C and this correlated with a significant decrease in pancreas-duodenum homeobox-1 protein levels. The attenuation of insulin secretion was, however, dissociated from the inhibition of proliferation and loss of viability, since expression of the P328L329del mutant led to a reduced rate of cell proliferation, but failed to induce apoptosis or to alter insulin secretion. Taken together, the present results suggest that mature rodent β -cells are sensitive to increased expression of WT HNF1 β and they imply that the levels of this protein are tightly regulated to maintain secretory competence and cell viability.

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Introduction

Maturity-onset diabetes of the young (MODY) is an early onset form of monogenic type II diabetes, which typically presents before 25 years (Fajans *et al.* 2001, Owen & Hattersley 2001) and is inherited in an autosomal dominant manner. MODY patients have a primary defect at the level of the β -cell caused by mutations in specific genes. Most of these genes encode transcription factors, including hepatocyte nuclear factor-(HNF)4 α (MODY1) (Yamagata *et al.* 1996a), HNF1 α (MODY3) (Yamagata *et al.* 1996b), pancreas-duodenum homeobox-1 (PDX-1; MODY4) (Stoffers *et al.* 1997), HNF1 β (MODY5) (Horikawa *et al.* 1997, Nishigori *et al.* 1998, Lindner *et al.* 1999, Bingham *et al.* 2000, Bingham & Hattersley 2004) and neuroD1/Beta2 (MODY6) (Malecki *et al.* 1999). The exception is MODY2, which is caused by mutations in the glucokinase gene (Froguel *et al.* 1993). In most cases, the MODY genes are known to be important for

the functional competence of the pancreatic β -cell and, as a consequence, mutations that lead to altered transcriptional or enzymatic activity are sufficient to cause β -cell defects and hence diabetes. As yet, the MODY5 gene, HNF1 β , has not been ascribed a clear role in pancreatic β -cells.

HNF1 α and 1 β are nuclear transcription factors of the homeodomain family. Their genes are located on different chromosomes in man, but the two may have arisen by an original gene duplication event during evolution (Bach *et al.* 1991). The proteins share a high degree of sequence homology, but are most divergent within the C-terminal transactivation domain. HNF1 α and -1 β bind to the same DNA consensus sequence and they can interact with this region either as homodimers or as an HNF1 α /1 β heterodimer (Mendel *et al.* 1991, Bach & Yaniv 1993, Cereghini 1996).

Despite the apparent similarities between HNF1 α and -1 β , it is likely that they undertake distinct functional roles within

the β -cell, as evidenced by the different phenotypes seen in MODY3 and MODY5 patients. Patients with mutations in HNF1 β have an impaired insulin secretory response to glucose and sulphonylureas (Nishigori *et al.* 1998, Bingham *et al.* 2000, Ryffel 2001, Pearson *et al.* 2004) and they exhibit a progressive loss in basal insulin secretion, suggesting a decline in β -cell mass. In contrast, MODY3 patients retain a robust insulin secretory response to sulphonylureas despite the attenuation of glucose-induced insulin secretion (Pearson *et al.* 2003, 2004). Thus, it seems likely that HNF1 α and -1 β exert differential effects in the β -cell and that the latter may regulate both secretory competence and cell viability.

Previous studies have established that HNF1 α is expressed in mature β -cells and that it promotes the transcription of a range of genes in these cells. These include several genes that are critical for the maintenance of the β -cell phenotype, such as Glut-2, PDX-1, L-type pyruvate kinase and possibly insulin (Wang *et al.* 1998, Ben-Shushan *et al.* 2001, Shih *et al.* 2001). HNF1 α may also be required for the proliferation of β -cells as it has been demonstrated to regulate genes involved in control of the cell cycle, such as cyclin E, p27 and insulin-like growth factor-I (Wobser *et al.* 2002, Yang *et al.* 2002). In contrast, little is known about the role of HNF1 β in β -cells. Homozygous HNF1 β knockout mice are non-viable, with death occurring soon after implantation of the embryo (7.5 embryo days in mice) (Barbacci *et al.* 1999, Coffinier *et al.* 1999), making the function of HNF1 β difficult to study using whole animal knockout approaches. In experiments where HNF1 β has been selectively deleted in mature mouse β -cells (using Cre-recombinase expressed under the control of the insulin promoter (RIP-Cre)), there was evidence of impaired glucose tolerance and reduced insulin secretion. These are correlated with alterations in the functional activity of other β -cell transcription factors such that PDX-1 and HNF1 α were increased and HNF4 α decreased (Wang *et al.* 2004). These results suggest that the expression of HNF1 β may be required to maintain the differentiation state and functional activity of mature β -cells (Coffinier *et al.* 1999, Wang *et al.* 2004). However, the experiments must be interpreted with caution, since it has subsequently been

revealed that alterations in glucose homeostasis can occur in RIP-Cre mice that are unrelated to changes in the gene of interest (Lee *et al.* 2006).

In order to investigate the function of HNF1 β in mature β -cells without the potential complications arising from RIP-Cre-recombinase-mediated knockout, we have used a clonal β -cell line (INS-1) to conditionally express either wild-type (WT) HNF1 β or one of two naturally occurring mutants identified in patients with MODY5 (A263insGG and P328L329del). The mutation P328L329del (abbreviated to P328del) leads to the synthesis of a protein having a severely truncated transactivation domain, but that retains the DNA binding and dimerisation domains (Fig. 1) (Bingham *et al.* 2000). From studies in HeLa cells, P328del has been reported to possess increased transcriptional activity compared with WT HNF1 β (Wild *et al.* 2000), although examination of the profile of genes that are up-regulated in response to the expression of this mutant in INS-1 cells suggests that it may be less active than the WT (Thomas *et al.* 2004). The A263insGG (A263ins) mutant has no transactivation domain and a truncated DNA-binding domain that is non-functional (Senkel *et al.* 2005). However, A263ins can still form dimers with WT HNF1 β and this has been suggested to result in dominant-negative activity against the native form in a variety of cell types, including the pancreatic β -cell line MIN6 (Nishigori *et al.* 1998, Tomura *et al.* 1999, Bai *et al.* 2002).

Materials and Methods

Cell culture

Pancreatic β -cell lines (INS-1) that conditionally express human WT HNF1 β or one of two mutant forms, designated P328del or A263ins, were used in these experiments. These cell lines were recently described in detail by Thomas *et al.* (2004) but, briefly, cDNAs encoding WT or the mutant forms of HNF1 β were cloned downstream of the Tet operator in the plasmid pcDNA5/FRT/TO. This plasmid was integrated by site-directed Flp recombination into the insulinoma cell

Figure 1 Structure of the HNF1 β protein. Schematic diagram of HNF1 β protein structure showing wild-type and the two truncated proteins encoded by MODY5 mutations. The native protein contains a dimerisation domain, a DNA-binding domain and a transactivation domain. The numbers refer to the amino acid positions in WT HNF1 β .

clone INS1-Flp-In-T-Rex. Stable cell lines containing the HNF1 β gene were obtained by hygromycin selection. The Tet operator inhibits the expression of HNF1 β gene and this can be alleviated by the addition of tetracycline.

Cells were cultured in RPMI 1640 medium (Invitrogen) containing 11 mM glucose, with 10% foetal bovine serum, 2 mM L-Gln and 50 μ M β -mercaptoethanol supplemented with 100 U/ml penicillin and 100 μ g/ml streptomycin, with 10 μ g/ml blasticidine and 150 μ g/ml hygromycin to maintain selection. Cells were cultured at 37 °C in 5% CO₂:95% air and grown and maintained in 75 cm³ flasks. They were used in experiments or passaged when approximately 80% confluent. To study the expression of the gene of interest, cells were seeded into 25 cm³ flasks, 24- or 6-well plates for 24 h before the addition of tetracycline at up to 1 μ g/ml.

Isolation of rat islets

Islets from Wistar rats were isolated by collagenase digestion of the pancreas. The dissected pancreas was distended with incubation buffer (Gey & Gey 1936) gassed with O₂/CO₂ (95:5), with 1 mM CaCl₂ and 4 mM glucose added just before use) and then finely chopped. Collagenase type XI was added and the pancreatic tissue shaken in a water bath (37 °C) for about 5 min until the exocrine component of the pancreas was digested, releasing free islets. The islets were hand picked using a finely drawn Pasteur pipette for use in individual experiments.

mRNA isolation

TRIZOL reagent (Invitrogen) was used to extract RNA from INS-1 cells, islets and tissue extracts. The cell lysates were passed through a pipette tip several times and the contents were transferred to sterile microfuge tubes. Chloroform (0.2 ml per 1 ml TRIZOL used) was added, the tubes vortexed, incubated at room temperature for 10 min and then spun at 12 000 g for 15 min. The upper aqueous phase containing the RNA was removed and transferred to a new tube. To this, 0.5 ml isopropanol was added for each 1 ml TRIZOL used and incubated at -20 °C for 1 h, before being centrifuged at 12 000 g for 20 min. The resulting pellet was washed twice in 75% ethanol, air-dried and resuspended in RNase-free (diethylpyrocarbonate) water.

Reverse transcriptase (RT)-PCR

The expression level of the HNF1 β gene was examined by a quantitative real-time PCR approach. cDNA was first generated from total RNA using an oligo dT primer by the Thermoscript first round cDNA synthesis kit (Invitrogen). Real-time PCR was then carried out using probes to WT HNF1 β and the endogenous control gene β -2-microglobulin (probe and primer sequences are described previously; Harries *et al.* 2004). Reactions contained 36 μ M each primer and

8 μ M probe in a total reaction volume of 20 μ l on the TaqMan 7000 platform. Expression levels of HNF1 β were measured relative to β -2-microglobulin and normalised to the expression levels in total rat kidney RNA using the $\Delta\Delta$ CT method described in Applied Biosystems User Bulletin number 2 (Relative Quantitation of Gene Expression, pp. 11–15; Warrington, UK).

Western blotting

To extract whole cell protein, INS-1 cells were washed in ice-cold PBS before the addition of 0.2 ml lysis buffer (20 mM Tris, 150 mM NaCl, 1 mM EDTA and 1% Triton X, with 10 μ l/ml protease inhibitor cocktail (Sigma) added just before use) per 25 cm³ flask, for 10 min on ice. The flasks were then scraped, the contents transferred to a microfuge tube (on ice) and vortexed (4× for 15 s), with 5 s on ice between each vortexing. The protein extract was then centrifuged at 1000 g for 10 min at 4 °C and the supernatant stored at -80 °C.

Equal amounts of denatured protein samples were run on a precast bis-Tris-HCl buffered 12% polyacrylamide gel (Invitrogen) at 200 V for 1 h in MOPS SDS running buffer (50 mM 3-(N-morpholino) propane sulphonic acid, 50 mM Tris base, 3.5 mM SDS, 1 mM EDTA). A prestained marker set (Amersham) was included to allow the sizes of relevant bands to be determined. Proteins were then transferred to a polyvinylidene fluoride membrane (Millipore, Watford, Herts, UK) using a 'wet' transfer tank (BioRad trans blot cell) for 4 h at 250 mA. The membrane was blocked overnight at 4 °C with Tris-buffered saline containing 0.05% Tween (TTBS) and 5% low fat dried milk. Primary antibodies raised against HNF1 β , GADD45 α and PAR4 were from Santa Cruz Biotech (sc-7411, sc-4100 and sc-1807 respectively; San Diego, CA, USA) anti-PDX-1 was a gift from Prof. C Wright, Vanderbilt University, Nashville, TN, USA, and anti-PTP-BL was a gift from Prof. K Erdmann, Ruhr-University Bochum, Germany. Antibodies were diluted 1 in 2000 in TTBS containing 1% milk and incubated with the membrane for 4 h at room temperature. An appropriate IgG-alkaline phosphatase conjugated secondary antibody was diluted 1 in 30 000 in TTBS containing 1% milk, added to the membrane and incubated for 1 h at room temperature. Immunoreactive bands were visualised using CPD-Star (Sigma) and exposure to X-ray film.

Insulin secretion

INS-1 cells were seeded into 24-well plates at 1×10^5 cells per well, 24 h before addition of tetracycline. At the end of the induction period, cells were washed and preincubated for 1 h in 500 μ l of incubation buffer (Gey & Gey 1936) containing 6 mM glucose and 0.1% BSA. Cells were acutely stimulated with the test reagents and the incubation medium was sampled after 1 h for the measurement of insulin by RIA.

Estimation of cell viability with Trypan Blue

For the determination of cell viability, vital dye staining was used. Experiments were carried out in six-well plates with 1×10^5 cells/well seeded 24 h before induction of HNF1 β expression. Floating and attached cells were collected from each well and stained with Trypan Blue. The number of viable and dead cells was counted using a haemocytometer.

Apoptosis assays

CaspACE FITC-VAD-FMK In situ Marker (Promega), a fluoroisothiocyanate conjugate of the cell permeable caspase substrate VAD-FMK, can be localised by fluorescence detection and acts as an *in situ* marker for cells undergoing apoptosis. Treated cells were labelled with 10 μ M CaspACE according to the manufacturer's instructions and viewed by fluorescence microscopy. The number of green fluorescent cells was counted in a field of about 100 cells and the percentage of apoptosis was calculated.

The Annexin V-Cy3 apoptosis detection kit (Sigma) uses two labels. The first, annexin-V Cy3-18 (AnnCy3), is a red fluorescent protein that binds to phosphatidylserine but cannot cross the plasma membrane. The second label, 6-carboxyfluorescein diacetate (6-CFDA), is cell permeable and is used as a measure of cell viability as it is hydrolysed by esterases present in living cells to produce the green fluorescent compound 6-carboxyfluorescein (6-CF). Dual staining with these two labels can distinguish between live, necrotic and apoptotic cells (Elliott *et al.* 2002). Following exposure to test reagents, INS-1 cells were labelled with the double-staining solution (AnnCy3 and 6-CFDA) according to the manufacturer's instructions and viewed by fluorescence microscopy.

Statistical analysis

All individual experiments were performed in at least duplicate and were repeated on a minimum of three separate occasions. The results were analysed by ANOVA and were considered significant when $P < 0.05$.

Results

Endogenous HNF1 β expression in β -cell lines and islets

In initial experiments, the expression levels of native HNF1 β in β -cells were examined. Using semi-quantitative RT-PCR, low levels (as compared to kidney) of HNF1 β mRNA were detectable in INS-1 cells and isolated rat islets (Fig. 2a). This is consistent with microarray data from INS-1 cells (Thomas *et al.* 2004) and with other studies in β -cells (Coffinier *et al.* 1999, Wang *et al.* 2004, Gunton *et al.* 2005). Despite the clear presence of mRNA encoding HNF1 β , the protein itself was not readily detectable by Western blotting in uninduced INS-1 cells or in primary rat and human islets, which confirms previous evidence (Maestro *et al.* 2003,

Figure 2 Expression of HNF1 β in β -cell lines and isolated islets. (a) Quantitative real-time RT-PCR of rat islet, INS-1 cells and kidney mRNA using HNF1 β specific primers and probes. Expression levels were measured relative to β -2microglobulin. (b) INS-1 cells conditionally expressing HNF1 β were treated in duplicate with 0, 20, 50 and 1000 ng/ml tetracycline for 24 h. Whole cell protein was extracted and HNF1 β protein levels determined using Western blotting with an HNF1 β specific antibody (upper panel). Relative expression levels were quantified by densitometry (den; lower panel).

Thomas *et al.* 2004) that HNF1 β is not highly expressed in mature pancreatic β -cells.

Conditional expression of HNF1 β in INS-1 cells

A tetracycline inducible system was used to conditionally express either WT HNF1 β or one of two naturally occurring MODY5 mutants, P328L329del (P328del) and A263insGG (A263ins) in INS-1 cells. Tetracycline treatment (24 h) of INS-1 cells stably transfected with WT HNF1 β resulted in a dose-dependent increase in HNF1 β protein levels (Fig. 2b) with 1000 ng/ml tetracycline yielding the highest levels of HNF1 β expression. Similar results were obtained for cells expressing the P328del and A263ins mutant forms of HNF1 β (not presented).

Effect of HNF1 β expression on insulin secretion

As MODY5 patients are characterised by impaired insulin secretory responses, the effect of increased expression of A263ins, P328del or WT HNF1 β on insulin secretion was studied.

Acute stimulation of the cells with a range of stimuli, including the metabolic fuels mono-methyl-succinate and

Figure 3 Effect of induced expression of HNF1 β on insulin secretion from INS-1 cells. (a) HNF1 β WT, (b) A263ins (c) P328del or protein expression was induced by exposure to 1 μ g/ml tetracycline. Controls were cultured in the absence of tetracycline. Following this culture period, the cells were washed and acutely stimulated with 5 mM mono-methyl succinate, 20 mM ketoisocaproate (KIC), 25 mM KCl or a combination of 200 μ M IBMX and 100 nM PMA for 1 h. Results are shown as means $\pm$ S.E.M. ($n=8$). * $P<0.01$ relative to the relevant uninduced stimulated cells.

α -ketoisocaproate, a depolarising concentration of KCl or a combination of isobutylmethyl xanthine (IBMX) and phorbol myristate acetate (PMA) caused a significant increase in insulin secretion (Fig. 3), although these cells did not respond to glucose stimulation (results not shown). Induction of WT HNF1 β protein for 24 h caused a significant decrease in insulin secretion caused by all stimuli tested (Fig. 3a). In contrast, expression of either of the mutant forms of HNF1 β

(A263ins or P328del) had no attenuating effect on insulin secretion in response to any of the stimuli used (Fig. 3b and c).

Role of PDX-1 in the impairment of insulin secretion mediated by HNF1 β

To study the genes that might be involved in HNF1 β -induced impairment of insulin secretion, microarray data from INS-1

Table 1 Microarray analysis of HNF1β-induced gene expression. INS-1 cells expressing either WT or the P328del mutant isoform of HNF1β were treated with 1000 ng/ml tetracycline for 24 h to induce protein expression. mRNA was then extracted and analysed on an Affymetrix gene chip (RAE230A). Genes that were altered in expression in two separate experiments (induced vs uninduced cells) were identified and candidate genes involved in insulin secretion, cell growth or apoptosis were selected for analysis. The table shows the fold changes of the genes of interest in response to HNF1β expression. Fold changes of > 1 represent an increase in gene expression, whereas changes of < 1 represent a decrease (as described in Thomas *et al.* 2004).

	Gene title	Function	Probe set	Fold change on HNF1β induction	
				WT	P328del
Gene symbol					
Ptpn13	Protein tyrosine phosphatase-basophil like (PTP-BL)	Involved in regulation of the cytoskeleton and cytokinesis	1374812	4.68	2.7
GADD45α	Growth arrest and DNA-damage-inducible 45α	Involved in apoptosis	1368947	3.9	NS
Pawr	PRKC, apoptosis, WT1, regulator (PAR4)	Up-regulated during apoptosis	1368702	2.85	NS
PDX-1	Pancreatic and duodenal homeobox gene 1	Regulation of insulin gene expression	1369516	0.17	NS

NS, no significant change

cells expressing HNF1β (detailed in Thomas *et al.* 2004) were analysed and it was noted that PDX-1 mRNA is markedly down-regulated upon induction of WT HNF1β (Table 1). Expression of PDX-1 protein was, therefore, monitored. As expected, PDX-1 protein was strongly expressed in control (uninduced) INS-1 cells and, in agreement with the microarray data, the levels were dramatically decreased within 24 h of WT HNF1β expression (Fig. 4a). It was notable that PDX-1 levels were not altered following the expression of P328del HNF1β (nor when A263ins was expressed), despite the fact that P328del acts as a gain of function mutant in some assay systems (Fig. 4b).

Effect of HNF1β expression on β-cell viability

A gradual decline in basal insulin levels (in addition to the loss of stimulated insulin secretion) has been observed in patients with HNF1β mutations, which could indicate a reduction in β-cell numbers during disease progression. The effect of expression of A263ins, P328del or WT HNF1β on β-cell growth and viability was, therefore, also investigated. Protein expression was induced for up to 96 h and changes in cell numbers were monitored (Fig. 5a–c). Uninduced INS-1 cells displayed a typical sigmoidal growth curve with the total cell number increasing almost fourfold over a 96 h period.

Figure 4 Effect of HNF1β expression on PDX-1 protein levels. (a) WT HNF1β was induced with or without 1 µg/ml tetracycline for 24 h, then total cell protein was extracted for Western blotting with anti-HNF1β and anti-PDX-1 serum. The results from triplicate samples in each case are presented. (b) P328del or A263ins HNF1β expression was induced with or without 1 µg/ml tetracycline for 24 h, then total cell protein was extracted for Western blotting with anti-PDX-1 serum. The results from duplicate samples in each case are presented.

Figure 5 Effects of HNF1 β expression on INS-1 cell viability. INS-1 cells conditionally expressing (a) HNF1 β WT, (b) A263ins and (c) P328del were treated with or without 1 μ g/ml tetracycline for up to 96 h. At each time point, cells were harvested and the number of viable cells counted with a haemocytometer. Results are shown as mean $\pm$ s.e.m. from triplicate experiments. * $P < 0.01$, compared with uninduced cells. (d) MEK 293 or INS-1 cells were induced with tetracycline and protein extracted for Western blotting to reveal the expression of HNF 1 β or β -actin. (e) HNF1 β WT or P328del protein expression was induced by incubation of INS-1 cells with tetracycline for 52 h. The cells were then harvested and stained with CaspACE reagent to identify apoptotic cells by fluorescence microscopy. * $P < 0.001$ relative to uninduced cells.

Unexpectedly, the induction of expression of WT HNF1 β dramatically inhibited the increase in β -cell number. This effect was evident within 48 h and it became increasingly marked as the experiment progressed. Even more strikingly, it was noted that, over 96 h, the total cell number had declined to a value that was below the 48h level. This suggests that not only had cell growth been inhibited, but that net cell death had also occurred. The latter effect was not observed with the P328del mutant since, although the expression of this mutant clearly attenuated cell growth, the total cell

number did not decline below its initial value. High-level induction of the A263ins mutant had no significant effect on the growth characteristics of the cells, confirming that overexpression of these proteins *per se* was not responsible for the altered responses measured. Expression of A263ins HNF1 β also failed to alter the number of dead cells recovered from the medium during the 96 h growth period, suggesting that it did not promote any reduction in cell viability.

To further confirm the specificity of the growth inhibitory effects of enforced expression of HNF1 β , a kidney cell line

(HEK293) was employed. Expression of WT HNF1 β in HEK293 cells using the same tetracycline induction system as in the INS-1 cells had no measurable effect on the viability of these cells (viability after 48 h induction of HNF1 β ; $96 \pm 4\%$ relative to control (not significant)), despite the finding that HNF1 β was overexpressed to a similar extent in both cell types (Fig. 5d).

To investigate the effects of WT and P328del HNF1 β on INS-1 cell death more directly, CaspACE (Fig. 5e) and annexin V (below) staining were employed to detect apoptotic cells. Both markers clearly revealed that expression of WT HNF1 β increased the extent of apoptosis in β -cells. By contrast, P328del did not cause any increase in apoptosis above the control levels. The proportion of apoptotic cells detected by annexin V was: WT-uninduced $0.97 \pm 0.38\%$, induced $13.33 \pm 0.4\%$ ($P < 0.001$); P328del-uninduced $0.87 \pm 0.3\%$, induced $1.2 \pm 0.3\%$ (NS).

Genes involved in HNF1 β induction of cell death

In order to identify candidate genes that were altered by HNF1 β induction and which might be involved in cell growth and apoptosis, microarray data were used to identify candidate genes. Three genes were selected for further study on the basis that their expression was altered significantly and that they might be expected to regulate growth or viability. The genes selected encoded growth arrest and DNA-inducible protein 45 α (GADD45 α), prostate apoptosis response-4 (PAR4) and protein tyrosine phosphatase-basophil like (PTP-BL) (see Table 1). Western blotting was then carried out to determine whether the corresponding proteins were expressed in INS-1 cells and to establish whether their expression was altered by HNF1 β induction.

GADD45 α protein was not detectable in control INS-1 cells, but its expression was clearly induced by NaF, a reagent that has previously been shown to cause apoptosis in islets and β -cell lines (Hollander *et al.* 1999, Sheikh *et al.* 2000, Elliott *et al.* 2002, Hildesheim *et al.* 2002). However, despite the evidence of increased GADD45 α mRNA expression in response to WT HNF1 β in microarray studies, no GADD45 α protein was detectable after either 24 or 48 h of induction of the transcription factor (results not shown).

PAR4 is a Leu zipper protein that is involved in the activation of apoptosis in many cell types (Sells *et al.* 1997, Rangnekar 1998, Chakraborty *et al.* 2001), but has not previously been identified in β -cells. Its expression was altered 2.7-fold by WT HNF1 β at the mRNA level and thus the protein levels of PAR4 were measured in INS-1 cells. Surprisingly, it was observed that INS-1 cells (and rat islets) express abundant amounts of PAR4 protein, even under control conditions, but this was not significantly increased in response to expression of HNF1 β (results not shown).

PTP-BL is a large soluble PTP that has been implicated in regulation of the cytoskeleton and cytokinesis (Erdmann 2003, Herrmann *et al.* 2003). It also interacts with several proteins

involved in the control of apoptosis, including Fas, adenomatous polyposis coli and nerve growth factor (Erdmann *et al.* 2000, Erdmann 2003, Herrmann *et al.* 2003). PTP-BL mRNA was increased by both WT and P328del HNF1 β as determined by microarray analysis. Uninduced INS-1 cells were found to express measurable amounts of PTP-BL protein by Western blotting and this was increased markedly by the induction of WT HNF1 β and to a lesser extent by P328del expression. By contrast, the expression of PTP-BL was not influenced by A263ins induction (Fig. 6).

To confirm these results, additional clones of INS-1 cells were derived that conditionally express PTP-BL in the absence of altered levels of HNF1 β . It was found that even small increases in PTP-BL expression led to an inhibition of cell growth, but had no effect on levels of cell death or apoptosis (Welters *et al.*, unpublished observations). Thus, PTP-BL may serve as an HNF1 β -controlled growth regulator in β -cells.

Discussion

HNF1 β has been defined as the protein responsible for MODY5 (Horikawa *et al.* 1997, Nishigori *et al.* 1998, Lindner *et al.* 1999, Bingham *et al.* 2000, Bingham & Hattersley 2004) and a variety of mutations have been identified within the sequence of its cognate gene (*TCF2*) in MODY5 pedigrees (Nishigori *et al.* 1998, Bingham *et al.* 2000, Ryffel 2001). MODY5 is characterised by impaired insulin secretion, accompanied by evidence of a gradual reduction in β -cell mass, suggesting that HNF1 β plays an important role in the maintenance of the differentiated phenotype of the β -cell and in the regulation of β -cell secretion and viability. In support of this, the selective deletion of HNF1 β from adult β -cells in RIP-Cre mice also

Figure 6 Effect of HNF1 β induction on PTP-BL protein expression in INS-1 cells. Cells expressing HNF1 β WT, P328del and A263ins were incubated in the absence (black bars) or presence (white bars) of 1 μ g/ml tetracycline for 24 h. Whole cell protein extracts were prepared and probed by Western blotting using a specific anti-PTP-BL antibody. Protein expression relative to uninduced cells was determined densitometrically. Results are shown as mean $\pm$ S.E.M. ($n=3$). * $P < 0.01$, relative to uninduced cells.

exerts a deleterious effect on insulin secretory capacity (Wang *et al.* 2004), although such experiments may be complicated by alterations in insulin secretion which occur independently of the changes in target gene expression (Lee *et al.* 2006).

In the present work, we have employed a conditional expression system to address the consequences of altered HNF1 β expression in fully differentiated β -cells derived from an INS-1 cell clone. The characteristics of this system have been described in detail in a recent study (Thomas *et al.* 2004) which concluded that it is well suited for the regulated expression and functional characterisation of β -cell transcription factors, including HNF1 β .

Initially, we examined the expression of HNF1 β in the parental INS-1 cell line (INS-1 Flp-In T-Rex) by RT-PCR and confirmed the presence of the transcript. However, HNF1 β protein was not detectable in these cells by Western blotting. A similar situation also pertained in both rat and human islets, where HNF1 β could not be detected by Western blotting in several islet preparations from either species. These data are consistent with previous observations (Coffinier *et al.* 1999, Maestro *et al.* 2003, Wang *et al.* 2004, Gunton *et al.* 2005) and imply that differentiated β -cells express HNF1 β mRNA, but that HNF1 β protein is maintained at a relatively low level.

We observed that enforced overexpression of WT HNF1 β in INS-1 cells caused a loss of insulin secretory response, a reduction in the rate of cell growth and a net decrease in cell viability. The latter was mediated by increased apoptosis, as judged by increased caspase activity and enhanced annexin-V staining of the overexpressing cells. This potential inhibition of cell growth by increased HNF1 β expression is consistent with situations where a reduction in HNF1 β levels leads to hyperproliferation of endothelial cells (Gresh *et al.* 2004, Haumaitre *et al.* 2005), suggesting that HNF1 β may act to regulate normal cell growth. Thus, a high level of expression of HNF1 β may be detrimental to the status and functional competence of mature β -cells and is consistent with the finding of limited expression of this protein in mature β -cells (Maestro *et al.* 2003). In this context, it is interesting to note that islets from patients with type 2 diabetes may express higher levels of HNF1 β mRNA than controls (Gunton *et al.* 2005). Although the increase in HNF1 β expression observed in that study (approximately twofold) was not statistically significant, it nevertheless raises the possibility that an elevation of HNF1 β might contribute to the loss of secretory function in the islets of some patients with type 2 diabetes. This suggestion certainly merits further consideration.

In parallel studies to those described above in INS-1 cells, WT HNF1 β protein was also overexpressed in HEK-293 cells to control for possible non-specific effects on cell viability. High-level induction of HNF1 β did not elicit any detrimental effects on proliferation or viability in these cells, suggesting that the responses observed in INS-1 cells were not mediated by non-specific mechanisms associated with protein overexpression. This conclusion is also supported by the fact

that differential effects were obtained in INS-1 cells by overexpression of WT versus mutant forms of the protein.

In an attempt to understand the molecular basis for the loss of insulin secretion seen in response to induction of WT HNF1 β , we examined the profile of genes that are influenced by this transcription factor in INS-1 cells (Thomas *et al.* 2004). This revealed that PDX-1 transcripts are dramatically reduced in response to WT HNF1 β induction. Since PDX-1 is essential for the regulation of a wide variety of important genes in β -cells (Waeber *et al.* 1996, Watada *et al.* 1996, Macfarlane *et al.* 2000), a reduction in expression would be expected to exert deleterious effects on insulin secretory responses. Expression of dominant-negative PDX-1 in INS-1 cells has been shown to inhibit nutrient- and KCl-induced insulin secretion (Wang *et al.* 2005). Monitoring of PDX-1 expression at the protein level in response to HNF1 β confirmed that it declined markedly within 24 h, suggesting that turnover of PDX-1 protein occurs rapidly in cells overexpressing WT HNF1 β .

The observation that HNF1 β induction causes a decreased expression of PDX-1 was unexpected, since it is known that the PDX-1 promoter contains an HNF1 consensus motif (Ben-Shushan *et al.* 2001), which might be expected to drive increased transcription of the gene. However, this motif appears to be regulated more efficiently by HNF1 α in β -cells (Ben-Shushan *et al.* 2001) and it is possible that, in the present studies, overexpression of HNF1 β gave rise to a net reduction in homodimeric HNF1 α (by promoting heterodimer formation between HNF1 α and 1 β) and thereby caused a decrease in the extent of HNF1 α -driven PDX-1 transcription. Irrespective of the mechanism, the finding that PDX-1 protein levels were reduced in cells expressing HNF1 β is in agreement with the report by Wang *et al.* (2004) that PDX-1 and HNF1 β are regulated in a reciprocal manner in β -cells.

Following these considerations, we also examined the results of the microarray analysis to identify additional candidate genes whose altered transcription might underlie the ability of HNF1 β to promote the loss of proliferation and viability of INS-1 cells. Several genes were identified and selected for further analysis. These included GADD45 α , PAR4 and PTP-BL, all of which are previously unstudied in the β -cell.

GADD45 α encodes a protein that is frequently induced by DNA damage and other cellular stresses in a variety of cells (Hollander *et al.* 1999, Sheikh *et al.* 2000, Hildesheim *et al.* 2002) and may play a role as a mediator of apoptosis. We were unable to detect GADD45 α at the protein level in uninduced INS-1 cells, although exposure of INS-1 cells to 5 mM NaF (which induces β -cell apoptosis (Elliott *et al.* 2002)) resulted in the appearance of immunoreactive GADD45 α . This suggests that, as in other cell types, GADD45 α may play a role in regulating the apoptotic response of β -cells to certain stimuli. However, despite the evidence for increased transcription of GADD45 α in cells expressing WT HNF1 β , this was not accompanied by a detectable increase in GADD45 α protein expression. Thus, we consider it unlikely that GADD45 α is primarily involved in mediating the apoptotic response to HNF1 β in INS-1 cells.

A second gene product investigated was PAR4, an immediate early response gene, which was first identified in prostate cancer cells as a gene whose transcription is rapidly up-regulated during the onset of apoptosis. When over-expressed, it can sensitise cells to apoptosis mediated by a range of stimuli (Sells *et al.* 1997, Rangnekar 1998, Chakraborty *et al.* 2001). In this study, we demonstrated that INS-1 cells (as well as rat and human islets) express relatively high levels of PAR4 protein, even under control conditions. Induction of HNF1 β expression did not cause any further significant increase in PAR4 levels and we conclude that changes in expression of this protein are unlikely to mediate the loss of β -cell viability caused by HNF1 β .

The third candidate, PTP-BL, is a soluble PTP, which serves as a central scaffolding protein for a range of cellular effectors. It contains a series of PSD 95 SA p90, discs large, ZO-1 (PDZ) domains which allow it to act as an adaptor to regulate various cellular functions, including cytoskeletal organisation and cytokinesis (Erdmann 2003, Herrmann *et al.* 2003). It can also control cell viability by regulating, for example, the surface expression of the death receptor, Fas, and the activity of pro-apoptotic transcription factors such as NF κ B (reviewed by Erdmann 2003). We observed that induction of the expression of WT or P328del HNF1 β in INS-1 cells caused an increase in PTP-BL protein expression, suggesting that altered expression of PTP-BL could underlie the effects of HNF1 β on INS-1 cell proliferation. This was confirmed by overexpression studies showing that a small increase in PTP-BL protein expression inhibited INS-1 cell growth (Welters *et al.* unpublished observations).

Overall, therefore, the results presented in this study reveal that HNF1 β protein is not highly expressed in pancreatic β -cells. Nevertheless, this gene product appears to play an important role in regulation of the functional competence of the cells, as in the case of other MODY genes. However, unlike the situation with other MODY genes, it appears that a low (rather than high) level of expression may be important for maintaining the function of mature β -cells, since our results reveal that increased levels of HNF1 β can have detrimental consequences on the secretory competence, proliferative potential and viability of differentiated pancreatic β -cells. This suggests that the levels of HNF1 β are tightly regulated in fully differentiated β -cells as a means to maintain their functional competence. Thus, the control of HNF1 β expression may be an important developmental feature of β -cells with foetal cells expressing high levels of the protein (Maestro *et al.* 2003) and adult cells much lower levels. It is possible that this pattern may correlate with the development of nutrient-sensitive insulin secretion in these cells.

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References

- Bach I & Yaniv M 1993 More potent transcriptional activators or a transdominant inhibitor of the HNF1 homeoprotein family are generated by alternative RNA processing. *EMBO Journal* **12** 4229–4242.
- Bach I, Mattei MG, Cereghini S & Yaniv M 1991 Two members of an HNF1 homeoprotein family are expressed in human liver. *Nucleic Acids Research* **19** 3553–3559.
- Bai Y, Pontoglio M, Hiesberger T, Sinclair AM & Igarashi P 2002 Regulation of kidney-specific Ksp-cadherin gene promoter by hepatocyte nuclear factor-1beta. *American Journal of Physiology. Renal Physiology* **283** F839–F851.
- Barbacci E, Reber M, Ott MO, Breillat C, Huetz F & Cereghini S 1999 Variant hepatocyte nuclear factor 1 is required for visceral endoderm specification. *Development* **126** 4795–4805.
- Ben-Shushan E, Marshak S, Shoshkes M, Cerasi E & Melloul D 2001 A pancreatic beta-cell-specific enhancer in the human PDX-1 gene is regulated by hepatocyte nuclear factor 3beta (HNF-3beta), HNF-1alpha, and SPs transcription factors. *Journal of Biological Chemistry* **276** 17533–17540.
- Bingham C & Hattersley AT 2004 Renal cysts and diabetes syndrome resulting from mutations in hepatocyte nuclear factor-1beta. *Nephrology Dialysis, Transplantation* **19** 2703–2708.
- Bingham C, Ellard S, Allen L, Bulman M, Shepherd M, Frayling T, Berry PJ, Clark PM, Lindner T, Bell GI *et al.* 2000 Abnormal nephron development associated with a frameshift mutation in the transcription factor hepatocyte nuclear factor-1 beta. *Kidney International* **57** 898–907.
- Cereghini S 1996 Liver-enriched transcription factors and hepatocyte differentiation. *FASEB Journal* **10** 267–282.
- Chakraborty M, Qiu SG, Vasudevan KM & Rangnekar VM 2001 Par-4 drives trafficking and activation of Fas and FasL to induce prostate cancer cell apoptosis and tumor regression. *Cancer Research* **61** 7255–7263.
- Coffinier C, Thepot D, Babinet C, Yaniv M & Barra J 1999 Essential role for the homeoprotein vHNF1/HNF1beta in visceral endoderm differentiation. *Development* **126** 4785–4794.
- Elliott J, Scarpello JH & Morgan NG 2002 Differential effects of genistein on apoptosis induced by fluoride and pertussis toxin in human and rat pancreatic islets and RINm5F cells. *Journal of Endocrinology* **172** 137–143.
- Erdmann KS 2003 The protein tyrosine phosphatase PTP-basophil/basophil-like, interacting proteins and molecular functions. *European Journal of Biochemistry/FEBS* **270** 4789–4798.
- Erdmann KS, Kuhlmann J, Lessmann V, Herrmann L, Eulenburg V, Muller O & Heumann R 2000 The adenomatous polyposis coli-protein (APC) interacts with the protein tyrosine phosphatase PTP-BL via an alternatively spliced PDZ domain. *Oncogene* **19** 3894–3901.
- Fajans SS, Bell GI & Polonsky KS 2001 Molecular mechanisms and clinical pathophysiology of maturity-onset diabetes of the young. *New England Journal of Medicine* **345** 971–980.
- Froguel P, Zouali H, Vionnet N, Velho G, Vaxillaire M, Sun F, Lesage S, Stoffel M, Takeda J & Passa P 1993 Familial hyperglycemia due to mutations in glucokinase: definition of a subtype of diabetes mellitus. *New England Journal of Medicine* **328** 697–702.
- Gey G & Gey M 1936 Maintenance of human normal cells in continuous culture; preliminary report; cultivation of mesoblastic tumours and normal cells and notes on methods of cultivation. *American Journal of Cancer* **27** 45–76.
- Gresh L, Fischer E, Reimann A, Tanguy M, Garbay S, Shao X, Hiesberger T, Fiette L, Igarashi P, Yaniv M *et al.* 2004 A transcriptional network in polycystic kidney disease. *EMBO Journal* **23** 1657–1668.

- Gunton JE, Kulkarni RN, Yim S, Okada T, Hawthorne WJ, Tseng YH, Roberson RS, Ricordi C, O'Connell PJ, Gonzalez FJ *et al.* 2005 Loss of ARNT/HIF1 β mediates altered gene expression and pancreatic-islet dysfunction in human type 2 diabetes. *Cell* **122** 337–349.
- Harries LW, Ellard S, Jones RW, Hattersley AT & Bingham C 2004 Abnormal splicing of hepatocyte nuclear factor-1 β in the renal cysts and diabetes syndrome. *Diabetologia* **47** 937–942.
- Haumaitre C, Barbacci E, Jenny M, Ott MO, Gradwohl G & Cereghini S 2005 Lack of TCF2/vHNF1 in mice leads to pancreas agenesis. *PNAS* **102** 1490–1495.
- Herrmann L, Dittmar T & Erdmann KS 2003 The protein tyrosine phosphatase PTP-BL associates with the midbody and is involved in the regulation of cytokinesis. *Molecular Biology of the Cell* **14** 230–240.
- Hildesheim J, Bulavin DV, Anver MR, Alvord WG, Hollander MC, Vardanian L & Fornace AJ Jr 2002 Gadd45a protects against UV irradiation-induced skin tumors, and promotes apoptosis and stress signaling via MAPK and p53. *Cancer Research* **62** 7305–7315.
- Hollander MC, Sheikh MS, Bulavin DV, Lundgren K, Augeri-Henmueller L, Shehee R, Molinaro TA, Kim KE, Tolosa E, Ashwell JD *et al.* 1999 Genomic instability in Gadd45a-deficient mice. *Nature Genetics* **23** 176–184.
- Horikawa Y, Iwasaki N, Hara M, Furuta H, Hinokio Y, Cockburn BN, Lindner T, Yamagata K, Ogata M, Tomonaga O *et al.* 1997 Mutation in hepatocyte nuclear factor-1 β gene (TCF2) associated with MODY. *Nature Genetics* **17** 384–385.
- Lee JY, Ristow M, Lin X, White MF, Magnuson MA & Hennighausen L 2006 RIP-Cre revisited: evidence for impairments of pancreatic β -cell function. *Journal of Biological Chemistry* **281** 2649–2653.
- Lindner TH, Njolstad PR, Horikawa Y, Bostad L, Bell GI & Sovik O 1999 A novel syndrome of diabetes mellitus, renal dysfunction and genital malformation associated with a partial deletion of the pseudo-POU domain of hepatocyte nuclear factor-1 β . *Human Molecular Genetics* **8** 2001–2008.
- Macfarlane WM, Shepherd RM, Cosgrove KE, James RF, Dunne MJ & Docherty K 2000 Glucose modulation of insulin mRNA levels is dependent on transcription factor PDX-1 and occurs independently of changes in intracellular Ca²⁺. *Diabetes* **49** 418–423.
- Maestro MA, Boj SF, Luco RF, Pierreux CE, Cabedo J, Servitja JM, German MS, Rousseau GG, Lemaigre FP & Ferrer J 2003 Hnf6 and Tcf2 (MODY5) are linked in a gene network operating in a precursor cell domain of the embryonic pancreas. *Human Molecular Genetics* **12** 3307–3314.
- Malecki MT, Jhala US, Antonellis A, Fields L, Doria A, Orban T, Saad M, Warram JH, Montminy M & Krolewski AS 1999 Mutations in NEUROD1 are associated with the development of type 2 diabetes mellitus. *Nature Genetics* **23** 323–328.
- Mendel DB, Hansen LP, Graves MK, Conley PB & Crabtree GR 1991 HNF-1 α and HNF-1 β (vHNF-1) share dimerization and homeo domains, but not activation domains, and form heterodimers *in vitro*. *Genes and Development* **5** 1042–1056.
- Nishigori H, Yamada S, Kohama T, Tomura H, Sho K, Horikawa Y, Bell GI, Takeuchi T & Takeda J 1998 Frameshift mutation, A263fsinsGG, in the hepatocyte nuclear factor-1 β gene associated with diabetes and renal dysfunction. *Diabetes* **47** 1354–1355.
- Owen K & Hattersley AT 2001 Maturity-onset diabetes of the young: from clinical description to molecular genetic characterization. *Best Practice and Research. Clinical Endocrinology and Metabolism* **15** 309–323.
- Pearson ER, Starkey BJ, Powell RJ, Gribble FM, Clark PM & Hattersley AT 2003 Genetic cause of hyperglycaemia and response to treatment in diabetes. *Lancet* **362** 1275–1281.
- Pearson ER, Badman MK, Lockwood CR, Clark PM, Ellard S, Bingham C & Hattersley AT 2004 Contrasting diabetes phenotypes associated with hepatocyte nuclear factor-1 α and -1 β mutations. *Diabetes Care* **27** 1102–1107.
- Rangnekar VM 1998 Apoptosis mediated by a novel leucine zipper protein Par-4. *Apoptosis* **3** 61–66.
- Ryffel GU 2001 Mutations in the human genes encoding the transcription factors of the hepatocyte nuclear factor (HNF)1 and HNF4 families: functional and pathological consequences. *Journal of Molecular Endocrinology* **27** 11–29.
- Sells SF, Han SS, Muthukumar S, Maddiwar N, Johnstone R, Boghaert E, Gillis D, Liu G, Nair P, Monnig S *et al.* 1997 Expression and function of the leucine zipper protein Par-4 in apoptosis. *Molecular and Cellular Biology* **17** 3823–3832.
- Senkel S, Lucas B, Klein-Hitpass L & Ryffel GH 2005 Identification of target genes of the transcription factors HNF-1 β and HNF-1 α in a human embryonic kidney cell line. *Biochimica et Biophysica Acta* **1731** 179–190.
- Sheikh MS, Hollander MC & Fornace AJ Jr 2000 Role of Gadd45 in apoptosis. *Biochemical Pharmacology* **59** 43–45.
- Shih DQ, Screenan S, Munoz KN, Philipson L, Pontoglio M, Yaniv M, Polonsky KS & Stoffel M 2001 Loss of HNF-1 α function in mice leads to abnormal expression of genes involved in pancreatic islet development and metabolism. *Diabetes* **50** 2472–2480.
- Stoffers DA, Ferrer J, Clarke WL & Habener JF 1997 Early-onset type-II diabetes mellitus (MODY4) linked to IPF1. *Nature Genetics* **17** 138–139.
- Thomas H, Senkel S, Erdmann S, Arndt T, Turan G, Klein-Hitpass L & Ryffel GU 2004 Pattern of genes influenced by conditional expression of the transcription factors HNF6, HNF4 α and HNF1 β in a pancreatic beta-cell line. *Nucleic Acids Research* **32** e150.
- Tomura H, Nishigori H, Sho K, Yamagata K, Inoue I & Takeda J 1999 Loss-of-function and dominant-negative mechanisms associated with hepatocyte nuclear factor-1 β mutations in familial type 2 diabetes mellitus. *Journal of Biological Chemistry* **274** 12975–12978.
- Waechter G, Thompson N, Nicod P & Bonny C 1996 Transcriptional activation of the GLUT2 gene by the IPF-1/STF-1/IDX-1 homeobox factor. *Journal of Molecular Endocrinology* **10** 1327–1334.
- Wang H, Maechler P, Hagenfeldt KA & Wollheim CB 1998 Dominant-negative suppression of HNF-1 α function results in defective insulin gene transcription and impaired metabolism-secretion coupling in a pancreatic beta-cell line. *EMBO Journal* **17** 6701–6713.
- Wang L, Coffinier C, Thomas MK, Gresh L, Eddu G, Manor T, Levitsky LL, Yaniv M & Rhoads DB 2004 Selective deletion of the Hnf1 β (MODY5) gene in beta-cells leads to altered gene expression and defective insulin release. *Endocrinology* **145** 3941–3949.
- Wang H, Iezzi M, Theander S, Antinozzi PA, Gauthier BR, Halban PA & Wollheim CB 2005 Suppression of Pdx-1 perturbs proinsulin processing, insulin secretion and GLP-1 signalling in INS-1 cells. *Diabetologia* **48** 720–731.
- Watada H, Kajimoto Y, Miyagawa J, Hanafusa T, Hamaguchi K, Matsuoka T, Yamamoto K, Matsuzawa Y, Kawamori R & Yamasaki Y 1996 PDX-1 induces insulin and glucokinase gene expressions in alphaTC1 clone 6 cells in the presence of betacellulin. *Diabetes* **45** 1826–1831.
- Wild W, Pogge von Strandmann E, Nastos A, Senkel S, Lingott-Frieg A, Bulman M, Bingham C, Ellard S, Hattersley AT & Ryffel GU 2000 The mutated human gene encoding hepatocyte nuclear factor 1 β inhibits kidney formation in developing *Xenopus* embryos. *PNAS* **97** 4695–4700.
- Wobser H, Dussmann H, Kogel D, Wang H, Reimertz C, Wollheim CB, Byrne MM & Prehn JH 2002 Dominant-negative suppression of HNF-1 α results in mitochondrial dysfunction, INS-1 cell apoptosis, and increased sensitivity to ceramide-, but not to high glucose-induced cell death. *Journal of Biological Chemistry* **277** 6413–6421.
- Yamagata K, Furuta H, Oda N, Kaisaki PJ, Menzel S, Cox NJ, Fajans SS, Signorini S, Stoffel M & Bell GI 1996a Mutations in the hepatocyte nuclear factor-4 α gene in maturity-onset diabetes of the young (MODY1). *Nature* **384** 458–460.
- Yamagata K, Oda N, Kaisaki PJ, Menzel S, Furuta H, Vaxillaire M, Southam L, Cox RD, Lathrop GM, Boriraj VV *et al.* 1996b Mutations in the hepatocyte nuclear factor-1 α gene in maturity-onset diabetes of the young (MODY3). *Nature* **384** 455–458.
- Yang Q, Yamagata K, Fukui K, Cao Y, Nammo T, Iwashashi H, Wang H, Matsumura I, Hanafusa T, Bucala R *et al.* 2002 Hepatocyte nuclear factor-1 α modulates pancreatic beta-cell growth by regulating the expression of insulin-like growth factor-1 in INS-1 cells. *Diabetes* **51** 1785–1792.

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