Functional Analysis of Two Promoters for the Human Mitochondrial Glycerol Phosphate Dehydrogenase Gene*

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Mitochondrial glycerol phosphate dehydrogenase (mGPD) is abundant in the normal pancreatic insulin cell, but its level is lowered 50% by diabetes. To evaluate mGPD expression, we cloned and characterized the 5′-flanking region of the human mGPD gene. The gene has two alternative first exons and two promoters. The downstream promoter (B) is 10 times more active than the upstream promoter (A) in insulin-secreting cells (INS-1) and HeLa cells. Promoter B has higher activity in INS-1 than in non-β cells. Deletion and mutation analysis suggested that a NRF-2 binding site at −94 to −101 and an E2F binding site at −208 to −215 are important regulatory cis elements in promoter B. Gel mobility shift assays indicated that the −94 to −101 region binds the NRF-2 protein. When INS-1 cells were maintained in the presence of high glucose (25 mM) for 7 days, mGPD was the only 1 of 6 enzyme activities lowered (53%). mGPD promoter B activity was reduced by 60% in INS-1 cells by the high glucose, but in HepG2 cells and HeLa cells, promoter B activity was unchanged or slightly increased. Deletion analysis indicated the glucose responsiveness was distributed across the region from −340 to −260 in promoter B. The results indicate that mGPD gene transcription in the beta cell is regulated differently from other cells and that decreased mGPD promoter B transcription is at least in part the cause of the decreased beta cell mGPD levels in diabetes.

Mitochondrial glycerol phosphate dehydrogenase (mGPD) is encoded in the nucleus and located in the outer surface of the inner mitochondrial membrane. mGPD along with the cytosolic NAD-linked glycerol phosphate dehydrogenase forms the glycerol phosphate shuttle that catalyzes the interconversion of glycerol phosphate and dihydroxyacetone phosphate to oxidize cytosolic NADH by transferring reducing equivalents from the cytosol to mitochondria. Since the enzyme activity of mGPD in the pancreatic beta cell is among the highest in the body (1, 2), it has been proposed that the enzyme plays a key role in glucose-induced insulin secretion (5). There are numerous reports that the activity of the enzyme is decreased in the pancreatic islet, but not in other tissues, of genetic models of non-insulin-dependent diabetes (NIDDM) (6–13). The enzyme is also reported to be decreased in the islet in humans with NIDDM (14). However, this decrease appears to be an acquired characteristic because it is present in experimentally induced models of NIDDM (7, 8) and because the enzyme level in the islet is restored to a normal level by normalizing the blood sugar with insulin (12).

In view of the various conditions that possibly influence the level of mGPD synthesis at the transcriptional or translational level, we cloned and characterized the 5′-flanking region of the human mGPD gene to begin to better understand the transcriptional regulation of mGPD expression. The results indicate that the human mGPD gene possesses two promoters and that the downstream promoter is more active in beta cells than in non-beta cells. The downstream promoter is 10-fold more active than the upstream promoter in transient expression assays. Deletion analysis, site-directed mutagenesis, and gel shift assays identified at least two known cis-acting regulatory elements in the downstream promoter. In the present work we show that the mGPD enzyme activity and mGPD promoter activity is lowered in INS-1 cells by long term exposure to a high concentration of glucose. Activities of other enzymes studied as controls were not lowered. However, the mGPD promoter activity is lower in beta cells (INS-1 cells) but is not lower in non-beta cells, such as human HepG2 hepatoma cells and HeLa cells, after long term exposure to a high concentration of glucose. The results of the current study suggest that transcription of the mGPD gene in the beta cell differs from other cells and that the negative transcriptional response to high glucose at least in part explains the low level of mGPD in hyperglycemic states.

EXPERIMENTAL PROCEDURES

Cell Culture—INS-1 cells were grown in RPMI 1640 medium (Life Technologies, Inc.) supplemented with 10% fetal calf serum, 50 μg/ml 2-mercaptoethanol, 1 mM pyruvic acid, and 10 mM HEPES. HeLa cells were grown in Dulbecco's modified Eagle's medium (Life Technologies, Inc.) supplemented with 10% fetal calf serum. H4-II-E cells were cultured in minimum essential Eagle's medium supplemented with 0.1 mM nonessential amino acids, 10% calf serum, and 10% fetal calf serum. All media contained 50 units/ml penicillin and 50 μg/ml streptomycin.

Determination of 5′-Terminal cDNA Sequence—The 5′-end of the human mGPD cDNA was extended and amplified using the 5′ rapid amplification of cDNA ends (RACE) according to the protocol provided (Life Technologies, Inc.). A mGPD gene-specific antisense primer 5′-GGTTGGCAGCCTCATAGG-3′ (bases 593 to 575 in exon 3) was used for reverse transcription of HeLa cell poly(A) + RNA. Polymerase chain reaction amplification of the 3′-tailed cDNA was carried out using the Abridged Anchor Primer (sense) provided by Life Technologies, Inc. and a nested antisense primer 5′-TCCCTGTAACTCGTCTGAAAT-3′ (bases 270 to 249 in exon 2 of the mGPD cDNA). The amplified products

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The abbreviations used are: mGPD, mitochondrial glycerol phosphate dehydrogenase; NIDDM, non-insulin-dependent diabetes; RACE, rapid amplification of cDNA ends; kb, kilobase(s); bp, base pair(s); GABP, GA-binding protein.

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were subcloned into the pCR2.1 vector using the TA cloning system (Invitrogen, San Diego, CA) and sequenced. Human mGPD Gene Promoter Constructs—A 3.4-kb fragment of exon 1A 5′-flanking sequence and a 1.3-kb fragment of exon 1B 5′-flanking sequence from genomic clones were cloned into the pGL3 Basic vector (Promega, Madison, WI) (Fig. 1A). Progressive deletions from the 5′ end of the 3.4-kb or 1.3-kb DNA fragment were prepared by cutting at restriction sites or by digestion with exonuclease III. In the mGPD-400 construct that contains a 400-bp fragment of the 5′-flanking region of exon 1B (Fig. 1B), the nucleotide sequence of 1.4 kb of the 5′-flanking region of the human mGPD was determined by 5′ RACE and the EST clones Alu rep (LTR8 rep, Alu rep). The locations matching the consensus sequences of exons 1A and 1B are identified by thin bars. Restriction enzymes sites are indicated as BstIII, E (EcoRI), K (KpnI), P (PstI), S (SmaI), Sa (SacI), X (XhoI), Xb (XbaI). B, the nucleotide sequence of 1.4 kb of the 5′-flanking region of the human mGPD. Lowercase characters show the sequences of exons 1A and 1B. The transcription start sites determined by 5′ RACE are indicated by an open triangle. The consensus sequences for AP1, AP2, E2F, E-box, and NRF-2 transcription factor binding sites are underlined.

**Fig. 1.** A, structure of the 5′-flanking region of the human mGPD gene. The exons 1A and 1B are identified by shaded boxes (ex1A, ex1B). The thick line identifies the 5.8 kb of this region that have been sequenced. Sequences showing homology to human repetitive elements are identified by striped bars (rep, LTR8 rep, Alu rep). The locations matching the consensus sequences for SP1, AP1, AP2, E2F, E-box, and NRF-2 transcription factor binding sites are underlined.

**B**

GAATTCGCGCGATTTAAGAGACATTAAATGAGTGAATCTAATACCATTTTAAAAAAATTACAC
CTTTTGTTCACACTAATTTTCTCCTTCTTGTGAAAGACCTTCCGCCCTCTTCCCTGATATT
TCCATACACAGATGGAGAAGATTATTTGACAAGGACAGAGCTCTGCGAACGAAAGCTGTGA
AGAACTGGGCTCCATTGAGCACCACCTTCCTGCTGGTCCCTGCTGCTCACTCTAA
GGATTTCAACAAAGTAAACGCTGCGATGCGCGAGTTGCGGAGGGTCGCGATTGAAAA

**Gel Mobility Shift Assay**—Nuclear extracts were prepared from HeLa cells and INS-1 cells for mobility shift DNA-binding protein assays (16, 17). A 30-mer wild type oligonucleotide 5′-AGCGGGGAGGAGGAATGGCGGGAGGAA-3′, which corresponds to the sequence from −110 to −81 in promoter B, and five oligonucleotides with single point mutations (G-100T, A-98C, G-96T, A-90C, and G-88T), which are indicated by bold letters in the wild type sequence, were synthesized. After end-labeling with [γ-32P]ATP and T4 kinase, the probe was annealed with an unlabeled complementary strand. Fifteen micrograms of crude nuclear extract was preincubated with poly(dI:dC) for 20 min at room temperature in binding buffer (8 mM Tris, 10 mM HEPES, pH 8.0, 1 mM EDTA). The activity of all enzymes except mGPD was estimated in homogenates of whole cells in a timed and stopped assay using continuous spectrophotometric assays at 37 °C. mGPD activity was estimated in homogenates of whole cells in a timed and stopped assay as described previously (2).
of the mGPD gene by the primer extension method and RNase protection assay were not successful because of the low abundance of mGPD mRNAs. Therefore, we used 5′ RACE to determine the transcriptional start sites of the two transcripts. HeLa cell poly(A) RNA was used as the template. Eighteen clones of various lengths were characterized. Seven of them contained sequences from exon 1A, and 11 of them contained sequences from exon 1B. Three transcriptional start sites were detected for exon 1A and four for 1B (Fig. 1B). The longest clones containing the sequence from exon 1A correspond to the last 159 nucleotides of 358 bp of exon 1A of the EST clone R15742 from human brain. The difference in length between the transcripts from HeLa cells and the EST, which was from brain, may be due to a failure of reverse transcriptase to read through regions of secondary structure in the RNA due to its high G/C content, or this difference in length could be due to differences in transcription start sites between the two tissues. The 5′ end from four other clones containing the sequence of 1B corresponds to position of +1 of the previously published cDNA sequence by our laboratory (20), and we have designated this position as +1 (Fig. 1B). The 5′ end of the longest clone-containing sequence from exon 1B corresponds to the position −48. In addition, we identified three clones with 5′ ends at position of +33 of exon 1B and one clone with a 5′ end at position of −29 of exon 1B.

Mapping of the mGPD Promoter A Region—To map promoter activity of the 5′-flanking region of exon 1A, various 5′ progressive deletions starting from −3400 were prepared, and their activities were measured. Fig. 2A shows the relative luciferase activities of these constructs in INS-1 and HeLa cells. The activities of the constructs in the forward orientation were 7–15% of the activity of the mGPD-600B, which was set at 100%.
times higher than the activity of the construct possessing the reverse orientation (R3400). The construct containing 460 nucleotides (460A) had the highest activity (Fig. 2A).

Mapping of the mGPD Gene Promoter B—To map promoter B of the mGPD gene, progressive deletions from the 5′ end as well as a 3′ deletion were prepared, and their activities were measured. Fig. 2B shows the relative luciferase activities of these constructs after transient transfection into INS-1 cells or HeLa cells. All constructs contain 6 bp of the 5′ end of exon 1B and the promoter B region that lies immediately upstream of exon 1B. The activity produced by the 600B construct, which contains the entire region of the promoter B, was comparable with that of the SV40/enhancer control vector when expressed in INS-1 cells. The mGPD promoter B inserted in the reverse orientation in the pGL3 vector was used as a control. Its activity was 9.6% and 3.2% that of the mGPD-600B construct in INS-1 cells and HeLa cells, respectively. The promoterless pGL3-Basic vector was also used as a control, and its activity was less than 5% of mGPD-600B in both INS-1 and HeLa cells.

Progressive 5′ deletions from −600 to −400 (400B) still retained 85 and 80% activity in INS-1 and HeLa cells, respectively. Further deletions resulted in a sharp stepwise decrease in activity. Deleting bases −6 to −140 and retaining bases −141 to −400 (−400 to −140B) eliminated about 90% of promoter activity, indicating that the proximal 140 bases contains strong positive elements.

Comparison of mGPD Promoter A and B Activities—The activities of two promoter A constructs (3400A and 460A) that have the highest activity were compared with the activities of two promoter B constructs that have the highest activity (600B and 400B). The promoter A constructs were 10% as active as the promoter B constructs in both INS-1 cells and HeLa cells (Fig. 3).

mGPD-400B Promoter Activity in INS-1 and Non-beta Cells—Since the activity of promoter B is much higher than that of promoter A, we further characterized promoter B. First we compared promoter B activity in non-beta cells including human HeLa cervical adenocarcinoma, human HepG2 hepatoblastoma, and rat H4-II-E hepatoma cells to that in INS-1 cells. The last cell line was derived from a rat pancreatic insulinoma (15) and expresses a high level of mGPD enzyme activity comparable with that of normal pancreatic islets.2 We compared the activity of pGL3-mGPD-400B with the activity of the pGL3

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2 M. J. MacDonald, unpublished data.
control vector, which contains the luciferase gene under control of the SV40 promoter in INS-1, HeLa, HepG2, and H4-II-E cells. Fig. 4A shows that the luciferase activity driven by mGPD-400B was 1.8-fold higher than that driven by the SV40 promoter in INS-1 cells, whereas the activities driven by mGPD-400B were 3, 5, and 43% that driven by SV40 in HeLa, HepG2, and H4-II-E cells, respectively. If the absolute light units of luciferase activity driven by mGPD-400B was normalized for the protein concentration of the cell lysates, the activity of the 400B construct expressed in INS-1 cells was 3- and 29-fold higher than that in HeLa and H4-II-E cells, respectively, and was slightly higher than in HepG2 cells (Fig. 4B).

**Internal Deletion Analysis of Promoter B**—Since the construct containing +1 to −400 nucleotides possesses 80% activity of the entire 600 bp of promoter B, a series of 25-nucleotide internal deletions from this region were prepared to attempt to identify important regulatory regions. Fig. 5 shows that removal of nucleotides −76 to −100 eliminated 50 and 70% of the promoter activity in INS-1 and HeLa cells, respectively, and that removal of sequence −201 to −225 eliminated 47 and 46% of the promoter activity in INS-1 and HeLa cells, respectively, indicating that these regions contain strong positive regulatory elements. The region from −252 to −276 also might contain important element(s) because removal of the region resulted in a 35 and 55% decrease in activity in INS-1 and HeLa cells, respectively. Examination of the sequence in this region revealed the presence of a Sp1 binding site between −280 and −275, suggesting this Sp1 may also contribute to promoter B activity, especially in HeLa cells.

To more explicitly identify potential regulatory elements in the region between −76 to −100 in promoter B, a series of 9-nucleotide internal deletions were prepared. The results suggest that the interval −94 to −102 contains an important regulatory element for the mGPD promoter B because removal of this sequence results in a 50% decrease in activity in INS-1 cells (Fig. 6A) and a 60% decrease in activity in HeLa cells (Fig. 6B). A similar study was also performed with the region −201 to −225. The deletion from −204 to −221 led to a decrease in relative activity of about 50% in both INS-1 cells (Fig. 6C) and HeLa cells (Fig. 6D). These data indicated that both the −94 to −102 and −204 to −221 regions contain regulatory sequences that significantly contribute to the activity of mGPD promoter B.

**Site-directed Mutagenesis of mGPD Promoter B Elements**—Examination of the DNA sequence of promoter B revealed the presence of a putative E2F (TTCGCGGT) binding site and a putative Sp1 (CCGCCC) binding site between −204 to −221 and a putative NRF-2 binding site (AGGAAG) between −94 and −102. To assess the contribution of these sites to promoter activity, the binding sites were altered by site-directed mutagenesis, and the activities of the mutant constructs were estimated. Fig. 7, A and B, shows that when the Sp1 site, CCGCCC, located at −220 to −225, was replaced by CAAAAC, the promoter construct still retained 90 and 85% activity in INS-1 and HeLa cells, respectively. However, when the E2F site, TTCGCGGT, located at −208 to −215 was mutated to TTCAAGGT, the promoter activity was decreased by 48 and 43% in INS-1 and HeLa cells, respectively. To study the role of the NRF-2 binding site in the −94 to −102 region, two point mutations (G-96T and A-98C) were created, and their activities were measured (Fig. 7, C and D). Adjacent to this NRF-2 site there is an NRF-2-like sequence (GGGAAG) at −93 to −88 that differs from the consensus sequence only in the first nucleotide (G instead of A). Two point mutations (G-88T and A-90C) were created in this region to serve as a control for the −94 to −102 region. The G-96T mutation resulted in 47 and 60% decreases in activity of INS-1 cells and HeLa cells, respectively, whereas the A-98C mutation resulted in 32 and 42% decreases in activity in INS-1 cells and HeLa cells, respectively, as compared with the wild type (400B) construct. The mutations G-88T and A-90C did not dramatically alter the promoter activity. In fact the G-88T mutation increased the activity to twice that of the wild type construct. These results suggest that the E2F consensus sequences in the −204 to −221 region and NRF-2 in −94 to −102 region significantly contribute to the activity of promoter B, but the Sp1 and NRF-2-like sequences in these regions do not.

**NRF-2 Protein Binds at the −102 to −94 Region in Promoter B**—To determine if the sequences at −102 to −94 and −93 to −88 are capable of binding proteins, we performed gel shift assays (Fig. 8). The wild type and mutated DNA sequences covering region −81 to −110 were used as probes and competitors in mobility gel shift studies as described under “Experimental Procedures.” As shown in Fig. 8, the wild type oligonucleotide caused retardation of two bands in INS-1 cells and three bands in HeLa cells, which were competed away by an excess of unlabeled oligonucleotide. The oligonucleotides that carried point mutations within the NRF-2 motif (G98T, A98C, and G100T) did not compete out the retarded bands in INS-1 or HeLa cell nuclear extracts. In contrast, the retarded bands were competed away by the oligonucleotides containing mutations G88T and A90C, which were at the NRF-2-like site. Taken together with the data from functional analysis of these point mutations in luciferase assays, these results suggest that the NRF-2 motif at −94 to −102 in mGPD promoter B plays an important role in the expression of the mGPD gene.

**Enzyme Activities in INS-1 Cells after Long Term Exposure to a High Concentration of Glucose**—Decreased activity of mGPD
in the pancreatic islets of several genetic models of NIDDM has been reported (6–13). However, in our laboratory the mGPD level in pancreatic islets cultured for 24 h at various of concentration of glucose did not change (21). To examine whether a longer term exposure of insulin cells to a high concentration of glucose causes a decrease in mGPD activity, we cultured the INS-1 cells in medium at 5 or 25 mM glucose for 4 or 7 days. Table I shows that mGPD activity in the cells exposed to 25 mM glucose for 4 days and for 7 days was 81 and 47%, respectively, that found in the cells cultured in 5 mM glucose for the same lengths of time. To discern whether this decrease in enzyme activity was a general phenomenon, activities of other enzymes were measured (Table I). The activities of isocitrate dehydrogenase, glucose-6-phosphate dehydrogenase, and glucokinase in INS-1 cells maintained in 25 mM glucose were similar to those in the cells maintained in 5 mM glucose, and the activity of cytosolic GPD in the cells cultured in 25 mM glucose was actually 3- and 5-fold higher than that in the cells cultured in 5 mM glucose for 4 and 7 days, respectively. The activity of malic enzyme was increased 1.5- and 2-fold after the cells were exposed to 25 mM glucose for 4 and 7 days, respectively.

**Effect of Long Term Exposure of Cells to Elevated Levels of Glucose on mGPD Promoter Activity**—The decrease in enzyme activity of mGPD in INS-1 cells after long term exposure to a high concentration of glucose may involve down-regulation of transcription of mGPD. To test this idea, we transiently transfected mGPD promoter constructs into INS-1 cells maintained in 5 or 25 mM glucose for 5 days and continued the exposure of the transfected cells to 5 or 25 mM glucose for another 2 days. Fig. 9A shows that culturing cells in 25 mM glucose decreased mGPD promoter B activity to about 40% that found in the cells cultured in 5 mM glucose, whereas the activities of mGPD promoter A and the SV40 promoter were not changed. This suggests that the glucose-induced down-regulation of mGPD activity may be a consequence of its influence on promoter B. The degree of decrease in luciferase activity showed a good correlation with the decrease in the enzyme activity in INS-1 cells treated with the high concentration of glucose. To investigate whether the effect of elevated glucose on the activity of mGPD promoter B was insulin cell-specific, non-beta cells, HepG2, and HeLa were transfected and cultured as described above. Fig. 9B shows that no decrease in the activity of mGPD promoter A or B was detected with either HeLa cells or HepG2 cells. In fact, the high concentration of glucose slightly increased the promoter activity of the 600B construct in both cell lines.

To define the region of promoter B involved in the glucose down-regulation of mGPD expression in INS-1 cells, the activities of a series of constructs carrying 5’ end deletions and one 3’ end deletion of promoter B were tested in INS-1 cells treated with 5 or 25 mM glucose (Fig. 10A). Deletions from the 5’ end to position –340 and from the 3’ end (position +6) to –140 had no significant effect upon the down-regulation of promoter B activity by glucose. Removal of the 5’ end to –260 completely abolished the down-regulation by high glucose. However, the activity of this construct in the presence of 5 mM glucose was already reduced to 30% that of the construct 400B. These results suggest that the sequence between –340 and –260 contains elements that may play a role in the down-regulation by high glucose of mGPD promoter B activity in beta cells.

To further map promoter B for glucose responsiveness, the promoter activity of the constructs with a series of 25-bp internal deletions from –376 to –201 were tested in INS-1 cells. Fig. 10B shows that deletions of single 25-bp fragments in this region did not abolish the response of the mGPD promoter B to a high concentration of glucose. It is possible that the regulation of mGPD expression in INS-1 cells by glucose is determined by multiple cis elements. Deletion of one or a few of them may not be sufficient to abolish the influence of glucose on promoter B activity. Since the NRF-2 element in the region –94 to –101 is a potential candidate for glucose responsiveness, we also assayed the construct with the deletion from –94 to –102, where the NRF-2 motif is located. Fig. 10B shows that deletion of the NRF-2 binding site did not abolish the responsiveness of the promoter B to high glucose. The construct with point mutation at NRF-2 site also did not abolish the response (data not shown). These results suggest that NRF-2 may not be involved in down-regulation of mGPD promoter B activity by glucose, although it is an important element for the basal activity of promoter B.
DISCUSSION

We cloned and sequenced the 5′-flanking region of the human mGPD gene. When this sequence and the sequence of the HeLa cDNA clone isolated by our laboratory (19) were compared with GenBank™, a match was found to EST clones R15742, T23788, and Z38279. A region of 358 bp of EST clone R15742 corresponded to a region in the mGPD gene located 605 bp upstream from the first exon of the HeLa cDNA clone (Fig. 1A). In the EST clone, the 358-bp region was spliced directly to exon 2 of the gene (20), indicating that this upstream region was an alternative first exon. The evidence for two alternative exons has been strengthened by a report from another group (4) of a human pancreatic islet mGPD cDNA containing this alternative first exon (1A) and more recently by a report of the sequence of another EST clone (AA169176) possessing the sequence identical to the portion of the region we designated exon 1B. We have designated the 5′-flanking region of exon 1A as promoter A and the 5′ flanking region of exon 1B as promoter B. No consensus TATA box or CAAT box were present in the first 100 bp upstream from the transcription initiation sites in either promoter A or promoter B. Promoters lacking a TATA box but containing GC boxes are frequently found in housekeeping genes, which usually have several transcription start sites (22). However, studies have demonstrated that some highly regulated genes, including immediate early genes, developmentally regulated genes, and tissue-specific genes also have promoters that lack TATA boxes (23). Sequence analysis of the immediate upstream of exon 1A of mGPD gene shows the presence of an E-box, but this region lacks the GC-box, AP1, AP2, E2F, and NRF-2 transcription factor binding sites that are present in promoter B (Fig. 1B). This together with the presence of human repeat elements in the more upstream region of promoter A may explain the lower activity of promoter A compared with promoter B. There is some evidence to suggest that Alu repeat elements function as either positive or negative regulatory elements to influence the expression of nearby genes (24–26).

Although the human mGPD gene uses two different promoters, the rat mGPD gene probably utilizes three promoters since the 5′ end of the rat mGPD gene contains three alternative first exons. These exons are transcribed from different promoters, and each of these is spliced to the common second exon (27). The sequence of rat exons 1a and 1b show homology to human exons 1A and 1B, respectively, whereas rat exon 1c does not show homology to the human transcripts (27, 28). The presence of this third promoter probably explains why the rat is the only animal known to express a high level of mGPD in testis (29, 30). In the rat, promoter A is utilized primarily in brain, promoter B is used ubiquitously, and promoter C is used only in the testis (27). Multiple and tissue-specific promoters have been reported for several other genes including pyruvate carboxylase, acyl-CoA synthetase, the glucocorticoid receptor, and macrophage colony-stimulating factor receptor (31–35). The enzyme activity of mGPD shows marked tissue differences. Highest enzyme activity of mGPD shows marked tissue differences. Highest activity is found in the pancreatic islet (1–3, 36–40), testis (rat) (27). Multiple and tissue-specific promoters have been reported for several other genes including pyruvate carboxylase, acyl-CoA synthetase, the glucocorticoid receptor, and macrophage colony-stimulating factor receptor (31–35). The enzyme activity of mGPD shows marked tissue differences. Highest activity is found in the pancreatic islet (1–3, 36–40), testis (rat only (30)), and brown adipose tissue (3); intermediate activity is found in brain and skeletal muscle (29); and low activity is found near genes (24–26).


table 1

| Enzyme activity | Time in presence of glucose | 7 Days |
|-----------------|----------------------------|-------|
| Glucose concentration in medium | 5 mm | 25 mm | 5 mm | 25 mm |
| nmol product/min/mg of protein | | | | |
| mGPD | 79 ± 6 | 63 ± 2 | 76 ± 2 | 37 ± 5<sup>a</sup> |
| Cytosolic GPD | 321 ± 6 | 927 ± 10<sup>b</sup> | 256 ± 20 | 1205 ± 171<sup>a</sup> |
| Malic enzyme | 24 ± 4 | 38 ± 2<sup>b</sup> | 22 ± 2 | 51 ± 5<sup>b</sup> |
| Isocitrate dehydrogenase | 148 ± 14 | 142 ± 10 | 145 ± 4 | 135 ± 12 |
| Glucose-6-phosphate dehydrogenase | 115 ± 10 | 151 ± 18 | 127 ± 29 | 136 ± 21 |
| Glucokinase | 43 ± 5 | 40 ± 4 | 44 ± 4 | 46 ± 2 |

<sup>a</sup> p < 0.005 compared with cells maintained in 5 mm glucose.
<sup>b</sup> p < 0.001 compared with cells maintained in 5 mm glucose.

3 L. J. Brown, unpublished data.
in other tissues (29). The existence of the alternate promoters in the mGPD gene is of potential interest because it could provide an explanation for the tissue-specific regulation of mGPD.

That previous studies (1–3, 36–40) showed mGPD enzyme activity to be extremely high in rodent and human insulin cells suggests the enzyme is important for insulin secretion. In the present work, we compared promoter B activity in the insulinoma cell line, INS-1, to that in non-beta cell lines, including human HeLa cervical adenocarcinoma, human HepG2 hepatoblastoma, and rat H4-II-E hepatoma cells, using the SV40 promoter as a control and found that the activity of promoter B is 1.8-fold higher than that of the SV40 promoter expressed in INS-1 cells, whereas in other cells the activity of the mGPD promoter B is 3 to 43% that of SV40 (Fig. 4).

Ferrer et al. (4) showed the presence of exons 1A and 1B in RNA from human pancreatic islets, insulinomas, and other tissues by the reverse transcription-polymerase chain reaction. Since the reverse transcription-polymerase chain reaction was not performed quantitatively, the proportions of the two transcripts within a single tissue and the relative level of these two transcripts in different tissues are not certain. However, the evidence of the usage of exon 1B in human pancreatic islets and insulinomas and the evidence of the high promoter activity in INS-1 cells may explain the differences in the enzyme activity of mGPD between the beta cells and non-beta cells.

To further characterize the cis elements involved in the transcriptional regulation of the mGPD gene, we performed deletion and mutation analysis. We determined that E2F binding site regions from –204 to –221 is important for promoter B activity in both INS-1 and HeLa cells. An alignment analysis of the human mGPD promoter with the rat promoter shows the
E2F site to be conserved. A NRF-2 sequence in the region between −96 to −101 also appears to be essential for promoter B activity. When a single nucleotide was mutated in this consensus NRF-2 sequence, the promoter activity was reduced 50% (Fig. 7, C and D). Gel mobility shift assays (Fig. 8) suggested that the NRF-2 binding site at the region between −94 and −101 of promoter B is capable of binding proteins. The two retarded bands detected with the INS-1 nuclear extract and three with the HeLa nuclear extract may arise through heteromultimers of NRF-2 subunits. A similar gel shift pattern has been reported by Virbasius et al. (41) when studying the promoter of cytochrome c oxidase subunit IV with HeLa cell nuclear extract. Human NRF-2 is composed of five subunits, α, β1, β2, γ1, and γ2. Only the α subunit has intrinsic DNA binding ability. The other subunits associate with the α subunit and participate in the formation of heteromeric complexes with distinct binding properties and regulate NRF-2-mediated transcription by competing with each other (41, 42). NRF-2 belongs to the Ets family of transcription factors (41, 43–48). There is evidence that GABP, a homologue of mouse GABP, and recent reports suggest that NRF-2 (GABP) regulates the expression of nuclear-enacting factors from tissue to tissue. In addition to mGPD, levels of glucose transporter 2 and pyruvate carboxylase are also decreased in the pancreatic islet of rodent models of NIDDM (12, 51–56). The coordinate decrease in a number of these key proteins of glucose metabolism could be the consequence of an adaptive decrease in the activities of transcription factors that influence genes that encode these proteins (57). This would have the effect of moderating the adverse effects of hyperglycemia on beta cell metabolism (13).

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