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Involvement of specific proteins (Sp1/Sp3) and nuclear factor Y in basal transcription of the distal promoter of the rat pyruvate carboxylase gene in β-cells☆

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Abstract

Pyruvate carboxylase plays diverse roles in different biosynthetic pathways, including glucose-induced insulin secretion in pancreatic β-cells. We have localized the control region of the P2 promoter by generating a series of 5′-nested deletion constructs, and both 25- and 9-bp internal deletion constructs, as well as by performing site-directed mutagenesis. Transient transfections of these constructs into INS-1 cells identified a CCAAT box and a GC box that are located at −65/−61 and −48/−41, respectively, as the important determinants. Disruption of the GC box resulted in a 4-fold reduction of the reporter activity, while disruption of the proximal CCAAT box (−65/−61) but not the distal CCAAT box (−95/−91) increased the reporter activity by 3-fold. Simultaneous disruptions of both the GC box and the CCAAT box reduced the reporter activity to a level that was close to that of the single GC box mutation. Electrophoretic mobility shift assays (EMSAs) and supershift EMSAs using nuclear extract from INS-1 cells demonstrated that Sp1 and Sp3 bind a GC box while the nuclear factor Y was shown to bind the proximal but not the distal CCAAT box.

Keywords: Pyruvate carboxylase; Biotin enzyme; Glucose-induced insulin release; Gene transcription; Sp1; Sp3; NF-Y

The pancreatic β-cell is the major fuel sensor in our body in that it quickly responds to an altered level of plasma glucose. After meals, an elevated level of absorbed glucose triggers the transportation of glucose via the glucose transporter (GLUT2) into β-cells. This allows glucose to enter glycolysis to produce pyruvate. Pyruvate then enters the mitochondria and is metabolized via two routes, i.e., oxidative decarboxylation by pyruvate dehydrogenase and carboxylation by pyruvate carboxylase (PC) [1,2]. This is strongly indicated by the substantial abundance of PC and pyruvate dehydrogenase mRNAs in pancreatic islets [1]. Short-term exposure to elevated concentrations of glucose also increases the transcription rates of both genes [3]. Inhibition of PC activity with phenylacetic acid results in the reduction of glucose-induced insulin release by both INS-1 cells and pancreatic islets, and further suggests the important role of PC in insulin secretion [4]. A recent study has also shown that the increased pyruvate flux via PC was essential for glucose-stimulated insulin secretion in β-cells [5]. Taken together, it has been proposed that the oxidation of pyruvate by pyruvate

☆ *Abbreviations:* PC, pyruvate carboxylase; Sp, specific protein; NF-Y, nuclear factor Y; P1, proximal promoter; P2, distal promoter; EMSA, electrophoretic mobility shift assay; WT, wild type.

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dehydrogenase and its carboxylation by PC allow an increase of ATP/ADP, NADPH/NADP, and malonyl CoA as the coupling factors that indirectly trigger insulin biosynthesis and secretion [6,7].

The physiological data on PC and insulin secretion were further supported in animal studies. In the genetically obese Zucker fatty rat and the GK rat, it has been shown that PC protein and activity in the pancreatic islets are 50% lower than in normal rats [8,9]. This has been suggested to be an adaptive response of β-cells to prevent self-destruction caused by overproduction of insulin to compensate for insulin resistance [8]. A recent study has shown that the hypoglycemia induced by a partial pancreatectomy causes a progressive hypertrophy of β-cells associated with the loss of insulin secretion machinery and decreased expression of β-cell metabolic enzymes including PC [10]. These data further support the link between PC and insulin secretion in β-cells.

Mammalian PC is located in the mitochondrial matrix [11], with its activity being highest in liver, kidney, adipose tissue, and pancreatic islets [12]. Characterization of the PC gene in rat demonstrated that it possesses two tissue-specific promoters, i.e., the proximal (P1) and the distal (P2) promoter, which are responsible for alternative transcription from a single gene [13]. Transcripts generated from both promoters share the same coding sequence but differ in their 5′-untranslated regions as a result of differential splicing [13,14]. The P1 promoter functions in gluconeogenic (liver and kidney) and lipogenic tissues. Conversely, the P2 promoter is highly active in pancreatic β cells where it serves the anaplerotic role mentioned above [15]. Here we have identified the region of the P2 promoter and the transcription factors that together regulate PC promoter activity. We demonstrate that the binding sites specifically recognized by the Sp-transcription factor family and NFY nuclear factor are important for the expression of a reporter gene in transfected insulin secreting cells (INS-1).

Materials and methods

Generation of reporter constructs. A 1.1 kb fragment of the rat PC distal promoter [P2] [13] was deleted from its 5′-end by using *Sac*I,

Table 1
Sequences of the oligonucleotides used for mutagenesis and EMSA

Oligonucleotide	Sequences
Mu1F	5′-CTCGAGGTCGACAGGGGTAGGTGG-3′
Mu1R	5′-CCACCTACCCCTGTCGACCTCGAG-3′
Mu2F	5′-CGCTTAAACGCGACCTGAAAGGGG-3′
Mu2R	5′-CCCCTTTCAGGTCGCGTTTAAGCG-3′
Mu3F	5′-TGCAGGGAGGAGTAGGGTCATTCA-3′
Mu3R	5′-TGAATGACCCTACTCCTCCCTGCA-3′
Mu4F	5′-AGCGGTGAATGAGGGGATGGGCTG-3′
Mu4R	5′-CAGCCCATCCCCTCATTCACCGCT-3′
Mu5F	5′-CCAATCTTTGGAAAAGTCTTACGG-3′
Mu5R	5′-CCGTAAGACTTTTCCAAAGATTGG-3′
Mu6F	5′-CTCAACCAATGGCTGCAGCAAGTT-3′
Mu6R	5′-AACTTGCTGCAGCCATTGGTTGAG-3′
Mu7F	5′-CTCAACCAATGGCGGGCGGAGCCA-3′
Mu7R	5′-TGGCTCCGCCCGCCATTGGTTGAG-3′
Mu8F	5′-AATGGAAAGTCTAGCCAGTGCTGC-3′
Mu8R	5′-GCAGCACTGGCTAGACTTTCCATT-3′
Mu9F	5′-AGTCTTACGGGCCTCCAGCAAGTT-3′
Mu9R	5′-AACTTGCTGCAGGCCCGTAAGACT-3′
Mu10F	5′-TGGAAAGTCTTACCAGTGCTGCAG-3′
Mu10R	5′-CTGCAGCACTGGTAAGACTTTCCA-3′
WT Sp1(−58/−35) [+]	5′-AAAGTCTTACGGGCGGAGCCAGTG-3′
WT Sp1 (−58/+35) [−]	5′-TGCAGCACTGGCTCCGCCCGTAAG-3′
Sp1m (+)	5′-AAAGTCTTACTTTTGGAGCCAGTG-3′
Sp1m (−)	5′-CACTGGCTCCAAAAGTAAGACTTT-3′
Cons Sp1 (+)	5′-TATTCGATCGGGGCGGGGCGAGC-3′
Cons Sp1 (−)	5′-TGCTCGCCCCGCCCCGATCGAAT-3′
WT CCAAT (−65/−61) [+]	5′-GGGCTGTCTCAACCAATGGAAAGT-3′
WT CCAAT (−65/−61) [−]	5′-GTAAGACTTTCCATTGGTTGAGAC-3′
WT CCAAT (−96/−92) [+]	5′-AGGGTCATTCATCCAATCTTTGGA-3′
WT CCAAT (−96/−92) [−]	5′-TCCCCTCCAAAGATTGGATGAATG-3′
MuNF-Y1F	5′-GCTGTCTCAAAACGCGGAAAGTCTT-3′
MuNF-Y1R	5′-AAGACTTTCCGCGTTTTGAGACAGC-3′
MuNF-Y2F	5′-GGTCATTCATAACGCCTTTGGAGG-3′
MuNF-Y2R	5′-CCCTCCAAAGGCGTTATGAATGACC-3′