Regulatory Elements Involved in Human *pdx-1* Gene Expression

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The pancreatic duodenal homeodomain transcription factor (PDX-1) is an essential regulator of pancreatic endocrine cell development and adult islet β-cell function. PDX-1 function appears to be well conserved, as its absence in humans and mice leads to agenesis of the pancreas (1,2). The gene is expressed both in endocrine and exocrine cells of the developing pancreas; however, in the adult pancreatic islet, its expression is restricted to the β-cells, where it acts as the glucose-sensitive transcription factor of the insulin gene (3,4). In mice, β-cell–selective disruption of *pdx-1* leads to diabetes associated with reduced insulin and GLUT2 expression (5). To identify key components that direct the expression of the *pdx-1* gene to β-cells, we performed deletion analysis of 7 kb of the 5′ flanking region of the human *pdx-1*. We identified a novel β-cell–specific distal enhancer element that showed no sequence homology with the mouse *pdx-1* gene.

Because it appears that PDX-1 function is similar in humans and rodents, we deduced that conserved sequences located within the 5′ flanking region of the gene would be of importance for its expression. Comparison of 4.5-kb upstream regions of the human and mouse *pdx-1* genes revealed that, in addition to the proximal promoter region described by Sharma et al. (6), only three relatively short sequences designated PH1, PH2, and PH3 (for *pdx-1* homology 1–3) were highly conserved between human and mouse. Using transient transfection experiments, we showed that PH1 and PH2 preferentially drive β-cell–specific expression of a reporter gene. Using DNAse I footprinting analyses and electrophoretic mobility shift assays, we demonstrate that PH1 element binds the transcription factor PDX-1 adjacent to the endodermal factor hepatocyte nuclear factor (HNF)-3β, where they act cooperatively to transactivate a PH1-driven reporter construct. Similar studies reveal that HNF-3β mediates the transcriptional activity of the PH2 domain. Our results suggest a possible feedback mechanism where PDX-1 could regulate its own expression.

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HNF, hepatocyte nuclear factor; PH, PDX-1 homology.

RESULTS

Human *pdx-1* sequences involved in β-cell–specific transcriptional activity. To delineate the putative DNA sequences controlling *pdx-1* gene expression, we performed a deletion analysis of the 5′ flanking region of human *pdx-1* extending from –7 to +0.117 kb. The fragment spanning 3.7 kb of the upstream region conferred strong β-cell–specific expression to the reporter gene, suggesting the presence of a strong positive regulatory element(s).

The human *pdx-1* sequence –3.7 to –3.4 kb acts as a β-cell–specific enhancer element. Detailed deletion analysis of 3.7-kb fragment allowed us to delineate a regulatory element spanning the region from –3.7 to –3.4 kb that exhibits no homology with the mouse *pdx-1* sequence. This element had the characteristics of an enhancer and strongly activated the minimal *pdx-1* promoter in a tissue-specific manner.

β-Cell expression of conserved sequences in the human *pdx-1* gene. Sequence comparison of 4.5 kb upstream of the human and mouse *pdx-1* promoter regions reveal three highly conserved elements, PH1, PH2, and PH3. The fragments extending from –2.8 to –2.6 kb (PH1), from –2.2 to –2.1 kb (PH2), and from –1.9 to –1.6 kb (PH3) present 94, 81, and 73% homology, respectively. When linked to the luciferase reporter gene driven by the thymidine kinase promoter and transiently transfected into HIT-T15 and CHO cells, the PH elements conferred β-cell–specific activation to a different extent. The PH1 region exhibits a 13-fold preferential induction in HIT-T15 vs. CHO cells compared with ~4- and 2.5- fold inductions for PH2 and PH3, respectively.

DNAse I footprinting of the human *pdx-1* PH elements. The transcriptional activity driven by the PH elements of human *pdx-1* gene suggested the presence of *cis*-acting–regulatory elements in these regions. To assess whether such putative elements interact with specific proteins, we performed DNAse I footprinting analysis using the PH fragments as probes and extracts from HIT-T15 and CHO cells. PH1 element contained two protected regions displaying a different digestion pattern in HIT-T15 and CHO cell extracts. PH2 element showed a hypersensitive site in the presence of HIT-T15 cell extracts.

The transcription factors PDX-1 and HNF-3β interact with the human PH1 and PH2 elements. To characterize the *trans*-acting factors binding to the protected regions within PH1 and PH2 elements, double-stranded oligonucleotides spanning these sequences were synthesized and used as probes with cell extracts from β- and non–β-cell lines in electrophoretic mobility shift assay. We demonstrated that two footprinted regions of PH1 element bind transcription factors present in β-cells. The analysis of putative binding sites allowed us to identify binding sites for

HNF-3β and PDX-1. Binding of these transcription factors to PH1 element was confirmed by specific antibodies. The same approach was used to identify factor binding to the PH2 element that appeared to be HNF-3β.

Combinatorial effect of PDX-1 and HNF-3β in the activation of the PH1 sequence in the human *pdx-1* gene. To investigate the effect of the PDX-1 and HNF-3β transcription factors in controlling gene expression driven by the PH1-conserved sequence, we performed transient transfection experiments in CHO cells, which endogenously lack both factors. It was shown that HNF-3β and PDX-1 separately activated the PH1-TK-luciferase construct in a dose-dependent manner. Most significantly, cotransfection with a constant amount of PDX-1 and increasing amounts of HNF-3β cooperatively stimulated the expression of the gene. Furthermore, both sites are necessary for the expression of the PH1 chimeric gene because mutations abolishing either PDX-1 or HNF-3β binding to their corresponding sequences significantly impaired the transcriptional activity of the gene in β-cells.

HNF-3β activates PH2-driven transcription. Transient transfection experiments in CHO cells demonstrated that increasing amounts of the HNF-3β expression plasmid transactivated the PH2-TK-luciferase construct in a dose-dependent manner. However, mutations that abolished HNF-3β binding to PH2 did not reduce the activity of this element in HIT-T15 cells.

SUMMARY

PDX-1 was shown to be expressed early during development in cells of both exocrine and endocrine origin; later it becomes restricted primarily to β-cells where it regulates the expression of β-cell–specific genes and mediates the glucose effect on insulin gene transcription. Therefore, it was important to identify the molecular mechanisms that specifically govern the expression of *pdx-1* in the mature β-cell. To address this question, we analyzed 7 kb of the 5′ flanking region of the human *pdx-1* gene. By transient transfections of β- and non–β-cell lines with different 5′ and 3′ deletions of that region, a strong β-cell-specific enhancer element located between –3.71 and –3.46 kb was revealed. We also sequenced about 4.5 kb of the human 5′ flanking region and compared it with that of the mouse *pdx-1* gene. This comparison revealed three short conserved regions, designated PH1, PH2, and PH3. We showed that HNF-3β can bind and stimulate the activity of the human PH1 and PH2 elements in non–β-cells. Results reported by Wu et al. (7) and Sharma et al. (6) also indicate that expression of the mouse *pdx-1* is controlled by an HNF-3–like element. Thus, it can be stated that at least some aspects of *pdx-1* expression rely on the transcription factor HNF-3β.

Because HNF-3β is not restricted to β-cells, the selective transcription of *pdx-1* is likely to rely on additional factors. Our findings that the PH1 enhancer element binds both HNF-3β and PDX-1 and that mutations in each individual site dramatically impair its transcriptional activity suggest that these factors cooperate with one another. We therefore propose that a possible feedback mechanism might control the expression of *pdx-1* at different stages during development.

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