β-Cell–Specific Expression of Insulin and PDX-1 Genes

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β-cell–restricted expression of the insulin gene relies on the interaction of 5′ flanking sequence motifs in the promoter region with a number of ubiquitous and islet-specific transcription factors. These interactions determine the temporal expression of the gene and its inducibility by physiological stimuli. Most of the studies on the regulation of insulin gene expression were conducted with rat insulin 1 and 2 and the human genes. They share a number of conserved *cis* elements in their 5′ flanking region and are probably regulated by similar *trans*-acting factors. Among such elements, the E and A boxes appear to be the major determinants of β-cell–specific insulin gene expression. The E boxes (E1 and E2), with the consensus CANNTG, bind transcription factors of the basic helix-loop-helix family. The A boxes (A1–A5) containing AT-rich sequences bind factors that belong to the homeodomain-containing protein family. The most conserved A1 and A3 boxes in the insulin promoter bind the pancreatic duodenal homeobox transcription factor (PDX-1), the mammalian homolog of the *Xenopus laevis* XlHbox8, previously described as insulin promoter factor-1, somatostatin transcription factor-1 (STF-1), islet duodenal homeobox-1 (IDX-1), glucose-sensitive factor (GSF), and insulin upstream factor-1 (IUF-1). Gene disruption experiments in mice showed that PDX-1 is needed for pancreas formation. Thus, tissue-specific expression of the insulin gene appears to depend on a combination of transcription factors, some of which are restricted to the β-cell.

Because of its central role in metabolic control, tight regulation of insulin production and release is critical. Inducible transcription may depend on the very same sequences in the promoter/enhancer regions that control the cell-type specificity of the insulin gene. In pancreatic β-cells, glucose is the major regulator of insulin biosynthesis and secretion. Experiments with rat and human islets, as well as β-cell lines, indicated that glucose regulates insulin gene expression by simultaneously stimulating transcription and inhibiting proinsulin mRNA degradation.

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Received for publication 9 June 2000 and accepted 19 June 2000.

This article is based on a presentation at a symposium. The symposium and the publication of this article were made possible by an unrestricted educational grant from Les Laboratoires Servier.

GSF, glucose-sensitive factor; HNF, hepatocyte nuclear factor; PH, PDX homology.

The glucose-responsiveness of the insulin gene is similarly mediated by *cis*-acting elements within the 5′ flanking region relative to the transcription start site. We have previously demonstrated the presence of an islet-specific GSF, whose DNA binding to the A3 motif of the rat I and human insulin promoters is modulated by extracellular glucose. A single mutation in the GSF binding site of the human insulin promoter abolishes the stimulation by high glucose in normal islets. The sequence of the purified protein was shown to correspond to PDX-1. Mapping of GSF/PDX-1 functional domains in normal islet cells showed that its transactivation domain is located within the NH_2-terminal region of the protein and is also regulated by extracellular glucose levels. Thus, in addition to its essential roles in the development and differentiation of pancreatic islets and in β-cell–specific gene expression, PDX-1 protein functions as a mediator of the glucose effect on insulin gene transcription in differentiated β-cells.

In type 2 diabetes, chronic hyperglycemia has been suggested to be detrimental to β-cell functions. Long-term in vitro exposure of human pancreatic islet cultures to high glucose concentrations led to dramatic decreases in glucose-induced insulin release and islet insulin content, with increased proportion of proinsulin-like peptides relative to insulin. The depletion in insulin stores correlated with the reduction in insulin mRNA levels and human insulin promoter transcriptional activity. High glucose dramatically lowered the binding activity of the glucose-sensitive transcription factor GSF/PDX-1. The loss in GSF/PDX-1 binding also appears to correlate with the decrease in its mRNA levels. Most of these β-cell impairments were partially reversible when islets were subsequently returned to normal glucose concentrations.

To characterize the regulatory elements and potential transcription factors necessary for PDX-1 gene expression in β-cells, we constructed a series of 5′ and 3′ deletion fragments of the 5′ flanking region of the human PDX-1 gene that were fused to the luciferase reporter gene. We identified by transient transfections in β-cells and non–β-cells a novel β-cell–specific distal enhancer element. DNAse I footprinting analysis revealed two protected regions that bind the identified transcription factors hepatocyte nuclear factor (HNF)-3β, HNF-1α, SP1, and SP3. Furthermore, mutations within each motif abolished the binding of the corresponding factor(s) and dramatically impaired the enhancer activity, therefore suggesting that these factors cooperate with one another. As PDX-1 function appears to be similar in humans and mice, we analyzed the functional conservation of homologous sequences important for the maintenance and the cell-specific regulation of the gene. Apart from the proximal promoter

region, three highly homologous (PH1–PH3) sequences were apparent in the human and mouse 5′ flanking regions of the gene. By transient transfections in β-cells and non–β-cells, we show that mainly PH1 and PH2 preferentially confer β-cell–specific activation on a heterologous promoter. DNAse I footprinting and binding analyses reveal that both bind and are transactivated by HNF-3β. Furthermore, the PH1 enhancer element also binds the PDX-1 transcription factor, suggesting a possible autoregulatory loop as a mechanism for PDX-1 to control its own expression.

The elucidation of the mechanism underlying glucose-regulated insulin biosynthesis may lead to a better understanding of the process of insulin production in both normal β-cells and states related to type 2 diabetes.