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Specific gene expression in pancreatic beta cells

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Objectives of research

Insulin is a crucial metabolic hormone produced exclusively in the pancreatic β cells. Impaired β cell function leads to inadequate insulin output, a key factor in the development of both major forms of diabetes (type 1 and type 2 diabetes). Our long term goal is to elucidate the molecular mechanisms that confer on the β cell its unique properties. We aim to identify and characterize the network of regulatory proteins responsible for activating the β cell phenotype, as well as their target genes, which are directly responsible for the biochemical activities of the β cell.

Recent findings

We have previously shown using RT-PCR analysis that the extent of selective expression of the insulin gene in β cells is greater than 100,000 fold. Transfection of non-β cells with expression vectors encoding the candidate insulin gene transcription factors E2A, β2 and PDX-1 (IPF1) (Fig. 1) indicated that these factors synergistically activate the insulin gene promoter by 150-200 fold. In order to explain the much greater extent of regulation observed in vivo, we examined whether additional transcription factors may be involved in insulin gene regulation. One such candidate transcription factor is HNF4α. MODY1 is a rare form of diabetes caused by mutation in HNF4α, a transcription factor of the nuclear receptor superfamily. To better understand the exact relationship between defective HNF4α function and the MODY phenotype, we tested whether HNF4α was able to modulate activity of the insulin gene promoter. Transfection of cultured 293T cells with an HNF4α expression vector led to 10-fold activation of a co-transfected reporter plasmid containing the rat insulin 1 gene promoter. Computer analysis indicated the presence of a potential HNF4α binding site between nucleotides -59 to -67 of the promoter (Fig. 1). Mutation of this sequence in the context of the insulin promoter led to reduced ability of HNF4α to activate the promoter. The ability of this sequence to bind HNF4α was

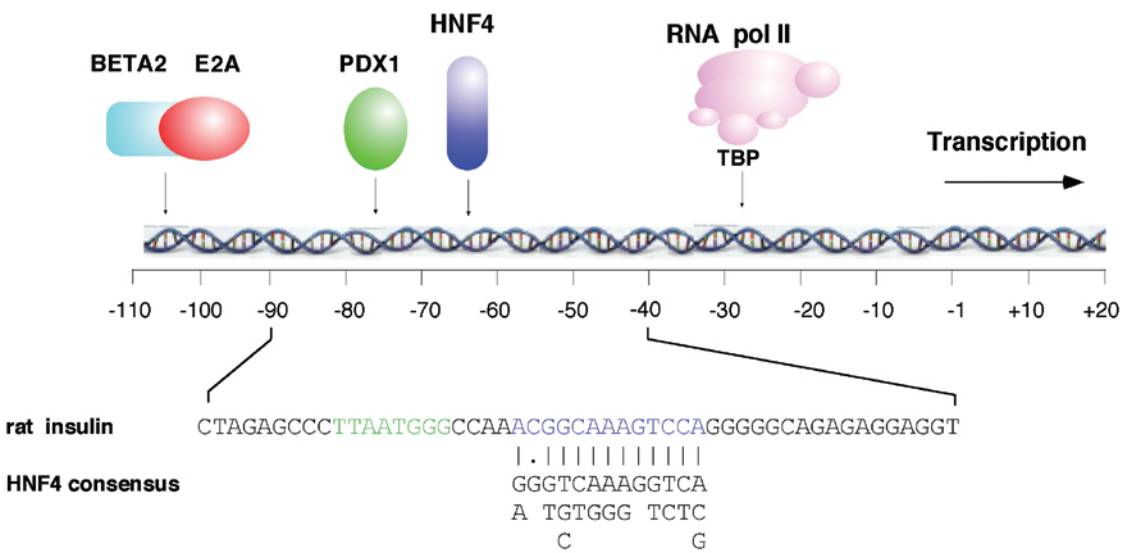

Fig. 1 Promoter of the rat insulin 1 gene, indicating key cis-acting elements together with BETA2, E2A and PDX1 (IPF1) the major transcription factors implicated in cell-specific transcription of the insulin gene. Also shown is HNF4α with its proposed binding site (shown in blue) and consensus binding sequence.

confirmed using gel shift analysis. The significance of HNF4α in β cells was examined by transfecting reporter plasmids bearing wild type or mutated insulin gene promoter to a line of β cells (INS-1) containing an inducible, dominant-negative (DN) form of HNF4α. Induction of DN-HNF4α led to significant reduction in the activity of wild type and both mutated promoters. Thus, in addition to the previously defined indirect action of HNF4α on the insulin gene promoter, HNF4α also activates the insulin gene directly, through a previously unrecognized cis-element. These results may contribute to a more complete understanding of the MODY1 phenotype.

A second transcription factor that we have investigated is Twist, a member of the helix-loop-helix (HLH) family of transcription factors, which play a key role in a wide range of developmental processes in mammals. Expression of Twist in both pancreatic β cell lines and non-β cell lines led to inhibition of insulin gene promoter activity. The inhibition was accompanied by a reduction of the concentration of β2-E2A heterodimers, dependent on both HLH dimerization domain and the adjacent DNA binding domain of Twist. We observed expression of Twist mRNA in a wide range of cells, including two types of pancreatic β cell lines. Thus Twist may play a role in the function of pancreatic β cells, perhaps by displacing β2 from β2-E2A complexes thereby modulating expression of the insulin gene.

One approach to better understanding β cell function is to define and characterize those genes expressed selectively in β cells. Using a differential cloning approach (RDA) we defined 26 cDNA fragments representing genes expressed preferentially in pancreatic β as compared with α cells. Of these 14 corresponded to known genes, only four of which were known to be selectively expressed in β cells. The known genes include transcription factors (STAT6) and mediators of important signaling pathways (guanylate cyclase, protein kinase A regulatory subunit). We are analyzing the unknown genes with a view to understanding their role in β cells. An example of the specificity of expression of one of the clones, clone 21y, is shown in Fig. 2, Our working hypothesis is that such differentially expressed genes play an important role in β cell function, and may be involved in β cell-specific autoimmune destruction observed in type 1 diabetes.

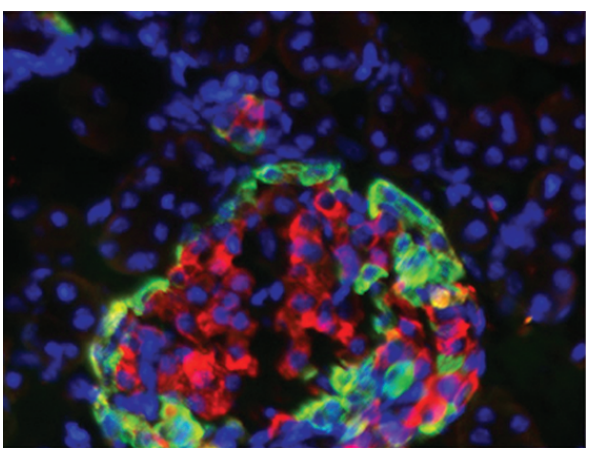

Fig. 2 *Immunofluorescence analysis of newborn mouse pancreas indicates that the protein encoded by gene 21y is expressed preferentially in beta cells within the pancreatic islet. Blue: DAPI staining indicating the location of all nuclei in the section including exocrine and endocrine cells. Green: staining for glucagon - identifying alpha cells located at the periphery of the islet. Red : anti-21y staining within the interior of the islet consistent with location of beta cells.*

Significance

Our research aims to characterize transcription factors and other regulatory proteins involved in β cell function. Although impaired β cell function is a hallmark of diabetes, a molecular understanding of the steps involved is not available: consequently current treatments are inadequate and fail to prevent the associated severe complications. By elucidating the events involved in the development and maintenance of the β cell, our approach may therefore lead to valuable new tools in the fight against diabetes.

Selected Publications

Argenton, F., Walker, M.D., Colombo, L. and Bortolussi, M. (1997) Functional characterization of the trout insulin promoter: implications for fish as a favorable model of pancreas development. FEBS Lett. 407, 191-196.

Arava, Y., Adamsky, K., Belleli, A., Shaltiel, S. and Walker, M.D. (1998) Differential expression of the protein kinase A regulatory subunit (RIα) in pancreatic endocrine cells. FEBS Lett. 425, 24-28.

Arava, Y., Adamsky, K., Ezerzer, C., Ablamunits, V. and Walker, M.D. (1999) Specific gene expression in pancreatic β-cells: cloning and characterization of differentially expressed genes. Diabetes 48, 552-556.

Arava, Y., Seger, R. and Walker, M.D. (1999) GRFβ, a novel regulator of calcium signaling, is expressed in pancreatic β cells and brain. J. Biol. Chem. 274, 24449-24452.

Glick, E., Leshkowitz, D. and Walker, M.D. (2000) Transcription factor β2 acts cooperatively with E2A and PDX1 to activate the insulin gene promoter. J. Biol. Chem. 275, 2199-2204.

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