Michael Walker

Sara Weiss
Gabriela Ridner
Nir Rubins
Hagit Shapiro
Reut Bartoov-Shifman

Specific gene expression in pancreatic beta cells

Department of Biological Chemistry

Tel. 972 8 934 **3597** **Fax.** 972 8 934 **4118**
E-mail: m.walker@weizmann.ac.il
Web page: www.weizmann.ac.il/Biological_Chemistry/scientist/Walker/michael_walker.html

Introduction

Insulin is a protein hormone that functions as the principal regulator of blood glucose homeostasis. The hormone is produced exclusively by the beta cells located within the endocrine islets of the pancreas. Secretion of insulin is controlled according to physiological need, principally responding to changes in blood glucose levels. Defective beta cell function plays an important role in the development of diabetes, a serious and common metabolic disease. The goal of our research is to understand how beta cells perform their unique functions. We focus on 1) the transcriptional mechanisms that permit selective expression of beta cell specific genes and 2) the signaling mechanisms that permit beta cells to couple the release of insulin to physiological needs.

Transcriptional regulation

The beta cell-specific expression of the insulin gene is controlled in large part at the transcriptional level through well defined elements located within the promoter region. Several transcription factors interact with these elements; strikingly, we were able to show that co-transfection of the factors PDX1, BETA2 and E2A synergistically activate transcription of a co-transfected insulin gene promoter by ~150 fold. On the other hand, measurement by quantitative RT-PCR of insulin mRNA levels in beta cells compared to non-beta cells indicates differential expression of at least 100,000 fold; thus, additional control mechanisms must be operating to confer the observed strict specificity of expression.

Target gene discrimination by PDX1

PDX1 is a homeodomain transcription factor essential for pancreatic development and mature beta cell function. Homeodomain proteins typically recognize short TAAT DNA motifs *in vitro*: this binding displays paradoxically low specificity and affinity, given the extremely high specificity of action of these proteins *in vivo*. To better understand how PDX1 selects target genes *in vivo*, we have examined the interaction of PDX1 with natural and artificial binding sites. Comparison of PDX1 binding sites in several target promoters revealed an evolutionarily conserved pattern of nucleotides flanking the TAAT core (Fig. 1). We defined three groups of binding sites displaying high, intermediate, and low affinity. Transfection experiments revealed a striking correlation between the ability of each sequence to activate transcription in cultured beta cells, and its ability to bind PDX1 *in vitro*. Site selection from a pool of oligonucleotides revealed a non-random preference for particular nucleotides at the flanking locations resembling natural PDX1 binding sites (Fig. 1). Taken together, the data indicate that the intrinsic DNA binding specificity of PDX1, in particular the bases adjacent to TAAT, plays an important role in determining the spectrum of target genes.

SAAB 1	SAAB 2	
A C C T A A T G A G	C G C T A A T T A C A	SAAB
A1	**A3**	
C C T T A A T G G G	A T C T A A T T A C	rat I
C C T T A A T G G G	C T C T A A T T A C	rat II
C C T T A A T G G G	C T A T A A T A A C	mouse I
C C T T A A T G G G	C T C T A A T T A C	mouse II
C C C T A A T G G G	C T C T A A T G A C	human

Fig. 1 *Preferred binding sites for PDX1 extend 5' and 3 to the TAAT core, and resemble the sequences found in natural target sequences. Site selection by the SAAB procedure yielded two consensus sequences (SAAB1 and 2) shown aligned with the natural PDX1 binding sites A1 and A3 located in the insulin gene promoters of several species. Conserved flanking bases are highlighted.*

Novel beta cell genes

To better understand beta cell function, we used a differential cloning procedure, by which we defined 26 cDNA fragments representing genes expressed selectively in pancreatic beta cells. These included genes of known function (e.g. protein kinase A regulatory subunit) and novel genes. Among the novel cDNAs, were GRFbeta, a splice isoform of GRF1, a Ca++-dependent guanine nucleotide exchange factor. GRFbeta appears to represent a novel dominant negative modulator of the Ras signaling pathway.

GPR40

RDA clone 21y is expressed exclusively in pancreatic beta cells. Using the RACE procedure, we isolated a full length clone, and determined that it corresponds to GPR40, a member of the family of G-protein coupled receptors. Recently it has been shown that fatty acids can activate GPR40, though the nature of its physiological ligand remains unclear. We have confirmed the specific expression of GPR40 within beta cells of the endocrine pancreas (Fig. 2), and developed an assay demonstrating GPR40 –dependent signaling in transfected 293T cells. This permitted structure-function analysis of the receptor including identification of a GPR40 point mutant displaying dominant negative action. We are generating lines of transgenic mice in which GPR40 is over-expressed and expressed ectopically to determine the role of the protein *in vivo*.

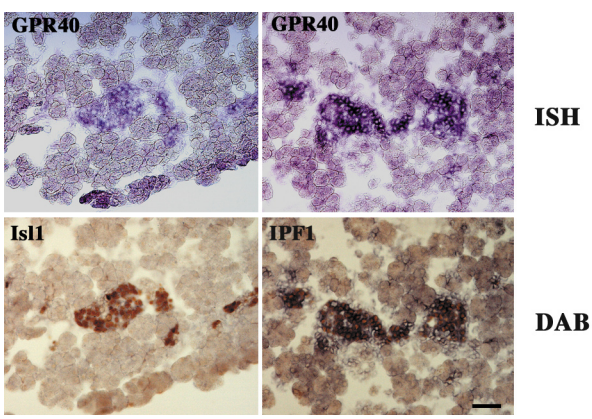

Fig. 2 *In situ hybridization (ISH) of GPR40 mRNA expression in mouse pancreas (top panels). Lower panels show immunohistochemistry (DAB) of the same sections using antibodies against the beta cell markers Isl1 and IPF1.*

Significance

It is now well established that the ongoing world-wide epidemic of diabetes is being driven by increasing obesity in the population. Although impaired beta cell function is clearly involved, the molecular basis for the underlying defects is unknown. By elucidating the factors involved in development and maintenance of beta cell function, particularly key transcription factors and receptors modulating insulin secretion, we believe our research will lead to valuable new tools for prevention and treatment of diabetes.

Selected Publications

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