Expression Profiling of Pancreatic β-Cells

Glucose Regulation of Secretory and Metabolic Pathway Genes

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The β-cell of the pancreatic islets of Langerhans is a primary component in vertebrate glucose homeostasis. β-Cells sense glucose levels directly via its metabolism and respond by secretion of insulin from storage granules formed in the regulated secretory pathway. This stimulus-secretion coupling takes place on the order of seconds/minutes and entails ATP production from glucose metabolism. β-Cells also respond to glucose in the longer term (minutes/hours) by increasing preproinsulin biosynthesis at the endoplasmic reticulum (ER) membrane (1,2). Derangement of this process leads to reduced insulin production and is a factor in the development of diabetes (3). Type 2 diabetes is characterized by reduced β-cell insulin stores, suggesting that the diabetic β-cell is unable to synthesize a sufficient quantity of insulin (4). Insulin production is primarily regulated by glucose at the level of preproinsulin mRNA translation (1,5), whereas regulation of transcription of the preproinsulin gene can provide longer-term enhancement (6). The mechanism by which glucose exerts translational control of preproinsulin mRNA is poorly understood.

The study of β-cell biology has been greatly advanced by the development of cultured β-cell lines (7). Because the pancreatic islet is a complex tissue composed of varied cell types, cell lines offer a pure cell population with which to work. MIN6 cells are one of the best-studied clonal β-cell lines and have been shown in early passages to maintain accurate β-cell function. This includes robust insulin secretion in response to physiological changes in glucose levels (8). Therefore, MIN6 cells provide a homogeneous β-cell population that responds synchronously and physiologically to changes in glucose concentration.

To identify pancreatic β-cell genes that are glucose-responsive, we carried out microarray analysis of murine β-cell line MIN6 cells exposed to either high (25 mmol/l) or low (5.5 mmol/l) glucose for 24 h. RNA, cDNA, and biotinylated cRNA were prepared, and the biotinylated cRNAs were hybridized to Affymetrix Mu6500 oligonucleotide arrays. Hybridization intensities were analyzed using Affymetrix Genechip Analysis Suite 3.2 software. This analysis indicated that 78 genes were up- or downregulated 2.2-fold or greater in high- versus low-glucose conditions.

These glucose-responsive genes and expressed sequence tags (ESTs) were clustered according to known cellular functions or by sequence similarity to genes of known function. Significant numbers of these genes were found to function in the secretory pathway and functions of metabolism, signaling, and transcriptional regulation. These clusters account for >75% of the identified genes and ESTs (Fig. 1).

The single largest cluster encompassed 21 transcripts that encode components of the early secretory pathway and the regulated secretory pathway. These included seven members of the ER translocon, transcripts encoding genes necessary for glycosylation, protein folding, and vesicle-mediated transport, sorting, and processing. The majority of these genes are involved in ER function, which suggests that regulation of the ER is important in β-cell glucose response. Our data demonstrate not only that the β-cell regulates the transcript abundance of multiple translocon components, but also that the transcript abundance leads to changes in the amounts of the encoded proteins. These changes in protein levels were most dramatically demonstrated for the SRα subunit. The levels of SRα protein were >20-fold upregulated in a high-glucose

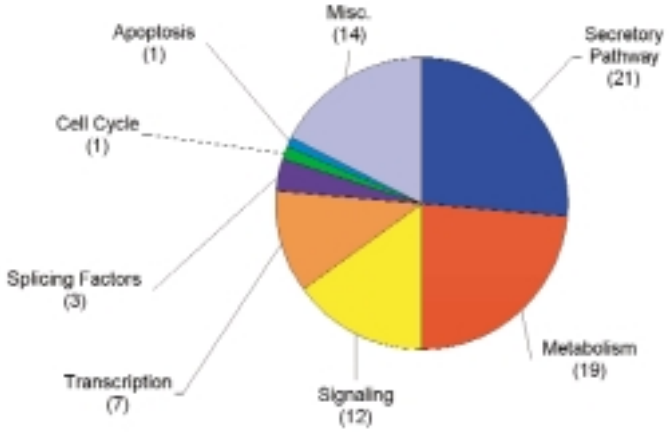

FIG. 1. Functional clusters of β-cell glucose-responsive genes.

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ER, endoplasmic reticulum; EST, expressed sequence tag.

medium. These data indicate that the translocon and ribosome docking are major regulatory targets of glucose in the β-cell.

The second largest cluster of glucose-responsive genes identified were enzymes involved in intermediary metabolism. These included upregulation of enzymes for forward movement through the glycolytic pathway and downregulation of enzymes for gluconeogenesis, nitrogen disposal, and mitochondrial protein import and synthesis. Although the effects of glycolytic flux and mitochondrial function on β-cells are known and partially characterized, we were intrigued by differential expression of genes that function in and around the disposal of nitrogen through the urea cycle. The expression profile of these genes (argininosuccinate synthetase, ornithine decarboxylase, ornithine aminotransferase, and spermidine synthase) indicated that low glucose brought about scavenging of free ornithine and the use of free ornithine in the urea cycle for disposal of ammonia. The primary source of ammonia in the cell is derived from amino acid catabolism. This conclusion was supported by observations of increased urea production under low-glucose conditions. We infer from these findings that β-cells utilize greater amounts of amino acids for energy production under low-glucose conditions.

In summary, our experiments on early-passage MIN6 cells identify glucose-responsive expression of multiple large functional clusters of genes. The two largest clusters encode secretory pathway components and enzymes of intermediary metabolism. In the secretory pathway, SRα is a major glucose responsive protein and indicates a mechanism by which β-cells regulate insulin biosynthetic levels. This is consistent with earlier findings of Welsh et al. (2) that demonstrated SRα was limiting for ribosome docking on the β-cell ER (2). Our present work demonstrates that β-cells actively regulate the amount of SRα in response to glucose. We further demonstrate regulation of metabolic pathways in response to glucose and show the regulation of nitrogen metabolism by glucose availability. Our findings also demonstrate that microarray-based technology provides a powerful tool for identifying genes involved in the function and regulation of pancreatic β-cells.

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