Checks and Balances—The Limits of β-Cell Endurance to ER Stress

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As James Madison remarked, "the great question now to be decided … is, whether checks and balances sufficient for the purposes of order, justice, and the general good, may not be created by a proper division and distribution of power among different bodies, differently constituted, but all deriving their existence from the elective principle, and bound by a responsible tenure of their trusts" (1). But which are the checks and balances that allow pancreatic β-cells to preserve their most differentiated function, i.e., the synthesis and release of insulin, and what are the limits of endurance of these cells when facing severe stress?

To evaluate this, Pappalardo et al. (2) made ingenious use of small interfering RNAs (siRNAs). RNA interference (RNAi) is triggered by endogenously produced or exogenously introduced RNA pairs that have complementarity with endogenous mRNAs, leading to their degradation and consequent "silencing" of the corresponding gene and its encoded protein. This provides the possibility of silencing individual genes in selected cells to understand their function(s). Instead of targeting individual genes, however, Pappalardo et al. (2) performed a whole-genome RNAi screen (targeting in parallel 22,000 mouse genes) to identify the genes that, when inhibited, would affect insulin transcription. This approach is based on the use of a mouse insulin-producing cell line, MIN6, engineered to express both a copy of the human insulin promoter driving a fluorescent EGFP reporter and the mCherry fluorescent protein under the control of a constitutively active viral promoter as an internal control. When one of the siRNAs introduced in the cells inhibits a positive regulator of the insulin promoter, the ratio of EGFP to mCherry decreases (3), indicating that this particular mRNA plays a direct or indirect role in the function of the insulin promoter. This is a challenging method, one which may be affected by off-target effects (i.e., interaction of the siRNA with a gene other than the target), variability of target knockdown, and the need for powerful bioinformatics approaches (4). By performing a stepwise approach, including the use of three to four different siRNAs per target and of different transfection reagents, by showing that the hits obtained point to mRNAs actually expressed in MIN6 cells and human islets as identified by RNA sequencing (5), and by a careful confirmation of identified hits, the present analysis (2) identified 26 novel regulators of insulin transcription (Fig. 1). While some of these hits and downstream pathways are related to direct and well-known regulators of the insulin gene, such as *Pdx1* and *Glis3*, many others are related to upstream pathways that may affect insulin expression in an indirect way, including oxidative phosphorylation (crucial for ATP production), vesicle traffic, endoplasmic reticulum (ER) stress, and the unfolded protein response (UPR) (2).

Pancreatic β-cells must increase their protein synthesis capacity severalfold during nutrient stimulation to face the increased demand for insulin synthesis and release. This poses a major burden on the ER, the organelle responsible for the synthesis and folding of secreted proteins, forcing β-cells to trigger check and balance mechanisms (known collectively as the UPR) that adapt the ER function to the demand. If the UPR fails in solving the ER stress, this may lead to β-cell apoptosis (6). The UPR signaling is mediated via three main transmembrane sensors: endoribonuclease inositol-requiring protein 1α (IRE1α), protein kinase RNA-like endoplasmic reticulum kinase (PERK), and activating transcription factor 6 (ATF6) (7). The activation of PERK attenuates global protein synthesis via eIF2α phosphorylation, consequently reducing the load of unfolded proteins into the ER (8). IRE1α dimerization and *trans* autophosphorylation activates its RNase domain, which cleaves and activates the transcription factor X-box binding protein 1 (XBP1). XBP1 is important for the expression of genes involved in protein folding, secretion, and ER-associated protein degradation (9). IRE1α also triggers the degradation of RNAs (known as regulated IRE1α-dependent decay or RIDD) (10), including insulin mRNAs in β-cells (11), thus reducing the amount of proteins translated in the ER.

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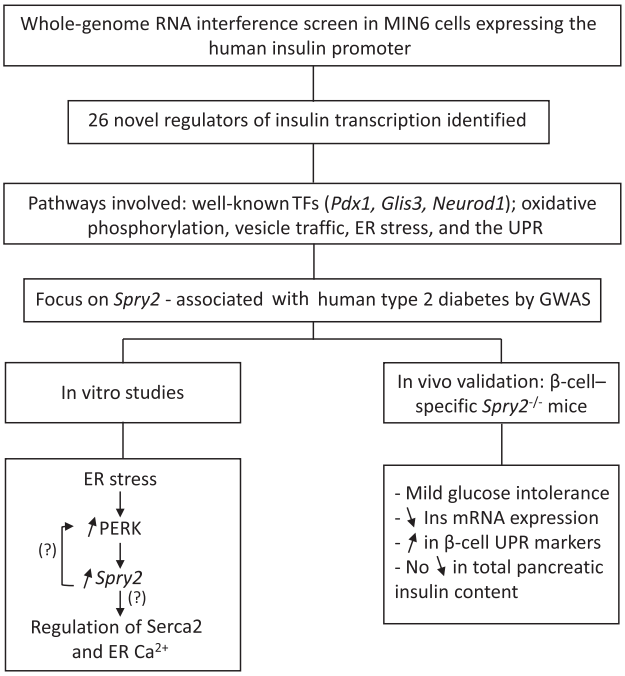

Figure 1—The research strategy of Pappalardo et al. (2) leading to the identification of *Spry2* and its function. Based on a whole-genome RNAi screen in insulin-producing MIN6 cells, 26 novel regulators of insulin transcription were identified, including both transcription factors that directly bind to the insulin promoter and mRNAs that regulate basic β-cell functions that interfere with insulin production. Functional studies focused on *Spry2*, a candidate gene for type 2 diabetes, and in vitro studies indicated that this gene is induced by ER stress, downstream of the PERK pathway, and regulates expression of the Ca^{2+} pump Serca2. These findings were partially validated in an in vivo mouse model of β-cell–specific knockout of *Spry2*. GWAS, genome-wide association study; Ins, insulin; TFs, transcription factors.

One of the novel potential regulators of insulin transcription identified by Pappalardo et al. (2), *Spry2*, was selected for additional studies. This gene is of particular interest in the context of type 2 diabetes, since it has been previously identified as a diabetes risk locus (12,13). In vitro studies indicated that chemical ER stressors induce *Spry2* expression in MIN6 cells and that mouse fibroblasts deficient for PERK fail to upregulate *Spry2* mRNA expression. Although it would have been important to confirm this finding in primary β-cells exposed to specific siRNAs or treated with available chemical blockers of PERK, these findings suggest that *Spry2* is downstream of the PERK pathway. It remains to be determined how PERK regulates *Spry2* expression, but additional experiments indicated that knockdown of *Spry2* aggravates ER stress, as suggested by increased expression of the activated form of PERK, spliced XBP1, and CHOP and augmented apoptosis. The main effect of *Spry2* seems to be mediated via regulation of the ER Ca^{2+} pump Serca2 (2). The Ca^{2+} concentration in the ER is crucial for the proper folding of proteins, and previous studies indicated that immune (14) or metabolic (15) challenges induce severe ER stress in β-cells via inhibition of Serca2b and consequent depletion of ER Ca^{2+}. Pappalardo et al. (2) observed that *Spry2* knockdown inhibited Serca2 protein expression by 50%, via a posttranscriptional mechanism that remains to be clarified, and increased the fraction of cells with low ER Ca^{2+}. These observations were partially validated in an in vivo mouse model of β-cell–specific depletion of *Spry2*. Thus, these mice presented mild glucose intolerance, a decrease in insulin mRNA expression, and a trend for higher expression of ER stress markers in the β-cells (2).

In conclusion, departing from a whole-genome RNAi study, Pappalardo et al. (2) identified a potential novel regulator of the UPR in pancreatic β-cells, *Spry2*, a target of PERK and a candidate gene for type 2 diabetes. β-Cells have evolved several checks and balances to avoid excessive IRE1α activation during ER stress (6,16,17). IRE1α regulates a key proapoptotic function, namely the activation of JNK, while a proper and well-balanced function of PERK is crucial for the main function of β-cells, namely the synthesis of insulin. Of interest, both mice and humans with deficiency in PERK or other genes relevant to the PERK pathway have severe diabetes (18–20). Further studies are now required to fully understand the checks and balances that allow pancreatic β-cells to function for decades in human beings, often in the face of severe insulin resistance caused by obesity.

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