# ID'ing a Novel Inhibitor of β-Cell Function, Id1

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It is well established that loss of β-cell mass and compromised β-cell function are telltale indicators of the development of type 2 diabetes (1). Even individuals at risk for developing diabetes show β-cell dysfunction such as reduction of first-phase insulin release (2). An early response to increased insulin demand, as brought about in states of insulin resistance such as obesity or pregnancy, is a compensatory upregulation of the pancreatic β-cell mass and function. However, when this compensation fails and an inadequate amount of insulin is released in response to a meal, diabetes develops. The inability of β-cells to compensate for insulin resistance is associated with several abnormalities in β-cell function such as mitochondrial stress and reactive oxygen species production, insufficient proinsulin-to-insulin processing, and increased expression of islet amyloid polypeptide. Many of these abnormalities are believed to be the result of a combination of genetic and environmental factors. In particular, the exposure of β-cells to high concentrations of free fatty acids in combination with hyperglycemia (glucolipotoxicity) have been shown to be detrimental to β-cells resulting in severe dysfunction, loss of differentiation markers, and apoptosis. The detailed cellular and molecular mechanism by which β-cell dysfunction develops leading to type 2 diabetes is yet to be fully understood.

In a search for genes with altered expression in islets from diabetic mice, it was found that the mRNA level for the gene encoding the transcriptional regulator Id1 (inhibitor of DNA binding-1) was increased in islets from *db/db* mice (3), and furthermore, *Id1* mRNA was increased in response to long-term exposure of β-cells to free fatty acids (4) as well as hyperglycemia (5). In order to investigate if Id1 plays a role in β-cell dysfunction in type 2 diabetes, Åkerfeldt and Laybutt (6) in this issue of *Diabetes* report the characterization of glucose metabolism in Id1-deficient mice. The major finding is that Id1-deficient mice are protected against diabetes following high-fat feeding, and that β-cell function in vivo and in vitro is enhanced in these mice devoid of Id1. Interestingly, insulin secretion is also enhanced in islets from mice on a standard diet suggesting that Id1 deficiency not only protects against the deleterious effects of high-fat feeding but also affects insulin secretion per se. In addition, gene expression analysis reveals reduced levels of stress-related genes in islets from Id1-deficient mice and preservation of β-cell–specific genes when mice are challenged with a high-fat diet. The authors conclude that Id1 functions as a negative regulator of insulin secretion and that induced expression of *Id1* might contribute to β-cell dysfunction in type 2 diabetes.

Several important questions arise from these findings. *1)* What are the factors that regulate the expression of *Id1* in normal physiology and more importantly in relation to type 2 diabetes? *2)* What is the mechanism of action of Id1 in β-cell physiology? *3)* Can pharmacological inhibition of Id1 be achieved and will this normalize β-cell function in type 2 diabetes? To address these questions, it is important to review the general knowledge of the Id proteins family. Id1 belongs to a family of four Id proteins (Id1–4), which are members of the helix-loop-helix (HLH) family of transcription factors (7,8). The HLH domain mediates the dimerization of individual HLH factors and is required for homo- or heterodimerization. Members of this large family of transcription factors are involved in the coordinated regulation of gene expression during cell lineage commitment and differentiation as well as cell proliferation. Two HLH factors, neurogenin3 and NeuroD, are crucial for correct β-cell development and function, and deletion of either one of these genes results in diabetes (9). The Id family of HLH protein is unique since Id proteins do not contain the otherwise conserved basic domain found *N*-terminal to the HLH domain, which is essential for the ability of HLH proteins to bind DNA and regulate transcription. However, Id proteins can dimerize with other HLH proteins and thereby inhibit their ability to bind DNA as homo- or heterodimeric complexes. Based on this ability, the Id proteins were named "inhibitor of DNA binding," and they function as dominant-negative regulators of HLH transcription factors (10). Subsequently it was observed that expression of Id proteins was often downregulated following differentiation, and the name Id sometimes refers to its role as inhibitor of differentiation. Expression of Id proteins is correlated with cell proliferation rates such that high levels of Id proteins are found in highly proliferating cells and low levels in quiescent cells. Id proteins affect several important cell cycle regulators and have been implicated in tumor formation (11).

Very little information is available concerning the role of Id1 protein in β-cell biology. In addition to the increased expression of *Id1* mRNA observed in islets from *db/db* mice (3) and in islets and MIN6 cells exposed to hyperglycemia (5), as mentioned above, immunohistochemical examination of the adult mouse pancreas has demonstrated expression of Id1 to be limited to the glucagon-producing α-cells with no apparent expression in β-cells (12). Furthermore, expression of *Id1* in islets was regulated by endogenous bone morphogenetic protein 4 (BMP4) and colocalized with BMP-receptor 2 expression. The *Id* genes are classical targets for the BMP signaling pathway, and *Id* transcription is induced by BMP activation of SMAD1/5/8 in many cells types. It is therefore of interest that BMP4 has been proposed to act as a factor required for normal β-cell function and to regulate many genes involved in the

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function of fully differentiated β-cells (13), while one of the classical target genes for BMP4, *Id1*, seems to be involved in the inhibition of differentiated β-cell function (6). However, BMP4 regulates many genes other than *Id1*, and the concerted outcome of these changes—whether in α- or β-cells—determine the final effect on β-cell function. While some information concerning the expression of *Id1* in islets exists, it will be important to elucidate if and how genetic and environmental factors known to affect β-cell function regulate *Id1* expression and to characterize the possible role of *Id1* expression in α-cells in relation to β-cell function. Since a global Id1-deficient animal model was used in this study, the generation of cell-specific gene ablation models to generate α- and β-cell–specific Id1-deficient mice will help answer some of the questions concerning islet cell–specific actions of Id1.

While the mechanism by which Id1 affects β-cell function remains largely unknown, most of the effects of Id proteins are mediated through their ability to inhibit the DNA binding and thus the action of basic HLH transcription factors. The two most prominent basic HLH factors in β-cells are neurogenin3 and NeuroD. While neurogenin3 expression is restricted to the developing immature β-cells, NeuroD expression has been shown to be required for mature β-cell function (14). Interactions between Id1 and NeuroD have been described, and Id1 was found to inhibit the DNA binding of a NeuroD/E47 dimer and to function as a negative regulator of NeuroD-dependent transcription (15). If such an interaction between Id1 and NeuroD also exists in β-cells remains to be determined, but it does offer a possible explanation of how Id1 could affect β-cell function. Id proteins have also be reported to interact with non-HLH transcription factors such as the paired-domain homeobox (PAX) family of transcription factors (7), and such interaction might also interfere with β-cell function since PAX4 and PAX6 in particular are known to play a role in β-cell function.

As β-cell dysfunction has been accepted as a major factor in the development of type 2 diabetes, it is very important to understand the molecular and cellular mechanisms behind the lack of proper β-cell function. The identification of the *Id1* gene as a potential factor for mediating the detrimental effects of high-fat feeding on β-cell function opens new possibilities for prevention of β-cell dysfunction by inhibition of Id1 expression or activity. A more detailed characterization of the factors involved in the regulation of Id1 expression might also provide possibilities for interfering with this pathway with the ultimate goal of preserving normal β-cell function.

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