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Distinct Role for ARNT/HIF-1β in Pancreatic Beta-Cell Function, Insulin Secretion and Type 2 Diabetes

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1. Introduction

Diabetes mellitus is a common metabolic syndrome that has become an epidemic in modern society and is characterized by either a near-complete lack of insulin production due to autoimmune destruction of pancreatic beta-cells as in type 1 diabetes or abnormal insulin secretion, beta-cell dysfunction and insulin resistance as in type 2 diabetes (T2D). T2D is a complex heterogeneous disease that is characterized by elevated fasting and postprandial blood glucose levels that can result in severe complications including renal failure, cardiovascular disease, blindness and slow wound healing (Lin and Sun, 2010). Pancreatic islet beta-cells play a critical role in maintaining blood glucose levels by secreting the hormone insulin following a meal. Insulin maintains blood glucose levels in the normal physiological range by promoting glucose uptake in muscles, liver and adipose tissue, and by inhibiting hepatic glucose production. Therefore, any defect in insulin secretion in response to a meal or defects in insulin action in peripheral tissues can lead to increased blood glucose levels (Tripathy and Chavez, 2010; Muoio & Newgard, 2008).

Abnormal insulin secretion is a hallmark of T2D. Despite the central role of insulin in maintaining glucose homeostasis, the fundamental biochemical mechanism regulating nutrient-stimulated insulin secretion from pancreatic beta-cells is still incompletely understood. Insulin secretion from the pancreatic beta-cells is regulated by nutrients, neurotransmitters and hormones. Among these three factors, nutrients, particularly glucose is the most dominant stimulatory signal for insulin secretion. Insulin secretion is biphasic with a first acute phase occurring within 10 minutes after a glucose load and a second more sustained phase that reaches a plateau very quickly as seen in mice or more gradually as seen in rats and humans (Gerich, 2002). Numerous models have been proposed over the last several decades to explain the mechanism governing glucose-stimulated insulin secretion (GSIS) from pancreatic beta-cells. The current model of GSIS holds that glucose enters beta-cells via the low affinity, high capacity glucose transporter 2 (GLUT2) and becomes phosphorylated by glucokinase (GK or hexokinase IV), which is the rate-limiting step in glycolysis. The glycolytic end product pyruvate then enters the tricarboxylic acid cycle (TCA), where oxidative phosphorylation occurs, leading to increased ATP production. The subsequent rise in the cytosolic ATP/ADP levels promotes closure of ATP-sensitive potassium channels (K_{ATP} channels) causing beta-cell membrane depolarization and

activation of voltage-dependent Ca^{2+} channels (VDCC). The opening of VDCCs facilitates influx of extracellular Ca^{2+}, leading to a rise in the beta-cell cytosolic Ca^{2+} levels, which triggers exocytosis of the insulin-containing secretory granules (Figure 1) (Jensen et al., 2008; Prentki & Matchinsky, 1987; Ashcroft & Rorsman, 1989; Newgard & Matchinsky, 2001; Newgard & McGarry, 1995). This so-called "K_{ATP} channel-dependent" mechanism appears to be particularly important for the first, acute phase of insulin release. However, in the second and more sustained phase of insulin secretion a "K_{ATP} channel-independent" pathway also appears to play a key role in the regulation of GSIS in conjunction with the K_{ATP} channel-dependent pathway (Henquin et al., 2003, Ravier et al., 2009). Important support for "K_{ATP} channel-independent" pathway of GSIS comes from studies showing that glucose still causes a significant increase in insulin secretion in conditions where K_{ATP} channels are held open by application of diazoxide followed by membrane depolarization with high K^+, or in animals lacking functional K_{ATP} channels (Nenquin et al., 2004; Shiota et al., 2002; Szollozi et al., 2007; Ravier et al., 2009). These and more recent studies suggest that mitochondrial metabolism of glucose generates signals other than changes in the ATP/ADP ratio that are important for normal insulin secretion. Several molecules, including glutamate, malonyl-CoA/LC-CoA and NADPH, have been proposed as candidate coupling factors in GSIS (Maechler & Wollheim, 1999; Ivarsson et al., 2005; Corkey et al., 1989; Prentki et al., 1992).

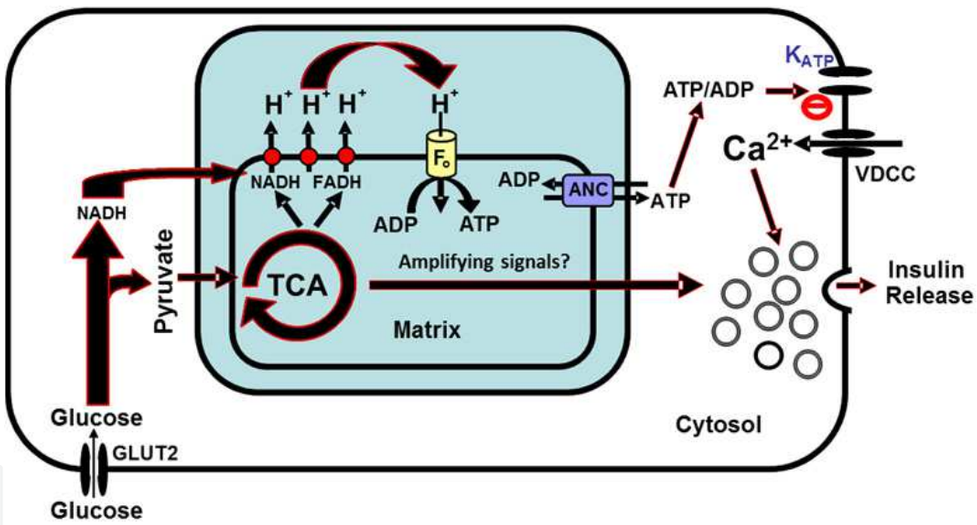

Fig. 1. Current model of glucose stimulated insulin secretion (GSIS) from pancreatic beta-cells. Glucose equilibrates across the plasma membrane through glucose transporter GLUT2, which initiates glycolysis. Pyruvate produced by glycolysis preferentially enters the mitochondria and is metabolized in the TCA cycle, producing reducing equivalents in the form of NADH and $FADH_2$. The transfer of electrons from these reducing equivalents through the mitochondrial electron transport chain is coupled with the pumping of protons from the mitochondrial matrix to the inter membrane space, leading to the generation of ATP. ATP is transferred to the cytosol through adenine nucleotide carrier (ANC), raising the ATP/ADP ratio. This results in the closure of the ATP sensitive K^+ channels (K_{ATP}), which in turn leads to membrane depolarization, opening of the voltage-sensitive Ca^{2+} channels, promoting calcium entry and increase in cytoplasmic Ca^{2+} leading to exocytosis of insulin granules. Glucose also generates amplifying signals other than ATP, which plays a significant role in the secretion of insulin from pancreatic beta-cells.

Maintenance of a functional mature beta-cell phenotype requires optimal expression of key transcription factors. Transcription factors regulate a variety of pancreatic beta-cell processes including cell differentiation, proliferation, cell signaling and apoptosis. By regulating the expression of specific sets of genes, transcription factors determine the spatio-temporal specificity of gene expression in most organisms, including mammals. Numerous studies have shown that transcription factors act synergistically to achieve normal beta-cell development and function (Cerf, 2006; Mitchell & Frayling, 2002; Lyttle et al., 2008). Development of the endocrine pancreas is initiated from multipotent precursor cells, which differentiate to form five different cell types in the pancreatic islet namely the α-cells (glucagon), β-cells (insulin), δ-cells (somatostatin), PP (pancreatic polypeptide) cells and ε-cells (ghrelin) (Steiner et al., 2010). The development of the islet architecture is regulated by an ordered system of transcriptional events activated by a hierarchy of transcription factors. Some of the major transcription factors represented in islets include several homeodomain factors like pancreatic and duodenal homeobox-1 (Pdx-1), paired box gene (Pax) Pax 4, Pax 6, Nkx 2.1 and Nkx 6.1 which are expressed in both progenitor as well as differentiated beta-cells. Pdx-1 and Nkx 2.2 are required for both early beta-cell differentiation and maintenance of a mature beta-cell phenotype (Habner et al., 2005). In addition, other transcription factors are important for maintenance of a mature beta-cell phenotype and their impairment may account for various pathophysiological abnormalities observed in type 2 diabetics. Among these, Pdx-1, neurogenin differentiation (NeuroD/BETA-2), foxhead box protein (FoxO-1), sterol regulatory element binding protein (SREBP-1c), and musculoaponeurotic fibrosarcoma oncogene homolog A (MafA) are the most studied (Johnson et al., 1994; Diraison et al., 2004; Kitamura et al., 2005).

In the context of T2D, it is a well-known fact that abnormal gene expression contributes to a myriad of beta-cell abnormalities. Support for this comes from studies of maturity-onset diabetes of the young (MODY), a monogenic form of T2D characterized by an early onset and defects in insulin secretion leading to hyperglycemia. With the exception of MODY-2, which is caused by a mutation in GK, MODY-1, 3, 4, 5 and 6 result from mutations in genes encoding transcription factors, hepatocyte nuclear factor (HNF) HNF-4α, HNF-1α, HNF-1β, Pdx-1, and NeuroD/BETA-2 respectively. These transcription factors regulate the expression of key genes involved in various aspects of beta-cell function (Stoffer & Zinkin, 1997; Habener et al., 1998; Fajans et al., 2001; Yamagata et al, 2003). Although there have been significant advancements in understanding the basic transcriptional network that exists in beta-cells, the exact mechanism of action of many of these factors still remains to be further defined. In this chapter we provide an overview of one of the recently described transcription factor in the context of impaired insulin secretion and beta-cell dysfunction, Aryl hydrocarbon receptor nuclear translocator (ARNT)/ hypoxia inducible factor 1β (HIF-1β), which is a master regulator of pancreatic beta-cell transcriptional network that regulates glucose metabolism and insulin secretion.

2. ARNT/HIF-1β

2.1 ARNT/HIF-1β structure and function

ARNT/HIF-1β belongs to a group of transcription factors, known as the basic helix loop helix - PER/ARNT/Sim (bHLH-PAS) family, which has a characteristic N-terminal bHLH

motif for DNA binding, a central PAS domain which facilitates heterodimerization and a C-terminal transactivation domain for the recruitment of transcriptional coactivators such as CBP/p300 (Jain et al., 1994; Kobayashi et al., 1997). Recent evidence suggest that the PAS domain may also provide an additional binding site for coactivators and thereby recruiting them in a step necessary for transcriptional responses to hypoxia (Partch & Gardener, 2011). ARNT/HIF-1β acts as a common binding partner for most of the bHLH-PAS family of transcription factors and bind specific DNA sequences in the regulatory regions of the responsive genes. The half-site for ARNT/HIF-1β is on the 3' side of the 5'-GTG-3' recognition sequence. The sequence of the other half of the binding site depends upon the identity of the ARNT/HIF-1β dimerization partner (Swanson et al., 1995). DNA binding of ARNT/HIF-1β is mediated by its bHLH region and may also involve the PAS region. Dimerization between ARNT/HIF-1β and other bHLH-PAS proteins is mediated by their bHLH and PAS regions (Jiang et al., 1996, Lindebro et al., 1995). The human ARNT/HIF-1β gene is about 65 Kb in size, has 22 exons and is well conserved on an evolutionary scale (Scheel & Schrenk, 2000).

ARNT/HIF-1β was originally cloned as a factor required for the activity of the aryl hydrocarbon receptor (AhR). AhR induces a transcriptional response to various environmental pollutants, such as polycyclic aromatic hydrocarbons, heterocyclic amines, and polychlorinated aromatic compounds (Reyes et al., 1992). ARNT/HIF-1β was also identified as the β-subunit of a heterodimeric transcription factor, hypoxia-inducible factor 1α (HIF-1α) (Wang et al., 1995 (a)). Similar to HIF-1α, ARNT/HIF-1β gene expression and protein levels are significantly increased under hypoxic conditions suggesting that this gene plays an important role in the transcriptional response to low oxygen tension (Wang et al., 1995 (b)). Consistent with this idea, it has been shown that ARNT/HIF-1β is essential for the hypoxic induction of vascular endothelial growth factor (VEGF) and the glycolytic enzymes aldolase A (ALDO) and phosphoglycerate kinase (PGK) in a mouse hepatoma (Hepa 1c1c7) cell line (Li et al., 1996; Salceda et al., 1996). Unlike HIF-1α, which is exclusively expressed under hypoxic conditions, ARNT/HIF-1β is constitutively expressed in a number of tissues, such as the brain, heart, kidney, muscles, thymus, retina, olfactory epithelium and beta-cells of pancreas (Hirose et al., 1996).

2.2 ARNT/HIF-1β localization, binding partners, mechanism of action and lessons from knockout animals

ARNT/HIF-1β is a nuclear protein in most cell types, although it may also be located in the cytosol, particularly during embryogenesis. Studies conducted by Holmes and Pollenz (1997) in hepatic and non-hepatic cell lines derived from rat, mouse, human, and canine tissues confirm ARNT/HIF-1β as a nuclear transcription factor and showed that its physical interaction with DNA requires entry into the nucleus.

ARNT/HIF-1β serves as an obligatory binding partner for a number of other bHLH-PAS proteins, whose activity is modulated either by exogenous chemicals (AhR), hypoxia (HIF-1α, HIF-2α and HIF-3α), or which show tissue-specific expression pattern (e.g. SIM-1) (Salceda et al., 1996; Swanson et., 1995; Woods & Whitelaw, 2002). In addition to forming heterodimers, ARNT/HIF-1β appears to be capable of forming homodimers and bind to an E-box sequence 5'-CACGTG-3' (Antonsson et al., 1995). It was also shown that ARNT/HIF-1β homodimer regulates the transcription of murine cytochrome P450 (Cyp) 2a5 gene

through a palindromic E-box element in the 5' regulatory region of Cyp2a5 gene in primary hepatocytes (Arpiainen et al., 2007). Two ARNT-related genes, ARNT-2 and ARNT-3 (also called BMAL-1 or MOP3) have been identified. ARNT-2 is more restricted in expression than ARNT/HIF-1β, but appears to dimerize with the same partner proteins as ARNT/HIF-1β (Hirose et al., 1996). ARNT-3 appears to have different dimerization potential than ARNT/HIF-1β (Ikeda & Nomura, 1997). The transactivation potential of ARNT/HIF-1β is not only determined through the recruitment of transcriptional cofactors, but also by signaling input from several protein kinases, such as PKC (Long et al., 1999).

The ARNT/HIF-1β/AhR heterodimer activates transcription of several genes involved in metabolism of foreign chemicals, including CYP1A1, CYP1B1, and NADP(H):oxidoreductase (NQO1) (Sogawa & Kuriayama, 1997; Beischlag et al., 2008). Transcriptional activation of these genes depends upon prior binding of AhR to xenobiotic ligands, including 2,3,7,8-tetrachlorodibenzo-p-dioxin (dioxin) and benzopyrene. The ARNT/HIF-1β/AhR heterodimer and ARNT/HIF-1β can have an impact on estrogen receptor (ER) activity. ARNT/HIF-1β interacts and functions as a potent coactivator of both ER-α and ER-β dependent transcription and it is believed that the C-terminal domain of ARNT/HIF-1β is essential for the transcriptional enhancement of ER activity (Lim et al., 2011;Rüegg et al., 2008).

ARNT/HIF-1β/HIF-1α heterodimer activity is primarily regulated by HIF-1α protein stability. Under normoxia, HIF-1α is hydroxylated by an oxygen requiring enzyme, prolyl hydroxylase (PHD), which is then targeted for ubiquitination by the E3 ubiquitin ligase, followed by binding to von Hippel-Lindau tumor suppressor (VHL) which leads to degradation of HIF-1α by the proteasome pathway. Conversely, under hypoxic conditions, a lack of oxygen inhibits hydroxylation, leading to stabilization of the HIF-1α protein and translocation of HIF-1α from the cytoplasm to the nucleus. In the nucleus, heterodimerization of HIF-1α with ARNT/HIF-1β is followed by binding to hypoxia response elements (HRE) in the promoter region of the target genes (Fedele et al., 2002) (Figure 2). Like HIF-1α, HIF-2α and 3α are stabilized by hypoxia and hypoglycemia, and activate transcription of genes involved in adapting to these adverse conditions, including the genes for erythropoietin (EPO), VEGF, and a number of enzymes of glycolysis including ALDO, phosphofructokinase (PFK) and lactate dehydrogenase (LDH) (Maltepe et al., 1997; Fraisl et al., 2009; Fedele et al., 2002). These studies suggest ARNT/HIF-1β is a central player in a number of signaling pathways and alterations in its activity can have serious impact on cellular responses to hypoxia, dioxin response and estrogen signaling in mammalian cells.

Observations from the ARNT/HIF-1β conditional knockout mice and whole body knockout mice have provided a wealth of information regarding the functional significance of this transcription factor in mammalian cells. Results obtained from the ARNT/HIF-1β null mice suggest that it plays a central role in embryonic development and physiological homeostasis as these mice are embryonic lethal due to severe defects in angiogenesis and placental development (Maltepe et al., 1997; Kozak et al., 1997). Data obtained from tissue specific ARNT/HIF-1β knockout mice demonstrates that disruption of ARNT/HIF-1β expression in liver and heart results in loss of AhR-stimulated gene transcription and that ARNT/HIF-1β is key to AhR function in these two mammalian tissues. It was also observed that ARNT/HIF-1β affects HIF-1α mediated target gene expression as several key genes including the expression of heme-oxygenase and glucose transporter-1 mRNA was abolished after treatment with $CoCl_2$, an agent that is thought to mimic hypoxia (Tomita et al., 2000).

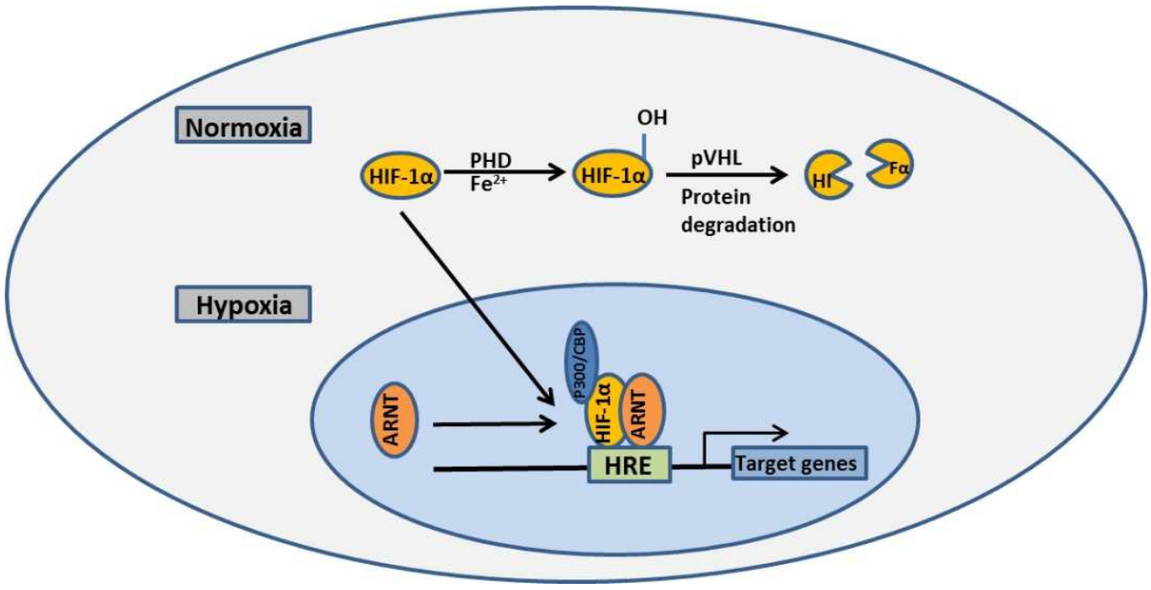

Fig. 2. Overview of gene regulation by ARNT/HIF-1β/HIF-1α complex in mammalian cells under normoxic and hypoxic conditions. In normoxic conditions, HIF-1α protein undergoes oxygen dependent hydroxylation by prolyl hydroxylases (PHD) and the hydroxylation site is recognized by pVHL, which targets the protein for ubiquitination by ubiquitin ligase, followed by degradation through ubiquitin proteasome pathway. During hypoxia, HIF-1α protein is not targeted for degradation and can translocate to the nucleus, where it heterodimerizes with ARNT/HIF-1β to form a stable transcriptional complex. The ARNT/HIF-1β/HIF-1α heterodimer then binds to the hypoxia response element (HRE) of target genes.

3. ARNT/HIF-1β and type 2 diabetes

3.1 ARNT/HIF-1β is reduced in human diabetic islets

In 2005, a study published in *Cell* (Gunton et al., 2005) suggested that ARNT/HIF-1β, a transcription factor with previously unknown functions in beta-cells, plays a significant role in mediating human beta-cell dysfunction in type 2 diabetics. Genome-wide gene expression profiling of islets obtained from human non-diabetics and type 2 diabetics revealed that the expression levels of ARNT/HIF-1β was reduced by 90% under the diabetic conditions. This was associated with reduced expression levels of several ARNT/HIF-1β target genes involved in glycolysis and insulin signaling. Several enzymes in glycolysis, including phosphoglucomutase (PGM), phosphoglucose isomerase (G6PI), PFK and ALDO were expressed at significantly lower levels as compared to those observed in normal islets. The low ARNT/HIF-1β expression levels observed under diabetic conditions was also associated with low gene expression levels of several key regulators in insulin signaling, such as the insulin receptor (IR), insulin receptor substrate 2 (IRS2), and protein kinase B (Akt2). Another interesting observation made in this study was that MODY genes, HNF-1α and HNF- 4α, were poorly expressed in human islets obtained from type 2 diabetics. HNF-4α, the gene mutated in MODY1, has been shown to interact with ARNT/HIF-1β possibly providing a connection between the two transcription factors (Tsuchiya et al., 2002).

In order to rule out the possibility that the profound ARNT/HIF-1β down regulation in pancreatic beta-cells is not caused by the diabetic environment, Gunton and co-workers demonstrated that an identical gene profile was observed in a beta-cell-specific ARNT/HIF-1β knockout mouse (β-ARNT KO). β-ARNT KO mice exhibited impaired GSIS and glucose intolerance, with no significant change in beta-cell insulin content and islet mass. The finding that ARNT/HIF-1β knockout mice have normal islet mass suggests that this transcription factor does not play a role in beta-cell differentiation.

Overall, a combination of *in vivo* and *in vitro* studies in humans and rodents have provided us with convincing evidence that reduction in ARNT/HIF-1β expression in human pancreatic beta-cells has negative consequences in terms of beta-cell function and insulin secretion. However, the extent of ARNT/HIF-1β mediated regulation of gene transcription is complex since it has the potential to bind with multiple partners affecting a multitude of signaling pathways.

3.2 ARNT/HIF-1β is reduced in human diabetic hepatic cells

In both rodents and humans, the liver plays a critical role in maintaining glucose and lipid homeostasis. During fasting, hepatic glucose production is critical for providing glucose for the brain, the kidneys and red blood cells. In liver, glucose is produced by glycogenolysis during the initial stages of fasting, however, after several hours of fasting, glucose production is primarily from gluconeogenesis, a process by which the liver produces glucose from precursors such as lactate and pyruvate (Michael et al., 2000; Saltiel & Kahn, 2001). Wang et al showed that ARNT/HIF-1β was severely reduced in the livers of human type 2 diabetics (Wang et al., 2009). Gene expression profiling of liver specimens from normal, obese and obese diabetic patients revealed a 30% reduction in the expression of ARNT/HIF-1β gene in obese diabetic individuals. The study demonstrated that the reduced expression of ARNT/HIF-1β in the livers of humans with T2D was associated with high glucose levels, high insulin levels, and insulin resistance. This study also suggested that insulin, not glucose regulates the expression of ARNT/HIF-1β gene and that ARNT/HIF-1β expression is reduced in both insulin-deficient and insulin-resistant states.

Wang et al (2009) also looked at the effects of liver-specific deletion of ARNT/HIF-1β gene in mice (L-ARNT KO) and demonstrated that there was an increase in gluconeogenesis, lipogenesis and increased serum insulin levels, all characteristic of human type 2 diabetics. The increase in hepatic gluconeogenesis and lipogenesis in L-ARNT KO mice was associated with the upregulation of several important gluconeogenic and lipogenic genes including PEPCK, G6Pase, SCD1 and FAS. Expression of C/EBPα and SREBP-1C, was also induced by 2-folds in L-ARNT KO mice. C/EBPα plays a major role in kick-starting hepatic glucose production at birth, and disruption of the C/EBPα gene in mice is known to cause hypoglycemia associated with the impaired expression of the gluconeogenic enzymes PEPCK and G6Pase (Pedersen et al., 2007; Qiao et al., 2006). SREBP-1C, on the other hand is a major player in lipogenesis (Horton et al., 2002). ARNT/HIF-1β may act as an upstream regulator of these transcription factors and play a key role in maintaining whole body glucose and lipid homeostasis. However, as seen in pancreatic beta-cells, the exact pathways targeted by ARNT/HIF-1β in liver cells are not clearly understood and is complicated by the fact that ARNT/HIF-1β has multiple binding partners.

3.3 ARNT/HIF-1β regulates glucose metabolism and insulin secretion in beta-cells

The central role played by ARNT/HIF-1β/HIF-1α heterodimer in the regulation of glucose homeostasis, particularly glycolysis has been well studied (Dery et al., 2005; Semenza et al., 1994) with a focus in cancer cell metabolism (Song et al., 2009; Semenza et al., 2000; Semenza, 2003). It is widely accepted that ARNT/HIF-1β/HIF-1α heterodimer plays a role in the Warburg effect, where cancer cells undergo a high rate of anaerobic glycolysis compared to normal cells. It has been suggested that the observed increase in glycolytic enzymes in these cancer cells is associated with increased HIF-1 activity, thus aiding in tumor formation and progression. Studies conducted in ARNT/HIF-1β mutant clonal cells indicate that it is an essential component of the HIF-1α complex and that absence of ARNT/HIF-1β leads to reduced cellular responses to stimuli such as hypoxia (Woods et al., 1996).

In pancreatic beta-cells, metabolism of glucose through aerobic glycolysis and oxidative phosphorylation plays a significant role in maintaining a normal secretory capacity. Beta-cells sense glucose and secrete appropriate amounts of insulin to promote glucose uptake by muscles and adipose tissue. Insulin also inhibits hepatic glucose production. Abnormal insulin secretion is one of the earliest detectable defects at the onset of T2D and despite its relevance, the mechanisms underlying GSIS are not completely understood. The generally accepted model of GSIS holds that metabolism of glucose in the beta-cells leads to a rise in the cytosolic ATP/ADP levels, which promotes closure of the K_{ATP} channel, increased cytosolic Ca^{2+} and triggers exocytosis of insulin-containing secretory granules (Henquin et al., 2003; Jensen et al., 2008). In beta-cells glucose derived pyruvate is directed mostly towards TCA for the production of ATP, since both the pentose phosphate pathway and anaerobic glycolysis is relatively inactive (Schuit et al., 1997). This exceptionally high dependence of beta-cells on the TCA cycle suggests that hypoxia or mechanisms reducing the aerobic capacity of beta-cells would probably have profound effects on GSIS.

It has been shown that down regulation of ARNT/HIF-1β in pancreatic beta-cells leads to loss of GSIS (Gunton et al., 2005; Pillai et al 2011). Our group has demonstrated that beta-cells with reduced ARNT/HIF-1β expression levels exhibit a 31% reduction in glycolytic flux without significant changes in glucose oxidation or the ATP/ADP ratio. Metabolomics analysis revealed that clonal beta-cells (832/13) treated with siRNAs against the ARNT/HIF-1β gene have lower levels of glycolytic, TCA cycle and fatty acid intermediates (Figure 3). It was also shown that the reduced levels of glycolysis, TCA and fatty acid intermediates were associated with a corresponding decrease in the expression of key genes in all three metabolic pathways including GLUT2, GK, PC, PDH, MEc, CIC DIC, CPT1a and FAS (Figure 4). The novel finding that reducing ARNT/HIF-1β levels leads to a profound reduction in PC, DIC, and OGC expression levels and a reduction in glycoslysis and TCA metabolites, even though glucose oxidation and ATP production were unaltered, is an unexpected result. These collective changes in metabolite levels suggest that the oxidative entry of pyruvate into the TCA cycle is preserved in the absence of ARNT/HIF-1β at the expense of a loss of anaplerosis. A key role for anaplerosis in insulin secretion is supported by the finding that pyruvate flows into mitochondrial metabolic pathways, in roughly equal proportions, through the anaplerotic (PC) and oxidative (PDH) entry points. Glucose carbon entering through the PC reaction leads to an increase in TCA intermediates (called anaplerosis) (Schuit et al., 1997; Khan et al., 1996). In addition, beta-cells contain enzymes that allow "cycling" of pyruvate via its PC-catalyzed conversion to oxaloacetate (OAA), metabolism of OAA to malate, citrate, or isocitrate in the TCA cycle, and subsequent recycling of these metabolites to pyruvate via several possible

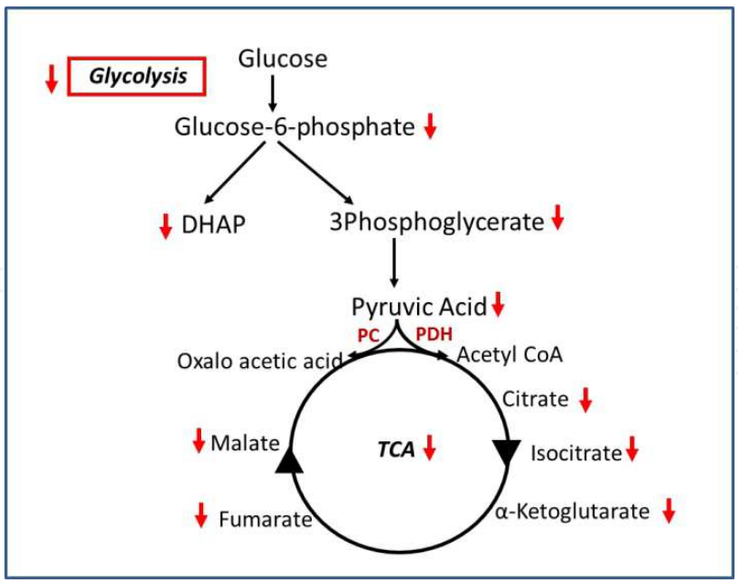

Fig. 3. Summary of the effects of siRNA mediated suppression of ARNT/HIF-1β in beta-cells. Several key metabolites in both glycolysis and TCA cycle were negatively affected by the knockdown of ARNT/HIF-1β. TCA, tricarboxylic acid cycle; DHAP, dihydroxyacetone phosphate; PC, pyruvate carboxylase; PDH, pyruvate dehydrogenase.

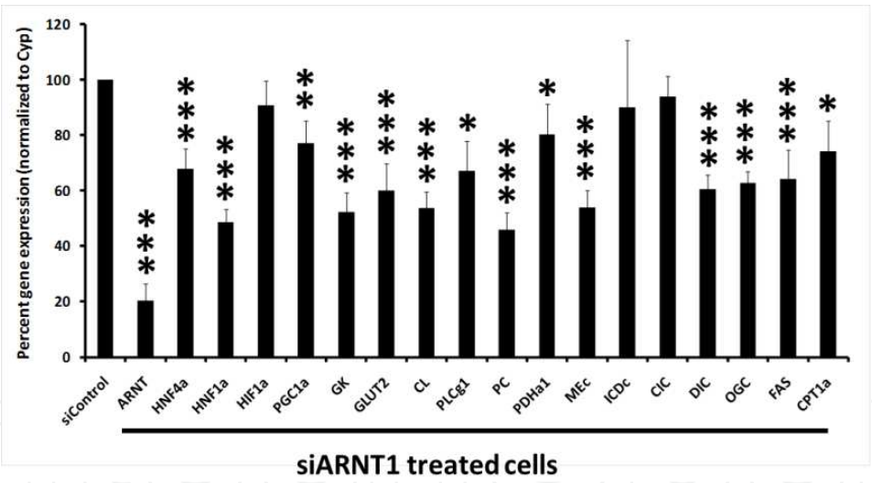

Fig. 4. Effects of siRNA mediated suppression of ARNT/HIF-1β on key genes involved in the metabolic regulation of β-cell function in 832/13 cells. Gene expression is expressed as a percentage of the target gene from siControl treated cells and corrected for by an internal control gene cyclophilin E (Cyp). There was no significant difference seen between treatment groups for Cyp (n=5). HNF4a, hepatocyte nuclear factor-4α; HNF1a, hepatocyte nuclear factor-1α; HIF1a, hypoxia inducible factor 1α; GK, glucokinase; GLUT2, glucose transporter-2; PLGg1, phospholipase γ-1; PC, pyruvate carboxylase; PDHa1, pyruvate dehydrogenase (α1 subunit); MEc, cytosolic malic enzyme; ICDc, cytosolic isocitrate dehydrogenase; CIC, citrate carrier; DIC, dicarboxylate carrier; OGC, α-ketoglutarate carrier; FAS, fatty acid synthase; CPT1a, Carnitine palmitoyl transferase 1α. * P<0.05, ** P<0.01, *** P<0.001 siControl vs siARNT1.

combinations of cytosolic and mitochondrial pathways (MacDonald et al., 1995). Numerous groups have shown that both pyruvate cycling and anaplerosis are important to maintain normal secretory capacity of beta-cells (Lu et al., 2002; Joseph et al., 2006; MacDonald et al., 2005; Ronnebaum et al., 2006). Since the amount of pyruvate is substantially lower in ARNT/HIF-1β depleted beta-cells, our data also suggests that this gene may play an important role in maintaining pyruvate cycling. However a direct link between ARNT/HIF-1β and pyruvate cycling has not yet been established. Figure 5 shows the diagrammatic representation of the transcriptional network regulated by ARNT/HIF-1β and its involvement glucose-stimulated anaplerosis and insulin release.

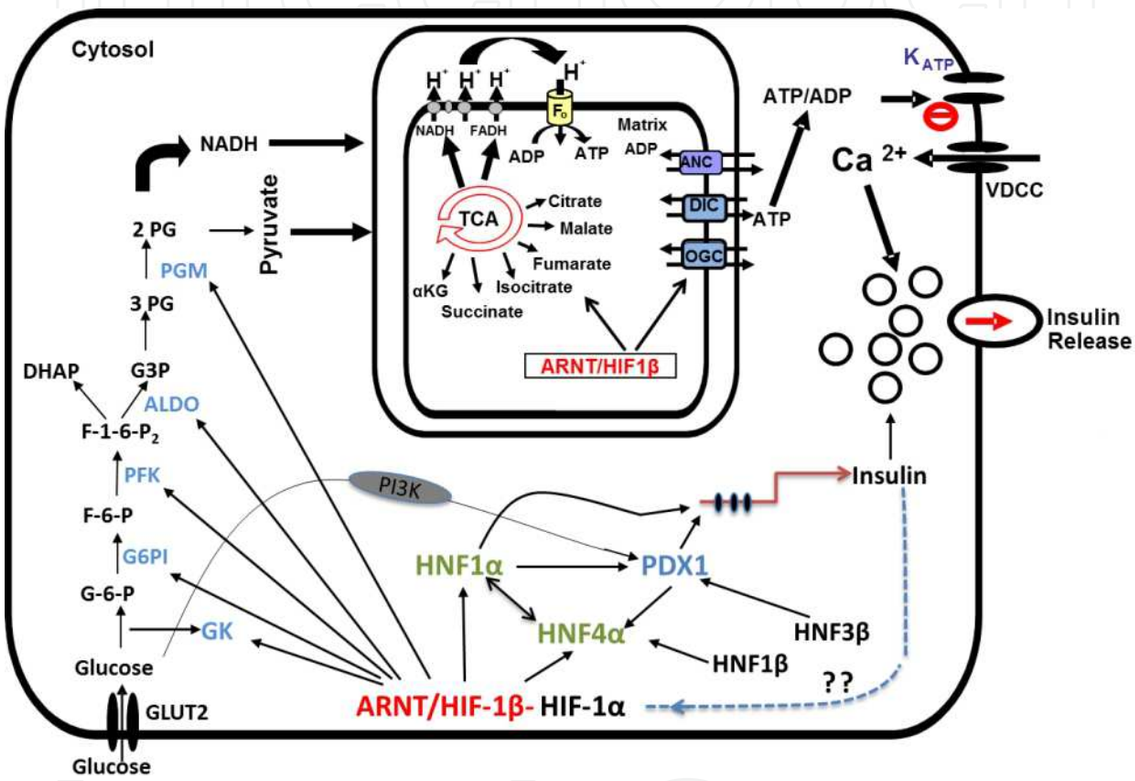

Fig. 5. Schematic of the transcriptional network regulated by ARNT/HIF-1β and its involvement in glucose-stimulated anaplerosis and insulin release from beta-cells. ARNT/HIF-1β regulates key genes in glycolysis and TCA cycle (shown in blue), including key metabolite carriers such as DIC and OGC. MODY genes regulated by ARNT/HIF-1β are shown in green. Interestingly, ARNT/HIF-1β does not seem to play a significant role in the regulation ATP production in beta-cells, however, it seems to be very important for glucose-induced anaplerosis, which provides crucial signals for GSIS.

3.4 ARNT/HIF-1β regulates beta-cell and hepatic transcriptional networks

Studies in ARNT/HIF-1β deficient beta-cells suggest that it plays a crucial role in the regulation of key genes involved in glucose metabolism and insulin secretion (Gunton et al., 2005, Pillai et al., 2011). ARNT/HIF-1β target genes in beta-cells include the MODY1 and MODY3 genes HNF4α and HNF1α, glucose metabolism genes GK, G6PI, PFK, aldolase, PC, PDH, MEc, DIC, OGC and insulin signaling genes IR, IRS2 and AKT2. In non-beta-cells it

has been shown that ARNT/HIF-1β is essential for the normal function of HIF-1α, HIF2α, and AhR. These heterodimeric complexes are required for cellular responses to hypoxia (HIF proteins) and environmental toxins (AhR), respectively (Kozak et al., 1997; Kewley et al., 2004). It has been estimated that there are more than 13,000 putative ARNT/HIF-1β binding sites in promoters in the human genome (Gunton et al., 2005). Many of the target gene promoters have multiple potential binding sites. Thus it is reasonable to estimate that a substantial decrease in ARNT/HIF-1β would affect the expression of a large number of genes in humans. Although there is a lack of direct biochemical evidence, many of the genes found to be altered in association with decreased ARNT/HIF-1β gene expression have putative ARNT/HIF-1β-dimer consensus binding sites in their promoters (including HNF4α, HNF1α, Akt2, G6PI, PFK, and aldolase), suggesting a direct role for ARNT/HIF-1β containing dimers in the regulation of their expression (Figure 6).

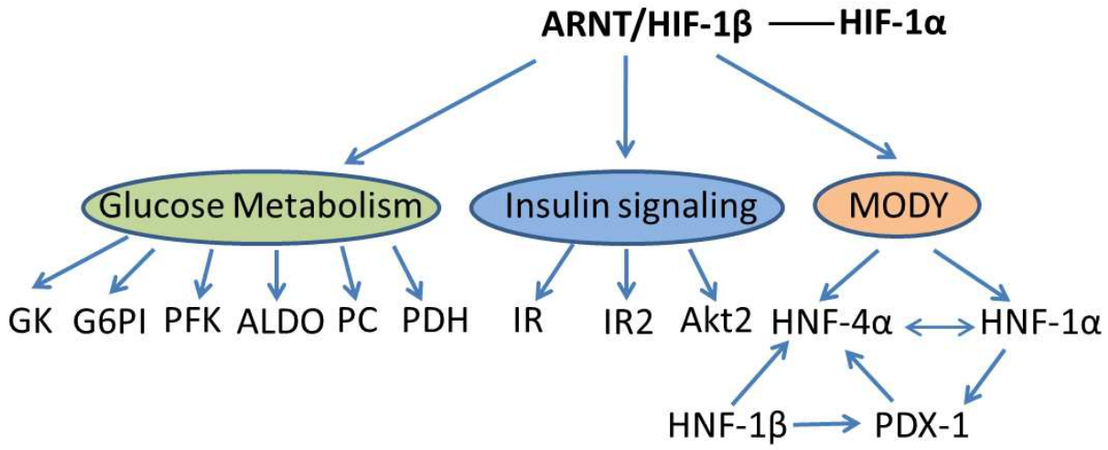

Fig. 6. Transcriptional network in pancreatic beta-cells regulated by ARNT/HIF-1β. ARNT/HIF-1β regulates several key genes involved in glucose metabolism, insulin signaling and MODY. Beta-cell specific knockout of ARNT/HIF-1β in mice leads to reduced expression of a number of important beta-cell genes including HNF-4α, HNF-1α, insulin receptor (IR), insulin receptor substrate-2 (IRS2), protein kinase b (Akt2), glucokinase (GK), glucose-6-phosphoisomerase (G6PI), phosphofructokinase (PFK), aldolase (ALDO), pyruvate carboxylase (PC) and pyruvate dehydrogenase (PDH).

In liver cells ARNT/HIF-1β has been shown to regulate the expression of several genes involved in glucose and lipid homeostasis. Support for the involvement of ARNT/HIF-1β in liver glucose homeostasis was provided by experiments showing that basal and insulin-induced expression of GLUT1, GLUT3, ALDO, PGK and VEGF were significantly reduced in ARNT/HIF-1β-defective HepG2 cells (Salceda et al., 1996). Wang *et al* (2009) demonstrated that a reduction of ARNT/HIF-1β in liver cells was associated with an increase in the expression of several important gluconeogenic and lipogenic genes including PEPCK, G6Pase, SCD1, FXR, C/EBPα, SREBP-1C, FBP-1 and FAS. The discovery that ARNT/HIF-1β may contribute to the regulation of beta-cell and hepatic genes suggests an essential role for this transcription factor in the regulation of glucose and lipid homeostasis (Figure 7).

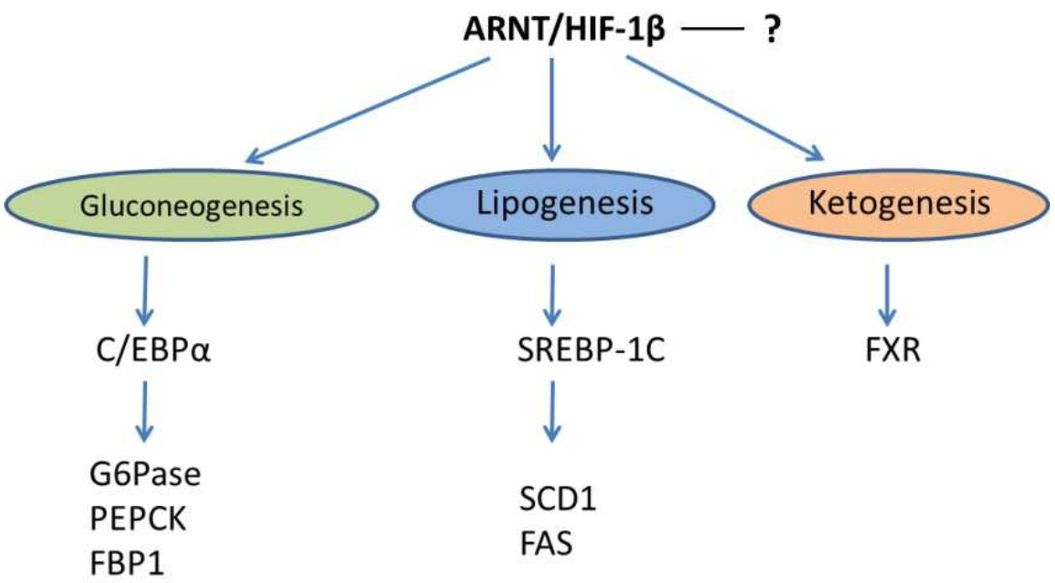

Fig. 7. ARNT/HIF-1β regulates the expression of several key genes involved in gluconeogenesis, lipogenesis and ketogenesis in the liver cells. Liver-specific knockout of ARNT/HIF-1β in mice leads to increased hepatic gluconeogensis and lipogenesis with a corresponding increase in the expression of phosphoenolpyruvate carboxykinase (PEPCK), Glucose-6-phosphatase (G6Pase), Fructose-1,6-biphosphatase (FBP1), Steroyl–CoA-desaturase (SCD1), Fatty acid synthase (FAS), CCAAT enhancer binding protein α (C/EBPα), sterol regulatory element binding protein (SREBP-1C) and Farsenoid X receptor (FXR). Adapted from Wang *et al.* (2009).

3.5 Regulation of ARNT/HIF-1β in beta-cells

Since ARNT/HIF-1β appears to be a major player in the pathogenesis of T2D, several attempts have been made to identify the upstream regulators of the gene in beta-cells. In 2008, Dror *et al.*, showed that glucose and endoplasmic reticulum Ca^{2+} channels regulate the expression of ARNT/HIF-1β in beta-cells via presinilin. Presinilin is a protein that has been implicated in the cellular response to reduced metabolic activity (Koo and Koppen, 2004). Overexpression of presenilin-1 in clonal Min6 beta-cells increased ARNT/HIF-1β suggesting that ARNT/HIF-1β may be a downstream target of presenilin (Dror et al., 2008). They demonstrated that this pathway is controlled by Ca^{2+} flux through intracellular channels. ARNT/HIF-1β has also recently been shown to be regulated by the carbohydrate-responsive element-binding protein (ChREBP), which is a transcription factor shown to regulate carbohydrate metabolism in the liver and pancreatic beta-cells in response to elevated glucose concentrations (Noordeen et al., 2009). In a genome-wide approach using high-density oligonucleotide arrays, the study showed that ChREBP binds directly to ARNT/HIF-1β promoter in Min6 clonal beta-cells. Accordingly, knockdown of ChREBP using siRNA resulted in an increase ARNT/HIF-1β mRNA levels whereas overexpression of ChREBP resulted in a decrease in ARNT/HIF-1β mRNA levels in rat. They also showed that incubating INS-1 (832/13) cells with glucose led to a substantial decrease in ARNT/HIF-1β mRNA levels.

Interestingly, it has also been shown that HIF-1α, the highly regulated binding partner for ARNT/HIF-1β, may be active under normoxic conditions in mouse and human beta-cells (Cheng et al., 2010). Coimmunoprecipitation studies demonstrated that HIF-1α was bound to ARNT/HIF-1β at the promoter region providing evidence for an interaction between HIF-1α and ARNT/HIF-1β in beta-cells. Treatment of diabetic mice with deferasirox (DFS), an agent that increases HIF-1α protein levels, improved glucose tolerance, normalized the expression of ARNT/HIF-1β and its target genes in human T2D islets. The same study also showed that HIF-2α, but not AhR, is another possible binding partner for ARNT/HIF-1β in pancreatic beta-cells. These studies provide a novel mechanism to regulate ARNT/HIF-1β gene expression in beta-cells.

Three studies published from independent laboratories studying the impact of increasing HIF-1α levels in beta-cells indicate that one has to be extremely cautious when using pharmacological agents, such as DFS, to activate HIF-1α in the islets (Puri et al., 2008; Zehetner et al., 2008; Cantley et al., 2009). These studies used the Cre-loxP system to conditionally delete VHL gene in beta-cells and showed that there were adverse effects associated with an increase in HIF-1α levels on beta-cell function. In all the three studies, increased HIF-1α levels were accompanied by severely impaired GSIS and increased lactate production, indicating a switch from aerobic to anaerobic glycolysis. Thus there appears to be a dose-response curve for the affects HIF-1α protein levels on beta-cell function (Cheng *et al.*, 2010). Although complete lack of HIF-1α seems deleterious to GSIS in mice and Min6 cells, milder increases are beneficial for beta-cell function. As seen in VHL knockout mice, very high levels of HIF-1α are detrimental for normal beta-cell function. Therefore, before we begin to develop a novel treatment regime that enhances HIF-1α or ARNT/HIF-1β activity in human diabetic islets, it is imperative that we understand the expected outcomes of such changes to avoid any detrimental effects.

4. Concluding remarks

It is well known that ARNT/HIF-1β plays a role in the cellular responses to hypoxia, however recent research has demonstrated a broader role for this transcription factor in maintaining glucose and lipid homeostasis in type 2 diabetics. It is now clear that a significant decrease in ARNT/HIF-1β gene expression in both the pancreatic beta-cells and the liver cells is deleterious and can result in T2D. Conversely, targeted disruption of ARNT/HIF-1β gene expression in the adipocytes followed by treatment of mice with a high fat diet improves insulin sensitivity and decreases adiposity (Jiang *et al.*, 2011). A central role for ARNT/HIF-1β in the regulation of key genes involved in glucose sensing, GSIS and insulin signaling in rodents as well in human islets suggest it plays an important role in maintaining normal beta-cell function. Current studies support the idea that ARNT/HIF-1β could act as an upstream regulator of many of the key genes involved in glucose and lipid homeostasis. Clearly, the transcriptional network regulated by ARNT/HIF-1β and genes that are under direct or indirect control of this transcription factor is very broad and hence any change in the regulation of ARNT/HIF-1β may have an impact on many signaling pathways. The fact that ARNT/HIF-1β is a binding partner for several other Per/ARNT/Sim transcription factor family members like HIF-1α, HIF-2α, HIF-3α and AhR makes it a significant member of this family of transcription factors. Improving our understanding of the beta-cell transcription factors, establishing their mechanism of action

and hierarchy and finding ways to regulate their expression could prove beneficial in developing novel tools to prevent or correct beta-cell dysfunction in T2D.

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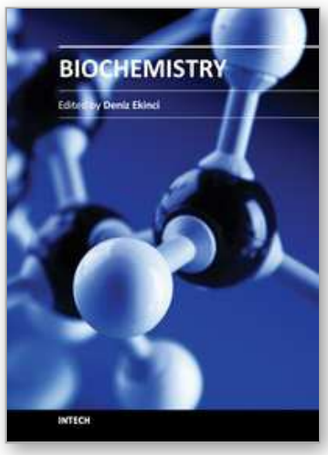

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Over the recent years, biochemistry has become responsible for explaining living processes such that many scientists in the life sciences from agronomy to medicine are engaged in biochemical research. This book contains an overview focusing on the research area of proteins, enzymes, cellular mechanisms and chemical compounds used in relevant approaches. The book deals with basic issues and some of the recent developments in biochemistry. Particular emphasis is devoted to both theoretical and experimental aspect of modern biochemistry. The primary target audience for the book includes students, researchers, biologists, chemists, chemical engineers and professionals who are interested in biochemistry, molecular biology and associated areas. The book is written by international scientists with expertise in protein biochemistry, enzymology, molecular biology and genetics many of which are active in biochemical and biomedical research. We hope that the book will enhance the knowledge of scientists in the complexities of some biochemical approaches; it will stimulate both professionals and students to dedicate part of their future research in understanding relevant mechanisms and applications of biochemistry.

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