REVIEW

Mechanisms of β -cell dedifferentiation in diabetes: recent findings and future research directions

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Abstract

Like all the cells of an organism, pancreatic β -cells originate from embryonic stem cells through a complex cellular process termed differentiation. Differentiation involves the coordinated and tightly controlled activation/repression of specific effectors and gene clusters in a time-dependent fashion thereby giving rise to particular morphological and functional cellular features. Interestingly, cellular differentiation is not a unidirectional process. Indeed, growing evidence suggests that under certain conditions, mature β -cells can lose, to various degrees, their differentiated phenotype and cellular identity and regress to a less differentiated or a precursor-like state. This concept is termed dedifferentiation and has been proposed, besides cell death, as a contributing factor to the loss of functional β -cell mass in diabetes. β -cell dedifferentiation involves: (1) the downregulation of β -cell-enriched genes, including key transcription factors, insulin, glucose metabolism genes, protein processing and secretory pathway genes; (2) the concomitant upregulation of genes suppressed or expressed at very low levels in normal β -cells, the β -cell forbidden genes; and (3) the likely upregulation of progenitor cell genes. These alterations lead to phenotypic reconfiguration of β -cells and ultimately defective insulin secretion. While the major role of glucotoxicity in β -cell dedifferentiation is well established, the precise mechanisms involved are still under investigation. This review highlights the identified molecular mechanisms implicated in β -cell dedifferentiation including oxidative stress, endoplasmic reticulum (ER) stress, inflammation and hypoxia. It discusses the role of *Foxo1*, *Myc* and inhibitor of differentiation proteins and underscores the emerging role of non-coding RNAs. Finally, it proposes a novel hypothesis of β -cell dedifferentiation as a potential adaptive mechanism to escape cell death under stress conditions.

Key Words

- ▶ β -cell
- ▶ dedifferentiation
- ▶ diabetes
- ▶ glucotoxicity
- ▶ islet

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Introduction

All the cells of an organism, including pancreatic β -cells, originate from embryonic stem cells through a complex cellular process termed differentiation (Keller 2005). β -cell differentiation involves the coordinated and tightly

controlled activation/repression of specific transcription factors and downstream gene clusters in a time-dependent manner. These complex signalling events drive the transition from definitive endoderm cells to mature

Figure 1

Schematic representation of the process of β -cell differentiation. This process involves coordinated and tightly controlled activation/repression of specific transcription factors that drive the transition from a stem cell to a mature β -cell during embryonic development and the postnatal period until weaning. Evidence from animal and human studies suggest that mature β -cells could regress backwards to a precursor-like stage and/or transdifferentiate into other islet cells in type 2 diabetes.

insulin-secreting β -cells with the progressive acquisition of specific morphological and functional cellular features (Puri & Hebrok 2010, Seymour & Sander 2011, Stanger & Hebrok 2013) (Fig. 1).

Interestingly, cellular differentiation is not unidirectional. Indeed, growing evidence suggests that under specific conditions, mature β -cells can lose, to various degrees, their differentiated phenotype and cellular identity and regress to a less differentiated or even a precursor-like state (Fig. 1). This concept, termed dedifferentiation, has been implicated in the pathogenesis of diabetes (Weir *et al.* 2013). Several *in vivo* studies in animal models and in humans over the last two decades suggest that, in addition to cell death (Butler *et al.* 2003, Jurgens *et al.* 2011), dedifferentiation (Jonas *et al.* 1999, Kjørholt *et al.* 2005, Talchai *et al.* 2012, Marselli *et al.* 2014, Cinti *et al.* 2016) may be an important contributing factor to the loss of functional β -cell mass in type 2 diabetes (Sakuraba *et al.* 2002, Yoon *et al.* 2003, Deng *et al.* 2004, Rahier *et al.* 2008).

β -cell dedifferentiation is manifested by reduced expression of β -cell-enriched genes, including key transcription factors, insulin, glucose metabolism genes, protein processing and secretory pathway genes and the concomitant upregulation of genes suppressed or expressed at very low levels in normal β -cells; the so-called β -cell disallowed or forbidden genes (Bensellam *et al.* 2012b, Schuit *et al.* 2012, Rutter *et al.* 2015, Lemaire *et al.* 2016, 2017). Moreover, the presence of progenitor cell markers has been observed in the dedifferentiated islets of diabetic animals (Talchai *et al.* 2012, Wang *et al.* 2014, Kim-Muller *et al.* 2016) and even markers of α -cells have been detected suggesting the transdifferentiation of β -cells to α -cells in diabetic animals and humans (Spijker *et al.* 2013, 2015, Brereton *et al.* 2014, Cinti *et al.* 2016). These alterations lead to metabolic and structural reconfiguration of β -cells and ultimately defective insulin secretion. While earlier and recent reports have well established the major role of hyperglycemia and the ensuing glucotoxicity in β -cell dedifferentiation (Bensellam *et al.* 2012b), the

precise molecular mechanisms involved are still under investigation. Understanding these mechanisms opens novel therapeutic horizons to preserve/restore the functional β -cell mass in diabetes.

In this extensive review, we first highlight the close relationship between the degree of β -cell differentiation and insulin secretory function. We then describe the known molecular mechanisms involved in β -cell dedifferentiation in diabetes with a focus on the role of glucotoxicity and its downstream pathways including oxidative stress, endoplasmic reticulum (ER) stress, inflammation and hypoxia. We depict the roles of the transcriptional regulators such as *Foxo1*, *Myc* and the inhibitors of differentiation and discuss the role of the failure of adaptive unfolded protein response (UPR). We also discuss the mounting role of non-coding RNAs in β -cell dedifferentiation. Finally, based on our and others emerging evidence, we propose a novel view of dedifferentiation as a potential adaptive mechanism to escape cell death under stress conditions.

Relationship of β -cell differentiation to insulin secretory function

Pancreatic β -cells play a fundamental role in the maintenance of glucose homeostasis in mammals. They synthesize and secrete insulin after meals to keep blood glucose levels within a tight range (between 4 and 6 mM in fasting adult humans). Glucose is the major physiological modulator of β -cell function and undeniable evidence indicates that glucose must be metabolized by β -cells in order to stimulate insulin secretion (Malaisse *et al.* 1979, Newgard & McGarry 1995, Jitrapakdee *et al.* 2010). Thus, upon a rise in its plasma concentration, glucose rapidly equilibrates across the β -cell plasma membrane via the high-capacity, low-affinity glucose transporter GLUT2 (also known as SLC2A2) (Thorens 2015). In humans, the high-affinity glucose transporter GLUT1 prevails instead of GLUT2 (De Vos *et al.* 1995, Ferrer *et al.* 1995). Nevertheless, in both human and rodent β -cells, glucose transport exceeds glucose utilization and is therefore not limiting (Newgard 1996). In the cytosol, glucose is then phosphorylated by glucokinase (GCK); a low-affinity type IV hexokinase. GCK regulates the rate of glucose utilization and is therefore considered as the glucose sensor of β -cells (Matschinsky 2009). Interestingly, β -cells present the remarkable feature of an almost complete repression of high-affinity type I–III hexokinases to avoid insulin release at low plasma glucose concentrations in the

fasted state (Sekine *et al.* 1994, Schuit *et al.* 1999). Glucose-6-phosphate is then oxidized by glycolysis to generate ATP, NADH and pyruvate. Because β -cells present another remarkable feature of expressing very low levels of lactate dehydrogenase (LDH) and monocarboxylate transporter (MCT) (Sekine *et al.* 1994, Ishihara *et al.* 1999, Zhao *et al.* 2001, Thorrez *et al.* 2011), almost all the pyruvate enters mitochondria to provide a substrate for the Krebs cycle (Fig. 2). This particular design also avoids exercise-induced insulin release (Otonkoski *et al.* 2007). To compensate for the low LDH activity and maintain the glycolytic flux, β -cells present a third notable characteristic of two highly active mitochondrial membrane electron shuttles: the glycerol-3-phosphate and the malate-aspartate shuttles that couple the regeneration of cytosolic NAD⁺ with the production of NADH or FADH₂ in the mitochondria (MacDonald 1981, 1982, Sekine *et al.* 1994, Eto *et al.* 1999). This NADH shuttle system allows tight coupling of glycolysis to mitochondrial metabolism (Fig. 2). In the mitochondria, pyruvate is either oxidized to acetyl-CoA (~50%) by pyruvate dehydrogenase (PDH) or carboxylated to oxaloacetate (~50%) by pyruvate carboxylase (PC) (MacDonald 1993, Khan *et al.* 1996). The latter enzyme replenishes Krebs cycle intermediates (anaplerosis) to compensate for their removal for biosynthetic purposes (cataplerosis) (Brun *et al.* 1996, Schuit *et al.* 1997, Farfari *et al.* 2000). Pyruvate metabolism in the Krebs cycle generates reducing equivalent NADH and FADH₂ which together with those generated by the mitochondrial shuttles transfer electrons to the respiratory chain to generate ATP (Duchen *et al.* 1993, Eto *et al.* 1999) (Fig. 2). The resulting atypical rise in ATP/ADP ratio (Detimary *et al.* 1996, 1998) leads to the closure of ATP-gated K⁺ (K_{ATP}) channels, plasma membrane depolarization, opening of voltage-gated Ca²⁺ channels (VDCC), Ca²⁺ influx and the elevation of cytosolic free Ca²⁺ concentration ([Ca²⁺]_i), which is the triggering signal for insulin secretion (Fig. 2). This sequence of events is referred to as the triggering pathway of glucose-stimulated insulin secretion (GSIS) (Henquin 2011). Besides, glucose can modulate insulin secretion independently from its action on K_{ATP} channels by generating signals that amplify the action of Ca²⁺ on insulin granule exocytosis, provided that Ca²⁺ influx is already stimulated and [Ca²⁺]_i is high. It is called the metabolic amplifying pathway of GSIS (Henquin 2011). This pathway requires glucose metabolism but the coupling factors have not been identified with certainty with a probable implication of NADPH (Ivarsson *et al.* 2005, Ferdaoussi *et al.* 2015) and other effectors (Fig. 2). Moreover, other nutrients (such as free fatty acids

Figure 2

Schematic summary of the stimulus-secretion coupling mechanism in mature differentiated β -cells with emphasis on key steps and principal components. Upon a rise in its extracellular concentration, glucose rapidly equilibrates across the plasma membrane via the glucose transporter GLUT2 (1) and is phosphorylated by glucokinase (GSK) (2). Glucose-6-phosphate (G-6-P) is further metabolized by glycolysis to generate pyruvate, ATP and NADH (3). The latter requires the mitochondrial shuttles glycerol-3-phosphate (GS) and malate-aspartate (MAS) to regenerate NAD^+ to compensate for low lactate dehydrogenase (LDH) activity. Because β -cells express low levels of monocarboxylate transporter (MCT), pyruvate is channelled to mitochondria where it is transformed into acetyl coenzyme A and oxaloacetate by pyruvate dehydrogenase (PDH) and pyruvate carboxylase (PC) respectively (4). Acetyl coenzyme A and oxaloacetate are used by the Krebs cycle (5) to generate NADH and FADH_2 that, together with those produced by the shuttles, transfer electrons to the electron transport chain (ETC) to generate ATP (6). The latter, together with ATP generated from glycolysis, increases the ATP/ADP ratio (7). This leads to the closure of ATP-dependent K^+ (K_{ATP}) channels (8), plasma membrane depolarization (9) and opening of voltage-dependent Ca^{2+} channels (VDCCs) (10). The ensuing Ca^{2+} influx and rise in cytosolic free Ca^{2+} concentration ($[\text{Ca}^{2+}]$) (11) is the triggering signal for insulin secretion (12) and this sequence of events is referred to as the triggering pathway. On the other hand, other metabolic coupling factors generated by glucose metabolism or other nutrient such as free fatty acids in addition to intracellular messengers affected by hormones such as glucagon-like peptide 1 (GLP1) and neurotransmitters such as acetylcholine (ACh) can amplify the effect of Ca^{2+} on insulin granule exocytosis (13). This is known as the amplifying pathway of insulin secretion. Green arrows: triggering pathway; red arrows: amplifying pathway. Effectors in green: β -cell-enriched genes; effectors in red: β -cell forbidden genes.

(Gravena *et al.* 2002, Itoh *et al.* 2003)), hormones (such as GLP1 (Campbell & Drucker 2013)) and neurotransmitters (such as acetylcholine (Gautam *et al.* 2007, Molina *et al.* 2014)) can also augment the action of Ca^{2+} on exocytosis (Fig. 2). The effect of hormones and neurotransmitters is known as the neurohormonal amplification of insulin secretion (Henquin 2011) (Fig. 2).

Adequate β -cell function relies on a specialized pattern of gene expression. Interestingly, the mature metabolic

phenotype of pancreatic β -cells is acquired only several weeks after birth (Asplund *et al.* 1969, Pildes *et al.* 1969, Lavine *et al.* 1971, Grill *et al.* 1981). The immaturity of neonatal β -cells and their poor GSIS have been correlated with low expression of key metabolic genes, high expression of the β -cell forbidden genes *Hk2*, *Ldha* and *Mct1* and changes in the expression of specific microRNAs (miRNAs) (Rorsman *et al.* 1989, Tan *et al.* 2002, Jermendy *et al.* 2011, Martens *et al.* 2014, Jacovetti *et al.* 2015).

Dietary differences between neonates and adults, in particular glucose, may drive the maturation of the β -cell phenotype. Indeed, strong evidence indicates that physiological glucose stimulation is a major regulator of the β -cell differentiated phenotype (Table 1). We and others have shown that the function and survival of rat islets and purified β -cells are optimally preserved by culture in the presence of 10 mM glucose (G10), whereas marked alteration accompanies culture at lower (G2–5) or higher glucose concentrations (G30; glucotoxicity will be discussed in ‘The loss of functional β -cell differentiation in diabetes: causes and mechanisms’ section) (Efanova *et al.* 1998, Flamez *et al.* 2002, Khalidi *et al.* 2004, Bensellam *et al.* 2009). The beneficial physiological glucose stimulation (G10 vs G2–5) is associated with increased mRNA levels of several critical genes including insulin, components of the triggering pathway of insulin secretion, such as *Glut2*, subunits of the K_{ATP} channel *Kir6.2* (also known as *Kcnj11*) and *Sur1* (also known as *Abcc8*) and metabolic genes including glycolysis and Krebs cycle genes, subunits of the mitochondrial electron transport chain, genes implicated in cataplerosis, fatty acid biosynthesis and cholesterol biosynthesis, in parallel with the upregulation of the mRNA levels of the transcription factors *Srebp1*, *Srebp2* and *Chrebp* (Flamez *et al.* 2002, Van Lommel *et al.* 2006, Bensellam *et al.* 2009) (Table 1). These findings are corroborated by the observation that prolonged fasting (72 h) markedly decreases insulin, *Glut2*, *Gck* and voltage-dependent L-type Ca^{2+} channel $\alpha 1$ subunit mRNA levels. Interestingly, these alterations were rapidly reversed after refeeding in association with the normalization of β -cell function (Iwashima *et al.* 1994).

In addition to metabolic genes, physiological glucose stimulation improves and maintains the β -cell differentiated phenotype by modulating several stress responses and prosurvival/proapoptotic effectors and pathways (Van de Casteele *et al.* 2003, Martens *et al.* 2005, Hou *et al.* 2008a, Bensellam *et al.* 2009, Roma *et al.* 2012, Sarre *et al.* 2012). Among them, the UPR occupies a central place (Elouil *et al.* 2007, Bensellam *et al.* 2009). β -cells are highly specialized secretory cells that synthesize large amounts of proinsulin in response to glucose stimulation (Schuit *et al.* 1988, Ling & Pipeleers 1996). To cope with this heavy ER load, β -cells are endowed with a fundamental adaptive response to prevent accumulation of misfolded or unfolded proteins in the ER when client protein load overwhelms the ER folding capacity. UPR activity is orchestrated by three sensors located in the membrane of the ER, namely the pancreatic ER kinase (PERK also known as EIF2AK3), the endoribonuclease/

kinase inositol requiring 1 (IRE1 also known as ERN1) and the activating transcription factor 6 (ATF6). Active PERK phosphorylates the α -subunit of the translation initiation factor 2 (eIF2 α) thereby leading to a transient and global attenuation of protein translation to decrease ER load in parallel with a paradoxical increase in the translation of special transcripts such as ATF4. This transcription factor upregulates the expression of chaperones and antioxidant genes as well as proapoptotic genes like *Ddit3* (also known as *Chop/Gadd153*), *Atf3* and *Trb3* (also known as *Trib3*) that contribute to β -cell death under prolonged or unresolved ER stress. Because eIF2 α can be phosphorylated by kinases other than PERK in response to other types of stress, we will refer to the eIF2 α -ATF4 arm of the UPR by Integrated Stress Response (ISR) if PERK activation has not been demonstrated. Activation of IRE1 leads to the unconventional splicing of *Xbp1* pre-mRNA and the ensuing upregulation of active X-Box-binding protein 1 (XBP1s). This key transcription factor, together with active ATF6, stimulates the expression of *Xbp1*, chaperones, foldases and genes of the ER-associated degradation (ERAD) pathway thereby leading to improved ER folding capacity and enhanced clearance of unfolded proteins from the ER (Schroder & Kaufman 2005). Interestingly, glucose stimulation exerts opposite effects on the PERK-eIF2 α -ATF4 and IRE1-XBP1s/ATF6 arms of the UPR. Thus, the PERK-eIF2 α -ATF4 arm is maximally activated at low glucose concentrations (PERK and eIF2 α phosphorylation, upregulation of ATF4 and downstream target genes such as *Ddit3*), where protein synthesis, ATP levels and $[Ca^{2+}]_{ER}$ are low, while the IRE1-XBP1 arm is maintained at basal level. Glucose stimulation rapidly repressed the ISR and triggered *Xbp1* pre-mRNA splicing leading subsequently to the upregulation of XBP1s and a wide array of downstream target genes to face the ER synthetic load (Elouil *et al.* 2007, Vander Mierde *et al.* 2007, Gomez *et al.* 2008, Bensellam *et al.* 2009, Jonas *et al.* 2009, Moore *et al.* 2011). Of note, an inverse relationship between blood glucose levels and eIF2 α phosphorylation in whole pancreatic extracts has been reported in the models of fasting mice and mice receiving an intraperitoneal glucose load (Zhang *et al.* 2002a). The fine-tuning of this adaptive response is vital for the preservation of the β -cell differentiated phenotype. Indeed, our and others recent evidence indicated that the failure of adaptive UPR is associated with the progression to diabetes and altered β -cell differentiation (Herbert & Laybutt 2016) (see ‘The loss of β -cell differentiation in diabetes: causes and mechanisms’ section).

Besides the role of nutrient-induced stimulation of gene expression, the acquisition and maintenance of

Table 1 Physiological glucose stimulation plays a key role in the maintenance of the differentiated β -cell phenotype.

Genes	Physiological glucose stimulation	Effect	Models	References
Islet hormones <i>Preproinsulin</i>	G10 vs G2–5	Up (also protein)	Cultured male Wistar rat islets (18 h) and purified β cells (3–10 days)	Schuit <i>et al.</i> (2002), Van Lommel <i>et al.</i> (2006), Bensellam <i>et al.</i> (2009)
	Fed vs fasted	Up	Fasted male C57BL6 mice (overnight) and male Sprague–Dawley rats (72 h)	Iwashima <i>et al.</i> (1994), Van Lommel <i>et al.</i> (2006)
<i>lapp</i>	G10 vs G2–5	Up	Cultured male Wistar rat islets (18 h)	Bensellam <i>et al.</i> (2009)
Transcription factors <i>Pdx1</i>	G10 vs G5	Up	Cultured C57BL6J mouse islets (24 h)	(Supplementary Fig. 1)
<i>Chrebp</i>	G10 vs G2–5	Up	Cultured male Wistar rat islets (18 h)	Bensellam <i>et al.</i> (2009)
<i>Srebp1 (Srebf1)</i>	G10 vs G2–5	Up	Cultured male Wistar rat islets (18 h) and purified β cells (24 h)	Bensellam <i>et al.</i> (2009), Flamez <i>et al.</i> (2002)
<i>Srebp2 (Srebf2)</i>	G10 vs G2–5	Up	Cultured male Wistar rat islets (18 h)	Bensellam <i>et al.</i> (2009)
Glucose transport and metabolism <i>Glut2 (Slc2a2)</i>	G10 vs G2–5	Up	Cultured male Wistar rat islets (18 h) and purified β cells (24–72 h) and cultured C57BL6J mouse islets (24 h)	Bensellam <i>et al.</i> (2009), Flamez <i>et al.</i> (2002), Martens & Pipeleers (2009), (Supplementary Fig. 1)
	Fed vs fasted	Up	Fasted male Sprague–Dawley rats (72 h)	Iwashima <i>et al.</i> (1994)
<i>Gck (Hk4)</i>	G10 vs G2–5	Up	Cultured male Wistar rat islets (18 h) and purified β cells (72 h)	Bensellam <i>et al.</i> (2009), Martens & Pipeleers (2009)
	Fed vs fasted	Up	Fasted male Sprague–Dawley rats (72 h)	Iwashima <i>et al.</i> (1994)
<i>Gapdh</i>	G10 vs G2–5	Up	Cultured male Wistar rat islets (18 h)	Bensellam <i>et al.</i> (2009)
<i>G6pd</i>	G10 vs G2–5	Up	Cultured male Wistar rat islets (18 h) and purified β cells (24 h)	Bensellam <i>et al.</i> (2009), Flamez <i>et al.</i> (2002)
<i>NADH:ubiquinone oxidoreductase subunits Ndufa1, Ndufa4, Ndufa11, Ndubf4, Ndufs5, Nd3</i>	G10 vs G2–5	Up	Cultured male Wistar rat islets (18 h)	Bensellam <i>et al.</i> (2009)
<i>Cyts</i>	G10 vs G2–5	Up	Cultured male Wistar rat islets (18 h)	Bensellam <i>et al.</i> (2009)
<i>Cytochrome c oxidase subunits Cox2, Cox3 (Cytb), Cox5b, Cox6b1, Cox7b, Cox17</i>	G10 vs G2–5	Up	Cultured male Wistar rat islets (18 h) and purified β cells (24 h)	Bensellam <i>et al.</i> (2009), Flamez <i>et al.</i> (2002)
<i>ATP synthase subunits Atp5a1, Atp5e, Atp5g1, Atp5i, Atp5j, Atp5o</i>	G10 vs G2–5	Up	Cultured male Wistar rat islets (18 h)	Bensellam <i>et al.</i> (2009)
<i>Ant2 (Slc25a5)</i>	G10 vs G2–5	Up	Cultured male Wistar rat islets (18 h)	Bensellam <i>et al.</i> (2009)
<i>Gpd2</i>	G10 vs G5	Up	Cultured C57BL6J mouse islets (24 h)	(Supplementary Fig. 1)
Anaplerosis/cataplerosis <i>Acly</i>	G10 vs G2–5	Up	Cultured male Wistar rat islets (18 h) and purified β cells (24 h)	Bensellam <i>et al.</i> (2009), Flamez <i>et al.</i> (2002)
<i>Mdh1</i>	G10 vs G2–5	Up	Cultured male Wistar rat islets (18 h)	Bensellam <i>et al.</i> (2009)
<i>Me1</i>	G10 vs G2–5	Up	Cultured male Wistar rat islets (18 h)	Bensellam <i>et al.</i> (2009)

(Continued)

Table 1 Continued.

Genes	Physiological glucose stimulation	Effect	Models	References
Fatty acid metabolism				
<i>Fasn</i>	G10 vs G2–5	Up	Cultured male Wistar rat islets (18h) and purified β cells (24h)	Bensellam et al. (2009) , Flamez et al. (2002)
<i>Hadh</i>	G10 vs G2–5	Up	Cultured male Wistar rat islets (18h) and purified β cells (72h)	Bensellam et al. (2009) , Martens & Pipeleers (2009)
<i>Scd1</i>	G10 vs G2–5	Up	Cultured male Wistar rat islets (18h)	Bensellam et al. (2009)
<i>Scd2</i>	G10 vs G2–5	Up	Cultured male Wistar rat islets (18h) and purified β cells (24h)	Bensellam et al. (2009) , Flamez et al. (2002)
Cholesterol metabolism				
<i>Hmgcs1</i> , <i>Hmgcr</i> , <i>Mvd</i> , <i>Idi1</i> , <i>Fdps</i> , <i>Fdft1</i> , <i>Sqle</i> , <i>Lss</i> , <i>Sc4mol</i> , <i>Nsdhl</i> , <i>Sc5dl</i> , <i>Dhcr7</i> , <i>Soat1</i> , <i>Scp2</i> , <i>Ldlr</i>	G10 vs G2–5	Up	Cultured male Wistar rat islets (18h) and purified β cells (24h)	Bensellam et al. (2009) , Flamez et al. (2002)
<i>Vldlr</i>	G10 vs G2–5	Down	Cultured male Wistar rat islets (18h) and purified β cells (24h)	Bensellam et al. (2009) , Flamez et al. (2002)
Ion channels, pumps and receptors				
<i>Kcnj11</i> (<i>Kir6.2</i>)	G10 vs G3	Up	Cultured purified male Wistar rat β cells (24h)	Flamez et al. (2002)
<i>Abcc8</i> (<i>Sur1</i>)	G10 vs G3	Up	Cultured purified male Wistar rat β cells (24h)	Flamez et al. (2002)
<i>Vdcca1D</i>	Fed vs fasted	Up	Fasted male Sprague–Dawley rats (72h)	Iwashima et al. (1994)
<i>Serca2b</i> (<i>Atp2a2</i>)	G10 vs G2–5	Up	Cultured male Wistar rat islets (18h)	Bensellam et al. (2009)
<i>Serca3</i> (<i>Atp2a3</i>)	G10 vs G2–5	Up	Cultured male Wistar rat islets (18h)	Bensellam et al. (2009)
<i>Gipr</i>	G10 vs G5	Up	Cultured purified male Wistar rat β cells (72h)	Martens & Pipeleers (2009)
<i>Gpr40</i> (<i>Ffar1</i>)	G10 vs G5	Up	Cultured C57BL6J mouse islets (24h)	(Supplementary Fig. 1)
<i>Slc30a1</i> (<i>ZnT-1</i>)	G10 vs G2–5	Down	Cultured male Wistar rat islets (18h)	Bensellam et al. (2009)
Proinsulin processing and insulin granule exocytosis				
<i>Pcsk1</i> , <i>Pcsk2</i> , <i>Cpe</i> , <i>Scg3</i> , <i>Syt13</i> , <i>Syt14</i> , <i>Snap25</i> , <i>Syp</i> , <i>Scfd1</i> , <i>Ykt6</i>	G10 vs G2–5	Up	Cultured male Wistar rat islets (18h) and purified β cells (72h)	Bensellam et al. (2009) , Martens & Pipeleers (2009)

the differentiated β -cell phenotype also relies on gene repression. The latter involves particular mechanisms including epigenetic and miRNA regulation. Thus, histone and DNA methylation are implicated in the metabolic maturation of β -cells in the postnatal period by repressing the expression of *Hk1*, *Mct1* and *Ldha* ([Thorrez et al. 2011](#), [Dhawan et al. 2015](#)). Moreover, a growing body of data suggests that miRNAs are implicated not only in the repression of β -cell forbidden genes ([Pullen et al. 2011](#), [Martinez-Sanchez et al. 2015](#)), but also in the modulation of insulin gene expression ([El Ouaamari et al. 2008](#), [Tang et al. 2009](#), [Melkman-Zehavi et al. 2011](#), [Zhao et al. 2012](#)), the metabolic reconfiguration of neonatal β -cells ([Jacovetti et al. 2015](#)) and insulin granule exocytosis

([Poy et al. 2004](#), [Lovis et al. 2008](#), [Latreille et al. 2014](#)). The switch from high-fat milk in newborns to carbohydrate-rich diet post-weaning has been suggested as an important driver of changes in miRNA expression that paralleled the maturation of the β -cell phenotype ([Jacovetti et al. 2015](#)). In agreement, glucose stimulation has been shown to regulate the expression of several miRNAs in β -cell lines and isolated islets ([El Ouaamari et al. 2008](#), [Esguerra et al. 2011](#)). Recent reports have revealed another mechanism of gene repression whereby the transcription factors NK2 homeobox 2 (NKX2.2), paired box 6 (PAX6) and Regulatory Factor X, 6 (RFX6) play a dual role by activating the expression of various β -cell-enriched genes and also by directly binding to and repressing the expression of

non- β -cell endocrine genes and other islet forbidden genes such as *Ldha* (Piccand *et al.* 2014, Gutierrez *et al.* 2017, Swisa *et al.* 2017). Of note, *Nkx2.2* mRNA levels were upregulated in rat islets cultured in the presence of G10 instead of G2–5 (Bensellam *et al.* 2009).

However, while physiological glucose stimulation plays a key role in the maintenance of the β -cell differentiated phenotype, exposure to supraphysiological glucose levels, such as in the context of diabetes, leads to β -cell dedifferentiation. This may contribute to the progressive loss of functional β -cell mass and the aggravation of the disease over time. Our understanding of this phenomenon has markedly expanded in the last two decades with the identification of various mechanisms and effectors.

The loss of β -cell differentiation in diabetes: causes and mechanisms

The loss of functional β -cell mass does not only result from apoptosis but also result from a loss of differentiation

It is now well recognized that the β -cell mass is reduced by 25–60% in T2D patients in comparison to weight-matched normal subjects (Sakuraba *et al.* 2002, Butler *et al.* 2003, Yoon *et al.* 2003, Deng *et al.* 2004, Rahier *et al.* 2008). The observation that β -cell mass decline correlated with the duration of T2D (Rahier *et al.* 2008) supports a role of chronic hyperglycemia and glucotoxicity. Indeed, prolonged or intermittent exposure of rodent and human islets to elevated glucose concentrations *in vitro* has been shown to increase β -cell apoptosis (Ling *et al.* 1994, Efanova *et al.* 1998, Piro *et al.* 2002, Khaldi *et al.* 2004, DelGuerra *et al.* 2007, Hou *et al.* 2008b, Bensellam *et al.* 2009, Jonas *et al.* 2009). These results are strongly supported by the observation of increased β -cell apoptosis in different animal models of T2D (Koyama *et al.* 1998, Pick *et al.* 1998, Donath *et al.* 1999, Finegood *et al.* 2001, Huang *et al.* 2007, Song *et al.* 2008) and in human patients (Butler *et al.* 2003, Jurgens *et al.* 2011). However, the rate of β -cell apoptosis is relatively low and may not fully explain the loss of β -cell mass in T2D. Thus, alternative mechanisms are possible. The concept of the loss of differentiation, introduced in the late nineties (Jonas *et al.* 1999), has been recently proposed as an important contributor to the reduced β -cell mass in T2D (Talchai *et al.* 2012, Marselli *et al.* 2014, Spijker *et al.* 2015, Cinti *et al.* 2016). Similar to apoptosis, chronic hyperglycemia has been shown to be a major inducer of β -cell dedifferentiation.

Role of hyperglycemia and glucotoxicity

In T2D, the complex interaction of genetic and environmental factors leads ultimately to defective insulin secretion. The latter is a consequence of the failure of β -cells to cope with the important metabolic demand imposed on them by insulin resistance. The ensuing chronic hyperglycemia exerts further damage on the β -cell phenotype thereby leading to the progressive decline of functional β -cell mass and deterioration of the disease over time. This is the concept of glucotoxicity (Bensellam *et al.* 2012b). Glucotoxicity-related loss of β -cell identity is characterised by alterations in the expression of many genes, with much initial and recent attention focused on the reduced expression of β -cell-enriched genes, in particular insulin.

Downregulation of insulin gene expression

Earlier studies in β -cell lines and isolated rodent and human islets have shown that prolonged exposure to elevated glucose concentrations markedly reduced the mRNA levels of insulin (Robertson *et al.* 1992, Olson *et al.* 1995, 1998, Briaud *et al.* 1999, Marshak *et al.* 1999). In agreement, a similar reduction was observed in the islets of Zucker diabetic fatty (ZDF) rats (Harmon *et al.* 2001) and 90% pancreatectomized (Px) rats (Jonas *et al.* 1999). Interestingly, in these animal models, insulin mRNA levels were restored by phlorizin treatment (an inhibitor of renal glucose reabsorption) and normalization of glycaemia (Jonas *et al.* 1999, Harmon *et al.* 2001). Reduced insulin mRNA levels have also been confirmed in β -cells of human T2D subjects (Marchetti *et al.* 2004, DelGuerra *et al.* 2005, Marselli *et al.* 2010, Guo *et al.* 2013). This reduction has been attributed to reduced expression and binding of the key transcription factors, pancreatic and duodenal homeobox 1 (PDX-1 also called IPF-1, STF-1, IUF-1, IDX-1 or GSF) and V-maf musculoaponeurotic fibrosarcoma oncogene homolog A (MAFA also known as RIPE3b1), to the insulin gene promoter (Olson *et al.* 1995, Sharma *et al.* 1995, Poitout *et al.* 1996, Moran *et al.* 1997, Harmon *et al.* 1998, Marshak *et al.* 1999, Gleason *et al.* 2000, Pino *et al.* 2005, Matsuoka *et al.* 2010) (Fig. 3 and Supplementary Fig. 1, see section on supplementary data given at the end of this article). Reduced mRNA levels of *Pdx1* and *Mafa* and nuclear expression of their proteins has also been reported in the islets of T2D patients (Guo *et al.* 2013). The reduction of the mRNA levels of *Pdx1* in the islets of Px rats and *Neurod1* in the islets of *db/db* mice was prevented by phlorizin treatment, thereby demonstrating the role of glucotoxicity (Jonas *et al.* 1999,

Figure 3

Schematic representation of the key effectors and mechanisms implicated in the loss of insulin gene expression in type 2 diabetes. Please see the text for details. Direction of the vertical black arrows close to proteins, mRNAs or stresses indicate the effect of glucotoxicity. C/EBP β , CCAAT/enhancer-binding protein β ; ChREBP, carbohydrate response element binding protein; ER, endoplasmic reticulum; JNK, c-Jun N-terminal kinase; ROS, reactive oxygen species; ETS1, V-ets avian erythroblastosis virus E26 oncogene homolog 1; IRE1 α , inositol requiring 1 α ; MAFA, V-maf musculoaponeurotic fibrosarcoma oncogene homolog A; MYC, myelocytomatosis viral oncogene homolog (avian); NEUROD1, neurogenic differentiation 1; PDX-1, pancreatic and duodenal homeobox 1; PTB, polypyrimidine tract binding protein; STAT3, signal transducer and activator of transcription 3; TXN, thioredoxin; TXNIP, thioredoxin-interacting protein.

Kjorholt *et al.* 2005). These results are further corroborated by a study showing that treatment of *db/db* mice with the sodium glucose cotransporter 2 (SGLT2) inhibitor luseogliflozin upregulated islet mRNA levels of insulin, *Pdx1* and *Mafa* (Okauchi *et al.* 2016).

The observation that the alteration of insulin gene expression was prevented by antioxidant treatment suggested an implication of oxidative stress (Tanaka *et al.* 1999, Robertson & Harmon 2006). In support of this idea, hydrogen peroxide treatment of β -cell lines and isolated islets has been shown to alter the nuclear expression and DNA-binding activity of MAFA and PDX1 (Kaneto *et al.* 2002b, Harmon *et al.* 2005, Guo *et al.* 2013). Moreover, reduced nuclear expression of MAFA in the islets of diabetic *db/db* mice was restored by transgenic expression of the antioxidant gene *Gpx1* (Harmon *et al.* 2009, Guo *et al.* 2013). Similarly, altered nuclear expression of PDX1

and MAFA in the islets of diabetic ZDF rats was markedly prevented by treatment with the glutathione peroxidase (GPX) mimetic ebselen (Mahadevan *et al.* 2013). Oxidative stress-mediated alteration of PDX1 nuclear localization and DNA-binding activity has been proposed to involve the activation of c-Jun N-terminal kinase (JNK) (Kaneto *et al.* 2002b, Kawamori *et al.* 2003) (Fig. 3). Besides oxidative stress, it has been proposed that chronic ER stress could affect the specificity of the endoribonuclease activity of IRE1, thus leading to the cleavage of ER-associated RNAs including that of insulin (Hollien & Weissman 2006, Pirot *et al.* 2007, Lipson *et al.* 2008, Han *et al.* 2009, Hollien *et al.* 2009) (Fig. 3). Moreover, other mechanisms have been identified implicating novel effectors, in particular miRNAs. Thus, it has been demonstrated that the high glucose-induced redox protein thioredoxin-interacting protein (TXNIP) (Shalev *et al.* 2002, Saxena *et al.* 2010),

via a STAT3-dependent pathway, upregulated miR-204 thereby leading to reduced *Mafa* mRNA levels (Xu *et al.* 2013) (Fig. 3). In addition, upregulation of miR-30a-5p by high glucose levels in INS1 cells and rat islets has been shown to inhibit *Neurod1* expression (Kim *et al.* 2013) (Fig. 3). Furthermore, in human islets exposed to high glucose, the induction of miR-133a targeted the polypyrimidine tract-binding protein and led to reduced insulin biosynthesis, although insulin mRNA levels were not significantly affected in this study (Fred *et al.* 2010) (Fig. 3). Another report has also shown that miR-124a is markedly upregulated in the islets of human T2D subjects and targeted *Foxa2* and *Neurod1* in MIN6 cells. Interestingly, overexpression of miR-124a blunted GSIS in MIN6 cells (Sebastiani *et al.* 2015). However, it is unclear whether miR-124a is regulated by glucotoxicity or another mechanism in T2D. On the other hand, the alteration of insulin gene expression under glucotoxic conditions also involves transcriptional repression by transcriptional repressor proteins. Thus, upregulation of CCAAT/enhancer-binding protein β (C/EBP β) in β -cell lines and islets of ZDF and Px rats has been implicated in the downregulation of insulin gene expression (Lu *et al.* 1997, Seufert *et al.* 1998). C/EBP β interacted with the basic helix-loop-helix transcription factor E47 thereby inhibiting its DNA binding to the insulin gene promoter and its transactivation ability (Fig. 3). High glucose also affected insulin gene transcription by upregulating the expression of myelocytomatosis viral oncogene homolog (avian) (MYC). MYC has been shown to inhibit neurogenic differentiation 1 (NEUROD1, also known as BETA2)-mediated transcriptional activation of the insulin gene promoter in β -cell lines and isolated rat islets (Kaneto *et al.* 2002a) (Fig. 3). Of note, *Myc* is also an oxidative stress-responsive gene (Elouil *et al.* 2005, Jonas *et al.* 2009) that has been implicated in β -cell dedifferentiation (Jonas *et al.* 2001, Laybutt *et al.* 2002c, Pascal *et al.* 2008, Robson *et al.* 2011). On the other hand, it has been proposed that *Myc* expression is negatively regulated by PDX1 (Chen *et al.* 2007). More recently, Vets avian erythroblastosis virus E26 oncogene homolog 1 (ETS1) has been shown to repress the expression of *Pdx1* under glucotoxicity by directly binding to its promoter and inhibiting its transcription in rodent β -cells (Chen *et al.* 2016) (Fig. 3). This study also suggested that ETS1 could interact with the transcription factor forkhead box O1 (FOXO1) and increase its binding to the *Pdx1* promoter (Chen *et al.* 2016). FOXO1 has previously been proposed to repress FOXA2-dependent *Pdx1* transcription and to be involved in the nucleocytoplasmic translocation of PDX1 under

oxidative stress (Kitamura *et al.* 2002, Kawamori *et al.* 2006). However, findings in the last decade revealed that the loss of FOXO1 under severe hyperglycemia is implicated in β -cell dedifferentiation thereby indicating a complex role of this transcription factor in β -cell pathophysiology (discussed in the next section).

Downregulation of β -cell-enriched genes

Besides the insulin gene and key upstream transcription factors, glucotoxicity alters the expression of additional genes that maintain the differentiated β -cell phenotype including other transcription factors such as *Nkx6.1*, *Hnf1 α* (also known as *Tcf1*) and *Hnf4 α* , glucose sensing and metabolism genes such as *Glut2*, *Gck* and *Gpd2* (Supplementary Fig. 1), stimulus-secretion coupling genes such as *Kcnj11* and *Abcc8*, amplification of insulin secretion genes such as *Glp1r*, *Chrm3* and *Gpr40* (Supplementary Fig. 1) and insulin granule exocytosis genes such as N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) complex proteins. A comprehensive and up to date list of the most important genes and the models in which they are affected are presented in Table 2. Many of these alterations, but not all, have also been confirmed in islets of human T2D subjects (Table 2). The normalization of these alterations in diabetic animals using phlorizin treatment, sulfonylureas or insulin therapy, but not bezafibrate treatment, strongly supports the implication of glucotoxicity rather than lipotoxicity (Jonas *et al.* 1999, Harmon *et al.* 2001, Gaisano *et al.* 2002, Kjørholt *et al.* 2005, Xu *et al.* 2007, Brereton *et al.* 2014, Wang *et al.* 2014). Several pathways downstream of glucotoxicity (Bensellam *et al.* 2012b) have been implicated in the loss of β -cell-enriched genes including oxidative stress (Tanaka *et al.* 1999, Kaneto *et al.* 2002b, Harmon *et al.* 2005, Robertson & Harmon 2006, Guo *et al.* 2013), ER stress (Hollien & Weissman 2006, Pirot *et al.* 2007, Lipson *et al.* 2008, Han *et al.* 2009, Szabat *et al.* 2011, Lombardi *et al.* 2012), the hexosamine pathway (Kaneto *et al.* 2001, Yoshikawa *et al.* 2002), inflammation (Nordmann *et al.* 2017) and hypoxia (Puri *et al.* 2013, Sato *et al.* 2014, 2017).

Downregulation of the β -cell-enriched genes is linked with altered expression of key transcription factors. Indeed, above and beyond the regulation of insulin gene expression, the β -cell key transcription factors PDX1, MAFA and NEUROD1, in addition to hepatocyte nuclear factors (HNF1 α , HNF4 α and HNF3 β /FOXA2) and PAX6, are implicated in a complex network of inter-regulation (Ben-Shushan *et al.* 2001, Samaras *et al.* 2002, 2003, Shih *et al.* 2002, Pedersen *et al.* 2005) and regulation of other β -cell-enriched genes including key metabolic genes such

Table 2 Downregulation of β -cell-enriched genes under glucotoxicity.

Genes	Glucotoxicity model		Confirmation in human T2D	Glucotoxic effect
	<i>In vitro/ex vivo</i>	<i>In vivo</i>		
Islet hormones <i>Preproinsulin</i>	HIT-T15 cells (Robertson <i>et al.</i> 1992, Sharma <i>et al.</i> 1995) β -TC6 cells (Poitout <i>et al.</i> 1996) INS1 cells (Olson <i>et al.</i> 1998) Wistar rat islets (Briaud <i>et al.</i> 1999) Human islets (Marshak <i>et al.</i> 1999)	Px rat islets (Zangen <i>et al.</i> 1997) ZDF rat islets (Tokuyama <i>et al.</i> 1995) Rat islets transplanted into diabetic rats (Laybutt <i>et al.</i> 2007) <i>Psammomys obesus</i> islets (Leibowitz <i>et al.</i> 2001) MKR mouse islets (Lu <i>et al.</i> 2008)	Yes: (Marchetti <i>et al.</i> 2004, DelGuerra <i>et al.</i> 2005, Masini <i>et al.</i> 2012, Guo <i>et al.</i> 2013)	mRNA, protein and volume density of insulin granules
<i>lapp</i>		Px rat islets (Jonas <i>et al.</i> 1999, Laybutt <i>et al.</i> 2003) Rat islets transplanted into diabetic rats (Laybutt <i>et al.</i> 2007)	Yes: (Marselli <i>et al.</i> 2010, Bugliani <i>et al.</i> 2013)	mRNA
Transcription factors <i>Pdx1</i>	INS1 cells (Wang <i>et al.</i> 2005) INS 832/13 (Yang <i>et al.</i> 2012) Sprague–Dawley rat islets (Hou <i>et al.</i> 2008b) C57BL6J mouse islets (Supplementary Fig. 1)	Px rat islets (Zangen <i>et al.</i> 1997, Jonas <i>et al.</i> 1999) Rat islets transplanted into diabetic rats (Laybutt <i>et al.</i> 2007) <i>db/db</i> mouse islets (Kjorholt <i>et al.</i> 2005, Chan <i>et al.</i> 2013) NZO mouse islets (Kluth <i>et al.</i> 2011)	Controversial Yes: (Ostenenson <i>et al.</i> 2006, Yang <i>et al.</i> 2012, Guo <i>et al.</i> 2013) Upregulated: (DelGuerra <i>et al.</i> 2005)	DNA methylation, mRNA, protein, and subcellular localization
<i>Mafa</i>	HIT-T15 cells (Sharma <i>et al.</i> 1995)	<i>db/db</i> mouse islets (Harmon <i>et al.</i> 2009, Chan <i>et al.</i> 2013, Guo <i>et al.</i> 2013) NZO mouse islets (Kluth <i>et al.</i> 2011) β V59M mouse islets (Brereton <i>et al.</i> 2014)	Yes: (Guo <i>et al.</i> 2013, Cinti <i>et al.</i> 2016)	mRNA, protein and subcellular localization
<i>Mafb</i>			Yes: (Bugliani <i>et al.</i> 2013, Guo <i>et al.</i> 2013)	mRNA, protein and subcellular localization
<i>Neurod1</i> (Beta2)		Px rat islets (Jonas <i>et al.</i> 1999, Laybutt <i>et al.</i> 2003) Rat islets transplanted into diabetic rats (Laybutt <i>et al.</i> 2007) <i>db/db</i> mouse islets (Kjorholt <i>et al.</i> 2005, Chan <i>et al.</i> 2013)		mRNA
<i>Nkx6.1</i>		Px rat islets (Jonas <i>et al.</i> 1999, Laybutt <i>et al.</i> 2003) Rat islets transplanted into diabetic rats (Laybutt <i>et al.</i> 2007) <i>db/db</i> mouse islets (Kjorholt <i>et al.</i> 2005, Chan <i>et al.</i> 2013, Guo <i>et al.</i> 2013) NZO mouse islets (Kluth <i>et al.</i> 2011) β V59M mouse islets (Brereton <i>et al.</i> 2014)	Yes: (Guo <i>et al.</i> 2013, Cinti <i>et al.</i> 2016)	mRNA, protein and subcellular localization
<i>Hnf1α</i> (Tcf1)		Px rat islets (Jonas <i>et al.</i> 1999, Laybutt <i>et al.</i> 2003)	Yes: (Marselli <i>et al.</i> 2010)	mRNA
<i>Hnf4α</i>		Px rat islets (Jonas <i>et al.</i> 1999, Laybutt <i>et al.</i> 2003)	Yes: (Gunton <i>et al.</i> 2005)	mRNA

(Continued)

Table 2 Continued.

Genes	Glucotoxicity model		Confirmation in human T2D	Glucotoxic effect
	<i>In vitro</i> <i>ex vivo</i>	<i>In vivo</i>		
<i>Hnf3β</i> (<i>Foxa2</i>) <i>Pax6</i>		Px rat islets (Jonas <i>et al.</i> 1999) Rat islets transplanted into diabetic rats (Laybutt <i>et al.</i> 2007) <i>db/db</i> mouse islets (Kjorholt <i>et al.</i> 2005, Swisa <i>et al.</i> 2017)		mRNA mRNA
<i>Foxo1</i>		GIRKO mouse islets (Talchai <i>et al.</i> 2012) <i>db/db</i> mouse islets (Kim-Muller <i>et al.</i> 2014)	Controversial Yes: (Marselli <i>et al.</i> 2010, Cinti <i>et al.</i> 2016) Upregulated: (DelGuerra <i>et al.</i> 2005)	mRNA and protein
<i>Tcf7l2</i> (<i>Tcf4</i>)		Vancouver Diabetic Fatty (VDF) Zucker rat islets (Shu <i>et al.</i> 2009) <i>db/db</i> mouse islets (Shu <i>et al.</i> 2009)	Yes: (Shu <i>et al.</i> 2009)	Protein
<i>Ppara</i>	INS 832/13 (Roduit <i>et al.</i> 2000) Wistar rat islets (Roduit <i>et al.</i> 2000)	Px rat islets (Laybutt <i>et al.</i> 2002b) ZDF rat islets (Wang <i>et al.</i> 1998)		mRNA and protein
Glucose transport and metabolism <i>Glut1</i>			Yes: (DelGuerra <i>et al.</i> 2005, Guo <i>et al.</i> 2013)	mRNA
<i>Glut2</i>	INS1 cells (Wang <i>et al.</i> 2005) C57BL6J mouse islets (Supplementary Fig. 1)	Px rat islets (Jonas <i>et al.</i> 1999, Laybutt <i>et al.</i> 2003) Rat islets transplanted into diabetic rats (Laybutt <i>et al.</i> 2007) <i>db/db</i> mouse islets (Thorens <i>et al.</i> 1992, Kjorholt <i>et al.</i> 2005, Chan <i>et al.</i> 2013, Guo <i>et al.</i> 2013) β V59M mouse islets (Brereton <i>et al.</i> 2014)	Yes: (DelGuerra <i>et al.</i> 2005, Ostenson <i>et al.</i> 2006, Marselli <i>et al.</i> 2010, Taneera <i>et al.</i> 2012, Guo <i>et al.</i> 2013)	mRNA and protein
<i>Gck</i>	INS1 cells (Wang <i>et al.</i> 2005)	Px rat islets (Jonas <i>et al.</i> 1999, Laybutt <i>et al.</i> 2003) ZDF rat islets (Tokuyama <i>et al.</i> 1995) Rat islets transplanted into diabetic rats (Laybutt <i>et al.</i> 2007) <i>db/db</i> mouse islets (Kjorholt <i>et al.</i> 2005, Chan <i>et al.</i> 2013)	Yes: (DelGuerra <i>et al.</i> 2005)	mRNA
<i>Pc</i>		Px rat islets (Jonas <i>et al.</i> 1999, Laybutt <i>et al.</i> 2003) ZDF rat islets (MacDonald <i>et al.</i> 1996) <i>db/db</i> mouse islets (Kjorholt <i>et al.</i> 2005, Chan <i>et al.</i> 2013) MKR mouse islets (Lu <i>et al.</i> 2008)	Yes: (MacDonald <i>et al.</i> 2009)	mRNA, protein and enzyme activity
<i>Gpd2</i> (<i>mGpdh</i>)	C57BL6J mouse islets (Supplementary Fig. 1)	Px rat islets (Jonas <i>et al.</i> 1999, Laybutt <i>et al.</i> 2003) ZDF rat islets (Tokuyama <i>et al.</i> 1995, MacDonald <i>et al.</i> 1996) <i>db/db</i> mouse islets (Kjorholt <i>et al.</i> 2005, Chan <i>et al.</i> 2013) MKR mouse islets (Lu <i>et al.</i> 2008)	Yes: (MacDonald <i>et al.</i> 2009, Marselli <i>et al.</i> 2010)	mRNA and enzyme activity

(Continued)

Table 2 Continued.

Genes	Glucotoxicity model		Confirmation in human T2D	Glucotoxic effect
	<i>In vitro</i> /ex vivo	<i>In vivo</i>		
<i>Acly</i>		GK rat islets (Homo-Delarche <i>et al.</i> 2006, Hasan <i>et al.</i> 2010)	Yes: (MacDonald <i>et al.</i> 2009, Marselli <i>et al.</i> 2010, Bugliani <i>et al.</i> 2013)	mRNA and enzyme activity
Receptors, ion channels and pumps				
<i>Glp1r</i>	Sprague–Dawley rat islets (Xu <i>et al.</i> 2007)	Px rat islets (Xu <i>et al.</i> 2007) GK rat islets (Homo-Delarche <i>et al.</i> 2006) Glucose-infused rat islets (Xu <i>et al.</i> 2007) <i>db/db</i> mouse islets (Chan <i>et al.</i> 2013)	Yes: (Shu <i>et al.</i> 2009, Taneera <i>et al.</i> 2012, Guo <i>et al.</i> 2013)	mRNA and protein
<i>Gipr</i>		Px rat islets (Xu <i>et al.</i> 2007) <i>db/db</i> mouse islets (Chan <i>et al.</i> 2013)	Yes: (Shu <i>et al.</i> 2009)	mRNA and protein
<i>Gpr40</i>	C57BL6J mouse islets (Supplementary Fig. 1)	<i>db/db</i> mouse islets (Chan <i>et al.</i> 2013)		mRNA
<i>Chrm3</i>	ICR mouse islets (Hauge-Evans <i>et al.</i> 2014)	<i>ob/ob</i> mouse islets (Hauge-Evans <i>et al.</i> 2014)		mRNA and protein
<i>Kcnj11</i> (<i>Kir6.2</i>)		Px rat islets (Jonas <i>et al.</i> 1999, Laybutt <i>et al.</i> 2003) ZDF rat islets (Tokuyama <i>et al.</i> 1995) <i>db/db</i> mouse islets (Kjorholt <i>et al.</i> 2005, Chan <i>et al.</i> 2013)	Yes: (Ostenson <i>et al.</i> 2006, Taneera <i>et al.</i> 2012)	mRNA
<i>Abcc8</i> (<i>Sur1</i>)			Yes: (Ostenson <i>et al.</i> 2006, Marselli <i>et al.</i> 2010)	mRNA
<i>Vdcca1D</i>		Px rat islets (Jonas <i>et al.</i> 1999) ZDF rat islets (Tokuyama <i>et al.</i> 1995)	Yes: (Marselli <i>et al.</i> 2010)	mRNA
<i>Vdccb</i>		Px rat islets (Laybutt <i>et al.</i> 2003)		mRNA
<i>Serca2b</i> (<i>Atp2a2b</i>)		Px rat islets (Jonas <i>et al.</i> 1999) <i>db/db</i> mouse islets (Kjorholt <i>et al.</i> 2005)	Yes: (Bugliani <i>et al.</i> 2013)	mRNA
<i>Serca3</i> (<i>Atp2a3</i>)		Px rat islets (Jonas <i>et al.</i> 1999) <i>db/db</i> mouse islets (Kjorholt <i>et al.</i> 2005)		mRNA
Hormone processing and insulin granule exocytosis				
<i>Pcsk1</i> (<i>Pc1</i>)		MKR mouse islets (Lu <i>et al.</i> 2008)	Yes: (Masini <i>et al.</i> 2012)	mRNA and protein
<i>Pcsk2</i> (<i>Pc2</i>)		MKR mouse islets (Lu <i>et al.</i> 2008)		Protein
<i>Cpe</i>		MKR mouse islets (Lu <i>et al.</i> 2008)	Yes: (Masini <i>et al.</i> 2012)	mRNA and protein
<i>Stx1a</i>	INS1E cells (Dubois <i>et al.</i> 2007) Sprague–Dawley rat islets (Torrejon-Escribano <i>et al.</i> 2011)	GK rat islets (Nagamatsu <i>et al.</i> 1999, Gaisano <i>et al.</i> 2002, Zhang <i>et al.</i> 2002b) <i>db/db</i> mouse islets (Do <i>et al.</i> 2014)	Yes: (Ostenson <i>et al.</i> 2006)	mRNA and protein
<i>Stxbp1</i> (<i>Munc18-1</i>)		GK rat islets (Zhang <i>et al.</i> 2002b)	Yes: (Ostenson <i>et al.</i> 2006)	mRNA and protein
<i>SNAP-25</i>		GK rat islets (Nagamatsu <i>et al.</i> 1999, Gaisano <i>et al.</i> 2002, Zhang <i>et al.</i> 2002b)	Yes: (Ostenson <i>et al.</i> 2006)	mRNA and protein

(Continued)

Table 2 Continued.

Genes	Glucotoxicity model		Confirmation in human T2D	Glucotoxic effect
	<i>In vitro/lex vivo</i>	<i>In vivo</i>		
<i>Vamp2</i>	INS1E cells (Dubois <i>et al.</i> 2007) Wistar (Gaisano <i>et al.</i> 2002) and Sprague–Dawley rat islets (Torrejon-Escribano <i>et al.</i> 2011) Human islets (Dubois <i>et al.</i> 2007)	Px rat islets (Torrejon-Escribano <i>et al.</i> 2011) GK rat islets (Gaisano <i>et al.</i> 2002, Zhang <i>et al.</i> 2002b) Rat islets transplanted into diabetic rats (Torrejon-Escribano <i>et al.</i> 2011) MKR mouse islets (Lu <i>et al.</i> 2008)	Yes: (Ostenson <i>et al.</i> 2006)	mRNA and protein
<i>Vamp3</i>	Sprague–Dawley rat islets (Torrejon-Escribano <i>et al.</i> 2011)	Px rat islets (Torrejon-Escribano <i>et al.</i> 2011) Rat islets transplanted into diabetic rats (Torrejon-Escribano <i>et al.</i> 2011)		Protein
<i>Syp</i>			Yes: (Ostenson <i>et al.</i> 2006)	mRNA and protein
<i>Syt14</i>		MKR mouse islets (Lu <i>et al.</i> 2008)		mRNA and protein

as *Glut2*, *Gck* and *Pc*, ion channel genes such as *Abcc8* and receptor genes such as *Glp1r* (Waeber *et al.* 1996, Cha *et al.* 2000, Shih *et al.* 2001, Sund *et al.* 2001, Kim *et al.* 2002, Moates *et al.* 2003, Zhang *et al.* 2005, Wang *et al.* 2007). Interestingly, PDX1, NKX6.1 and PAX6 have also been shown to play a key role in preserving β -cell identity by repressing the expression of other islet cell type genes (Schaffer *et al.* 2013, Taylor *et al.* 2013, Gao *et al.* 2014, Mitchell *et al.* 2017, Swisa *et al.* 2017). Besides these canonical transcription factors, recent evidence has suggested that reduced expression of sex-determining region Y-box 5 (SOX5) may also contribute to the downregulation of β -cell-enriched genes in T2D (Axelsson *et al.* 2017). Adequate regulation of β -cell-enriched gene expression by these transcription factors depends on correct binding to specific promoters, but also on promoter-enhancer looping of chromatin and formation of multimeric transcriptional complexes. This has been shown to involve LIM domain-binding protein 1 (LDB1). LDB1, together with its binding partner islet 1 (ISL1), was enriched at DNA regions occupied by PDX1, NKX2.2, NKX6.1 and FOXA2 and LDB deletion in adult murine β -cells markedly affected the expression of β -cell-enriched genes including *Mafa*, *Pdx1*, *Nkx6.1*, *Glut2*, *Kcnj11*, *Abcc8* and *Glp1r*. Interestingly, reduced LDB1 enrichment in a putative active enhancer nearby *GLUT2* gene has been observed in islets from T2D donors (Ediger *et al.* 2017). But whether dysregulation of LDB1 is involved in downregulation of other β -cell-enriched genes in human T2D requires further exploration.

Gene inhibition/overexpression strategies *in vitro* and *in vivo* have allowed a better understanding of the

implication of these genes in the alteration of β -cell identity and function. The numerous forms of the maturity-onset diabetes of the young (MODY) best illustrate this in humans. These monogenic disorders result from mutations in transcription factor genes such as *Hnf4a* (MODY1), *Hnf1a* (MODY3), *Pdx1* (MODY4), *Hnf1b* (MODY5) and *Neurod1* (MODY6), and mutations in ion channel and metabolic genes such as *Gck* (MODY2), *Abcc8* (MODY12) and *Kcnj11* (MODY13) (Hattersley & Patel 2017). However, despite important advances in this field, there are still significant gaps of knowledge with regards to the precise targets of these transcription factors, their complex interaction network and the identification of novel transcription factors implicated in the loss of β -cell identity.

In recent years, FOXO1 has received much attention as a potential transcription factor linking metabolic stress with β -cell dedifferentiation in T2D. FOXO1 is a multifunctional transcription factor that has been shown to play complex adaptive/deleterious roles in β -cells under stress depending on the context. Thus, evidence showed that FOXO1 translocates to the nucleus under oxidative stress and promotes the expression of *Neurod1* and *Mafa* (Kitamura *et al.* 2005). In transgenic mouse models, FOXO1 has been shown to play a role in β -cell compensation during insulin resistance via increasing β -cell proliferation, mass, function and antioxidant gene expression (Okamoto *et al.* 2006, Zhang *et al.* 2016). Interestingly, *Foxo1* somatic deletion in β -cells resulted in β -cell dedifferentiation in ageing male mice and multiparous female mice in parallel with the upregulation of progenitor and pluripotency markers (Talchai *et al.* 2012). Furthermore, loss of FOXO1

has been observed in the islets of severely hyperglycemic *db/db* mice and insulin-resistant GIRKO mice (Talchai *et al.* 2012, Kim-Muller *et al.* 2014). This observation was confirmed by the same group in human T2D islets and was associated with altered expression and nuclear localization of MAFA and NKX6.1 (Cinti *et al.* 2016). In contrast, FOXO1 nuclear translocation in response to glucose starvation or palmitate treatment has been implicated in β -cell apoptosis, at least in part, via the stimulation of *Ddit3* gene expression (Martinez *et al.* 2006, 2008). FOXO1 subcellular localization and activity are modulated by posttranslational modifications including phosphorylation, ubiquitination, O-GlcNAcylation and acetylation (Kitamura 2013). In the NZO mouse model, a dietary carbohydrate challenge and the ensuing hyperglycemia were associated with rapid and marked dephosphorylation of FOXO1 (activation) followed by reduced GLUT2, PDX1, MAFA and NKX6.1 protein levels and increased apoptosis (Kluth *et al.* 2011), in agreement with the previously reported role of FOXO1 in the inhibition of *Pdx1* transcription (Kitamura *et al.* 2002). However, in this model, it is unclear whether nuclear expression of active FOXO1 is upregulated and whether it declines thereafter or not (Kluth *et al.* 2011). Another study has shown increased nuclear expression of FOXO1 in β -cells of diabetic *db/db* mice in association with downregulation of PDX1, MAFA and NKX6.1. Interestingly, caloric restriction for three months markedly reversed these alterations and normalized glycaemia and β -cell function (Sheng *et al.* 2016). Furthermore, increased mRNA levels of *Foxo1* have been reported in the islets of T2D subjects (DelGuerra *et al.* 2005). All in all, although undeniable evidence supports an important adaptive role of FOXO1 in the preservation of β -cell identity, especially under hyperglycemia and subsequent oxidative stress, additional work is required to further elucidate the mechanisms and timing of activation/inhibition of FOXO1 in diabetes and identify its precise targets and interacting network. The use of models of inducible expression/deletion in adult animals could be useful.

Besides altered expression and subcellular localization of transcription factors, downregulation of β -cell-enriched genes also involves gene repression by epigenetic mechanisms and miRNAs. Thus, in the islets of human T2D patients, increased DNA methylation in the promoter and enhancer regions of *Pdx1* has been proposed to be involved in its downregulation under hyperglycemia (Yang *et al.* 2012). Similarly, increased DNA methylation in the *Glp1r* promoter has been observed in

the islets of human T2D subjects (Hall *et al.* 2013). We have highlighted in the previous section the implication of several miRNAs in the inhibition of insulin gene expression and upstream transcription factors (Fred *et al.* 2010, Kim *et al.* 2013, Xu *et al.* 2013, Sebastiani *et al.* 2015). In addition, various miRNAs upregulated in the islets of GK rats have been shown to target transport and secretory pathway genes (Esguerra *et al.* 2011). Moreover, it has recently been shown that upregulation of miR-130a-3p, miR-130b-3p and miR-152-3p in the islets of GK rats was associated with reduced protein expression of GCK and pyruvate dehydrogenase E1- α and reduced intracellular ATP levels. Upregulation of these miRNAs has also been confirmed in the islets of hyperglycemic human donors (Ofori *et al.* 2017). Furthermore, upregulation of miR-187 in the islets of human T2D subjects was associated with altered GSIS and has been proposed to target *Hipk3* (Locke *et al.* 2014), a gene encoding for a serine-threonine kinase implicated in the regulation of β -cell function and survival (Shojima *et al.* 2012). However, transfection of INS1 cells with miR187 mimetic had only a minor effect on *Hipk3* mRNA levels and promoter activity, thereby suggesting the implication of other effectors (Locke *et al.* 2014). Thus, growing evidence supports an important role of miRNAs in the alteration of β -cell identity. Future studies are needed in this emerging field of research to identify which miRNAs are directly implicated in the loss of β -cell identity in T2D, map their precise targets and determine the upstream mechanisms of induction. A special attention in the *in vitro* studies must be paid to the duration of exposure to elevated glucose levels when evaluating the effects of glucotoxicity. Indeed, acute and sub-acute/chronic exposure may have different/opposite effects on the expression of some miRNAs (El Ouamari *et al.* 2008, Esguerra *et al.* 2011, Sun *et al.* 2011). Altogether, these findings show the multiple levels at which glucotoxicity affects the β -cell phenotype as well as the various potential mechanisms of β -cell dedifferentiation in T2D. More large-scale studies, especially using human islets, human cell lines and/or human β -cells derived from induced pluripotent stem cells, are needed to validate and extend the findings in rodents and to understand the pathophysiological role of β -cell dedifferentiation in T2D.

In addition to the loss of insulin, transcription factor, metabolism and secretory pathway genes, a growing body of data indicates that the loss of the adaptive UPR is a key event that accompanies β -cell decompensation and progression towards diabetes.

Failure of the adaptive UPR and progression towards diabetes

β -cells are vulnerable to ER stress due to their elevated rate of insulin biosynthesis in response to glucose stimulation. Therefore, an operational and fine-tuned UPR is fundamental for the preservation of β -cell function and survival under physiological conditions, and even more under conditions of high insulin demand, such as obesity and pregnancy. Indeed, genetic disruption of several UPR components, including *Perk*, *eIF2 α* , *Ire1*, *Xbp1* and *Dnajc3* leads to β -cell demise and the development of insulin-dependent diabetes in man and rodents (Delepine *et al.* 2000, Harding *et al.* 2001, Zhang *et al.* 2002a, Ladiges *et al.* 2005, Back *et al.* 2009, Iwawaki *et al.* 2010, Julier & Nicolino 2010, Lee *et al.* 2011, Synofzik *et al.* 2014, Xu *et al.* 2014, Han *et al.* 2015, Hassler *et al.* 2015). The UPR is thus an important trait of differentiated β -cells. Until recently, the accepted concept was that excessive stimulation of the UPR under chronic/unresolved ER stress plays an important role in β -cell decompensation and failure. This view was supported by several studies showing increased activation of ER stress sensors and downstream effectors and target genes in different *in vitro* and *in vivo* models in addition to human T2D (reviewed in Bensellam *et al.* 2012b). Furthermore, ablation of the ISR gene *Ddit3* in different animal models of diabetes has been shown to be protective (Oyadomari *et al.* 2002, Song *et al.* 2008), while overexpression of *Trib3* altered glucose tolerance and GSIS and increased β -cell apoptosis (Qian *et al.* 2008, Liew *et al.* 2010, Fang *et al.* 2014). Moreover, adenovirus-mediated overexpression of *Atf6* in INS1 cells was associated with altered GSIS and reduced expression of insulin, *Pdx1* and *Mafa* (Seo *et al.* 2008). Likewise, overexpression of *Xbp1* in primary rat β -cells was correlated with impaired function, increased apoptosis and reduced expression of insulin, *Pdx1*, *Mafa* and *Glut2* (Allagnat *et al.* 2010). As described earlier, chronic IRE1 activation has also been shown to alter β -cell differentiation via the degradation of ER-localized insulin mRNA (Pirot *et al.* 2007, Lipson *et al.* 2008, Han *et al.* 2009).

However, emerging evidence has challenged this concept by showing in different models of obesity and insulin resistance that the loss of β -cell differentiation and progression towards diabetes is accompanied by failure of the adaptive UPR. This led to the hypothesis that β -cell dysfunction in T2D may result from a failure of the UPR to adequately adapt rather than from a maladaptive UPR (Herbert & Laybutt 2016). By comparing time-dependent gene expression changes in islets of diabetes-prone (*db/db* mice on C57BL/KsJ genetic background)

and diabetes-resistant (*ob/ob* mice on C57BL/6J genetic background) mouse models of obesity, we observed in *ob/ob* mice a progressive upregulation of adaptive UPR genes, including *Hspa5*, *Fkbp11* and *Dnajc3*, in parallel with the maintenance of increased spliced/total *Xbp1* (*Xbp1s/t*) mRNA ratio. In contrast, adaptive UPR genes were upregulated in prediabetic *db/db* mouse islets (6 weeks of age) but declined in diabetic mice (16 weeks of age) in parallel with a similar decline in *Xbp1s/t* mRNA ratio. Moreover, despite partial downregulation of some β -cell key transcription factor genes and other β -cell-enriched genes in prediabetic *db/db* mice and age-matched *ob/ob* mice, the β -cell phenotype recovered in 16-week-old *ob/ob* mice. On the contrary, there was a further loss of these genes in 16-week-old diabetic *db/db* mice. Interestingly, downregulation of β -cell-enriched genes in *db/db* mouse islets was partially restored with the chemical chaperone 4-phenylbutyrate suggesting that the inactivation of the adaptive UPR plays a role in β -cell dedifferentiation (Chan *et al.* 2013). Downregulation of adaptive UPR gene expression is unlikely to result from reduced proinsulin biosynthesis since the latter has been shown to be increased in diabetic *db/db* mice (Alarcon *et al.* 2016). These results were corroborated by similar observations in the islets of female ZDF (fZDF) rats. Thus, adaptive UPR genes were upregulated in the islets of obese, prediabetic fZDF rats fed a chow diet (model of β -cell compensation) in comparison to age-matched lean controls. However, UPR gene expression was downregulated in diabetic fZDF rats fed a high-fat diet (model of β -cell decompensation) in comparison to age-matched obese, prediabetic fZDF rats fed a chow diet, in parallel with altered β -cell differentiation (Omikorede *et al.* 2013). Noteworthy, *in vitro*, proteomic analysis of glucose-responsive (low passage; more differentiated) vs glucose non-responsive (high passage; less differentiated) MIN6 cells also revealed downregulation of HSPA5, HSP90B1, PDI and ERP29 (Dowling *et al.* 2006). In humans, a previous microarray study has revealed a significant downregulation of *Hsp90b1* in the islet of T2D donors (Gunton *et al.* 2005). A more recent study has confirmed and extended this finding by showing that numerous UPR, ER-Golgi (retrograde)transport, ER quality control, ERAD and (retro)translocon-related genes were significantly downregulated in islets of T2D donors vs BMI-matched non-diabetic subjects, including *Perk*, *Hsp90b1*, *Hspa8*, *Hspa13*, *Ero1 β* , *Pdia6*, *Ppib*, *Erp44*, *Dnaja2*, *Dnajb9*, *Dnajc6*, *Dnajc9*, *Dnajc10*, *Dnajc12*, *Serp1*, *Man1a1*, *Cnx*, *Herpud1*, *Ssr1*, *Ssr3*, *Sec11a*, *Sec13*, *Sec23a*, *Sec23b*, *Sec23ip*, *Sec24a*, *Sec24b*, *Sec24d*, *Sec62*, *Sec63*,

Copb1, *Copb2* and *Copz1*, in addition to downregulation of the ubiquitin-proteasome system genes and reduced proteasome activity (Bugliani *et al.* 2013). A following report examining protein expression of XBP1s, ATF6 and phosphorylated-eIF2 α in pancreatic sections from non-BMI-matched normal, prediabetic and diabetic women with different durations of diabetes has shown an overall decreased staining in the islets of diabetic vs control and prediabetic women for the three markers (Engin *et al.* 2014). Collectively, these observations support the notion that adaptive UPR inactivation occurs during the progression towards diabetes in rodents and humans and may play an important role in β -cell dedifferentiation. But what could be the mechanism(s) behind this inactivation?

Hyperglycemia and the ensuing glucotoxicity are primary candidates. Indeed, downregulation of adaptive UPR and ISR gene expression has been observed in

mouse islets transplanted into diabetic mice with a non-sufficient islet number, in comparison to those transplanted into non-diabetic control mice. These alterations were paralleled by similar changes in the expression of β -cell key transcription factors and other β -cell-enriched genes, thereby further supporting a link between β -cell dedifferentiation and failure of the UPR. Interestingly, these alterations were prevented in mouse islets transplanted into diabetic mice with a sufficient number to restore normoglycaemia, thereby suggesting that hyperglycaemia contributes to the downregulation of the UPR (Walters *et al.* 2013). Glucotoxicity exerts its deleterious effects via myriad mechanisms, including hypoxia (Sato *et al.* 2011, 2014, Bensellam *et al.* 2012a,b). We have recently provided evidence that hypoxia could be an important cellular mechanism for the inactivation of the adaptive UPR in T2D. Thus, we have shown that

Figure 4

Schematic representation of the role of hypoxia and inflammation in the loss of adaptive UPR under chronic hyperglycemia. Under chronic hyperglycemia, the acceleration of mitochondrial metabolism and the stimulation of ATP consuming cellular processes, increases β -cell O_2 consumption. On the other hand, chronic hyperglycemia negatively affects islet vasculature thereby resulting in reduced O_2 supply. These events lead to β -cell hypoxia and activation of downstream pathways including c-Jun N-terminal kinase (JNK), double-stranded RNA-dependent protein kinase (PKR) and downstream DNA damage inducible transcript 3 (DDIT3). Activation of these effectors plays an important role in the inhibition of the adaptive unfolded protein response (UPR) under hypoxia. Inflammation induced by chronic hyperglycemia, but also by hypoxia, can also increase JNK phosphorylation and has been proposed to play a role in the failure of adaptive UPR. The timing of these complex events is represented in the insert. In the prediabetic phase (β -cell compensation), the UPR is markedly upregulated in parallel with a moderate activation of inflammatory signaling in the absence of signs of islet hypoxia. However, in the diabetic phase (β -cell decompensation), the adaptive UPR declines concomitantly with strong upregulation of inflammatory and hypoxia-response genes.

hypoxia-response genes were upregulated in the islets of diabetic, but not prediabetic, *db/db* mice in an inverse relationship with UPR gene expression (Fig. 4). We have demonstrated that hypoxia inhibits the adaptive UPR specifically in β -cells and that this inhibition was associated with impaired ER-to-Golgi protein trafficking and was implicated in increased apoptosis under hypoxic stress. These effects were mediated by the activation of JNK and *Ddit3*, but were independent of hypoxia-inducible factor 1 α (HIF1 α) (Bensellam *et al.* 2016) (Fig. 4). Interestingly, JNK activation has also been observed in the islets of diabetic *db/db* mice and its inhibition increased adaptive UPR gene expression and reduced apoptosis (Chan *et al.* 2015).

In addition to hypoxia, inflammation is another potential mechanism that may be involved in UPR inactivation (Fig. 4). T2D is characterized by a low-grade inflammation and proinflammatory cytokines released by immune cells, endothelial cells and adipose tissue under chronic hyperglycemia have been proposed to contribute to islet inflammation and β -cell dedifferentiation (Donath & Shoelson 2011, Nordmann *et al.* 2017). Interestingly, cytokine treatment of mouse islets downregulated the mRNA levels of several adaptive UPR genes including *Hspa5*, *Hsp90b1*, *Fkbp11* and *Dnajc3* (Chan *et al.* 2011). Importantly, the progression towards diabetes in *db/db* mice was accompanied by a progressive upregulation of inflammatory genes including *Il6*, *Il1 β* , *Cxcl1*, *Tnfa*, *Ccl2* and the macrophages marker gene *Cd68* (Fig. 4). This inflammatory signature was however absent in the islets of *ob/ob* mice (Chan *et al.* 2013). Moreover, assessment of time-dependent changes in XBP1s and ATF6 protein expression in the islets of mouse models of type 1 diabetes (T1D), which is an immune-mediated disease, revealed a progressive downregulation. These alterations were also observed on pancreatic section of humans T1D donors (Engin *et al.* 2013). Of note, hypoxia also upregulated inflammatory genes in mouse islets (Bensellam *et al.* 2016) (Fig. 4). Altogether, these observations suggest a role for inflammatory signalling in the downregulation of the adaptive UPR in T2D.

Very interestingly, in rat models of hypertrophic and failing heart, myocardial XBP1s and HSPA5 protein levels were upregulated in the early phase of the disease (adaptive phase) but declined in the late phase (maladaptive phase), very similarly to what has been observed in *db/db* mouse islets. In the cardiac context, it has been shown that miR-30-p3 family and miR-214 were implicated in XBP1s downregulation (Duan *et al.* 2015). Likewise, miRNAs may also be involved in UPR inactivation in β -cells. The expression of various miRNAs is affected in

prediabetic and diabetic *db/db* mouse islets in comparison to their age-matched lean controls (Nesca *et al.* 2013), and miR-200 has been shown to negatively regulate *Dnajc3* expression (Belgardt *et al.* 2015). Studies comparing the time-dependent changes in miRNA expression in obese models of β -cell compensation and decompensation are needed to shed more light on this possibility.

In sum, there is growing evidence that failure of the adaptive UPR in T2D is linked with β -cell dedifferentiation and dysfunction. Our evidence strongly supports the implication of hypoxia and very likely inflammatory signalling in UPR inactivation. But how the inactivation of the UPR could affect the differentiated β -cell phenotype is unclear at this stage. One possibility is that failure of the adaptive UPR exacerbates hyperglycemia-induced oxidative stress. Not only is the role of the latter in β -cell dedifferentiation well established, but oxidative stress is also linked with ER stress. Indeed, defective ER protein folding machinery and subsequent accumulation of unfolded proteins in the ER has been shown to trigger oxidative stress and damage in association with altered β -cell differentiation (Back *et al.* 2009, Han *et al.* 2015). Moreover, expression of a mutant form of proinsulin that is prone to misfolding in Akita mouse islets has also been associated with increased oxidative stress and damage (Yuan *et al.* 2012). Furthermore, treatment of glucose-infused rats with chemical chaperones reduced islet superoxide anion generation and preserved β -cell function (Tang *et al.* 2012). Thus, it is postulated that the inactivation of the UPR in T2D leads to progressive accumulation of unfolded proteins in the ER; this potentiates hyperglycemia-induced reactive oxygen species (ROS) production and oxidative stress, which leads to a gradual loss of β -cell identity. Future work in this field is needed to verify this hypothesis and explore the mechanism(s) by which protein misfolding in the ER induces/aggravates oxidative stress and whether a crosstalk between the ER and mitochondria may be involved.

Upregulation of β -cell forbidden genes

As well as the downregulation of β -cell-enriched genes (addressed above), β -cell dedifferentiation in T2D features upregulation of genes that are either suppressed or expressed at very low levels in mature β -cells under physiological conditions. These genes that we term as β -cell forbidden genes enclose various types including metabolic genes, transcriptional regulators, signalling effectors and stress response genes (Table 3 and Bensellam *et al.* 2012b). Upregulation of these genes under hyperglycemia has important consequences on β -cell identity and function.

Table 3 Upregulation of β -cell forbidden genes under glucotoxicity.

Genes	Glucotoxicity model		Confirmation in human T2D	Glucotoxic effect
	<i>In vitro/ex vivo</i>	<i>In vivo</i>		
Transcriptional regulators				
<i>C/EBPβ</i>	HIT-T15 cells (Lu <i>et al.</i> 1997) INS1 cells (Lu <i>et al.</i> 1997)	Px rat islets (Seufert <i>et al.</i> 1998) ZDF rat islets (Seufert <i>et al.</i> 1998)		mRNA and protein
<i>Crem</i>	Wistar and Sprague–Dawley rat islets (Bensellam <i>et al.</i> 2009, Zhou <i>et al.</i> 2003)			mRNA
<i>Myc</i>	Wistar rat islets (Bensellam <i>et al.</i> 2009, Elouil <i>et al.</i> 2005, Kaneto <i>et al.</i> 2002a)	Px rat islets (Jonas <i>et al.</i> 2001, Jonas <i>et al.</i> 1999) Gk rat islets (Lacraz <i>et al.</i> 2010)		mRNA
<i>Id1</i>	Human islets (Wice <i>et al.</i> 2001)	<i>db/db</i> mouse islets (Kjorholt <i>et al.</i> 2005, Chan <i>et al.</i> 2013, Bensellam <i>et al.</i> 2015)		mRNA and protein
<i>Id2</i>		GK rat islets (Homo-Delarche <i>et al.</i> 2006) <i>db/db</i> mouse islets (Bensellam <i>et al.</i> 2015)		mRNA
<i>Id3</i>		<i>db/db</i> mouse islets (Bensellam <i>et al.</i> 2015)		mRNA and protein
<i>Id4</i>		<i>db/db</i> mouse islets (Bensellam <i>et al.</i> 2015)		mRNA
<i>Ngn3</i>		<i>db/db</i> mouse islets (Talchai <i>et al.</i> 2012) K _{ATP} -GOF mice (Wang <i>et al.</i> 2014) GIRKO mouse islets (Talchai <i>et al.</i> 2012) Multiparous and ageing FoxO1-KO mice (Talchai <i>et al.</i> 2012)		Protein
<i>Nanog</i>		Multiparous FoxO1-KO mice (Talchai <i>et al.</i> 2012)		Protein
<i>Oct4</i>		<i>db/db</i> mouse islets (Talchai <i>et al.</i> 2012) GIRKO mouse islets (Talchai <i>et al.</i> 2012) Multiparous FoxO1-KO mice (Talchai <i>et al.</i> 2012)		Protein
<i>L-Myc</i>		<i>db/db</i> mouse islets (Talchai <i>et al.</i> 2012) GIRKO mouse islets (Talchai <i>et al.</i> 2012) Multiparous FoxO1-KO mice (Talchai <i>et al.</i> 2012)		Protein
<i>Sox9</i>			Yes: (Marselli <i>et al.</i> 2010, Bugliani <i>et al.</i> 2013)	mRNA
Metabolic genes				
<i>Hk1</i>		Px rat islets (Jonas <i>et al.</i> 1999) ZDF rat islets (Cockburn <i>et al.</i> 1997) <i>db/db</i> mouse islets (Kjorholt <i>et al.</i> 2005) <i>Psammomys obesus</i> (Nesher <i>et al.</i> 1999)		mRNA and enzymatic activity
<i>Hk2</i>	Rat islets (Ghanaat-Pour <i>et al.</i> 2007)			mRNA
<i>G6pase</i>		Px rat islets (Laybutt <i>et al.</i> 2002b) GK rat islets (Ling <i>et al.</i> 2001)		mRNA and enzymatic activity
<i>Fbp1</i>		Px rat islets (Laybutt <i>et al.</i> 2002b)		mRNA
<i>Pck1</i>	Human islets (Shalev <i>et al.</i> 2002)		Yes: (Marselli <i>et al.</i> 2010)	mRNA
<i>Ldha</i>	Wistar rat islets (Bensellam <i>et al.</i> 2009, Bensellam <i>et al.</i> 2012a)	Px rat islets (Jonas <i>et al.</i> 1999, Laybutt <i>et al.</i> 2003) GK rat islets (Homo-Delarche <i>et al.</i> 2006, Sasaki <i>et al.</i> 2013) ZDF rat islets (Li <i>et al.</i> 2006b) Rat islets transplanted into diabetic rats (Laybutt <i>et al.</i> 2007)	Yes: (Marselli <i>et al.</i> 2010)	mRNA, protein and lactate production
<i>Mct1</i>		Px rat islets (Laybutt <i>et al.</i> 2002b)		mRNA
<i>Mct2</i>		Px rat islets (Laybutt <i>et al.</i> 2002b)		mRNA

(Continued)

Table 3 Continued.

Genes	Glucotoxicity model		Confirmation in human T2D	Glucotoxic effect
	<i>In vitro/ex vivo</i>	<i>In vivo</i>		
<i>Mct4</i>	Wistar rat islets (Bensellam <i>et al.</i> 2012a)	Px rat islets (Laybutt <i>et al.</i> 2002b)		mRNA
<i>Ucp2</i>	Wistar rat islets (Khaldi <i>et al.</i> 2004)	Glucose-infused rats (Kassis <i>et al.</i> 2000) Px rat islets (Laybutt <i>et al.</i> 2002b) ZDF rat islets (Oberkofler <i>et al.</i> 2009) <i>db/db</i> mouse islets (Kjorholt <i>et al.</i> 2005) <i>ob/ob</i> mouse islets (Zhang <i>et al.</i> 2001) ZDF rat islets (Parton <i>et al.</i> 2006)		mRNA and protein
<i>Acot7</i>			Yes: (Marselli <i>et al.</i> 2010)	mRNA
<i>Aldh1a3</i>		<i>db/db</i> mouse islets (Ishida <i>et al.</i> 2017, Kim-Muller <i>et al.</i> 2016) <i>Abcc8</i> -KO mice (Stancill <i>et al.</i> 2017)	Yes: (Cinti <i>et al.</i> 2016)	mRNA, protein and enzymatic activity

Metabolic genes A major earlier and recent focus in this field has been on metabolic genes, in particular *Ldha* and *Mct1*. Pioneering studies have shown that LDHA activity is markedly lower in β -cells in comparison to other islet cells, exocrine cells and hepatocytes (Hellman & Taljedal 1967, Sekine *et al.* 1994). Ensuing large-scale studies comparing gene expression patterns of different mouse tissues with pancreatic islets confirmed low expression of *Ldha* and *Mct1* and identified other β -cell forbidden genes (Pullen *et al.* 2010, Thorrez *et al.* 2011). Interestingly, *Ldha* and various *Mct* isoform genes were upregulated in the islets of rodent models of hyperglycemia and diabetes (Jonas *et al.* 1999, Laybutt *et al.* 2002b, 2007, Homo-Delarche *et al.* 2006, Li *et al.* 2006b). Upregulation of *Ldha* has also been reported in β -cells of human T2D subjects (Marselli *et al.* 2010) (Table 3). The role of hyperglycemia in this induction has been demonstrated by phlorizin treatment experiments in Px rats (Jonas *et al.* 1999, Laybutt *et al.* 2002b). In addition, we have previously reported upregulation of *Ldha* mRNA levels, along with other glycolytic and hypoxia-response genes, in cultured rat islets exposed to elevated glucose levels (Bensellam *et al.* 2009). The prevention of this induction by hyperoxia suggested an implication of high glucose-induced β -cell hypoxia and activation of HIF1/2 α (Bensellam *et al.* 2012a). Another pathway implicating oxidative stress may be involved in LDHA induction in the islets of GK rats (Sasaki *et al.* 2013). Evidence has also shown a role of miRNAs in the regulation of *Mct1* expression (Pullen *et al.* 2011, Martinez-Sanchez *et al.* 2015). But whether altered expression of specific miRNAs in T2D is involved in the upregulation of *Mct1* requires further proof. Upregulation of *Ldha* and *Mct* genes may alter β -cell function by diverting the glycolytic flux from mitochondrial pyruvate oxidation to lactate production and transport. This could

alter glucose homeostasis by sensitising β -cells to secrete insulin in response to non-physiological stimuli (Ishihara *et al.* 1999, Ainscow *et al.* 2000, Otonkoski *et al.* 2007, Pullen *et al.* 2012).

As explained in 'Relationship of β -cell differentiation to insulin secretory function' section, the repression of high-affinity type I–III hexokinases in β -cells prevents insulin release in response to low non-stimulatory glucose levels such as in the fasted state (Sekine *et al.* 1994, Schuit *et al.* 1999). In the context of diabetes, increased mRNA levels of *Hk1* have been reported in Px rats and *db/db* mouse islets (Jonas *et al.* 1999, Kjorholt *et al.* 2005). In agreement, HK enzymatic activity was upregulated in the islets of ZDF rats (Cockburn *et al.* 1997), DBA/2 mice (Kooptiwut *et al.* 2002) and *Psammomys obesus* on high energy diet (Nesher *et al.* 1999). Moreover, exposure of rat islets to elevated glucose levels *in vitro* upregulated the mRNA levels of *Hk2* (Ghanaat-Pour *et al.* 2007) (Table 3). Upregulation of *Hks*, and likely other glycolytic genes, may explain the reduced threshold glucose concentration for the stimulation of insulin secretion (glucose hypersensitivity) observed in INS1 cells (Roche *et al.* 1997), rat and mouse islets (Khaldi *et al.* 2004, Bensellam *et al.* 2009, Pascal *et al.* 2010) and purified rat and human β -cells (Ling & Pipeleers 1996, Ling *et al.* 1996) cultured in the presence of supraphysiological glucose levels, as well as in the islets of animal models of diabetes, including glucose-infused rats (Ammon *et al.* 1998), Px rats (Leahy *et al.* 1993, Hosokawa *et al.* 1995), ZDF rats (Tokuyama *et al.* 1995), *ob/ob* mice (Chen *et al.* 1993) and *Psammomys obesus* (Pertusa *et al.* 2002). In support of this hypothesis, overexpression of *Hk1* in MIN6 cells and primary rat islets has been shown to increase the basal glycolytic flux and insulin release along with altered GSIS (Becker *et al.* 1994, Ishihara *et al.* 1994).

Other metabolic genes presenting low expression under physiological conditions and upregulated in diabetes include the gluconeogenic genes *G6pase* (Ling *et al.* 2001, Laybutt *et al.* 2002b), *Fbp1* (Laybutt *et al.* 2002b) and *Pck1* (Marselli *et al.* 2010). The latter gene was also upregulated in isolated human islets cultured in the presence of high glucose levels (Shalev *et al.* 2002) (Table 3). Upregulation of *G6pase* may divert glucose from its classical metabolic route by inducing glucose cycling (GC). GC is a futile cycle where glucose is phosphorylated to glucose-6-phosphate by GCK and then dephosphorylated to glucose by glucose-6-phosphatase (G6Pase) with consumption of an ATP molecule. Indeed, *G6pase* overexpression in INS1 cells has been shown to increase GC and reduce GSIS (Trinh *et al.* 1997). Upregulation of *G6pase* mRNA and enzymatic activity in the islets of Px and GK rats was reversed by phlorizin treatment thereby underscoring the role of hyperglycemia (Ling *et al.* 2001, Laybutt *et al.* 2002b).

Another β -cell forbidden gene, *Acot7* (Pullen *et al.* 2010), which encodes for mitochondrial and cytosolic isoforms of acyl-CoA thioesterase 7 (ACOT7), hydrolyses long-chain acyl-CoA esters. Its expression was upregulated in the islets of ZDF rats and β -cells of human T2D patients (Parton *et al.* 2006, Marselli *et al.* 2010) (Table 3). Interestingly, repression of *Acot7* in mouse has been proposed in a recent study to be necessary for adequate insulin secretion. Indeed, selective overexpression of the mitochondrial *Acot7* in β -cells of adult mice impaired glucose tolerance as a consequence of altered insulin secretion (Martinez-Sanchez *et al.* 2016). However, further work is needed to determine the precise mechanism(s) involved.

Recently, *Aldh1a3* has been proposed as a novel β -cell forbidden gene and putative marker of dedifferentiated β -cells (Kim-Muller *et al.* 2016). Aldehyde dehydrogenase family 1 member A3 (ALDH1A3) expression and activity were upregulated in the islets of various animal models including *db/db* mice (Kim-Muller *et al.* 2016, Ishida *et al.* 2017, Stancill *et al.* 2017). Increased ALDH1A3 immunostaining has also been confirmed in the islets of diabetic human subjects and was associated in some β -cells with cytoplasmic expression of NKX6.1 (Cinti *et al.* 2016) (Table 3). However, overexpression of *Aldh1a3* in MIN6 cells did not alter the expression of β -cell-enriched genes and was even associated in mouse islets with a slight potentiation of GSIS thereby ruling out a causative role of ALDH1A3 in β -cell dedifferentiation (Kim-Muller *et al.* 2016). *Aldh1a3* is a detoxifying enzyme that catalyses the oxidation of retinal to retinol using NAD⁺. It is also

involved in other metabolic processes including amino acid metabolism and the metabolism of lipid peroxidation products (Duan *et al.* 2016). Therefore, its upregulation in the islets of diabetic animals and T2D subjects could be a response to oxidative stress and the ensuing damage.

Transcriptional regulators Besides metabolic genes, β -cell forbidden genes also enclose transcriptional regulators. We have described in 'Down regulation of insulin gene expression' section the role of C/EBP β , MYC and ETS1 in the alteration of insulin gene expression. In addition, several transcription factors typically expressed in progenitor cells at the embryonic stage and repressed in adult β -cells were upregulated in β -cells of various diabetic animal models, including neurogenin 3 (NGN3), nanog homeobox (NANOG), octamer-binding transcription factor 4 (OCT4 also known as POU5F1) and v-myc avian myelocytomatosis viral oncogene homolog 1, lung carcinoma derived (L-Myc) (Talchai *et al.* 2012, Brereton *et al.* 2014, Wang *et al.* 2014) (Table 3). Lineage tracing studies confirmed that these dedifferentiated cells originate from cells that expressed insulin and also suggested their transdifferentiation in other islet non- β -cells, including α -cells (Talchai *et al.* 2012, Wang *et al.* 2014) (Fig. 2). Interestingly, NGN3 reexpression in the islets of a diabetic mouse model with K_{ATP} channel mutation was reversed by insulin therapy (Wang *et al.* 2014). This observation is of importance. Together with the phlorizin and caloric restriction studies mentioned in previous sections, these reports highlight the astonishing plasticity of the β -cell phenotype and indicate the possibility to reverse β -cell dedifferentiation, at least in rodents. Furthermore, a recent study has also shown the possibility to prevent downregulation of some β -cell key transcription factor genes *in vitro* under cytokine treatment and *in vivo* in *db/db*, *ob/ob* and Akita mice using small-molecule inhibitors of the tumour growth factor β (TGF β) pathway that target the TGF β receptor I (also known as ALK5) (Blum *et al.* 2014). In humans, to date and to our knowledge, there is no confirmation of such expression of endocrine progenitor transcription factors in β -cells of T2D patients, except for upregulation of *POU5F1* mRNA levels (Guo *et al.* 2013). However, two studies have reported increased mRNA levels of the pre-endocrine gene *SOX9* (Marselli *et al.* 2010, Bugliani *et al.* 2013). Evidence suggests that *SOX9* upregulation in T2D may result from hypoxia and activation of HIF1 and could play a role in the downregulation of β -cell-enriched genes (Puri *et al.* 2013).

Inhibitor of differentiation (ID) proteins are another class of transcriptional regulators induced in response to glucose stimulation in β -cell lines and human islets (Wice *et al.* 2001) and upregulated in the islets of diabetic *db/db* mice and GK rats (Kjorholt *et al.* 2005, Homo-Delarche *et al.* 2006, Bensellam *et al.* 2015) (Table 3). They have been proposed to act as repressors of basic helix-loop-helix (bHLH) transcription factors thereby modulating cell differentiation and proliferation (Lasorella *et al.* 2014, Nair *et al.* 2014). Upregulation of *Id1* under high-fat diet has been implicated in the inhibition of insulin secretion and alteration of glucose homeostasis (Akerfeldt & Laybutt 2011). We have subsequently demonstrated that IDs are a novel class of oxidative stress-responsive genes in β -cells. Surprisingly, under oxidative stress conditions, deletion of *Id1* and/or *Id3* resulted in mitochondrial impairment and induced a global attenuation of antioxidant gene expression in association with a further increase in intracellular ROS levels and apoptosis. These effects involved a novel interaction of IDs with the NFE2L2-small Maf antioxidant pathway (Bensellam *et al.* 2015). These findings suggest that IDs are key components of the antioxidant response and play an important role in the promotion of β -cell survival under oxidative stress. However, these observations also raise the question of whether dedifferentiation could be an adaptive mechanism to escape cell death under stress conditions (discussed in the next section).

Stress response genes Chronic hyperglycemia exposes β -cells to higher levels of stress than under physiological conditions. To face such threat, β -cells upregulate endogenous stress responses to uphold homeostasis. Thus, the induction and the regulatory mechanisms of the antioxidant and the ER stress responses have received much attention given the important role of these pathways in β -cell pathophysiology (reviewed in Bensellam *et al.* 2012b). We have described in 'Failure of the adaptive UPR and progression towards diabetes' section the regulation of the UPR in diabetes and the emerging evidence linking the failure of this response with the progression towards diabetes and β -cell dedifferentiation. In the following paragraphs, we will focus on the antioxidant response and its potential implication in β -cell dedifferentiation.

Upregulation of antioxidant gene expression in response to elevated glucose levels has been well documented *in vitro* and in diabetic animals, and has also been confirmed in human T2D for some antioxidant genes (reviewed in Bensellam *et al.* 2012b). The primary role of the antioxidant response is to relieve hyperglycemia-induced

oxidative stress and promote cell survival. Nevertheless, β -cell mass is reduced by about 60% in GK rats although the mRNA and protein levels of *Nfe2l2* and several other antioxidant genes were markedly upregulated (Movassat *et al.* 1997, Lacraz *et al.* 2009). Moreover, the β -cell differentiated phenotype is noticeably altered in Px rats despite strong upregulation of the mRNA levels of *Hmox1* and *Gpx1* (Jonas *et al.* 1999, Laybutt *et al.* 2002a). Similarly, β -cell mass is reduced in human T2D patients even with upregulated mRNA levels of several antioxidant genes including various isoforms of metallothioneins (Marchetti *et al.* 2004, Marselli *et al.* 2010). These observations suggest that either the endogenous antioxidant response triggered by hyperglycemia is not robust enough to efficiently protect the β -cell differentiated phenotype or its strong and sustained activation under chronic hyperglycemia is deleterious and may play a role in β -cell dedifferentiation.

In support of the first possibility, previous evidence has shown that rat islets express low levels of several antioxidant genes in comparison to other tissues (Lenzen *et al.* 1996, Tiedge *et al.* 1997). The H_2O_2 detoxifying enzyme catalase is specifically repressed in adult mouse β -cells in comparison to other tissues (Thorrez *et al.* 2011, Pullen *et al.* 2017). Lower GPX1 expression and activity has also been confirmed in isolated human islets (Tonooka *et al.* 2007). In highly metabolically active β -cells, such low antioxidant gene expression signature is undoubtedly an Achilles' heel, especially under prolonged hyperglycemia. Interestingly, β -cell-specific overexpression of *Gpx1* in *db/db* mice prevented the loss of the β -cell-enriched genes MAFA, NKX6.1 and GLUT2 (Harmon *et al.* 2009, Guo *et al.* 2013). Similarly, antioxidant supplementation has been proven beneficial, at least *in vitro* and in rodent models (reviewed in Bensellam *et al.* 2012b). Why β -cells exhibit lower antioxidant gene expression in comparison to other tissues is unclear. However, some studies have proposed that physiological levels of H_2O_2 may play the role of a coupling factor for GSIS (Pi *et al.* 2007, Leloup *et al.* 2009). In addition, it has been shown that catalase or metallothionein overexpression in β -cells, while reducing cytokine-induced ROS generation, accelerated diabetes onset in male NOD mice in association with increased β -cell death and reduced PDX1 protein levels (Li *et al.* 2006a). It should be emphasized here that the antioxidant arsenal of β -cells is not limited to the classically studied genes such as *Gpx1* and catalase but encloses many other players including those of the glutathione system, the peroxiredoxin system, the thioredoxin system, haem oxygenases and metallothioneins and many of these genes are either expressed at high levels under

basal conditions or markedly upregulated under stress (Bensellam *et al.* 2009). The scenery is even more complex as some antioxidant genes can play other roles and be involved in different cellular processes such as the zinc buffering properties of metallothioneins (Chabosseau & Rutter 2016) or the involvement in iron metabolism for *Hmox1* (Gozzelino & Soares 2014). Therefore, one may propose that sustained upregulation of this wide battery of antioxidant genes under chronic hyperglycemia may exert a negative impact on the β -cell differentiated phenotype by yet to be defined mechanisms.

Dedifferentiation as a potential adaptive mechanism

Despite marked alteration of the β -cell differentiated phenotype and reduction of β -cell mass in human T2D, the β -cell apoptosis rate remains relatively low. This phenomenon is also observed in experimental models such as Px rats, *db/db* mice, *Foxo1*-KO mice and K_{ATP} -GOF mice. An emerging body of data suggests that β -cell dedifferentiation could be an adaptive mechanism to escape cell death under chronic hyperglycemia at the price of altered identity and function.

As mentioned in the previous section, we found that upregulation of IDs (inhibitors of bHLH transcription factors) during oxidative stress played a key role in maintaining the antioxidant response and mitochondrial integrity and thereby promoting β -cell survival (Bensellam *et al.* 2015). In addition, previous reports have shown that β -cell line maturation from a glucagon-producing phenotype to an insulin-producing phenotype was associated with increased sensitivity to several toxic agents such as cytokines, streptozotocin and alloxan (Nielsen *et al.* 1999). Further studies revealed an important role of increased expression of *Pdx1* and *Nkx6.1* in this acquired sensitivity (Nielsen *et al.* 2004). The potential link between dedifferentiation and adaptation/resistance to stress seems to be a conserved biological mechanism that has also been described in other cell types and models including cancer cells (Del Vecchio *et al.* 2014, Li *et al.* 2016, Roesch *et al.* 2016) and even plant cells (Grafi & Barak 2015). Future work is needed to validate this hypothesis in β -cells and to fully characterize the response of dedifferentiated β -cells to different types of stress.

From a therapeutic perspective, alleviating β -cell stress may provide a novel strategy to reverse the process of dedifferentiation and restore functional β -cell mass. Previous β -cell rest approaches have shown promising

results *in vitro* and in animal models (Grill *et al.* 2009). Noteworthy, in isolated rat islets, high glucose-induced upregulation of several β -cell forbidden genes and other stress response genes such as *Ldha*, *Crem*, *Myc*, *Adm* and *Hmox1* was markedly prevented by diazoxide treatment (Jonas *et al.* 2001, 2003, Ma *et al.* 2007). However, until now, these methods failed to improve glycemic control in T2D patients (reviewed in Bensellam *et al.* 2012b). Given the key role of hyperglycemia in β -cell dedifferentiation and the demonstrated beneficial effects of the glucose lowering strategies in rodents, SGLT2 inhibition by gliflozins merits further attention in humans (Abdul-Ghani *et al.* 2017). The use of these drugs in combination with other therapies such as incretins may exert beneficial effects on the β -cell phenotype (Busch & Kane 2017, Kaneto *et al.* 2017). The potential combination with antioxidants such as GPX mimetics and/or chemical chaperones such as the recently identified small-molecule azoramide (Fu *et al.* 2015) merits further experimental and clinical testing.

Conclusion

Growing evidence indicates that β -cell dedifferentiation plays a key role in the loss of functional β -cell mass in T2D. Over the last decades, *in vitro* and animal studies implicated glucotoxicity and downstream pathways in this process. Dysregulation of numerous – and not fully identified – transcriptional regulators, genes, miRNAs, stress responses, metabolic processes and epigenetic mechanisms converge to compromise β -cell identity in T2D. Based on emerging data and observations in other cell types and models, we proposed that β -cell dedifferentiation could be an adaptive mechanism to escape cell death under stress conditions. Interestingly, animal studies suggest the possibility to reverse β -cell dedifferentiation by reducing β -cell stress strategies. More large-scale studies using human β -cells are needed to validate the findings in rodents and shed more light on the different signalling effectors and pathways involved in the alteration of the β -cell differentiated phenotype. A better understanding of the physiological and pathological regulation of these pathways may pave the way to reverse β -cell dedifferentiation and restore the functional β -cell mass in human T2D.

Supplementary data

This is linked to the online version of the paper at <https://doi.org/10.1530/JOE-17-0516>.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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