Review

MOLECULAR METABOLISM

# Transcribing β-cell mitochondria in health and disease

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## ABSTRACT

**Background:** The recent genome-wide association studies (GWAS) of Type 2 Diabetes (T2D) have identified the pancreatic β-cell as the culprit in the pathogenesis of the disease. Mitochondrial metabolism plays a crucial role in the processes controlling release of insulin and β-cell mass. This notion implies that mechanisms controlling mitochondrial function have the potential to play a decisive pathogenetic role in T2D.
**Scope of the review:** This article reviews studies demonstrating that there is indeed mitochondrial dysfunction in islets in T2D, and that GWAS have identified a variant in the gene encoding transcription factor B1 mitochondrial (*TFB1M*), predisposing to T2D due to mitochondrial dysfunction and impaired insulin secretion. Mechanistic studies of the nature of this pathogenetic link, as well as of other mitochondrial transcription factors, are described.
**Major conclusions:** Based on this, it is argued that transcription and translation in mitochondria are critical processes determining mitochondrial function in β-cells in health and disease.


**Keywords** β-Cell; Mitochondria; Islets; Genome-wide association study (GWAS); Insulin secretion; Expression quantitative trait locus (eQTL)

## 1. INTRODUCTION

Mitochondria are at center stage in whole body metabolic control in health and disease. This is undisputed given their critical functions in the cellular machinery providing fuel, reducing equivalents and building blocks for basic molecular processes. This notwithstanding, how dysfunction of this system evolves and contributes to, as well as causes, common metabolic diseases is still less well understood. This review will focus on the role of mitochondria in the insulin-secreting β-cells under normal conditions and the events culminating in Type 2 Diabetes (T2D). The emphasis on β-cell mitochondria is prompted by the fact that the genome-wide association studies (GWAS) of T2D and related traits have shown that the β-cell is the main culprit in T2D [1]. A potential role for mitochondrial dysfunction in insulin target tissues is heavily debated but will not be dealt with here; excellent reviews are available elsewhere [2,3].
I will begin by outlining the critical role of mitochondria in β-cell function, with a focus on glucose-stimulated insulin secretion (GSIS). Then I will describe the fundamental processes whereby mitochondrial function is controlled, with an emphasis on transcriptional and translational regulation in mitochondria. I will highlight how dysregulation of mitochondria has been implicated in the pathogenesis of T2D. To this end, I will review human and experimental studies linking mitochondrial dysfunction causally to β-cell dysfunction and subsequently diabetes.

## 2. MITOCHONDRIA AND β-CELLS: STIMULUS-SECRETION COUPLING

The primary role of the pancreatic β-cell is to release insulin in response to a rise in blood glucose levels after a meal. To carry out this task, β-cells have developed an elaborate machinery that translates fluctuations in ambient glucose concentrations into cellular processes that signal to the exocytotic machinery, leading to transport, priming, fusion and emptying of insulin granules. Moreover, insulin secretion in response to a strong stimulation by glucose is biphasic, i.e., a rapid initial peak is followed by a slower but sustained second phase of insulin secretion. The critical role of mitochondria is to provide the signals controlling these processes. These signals are generated from fuel metabolism (Figure 1).

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*Abbreviations:* ATGL, adipocyte triglyceride lipase; AMPK, AMP-dependent protein kinase; COX, Cytochrome *c* oxidase; CYTB, Cytochrome *b*; ERR-α, Estrogen-related receptor-α; eQTL, Expression quantitative trait locus; GDH, Glutamate dehydrogenase; GSIS, Glucose-stimulated insulin secretion; GWAS, Genome-wide association study; HSL, Hormone-sensitive lipase; $ICD_c$, Cytosolic isocitrate dehydrogenase; $K_{ATP}$, ATP-dependent $K^+$-channel; MTERF, Mitochondrial transcription termination factor; ND, NADH dehydrogenase; NRF, Nuclear respiratory factor; NSUN4, NOP2/Sun RNA methyltransferase family member 4; OXPHOS, Oxidative phosphorylation; PC, Pyruvate carboxylase; PDH, pyruvate dehydrogenase; PGC, Peroxisome proliferator-activated receptor-γ co-activator; POLγ, DNA polymerase-γ; POLRMT, Mitochondrial RNA polymerase; PPARγ, Peroxisome proliferator-activated receptor-γ; PRC, PGC1-related coactivator; SENP1, Sentrin/SUMO-specific protease-1; SNP, Single Nucleotide Polymorphism; SUR1, Sulphonylurea receptor-1; T2D, Type 2 Diabetes; TCA, Tricarboxylic acid; TEFM, Mitochondrial transcription elongation factor; TFAM, Transcription factor A mitochondrial; TFB1M, Transcription factor B1 mitochondrial; TFB2M, Transcription factor B2 mitochondrial

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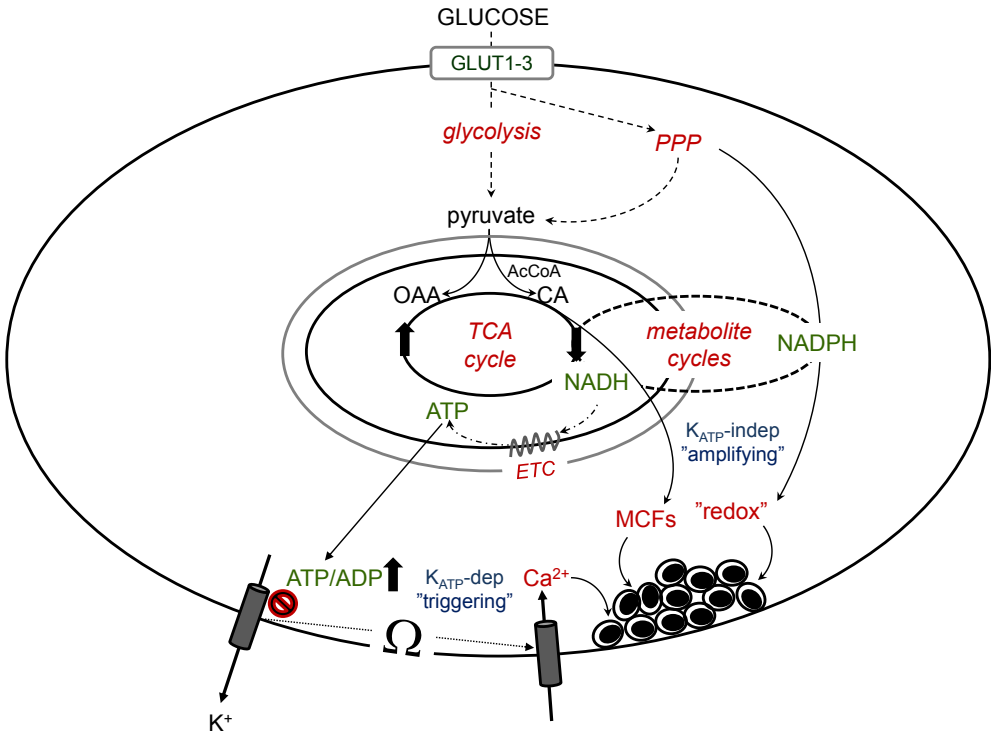


**Figure 1: Stimulus-secretion coupling in the pancreatic β-cell.** Glucose is transported into the β-cell via facilitated diffusion in proportion to its extracellular concentration. Metabolism in glycolysis and the tricarboxylic acid (TCA) cycle ensues. This leads to a rise in the ATP/ADP ratio and closing of an ATP-dependent $K^+$-channel ($K_{ATP}$) in the plasma membrane. A voltage-dependent $Ca^{2+}$-channel opens and triggers exocytosis of insulin. In addition, metabolic coupling factors (MCF) amplify insulin secretion. These are thought to be generated by metabolite cycles associated with the TCA cycle and the pentose phosphate pathway (PPP). MCFs may be NADPH, perhaps mediating its effect via cellular redox, glutamate or lipid moieties. AcCoa — acetyl-CoA; CA — citrate; ETC — electron transport chain; GLUT — glucose transporters; OAA — oxaloacetate; Ω — membrane polarization.

### 2.1. Triggering phase of insulin release

The consensus model of β-cell stimulus-secretion coupling holds that glucose is transported into the β-cell by facilitated transport in proportion to its extracellular concentration [4,5] (Figure 1). This is carried out by GLUT2 in rodents [6] and predominantly by GLUT1 and 3 in humans [7]. After phosphorylation by glucokinase, glycolysis ensues, resulting mainly in production of pyruvate; lactate formation occurs, if at all, at a slow rate owing to very low expression of lactate dehydrogenases under normal conditions [8—10]. Pyruvate enters the mitochondria and is either decarboxylated by pyruvate dehydrogenase (PDH) or carboxylated by pyruvate carboxylase (PC); hence, acetyl-CoA and oxaloacetate are formed, respectively, adding carbons to the tricarboxylic acid (TCA) cycle in the mitochondrial matrix. Oxidation of citrate back to oxaloacetate generates two molecules of $CO_2$, the reducing equivalents NADH, as well as one molecule GTP. NADH donates electrons to the electron transport chain in the inner mitochondrial membrane. A proton-motive force is created, driving extrusion of protons through complexes I, III, and IV into the intermembrane space. Hereby, an electrochemical proton gradient is generated. The ATP synthase allows flow of protons back into the matrix. The energy harnessed by this flow drives phosphorylation of ADP to ATP — oxidative phosphorylation (OXPHOS).

The ATP formed is the critical energy required in any cell. It is transported out of the mitochondria by the adenine nucleotide transporter. In addition to energizing the major functions of the cell, ATP serves a specialized function in the β-cell. A rise in the cellular ATP/ADP ratio is sensed by sulphonylurea receptor-1 (SUR1) [11]. SUR1 controls an outward-rectifying $K^+$-channel in the plasma membrane, which maintains a negative resting membrane potential in the β-cell [12]. When this ATP-dependent $K^+$-channel ($K_{ATP}$) is closed by increased ATP production in the β-cell, i.e., a rise in the ATP/ADP ratio, the plasma membrane depolarizes [13]. This activates voltage-gated $Ca^{2+}$-channels, leading to a rapid rise in intracellular $Ca^{2+}$, and consequently triggering of exocytosis of insulin granules. This mode of stimulus-secretion coupling is sometimes referred to as the $K_{ATP}$-dependent or triggering pathway of insulin secretion [14] (Figure 1). Clearly, mitochondrial function is of paramount importance for this process [4,15].

### 2.2. Amplifying phase of insulin release

Raising intracellular $Ca^{2+}$ by other means, e.g., via depolarization of the plasma membrane by KCl, produces a rapid, but transient, release of insulin [16]. This observation led to the recognition of processes required to sustain and amplify secretion of the hormone [14]. Importantly, it was found that some of these processes work independently of the $K_{ATP}$-channel [17]; they are referred to as $K_{ATP}$-independent or amplifying pathways of insulin secretion (Figure 1).

Arguably, the most important $K_{ATP}$-independent stimulator of insulin secretion is glucose itself [18]. A seminal discovery was that the hexose exerts a stimulatory effect on insulin release in the presence of high extracellular $K^+$ levels (depolarizes the plasma membrane) and diazoxide (maintains the $K_{ATP}$-channel in an open state) [17]. It was suggested that this amplifying effect on insulin release is exerted by a direct effect on the exocytotic machinery by signals generated in cellular metabolism, i.e., metabolic coupling factors [19,20]. Although widely accepted, this paradigm has recently been challenged, largely

  

owing to the lack of stringent mechanistic evidence for how metabolic coupling factors are formed proportionally to fuel metabolism in the β-cell, and, further, how they interact with the exocytotic machinery [21].

### 2.3. Anaplerosis

Levels of intermediates in the TCA cycle are maintained by the balanced additions and losses of carbons, i.e., anaplerosis and cataplerosis. Thus, formation of a metabolic coupling factor by mitochondrial metabolism, foremost the TCA cycle, requires the net addition of carbons; otherwise, the TCA cycle will come to a halt due to depletion of intermediates. Fluxes through PDH and PC are of equal magnitude in β-cells [22], but the latter provides three carbons to the TCA cycle. This means that an excess of carbons is provided (anaplerosis), since two carbons are lost as two molecules of $CO_2$. This sets the stage for an exit of carbons (cataplerosis), a prerequisite for mitochondria-derived coupling factors interacting with the secretory machinery in the cytosol [23,24]. In fact, the anaplerotic/cataplerotic activity of clonal insulin-producing cell lines correlates positively with glucose-responsiveness [25].

### 2.4. Metabolic coupling factors

Ever since the discovery of the $K_{ATP}$-independent pathway of insulin secretion, much effort has been devoted to identifying metabolic coupling factors [4,15,19,20,26]. These putative factors are thought to sustain and amplify secretion of the hormone and believed to derive mainly from mitochondrial metabolism. An exhaustive review of metabolic coupling factors is beyond the scope of this review but a few examples will be given.

When glucose is metabolized in β-cell mitochondria, carbons in the form of citrate may leave the TCA cycle; a citrate carrier in the inner mitochondrial membrane permits this transport. Citrate is cleaved by citrate lyase in the cytosol, forming oxaloacetate and acetyl-CoA. This is essentially the first step in de novo lipogenesis, a pathway which is not thought to be highly active in β-cells [27]. Instead, malonyl-CoA, formed by acetyl-CoA carboxylase, serves as an inhibitor of carnitine palmitoyl transferase 1, thereby blocking transport of acyl-CoA into the mitochondria and inhibiting fatty acid oxidation [28]. Long chain acyl-CoA levels rise in the cytosol as a consequence hereof [29]. They have been postulated as coupling factors, linking glucose metabolism directly to exocytosis [30]. Exactly how these lipid moieties stimulate exocytosis has not been determined. The fact that β-cells express hormone-sensitive lipase (HSL) [31], the rate-limiting enzyme in lipolysis, as well as the adipocyte triglyceride lipase (ATGL) [32], suggests that endogenous triglycerides may be a source of long chain acyl-CoA in the β-cell. Indeed, β-cell-specific knock out mice for HSL or ATGL exhibit impaired GSIS [33,34].

A modified model of lipid coupling of insulin secretion has been based on observations of a glycerolipid/fatty acid cycle in β-cells [35]. It is a futile cycle in terms of energy production, but it was suggested that input of monoacylglycerol drives a lipogenic arm of the cycle. This would lead to generation of lipid species, amplifying insulin secretion. Indeed, genetic targeting of membrane-bound monoacylglycerol lipase (α/β-hydrolase domain-6) increases insulin secretion in mice, perhaps by preventing formation of monoacylglycerol, which is destined to bind the exocytotic protein Munc13 [36].

Another line of research showed that glutamate may be a metabolic coupling factor in GSIS [37]. Upon mitochondrial metabolism of glucose-derived carbons, glutamate is formed from α-ketoglutarate, a TCA cycle intermediate, by the action of glutamate dehydrogenase (GDH). This research has been extensively reviewed [38]. In brief, this role of glutamate is supported by the existence of activating mutations in GDH, which result in neonatal hyperinsulinism [39]. Targeting of GDH in mouse β-cells impairs insulin secretion [40], while overexpression of GDH in β-cells results in increased GSIS [41]. A controversy in the field is whether glutamate or α-ketoglutarate is formed by the actions of GDH, thereby leading to stimulation of insulin secretion [42]. It is also plausible that an anaplerotic action of GDH, increasing TCA cycle activity could lead to enhanced GSIS.

### 2.5. A role for glycolysis in stimulus-secretion coupling?

Although this review is focused on the role of mitochondria in stimulus-secretion coupling, glycolysis cannot be entirely ignored, if nothing else, owing to the fact that its metabolism of glucose provides intermediates for mitochondrial metabolism [43].

Recent interest has been placed on the role of the reducing equivalent NADPH. It was shown that a rise in glucose increases the NADPH/$NADP^+$ ratio and, in parallel, enhances insulin secretion [44]. Intracellular provision of NADPH stimulates insulin exocytosis. NADPH is mainly formed in the cytosol by the pentose phosphate shunt, malic enzyme (oxidizing malate to pyruvate) and the action of cytosolic isocitrate dehydrogenase ($ICD_c$; producing α-ketoglutarate). The latter two reactions have been suggested as part of the cycling of pyruvate, i.e., anaplerotic/cataplerotic processes [45,46], the extent of which has been correlated with glucose-responsiveness of clonal insulin-producing cells [25]. Upon glucose stimulation, both clonal insulin-producing cells and rat islets show a rapid increase in ribose-6-phosphate levels [47], a central intermediate in the pentose phosphate pathway; this rise parallels an increase in insulin release and the NADPH/$NADP^+$ ratio. Silencing of $ICD_c$ in clonal insulin-producing cells and rat islets abrogates GSIS, lowers NADPH levels and retards pyruvate cycling activity [45]. While silencing of malic enzyme abrogates GSIS and formation of NADPH in clonal insulin-producing cells, somewhat surprisingly, silencing or knock out of malic enzyme in rat islets has no effect on GSIS [46].

Exactly how NADPH controls exocytosis is unclear but the cellular redox state has been implicated [47,48]. Silencing of glutaredoxin inhibits GSIS and single cell exocytosis in clonal insulin-producing cells, as well as in rat islets, while overexpression of the protein enhances exocytosis [49]. Blocking the pentose phosphate pathway lowers ribose 5-phosphate levels, as well as reduced glutathione, in parallel with reduced GSIS, but has no effect on ATP production [47]. Recently, metabolism-linked reduction of glutathione, via NADPH formation, was linked to a protein modification termed SUMOylation [48]. Thus, redox-dependent activation of sentrin/SUMO-specific protease-1 (SENP1) is linked to amplification of GSIS via deSUMOylation of exocytotic proteins. Another mechanism linking the pentose phosphate pathway to exocytosis is formation of purines from ribose 5-phosphate [50]. Specifically, formation of adenylosuccinate correlates with GSIS and is dependent on SENP1.

## 3. TRANSCRIPTION IN MITOCHONDRIA

Regulation of transcription in mitochondria is unconventional. It requires the integrated action of two genomes and their control machineries, i.e., transcription factors, repressors, enhancers, and co-activators. The mitochondrial DNA does not encode any such factors — they are all derived from the nuclear genome. Hence, transcriptional control of mitochondrial DNA starts with the transcription and translation of mitochondrial RNA polymerases and transcription factors in the nucleus. Another level of complexity is that most mitochondrial proteins are neither encoded by mitochondrial DNA nor synthesized in the organelle. Instead, like the mitochondrial transcription factors, they are under

 

nuclear genomic control and imported from the cytosol. This process is regulated by nuclear encoded transcription factors and co-activators, such as nuclear respiratory factor (NRF) 1 and 2, and peroxisome proliferator-activated receptor (PPAR)-γ co-activators (PGC). Mouse genomics have shown that the transcriptional machinery in mitochondria is absolutely critical for mitochondrial and, consequently, cellular function, whereas the nuclear factors controlling expression of mitochondrial proteins are essential but redundant to some extent.

### 3.1. Mitochondrial DNA

Mitochondria are unique in that they possess their own DNA. This is likely a remnant of a primitive bacterial DNA derived from an ancestral α-proteobacterium. It was engulfed by an archaebacterium in an endosymbiotic event postulated to have occurred during early evolution [51]. Like bacterial DNA, mitochondrial DNA (16.5 kB) is a double-stranded circular molecule, lacking histones and introns, and packed together as a nucleoid. It is replicated by a dedicated mitochondrial DNA polymerase, the DNA polymerase-γ (POLγ) [52], encoded by *POLG1* in the nucleus and imported into mitochondria. Mutations in *POLG1* have been reported in humans [53]: a spectrum of phenotypes is evident and includes diabetes in a subset of individuals. A mouse carrying a mitochondrial DNA polymerase with impaired proof-reading capacity has been created as a model to study aging [54]. This model does not exhibit an increased incidence of diabetes. Surprisingly, when crossbred with the diabetes-prone Akita mouse, the diabetes phenotype in male Akita mice carrying the deficient POLγ is ameliorated, which was attributed to reduced food intake [55].

The two DNA strands are referred to as the light and heavy strand, owing to the number of guanines in the sequence, and are transcribed as polycistronic mRNAs. The vast majority of the original genes from the ancestral prokaryote have transmigrated into the nuclear DNA of the cell [56]. Only 37 genes remain; they encode 13 critical proteins in the respiratory chain, as well as two mitochondrial rRNAs and 22 tRNAs (Table 1). This implies that mitochondria have retained an important level of transcriptional and translational control of their own mRNA and protein.

In contrast to nuclear DNA, many copies of the mitochondrial DNA exist in the cell; each mitochondrion contains several nucleoids in which the DNA and the accessory proteins are packed [57]. This also explains why mitochondrial DNA is more prone to mutation; every time new mitochondria form, mitochondrial DNA is replicated, increasing the likelihood of a mistake in replication. That mitochondrial DNA in cells is variable is termed heteroplasmy [58]. Contrary to nuclear DNA, where mutation of one base in one DNA copy is sufficient for a profound impact, a certain level of heteroplasmy is required for a mitochondrial DNA mutation to become penetrant. Of note, mitochondria and consequently mitochondrial DNA are exclusively maternally derived. Hence, mitochondrial genetic diseases have a typical maternal pattern of inheritance.

**Table 1** — The 13 mitochondrial genes encoding respiratory proteins in the electron transport chain.

| Mitochondrial genes encoding proteins | | | |
|---|---|---|---|
| Gene name | Gene Bank ID | Protein | Complex |
| *MT-ND1* | 4535 | NADH dehydrogenase 1 | I |
| *MT-ND2* | 4536 | NADH dehydrogenase 2 | I |
| *MT-ND3* | 4537 | NADH dehydrogenase 3 | I |
| *MT-ND4* | 4538 | NADH dehydrogenase 4 | I |
| *ND4L* | 4539 | NADH 4L dehydrogenase | I |
| *MT-ND5* | 4540 | NADH dehydrogenase 5 | I |
| *MT-ND6* | 4541 | NADH dehydrogenase 6 | I |
| *MT-CYB* | 4519 | Cytochrome *b* | III |
| *MT-CO1* | 4512 | Cytochrome *c* oxidase I (COX1) | IV |
| *MT-CO2* | 4513 | Cytochrome *c* oxidase II (COX2) | IV |
| *MT-CO3* | 4514 | Cytochrome *c* oxidase III (COX3) | IV |
| *MT-ATP6* | 4508 | ATP synthase 6 | V |
| *ATP8* | 4509 | ATP synthase 8 | V |

Information derived from NCBI Gene Bank. Human mitochondrial gene names are given.

### 3.2. Mitochondrial RNA polymerase (POLRMT)

Mitochondria contain a dedicated RNA polymerase [59]. POLRMT is a single subunit protein encoded by the nucleus and imported into mitochondria. While it binds sequence-specifically to elements in the heavy and light strand promoters (HSP/LSP), it cannot initiate transcription without the assistance of the mitochondrial transcription factors A and B2 [60]. A prerequisite for mitochondrial RNA synthesis is primer formation, a process for which POLRMT is crucial [61].

### 3.3. Transcription factor A mitochondrial (TFAM)

TFAM is a high mobility group-box protein that controls transcription of both the heavy and light strands of mitochondrial DNA, resulting in bi-directional transcription [62]. It appears to exert several critical functions in transcription of mitochondrial DNA: it binds to a high affinity site just upstream of the transcription start site, recruiting POLRMT [63]. In this process, a bending of the mitochondrial DNA has been suggested to be an important event [64]. TFAM can also induce negative supercoils in DNA. Binding of TFAM monomers to the DNA helps to unwind it, facilitating transcription [65]. TFAM initiates formation of nucleoids via aggregation and cross-strand binding of mitochondrial DNA [66].

Genetic targeting of *Tfam* in mouse supports a critical role for the protein in mitochondrial function: a general knock out of the gene is embryonically lethal, due to extensive mitochondrial disruption [67]. Conditional targeting of *Tfam* in heart results in a progressive respiratory chain deficiency, subsequent cardiomyopathy, and premature death after 2–4 weeks [68].

The transcription factor PDX1 controls both embryonic development of the pancreas and function of mature β-cells. A regulatory link between PDX1 and TFAM has been established: disruption of PDX1-activity in β-cells reduces *Tfam* expression and causes mitochondrial dysfunction, a phenotype that is rescued by *Tfam* expression [69].

### 3.4. Transcription factor B1 mitochondrial (TFB1M)

TFB1M was initially thought to be a transcription factor, hence its name; it facilitates transcription at high concentrations in an in vitro system [70]. Subsequent examinations showed that TFB1M is part of a larger family of conserved rRNA methyltransferases [71]. TFB1M is also known as mitochondrial dimethyladenosine transferase 1. Indeed, it was shown to effectively methylate two adjacent adenines in the 3′-end of mitochondrial 12S rRNA — loss of this methylation impairs biogenesis of mitochondrial ribosomes [71]. Consequently, protein translation in mitochondria will be severely impaired. Accordingly, a general knock out of *Tfb1m* is embryonically lethal. Conditional targeting of *Tfb1m* in heart muscle leads to perturbed assembly of mitochondrial ribosomes and, consequently, a block in protein translation [71].

### 3.5. Transcription factor B2 mitochondrial (TFB2M)

On the other hand, TFB2M, a paralogue of TFB1M, is a bona fide transcription factor [70,72]. It is part of the initiation process of mitochondrial transcription. After the binding of POLRMT to TFAM, the

  

polymerase changes its confirmation, allowing binding of TFB2M to the complex and formation of the first phosphodiester bonds in the growing RNA molecule [72]; after initiation of transcription, TFB2M detaches from the transcriptional unit [73].

### 3.6. Additional mitochondrial control factors

Mitochondrial transcription elongation factor (TEFM) interacts with the C-terminal portion of POLRMT, assisting POLRMT with transcription of longer RNA stretches, but also with that of structurally more complex DNA regions [74].

Conversely, there are mitochondrial transcription termination factors, of which MTERF1 was the first to be identified [75]. Several observations support the notion that transcription of the heavy strand halts at the $tRNA^{Leu(UUR)}$ gene, downstream of the 16S rRNA gene [76]. This action has been suggested to be mediated by MTERF1. An alternative hypothesis is that MTERF1 blocks transcription of the opposite strand, thereby hindering transcriptional interference at the LSP [77].

Three homologs of MTERF1 have been identified but none of them have been shown definitively to terminate mitochondrial DNA transcription [78]. MTERF2 localizes to nucleoids but a functional role remains to be identified. MTERF3 and 4 have been implicated both in mitochondrial DNA transcription and assembly of ribosomes; deletion of either *Mterf3* or *Mterf4* in mouse is embryonically lethal.

NOP2/Sun RNA methyltransferase family member 4 (NSUN4) is yet another important protein in mitochondria [79]; like TFB1M, it is a methyltransferase, methylating cytosine 911 in 12S rRNA. A general knock out of *Nsun4* is embryonically lethal, while a conditional knock out in heart leads to mitochondrial dysfunction, due to impaired formation of the respiratory complexes secondary to disrupted protein translation.

## 4. NUCLEAR TRANSCRIPTIONAL CONTROL OF MITOCHONDRIA

The transcriptional machinery described in 3 is confined to mitochondria but is ultimately under nuclear control. Thus, a nuclear control machinery ensures that mitochondria are optimally equipped for the metabolic processes that cells rely on. If this fails, apoptosis and cell death may occur, and metabolic diseases evolve. An important part of this is controlling mitochondrial number, also known as mitochondrial biogenesis. It is perhaps more appropriate to term this mitochondrial volume rather than number. The classical view of mitochondria as discrete organelles is being replaced by the realization that mitochondria form a dynamic compartment of the cell, and they are continually being reshaped by fusion and fission. This important and expanding research field has been excellently reviewed elsewhere [80–82].

### 4.1. Nuclear transcription factors

The most ubiquitous and well-known mitochondrial transcription factor is NRF1. It mainly controls expression of genes encoding the respiratory proteins but extends to numerous genes encoding proteins required for mitochondrial transcription and translation, heme biosynthesis, and mitochondrial protein import and assembly [83]. In fact, NRF1 has been found to bind to promoters in a large group of genes, many of which are housekeeping genes [84]. Importantly, among these are *TFAM*, *TFB1M* and *TFB2M*.

NRF1 responds to the metabolic state by cues derived from, e.g., growth, replication, exercise, and, possibly, inflammation [83]. Exactly how this control is exerted is unclear but cAMP-protein kinase A-dependent mechanisms appear to play a role [85]. Thus, there is a binding site for cAMP-responsive element binding protein in the promoter of *NRF1*. AMP-dependent protein kinase (AMPK) is another regulator of NRF1, likely via activation of PGC1α [86]. As cellular energy is compromised, AMPK will initiate a gene program serving to provide the cell with energy. Moreover, the estrogen-related receptor α (ERR-α) has also been demonstrated to exert transcriptional control over *NRF1* [87].

*Nrf1* has been targeted in mouse β-cells [88]. These mice exhibit euglycemia in the face of marked hyperinsulinemia. While this suggests that the mice are insulin-resistant, it was not determined in the study. Despite the hyperinsulinemia, *Nrf1*-deficient islets are unresponsive to glucose ex vivo. The most striking change in gene expression is a dramatic increase in *Hk1* expression. Accordingly, the authors suggest that the functional consequences of *Nrf1*-deficiency are explained by enhanced glycolytic metabolism upon an increase in low $K_M$ glucose transport. Similar findings were made in MIN-6 cells, where *Nrf1* had been stably silenced; mitochondrial mass is unchanged as is expression of most mitochondrial proteins. Given the essential role of NRF1 in control of mitochondrial genes, these results are unexpected and require further study.

NRF2, also known as GA-binding protein transcription factor-α, is, like NRF1, part of the same family of transcription factors [89]. Its targets overlap significantly with that of NRF1 but NRF2 seems to play a more important role in antioxidant processes, and has thereby been implicated in longevity. In contrast to *Nrf1*, knock out of *Nrf2* in mouse β-cells has no effect on insulin secretion from isolated islets [88]. Instead, *Nrf2*-deficiency results in a curtailed anti-oxidant response, as a result of reduced expression of anti-oxidant proteins upon oxidative stress [90]. Induction of NRF may thus restore insulin secretion and enhance β-cell viability perturbed by oxidative stress [91,92].

### 4.2. Nuclear transcriptional coactivators

Transcriptional coactivators are unable to regulate transcription on their own. Instead they facilitate transcription in many different ways, thereby exerting powerful control over gene transcription. This may include recruitment of histone-modifying enzymes, juxtaposing transcription factors to transcriptional initiation complexes, and taking part in the processing of the generated transcripts.

With respect to mitochondrial function, the PGCs have taken center stage [83]. They integrate metabolic cues derived from signaling pathways, foremost adrenergic, involved in growth, cell differentiation, and metabolic processes, particularly cold exposure, fasting/starvation, and exercise [93]. PGC1α was the first one to be identified [94]; it was found to be highly expressed in brown adipose tissue, brain, cardiac and skeletal muscle, as well as in kidney.

The role of PGC1α in islets is controversial. *PPARGC1A* mRNA expression in islets from patients with T2D is reduced by 90% [95], correlating with lower insulin secretion. Moreover, silencing of *PPARGC1A* in healthy human islets leads to a reduction in insulin secretion [95]. In contrast, *Pgcarg1a* is overexpressed in islets from diabetic animal models and adenoviral-mediated overexpression of *Pgcarg1a* leads to blunted ATP production and reduced insulin secretion [96].

PGC1β is a homolog of PGC1α, exhibiting quite similar effects [97]. PGC1-related coactivator (PRC) is a more distant relative [98]; it seems to be regulated in a more rapid fashion but exhibits a similar expression distribution as the other PGCs. The PGCs appear to be relatively redundant but PGC1β is not induced by cold exposure in brown fat [97]. PGC1α and 1β exert a strong positive effect on mitochondrial DNA and as well as nuclear and mitochondrial-encoded genes [99]. Jointly, this leads to an increase in mitochondrial biogenesis and enhancement of oxidative metabolism. Specifically,

 

PGC1α increases expression of *TFAM*, *TFB1M*, and *TFB2M* [100]; this is, in part, mediated by increasing NRF1 and 2 and ERRα but also via a direct effect on said genes.

## 5. MITOCHONDRIAL FUNCTION IN β-CELLS IN TYPE 2 DIABETES

The most obvious and clear support for the pathogenetic role of mitochondrial dysfunction in diabetes comes from mitochondrial diabetes, a disorder caused by maternally inherited mutations in the mitochondrial DNA [101]. As a consequence, functions of the 13 genes encoding components of the respiratory chain or the rRNAs and tRNAs are perturbed, leading to impaired OXPHOS and mitochondrial dysfunction. In fact, most known forms of mitochondrial diabetes arise due to mutations in the mitochondrial tRNA genes. A prime example of this is maternally inherited diabetes and deafness, which is caused by a 3243 A>G mutation, impacting on a gene for Leu tRNA. While this is theoretically very important, it is less of a clinical concern since mitochondrial diabetes is a rare disorder (<1% of clinical T2D). Mitochondrial diabetes has been reviewed elsewhere and will not be further discussed here [101,102]. Instead, the focus of this article is if and how mitochondrial dysfunction in β-cells causes and contributes to human T2D.

### 5.1. Islet mitochondrial function in human T2D

To determine whether mitochondrial dysfunction occurs in T2D requires access to human islets from patients with the disease. This pressing requirement has not been fulfilled until recently. The establishment of centers for clinical islet transplantation around the world has made islets from patients with T2D available to researchers. This very precious material is scarce but has provided insight into the role of mitochondria in β-cell dysfunction.

Early work reported that metabolites stimulating mitochondrial metabolism evoke insulin release from T2D islets [103], while that induced by glucose is impaired. This actually suggests that mitochondrial function is maintained. GSIS, but not arginine- and glibenclamide-stimulated insulin release, is impaired in islets from patients with T2D [104]. Mitochondrial fuels were not tested.

Metabolic dysfunction in islets from T2D patients is further suggested by reduced utilization [103] and oxidation [105] of glucose, as well as increased oxidative stress [104]. A subsequent, directed, analysis of mitochondria revealed that ATP levels and the ATP/ADP ratio stimulated by glucose are lower in islets from T2D patients [106]. This is accompanied by impaired hyperpolarization of the mitochondrial membrane, indicative of a diminished response of mitochondria to glucose. The activities of glycerol phosphate dehydrogenase, PC and succinyl-CoA:3-ketoacid-CoA transferase are significantly lower in islets from patients with T2D [107], as is expression of the FAD-linked glycerophosphate dehydrogenase [105]; this enzyme links glycolysis with TCA cycle metabolism.

Increased protein expression of uncoupling protein-2, as well as of complex I and ATP synthase, is observed in islets from patients with T2D [106]. A higher level of nitrotyrosine is also evident. β-cells from patients with T2D and from non-diabetic donors possess similar numbers of mitochondria but the mitochondrial density volume is significantly higher in T2D [106].

Together, these data suggest that mitochondrial dysfunction occurs in islets from patients with T2D. This notwithstanding, the data do not resolve whether mitochondrial dysfunction causes β-cell dysfunction or whether mitochondrial dysfunction is a consequence of β-cell dysfunction.

Another aspect of mitochondrial dysfunction is whether it may trigger apoptosis, thus contributing to β-cell apoptosis in T2D. Whether loss of β-cell mass occurs in T2D or not has been controversial over the years [108,109]. A balanced view may be that islets from T2D patients do exhibit lower β-cell mass, particularly in view of that increased demands on islets should result in increased β-cell mass, but that this deficiency in itself is not sufficient to impair GSIS in the patient. Indeed, Type 1 Diabetes does not become apparent until 85% of β-cell mass is lost. Nevertheless, several groups have demonstrated increased rates of apoptosis [108,110], albeit at a most moderate level, in islets from T2D. The role of mitochondria in β-cell apoptosis, however, is unclear.

### 5.2. Animal models of T2D with islet mitochondrial dysfunction

Mitochondrial function in mouse islets appears to be critical for maintaining glucose homeostasis. Fex et al. showed that euglycemia is maintained in mice made insulin resistant by high fat diet [111]. This is due to enhanced β-cell function accounted for, at least in part, by altered mitochondrial function; e.g., islets released insulin in response to glutamine, a fuel normally ineffective as secretagogue in the absence of leucine.

MKR mice, which express a dominant-negative IGF-1 receptor in skeletal muscle, are severely insulin-resistant and progressively develop hyperglycemia [112]. This becomes apparent as insulin secretion is attenuated, which is associated with reduced hyperpolarization of the inner mitochondrial membrane and impaired mitochondrial $Ca^{2+}$ uptake in islets [113]. Mitochondrial dysfunction subsequently progresses with swelling of mitochondria, exhibiting disrupted cristae, and a reduction in oxygen consumption and the ATP/ADP ratio. Proteomics analysis revealed that expression of 36 mitochondrial proteins, including respiratory proteins, is altered in islets from the MKR mice.

## 6. THE GENETICS OF MITOCHONDRIAL FUNCTION IN DIABETES

GWAS of T2D have, arguably, provided a major leap forward in the understanding of the etiology and pathogenesis of the disease [1,114]. The scale on which these analyses have been performed has permitted the establishment of robust statistical associations between variants of genes and metabolic traits in humans. More recently, novel technologies have also allowed other aspects of genomic regulation to be linked to T2D and associated traits, such as fasting plasma glucose, body mass index, glucose intolerance, insulin sensitivity, and many more. Regulation of this sort includes epigenetic alterations of the methylation and acetylation state of DNA, micro- and linker-RNAs, histone modifications, as well as the open/closed state of chromatin.

In spite of these advances, GWAS have also been criticized for the lack of mechanistic understanding of how gene variants actually contribute to disease. While there is considerable consensus that most gene variants identified to date seem to affect β-cell function [1], only a few examples have been presented, in which a robust mechanism has been established [115,116]. In fact, in some cases, even the identity of the gene involved may be unclear; although a single nucleotide polymorphism (SNP) is located in a "gene" — exon, intron, or regulatory region — it may still be so that another gene, distant from the locus, is the actual target of the SNP [117]. Furthermore, the 100 plus list of gene variants that have been convincingly associated with T2D and its traits only account for, at most, 20% of the heritability of T2D [1]. So far, it has not been possible to identify rare gene variants, which were hoped to have a much stronger impact on the risk of developing T2D [118]. Possibly, other genomic factors, such as epigenetics and regulatory RNAs, may make up for some of the "missing heritability" of T2D.

  

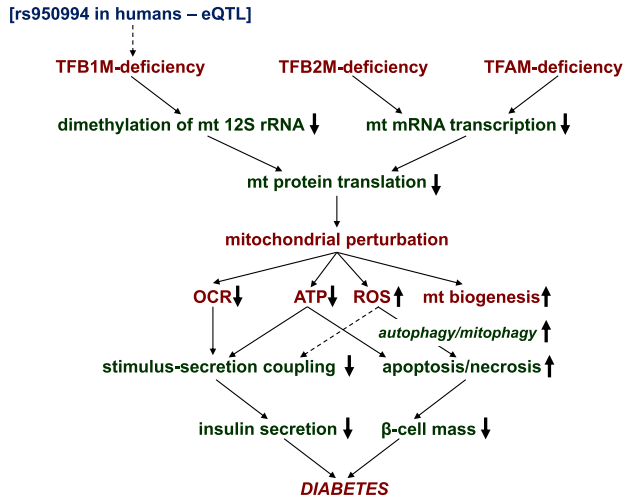


**Figure 2: Model for how deficient transcription and translation in mitochondria cause β-cell dysfunction and diabetes.** Deficiencies of Transcription factors A, B1, and B2 mitochondrial (TFAM; TFB1M; TFB2M) are thought to cause mitochondrial and β-cell dysfunction via largely similar pathways; italics and hatched lines indicate that functional links are not definitive but possible. eQTL — expression trait quantitative locus; mt — mitochondrial; OCR — oxygen consumption rate; ROS — reactive oxygen species; rRNA — ribosomal RNA.

### 6.1. Gene variants in mitochondrial DNA and Type 2 diabetes

Hypothetically, SNPs in mitochondrial DNA could be linked to T2D and metabolic traits just as SNPs in genomic DNA. Whereas several SNPs in the coding region of mitochondrial DNA, which cause mutations of genes encoding proteins, rRNA and tRNA, have been reported, SNPs which do not affect protein sequence and which associate with metabolic traits have yet to be described [119]. This suggests that the mitochondrial genome, which obviously differs from the nuclear genome in many ways, also is different in the way that genetic variants are inherited and penetrate.

### 6.2. Gene variants in nuclear DNA and Type 2 diabetes — the *TFB1M* locus

We mined the Diabetes Genetics Initiative (DGI) GWAS for SNPs associating with insulin secretion. The SNP with the strongest negative association with insulinogenic index, i.e., insulin secretion corrected for plasma glucose in an oral glucose tolerance test (OGTT), was rs950994, which is located in the 2nd intron of *TFB1M* [120]. The association is found in several cohorts but is sex-specific since it seems to be determined by the female study participants. There is also an association with 2-h glucose in an OGTT. Carriers of an rs950994 A-allele have a nominally increased risk of T2D in DGI; analysis of a prospective cohort revealed that the risk variant also predicts future T2D.

The A-allele of rs950994 in *TFB1M* confers lower expression of *TFB1M* mRNA levels in human islets, i.e., it is an expression quantitative trait locus (eQTL) [120]. This was recently confirmed and expanded upon, using RNA sequencing [121]: the number of A-alleles in rs950994 exerts a negative gene-dose effect on *TFB1M* mRNA in human islets. Moreover, the number of A-alleles, as well as *TFB1M* mRNA, correlates negatively and positively, respectively, with insulin secretion in human islets. Moreover, TFB1M protein levels, as well as those of ND1 and ND5, respiratory proteins encoded by mitochondrial DNA (Table 1), are reduced in islets from *TFB1M* risk variant carriers [120]. In contrast, levels of NDUFB8, QCR2, and ATPA, proteins encoded by the nuclear genome, are unchanged.

Taken together, these results support a pathogenetic effect of the *TFB1M* variant in individuals carrying an A-allele. These carriers have lower levels of the encoded protein in islets, which results in abrogated translation of mitochondrial proteins. Hence, the mitochondrial contribution to β-cell stimulus-secretion coupling is impaired, explaining the reduction in insulin secretion and the greater risk of T2D (Figure 2).

## 7. MANIPULATING TRANSCRIPTION AND TRANSLATION IN β-CELL MITOCHONDRIA

Mouse genetics has proven to be a powerful tool to elucidate mitochondrial function [122]. This can be attributed to the fact that mitochondrial function is rather well-conserved, and even more so in mammals. Whether mitochondrial diseases are as useful for elucidation with mouse genetics may need more investigation. Here, I will review experimental models where mitochondrial function has been specifically targeted (summarized in Table 2). There are a number of knock out mouse lines, in which metabolic and bioenergetical enzymes and proteins have been targeted and mitochondrial function impacted,

**Table 2** — Phenotype of mice with targeted disruptions of mitochondrial transcription and translation factors.

| Phenotype | β-*Tfam*$^{-/-}$ | *Tfb1m*$^{+/-}$ | β-*Tfb1m*$^{-/-}$ | β-*Tfb2m*$^{-/-}$ | β-*Tfb2m*$^{+/-}$ |
|---|---|---|---|---|---|
| mt DNA | - | - | - | ↓ | - |
| mt transcription | ↓ | - | - | ↓ | ↓ |
| mt translation | - | ↓ | ↓ | - | - |
| mt number | | - | ↑ | | |
| mt dysmorphology | - | - | ↑ | ↑ | - |
| MMP | ↓ | - | ↓ | ↓ | ↓ |
| OCR | - | - | ↓ | | - |
| ATP | | ↓ | ↓ | ↓ | ↓ |
| ROS | - | - | ↑ | ↑ | - |
| Autophagy | - | - | - | ↑ | - |
| Mitophagy | - | - | - | ↑ | - |
| Apoptosis | - | - | ↑ | ↑ | |
| Insulin secretion | ↓ | ↓ | ↓ | ↓ | ↓ |
| Insulin content | → | - | - | - | ↓ |
| β-cell mass | ↓ | - | ↓ | ↓ | - |
| Glucose intolerance | ↑ | ↑ | ↑ | - | ↑ |
| Diabetes | ↑ | - | ↑ | ↑ | - |

mt — mitochondria; MMP — mitochondrial membrane potential; OCR — oxygen consumption rate; ROS — reactive oxygen species.

 

but these are beyond the scope of this review, which is transcriptional and translational control of mitochondria (Table 2).

### 7.1. Targeting of *Tfam* in mouse β-cells

A β-cell-specific knock out of *Tfam* results in extensive depletion of mitochondrial DNA and impaired OXPHOS in islets [123]. Mitochondria display abnormal morphology. Insulin secretion from *Tfam*-deficient islets is impaired to due to reduced hyperpolarization of the inner mitochondrial membrane and a less vigorous rise in cytosolic calcium. Ultimately, β-cell mass is reduced and diabetes ensues due to a loss of insulin secretion.

### 7.2. Targeting of *Tfb1m* in mice

In view of the genetic data on the diabetogenic *TFB1M* variant, mouse genetics was used to create a disease model in vivo. As mentioned, *Tfb1m* null mice are embryonically lethal while heterozygous *Tfb1m* knock out mice ($Tfb1m^{+/-}$) have no overt phenotype [71]. However, a detailed metabolic analysis of these mice revealed that $Tfb1m^{+/-}$ mice become glucose intolerant due to reduced insulin secretion [120]. $Tfb1m^{+/-}$ islets release less insulin ex vivo in response to either glucose or mitochondrial secretagogues. Notably, knock out of one *Tfb1m* allele led to a 50% reduction in TFB1M protein in islets, as well as reduced ND5, suggesting that translation of mitochondrial encoded proteins is reduced. Consequently, ATP production is significantly lower in $Tfb1m^{+/-}$ islets [120].

The phenotype of a general knock out reflects that the gene and protein are lost from all cells where they are expressed. Therefore, we created a murine β-cell-specific knock out of *Tfb1m* (β-$Tfb1m^{-/-}$) [121]. The phenotype of these mice largely reflects that of $Tfb1m^{+/-}$ mice but is more pronounced. β-$Tfb1m^{-/-}$ remain normoglycemic until 3 months, when plasma glucose levels rise; the mice are severely diabetic at 5 months of age. Methylation of 12S rRNA in mitochondria is reduced in β-$Tfb1m^{-/-}$ islets, which also exhibit lower protein levels of ND1, NDUFB8, and COX1. Consequently, mitochondrial function, reflected by inner mitochondrial membrane hyperpolarization, oxygen consumption rate, and ATP production, is perturbed, as is insulin secretion from β-$Tfb1m^{-/-}$ islets ex vivo. Interestingly, it seems like β-$Tfb1m^{-/-}$ β-cells compensate for perturbed function by increasing mitochondrial mass. However, the β-$Tfb1m^{-/-}$ mitochondria appear swollen with disrupted cristae. Moreover, β-$Tfb1m^{-/-}$ mice exhibit loss of β-cell mass, likely as a consequence of increased oxidative stress.

### 7.3. Targeting of *Tfb1m* in insulin-producing cells

To gain further mechanistic detail, we used the insulin-producing cell line INS-1 832/13 [124], in which *TFB1M* mRNA and protein levels were reduced by RNA interference [120]. This had a profound negative effect on levels of mitochondrial encoded proteins, as well as respiratory complex activities. In addition, oxygen consumption rate, ATP production and, ultimately, insulin secretion in response to glucose and mitochondrial secretagogues become perturbed. Thus, the in vitro model of *TFB1M*-deficiency faithfully mimics that of the analogous situation in the human and mouse.

### 7.4. Targeting of *Tfb2m* in mice

After having established the essential role of TFB1M in control of mitochondrial protein translation and mitochondrial function, we examined whether deficiency of mitochondrial transcription in mouse β-cells would impact mitochondrial function and insulin secretion similar to *TFB1M*-deficiency. To this end, a murine β-cell specific knock out of *Tfb2m* was created [125].

β-$Tfb2m^{-/-}$ mice become hyperglycemic promptly after weaning due to loss of insulin secretion [125]. Islets from β-$Tfb2m^{-/-}$ mice pre-weaning exhibit perturbed insulin secretion in response to glucose and mitochondrial secretagogues but not KCl, with the perturbation remaining after weaning (7 weeks of age). The inner mitochondrial membrane potential is less polarized in response to glucose in β-$Tfb2m^{-/-}$ islets. As expected, perturbed transcription in mitochondria reduces levels of most mitochondrial encoded mRNAs and proteins, as well as mitochondrial DNA. Close to 80% of mitochondria exhibit morphological alterations and signs of increased mitophagy, and there is also a marked negative effect on β-cell mass.

Since the β-$Tfb2m^{-/-}$ mice exhibit such a profound phenotype, limiting certain functional analyses, we also examined mice where one *Tfb2m* allele was inactivated in β-cells [125]. Similar to $Tfb1m^{+/-}$ mice, β-$Tfb2m^{+/-}$ mice exhibit a loss of approximately 50% of the targeted transcript. Consequently, expression of some mitochondrial-encoded genes, such as *Atp8* and *Nd5/6*, decreases in β-$Tfb2m^{+/-}$ islets, while expression of *Co1*, *Cyb*, and *Nd1* remains unchanged. Accordingly, the phenotype of β-$Tfb2m^{+/-}$ mice was less pronounced and delayed compared with that of β-$Tfb2m^{-/-}$ mice. At 7 months of age, β-$Tfb2m^{+/-}$ mice show glucose intolerance during an OGTT due to less release of insulin; plasma glucose levels in the fed state are slightly elevated while insulin levels are reduced. Ex vivo, β-$Tfb2m^{+/-}$ islets secrete less insulin in response to glucose and mitochondrial secretagogues, and the inner mitochondrial membrane potential is less polarized in response to glucose.

### 7.5. Targeting of *Tfb2m* in insulin-producing cells

Silencing of *TFB2M* in INS-1 832/13 cells results in a pronounced reduction in mitochondrial encoded mRNA, proteins and mitochondrial DNA [125]. Citrate synthase activity, a marker of mitochondrial number, is reduced. Functionally, these alterations had a profound effect: oxygen consumption rate, ATP content, and insulin secretion, in response to glucose and mitochondrial secretagogues, become impaired. Detailed analysis of the *TFB2M*-deficient 832/13 cells revealed that mitophagy and autophagic flux are impaired. This is likely driven by increased oxidative stress, as evidenced by increased levels of ROS, all of which underlie increased activation of apoptosis.

### 7.6. Key pathogenetic mechanisms in β-cells subject to impaired mitochondrial transcription and translation

Together, the data from the experimental models in which mitochondrial transcription and translation have been perturbed show that β-cell dysfunction evolves due to a combined insult of deficient function and control of cell number (Figure 2). Loss of mitochondrial proteins, either due to disruption of transcription or translation in mitochondria, perturbs β-cell stimulus-secretion coupling. This includes effects on electron transport and OXPHOS but there is also likely to be an impaired defense to oxidative stress. Consequently, energy deprivation and increased levels of ROS increase mitophagy, disrupt autophagy, and, ultimately, programmed cell death ensues, leading to a loss of β-cell mass. All these features are present, to varying extents, in the experimental models reviewed here and are likely to contribute to β-cell dysfunction and loss (Figure 2). Mitophagy is an autophagic process, wherein mitochondria are specifically targeted, a phenomenon also observed in insulin-producing cells [126]. Autophagy has been reported in islets in T2D [127]. Mitophagy is controlled, at least in part by PDX1 in mouse β-cells [128]. Whether any of the mitochondrial transcription factors were involved, being downstream of PDX1, is likely but not proven [128].

  

## 8. CONCLUSIONS AND FUTURE DIRECTIONS

It is undisputed that fuel-stimulated insulin secretion, regardless of whether it is triggered or amplified, depends on mitochondrial metabolism [4,129] (Figure 1). This has been further supported by the fact that mutations in mitochondrial DNA, impacting on mitochondrial function, and, hence, β-cell stimulus-secretion coupling, cause a rare form of diabetes, i.e., mitochondrial diabetes [102]. This notwithstanding, it has, until recently [120], been unclear whether mitochondrial dysfunction is part of the pathogenesis of common T2D.

Here, I have reviewed work demonstrating that mitochondrial dysfunction in islets is part of the pathogenetic processes in human T2D [106], as well as in experimental models of the disease [120,123,125] (Figure 2). Moreover, I described recent studies revealing that a common genetic variant in *TFB1M*, a gene encoding a crucial protein controlling mitochondrial protein synthesis, is associated with T2D [120], as well as mechanistic studies elucidating the mechanisms of this link [121].

Future work should consider two main things. **First**, more genes involved in mitochondrial function, and mechanistic processes by which they cause disease, must be identified. Clearly, most of the "low-hanging fruit" have been harvested already by GWAS. Few of the identified genes appear to play a clear role in mitochondrial function. Having stated this, it must be emphasized that although a host of SNPs associated with T2D have been identified, it is still not resolved how many of them actually cause disease [115]. It is not impossible that several of them may actually be essential for mitochondrial function. Furthermore, it is also possible that nuclear genes involved in control of mitochondria may be targets of micro- and linker-RNAs, or histone modifications, research which is still in its infancy.

**Second**, it is not trivial to target mitochondrial function therapeutically in a fashion that delivers desired effects. How shall we target mitochondria in β-cells but leave those in other tissues unaffected? To exemplify, uncoupling is an attractive strategy to help the body to burn excess energy instead of accumulating it as fat. In β-cells, uncoupling will likely be detrimental, short-circuiting β-cell stimulus-secretion coupling, and thereby abrogating insulin secretion. Cell- and tissue-targeting may be the solution here but robust modalities for this remain to be established.

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## CONFLICT OF INTEREST

None declared.

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