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The aetiology of type 2, or non-insulin-dependent, diabetes mellitus has been characterized in only a limited number of cases. Among these, mitochondrial diabetes, a rare subform of the disease, is the consequence of pancreatic β-cell dysfunction caused by mutations in mitochondrial DNA, which is distinct from the nuclear genome. The impact of such mutations on β-cell function reflects the importance of mitochondria in the control of insulin secretion. The β-cell mitochondria serve as fuel sensors, generating factors that couple nutrient metabolism to the exocytosis of insulin-containing vesicles. The latter process requires an increase in cytosolic Ca^{2+}, which depends on ATP synthesized by the mitochondria. This organelle also generates other factors, of which glutamate has been proposed as a potential intracellular messenger.

Mitochondria are present in most eukaryotic cells, varying in number from hundreds to thousands[1], and have also been visualized as a continuous network[2]. Their origin is generally thought to lie in the endosymbiotic association of oxidative bacteria and glycolytic proto-eukaryotic cells[3]. The phenotype resulting from the absence of mitochondria is illustrated by mammalian peripheral red blood cells, which depend entirely on glycolysis for their energy supply. The endosymbiotic hypothesis of mitochondrial origin is supported, for example, by the double membrane surrounding the organelle and a unique genome in the form of circular mitochondrial DNA (mtDNA) with bacterial characteristics[3]. Human mtDNA comprises only 37 genes (16,569 base pairs (bp)), the most notable of which are those encoding 13 polypeptides (Fig. 1) that are part of the multisubunit enzyme complexes of the respiratory chain[1].

The mtDNA is transcribed and translated within the mitochondrion. The nuclear genome specifies the remainder (the majority) of the enzyme subunits and the other

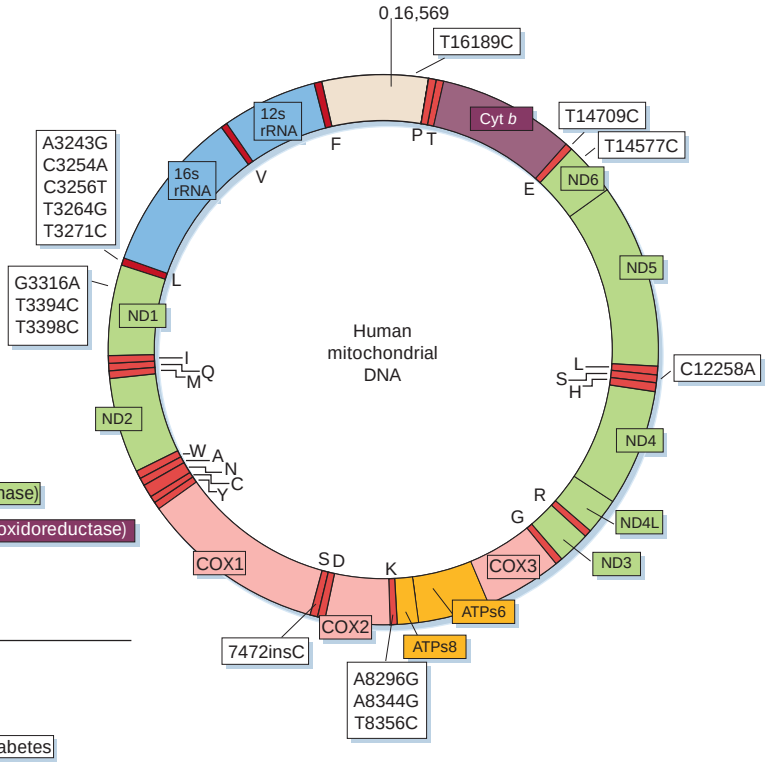

Figure 1 A map of human mitochondrial DNA indicating diabetes-associated mutations. The mitochondrial genome encodes 37 genes (16,569 bp): 13 polypeptides, 22 tRNAs and 2 ribosomal RNAs. The polypeptides are constituents of the respiratory-chain complexes: 7 complex I subunits (NADH dehydrogenase), 1 subunit of complex III (ubiquinol: cytochrome *c* oxidoreductase), 3 subunits of complex IV (cytochrome *c* oxidase) and 2 subunits of complex V (ATP synthase). The genes for tRNAs are presented as one-letter symbols. Mutations in four of these tRNA genes are associated with diabetes: those for leucine (L), serine (S), lysine (K) and glutamic acid (E) tRNAs.

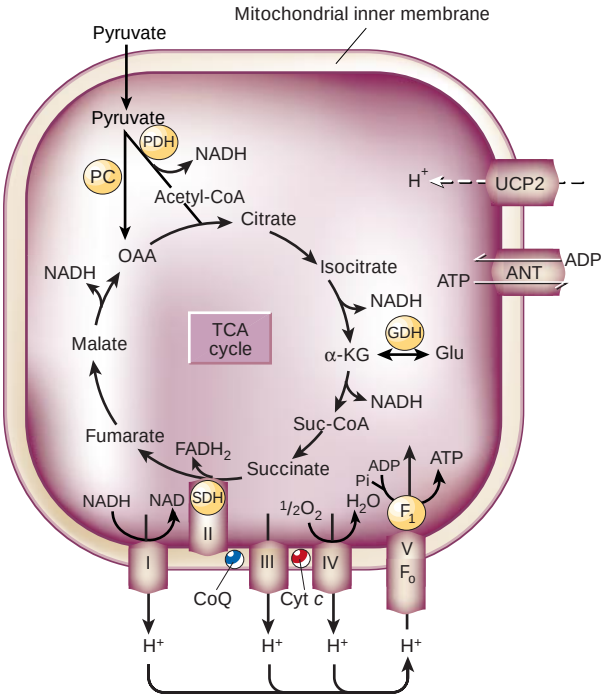

Figure 2 The TCA cycle and respiratory chain in a mitochondrion. Substrate oxidation in the TCA cycle activates the respiratory chain, leading to the generation of ATP, which is subsequently translocated to the cytosol. ANT, adenine nucleotide translocator; GDH, glutamate dehydrogenase; Glu, glutamate; KG, ketoglutarate; OAA, oxaloacetate; PC, pyruvate carboxylase; PDH, pyruvate dehydrogenase; Suc-CoA, succinyl-CoA; SDH, succinate dehydrogenase; TCA, tricarboxylic acid; UCP2, uncoupling protein 2.

mitochondrial proteins; these are synthesized in the cytosol and imported into the mitochondrion[4]. The nucleus also controls the transcriptional activity of mtDNA through regulatory proteins such as mitochondrial transcription factor A (TFAM), which are encoded by nuclear genes[5]. The mtDNA is maternally inherited because of the non-persistence of sperm mtDNA in the zygote after fertilization. The mitochondrial genome exists in multiple copies in every cell. It is highly susceptible to mutation as, in contrast to nuclear DNA, mtDNA consists only of coding sequences and its repair mechanisms are poor. Consequently, it is particularly sensitive to oxidative stress. Moreover, it is juxtaposed to the respiratory chain, which generates mutagenic oxygen derivatives[6].

The mitochondria are the main source of energy, essentially ATP, which is required for such vital cellular functions as the maintenance of transmembrane ion gradients, protein synthesis and vesicular transport. Additionally, in the pancreatic β-cell, ATP and other mitochondrial factors accomplish the coupling of glucose metabolism to insulin secretion. The mitochondria can be activated by the three classes of fuel: amino acids, fatty acids and carbohydrates, the latter being of most relevance in β-cells under physiological conditions. The principal mitochondrial substrate is pyruvate, formed essentially by glycolysis. Pyruvate carbons enter the tricarboxylic acid cycle (TCA cycle) in the mitochondrial matrix, in which substrates are oxidized with the formation of CO_2 and the reduction of NAD^+ and FADH to NADH and $FADH_2$, respectively. These provide electrons to the respiratory chain upon their reoxidation (Fig. 2).

The electron flow along the respiratory chain drives the extrusion of protons from the mitochondrial matrix, which establishes a steep electrochemical gradient across the inner mitochondrial membrane. The mitochondrial membrane potential is created by complexes I, III and IV of the respiratory chain and is negative inside. ATP synthase (complex V) in the mitochondrial membrane catalyses the condensation of ADP with inorganic phosphate to yield ATP. The generation of this 'high-energy bond' is powered by the diffusion of protons back into the matrix through ATP synthase. Finally, ATP is transferred to the cytosol in exchange for ADP by the adenine nucleotide translocator (ANT). Electrons can enter the respiratory chain at both complexes I (NADH) and II ($FADH_2$). The latter complex, succinate dehydrogenase, is also an integral part of the TCA cycle. The entire bioenergetic process is regulated not only by substrate flux but also by Ca^{2+}, which increases the activity of several mitochondrial dehydrogenases. An increase in free cytosolic Ca^{2+} that occurs at cell activation is relayed to the mitochondrial matrix by way of a Ca^{2+} uniporter, thus ensuring that the energy requirements of the cell are covered[7,8]. Ca^{2+} entry is favoured by activation of the respiratory chain, for instance by glucose in the β-cell.

Stimulus–secretion coupling of insulin release

Blood glucose level is tightly controlled by insulin secretion from pancreatic β-cells and insulin action on liver, muscle and other target tissues. The β-cell is poised to adapt insulin secretion to the fluctuations in blood glucose concentration (Fig. 3). Glucose equilibrates across the plasma membrane and its phosphorylation by glucokinase to glucose-6-phosphate determines the rate of glycolysis and the rate of pyruvate generation[9,10]. Thus, when blood glucose is high, the rate of glycolysis in the β-cell will increase. In the β-cell, pyruvate is the main product of glycolysis, as little lactate is produced[11]. The supply of cytosolic NAD^+, necessary to maintain high rates of glycolysis, is therefore ensured by mitochondrial shuttles[9]. Compared to other cell types, an unusually high proportion of glucose-derived carbon enters the mitochondria in the form of pyruvate and enters the TCA cycle.

Subsequent oxidative metabolism provides the link between the glucose stimulus and insulin secretion[12,13]. In the mitochondria, pyruvate is a substrate for both pyruvate dehydrogenase and pyruvate carboxylase, thereby ensuring anaplerosis (provision of carbons) to the TCA cycle. Electron transfer from the TCA cycle to the respiratory chain by NADH and $FADH_2$ promotes the generation of ATP, which is exported to the cytosol. The increase in the ATP:ADP ratio in the cytosol causes depolarization of the plasma membrane by the closure of ATP-sensitive K^+ channels (K_{ATP})[14]. This allows the opening of voltage-sensitive Ca^{2+} channels[14,15], similar to those expressed in other excitable cells. This is the key step by which glucose stimulates insulin secretion, as the increase in cytosolic Ca^{2+} is the main trigger for exocytosis, the process by which the insulin-containing secretory granules fuse with the plasma membrane[15,16].

The importance of membrane-potential control is illustrated by the syndrome of persistent hyperinsulinaemic hypoglycaemia of infancy (PHHI). It is most frequently caused by mutations in one of the two subunits (the sulphonylurea receptor and KIR6.2) of the K_{ATP} channel, resulting in uncontrolled Ca^{2+}-mediated hypersecretion of insulin[17]. However, PHHI patients often retain some glucose-stimulated insulin secretion above the constitutively increased basal rate[18]. This supports *in vitro* observations that suggest the existence of a K_{ATP} channel-independent effect of glucose[19]. Glucose is thus capable of eliciting a partial secretory response under conditions of clamped, elevated cytosolic Ca^{2+} concentration without affecting the plasma membrane potential. It can be concluded that ATP generated in the mitochondria is the main coupling messenger in insulin secretion, but that other metabolic factors are necessary for the full development of the secretory response.

Signals and messengers for insulin exocytosis

Ultrastructural examination of the β-cell has suggested that the mitochondria are often in close proximity to the secretory insulin

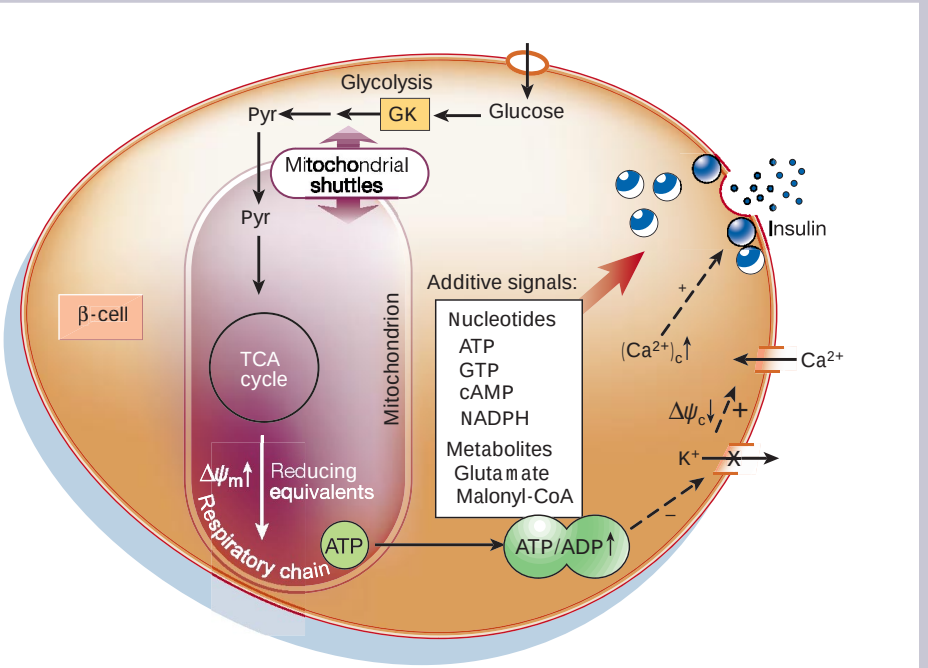

Figure 3 Model for coupling of glucose metabolism to insulin secretion in the β-cell. Glucose is phosphorylated by glucokinase (GK) and converted to pyruvate (Pyr) by glycolysis. Pyruvate preferentially enters the mitochondria and fuels the TCA cycle, resulting in the transfer of reducing equivalents to the respiratory chain, leading to hyperpolarization of the mitochondrial membrane ($\Delta\Psi_m\uparrow$) and generation of ATP. Subsequently, closure of K_{ATP}-channels depolarizes the cell membrane ($\Delta\Psi_c\downarrow$). This opens voltage-gated Ca^{2+} channels, raising the cytosolic Ca^{2+} concentration ($[Ca^{2+}]_c$), which triggers insulin exocytosis. Several putative messengers, or additive signals, proposed to participate in the metabolism–secretion coupling are indicated.

granules (Fig. 4). This may facilitate metabolism–secretion coupling, as ATP is a major permissive factor for movement of insulin granules and for priming of exocytosis[16,20]. This action of ATP is distinct and complementary to its action on the K_{ATP} channel. Thus, channel activity and exocytosis are both determined by the cytosolic ATP:ADP ratio. Glucose also generates GTP, which could trigger insulin exocytosis through GTPases[21,22]. GTP is formed in mitochondria by the TCA cycle, but is trapped in the organelle. In the cytosol, GTP is formed mainly through the action of nucleoside diphosphate kinase. In contrast to ATP, GTP is capable of initiating insulin exocytosis in a Ca^{2+}-independent fashion, which qualifies it as a messenger molecule[22–24]. It is not known whether GTP acts by way of a monomeric or heterotrimeric G protein that directly controls exocytosis[16,25].

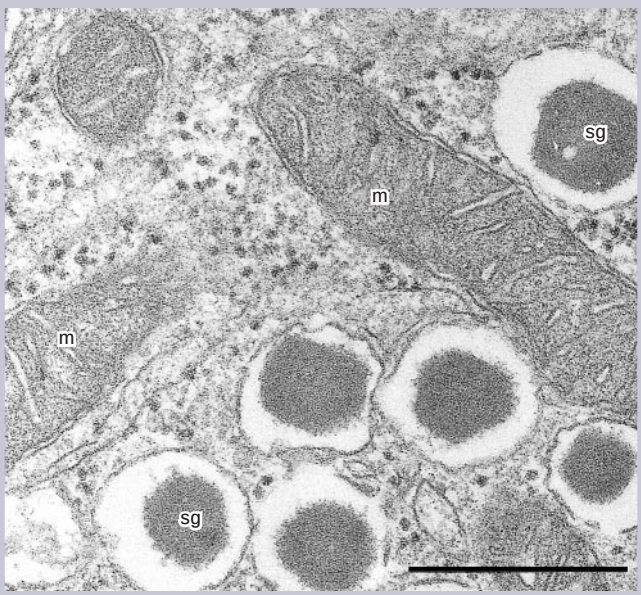

Figure 4 Electron micrograph of part of a rat β-cell showing mitochondria (m) and insulin-containing secretory granules (sg). The scale bar represents 0.5 μm. Reprinted with permission from ref. 13.

Cyclic AMP (cAMP) — the universal second messenger — is generated at the plasma membrane from ATP and potentiates glucose-stimulated insulin secretion. Many neurotransmitters and hormones, including glucagon and the intestinal hormones glucagon-like peptide 1 (GLP-1) and gastric inhibitory polypeptide (GIP), increase cAMP levels in the β-cell by activating adenylyl cyclase[26]. Although glucose itself is inefficient in stimulating the production of cAMP, permissive levels of cAMP are necessary for normal responsiveness of secretion[27]. The most important target of cAMP is the exocytotic machinery, where the messenger acts in both a protein kinase A-dependent and independent manner[22,23,28–30]. Among other putative nucleotide messengers, NADH and NADPH are generated by glucose metabolism[9]. The rise in pyridine nucleotides precedes the increase in cytosolic Ca^{2+} (ref. 31) and their levels rise more rapidly in the cytosol than in the mitochondria[32]. It remains to be determined whether pyridine nucleotides participate in β-cell activation through effects on ion fluxes or on the process of exocytosis.

In glucose-stimulated β-cells, the TCA cycle intermediate citrate is exported from mitochondria. In the cytosol, citrate carbons are transferred to coenzyme A (CoA) to form malonyl-CoA, which is a lipid precursor. Malonyl-CoA inhibits transport of fatty acids into the mitochondria and their subsequent oxidation, thereby favouring the synthesis of long-chain acyl-CoAs in the cytosol. This metabolic switch led to the proposal that malonyl-CoA acts as a metabolic coupling factor in insulin secretion[33]. The long-chain acyl-CoA hypothesis was substantiated by the observation that palmitoyl-CoA enhances Ca^{2+}-evoked insulin exocytosis[34]. Disruption of malonyl-CoA accumulation during glucose stimulation did not, however, attenuate the secretory response[35]. Therefore, the role of long-chain acyl-CoA derivatives in metabolism–secretion coupling remains controversial.

Studies in a permeabilized β-cell model have shown a direct link between mitochondrial activation and insulin exocytosis[36]. Under conditions of permissive Ca^{2+} concentrations, stimulation of mitochondrial metabolism[37] indicated the generation of a coupling factor[36]. The factor was subsequently identified as glutamate[38], which can be produced from the TCA cycle intermediate α-ketoglutarate or

by transamination reactions[39]. In non-permeabilized cell preparations, a membrane-permeant derivative of glutamate was also shown to enhance the action of glucose in insulin secretion[38,40]. Further studies demonstrated a positive correlation between cellular glutamate concentrations and the secretory response to glucose[41]. At present, the mechanism of glutamate action on exocytosis is unknown. In this scenario the classical neurotransmitter glutamate is allocated a new role, that of an intracellular messenger or cofactor in insulin secretion[42].

Mitochondrial dysfunction in the β-cell

It was established several decades ago that blockade of the respiratory chain inhibits glucose-stimulated insulin secretion. This conclusion was based on the use of various mitochondrial poisons and lowering of the oxygen supply to the β-cell[43]. More recently, the activity of the respiratory chain was impaired by suppressing the production of those enzyme subunits encoded by mtDNA, which creates so-called rho^0 cells[44]. In this way, ethidium bromide and other chemical treatment of β-cell lines resulted in depletion of mtDNA with preservation of insulin biosynthesis and cell viability, albeit with a reduced proliferation rate[45–48]. In such cells, glucose-induced insulin release is absent, as a result of defective respiratory-chain activation and the loss of mitochondrial ATP production[45–47]. The defect is of mitochondrial origin, as specific mitochondrial substrates were equally inefficient, whereas the secretory response to Ca^{2+}-raising agents was still present[46–48]. Elegant experiments showed that the secretory response to glucose could be restored by replenishing rho^0 β-cells with normal mitochondria[45].

Expression of mtDNA is controlled by a nucleus-encoded transcription factor, TFAM, and disruption of this gene in the mouse is lethal[5]. The phenotype of the heterozygous knockout mouse revealed that the heart is highly sensitive to respiratory-chain deficiency[5]. The β-cell-specific deletion of the *Tfam* gene caused a diabetic phenotype[49]. The islets of these mice showed attenuated respiratory-chain activation and diminished secretory response to glucose. These transgenic animals represent the first model of human mitochondrial diabetes, which, it should be noted, has been linked mostly to mutations in tRNA genes (Fig. 1). Taken together, the *in vitro* and *in vivo* studies highlight the pivotal role of mitochondria in stimulus–secretion coupling in the β-cell.

mtDNA mutations and diabetes

Point mutations or deletions in mtDNA have been associated with a large spectrum of diseases, with symptoms such as muscle weakness, cardiomyopathy, optic nerve atrophy, retinal dystrophy, impaired hearing and hyperglycaemia (diabetes mellitus). Point mutations in the mitochondrial tRNA genes are the primary cause of these pathological manifestations (Fig. 1). A specific, maternally inherited form of diabetes mellitus was first linked to mutations in the mtDNA in 1992 (refs 50, 51). Often associated with neurosensory deafness, it is also called maternally inherited mitochondrial diabetes and deafness (MIDD). The most frequent mutation encountered is the A3,243G mutation in the gene for $tRNA^{Leu}$ (bearing the anticodon UUR)[51,52]. Altogether, mitochondrial diabetes accounts for approximately 1% of all cases of diabetes[53]. The mutation reduces the stability of mtDNA-encoded proteins and the binding of leucine to its tRNA, resulting in decreased synthesis of certain mitochondrial proteins[53]. The same mutation causes the MELAS syndrome (mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes)[54], which may be associated with diabetes[53].

How is it possible that the same A3,243G mutation in mtDNA can give rise to two different phenotypes affecting different tissues? On the one hand, this is due to tissue-specific segregation of the mutant mtDNA copies. In the course of cell division, an increasing proportion of mutant mtDNA substituting for wild-type copies is referred to as heteroplasmy, a process aggravated in post-mitotic cells. On the other hand, the decreased mitochondrial function will impact differently on cell viability according to tissue sensitivity to apoptosis. The A3,243G mutation has been associated with reduction of islet mass involving both β-cells and the neighbouring glucagon-producing α-cells[55,56]. In post-mortem studies, diabetic patients with A3,243G mutation showed a degree of heteroplasmy ranging from 32% to 63% (refs 55, 56). It is possible that the extent of heteroplasmy may participate in the lowering of the cellular energy capacity below the bioenergetic threshold[1]. The consequence of this would be that the organelle could no longer fulfil the energetic and signalling requirements for glucose-stimulated insulin secretion, a concept also supported by clinical observations[57]. In contrast, secretion induced by the receptor agonist glucagon, acting by way of cAMP effects on exocytosis, was preserved in A3,243G patients[57]. This form of diabetes may or may not require insulin injections, and is usually not associated with resistance of muscle and other tissues to the action of the hormone[52,53,57].

The molecular diagnosis of mitochondrial diabetes is complicated by an invariably low degree of heteroplasmy in the peripheral white blood cells usually used for genetic analysis. *Ex vivo* mitochondrial dysfunction associated with the A3,243G mutation was demonstrated in skin fibroblasts[58] or in cells enriched for patient mutant mitochondria[59]. A similar conclusion was drawn from a study on another mtDNA mutation associated with a neurodegenerative disease[60]. The mitochondrial diabetes phenotype illustrates the importance of normal respiratory-chain function in the β-cell for glucose homeostasis.

Other pathophysiological considerations

In contrast to the rare monogenic mitochondrial diabetes, type 2 or non-insulin-dependent diabetes mellitus is common and polygenic in nature[61]. Patients usually display both resistance to insulin at its target tissues (mainly skeletal muscle) as well as defective insulin secretion[62]. Although the contribution of variations in mtDNA to the development of type 2 diabetes is unknown, a 50% decrease in mtDNA copy number in skeletal muscle of type 2 diabetics was observed[63]. A reduced mtDNA content was also reported in peripheral blood cells in such patients even before the onset of the disease[64]. The corresponding information for β-cells is lacking.

The diabetic state is generally characterized by accelerated tissue ageing, perhaps related to mitochondrial dysfunction. Accumulation of point mutations in mtDNA has been reported to occur in an age-dependent manner in humans[65]. There is an age-related increase in the production of reactive oxygen species (ROS), while concurrently the defence mechanisms against these free radicals are diminishing[6]. The mitochondria are the principal source of ROS resulting from imperfect electron transport. Normally, only 0.1% of total oxygen consumption leaks to ROS generation, but the percentage becomes greater in the ageing tissue[6]. This deleterious process is amplified by the diminishing natural enzymatic defences (for example, catalase and superoxide dismutase). The low expression of these protective enzymes[66] makes the β-cell particularly susceptible to ROS action[67]. In addition to their acute effects, ROS may also lead to increased mutation in mtDNA, exacerbated by the limited repair capacity. These considerations have prompted the suggestion that ROS may participate in the impairment of glucose-induced insulin secretion seen both in ageing and in type 2 diabetes[68].

Further clues on β-cell function come from other forms of monogenic diabetes. Different subclasses of maturity-onset diabetes of the young (MODY) represent such monogenic forms of diabetes with autosomal dominant transmission. They are characterized by β-cell dysfunction as a result of mutations in nuclear genes[69]. MODY1 and MODY3 have been linked to mutations in the transcription factors hepatocyte nuclear factor HNF-4α and HNF-1α, respectively[69]. MODY3 is the most common form of this inherited disease and explains 2–5% of diabetic cases. Suppression of the *HNF-1α* gene in mice results in diabetes and impairment of glucose-induced insulin secretion assessed *in vitro*[70]. In cellular model systems, the molecular

basis of the defect has been attributed to deranged mitochondrial metabolism[71,72]. In particular, the defective respiratory-chain activation correlated with downregulation of the TCA cycle enzyme α-ketoglutarate dehydrogenase, accompanied by an upregulation of uncoupling protein 2 (UCP2)[72].

UCP2 is an inner mitochondrial membrane protein that tends to diminish the proton gradient generated by the respiratory chain. Its overexpression in β-cells attenuates ATP generation and insulin secretion in response to glucose[73]. It is of interest that deletion of the *UCP2* gene in mice enhances islet ATP generation and insulin secretion during glucose stimulation[74]. In obese, diabetic hyperlipidaemic rodent models, UCP2 levels in islets were reported to be either lower[75] or higher[74] than lean controls. Thus, there is no simple relationship *in vivo* between circulating lipids and UCP2 function. Type 2 diabetics usually have both hyperglycaemia and hyperlipidaemia. This is thought to induce the phenomenon of 'glucolipotoxicity' in the β-cell, leading to lipid accumulation, impaired glucose metabolism and alterations in mitochondria[76,77]. Chronic exposure of β-cells to fatty acids induces changes in the expression of numerous genes[76]. Among them, *UCP2* is induced, which correlates with reduced glucose-evoked insulin secretion[78]. This may be part of an adaptive mechanism protecting the β-cell against oxidants, as indicated by *in vitro* experiments[79]. Elucidation of the adaptation of the mitochondrial machinery is complicated by the multiple influences on the β-cell in the course of the development of the diabetic state.

Possible therapeutic interventions and perspectives

Patients with mitochondrial diabetes or with one of the MODY subforms are treated like any other case of type 2 diabetes. Treatment begins with diet, but usually needs to be supplemented with oral hypoglycaemic agents, in particular the sulphonylureas. Eventually, blood glucose control may require insulin injections.

Mitochondrially targeted therapy of the insulin secretory defect in a rat model of type 2 diabetes has been proposed. Dimethylsuccinate, a precursor of the TCA cycle intermediate succinate, was found to improve the secretory response[80]. Diabetic patients with a mitochondrial DNA mutation have been given long-term treatment with coenzyme Q10, a component of the respiratory chain[81]. This resulted in improved insulin secretion but, disappointingly, did not affect diabetic complications (nephropathy, retinopathy and neuropathy). More efficient treatments should be envisaged for such patients. The ultimate goal is the replacement of mutated DNA with normal mtDNA by either gene or cell therapy. Despite much research, these techniques are not yet available. It was, however, shown recently that complementation of normal mtDNA in mice carrying mutated mtDNA restores mitochondrial function[82]. This may open new perspectives for gene therapy of mitochondrial diseases.

In view of the requirement for optimally functioning mitochondria, measures directed to protecting these organelles should be envisaged in diabetes prevention. Even after the disease is manifest, such therapy could preserve partial β-cell sensitivity to glucose. Further definition of the molecular mechanisms underlying the role of mitochondria in cell activation will help to target interventions in diabetes and other metabolic diseases. □

1. Wallace, D. C. Mitochondrial diseases in man and mouse. *Science* **283,** 1482–1488 (1999).
2. Rizzuto, R. *et al.* Close contacts with the endoplasmic reticulum as determinants of mitochondrial Ca^{2+} responses. *Science* **280,** 1763–1766 (1998).
3. Gray, M. W., Burger, G. & Lang, B. F. Mitochondrial evolution. *Science* **283,** 1476–1481 (1999).
4. Neupert, W. Protein import into mitochondria. *Annu. Rev. Biochem.* **66,** 863–917 (1997).
5. Larsson, N. G. *et al.* Mitochondrial transcription factor A is necessary for mtDNA maintenance and embryogenesis in mice. *Nature Genet.* **18,** 231–236 (1998).
6. Beckman, K. B. & Ames, B. N. The free radical theory of aging matures. *Physiol. Rev.* **78,** 547–581 (1998).
7. McCormack, J. G., Halestrap, A. P. & Denton, R. M. Role of calcium ions in regulation of mammalian intramitochondrial metabolism. *Physiol. Rev.* **70,** 391–425 (1990).
8. Duchen, M. R. Contributions of mitochondria to animal physiology: from homeostatic sensor to calcium signalling and cell death. *J. Physiol.* **516,** 1–17 (1999).
9. Newgard, C. B. & McGarry, J. D. Metabolic coupling factors in pancreatic beta-cell signal transduction. *Annu. Rev. Biochem.* **64,** 689–719 (1995).
10. Matschinsky, F. M. A lesson in metabolic regulation inspired by the glucokinase glucose sensor paradigm. *Diabetes* **45,** 223–241 (1996).
11. Ishihara, H., Wang, H., Drewes, L. R. & Wollheim, C. B. Overexpression of monocarboxylate transporter and lactate dehydrogenase alters insulin secretory responses to pyruvate and lactate in β cells. *J. Clin. Invest.* **104,** 1621–1629 (1999).
12. Schuit, F. *et al.* Metabolic fate of glucose in purified islet cells. Glucose-regulated anaplerosis in beta cells. *J. Biol. Chem.* **272,** 18572–18579 (1997).
13. Wollheim, C. B. Beta-cell mitochondria in the regulation of insulin secretion: a new culprit in Type II diabetes. *Diabetologia* **43,** 265–277 (2000).
14. Ashcroft, F. M. *et al.* Stimulus-secretion coupling in pancreatic beta cells. *J. Cell Biochem.* **55,** 54–65 (1994).
15. Rorsman, P. The pancreatic beta-cell as a fuel sensor: an electrophysiologist's viewpoint. *Diabetologia* **40,** 487–495 (1997).
16. Lang, J. Molecular mechanisms and regulation of insulin exocytosis as a paradigm of endocrine secretion. *Eur. J. Endocrinol.* **259,** 3–17 (1999).
17. Dunne, M. J. *et al.* Familial persistent hyperinsulinemic hypoglycemia of infancy and mutations in the sulfonylurea receptor. *N. Engl. J. Med.* **336,** 703–706 (1997).
18. Grimberg, A. *et al.* Dysregulation of insulin secretion in children with congenital hyperinsulinism due to sulfonylurea receptor mutations. *Diabetes* **50,** 322–328 (2001).
19. Henquin, J. C. Triggering and amplifying pathways of regulation of insulin secretion by glucose. *Diabetes* **49,** 1751–1760 (2000).
20. Eliasson, L., Renstrom, E., Ding, W. G., Proks, P. & Rorsman, P. Rapid ATP-dependent priming of secretory granules precedes Ca^{2+}-induced exocytosis in mouse pancreatic B-cells. *J. Physiol.* **503,** 399–412 (1997).
21. Detimary, P., Van den Berghe, G. & Henquin, J. C. Concentration dependence and time course of the effects of glucose on adenine and guanine nucleotides in mouse pancreatic islets. *J. Biol. Chem.* **271,** 20559–20565 (1996).
22. Wollheim, C. B., Ullrich, S., Meda, P. & Vallar, L. Regulation of exocytosis in electrically permeabilized insulin-secreting cells. Evidence for Ca^{2+} dependent and independent secretion. *Biosci. Rept.* **7,** 443–454 (1987).
23. Vallar, L., Biden, T. J. & Wollheim, C. B. Guanine nucleotides induce Ca^{2+}-independent insulin secretion from permeabilized RINm5F cells. *J. Biol. Chem.* **262,** 5049–5056 (1987).
24. Proks, P., Eliasson, L., Ammala, C., Rorsman, P. & Ashcroft, F. M. Ca^{2+}- and GTP-dependent exocytosis in mouse pancreatic beta-cells involves both common and distinct steps. *J. Physiol.* **496,** 255–264 (1996).
25. Iezzi, M., Regazzi, R. & Wollheim, C. B. The Rab3-interacting molecule RIM is expressed in pancreatic beta-cells and is implicated in insulin exocytosis. *FEBS Lett.* **474,** 66–70 (2000).
26. Schuit, F. C., Huypens, P., Heimberg, H. & Pipeleers, D. G. Glucose sensing in pancreatic beta-cells: a model for the study of other glucose-regulated cells in gut, pancreas, and hypothalamus. *Diabetes* **50,** 1–11 (2001).
27. Huypens, P., Ling, Z., Pipeleers, D. & Schuit, F. Glucagon receptors on human islet cells contribute to glucose competence of insulin release. *Diabetologia* **43,** 1012–1019 (2000).
28. Jones, P. M., Fyles, J. M. & Howell, S. L. Regulation of insulin secretion by cAMP in rat islets of Langerhans permeabilised by high-voltage discharge. *FEBS Lett.* **205,** 205–209 (1986).
29. Ammala, C. *et al.* Activation of protein kinases and inhibition of protein phosphatases play a central role in the regulation of exocytosis in mouse pancreatic beta cells. *Proc. Natl Acad. Sci. USA* **91,** 4343–4347 (1994).
30. Ozaki, N. *et al.* cAMP-GEFII is a direct target of cAMP in regulated exocytosis. *Nature Cell Biol.* **2,** 805–811 (2000).
31. Pralong, W. F., Bartley, C. & Wollheim, C. B. Single islet beta-cell stimulation by nutrients: relationship between pyridine nucleotides, cytosolic Ca^{2+} and secretion. *EMBO J.* **9,** 53–60 (1990).
32. Patterson, G. H., Knobel, S. M., Arkhammar, P., Thastrup, O. & Piston, D. W. Separation of the glucose-stimulated cytoplasmic and mitochondrial NAD(P)H responses in pancreatic islet beta cells. *Proc. Natl Acad. Sci. USA* **97,** 5203–5207 (2000).
33. Prentki, M. *et al.* Malonyl-CoA and long chain acyl-CoA esters as metabolic coupling factors in nutrient-induced insulin secretion. *J. Biol. Chem.* **267,** 5802–5810 (1992).
34. Deeney, J. T. *et al.* Acute stimulation with long chain acyl-CoA enhances exocytosis in insulin-secreting cells (HIT T-15 and NMRI beta-cells). *J. Biol. Chem.* **275,** 9363–9368 (2000).
35. Antinozzi, P. A., Segall, L., Prentki, M., McGarry, J. D. & Newgard, C. B. Molecular or pharmacologic perturbation of the link between glucose and lipid metabolism is without effect on glucose-stimulated insulin secretion. A re-evaluation of the long-chain acyl-CoA hypothesis. *J. Biol. Chem.* **273,** 16146–16154 (1998).
36. Maechler, P., Kennedy, E. D., Pozzan, T. & Wollheim, C. B. Mitochondrial activation directly triggers the exocytosis of insulin in permeabilized pancreatic β-cells. *EMBO J.* **16,** 3833–3841 (1997).
37. Maechler, P., Kennedy, E. D., Wang, H. & Wollheim, C. B. Desensitization of mitochondrial Ca^{2+} and insulin secretion responses in the beta cell. *J. Biol. Chem.* **273,** 20770–20778 (1998).
38. Maechler, P. & Wollheim, C. B. Glutamate acts as a mitochondrially derived messenger in glucose-induced insulin exocytosis. *Nature* **402,** 685–689 (1999).
39. Nissim, I. Newer aspects of glutamine/glutamate metabolism: the role of acute pH changes. *Am. J. Physiol.* **277,** F493–F497 (1999).
40. Sener, A. *et al.* Insulinotropic action of glutamic acid dimethyl ester. *Am. J. Physiol.* **267,** E573–E584 (1994).
41. Rubi, B., Ishihara, H., Hegardt, F. G., Wollheim, C. B. & Maechler, P. GAD65-mediated glutamate decarboxylation reduces glucose-stimulated insulin secretion in pancreatic beta cells. *J. Biol. Chem.* **276,** 36391–36396 (2001).
42. Maechler, P. & Wollheim, C. B. Mitochondrial signals in glucose-stimulated insulin secretion in the beta cell. *J. Physiol.* **529,** 49–56 (2000).
43. Malaisse, W. J. *et al.* The stimulus–secretion coupling of glucose-induced insulin release. XXXV. The links between metabolic and cationic events. *Diabetologia* **16,** 331–341 (1979).
44. King, M. P. & Attardi, G. Human cells lacking mtDNA: repopulation with exogenous mitochondria by complementation. *Science* **246,** 500–503 (1989).
45. Soejima, A. *et al.* Mitochondrial DNA is required for regulation of glucose-stimulated insulin secretion in a mouse pancreatic beta cell line, MIN6. *J. Biol. Chem.* **271,** 26194–26199 (1996).
46. Kennedy, E. D., Maechler, P. & Wollheim, C. B. Effects of depletion of mitochondrial DNA in metabolism secretion coupling in INS-1 cells. *Diabetes* **47,** 374–380 (1998).
47. Tsuruzoe, K. *et al.* Creation and characterization of a mitochondrial DNA-depleted pancreatic beta-cell line: impaired insulin secretion induced by glucose, leucine, and sulfonylureas. *Diabetes* **47,** 621–631 (1998).

48. Hayakawa, T. *et al.* Ethidium bromide-induced inhibition of mitochondrial gene transcription suppresses glucose-stimulated insulin release in the mouse pancreatic β-cell line βHC9. *J. Biol. Chem.* **273,** 20300–20307 (1998).
49. Silva, J. P. *et al.* Impaired insulin secretion and β-cell loss in tissue-specific knockout mice with mitochondrial diabetes. *Nature Genet.* **26,** 336–340 (2000).
50. Ballinger, S. W. *et al.* Maternally transmitted diabetes and deafness associated with a 10.4 kb mitochondrial DNA deletion. *Nature Genet.* **1,** 11–15 (1992).
51. van den Ouweland, J. M. *et al.* Mutation in mitochondrial $tRNA^{Leu(UUR)}$ gene in a large pedigree with maternally transmitted type II diabetes mellitus and deafness. *Nature Genet.* **1,** 368–371 (1992).
52. Kadowaki, T. *et al.* A subtype of diabetes mellitus associated with a mutation of mitochondrial DNA. *N. Engl. J. Med.* **330,** 962–968 (1994).
53. Maassen, J. A., van Essen, E., van den Ouweland, J. M. & Lemkes, H. H. Molecular and clinical aspects of mitochondrial diabetes mellitus. *Exp. Clin. Endocrinol. Diabetes* **109,** 127–134 (2001).
54. Goto, Y., Nonaka, I. & Horai, S. A mutation in the $tRNA^{Leu(UUR)}$ gene associated with the MELAS subgroup of mitochondrial encephalomyopathies. *Nature* **348,** 651–653 (1990).
55. Kobayashi, T. *et al. In situ* characterization of islets in diabetes with a mitochondrial DNA mutation at nucleotide position 3243. *Diabetes* **46,** 1567–1571 (1997).
56. Otabe, S. *et al.* Molecular and histological evaluation of pancreata from patients with a mitochondrial gene mutation associated with impaired insulin secretion. *Biochem. Biophys. Res. Commun.* **259,** 149–156 (1999).
57. Suzuki, Y. *et al.* Diabetes mellitus associated with the 3243 mitochondrial tRNA(Leu)(UUR) mutation: insulin secretion and sensitivity. *Metabolism* **46,** 1019–1023 (1997).
58. James, A. M., Wei, Y. H., Pang, C. Y. & Murphy, M. P. Altered mitochondrial function in fibroblasts containing MELAS or MERRF mitochondrial DNA mutations. *Biochem. J.* **318,** 401–407 (1996).
59. van den Ouweland, J. M., Maechler, P., Wollheim, C. B., Attardi, G. & Maassen, J. A. Functional and morphological abnormalities of mitochondria harbouring the tRNA(Leu)(UUR) mutation in mitochondrial DNA derived from patients with maternally inherited diabetes and deafness (MIDD) and progressive kidney disease. *Diabetologia* **42,** 485–492 (1999).
60. Brini, M. *et al.* A calcium signaling defect in the pathogenesis of a mitochondrial DNA inherited oxidative phosphorylation deficiency. *Nature Med.* **5,** 951–954 (1999).
61. Froguel, P. & Velho, G. Genetic determinants of type 2 diabetes. *Recent Prog. Horm. Res.* **56,** 91–105 (2001).
62. Polonsky, K. S., Sturis, J. & Bell, G. I. Non-insulin-dependent diabetes mellitus—a genetically programmed failure of the beta cell to compensate for insulin resistance. *N. Engl. J. Med.* **334,** 777–783 (1996).
63. Antonetti, D. A., Reynet, C. & Kahn, C. R. Increased expression of mitochondrial-encoded genes in skeletal muscle of humans with diabetes mellitus. *J. Clin. Invest.* **95,** 1383–1388 (1995).
64. Lee, H. K. *et al.* Decreased mitochondrial DNA content in peripheral blood precedes the development of non-insulin-dependent diabetes mellitus. *Diabetes Res. Clin. Pract.* **42,** 161–167 (1998).
65. Michikawa, Y., Mazzucchelli, F., Bresolin, N., Scarlato, G. & Attardi G. Aging-dependent large accumulation of point mutations in the human mtDNA control region for replication. *Science* **286,** 774–779 (1999).
66. Tiedge, M., Lortz, S., Drinkgern, J. & Lenzen, S. Relation between antioxidant enzyme gene expression and antioxidative defense status of insulin-producing cells. *Diabetes* **46,** 1733–1742 (1997).
67. Maechler, P., Jornot, L. & Wollheim, C. B. Hydrogen peroxide alters mitochondrial activation and insulin secretion in pancreatic beta cells. *J. Biol. Chem.* **274,** 27905–27913 (1999).
68. Coordt, M. C., Ruhe, R. C. & McDonald, R. B. Aging and insulin secretion. *Proc. Soc. Exp. Biol. Med.* **209,** 213–222 (1995).
69. Hattersley, A. T. Maturity-onset diabetes of the young: clinical heterogeneity explained by genetic heterogeneity. *Diabet. Med.* **15,** 15–24 (1998).
70. Pontoglio, M. *et al.* Defective insulin secretion in hepatocyte nuclear factor 1α-deficient mice. *J. Clin. Invest.* **101,** 2215–2222 (1998).
71. Wang, H., Maechler, P., Hagenfeldt, K. A. & Wollheim, C. B. Dominant-negative suppression of HNF-1α function results in defective insulin gene transcription and impaired metabolism-secretion coupling in a pancreatic β-cell line. *EMBO J.* **17,** 6701–6713 (1998).
72. Wang, H., Antinozzi, P. A., Hagenfeldt, K. A., Maechler, P. & Wollheim, C. B. Molecular targets of a human HNF1α mutation responsible for pancreatic β-cell dysfunction. *EMBO J.* **19,** 4257–4264 (2000).
73. Chan, C. B. *et al.* Increased uncoupling protein-2 levels in beta-cells are associated with impaired glucose-stimulated insulin secretion: mechanism of action. *Diabetes* **50,** 1302–1310 (2001).
74. Zhang, C. Y. *et al.* Uncoupling protein-2 negatively regulates insulin secretion and is a major link between obesity, beta cell dysfunction, and type 2 diabetes. *Cell* **105,** 745–755 (2001).
75. Wang, M. Y. *et al.* Adenovirus-mediated overexpression of uncoupling protein-2 in pancreatic islets of Zucker diabetic rats increases oxidative activity and improves beta-cell function. *Diabetes* **48,** 1020–1025 (1999).
76. Unger, R. H., Zhou, Y. T. & Orci, L. Regulation of fatty acid homeostasis in cells: novel role of leptin. *Proc. Natl Acad. Sci. USA* **96,** 2327–2332 (1999).
77. Roduit, R. *et al.* Glucose down-regulates the expression of the peroxisome proliferator-activated receptor-alpha gene in the pancreatic beta-cell. *J. Biol. Chem.* **275,** 35799–35806 (2000).
78. Lameloise, N., Muzzin, P., Prentki, M. & Assimacopoulos-Jeannet, F. Uncoupling protein 2: a possible link between fatty acid excess and impaired glucose-induced insulin secretion? *Diabetes* **50,** 803–809 (2001).
79. Li, L. X., Skorpen, F., Egeberg, K., Jorgensen, I. H. & Grill, V. Uncoupling protein-2 participates in cellular defense against oxidative stress in clonal beta-cells. *Biochem. Biophys. Res. Commun.* **282,** 273–277 (2001).
80. Garcia-Martinez, J. A., Cancelas, J., Villanueva-Penacarrillo, M. L., Valverde, I. & Malaisse, W. J. Prolongation of the insulinotropic action of glucagon-like peptide 1 by the dimethyl ester of succinic acid in an animal model of type-2 diabetes. *Int. J. Mol. Med.* **6,** 319–321 (2000).
81. Suzuki, S. *et al.* The effects of coenzyme Q10 treatment on maternally inherited diabetes mellitus and deafness, and mitochondrial DNA 3243 (A to G) mutation. *Diabetologia* **41,** 584–588 (1998).
82. Nakada, K. *et al.* Inter-mitochondrial complementation: mitochondria-specific system preventing mice from expression of disease phenotypes by mutant mtDNA. *Nature Med.* **7,** 934–940 (2001).

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