

# Mitochondrial damages and the regulation of insulin secretion

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## Abstract

Pancreatic  $\beta$ -cells are able to respond to nutrients, principally glucose, as the primary stimulus for insulin exocytosis. This unique feature requires translation of metabolic substrates into intracellular messengers recognized by the exocytotic machinery. Central to this signal transduction mechanism, mitochondria integrate and generate metabolic signals, thereby coupling glucose recognition with insulin secretion. In response to a glucose rise, nucleotides and metabolites are generated by mitochondria and participate, together with cytosolic  $\text{Ca}^{2+}$ , in the stimulation of insulin exocytosis. Mitochondrial defects, such as mutations and ROS (reactive oxygen species) production, might be associated with  $\beta$ -cell failure in the course of diabetes. mtDNA (mitochondrial DNA) mutation A3243G is associated with MIDD (mitochondrial inherited diabetes and deafness). A common hypothesis to explain the link between the genotype and the phenotype is that the mutation might impair mitochondrial metabolism expressly required for  $\beta$ -cell functions, although this assumption lacks direct demonstration. mtDNA-deficient cellular models are glucose-unresponsive and are defective in mitochondrial function. Recently, we used clonal cytosolic hybrid cells (namely cybrids) harbouring mitochondria derived from MIDD patients. Compared with control mtDNA from the same patient, the A3243G mutation markedly modified metabolic pathways. Moreover, cybrid cells carrying patient-derived mutant mtDNA exhibited deranged cell  $\text{Ca}^{2+}$  handling and elevated ROS under metabolic stress. In animal models, transgenic mice lacking expression of the mitochondrial genome specifically in  $\beta$ -cells are diabetic and their islets are incapable of releasing insulin in response to glucose. These various models demonstrate the fragility of nutrient-stimulated insulin secretion, caused primarily by defective mitochondrial function.

## Introduction

Blood glucose homeostasis is chiefly maintained by adequate insulin release from pancreatic  $\beta$ -cells, which serve as a glucosensor (Figure 1). The intracellular pathways linking glucose recognition to regulated insulin exocytosis are initiated by the passive entry of glucose into the  $\beta$ -cell and its phosphorylation by glucokinase, thereby initiating glycolysis [1]. As low lactate is produced in  $\beta$ -cells [2], most of the glycolysis-derived pyruvate feeds mitochondrial metabolism through both pyruvate dehydrogenase and pyruvate carboxylase [3,4]. The latter pathway is remarkably significant in  $\beta$ -cells, pointing to important equilibrium between anaplerosis and cataplerosis in metabolism–secretion coupling [5]. Mitochondria primarily generate ATP, promoting the closure of  $\text{K}_{\text{ATP}}$ -channel (ATP-sensitive  $\text{K}^+$  channels) and, as a consequence, depolarization of the plasma membrane [6]. This leads to  $\text{Ca}^{2+}$  influx through voltage-gated  $\text{Ca}^{2+}$  channels and to subsequent rise in cytosolic

$\text{Ca}^{2+}$  concentrations triggering insulin exocytosis [6,7]. In order to reproduce the sustained secretion elicited by glucose, additional signals are required, participating in the so-called amplifying pathway of stimulation of insulin secretion [8]. In this regard, mitochondria ensure efficient coupling of glucose recognition with insulin secretion by integrating and generating metabolic signals. In particular, mitochondrial metabolism is the source of coupling factors participating in the sustained second phase of the secretory response [9].

Mitochondrial defects in  $\beta$ -cells perturb glucose-stimulated insulin secretion and might be associated with diabetes [10,11]. At the clinical level, one of the most direct indications that mitochondrial dysfunction could impair insulin release comes from the association between mutated mitochondrial genome and maternally inherited diabetes [12].

## mtDNA (mitochondrial DNA) mutations and $\beta$ -cell function

mtDNA carries only 37 genes (16 569 bp) encoding 13 polypeptides, 22 tRNAs and two ribosomal RNAs [13]. Mitochondrial protein biogenesis is determined by both nuclear and mitochondrial genomes, and the few polypeptides encoded by the mtDNA are all subunits of the electron transport chain [14]. Transgenic mice lacking expression of the mitochondrial genome specifically in the  $\beta$ -cells are diabetic and their islets exhibit impaired glucose-stimulated

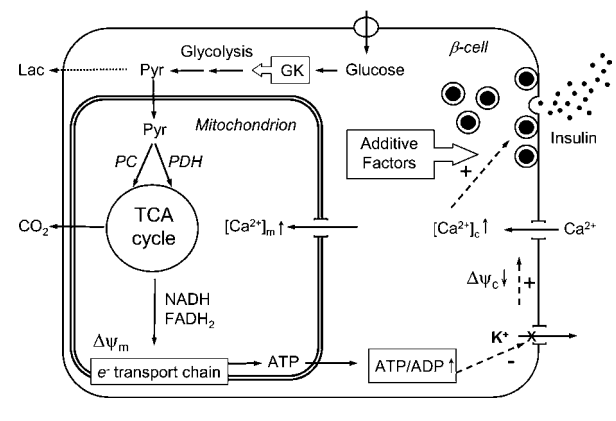
**Key words:** cybrid, diabetes, insulin secretion, mitochondrial DNA (mtDNA), oxidative stress, pancreatic  $\beta$ -cell.

**Abbreviations used:** COX, cytochrome c oxidase;  $\text{K}_{\text{ATP}}$ -channel, ATP-sensitive  $\text{K}^+$  channel; LHON, Leber's hereditary optic neuropathy; mtDNA, mitochondrial DNA;  $\rho^0$  cell, mtDNA-depleted cell; MELAS, mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes; MERRF, myoclonus epilepsy with ragged-red fibres; MIDD, mitochondrial inherited diabetes and deafness; ROS, reactive oxygen species; SOD, superoxide dismutase.

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### Figure 1 | Model for coupling of glucose metabolism with insulin secretion in the $\beta$ -cell

Glucose equilibrates across the plasma membrane and is phosphorylated by glucokinase (GK). Further glycolysis produces pyruvate (Pyr). Pyr preferentially enters the mitochondria, as low lactate (Lac) is produced, and fuels the tricarboxylic acid (TCA) cycle through both pyruvate dehydrogenase (PDH) and pyruvate carboxylase (PC). This results in the transfer of reducing equivalents (NADH and FADH<sub>2</sub>) to the respiratory chain, leading to hyperpolarization of the mitochondrial membrane potential ( $\Delta\Psi_m$ ) and generation of ATP. ATP is then transferred to the cytosol, raising the ATP/ADP ratio. Subsequently, closure of K<sub>ATP</sub>-channels depolarizes the cell-membrane potential ( $\Delta\Psi_c$ ). This opens voltage-dependent Ca<sup>2+</sup> channels, increasing cytosolic Ca<sup>2+</sup> concentration ([Ca<sup>2+</sup>]<sub>c</sub>), which triggers insulin exocytosis.



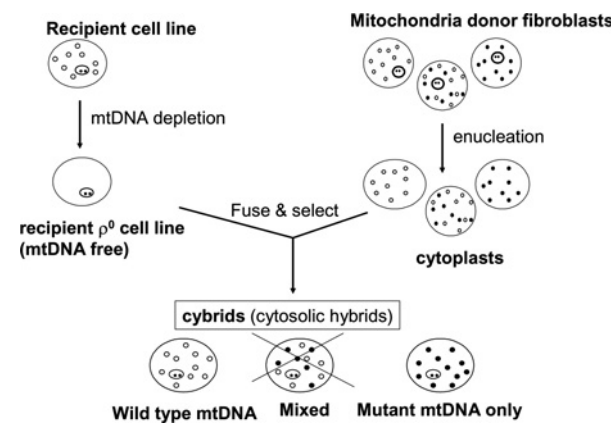
insulin secretion [15]. Moreover, mtDNA-deficient  $\beta$ -cell lines are glucose-unresponsive and carry defective mitochondria, although they still exhibit secretory responses to Ca<sup>2+</sup>-raising agents [16–18].

MIDD (mitochondrial inherited diabetes and deafness) is often associated with mtDNA A3243G point mutation on the tRNA<sup>Leu</sup> gene [19,20], usually in the heteroplasmic form, i.e. a mixture of wild-type and mutant mtDNA in patient cells. Mitochondrial diabetes usually appears during adulthood with maternal transmission and often in combination with bilateral hearing impairment [12]. The aetiology of diabetes may not be primarily associated with  $\beta$ -cells, rendering the putative link between mtDNA mutations and  $\beta$ -cell dysfunction still hypothetical [11]. Moreover, pancreatic islets of such patients may carry low heteroplasmy percentage of the mutation [21] and, accordingly, the pathogenicity of this mutation is hardly detectable in the endocrine pancreas [21,22].

Some clinical studies strongly suggest a direct link between mtDNA mutations and  $\beta$ -cell dysfunction. Diabetic patients carrying mtDNA mutations exhibit marked reduction in insulin release upon intravenous glucose tolerance tests and hyperglycaemic clamps compared with non-carriers [23–25]. In addition, individuals with Pearson syndrome or Kearns-Sayre syndromes due to large deletions in mtDNA exhibit an insulin-deficient diabetes phenotype [26,27]. It is generally hypothesized that mtDNA mutations could result in mito-

### Figure 2 | Generation of cybrid cells

A recipient cell line for mitochondria is depleted of mtDNA by chemical treatment, producing  $\rho^0$  cells. In parallel, fibroblasts collected from a patient with specific mtDNA mutation are enucleated, producing cytoplasts. Cell line-derived  $\rho^0$  cells are then fused with patient-derived cytoplasts before selection of the resulting cytosolic hybrids, i.e. cybrids. As most of the cybrids would exhibit heteroplasmy (mixed wild-type and mutated mtDNA), further subcloning selects cybrid clones carrying only either wild-type or mutated mtDNA.



chondrial impairment associated with  $\beta$ -cell dysfunction as a primary abnormality in carriers of the mutation [23]. Alternatively, impaired mitochondrial metabolism in cells of individuals carrying mtDNA mutations might rather predispose for  $\beta$ -cell dysfunction, explaining late onset of the disease. Due to technical limitation of  $\beta$ -cell accessibility in individuals, the putative impact of mtDNA mutations on insulin secretion still lacks direct demonstration.

In cellular models, direct investigation of  $\beta$ -cell functions carrying specific mtDNA mutations also faces technical obstacles. Indeed, as opposed to genomic DNA, specific mtDNA manipulations are not feasible. The alternative commonly used is to introduce patient-derived mitochondria into cell lines by fusing enucleated cells carrying mitochondria of interest with  $\rho^0$  cells (mtDNA-depleted cells), resulting in cytosolic hybrids, namely cybrids (Figure 2). However, until very recently human  $\beta$ -cell lines were not available and the alternative, mixing human mitochondria with rodent genomic DNA (e.g. with available rodent  $\beta$ -cell lines), is not possible.

In a previous study, we investigated patient-derived mitochondria in a human osteosarcoma cell line [28]. The corresponding  $\rho^0$  cells were used as recipients for mtDNA from a patient with the diabetes-associated MIDD A3243G mutation. The resulting clonal cell lines contained either exclusively patient-derived mutated mtDNA or wild-type mtDNA from the same patient [28]. Recently, we measured key parameters for metabolism–secretion coupling in responses to glucose, thereby establishing a link between clinical observation and molecular events. The study shows that mitochondrial A3243G mutation is responsible for defective mitochondrial metabolism associated with impaired

Ca<sup>2+</sup> homeostasis [29]. In particular, cytosolic Ca<sup>2+</sup> rises are prolonged in mtDNA mutant cells, an effect associated with lowered Ca<sup>2+</sup> retention capacity in the mitochondrial matrix. Deranged Ca<sup>2+</sup> homeostasis may vary according to specific mtDNA mutations. In cybrid cells with the T8356C mtDNA mutation, which is associated with the disease MERRF (myoclonus epilepsy with ragged-red fibres) [30], mitochondrial Ca<sup>2+</sup> homeostasis is also altered [31]. However, in contrast with A3243G cybrids, T8356C cells exhibit normal cytosolic Ca<sup>2+</sup> responses. Such a difference might possibly contribute to mtDNA-specific phenotypes.

The A3243G mutation induces a shift towards dominantly glycolytic metabolism, while glucose oxidation is reduced [29]. The levels of reducing equivalents in the form of NAD(P)H cannot be elevated upon glucose stimulation in mtDNA mutant cells, reflecting the impact of this mutation on the electron transport chain activity [28]. The resulting deficient NADH reoxidation leads to mitochondrial accumulation of its reduced form, slowing down NADH-generating coupled reactions in the tricarboxylic acid cycle. As a metabolic consequence, we observed a switch to anaerobic glucose utilization accompanied by increased lactate generation [29]. Accordingly, ATP supply is totally dependent on high glycolytic rates, enabling the mtDNA mutant cells to only reach basal normal ATP levels at the expense of stimulatory glucose concentrations. Such a phenotype is well known to dramatically impair glucose-stimulated insulin secretion in  $\beta$ -cells.

### ROS (reactive oxygen species) and $\beta$ -cell function

ROS refer to a variety of molecules and free radicals derived from molecular oxygen, superoxide anion (O<sub>2</sub><sup>•-</sup>) being the precursor of most ROS. Mitochondria are known to be the main source and target of ROS and the imbalance between excessive formation of ROS and limited antioxidant defences leads to oxidative stress [32].  $\beta$ -Cells are particularly susceptible to oxidative stress [33] due to the weak expression of natural enzymatic defences, e.g. catalase and SOD (superoxide dismutase) [34]. This might trigger  $\beta$ -cell apoptosis and decrease  $\beta$ -cell mass [35]. ROS content in isolated islets of Zucker diabetic fatty rats is higher under resting conditions and their ROS production is linked to mitochondrial metabolism [36]. In a rat model of intrauterine growth retardation that leads to diabetes in adulthood [37,38], increased ROS production is associated with damaged mtDNA. This causes further production of ROS, ultimately leading to progressive loss of  $\beta$ -cell function and development of Type 2 (non-insulin-dependent) diabetes in the adult [39]. Altogether, these observations indicate that ROS may participate in the impairment of glucose-stimulated insulin secretion observed in association with aging and Type 2 diabetes [40]. It was speculated that antioxidant therapeutic interventions might contribute to preserving  $\beta$ -cell function [41], although the direct contribution of oxidative stress to the aetiology of diabetes remains uncertain

[42]. To shed light on mitochondrial genome contribution to ROS homeostasis, some studies were conducted using cells originating from different tissues and depleted of mtDNA, i.e.  $\rho^0$  cells. Yet, it is still questioned whether  $\rho^0$  cells generate more ROS compared with control cells and ROS production in cybrid cells might be tissue-dependent. For instance, bone, lung and muscle  $\rho^0$  cells differ considerably both in their antioxidant defences and ROS homeostasis [43]. Osteosarcoma  $\rho^0$  cells exhibit reduced total SOD activity and, under glucose deprivation, generate 2-fold more ROS compared with controls, a situation reversed under standard growth conditions [43]. SK-Hep1  $\rho^0$  cells show more resistance to cell death, low basal ROS levels, increased manganese SOD (SOD2) expression and no changes in Cu/Zn SOD (SOD1) expression compared with controls [44]. Accordingly, it was proposed that upstream inhibition of the electron transport chain or its dysfunction by mtDNA depletion would prevent ROS accumulation and protect cells against programmed cell death (reviewed in [45]). To date, only few studies looked at ROS homeostasis in cells carrying mtDNA mutations [46–48]. It was reported that cybrid cells carrying mutant mtDNA exhibit either increased [46–48] or unchanged [47] ROS levels. Cybrid cells harbouring mtDNA mutations associated with LHON (Leber's hereditary optic neuropathy) cybrids exhibit reduced antioxidant efficiency compared with controls, and this situation is magnified by stressful metabolic conditions [49]. However, these cells are able to handle ROS generation when kept under glucose-supplemented medium. Another study investigated the mutation-specific profiles of ROS production and antioxidant defences in cybrids harbouring A8344G (MERRF), A3243G [MIDD and MELAS (mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes)] and G6930A COX (cytochrome c oxidase) mutations [48]. An increase was found in both ROS levels and antioxidant enzyme activities in MERRF and MELAS cybrids, but neither ROS nor antioxidant enzyme activities were augmented in COX cybrids, suggesting that different mtDNA mutations might lead to diverse ROS generation and antioxidant defence profiles. It was also shown that cybrids carrying different proportions of mutant mtDNA (A3243G) have lower mitochondrial membrane potential and reduced COX activity, although basal ROS levels in mutant cybrids are similar to controls [47].

Recently, mtDNA mutator mice were used as a model to investigate the relationship between ROS generation, mitochondrial damage and aging [50]. Although aging was accelerated in these mice, primary cultures of wild-type and mutant mouse embryonic fibroblasts revealed no difference in H<sub>2</sub>O<sub>2</sub> and superoxide levels. This study demonstrates that ROS generation can be dissociated from mtDNA mutations, being more likely that a defective respiratory chain would trigger aging by bioenergy insufficiency. Therefore the contribution of mtDNA defects/mutations to ROS generation remains a matter of debate.

In permeabilized insulin-secreting  $\rho^0$  cells (INS-1  $\rho^0$  cells), mitochondrial substrates do not raise insulin secretion

compared with controls, stressing that the secretion defect is not at exocytosis level but is due to mitochondrial failure [17]. We reported recently that the diabetes-associated A3243G mtDNA mutation was associated with higher intracellular ROS levels compared with control cells under glucose deprivation, which was reversed upon glucose provision [29]. Particularly, glucose deprivation forced mtDNA mutant cells to rely exclusively on oxidative metabolism, which is negligible in these cells, associated with ATP depletion and overall metabolic stress. SOD2 mRNA levels were reduced in cells carrying the A3243G mutation compared with controls, similarly to LHON cybrids [49]. Taken together, these results indicate that diabetes-associated A3243G mtDNA mutation renders cells more susceptible to oxidative stress damage under metabolic insult.

## Conclusions

As discussed in this review, the interplay between mtDNA mutations and ROS has not been yet fully clarified. In particular, it remains debated whether mutated mtDNA would contribute to ROS generation. On the one hand, elevated ROS levels can by themselves perturb  $\beta$ -cell function. On the other hand, mtDNA damages are also directly associated with impaired glucose-stimulated insulin secretion. Future investigations might shed light on complex equilibrium between mtDNA preservation and ROS homeostasis. As these factors are thought to participate on the long term in the aetiology of late onset diabetes, elucidating the respective contribution of mtDNA mutations and ROS generation will represent a major challenge.

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## References

- Matschinsky, F.M. (1996) *Diabetes* **45**, 223–241
- Ishihara, H., Wang, H., Drewes, L.R. and Wollheim, C.B. (1999) *J. Clin. Invest.* **104**, 1621–1629
- Schuit, F., De Vos, A., Farfari, S., Moens, K., Pipeleers, D., Brun, T. and Prentki, M. (1997) *J. Biol. Chem.* **272**, 18572–18579
- Cline, G.W., Lepine, R.L., Papas, K.K., Kibbey, R.G. and Shulman, G.I. (2004) *J. Biol. Chem.* **279**, 44370–44375
- Maechler, P., Carobbio, S. and Rubi, B. (2006) *Int. J. Biochem. Cell Biol.* **38**, 696–709
- Rorsman, P. (1997) *Diabetologia* **40**, 487–495
- Lang, J. (1999) *Eur. J. Biochem.* **259**, 3–17
- Henquin, J.C. (2000) *Diabetes* **49**, 1751–1760
- Maechler, P. (2002) *Cell. Mol. Life Sci.* **59**, 1803–1818
- Maechler, P. and Wollheim, C.B. (2001) *Nature* **414**, 807–812
- Lowell, B.B. and Shulman, G.I. (2005) *Science* **307**, 384–387
- Maassen, J.A., Janssen, G.M. and t Hart, L.M. (2005) *Ann. Med.* **37**, 213–221
- Wallace, D.C. (1999) *Science* **283**, 1482–1488
- Buchet, K. and Godinot, C. (1998) *J. Biol. Chem.* **273**, 22983–22989
- Silva, J.P., Kohler, M., Graff, C., Oldfors, A., Magnuson, M.A., Berggren, P.O. and Larsson, N.G. (2000) *Nat. Genet.* **26**, 336–340
- Soejima, A., Inoue, K., Takai, D., Kaneko, M., Ishihara, H., Oka, Y. and Hayashi, J.I. (1996) *J. Biol. Chem.* **271**, 26194–26199
- Kennedy, E.D., Maechler, P. and Wollheim, C.B. (1998) *Diabetes* **47**, 374–380
- Tsuruzoe, K., Araki, E., Furukawa, N., Shirotani, T., Matsumoto, K., Kaneko, K., Motoshima, H., Yoshizato, K., Shirakami, A., Kishikawa, H. et al. (1998) *Diabetes* **47**, 621–631
- Ballinger, S.W., Shoffner, J.M., Hedaya, E.V., Trounce, I., Polak, M.A., Koontz, D.A. and Wallace, D.C. (1992) *Nat. Genet.* **1**, 11–15
- van den Ouweland, J.M., Lemkes, H.H., Ruitenbeek, W., Sandkuijl, L.A., de Vijlder, M.F., Struyvenberg, P.A., van de Kamp, J.J. and Maassen, J.A. (1992) *Nat. Genet.* **1**, 368–371
- Lynn, S., Borthwick, G.M., Charnley, R.M., Walker, M. and Turnbull, D.M. (2003) *Diabetologia* **46**, 296–299
- Maassen, J.A., van Essen, E., van den Ouweland, J.M. and Lemkes, H.H. (2001) *Exp. Clin. Endocrinol. Diabetes* **109**, 127–134
- Velho, G., Byrne, M.M., Clement, K., Sturis, J., Pueyo, M.E., Blanche, H., Vionnet, N., Fiet, J., Passa, P., Robert, J.J. et al. (1996) *Diabetes* **45**, 478–487
- Brandle, M., Lehmann, R., Maly, F.E., Schmid, C. and Spinas, G.A. (2001) *Diabetes Care* **24**, 1253–1258
- Maassen, J.A., t Hart, L.M., van Essen, E., Heine, R.J., Nijpels, G., Jahangir Tafrechi, R.S., Raap, A.K., Janssen, G.M. and Lemkes, H.H. (2004) *Diabetes* **53** (Suppl. 1), S103–S109
- De Block, C.E., De Leeuw, I.H., Maassen, J.A., Ballaux, D. and Martin, J.J. (2004) *Exp. Clin. Endocrinol. Diabetes* **112**, 80–83
- van den Ouweland, J.M., de Klerk, J.B., van de Corput, M.P., Dirks, R.W., Raap, A.K., Scholte, H.R., Huijmans, J.G., Hart, L.M., Bruining, G.J. and Maassen, J.A. (2000) *Eur. J. Hum. Genet.* **8**, 195–203
- van den Ouweland, J.M., Maechler, P., Wollheim, C.B., Attardi, G. and Maassen, J.A. (1999) *Diabetologia* **42**, 485–492
- de Andrade, P.B., Rubi, B., Frigerio, F., van den Ouweland, J.M., Maassen, J.A. and Maechler, P. (2006) *Diabetologia* **49**, 1816–1826
- Silvestri, G., Moraes, C.T., Shanske, S., Oh, S.J. and DiMauro, S. (1992) *Am. J. Hum. Genet.* **51**, 1213–1217
- Brini, M., Pinton, P., King, M.P., Davidson, M., Schon, E.A. and Rizzuto, R. (1999) *Nat. Med.* **5**, 951–954
- Turrens, J.F. (2003) *J. Physiol.* **552**, 335–344
- Maechler, P., Jornot, L. and Wollheim, C.B. (1999) *J. Biol. Chem.* **274**, 27905–27913
- Tiedge, M., Lortz, S., Drinkgern, J. and Lenzen, S. (1997) *Diabetes* **46**, 1733–1742
- Mandrup-Poulsen, T. (2001) *Diabetes* **50** (Suppl. 1), S58–S63
- Bindokas, V.P., Kuznetsov, A., Sreenan, S., Polonsky, K.S., Roe, M.W. and Philipson, L.H. (2003) *J. Biol. Chem.* **278**, 9796–9801
- Ogata, E.S., Bussey, M.E. and Finley, S. (1986) *Metabolism* **35**, 970–977
- Simmons, R.A., Templeton, L.J. and Gertz, S.J. (2001) *Diabetes* **50**, 2279–2286
- Simmons, R.A., Suponitsky-Kroyter, I. and Selak, M.A. (2005) *J. Biol. Chem.* **280**, 28785–28791
- Coordt, M.C., Ruhe, R.C. and McDonald, R.B. (1995) *Proc. Soc. Exp. Biol. Med.* **209**, 213–222
- Robertson, R.P., Harmon, J., Tran, P.O., Tanaka, Y. and Takahashi, H. (2003) *Diabetes* **52**, 581–587
- Scott, J.A. and King, G.L. (2004) *Ann. N. Y. Acad. Sci.* **1031**, 204–213
- Vergani, L., Floreani, M., Russell, A., Ceccon, M., Napoli, E., Cabrelle, A., Valente, L., Bragantini, F., Leger, B. and Dabbeni-Sala, F. (2004) *Eur. J. Biochem.* **271**, 3646–3656
- Park, S.Y., Chang, I., Kim, J.Y., Kang, S.W., Park, S.H., Singh, K. and Lee, M.S. (2004) *J. Biol. Chem.* **279**, 7512–7520
- Mignotte, B. and Vayssiere, J.L. (1998) *Eur. J. Biochem.* **252**, 1–15
- Beretta, S., Mattavelli, L., Sala, G., Tremolizzo, L., Schapira, A.H., Martinuzzi, A., Carelli, V. and Ferrarese, C. (2004) *Brain* **127**, 2183–2192
- Sandhu, J.K., Sodja, C., McRae, K., Li, Y., Rippstein, P., Wei, Y.H., Lach, B., Lee, F., Bucurescu, S., Harper, M.E. and Sikorska, M. (2005) *Biochem. J.* **391**, 191–202
- Vives-Bauza, C., Gonzalo, R., Manfredi, G., Garcia-Arumi, E. and Andreu, A.L. (2006) *Neurosci. Lett.* **391**, 136–141
- Floreani, M., Napoli, E., Martinuzzi, A., Pantano, G., De Riva, V., Trevisan, R., Bisetto, E., Valente, L., Carelli, V. and Dabbeni-Sala, F. (2005) *FEBS J.* **272**, 1124–1135
- Trifunovic, A., Hansson, A., Wredenberg, A., Rovio, A.T., Dufour, E., Khvorostov, I., Spelbrink, J.N., Wibom, R., Jacobs, H.T. and Larsson, N.G. (2005) *Proc. Natl. Acad. Sci. U.S.A.* **102**, 17993–17998

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