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Review

Mitochondrial diseases and ATPase defects of nuclear origin

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Abstract

Dysfunctions of the F_1F_o-ATPase complex cause severe mitochondrial diseases affecting primarily the paediatric population. While in the maternally inherited ATPase defects due to mtDNA mutations in the *ATP6* gene the enzyme is structurally and functionally modified, in ATPase defects of nuclear origin mitochondria contain a decreased amount of otherwise normal enzyme. In this case biosynthesis of ATPase is down-regulated due to a block at the early stage of enzyme assembly—formation of the F_1 catalytic part. The pathogenetic mechanism implicates dysfunction of Atp12 or other F_1-specific assembly factors. For cellular energetics, however, the negative consequences may be quite similar irrespective of whether the ATPase dysfunction is of mitochondrial or nuclear origin.

Keywords: Mitochondrial disease; Cardiomyopathy; ATP synthase; Oxidative phosphorylation; Respiratory chain complex

1. Introduction

Last decade of bioenergetic research on mitochondrial diseases has uncovered an increasing number of human mitochondrial disorders that are caused by mutations in nuclear genes encoding the subunits of oxidative phosphorylation complexes (OXPHOS), or other proteins that are essential either for the biosynthesis of specific cofactors (such as heme) or assembly of heterooligomeric OXPHOS complexes from individual subunits (for review, see Ref. [1]). Up to now, numerous mutations in nuclear genes have been shown to cause a dysfunction of all mitochondrial respiratory chain complexes, NADH dehydrogenase (Complex I), succinate dehydrogenase (Complex II), bc_1 complex (Complex III), cytochrome *c* oxidase (Complex IV) and also F_1F_o-ATP synthase (ATPase, Complex V). Very often, these defects manifest rather early and affect paediatric population. Interestingly, the Complex I defects are mostly connected with mutations in genes encoding the subunits of the complex, while Complex IV and Complex III defects are caused by mutations in specific assembly proteins or biosynthetic factors. To the same category apparently belong the ATPase deficiencies of nuclear origin that are rather rare and biochemically manifest as a reduction of cellular content of ATPase complex that is otherwise structurally and functionally normal.

2. Selective defects of mitochondrial ATPase

Mitochondrial ATPase is the key enzyme of cellular energy conversion. ATPase uses the H^+ gradient generated by the respiratory chain as a driving force for the synthesis of ATP from ADP and phosphate. The mammalian ATPase complex is formed by 16 different subunits [2] and consists of the globular F_1 catalytic part connected by two stalks to the membrane-embedded F_o moiety, which translocates protons across the inner mitochondrial membrane. Two F_o subunits, subunit a (ATP6) and A6L (ATP8), are coded for by mitochondrial DNA (mtDNA) [3]; all other subunits are nuclearly encoded. Mitochondrial encephalomyopathies due to selective defects in mitochondrial ATPase are less frequent than the disorders of the respiratory chain complexes. They are mostly very severe and can be caused by

Abbreviations: OXPHOS, oxidative phosphorylation; ATPase, mitochondrial F_1F_o-ATP synthase; mtDNA, mitochondrial DNA

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mtDNA mutations as well as by mutations in nuclear genes.

3. *ATP6* mutations

All maternally inherited primary ATPase defects are associated with subunit a (see Ref. [4]), an essential component, together with multiple copies of the subunit c of the ATPase proton channel [5,6]. The *ATP6* gene is a hotspot of pathogenic mtDNA mutations affecting ATPase; no mutations have been found in the *ATP8* gene. *ATP6* mutations are mostly missense heteroplasmic mtDNA mutations affecting the protonophoric function of the subunit a. Higher prevalence show T8993G(C) mutations [7,8] which change Leu^{156} to Arg or Pro. At a lower mutation load they are manifested as a NARP syndrome (Neurogenic muscle weakness, Ataxia, Retinitis Pigmentosa), at heteroplasmy exceeding 90% they present as maternally inherited Leigh syndrome (severe and fatal encephalopathy). Less frequent are T9176G(C) mutations which change Leu^{217} [9,10] or a T8851G mutation [11] affecting Trp^{109}, both manifesting also as striatal necrosis syndromes (see Ref. [4]). Impairment of the ATPase H^+ channel by different *ATP6* mutations results often, but not always, in decreased ATP production, while the ATPase hydrolytic activity remains unchanged [12,13]. Mitochondria from T8993G cells are capable of ATP-dependent proton translocation [14] indicating that at least the vectorial proton transport by the enzyme from matrix to cytosol is unaffected. Increased lability of the ATPase complex, possibly due to altered assembly of subunit a, apparent as accumulation of incomplete ATPase assemblies, has been described in T8993G mutation [13,15]. Interestingly, these assembly intermediates could not be found in some other cases [16], which indicates that, similarly as in segregation of *ATP6* mutations [17], a different nuclear background and participation of putative regulatory factor(s) may be involved in their pathogenetic mechanism.

A completely different type of pathogenic mechanism is represented by homoplasmic mtDNA 2-bp microdeletion 9205delTA, so far found in two cases only [18,19]. This mutation removes the stop codon of the *ATP6* gene and affects the cleavage site between *ATP6* and *COXIII* transcripts. The biochemical and clinical presentations of these two cases are, however, strikingly different [20–22]. An involvement of some nuclear-encoded factor operating at the level of mitochondrial RNA processing is to be expected. Several proteins have been described in yeast (NCA2, NCA3, NAM1/MTF2, Aep3p) that are essential for proper processing of mitochondrial RNAs, namely of the *ATP8–ATP6* cotranscript (see Ref. [23]), but their mammalian orthologues were not found, possibly reflecting differences in the structure of mammalian mitochondrial RNAs that, in comparison with yeast, lack introns and 3- and 5-prime untranslated regions. Another group of factors involved in translation of mitochondrial RNAs is represented by proteins mediating mRNA–ribosome interactions. Search for mammalian orthologues in this group was more successful and an LRPPRC protein was identified using functional genomics approach [24]. It was shown that mutation in the *LRPPRC* gene causes the Leigh syndrome of French–Canadian type, which is a human mitochondrial COX deficiency [25]. Identification of additional mammalian factors specific for other mitochondrial transcripts can be foreseen.

4. ATPase defects of nuclear origin

ATPase defects due to a nuclear genome mutations where an alteration of mtDNA genes was excluded are characterised as selective decrease of ATPase content that is caused by diminished biosynthesis of the ATPase complex. An increasing number of cases diagnosed recently (Table 1) indicates that these defects may be more frequent than originally expected. ATPase deficiency of possible non-mitochondrial origin was first described in a child with 3-methylglutaconic aciduria and severe lactic acidosis [26]. An extremely low ATPase activity and low, tightly coupled, respiration rates were observed in muscle mitochondria, but no mutation was found in mtDNA genes encoding ATPase subunits. The nuclear origin of ATP synthase deficiency was demonstrated for the first time in 1999 [27] in a new type of fatal mitochondrial disorder. A child with severe lactic acidosis, cardiomyopathy and hepatomegaly died 2 days

Table 1
Patients with mitochondrial ATPase deficiency

Case	ATPase (%)	Onset/ survival	Biochemical data and phenotype	Reference
I	<10	neonate/15 months	LA, methylglutaconic aciduria, CM, developmental delay	[26]
IIa	<30	neonate/2 days	fatal LA, CM, hepatomegaly	[27]
IIb	<30	neonate/2 days	fatal LA, CM	
IIIa	<20	neonate/32 days	LA, CM, respiratory failure	[22]
IIIb	<30	neonate/3 years	LA, CM, short statute, failure to thrive, developmental delay	
IV	<30	neonate/8 years	LA, methylglutaconic aciduria, CM, developmental delay	[22]
V	<10	neonate/3 years	LA, CM, developmental delay	[28]
VI	<20	neonate/14 months	LA, methylglutaconic aciduria, dysmorphism, enlarged liver, marked brain atrophy	[29]
VII	<30	neonate/3 days	fatal LA, enlarged liver, small left ventricle, intracranial haemorrhage	[29]

LA—lactic acidosis, CM—cardiomyopathy.

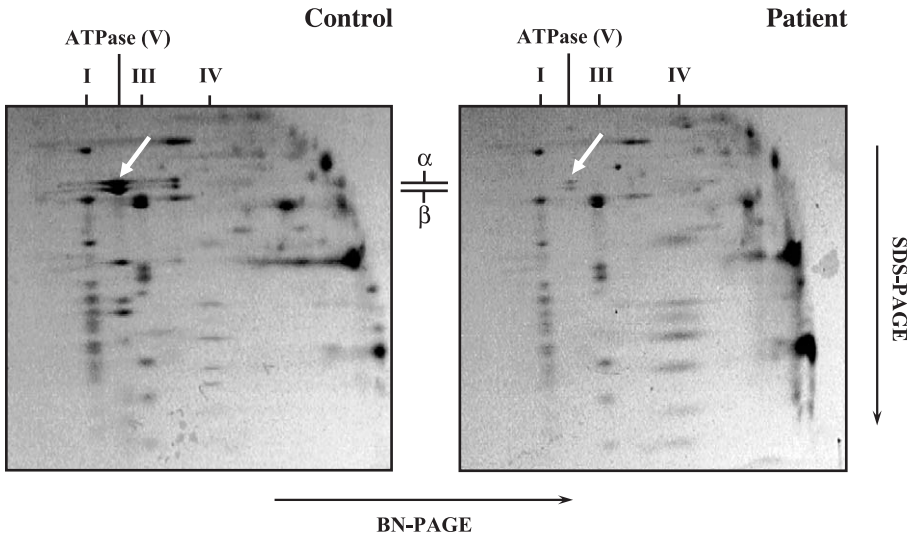

Fig. 1. OXPHOS complexes in a patient with ATPase deficiency. Blue-Native/SDS two-dimensional electrophoresis was performed using laurylmaltoside-solubilised proteins from heart mitochondria from patient (IIa in Table 1) and control, the gel was stained with Coomassie blue. The position of ATPase complex and of respiratory chain complexes I, III and IV is indicated; white arrows point to α/β F_1-ATPase subunits.

after birth. A generalised, 70–80% decrease in ATPase activity and ATP production was associated with corresponding selective decrease of the content of the ATPase complex, which had normal size and subunit composition (Fig. 1). Transmitochondrial cybrid cells made of patient's fibroblasts fully complemented the ATPase defect and confirmed the nuclear origin of impaired biogenesis of the enzyme complex [27]. Later on, two other similar cases of selective ATPase deficiency were found in the Czech Republic [22] and one in Austria [28]. Most recently, two patients with selective ATPase deficiency were described in Belgium [29]. In one of them, De Meirleir et al. located the pathogenic mutation in the *ATP12* gene for the first time (see further). As summarised in Table 1, most of the cases showed a reduction of ATPase content to <30% of the control, early onset of the disease, cardiomyopathy and survival of several days or weeks. Interestingly, methylglutaconic aciduria was found in several longer surviving patients. One of them showed a different phenotype of degenerative encephalopathy characterised by cortical and subcortical atrophy [29].

5. Altered biosynthesis of ATPase

The mechanism of ATPase biosynthesis is still not very well understood. The mammalian enzyme is expected to assemble stepwise (Fig. 2). Several transient assembly intermediates have been identified with initial formation of the F_1 catalytic part to which the nuclear encoded membrane sector subunits are added, followed by mtDNA-encoded subunits [30–32]. Native forms of ATPase can be easily revealed by Blue-Native electrophoresis [33] that became a very powerful approach to diagnostics of mitochondrial OXPHOS defects today. We have performed detailed studies on most of the diagnosed cases of selective ATPase deficiency and always found only a full-size ATPase complex to be present in cells and isolated mitochondria from the patient's tissues [27]. No accumulation of assembly intermediates analogous to subcomplexes observed in *ATP6* mutations or cells with doxycycline-inhibited translation of mitochondrial proteins [13,15,32] could be detected in fibroblasts with ATPase defects of nuclear origin. In [^{35}S]-methionine labelling experiments, decreased biosynthesis of the assembled ATPase has been found (Fig. 3). It contrasted with the increased biosynthesis of the β subunit of the F_1 catalytic part that had a very short half-life [27]. The cells also showed extramitochondrial accumulation of the β subunit, supporting the view that the biogenesis of ATPase is disturbed at an early stage when the F_1 catalytic part is formed.

6. F_1-ATPase assembly and affected nuclear factor(s)

The possible cause of insufficient ATPase biosynthesis can be a limited production or mutation of some of the

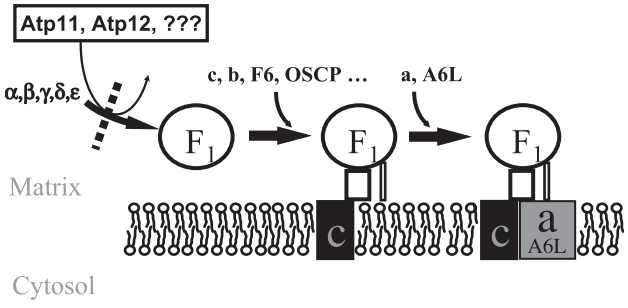

Fig. 2. ATPase assembly scheme.

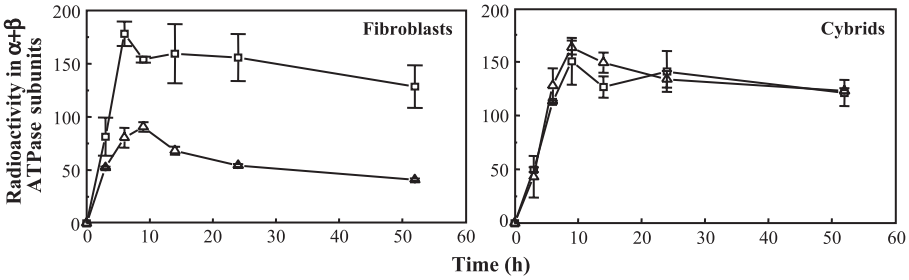

Fig. 3. Metabolic labelling of ATPase-deficient fibroblasts and derived transmitochondrial cybrids. Cells from patient (△) and control (□) were labelled for 3 h with [^{35}S]methionine, followed by 3–48-h chase with excess cold methionine. Aliquots of solubilised OXPHOS complexes were analysed by two-dimensional electrophoresis and radioactivity was determined by phosphorimaging (patient IIa in Table 1).

ATPase subunits. The cellular content of ATPase differs in mammalian tissues and it may vary also during ontogenetic development. Physiologically ATPase is down-regulated about 10-fold, relative to other OXPHOS complexes, in thermogenic brown fat where the electrochemical potential of proton gradient is dissipated in the form of heat by the uncoupling protein UCP1 [34]. Interestingly, the two-dimensional electrophoretic pattern of OXPHOS proteins in brown fat is almost identical with that found in patients with ATPase deficiency. In brown fat a selective transcriptional down-regulation of subunit *c* isogenes *P1* and *P2* and availability of the subunit c has been found as a limiting step for de novo synthesis of ATPase [35]. The transcriptional control of subunit c genes has been implicated in control of the cellular content of ATPase also in other mammalian tissues [36,37]. However, in ATPase-deficient patients expression of subunit c isogenes was normal or even increased [27] and the mRNA levels for other ATPase subunits did not show a significant decrease. As also sequencing of cDNAs of F_1 and F_o subunits from patient cells was unable to detect any pathogenic mutation, the possible cause of the disease might be associated with a dysfunction of an ATPase-specific assembly factor.

Biosynthesis of eukaryotic ATPase is a highly ordered process, which involves several ATPase-specific assembly proteins. Studies in yeast identified five chaperone-type factors necessary for the assembly of the functional enzyme. Atp10p and Atp22p were found to mediate F_o assembly [38,39] while Atp11p, Atp12p and Fmc1p are required for the F_1 part [40,41]. Fmc1p is essential at elevated growth temperatures but *FMC1* deletion could be rescued by overexpression of Atp12p [41]. Recently, human orthologues of Atp11p and Atp12p (Atpaf1p and Atpaf2p according to new nomenclature) have also been identified. Like the yeast proteins, human Atp11p has chaperone-like activity toward the β subunit and human Atp12p interacts with the α subunit [42,43]. Expression of *ATP11* and *ATP12* in mammalian tissues is about two orders of magnitude lower than expression of genes for the corresponding F_1 subunits, in accordance with the chaperone function of these proteins. Interestingly, *ATP11* expression in mouse is nearly constant in all tissues, indicating that *ATP11* rather behaves like a maintenance gene. In contrast, *ATP12* expression differed up to 30-fold in diferent tissues and it was found to correlate well with mRNA levels of both F_1-α and F_1-β (BAT≫kidney, liver>-heart, brain>skeletal muscle), showing the highest mRNA level in the thermogenic, ATPase-poor brown adipose tissue [44].

The most recent molecular genetic studies by De Meirleir et al. [29] in two patients from Belgium with ATPase deficiency identified in one case the first mutation in *ATP12* (patient 6 in Table 1), which was homozygous TGG-AGG missense mutation in exon 3 changing Trp^{94} to Arg. No mutation in *ATP11* and *ATP12* was present in the second case. Similarly, we were unable to find any mutation in other cases that have been analysed (patients II–V in Table 1). Moreover, we were also unable to detect any decrease in *ATP11* and *ATP12* transcripts in these patients that would indicate their decreased content. Taken together, the available data suggest an involvement of another, yet unidentified ATPase assembly factor. This view might also be supported by the fact that the phenotype of the patient with *ATP12* mutation—marked brain atrophy, significantly differs from other patients presenting with cardiomyopathy (Table 1).

7. Biochemical changes in nuclear ATPase defects

Pronounced reduction of ATPase content in patient's fibroblasts strongly decreased the synthesis of ATP, ADP-stimulated respiration, and the discharge of mitochondrial membrane potential which showed even higher steady state values than the control cells [22,27,28]. However, even in cells with 90% decrease of ATPase content, a significant ATP production could be seen [28]. As apparent from inhibitor titration studies [45,46], individual OXPHOS enzymes of mitochondrial energy metabolism can be inhibited to a certain extent without noticeable reduction of the mitochondrial coupled respiration rate. These threshold effects are different for individual OXPHOS complexes and they also display tissue specificity. In the case of ATPase, some 10% of normal activity of the enzyme was found to be sufficient for 30–60% functionality of the whole respiratory chain, depending on the type of tissue. This would mean that, with respect of mitochondrial energy

provision, a significant decrease of ATPase capacity can be tolerated, at least under conditions when the energetic demands of the patient's organism are rather low.

For the pathogenetic mechanism of ATPase deficiency thus may be more relevant the mitochondrial ROS production related to the higher levels of $\Delta\Psi_m$, observed in patient's fibroblasts. An exponential increase of mitochondrial ROS production occurs at high levels of $\Delta\Psi_m$ above 140 mV [47,48]. On the contrary, decrease of $\Delta\Psi_m$ via stimulation of ATP synthase activity, a low ATP/ADP ratio, and substrate limitation or increased proton permeability due to external or internal uncoupling lower the amount of

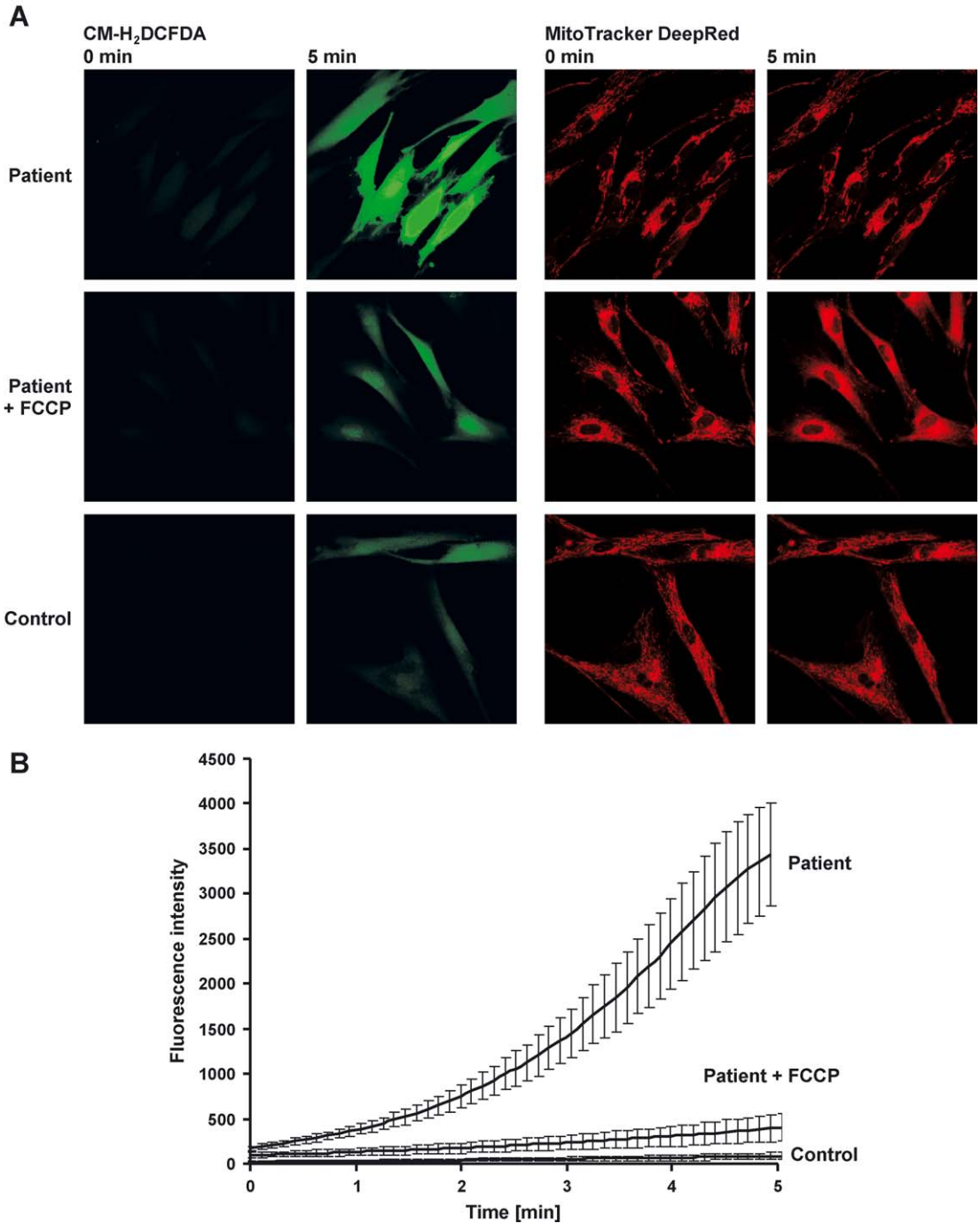

Fig. 4. ROS production analysed by confocal microscopy in ATPase-deficient cells. Intact fibroblasts (patient IV in Table 1) were labelled with 1 μM CM-H_2DCFDA (5-(and-6)-chloromethyl-2′,7′-dichlorodihydrofluorescein diacetate) and 50 nM Mitotracker DeepRed and analysed on a Leica TCS SP2 microscope. (A) Increase in green fluorescence serves as a measure of ROS production in cell, as non-fluorescent CM-H_2DCFDA is oxidized by ROS to fluorescent CM-DCFDA (fluorescein derivative). (B) Quantification of confocal images is shown. To compensate for mitochondrial content in patient and control cells, CM-DCFDA fluorescence was related to Mitotracker fluorescence.

ROS produced (see Ref. [49]). Our direct measurements in intact fibroblasts from patients with ATPase deficiency revealed pronounced activation of ROS production that was prevented by an uncoupler (Fig. 4). Interestingly, cells with *ATP6* mutations also show an altered discharge of $\Delta\Psi_m$ [22,50]. Therefore, it appears that the mitochondrial membrane potential is generally increased in both types of ATPase defects, irrespective of whether the inner mitochondrial membrane contains decreased amounts of normal ATPase complexes or normal content of altered ATPase complexes that are unable to operate as ATP synthase. In both situations, therefore, up-regulation of ROS production is to be expected to play a key role in the pathogenesis of the disease.

8. Conclusions

Involvement of nuclear genes in the pathogenesis of primary ATPase defects resulting in severe mitochondrial encephalo-cardiomyopathies may be rather complex. While selective ATPase deficiency is caused by a dysfunction of F_1-specific, nuclear-encoded assembly factor(s), other nuclear genes are likely to be involved in transmission and manifestation of mtDNA mutations in the *ATP6* gene that impair the function of the ATPase proton channel.

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