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Mitochondrial Calcium Signalling: Role in Oxidative Phosphorylation Diseases

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1. Introduction

Mitochondria are double membrane-bound organelles that not only constitute the "cellular power plants" but also are crucially involved in cell survival, apoptosis, redox control, Ca^{2+} homeostasis and many metabolic and biosynthetic pathways.

The mitochondria generate energy by oxidizing hydrogen derived from dietary carbohydrate (TCA: tricarboxylic acid cycle) and lipids (beta-oxidation) with oxygen to generate heat and energy in the form of ATP (Adenosine triphosphate). Energy generation in mitochondria occurs primarily through oxidative phosphorylation (OXPHOS), a process in which electrons are passed along a series of carrier molecules called the electron transport chain (ETC). This chain is composed of four multisubunit assemblies that are embedded in the mitochondrial inner membrane: complex I (NADH:ubiquinone oxidoreductase; EC 1.6.5.3), complex II (succinate:ubiquinone oxidoreductase; EC 1.3.5.1), complex III (ubiquinol:cytochrome-*c* oxidoreductase; EC 1.10.2.2) and complex IV (cytochrome-*c* oxidase; EC1.9.3.1). Complexes I, III and IV actively translocate protons from the matrix into the intermembrane space using energy extracted from electrons passing through the chain. These electrons are liberated from NADH and $FADH_2$, at complexes I and II, respectively, where they are donated to the lipophilic electron carrier coenzyme Q for further transport to complex III. From there, electrons are shuttled to complex IV by cytochrome-*c*. At this complex, electrons are finally used for the reduction of oxygen to water (Hatefi, 1985; Saraste, 1999) (Figure 1 A).

The energy released by the flow of electrons through the ETC and the flux of protons out of the mitochondrial inner membrane creates a capacitance across the mitochondrial inner membrane, the electrochemical gradient (ΔP) composed of an electrical potential ($\Delta\psi$) and a concentration ratio (ΔpH). The potential energy stored in ΔP is coupled to ATP synthesis by complex V (F_0/F_1-ATP-synthase; EC 3.6.1.34). As protons flow back into mitochondrial

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matrix through complex V, ADP and Pi are bound, condensed and released as ATP. With Complex V, the ETC complexes constitute the OXPHOS system. The OXPHOS system generates the vast majority of cellular ATP during oxidative metabolism. Some of the ATP is used for the mitochondrion's own needs, but most of it is transported outside the organelle by the adenine nucleotide translocator (ANT) and used for diverse cell functions (Hatefi, 1985; Saraste, 1999) (Figure 1 A).

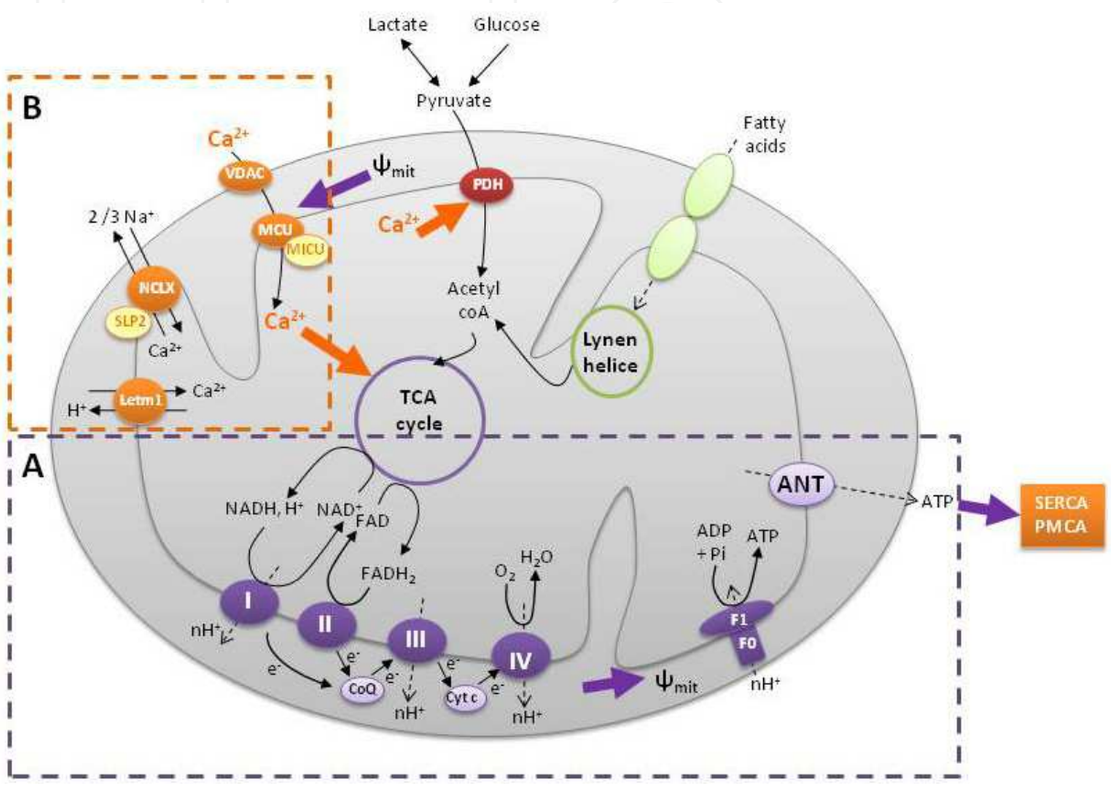

Fig. 1. Schematic view of of mitochondrial OXPHOS system (A), and. mitochondrial Ca^{2+} influx and efflux mechanisms (B). Large size arrows indicate the interplay between mitochondrial Ca^{2+} signaling and OXPHOS.

2. Genetics and pathology of OXPHOS

2.1 Genetics of OXPHOS system

The OXPHOS system is composed of more than 80 different proteins, 13 of which are encoded by the mitochondrial DNA (mtDNA) and the others by the nuclear genome (nDNA) (Chinnery & Turnbull, 2001; Wallace, 1992). There are seven mtDNA-encoded subunits in complex I, one in complex III, three in complex IV and two in complex V. Complex II consists solely of nDNA-encoded subunits.

The structure of human mitochondrial DNA (mtDNA) was reported ≈ 30 years ago (Clayton & Vinograd, 1967). The transcription products of mtDNA include 2 ribosomal RNA species (12S and 16S rRNA), 13 messenger RNAs and 22 transfer RNAs. Replication of mtDNA occurs independently from cell cycle phase and from replication of nuclear DNA. Mitochondrial DNA is present in 10^3–10^4 identical copies in each cell, with the exception of

sperm and mature oocytes, in which mtDNA copy numbers are ≈ 10^2 and ≈ 10^5, respectively. In general, there are believed to be two to ten copies of DNA per mitochondrion. The sequences of mtDNAs from unrelated individuals in human populations typically differ by about 0.3 %. Most individuals, however, have a single mtDNA sequence variant in all their cells (homoplasmy). mtDNA transmission occurred exclusively through the maternal lineage.
Almost all of the nDNA-encoded OXPHOS subunits have been characterized at the cDNA level and several at the genomic level in humans. In general, the chromosomal distribution of the genes seems to be random, and expression of most gene products is ubiquitous but predominates in tissues or organs with a high energy demand.
Richard Scarpulla and co-workers have provided important insight into the regulatory mechanisms that are involved in the transcriptional control of OXPHOS genes (Gugneja et al., 1996; Huo & Scarpulla, 1999; Wu et al., 1999). They identified the nuclear respiratory factors NRF1 and NRF2, which act on overlapping subsets of nuclear genes that are involved in the biogenesis of the respiratory chain. Recent mammalian studies have identified PGC1 as a crucial regulator of cardiac mitochondrial number and function in response to energy demand (Lehman et al., 2000). Analysis of the expression pattern of OXPHOS genes revealed that their regulation might also be exerted post-transcriptionally (Di Liegro et al., 2000).

2.2 OXPHOS diseases

Among the inborn errors of metabolism, mitochondrial disorders are the most frequent with an estimated incidence of at least 1 in 10,000 births (reviewed in (Smeitink et al., 2001)). Although the term mitochondrial disorder is very broad, it usually refers to diseases that are caused by disturbances in the OXPHOS system. After the first description, ≈ 40 years ago, of a patient with "loose coupling" — a defect in the coupling between mitochondrial respiration and phosphorylation — by Luft and collaborators (Luft et al., 1962), thousands of patients have been diagnosed by measurement of OXPHOS-system enzyme activities. The great complexity of the OXPHOS system, which consists of proteins, some encoded by the mitochondrial genome and others by the nuclear genome, may explain the wide variety of clinical phenotypes that are associated with genetic defects in oxidative phosphorylation. Disease-causing defects can occur in a single OXPHOS complex (isolated deficiency) or multiple complexes at the same time (combined deficiency). OXPHOS diseases give rise to a variety of clinical manifestations, particularly in organs and tissues with high-energy demand such as brain (encephalopathies), heart (cardiomyopathies), skeletal muscle (myopathies) and liver (hepatopathies) (reviewed in (Finsterer, 2006a, 2006b; Schaefer et al., 2004)).
We have also to consider the presence of fundamental differences between mitochondrial genetics and Mendelian genetics when studying human OXPHOS diseases. These differences are linked to maternal inheritance of mtDNA, polyplasmy, heteroplasmy and the threshold effect, whereby a critical number of mutated mtDNAs must be present for the OXPHOS system to malfunction (Wallace, 2005).
One of the frequent OXPHOS disorders is Leigh Syndrome (OMIM 256000), an early-onset progressive neurodegenerative disorder, leading to death mostly within a few years after the onset of the symptoms. This disorder is characterized by lesions of necrosis and capillary proliferation in variable regions of the central nervous system. Clinical signs and symptoms comprise muscular hypotonia, developmental delay, abnormal eye movements, seizures, respiratory irregularities and failure to thrive. Other mitochondrial disorders caused by

OXPHOS defects include Mitochondrial Encephalopathy, Lactic Acidosis and Stroke-like episodes (MELAS; OMIM 540000), Myoclonic Epilepsy with Ragged Red Fibers (MERRF; OMIM 545000), Neurogenic weakness, Ataxia, Retinitis Pigmentosa/Maternally Inherited Leigh Syndrome (NARP/MILS; OMIM 516060), Leber's Hereditary Optic Neuropathy (LHON; OMIM 535000), and Mohr–Tranebjaerg syndrome (a.k.a. Deafness Dystonia Syndrome; OMIM 304700).

Because of the genetic complexity of the energy-generating system, many other diseases have been shown to be associated with defect in mitochondrial function (DiMauro & Moraes, 1993; DiMauro & Schon, 2003). For example, there is increasing evidence that inherited OXPHOS dysfunction is also implicated in diabetes, age-related neurodegenerative diseases, such as Parkinson's, Alzheimer and Huntington's diseases, and various forms of cancers (Shoubridge, 2001; Zeviani & Carelli, 2007).

2.2.1 Mitochondrial DNA mutations linked to OXPHOS diseases

The complexity of mitochondrial DNA mutations linked to OXPHOS diseases is that one mutation can cause a broad spectrum of clinical manifestations. Conversely, different mutations can be associated with the same clinical phenotype. Specific phenotypes include forms of blindness, deafness, movement disorders, dementia, cardiovascular diseases, muscle weakness, renal dysfunction, and endocrine disorders including diabetes. In the past 20 years, more than 100 point mutations and innumerable rearrangements have been associated with human mitochondrial diseases. In this context, it is worth mentioning, however, that we still lack comprehensive and unbiased epidemiological data about the frequency of known mtDNA mutations. Although tRNA genes as a whole represent ≈ 10% of the mtDNA, mutations in these genes account for ≈ 75% of mtDNA-related diseases.

We can identify three categories of pathogenic mtDNA mutations: rearrangement mutations, polypeptide gene missense mutations, and protein synthesis (rRNA and tRNA) gene mutations (reviewed in (Wallace, 2005)).

- Rearrangement mutations of mtDNA can be either inherited or spontaneous. Inherited mtDNA rearrangements are primarily insertions. The first inherited insertion mutation to be identified caused maternally inherited diabetes and deafness (Ballinger et al., 1992, 1994). Spontaneous mtDNA deletions result in a related spectrum of symptoms, irrespective of the position of the deletion end points. This is because virtually all deletions remove at least one tRNA and thus inhibit protein synthesis (Moraes et al., 1989). Thus the nature and severity of the mtDNA deletion rearrangement is not a consequence of the nature of the rearrangement, but rather of the tissue distribution of the rearranged mtDNAs.
- Missense mutations in mtDNA polypeptide genes can also result in an array of clinical manifestations. Three relatively frequently observed point mutations are A3243G in the tRNA(Leu)(UUR) gene, A8344G in the tRNA(Lys) gene and T8993G in the ATPase 6 gene and are associated with NARP when present at lower percentage of mutants or with lethal Leigh syndrome when present at higher percentage of mutants (Holt et al., 1990; Tatuch et al., 1992). Mutations have also been identified in mtDNA genes that encode proteins of the OXPHOS system, such as the cytochrome b gene and the mitochondrial complex I genes. A prominent example of the latter group of mtDNA protein-coding gene mutations is LHON, which is a common cause of subacute bilateral optic neuropathy that usually presents in early adult life and that predominantly affects

males. Most LHON patients harbor one of three point mutations that affect mtDNA complex I, or the NADH:ubiquinone oxidoreductase (ND) genes: G3460A in ND1, G11778A in ND4 and T14484C in ND6. Patrick Chinnery and colleagues showed that the mitochondrial ND6 gene is a hot spot for LHON mutations and suggested that the ND6 gene should be sequenced in all LHON patients who do not harbour one of the three common LHON mutations (Chinnery et al., 2001). Rare nonsense or frameshift mutants in Cytochrome oxydase subunit I (COI) have been associated with encephalomyopathies (Bruno et al., 1999; Comi et al., 1998).

- Pathogenic mtDNA proteins synthesis mutations can also result in multisystem disorders with wide range of symptoms. The most common mtDNA protein synthesis mutation is A3243G in the tRNA(Leu). This mutation is linked to a variety of clinical symptoms. When present at relatively low level (10%-30%) in the blood, the patient may manifest only type II diabetes. By contrast, when the mutation is present in > 70% of the mtDNA, it causes more severe symptoms including short stature, cardiomyopathy, Chronic Progressive External Ophthalmoplegia (CPEO; OMIM157640) and MELAS (Goto et al., 1990; van den Ouweland et al., 1994).

2.2.2 Nuclear DNA mutations linked to OXPHOS diseases

Nuclear DNA mutations linked to OXPHOS diseases includes defects in structural OXPHOS genes, faulty inter-genomic communication, and defects in OXPHOS assembly, homeostasis and import. Most nuclear gene mutations affect various protein subunits of complex I and complex II.

The first structural OXPHOS-gene mutation was reported in two sisters with Leigh syndrome and isolated complex II deficiency (Bourgeron et al., 1995). The pathogenic mutation was in the gene that encodes the flavoprotein: SDHA (succinate dehydrogenase subunit A). Subsequently, another family was found to have mutations in this subunit (Parfait et al., 2000). Very interestingly, two groups independently reported mutations of the complex II subunit D and C genes in hereditary paraganglioma — usually benign, vascularized tumours in the head and in the neck (Baysal et al., 2000; Niemann & Muller, 2000). This work has uncovered a new and surprising association between mitochondrial defects and carcinogenesis. Genetic characterization of Complex I deficiency in a patient with a Leigh-like presentation revealed a 5-base-pair (bp) duplication in NDUFS4 (NADH dehydrogenase (ubiquinone) Fe–S protein 4) that destroys the consensus phosphorylation site in the gene product and extends the length of the protein by 14 amino acids (van den Heuvel et al., 1998). Further studies have revealed that this duplication abolishes cyclic-AMP-dependent phosphorylation of NDUFS4, thereby impairing activation of the complex. Further complex I mutations have been identified and ≈ 40% of complex I deficiencies in children, in which the defect is detected in cultured skin fibroblasts, can now be explained by mutations in structural nuclear genes (Loeffen et al., 1998, 2000).

OXPHOS defects caused by defective interplay between the mitochondrial and nuclear genomes have also been described. The clinical features of the Mitochondrial Neuro-GastroIntestinal Encephalomyopathy syndrome (MNGIE) include ophthalmoparesis, peripheral neuropathy, leucoencephalopathy and gastrointestinal symptoms (chronic diarrhea and intestinal dysmotility). Muscle biopsy shows ragged red fibers (RRFs) and COX-negative fibers and either partial isolated complex IV deficiency or combined OXPHOS-complex deficiencies (Hirano et al., 1994). Mitochondrial DNA analysis in this

autosomal recessive syndrome showed mtDNA deletions, depletion, or both. The MNGIE locus was mapped to chromosome 22q13.32-qter, a region that contains the thymidine phosphorylase (TP) gene (gene symbol ECGF1). Studies on patients showed that TP activity was markedly decreased. Ichizo Nishino and collaborators found various homozygous as well as compound heterozygous ECGF1 mutations in the genomic DNA of MNGIE patients (Nishino et al., 1999). The precise mechanism by which TP deficiency leads to mtDNA rearrangements have still to be explained, but imbalance of the mitochondrial nucleotide pool is likely to have a role. Autosomal dominant Progressive External Ophtalmogia (adPEO) is an adult-onset mitochondrial disorder that is characterized by progressive external ophthalmoplegia and variable additional features, including exercise intolerance, ataxia, depression, hypogonadism, hearing deficit, peripheral neuropathy and cataract (Zeviani et al., 1990). Some patients carry mtDNA deletions, although the disease is inherited in an autosomal fashion. Of the two autosomal loci for this disorder, the 4q-adPEO locus includes the gene for the heart and skeletal muscle isoform of the ANT1. Kaukonen and collaborators (Kaukonen et al., 2000) identified two heterozygous missense mutations in this gene in several families and in one sporadic patient with adPEO.

Enzyme complex I and IV deficiencies are by far the most frequently observed abnormalities of the OXPHOS system. In sharp contrast to isolated complex I deficiencies, no mutations have been found as yet in the ten nuclear genes that encode the structural proteins of complex IV (Adams et al., 1997). The discovery of mutations in a nuclear assembly gene that is associated with COX deficiency resulted from chromosomal transfer experiments. This approach identified mutations in the SURF1 gene in patients with COX-deficient Leigh syndrome (Tiranti et al., 1998; Zhu et al., 1998). SURF1 is part of a cluster of unrelated housekeeping genes and is the only gene of this cluster that is known or believed to be involved in COX assembly (Tiranti et al., 1999). Nuclear gene defects that are associated with isolated complex III or complex V deficiencies have not yet been discovered. In recent years, four inherited neurodegenerative diseases, Friedreich ataxia, hereditary spastic paraplegia, human DDP syndrom (deafness/dystonia peptide) and dominant optic atrophy (OPA1) have also been shown to be mitochondrial disorders that are caused by nuclear DNA mutations in the genes for frataxin, paraplegin, DDP and OPA1, respectively. Mitochondria obtained from heart biopsies of Friedreich ataxia patients disclosed specific defects in the citric-acid cycle enzyme aconitase, and complex I–III activities (Rotig et al., 1997). The causative Friedreich ataxia protein, frataxin, has an essential role in mitochondrial iron homeostasis, and Friedreich ataxia can therefore be considered as an OXPHOS homeostasis defect. Muscle biopsies from the autosomal recessive form of patients with hereditary spastic paraplegia revealed histochemical signs of a mitochondrial disorder, namely RRFs, COX-negative fibers and succinate dehydrogenase-positive hyperintense fibers (Casari et al., 1998). Linkage and subsequent mutation analysis revealed large deletions in a gene dubbed paraplegin (Casari et al., 1998). Owing to the homology with a yeast mitochondrial ATPase with both proteolytic and chaperone-like activities, it has been suggested that this form of hereditary spastic paraplegia could be a neurodegenerative disorder due to OXPHOS deficiency, attributing a putative function in the assembly or import of respiratory chain subunits or cofactors to paraplegin (Di Donato, 2000). The DDP syndrome, an X-linked recessive disorder also known as the Mohr–Tranebjaerg syndrome, is associated with a novel defect in mitochondrial protein import (Koehler et al., 1999). The defective gene is homologous to the yeast protein Tim8, which belongs to a family of

proteins that are involved in intermembrane protein transport in mitochondria. Therefore, the DDP syndrome should be considered as the first example of a new group of mitochondrial import diseases (Koehler et al., 1999). Finally, OPA1 is caused by defects in a dynamin-related protein that is targeted to mitochondria and might exert its function in mitochondrial biogenesis and in stabilization of mitochondrial membrane complexes (Delettre et al., 2000).

3. Models to study OXPHOS diseases

3.1 Cybrids and Rho^0 cells

Cybrids, or "cytoplasmic hybrids," are cultured cells manipulated to contain introduced mitochondrial DNA (mtDNA). Cybrids have been a central tool to unravel effects of mtDNA mutations in OXPHOS diseases. In this way, the nuclear genetic complement is held constant so that observed effects on OXPHOS can be linked to the introduced mtDNA. The cybrids are produced by first treating mitochondrial donor cells with cytochalasin B to weaken the cytoskeleton, before subjecting the cells to a centrifugal force, either as attached cells or in suspension. The dense nuclei are extruded, leaving plasma membrane-bound "cytoplasts" containing cell cytoplasm and organelles, including mitochondria. These cytoplasts are then fused with a nuclear donor cell line. The first mammalian cultured cell phenotype identified to segregate with mtDNA was in human (HeLa) cells, where mtDNA imparted resistance to the antibiotic chloramphenicol (Spolsky & Eisenstadt, 1972). Several other mtDNA-linked drug-resistant phenotypes were identified in mammalian cells in the 1970s and 1980s, including resistance to the complex III inhibitors antimycin and myxothiazol (Howell & Gilbert, 1988) and to the complex I inhibitor rotenone (Bai & Attardi, 1998). The development of robust DNA-sequencing methods leads to the identification of single-base substitutions in the 16S rRNA gene of the mtDNA of independently derived yeast, mouse, and human chloramphenicol cell lines (Blanc & Dujon, 1980; Kearsey & Craig, 1981). These pioneering studies were in turn followed by identification of the first cytochrome b mutants (Howell & Gilbert, 1988) and more recently ND5, ND6, and COI mutants.

The second cellular OXPHOS model corresponds to the isolation of a human cell line without mtDNA (called Rho^0 cells). Employing an approach first used in yeast (Slonimski et al., 1968), cells were incubated with low levels of the drug ethidium bromide, which intercalates DNA. Low levels of the drug selectively inhibits the gamma-DNA polymerase responsible for mtDNA replication, and with ongoing cell division, the mtDNAs are ''diluted'' to the point where clones can be isolated without detectable organelle genomes. King and Attardi (King & Attardi, 1989) also discovered the absolute requirement for pyruvate gained by these cells and confirmed the previous observation from Paul Desjardins and collaborators (Desjardins et al., 1985) that mtDNA-less cells also required added uridine for growth. This allowed a selection regime to be used after cytoplast– Rho^0 cell fusion so that unfused Rho^0 cells could be eliminated and cybrids selected with the use of an appropriate nuclear drug-resistant marker (King & Attardi, 1989). Apart from their value in cybrid experiments, such Rho^0 cells represent a unique research tool by themselves. They are a surprising reminder that OXPHOS is dispensable, at least for some differentiated mammalian cell types. In this instance, ATP production is 100% from glycolysis, so the cells acidify culture media very rapidly by producing large quantities of lactate. They retain functional mitochondria (except lacking OXPHOS), which show a transmembrane potential

(probably from the electrogenic exchange of ATP for ADP) and can import the hundreds of other proteins needed for non-OXPHOS functions. The pioneering cybrid work using the selectable markers was limited in the sense that endogenous mtDNAs were also present; that is, the cybrids were heteroplasmic. The Rho^0 cell approach allowed creation of homoplasmic or heteroplasmic cells, depending on the mtDNA donor cell(s) used.

3.2 Human fibroblasts

The use of individual patient's cells in tissue cultures enables the study of specific defects. With respect to cell type, myoblasts are most likely to express the phenotype observed in muscle, but it is generally not feasible to derive myoblasts for each diagnostic muscle biopsy, because most of the muscle tissue is used up for enzymatic, pathological and molecular workup. Moreover, myoblasts are not representative of some liver-specific phenotypes. An alternative to myoblasts, are fibroblasts, which are much easily obtained during a muscle biopsy or after (Robinson, 1996). Fibroblasts cultures are in general, the most obtainable and renewable source of cells for both diagnosis and research. The major drawback with fibroblasts in culture is that they sometimes fail to maintain the diseased phenotype. This is especially true for fibroblast cultures derived from tissue specific forms of mitochondrial diseases. Nevertheless, many patients do express mitochondrial dysfunction in primary fibroblasts albeit the defect is sometimes unmasked only under stressful growth conditions in culture media, devoid of glucose or serum (Iuso et al., 2006; Robinson, 1996; Taanman et al., 2003). Therefore, patient's fibroblast harboring nuclear encoded mutations can be a suitable tool to study OXPHOS diseases and a platform for the search for treatments by small molecules, using individual approaches tailored to a specific defect.

3.3 Mouse models

Despite some obvious limitations, our ability to mimic human disease in animal models is undoubtedly one of the most important technological breakthroughs in modern genetics.
Since the first knockout mice with impaired OXPHOS were generated in 1995 (reviewed in (Smeitink et al., 2001) and (Larsson & Rustin, 2001)), eight others have been described.
Classical knockout (KO) technology has been achieved for the manganese superoxide dismutase gene (SOD2) and the ANT1. These mice can be considered as secondary OXPHOS-deficient mice because the genes are only indirectly related to the OXPHOS system. SOD2 is an oxygen radical scavenger in the mitochondrial matrix, which acts as a first line of defense against the superoxide that is produced as a by-product of OXPHOS (Li et al., 1995). To gain further insight into the effects of the ANT1 mutation in particular, study the regulation of nuclear and mitochondrial genes in the skeletal muscle of mice KO of ANT1 (Murdock et al., 1999) revealed upregulation of 17 genes that fall into four categories: nuclear and mitochondrial genes that encode OXPHOS components; mitochondrial tRNA and ribosomal RNA genes; genes involved in intermediary metabolism; and an eclectic group of other genes, among which are genes previously unknown to be related to mitochondrial function.
Knockout mice for the mitochondrial transcription factor A (TFAM) can be considered primary OXPHOS mice, because TFAM has a direct role in the regulation of OXPHOS gene expression. Using a conditional knockout approach, three distinct TFAM knockout mice have been created (Larsson et al., 1998; Wang et al., 1999): one for skeletal and cardiac muscle; one for cardiac muscle alone; and one for pancreatic β-cells. TFAM is essential for

mitochondrial biogenesis and embryonic development, and the conditional knockouts have indicated that the OXPHOS system is crucial for normal heart function and insulin secretion. Five mouse models that were specifically designed to mimic isolated complex I deficiency in humans involve the NDUFS4 gene. This gene constitutes a mutational hotspot in humans. Four models are KO or conditioned KO for NDUFS4, the fifth one corresponds to a point mutation in NDUFS4. The whole-body and neuron-targeted NDUFS4 KO mice displayed small size and displayed weight loss. This was accompanied by ataxia, blindness, hearing loss, loss of motor skills and death from a fatal encephalomyopathy. The Purkinje cell specific KO mice only manifested mild behavioral and neuropathological abnormalities. Homozygote point mutation NDUFS4 mice were not viable, demonstrating that the presence of mutated NDUFS4 protein leads to a much more severe phenotype than complete absence of NDUFS4 (reviewed in (Roestenberg et al., 2011)).

Two mouse models for Friedreich ataxia have also been created (Puccio et al., 2001). Like the ANT1- and SOD2-deficient mice, these mice can also be considered as secondary OXPHOS-deficient mice. The frataxin-deficient mammals showed time-dependent iron accumulation and will allow the detailed study of the mechanism of frataxin involvement in iron metabolism and iron–sulphur biogenesis.

Finally, Jun-Ichi Hayashi's group, using a completely different approach, generated mice that carry large-scale mtDNA deletions (Inoue et al., 2000). Synaptosomes from mouse brains with naturally occurring somatic mtDNA mutations were fused with Rho^0 cells. Each fusion event introduced a variable number of mutant and wild-type mtDNAs, which then repopulate the Rho^0 cell, creating a cybrid cell line. Enucleated cybrid cells were fused to donor embryos and implanted in pseudo pregnant females. In this way, they generate heteroplasmic founder female animals in which mtDNA deletion transmission was obtained for three generations (Inoue et al., 2000).

4. Calcium signalling and mitochondrial OXPHOS physiology

Calcium (Ca^{2+}) is one of the most common second messengers in intracellular signalling networks. Periodic fluctuations in cytosolic calcium concentration ($[Ca^{2+}]_{cyt}$) is driven by electrical activation of voltage-gated Ca^{2+} channels (VGCC) or by agonist stimulation of plasma membrane receptors and the subsequent formation of Ca^{2+}-mobilizing second messengers, such as inositol 1,4,5-trisphosphate (IP_3). IP_3 binds to its receptor the IP_3R (inositol 1,4,5-trisphosphate) on the endoplasmic reticulum (ER) membrane leading to Ca^{2+} release from the ER to the cytosol. In excitable cells, Ca^{2+} release from the ER occurs also through ryanodine receptors (RyR) that function as Ca^{2+}-activated Ca^{2+} channels which further amplify Ca^{2+} signals originating from other sources.

The frequency, amplitude and/or duration of cytosolic $[Ca^{2+}]_{cyt}$ spikes can be detected and decoded by downstream Ca^{2+}-sensitive proteins providing a versatile pathway for extracellular stimuli to exert control over a wide range of metabolic pathways (Berridge et al., 2000).

Complex buffering systems that include multiple Ca^{2+}-buffering proteins, ATP-dependent Ca^{2+} pumps (SERCA (sarco-endoplasmic Reticulum Ca^{2+} ATPase) accumulating Ca^{2+} from the cytosol to the ER, and PMCA (Plasma membrane Ca^{2+} ATPase) extruding Ca^{2+} from cytosol to the extracellular space), and the sodium-Ca^{2+} exchanger (Na^+/Ca^{2+}), work together to restore $[Ca^{2+}]$ back to resting levels. Mitochondria also play an important role in shaping Ca^{2+} signals by utilizing potent mitochondrial Ca^{2+} uptake mechanisms. Ca^{2+}

uptake into mitochondria plays an important role in cellular physiology by stimulating mitochondrial metabolism and increasing mitochondrial energy production (Duchen, 1992). However, excessive Ca^{2+} uptake into mitochondria can lead to opening of a permeability transition pore (PTP) and apoptosis.

4.1 Interplay between Ca^{2+} and OXPHOS

Mitochondrial bioenergetics and Ca^{2+} shaping are mutually regulated. Indeed, on the one hand, mitochondria Ca^{2+} accumulation enables the activity of OXPHOS and ATP production; on the other hand, mitochondrial ATP favours the effective functioning of the two major Ca^{2+} pumps PCMA and SERCA and actively participates in shaping cytosolic Ca^{2+} signals (Figure 1 A and B).

One important target for Ca^{2+} signals is the activation of mitochondrial oxidative metabolism and the consequent increase in the formation of ATP. Studies performed in 1960-1970 led to the demonstration that four mitochondrial dehydrogenases are activated by Ca^{2+} ions. These are FAD-glycerol phosphate dehydrogenase, pyruvate dehydrogenase, NAD^+-isocitrate dehydrogenase and α-ketoglutarate dehydrogenase. FAD-glycerol phosphate dehydrogenase is located on the outer surface of the inner mitochondrial membrane and is influenced by changes in cytoplasmic Ca^{2+} ions concentrations. The other three enzymes are located within mitochondria and are regulated by matrix Ca^{2+} ions concentration. The effects of Ca^{2+} ions on FAD-isocitrate dehydrogenase involve binding to an EF-hand binding motif within this enzyme, leading to lowering of the K_m for glycerol phosphate very substantially (reviewed in (Denton, 2009)). Mitochondrial Ca^{2+} ions bind also directly to NAD^+-isocitrate dehydrogenase and α-ketoglutarate dehydrogenase to decrease the K_m for their respective substrates, whereas an increase in the dephosphorylated and active form of pyruvate dehydrogenase is regulated by a Ca^{2+}-sensitive phosphatase (Bulos et al., 1984; Denton & Hughes, 1978; Denton et al., 1972, 1978, 1996; McCormack et al., 1990; McCormack & Denton, 1979; Robb-Gaspers et al., 1998). Extramitochondrial Ca^{2+} regulates the glutamate-dependent state 3 respiration by the supply of glutamate to mitochondria via aralar, a mitochondrial glutamate/aspartate carrier (Gellerich et al., 2010).

A very recent finding suggests a novel paradigm in which the transcription of genes for mitochondrial enzymes that produce ATP and the genes that consume ATP is coordinately regulated by the same transcription factors (Watanabe et al., 2011). Thus, TFAM and TFB2M, recognized as mtDNA-specific transcription factors, were shown to regulate transcription of the SERCA2 gene (Watanabe et al., 2011).

It was also demonstrated that metabolites generated during energy production may influence IP_3R-mediated Ca^{2+} dynamics. Indeed, it was shown that reduced Nicotinamide adenine dinucleotide selectively stimulates the release of Ca^{2+} mediated by IP_3R (Kaplin et al., 1996). Another evidence of communication between cellular metabolism and Ca^{2+} signalling was reported recently by Bakowski and Parekh who showed that pyruvate, the precursor substrate for the Krebs cycle, directly increases the native I_{CRAC} (store operated Ca^{2+} influx channels at the plasma membrane) by reducing inactivation of the channel, thereby coupling oxidation of glucose and its own metabolism in the mitochondria to Ca^{2+} influx by the CRAC channel (Bakowski & Parekh, 2007).

In addition to serving as a target of Ca^{2+} signalling, the uptake of Ca^{2+} by mitochondria has important feedback effects to shape cytosolic Ca^{2+} signals. Rosario Rizzuto and collaborators (Rizzuto et al., 1993) were the first to make direct *in situ* measurements of mitochondrial

Ca^{2+}. They showed that receptor-activated Ca^{2+} signals caused rapid and large Ca^{2+} signals in the mitochondrial matrix (mechanisms of mitochondrial Ca2+ influx and efflux are detailed below).

4.2 Mechanisms of mitochondrial calcium influx and efflux

4.2.1 Mechanisms of mitochondrial calcium influx

Mitochondrial Ca^{2+} uptake is dependent on the strong driving force ensured by their membrane potential (-180 mV, negative inside) built by the respiratory chain (for review see (Bianchi et al., 2004)). It has been assumed that $[Ca^{2+}]_{cyt}$ far exceeding the micromolar range is required for net Ca^{2+} uptake, however, such $[Ca^{2+}]_{cyt}$ values have not been observed experimentally in the bulk cytoplasm. Ca^{2+} diffusion in the cytoplasm is also controlled by protein binding (Allbritton et al., 1992). Thus, local Ca^{2+} transients with amplitudes far exceeding those measured over the global cytoplasm are confined in cytosolic microdomains at the mouth of Ca^{2+} channels beneath the plasma membrane or ER internal store. This concept was consolidated by the demonstration that mitochondria, forming a complex cytoplasmic tubulovesicular system (Tinel et al., 1999), are frequently apposed to the smooth as well as the rough ER. These contact points, have been observed in several cell types by means of electron microscopy or tomography (Mannella et al., 1998). The experiments by Rosario Rizzuto and Tulio Pozzan definitively demonstrated that Ca^{2+} released through IP_3R in these microdomains, induce supramicromolar, or even submillimolar Ca^{2+} signals (Rizzuto et al., 1993).

Accordingly, the group of György Hajnoczky demonstrates that maximal activation of mitochondrial Ca^{2+} uptake is evoked by IP_3-induced perimitochondrial $[Ca^{2+}]$ elevations, which appear to reach values >20-fold higher than the global increases of $[Ca^{2+}]_{cyt}$. Incremental doses of IP_3 elicited $[Ca^{2+}]_{mit}$ elevations that followed the quantal pattern of Ca^{2+} mobilization, even at the level of individual mitochondria. These results and others by the same group allow concluding that each mitochondrial Ca^{2+} uptake site faces multiple IP_3R, a concurrent activation of which is required for optimal activation of mitochondrial Ca^{2+} uptake (Csordas et al., 1999; Hajnoczky et al., 1995) and reviewed in (Csordas et al., 2006). Targeting aequorin to the outer surface of the IMM in HeLa cells made the measurement of $[Ca^{2+}]$ in the mitochondrial intermembrane space possible. After stimulation with histamine $[Ca^{2+}]$ rose in the intermembrane space to significantly higher values than in the global cytosol (Rizzuto et al., 1998). This observation has given a strong support to the concept that net mitochondrial Ca^{2+} uptake occurs from high-Ca^{2+} peri-mitochondrial microdomains.

The existence of physical support for the ER–mitochondrial interface has been indicated by co-sedimentation of ER particles with mitochondria and electron microscopic observations of close associations between mitochondria and ER vesicles (Mannella et al., 1998; Meier et al., 1981; Shore & Tata, 1977). At these sites the shortest ER-OMM distance varies from 10 nm to 100 nm. In cells exposed to ER stress (serum starvation, tunicamycin) an increase in the ER–mitochondrial interface has been observed (Csordas et al., 2006). Also, coupling of the two organelles with a fusion protein increased the ER–mitochondria interface area, reduced the ER–mitochondrial distance to about 6 nm and greatly facilitated the transfer of cytosolic Ca^{2+} signal into the mitochondria of RBL-2H3 cells (Csordas et al., 2006). Accordingly, our team showed that the truncated variant of the sarco-endoplasmic

reticulum Ca^{2+}-ATPase 1 (S1T) is induced under ER stress conditions. S1T is localized in the ER-mitochondria microdomains, increases number of ER-mitochondria contact sites, and inhibits mitochondria movements thus determining a privileged Ca^{2+} transfer from the ER to mitochondria leading to the activation of the mitochondrial apoptotic pathway (Chami et al., 2008).

Mitochondrial fission and fusion is another essential phenomenon for maintaining the metabolic function of these organelles as well as regulating their roles in cell signalling (Tatsuta & Langer, 2008; Yaffe, 1999; Chan, 2006). Changes in the relative rates of fusion and fission alter the overall morphology of the mitochondria affecting the function of the organelles both as regulators of survival/apoptosis and in Ca^{2+} handling. It has been shown that fusion is blocked (Karbowski & Youle, 2003) and mitochondria become fragmented during apoptosis (Frank et al., 2001). However, enhanced fission alone does not induce apoptosis and has even been shown to protect against Ca^{2+}-dependent apoptosis by preventing the propagation of harmful Ca^{2+} waves through the mitochondrial reticulum (Szabadkai et al., 2004).

The outer mitochondrial membrane is permeable to solutes and the inner mitochondrial membrane is impermeable to solutes that harbor the respiratory chain complexes. As described in chapter 1, the respiratory chain pumps protons against their concentration gradient from the matrix of the mitochondrion into the inter-membrane space, generating an electrochemical gradient in the form of a negative inner membrane potential and of a pH gradient, the matrix being more alkaline than the cytosol (Bernardi et al., 1999; Poburko et al., 2011).

Ca^{2+} import across the outer mitochondrial membrane (OMM) occurs through the voltage-dependent anion channels (VDAC) (Simamura et al., 2008). VDAC is as a large voltage-gated channel, fully opened with high-conductance and weak anion-selectivity at low transmembrane potentials (< 20–30 mV), but switching to cation selectivity and lower conductance at higher potentials (Colombini, 2009; Shoshan-Barmatz et al., 2010). The precise mechanisms of VDAC conductance are however still under debate.

Ca^{2+} import across the inner mitochondrial membrane (IMM) occurs through a Ca^{2+}-selective channel known as the mitochondrial Ca^{2+} uniporter (MCU) (Kirichok et al., 2004). Electrophysiological recordings of mitoplasts, small vesicles of inner mitochondrial membrane, revealed that the MCU is a highly Ca^{2+}-selective inward-rectifying ion channel (Kirichok et al., 2004). The MCU has a relatively low Ca^{2+} affinity (Kd ~10 µM in permeabilized cells (Bernardi, 1999)). The activity of the MCU had been known for decades to be inhibited by ruthenium red and its derivative Ru360 (Vasington et al., 1972), but its molecular identity has only been unraveled very recently. It has been reported recently that the process of Ca^{2+} accumulation undergoes complex regulation by Ca^{2+} itself. Thus mitochondrial uptake of Ca^{2+} was significantly reduced by inhibitors of calmodulin, suggesting that a Ca^{2+}–calmodulin-mediated process is necessary for activation of the uniporter but Ca^{2+} also appeared to inhibit its own uptake. However, in contrast to the sensitization of mitochondrial Ca^{2+} uptake, the Ca^{2+}-dependent inactivation was not sensitive to calmodulin blockers (Moreau & Parekh, 2008).

In recent years, several molecules have been proposed to be either an essential or an accessory component of the MCU. In 2007, the uncoupling proteins (UCP) 2 and 3 (Trenker et al., 2007) were proposed to be essential for the MCU. Indeed, UCP2/3 overexpression

increased mitochondrial Ca^{2+} elevations and the contrary is observed upon UCP2/3 depletion. In addition, mice lacking UCP2 exhibited a reduced sensitivity to the Ca^{2+} uptake inhibitor ruthenium red. However, these findings were disputed by another study that reported normal mitochondrial Ca^{2+} uptake in mice genetically ablated for UCP2 and UCP3 (Brookes et al., 2008). Furthermore, it was recently showed that UCP3 modulates the activity of sarco/endoplasmic reticulum Ca^{2+} ATPases by decreasing mitochondrial ATP production (De Marchi et al., 2011). The mitochondrial Ca^{2+} alterations associated with changes in UCP3 levels therefore reflect the exposure of mitochondria to abnormal cytosolic Ca^{2+} concentrations and do not reflect changes in MCU activity. These data indicate that UCP3 is not the mitochondrial Ca^{2+} uniporter. In 2009, Jiang and collaborators identified the leucine zipper EF hand containing transmembrane protein 1(Letm1) as a molecule that regulate both mitochondrial Ca^{2+} and H^+ concentrations (Jiang et al., 2009). Letm1 was reported to be a high-affinity mitochondrial Ca^{2+}/H^+ exchanger able to import Ca^{2+} at low (i.e. sub-micromolar) cytosolic concentrations into energized mitochondria. Earlier studies had however linked Letm1 to mitochondrial K^+/H^+ exchange and to the maintenance of ionic mitochondrial balance, the integrity of the mitochondrial network and cell viability (Dimmer et al., 2008; Nowikovsky et al., 2004). The high-affinity of Letm1 for Ca^{2+} and its postulated $1Ca^{2+}/1\ H^+$ stoichiometry are at odds with the known properties of the MCU. Thus, Letm1 is not the dominant mechanism of mitochondrial Ca^{2+} uptake. Instead, Letm1 might contribute to an alternate mode of mitochondrial Ca^{2+} uptake, known as rapid mode of uptake (RaM), that was first reported in isolated rat liver mitochondria by Gunter's group. It was reported that mitochondrial Ca^{2+} sequestration via a the RaM occurred at the beginning of each pulse and was followed by a slower Ca^{2+} uptake characteristic of the MCU (Sparagna et al., 1995; Szabadkai et al., 2001). The implications of the coexistence of low and high-affinity modes of Ca^{2+} uptake have been recently reviewed (Santo-Domingo & Demaurex, 2010).

In 2010, Palmer and Mootha reported that a new mitochondrial EF hand protein MICU1 (for mitochondrial Ca^{2+} uptake 1) was required for high capacity mitochondrial Ca^{2+} uptake, and proposed that MICU1 acts as a Ca^{2+} sensor that controls the entry of Ca^{2+} across the uniporter (Perocchi et al., 2010). Building up on this discovery, the same group and another simultaneously identified the mitochondrial Ca^{2+} uniporter (Baughman et al., 2011; De Stefani et al., 2011). Using *in silico* analysis combined with phylogenetic profiling and analysis of RNA and protein co-expressed with MICU1, the group of Vamsi Mootha isolated a novel protein that co-immunoprecipitated with MICU1 (Baughman et al., 2011). Using the same database, the group of Rosario Rizzuto independently identified the same protein. From the 14 proteins characterized by two or more transmembrane domains and known to exhibit or lack uniport activity domains, these authors identified a protein with a highly conserved domain encompassing two transmembrane regions separated by a loop bearing acidic residues. Functional analysis confirmed that this protein behaves as expected for the mitochondrial uniporter, and it was therefore assigned the defining name of MCU. Mitochondrial Ca^{2+} uptake was strongly reduced by MCU silencing in cultured cells and in purified mouse liver mitochondria, whereas MCU overexpression enhanced ruthenium red-sensitive mitochondrial Ca^{2+} uptake in intact and permeabilized cells (De Stefani et al., 2011). The MCU is a 45 kDa protein that can forms oligomers (Baughman et al., 2011). Both studies mapped the MCU to the inner mitochondrial membrane, but disagreed on whether the N and C termini face the matrix of the inter-membrane space (Baughman et al., 2011; De

Stefani et al., 2011). Mutations of conserved acidic residues within the short sequence linking the two transmembrane domains abrogated the ability of MCU to reconstitute mitochondrial Ca^{2+} uptake, whereas mutation of a nearby serine residue (S_{259}) conferred resistance to Ru360, indicating that the acidic residues are required for Ca^{2+} uptake and that S_{259} is critical for MCU sensitivity to ruthenium red (Baughman et al., 2011). Finally, and most convincingly, expression of the purified protein in planar lipid bilayers was sufficient to reconstitute ion channel activity in solutions containing only Ca^{2+} (De Stefani et al., 2011). The currents were carried by a channel of small conductance (6–7 pS), fast opening/closing kinetics, and low opening probability, and were inhibited by ruthenium red, as expected for the MCU. Proteins mutated at two of the conserved acidic residues failed to generate Ca^{2+} currents when inserted into bilayers and acted as dominant negative when expressed in HeLa cells. These data clearly identified MCU as mitochondrial Ca^{2+} uniporter. In accordance to the notion that mitochondrial Ca^{2+} overload enhances the sensitivity to apoptosis, it was also demonstrated that cells overexpressing MCU were more sensitive to apoptosis after treatment with ceramide and H_2O_2 (De Stefani et al., 2011) (Figure 1B).

4.2.2 Mechanisms of mitochondrial calcium efflux

Compared to the MCU, the proteins that catalyze the efflux of Ca^{2+} from mitochondria have received much less attention. The extrusion of Ca^{2+} from mitochondria is coupled to the entry of Na^+ across an electrogenic $1Ca^+$:$3Na^+$ exchanger (Dash & Beard, 2008) that is inhibited by the benzothiazepine derivative CGP37157 ((Cox et al., 1993), and reviewed in (Bernardi, 1999)). The subsequent efflux of sodium ions by the mitochondrial $1Na^+$:$1H^+$ exchanger (mNHE) eventually results in the entry of three protons into the matrix for each Ca^{2+} ion that leaves mitochondria. Ca^{2+} extrusion thus has a high energetic cost, as it dissipates the proton gradient generated by the respiratory chain (reviewed in (Bernardi, 1999)). The molecule catalyzing mitochondrial Na^+/Ca^{2+} exchange has been recently identified as NCLX/NCKX6, a protein localized in mitochondrial cristae (Palty et al., 2010), whereas stomatin-like protein 2 (SLP-2), an inner membrane protein, was shown to negatively modulate the activity of the mitochondrial Na^+/Ca^{2+} exchanger (Da Cruz et al., 2010). Functional evidence from knock-down and overexpression studies indicate that NCLX is an essential part of the mitochondrial sodium Ca^{2+} exchanger whereas SLP-2 is an accessory protein that negatively regulates mitochondrial Ca^{2+} extrusion (Figure 1B).

4.2.3 Mitochondrial calcium overload: Activation of the permeability transition pore

When mitochondrial Ca^{2+} loads exceed the buffering capacity of inner membrane exchangers, an additional pathway for Ca^{2+} efflux from mitochondria may exist through opening of the permeability transition pore (PTP). The PTP is a voltage-dependent, cyclosporin A (CsA)-sensitive, high-conductance channel of the inner mitochondrial membrane (for reviews, see (Bernardi et al., 2006; Rasola & Bernardi, 2007)). Indeed, the interplay between the rate of mitochondrial Ca^{2+} influx and efflux modulates mitochondrial matrix Ca^{2+}, which in turn is widely considered to be a key factor for the regulation of the PTP open–closed transitions (Bernardi et al., 1999). Although opening of the PTP in response to Ca^{2+} has been documented in isolated mitochondria and permeabilized cells (Bernardi et al., 2006; Rasola & Bernardi, 2007), assessing opening of the PTP in intact neurons and other

primary cells in response to physiological activators that dictate cytosolic Ca^{2+} has remained a major challenge. Yet, opening of the PTP is often thought to be associated with pathophysiological processes (for reviews see (Hajnoczky et al., 2006; Rizzuto et al., 2003)). In these scenarios, activation of the PTP leads to respiratory inhibition, and thus ATP depletion, and the release of mitochondrial Ca^{2+} stores and apoptotic activators, ultimately resulting in cell death (Bernardi et al., 1999; Di Lisa & Bernardi, 2009). These have led to the idea that opening of the PTP by elevated mitochondrial Ca^{2+} is a terminal, pathologic event. However, it has been reported recently that CyPD-dependent PTP may participate in non-lethal Ca^{2+} homeostasis in cells and neurons (Barsukova et al., 2011).

5. Calcium deregulation in OXPHOS diseases

The direct consequences of OXPHOS defects include alteration of mitochondrial membrane potential, ATP/ADP ratio, ROS production and mitochondrial Ca^{2+} homeostasis. The varied biochemical changes that occur in cases of OXPHOS deficiencies have a direct effect on cellular functions. Yet, they are also key underlying mediators of the (retrograde) communication between the mitochondrion and the nucleus, which results in specific gene expression of both nuclear and mitochondrial genomes (see review (Reinecke et al., 2009)).

We will review in this chapter only Ca^{2+} deregulation in OXPHOS. We will discuss the consequences of such deregulation on mitochondrial function and the cross regulation between Ca^{2+} and bioenergetics in the development of cellular pathology. We summarized in Table 1 the alterations of subcellular Ca^{2+} signals in OXPHOS related diseases (Table 1).

Decreased proton pumping due to respiratory chain defects can result in reduced mitochondrial membrane potential and proton gradient, which are used to generate ATP. Deregulation of the membrane potential secondary to a deficiency in the respiratory chain may modify the kinetics and/or accumulation capacity of Ca^{2+} in the mitochondria, with possible consequences not only at the level of respiratory chain function (loop effect) and of the mitochondria in general, but also at the level of the ER function, which is largely dependent on Ca^{2+} concentrations, and at the level of cytosolic Ca^{2+} signalling, which plays a major role in regulating cell functions. Deficiencies of OXPHOS also result in other immediate and downstream metabolic, structural, and functional effects. These effects are closely associated with mitochondrial dysfunction. The nicotinamide dinucleotide (NAD) redox balance, which is converted to the reduced state in OXPHOS deficiencies, is a fundamental mediator of several biological processes, such as energy metabolism, Ca^{2+} homeostasis, cellular redox balance, immunological function, and gene expression (Munnich & Rustin, 2001; Ying, 2008).

It is important to mention that analyses of Ca^{2+} signalling targeting OXPHOS diseases are sporadic, partial and incomplete. This situation can be explained by : 1) the recent development of new techniques permitting detailed and specific subcellular Ca^{2+} analyses such as recombinant "aequorin" probes developed by the group headed by Professors Rizzuto and Pozzan, and the latest generation of GFP-based Ca^{2+} probes (camgaroos, cameleons and pericams) characterized by a great potential to analyse Ca^{2+} dynamics in mitochondria at the single cell level; 2) Absence of suitable “easy” study models (see chapter 3); and 3) the difficulty in the characterization of OXPHOS deficiencies (see chapter 2-2).

Disease	Gene	Mutation/ Deficiency	Study model	Mitochondrial/ Cellular pathology	Ca^{2+} deregulation	Ca^{2+} probe	References
MERRF	$tRNA_{Lys}$	nt 8356 T/C	Cybrids	↓ $[ATP]_{mit}$	↓ $[Ca^{2+}]_{mit}$; unchanged $[Ca^{2+}]_{cyt}$	Aequorin	(Brini, 1999)
NARP	*ATPase6*	nt 8993 T/G	Cybrids	↓ $[ATP]_{mit}$	Unchanged $[Ca^{2+}]_{mit}$ & $[Ca^{2+}]_{cyt}$	Aequorin	(Brini, 1999)
NARP	*ATPase6*	nt 8993 T/G Rho^0	Cybrids	Disturbed mitochondrial network and Actin cytoskeleton organization;	↓ Ca^{2+} influx in NARP & Rho^0	Fura 2, AM	(Szczepanowska, 2004)
MELAS	$tRNA_{Leu}$	nt 3243 A/G nt 3271 T/C nt 8344 A/G	Fibroblasts	↓Δ_{mit}	↑ baseline level of $[Ca^{2+}]_{cyt}$; ↓sequestration of $[Ca^{2+}]_{mit}$	Fura 2, AM	(Moudy, 1995)
MELAS	$tRNA_{Leu}$	nt 3243 A/G nt 3202 A/G Rho^0	Cybrids	Complex I, III, IV and V deficiencies; ↓Δ_{mit}	↑ time to clear up $[Ca^{2+}]_{cyt}$	Indo 1, AM ; Aequorin	(von Kleist-Retzow, 2007)
Leigh	NDUFS7	nt G364A	Fibroblasts	↓ $[ATP]_{mit}$	↓ $[Ca^{2+}]_{ER}$; ↓ $[Ca^{2+}]_{mit}$; ↓ $[Ca^{2+}]_{cyt}$	Fura 2, AM; Aequorin	(Visch, 2004)
NC	*NDUFS1*	nt G1854A nt C1668T	Fibroblats	↓ $[ATP]_{mit}$	↓ $[Ca^{2+}]_{mit}$; ↓$[Ca^{2+}]_{cyt}$; ↓ $[Ca^{2+}]_{ER}$	Fura 2, AM; Aequorin	(Visch, 2004)
NC	*NDUFS2*	nt T1237C	Fibroblasts	↓ $[ATP]_{mit}$	↓$[Ca^{2+}]_{cyt}$	Fura 2, AM; Aequorin	(Visch, 2004)
NC	*NDUFS2*	nt C686A	Fibroblasts	↓ $[ATP]_{mit}$	↓ $[Ca^{2+}]_{mit}$; unchanged $[Ca^{2+}]_{cyt}$ and $[Ca^{2+}]_{ER}$	Fura 2, AM; Aequorin	(Visch, 2006)
NC	*NDUFS2*	nt G683A	Fibroblasts	Unchanged $[ATP]_{mit}$	unchanged $[Ca^{2+}]_{mit}$, $[Ca^{2+}]_{cyt}$ and $[Ca^{2+}]_{ER}$	Fura 2, AM; Aequorin	(Visch, 2006)
NC	*NDUFS2*	NC	Fibroblasts	↓ $[ATP]_{mit}$	↓[$Ca^{2+}]_{mit}$; ↓ $[Ca^{2+}]_{cyt}$; ↓ $[Ca^{2+}]_{ER}$	Fura 2, AM; Aequorin	(Visch, 2006)
NC	*NDUFS2*	NC	Fibroblasts	Unchanged $[ATP]_{mit}$	unchanged $[Ca^{2+}]_{mit}$, $[Ca^{2+}]_{cyt}$ & $[Ca^{2+}]_{ER}$	Fura 2, AM; Aequorin	(Visch, 2006)
NC	*NDUFS4*	AAGTC471(1)	Fibroblasts	Unchanged $[ATP]_{mit}$	unchanged $[Ca^{2+}]_{mit}$, $[Ca^{2+}]_{cyt}$ & $[Ca^{2+}]_{ER}$	Fura 2, AM; Aequorin	(Visch, 2006)
NC	*NDUFS4*	nt C316T	Fibroblasts	↓ $[ATP]_{mit}$	↓[$Ca^{2+}]_{cyt}$	Fura 2, AM; Aequorin	(Visch, 2006)
NC	*NDUFS4*	nt C316T	Fibroblasts	↓ $[ATP]_{mit}$	↓ $[Ca^{2+}]_{mit}$; ↓[$Ca^{2+}]_{cyt}$; ↓ $[Ca^{2+}]_{ER}$	Fura 2, AM; Aequorin	(Visch, 2006)
NC	*NDUFS4*	C202G/G203(2)	Fibroblasts	↓ $[ATP]_{mit}$	↓ $[Ca^{2+}]_{mit}$; ↓[$Ca^{2+}]_{cyt}$; ↓ $[Ca^{2+}]_{ER}$	Fura 2, AM; Aequorin	(Visch, 2006)
NC	*NDUFS7*	nt G364A	Fibroblasts	↓ $[ATP]_{mit}$	↓ $[Ca^{2+}]_{mit}$; ↓[$Ca^{2+}]_{cyt}$; ↓ $[Ca^{2+}]_{ER}$	Fura 2, AM; Aequorin	(Visch, 2006)
NC	*NDUFS8*	nt C280T	Fibroblasts	↓ $[ATP]_{mit}$	↓ $[Ca^{2+}]_{mit}$; ↓[$Ca^{2+}]_{cyt}$; ↓ $[Ca^{2+}]_{ER}$	Fura 2, AM; Aequorin	(Visch, 2006)
NC	*NDUFV1*	nt C175T/ nt C1268T	Fibroblasts	↓ $[ATP]_{mit}$	↓ $[Ca^{2+}]_{mit}$; ↓[$Ca^{2+}]_{cyt}$; ↓ $[Ca^{2+}]_{ER}$	Fura 2, AM; Aequorin	(Visch, 2006)
NC	*NDUFV1*	nt C175T/ nt C1268T	Fibroblasts	Unchanged $[ATP]_{mit}$	unchanged $[Ca^{2+}]_{mit}$, $[Ca^{2+}]_{cyt}$ & $[Ca^{2+}]_{ER}$	Fura 2, AM; Aequorin	(Visch, 2006)
Leigh	SDHA	nt C1684T	Fibroblasts	↓ $[ATP]_{mit}$; ↓Δ_{mit} ; ↑ROS ; ↓ mitochondrial movement; ↑ ER-mitochondria contact sites	↑ $[Ca^{2+}]_{mit}$; ↑[$Ca^{2+}]_{cyt}$; ↓ $[Ca^{2+}]_{ER}$;	Fluo 4, AM; X-Rhod-1, AM; Aequorin	(M'Baya, 2010)
Leigh	SURF1	KO	SURF-/- mouse	COX deficiency; increased lifespan; protection from Ca^{2+}-dependent neurotoxicity;	↓ $[Ca^{2+}]_{mit}$; ↓[$Ca^{2+}]_{cyt}$	Fura, FF ; Aequorin	(Dell'agnello, 2007)
Leigh	SURF1		Fibroblasts	COX deficiency	↓SOC	Fura 2, AM	(Wasniewska, 2001)
Freidreich ataxia	Frataxin	GAA(3)	Fibroblasts	ND	↑[$Ca^{2+}]$	BAPTA-AM	(Wong & Cortopassi, 1997)
Huntigton	Htt	CAG(3)	Lymphoblasts Brain from Tg mice	↓Δ_{mit}	↓ $[Ca^{2+}]_{mit}$	Green-5N	(Panov, 2002)
Huntigton	Htt	CAG(3)	Immortalized striatal cells from Tg mouse	Normal mitochondrial function ; increased ROS	↓ basal $[Ca^{2+}]$; ↓ $[Ca^{2+}]mit$; ↓ IP_3, ↓ P2Y1/2 expression ; ↑ BK 1/2 expression	Fura 2, AM; Aequorin	(Lim, 2008)
NC	NC	COX deficiency	Fibroblasts	COX deficiency	Unchanged resting $[Ca^{2+}]$	Fura 2, AM	(Handran, 1997)
NC	NC	PDH deficiency	Fibroblasts	↓Δ_{mit}	↓[$Ca^{2+}]_{mit}$	Aequorin	(Padua, 1998)

BK: bradykinin; COX: cytchrome oxidase Htt: Huntingtin; NC: non communicated; ND: not determined; ROS: reactive oxygen species; SOC: store operated Ca^{2+} entry; PDH: Pyruvate dehydrogenase; KO: knock out; $[Ca^{2+}]$cyt, cytosolic calcium-concentration; $[Ca^{2+}]$er, endoplasmic reticulum calcium-concentration; $[Ca^{2+}]_{mt}$, mitochondrial calcium-concentration; Ca^{2+}, calcium. (1) Insertion; (2) Deletion; (3) repeat.

Table 1. Calcium deregulation in OXPHOS diseases

5.1 Calcium deregulation in MELAS, MERRF, NARP and LHON

Calcium deregulation was first reported in OXPHOS diseases linked to mitochondrial mutation. Brini and collaborators monitored subcellular Ca^{2+} signalling in cybrid cells with 0% and 100% of the MERRF (nt 8356 T/C) and NARP (nt 8993 T/G) mutations using cytosolic aequorin and aequorin probe targeted to the mitochondria. They showed a reduced mitochondrial [Ca^{2+}] ($[Ca^{2+}]_{mit}$) transient in MERRF cells but not in NARP cells upon stimulation with IP3-generating agonist, whereas cytosolic Ca^{2+} responses ($[Ca^{2+}]_{cyt}$) were normal in both cell types (Brini et al., 1999).

In another study, cybrid cells with 98 % of NARP mutation (nt 8993 T/G) and Rho^0 cells show a disturbed mitochondrial network and actin cytoskeleton. These cells show also a slower Ca^{2+} influx rates in comparison to parental cells. Authors postulate that proper actin cytoskeletal organization is important for CCE (capacitative Ca^{2+} entry) in these cells (Szczepanowska et al., 2004).

Abnormal Ca^{2+} homeostasis and mitochondrial polarization was also reported in fibroblasts from patients with MELAS syndrome. These cells showed an increased Ca^{2+} influx associated to a decreased mitochondrial potential (Moudy, 1995).

A comparative study was performed to establish sensitivity to oxidant in cybrid cells bearing the LHON, MELAS, or MERRF. The order of sensitivity to H_2O_2 exposure was MELAS>LHON>MERRF>controls. Consistent with the hypothesis that death induced by oxidative stress is Ca^{2+} dependent, depletion of Ca^{2+} from the medium protected all cells from cell death. This study reveals indirectly that LHON as well as MELAS and MERRF show an increased basal Ca^{2+} load (Wong & Cortopassi, 1997).

In 2007, another study performed on cybrid cells incorporating two pathogenic mitochondrial mutations (nt 3243 A/G, nt 3202 A/G) reveal that the decreased ATP production by oxidative phosphorylation was compensated by a rise in anaerobic glycolysis. Regarding Ca^{2+} homeostasis, these cells did not show any alteration of Ca^{2+} signals in the cytosol but take longer to clear up the histamine induced Ca^{2+} signal in the mitochondria (von Kleist-Retzow et al., 2007).

All over, these studies revealed a deranged Ca^{2+} homeostasis in OXPHOS diseases linked to mitochondrial mutations. These alteration are not solely at the level of mitochondria but were also observed in the cytosol. Depending on the study model and/or mutation, increased cytosolic Ca^{2+} levels are linked to increased Ca^{2+} influx through the plasma membrane or reduced Ca^{2+} uptake capacity by the mitochondria.

5.2 Calcium deregulation in Complex I deficiency

The consequences of mitochondrial complex I deficiency on Ca^{2+} homeostasis was first studied in a genetically characterized human complex I deficient fibroblast cell lines harbouring nuclear NDUFS7 (nt 364G/A) mutation linked to Leigh's syndrome. These cells show a reduced mitochondrial Ca^{2+} accumulation and consequent ATP synthesis (Visch et al., 2004). In 2006, the same group investigated the mechanism(s) underlying this impaired response. The study was conducted in fibroblasts from 6 healthy subjects and 14 genetically characterized patients expressing mitochondria targeted luciferase. The results revealed that the agonist-induced increase in mitochondrial ATP ($[ATP]_{mit}$) was significantly, but to a variable degree, decreased in 10 patients. They also reported a reduced agonist-evoked mitochondrial [Ca^{2+}] signal, measured with mitochondria targeted aequorin, and cytosolic [Ca^{2+}] signal, measured with Fura-2, AM. Measurement of Ca^{2+} content of the ER, calculated from the increase in $[Ca^{2+}]_{Cyt}$ evoked by thapsigargin, an inhibitor of the ER Ca^{2+} ATPase

revealed also a decrease in mutated cells as compared to controls. Regression analysis revealed that the increase in $[ATP]_{mit}$ was directly proportional to the increases in $[Ca^{2+}]_{cyt}$ and $[Ca^{2+}]_{mit}$ and to the ER Ca^{2+} content. This was the first report showing a pathological ER Ca^{2+} homeostasis in OXPHOS disease models. The authors postulated that the reduced ER Ca^{2+} content could be the direct cause of the impaired agonist-induced increase in $[ATP]_{mit}$ in human complex I deficiency (Visch et al., 2006). However, the molecular mechanisms underlying ER Ca^{2+} deregulation were not revealed.

Another key cellular feature that was extensively investigated in patient fibroblasts harboring complex I deficiency is mitochondrial morphology. The quantification of mitochondrial morphology in a cohort of 14 patients fibroblast cell lines revealed two distinct classes of patient fibroblasts, one in which the cells mainly contained short circular fragmented mitochondria, and one in which the cells displayed a normal filamentous mitochondrial morphology (Koopman et al., 2007). Authors postulated that these differences are linked to ROS levels (Koopman et al., 2007). In a second report, the authors analyzed the relationship between mitochondrial dynamics and structure and Ca^{2+}/ATP handling in the same cohort. Regression analysis of the agonist-induced Ca^{2+}/ATP handling and mitochondrial morphology shows that increased mitochondrial number is associated to reduced Ca^{2+}-stimulated mitochondrial ATP and reduced stimulation of cytosolic Ca^{2+} removal rate (Willems et al., 2009).

5.3 Calcium deregulation in Complex II deficiency

The investigation of Ca^{2+} deregulation linked to complex II deficiency were largely performed upon complex II inhibition by 3-nitropropionic acid (3NP) . The inhibition of complex II by 3NP is related to neuronal death, anatomic and neurochemical changes similar to those occurring in Huntington's disease (HD).

In primary cultures of rodent central nervous system, 3NP elicits an early increase in neuronal $[Ca^{2+}]_{cyt}$, and both apoptotic and necrotic neuronal death (Greene et al., 1998). 3NP treatment produces a long term potentiation of the NMDA-mediated synaptic excitation in striatal spiny neurons. This also involves increased intracellular Ca^{2+} (Calabresi et al., 2001).

To the mechanisms underlying increased $[Ca^{2+}]_{cyt}$ upon 3NP treatment, it was shown that short treatment-induced $[Ca^{2+}]_{cyt}$ increase occurs through NMDA-GLUR (Glutamate receptor) and VGCC and implicates also internal stores (Lee et al., 2002). In astrocyte cultures, Tatiani, R. Rosenstock and collaborators showed that 3NP is also able to release mitochondrial Ca^{2+} independently from internal stores and from Ca^{2+} entry through the plasma membrane (Rosenstock et al., 2004). Another group showed that 3NP-induced necrosis in primary hippocampal neurons is associated with an increase in both cytosolic and mitochondrial $[Ca^{2+}]$, decreased ATP and rapid mitochondrial potential depolarization. In this context, the increased $[Ca^{2+}]$ was shown to result from Ca^{2+} influx through NMDA receptors (Nasr et al., 2003).

The occurrence of mitochondrial permeability transition (PT) was shown to be the cause of the loss of neuronal viability induced by complex II inhibition (Maciel et al., 2004). This is in line with studies showing increased susceptibility of striatal mitochondria to Ca^{2+}- induced PT (Brustovetsky et al., 2003) and that cyclosporine A (inhibitor of PT) protects against 3NP toxicity in striatal neurons (Leventhal et al., 2000) and astrocytes (Rosenstock et al., 2004). Accordingly, inhibition of mitochondrial Ca^{2+} influx by ruthenium red significantly reduces 3NP-induced cell death (Ruan et al., 2004).

The data obtained upon complex II inhibition by 3NP are in accordance with those obtained from Huntington's patients and transgenic mice. Mitochondria isolated from lymphoblasts of individuals with HD showed reduced mitochondrial potential and increased sensitivity to depolarization upon Ca^{2+} addition. Similar results were obtained in transgenic HD mice expressing mutated huntingtin (Panov et al., 2002). In addition, mitochondria from HD mice showed lower Ca^{2+} retention capacity. These mitochondrial abnormalities preceded the onset of pathological or behavioural tract by months, suggesting that mitochondrial Ca^{2+} deregulation occurs early in HD (Panov et al., 2002). In a recent study, Lim and collaborators explore Ca^{2+} homeostasis and mitochondrial dysfunction in clonal striatal cell lines established from a transgenic HD mouse model and showed transcriptional changes in the components of the phosphatidylinositol cycle and in receptors for myo-inositol triphosphate-linked agonist. The overall result of such changes is to decrease basal Ca^{2+} in mutant cells. Mitochondria from mutant cells failed to handle large Ca^{2+} loads and this seems to be due to increased Ca^{2+} sensitivity of the permeability transition. This study reveals a compensatory attempt to prevent the Ca^{2+} stress that would exacerbate mitochondrial damage in HD (Lim et al., 2008).

Our group was the first to investigate Ca^{2+} homeostasis in human fibroblasts isolated from a patient with Leigh's syndrome harbouring a homozygous R554W substitution in the flavoprotein subunit of the complex II (SDHA). Our study was conducted in parallel in control fibroblasts and in neuroblastoma SH-SY5Y cells upon inhibition of complex II with 3NP or Atpenin A5 at doses which did not induce cell death, thus affording to study complex II deficiency independently from cell death. We showed that mutation or chronic inhibition of complex II determined a large increase in basal and agonist-evoked Ca^{2+} signals in the cytosol and mitochondria, in parallel with mitochondrial dysfunction (membrane potential loss, ATP reduction and increased ROS). Cytosolic and mitochondrial Ca^{2+} overload are linked to increased ER Ca^{2+} leakage, and to PMCA and SERCA2b proteasome-dependent degradation. Increased mitochondrial Ca^{2+} load is also contributed by decreased mitochondrial motility and increased ER-mitochondrial contacts. These findings are interesting since they link for the first time OXPHOS-related mitochondrial pathology to the regulation of the stability of two major actors in Ca^{2+} signalling regulation, namely PMCA and SERCA. We postulate that SERCA2b and PMCA degradation is predictably related to a decrease of mitochondrial ATP production, since SERCA2b and PMCA degradation was also observed upon ATP synthase inhibition by rotenone. This phenomenon could be interpreted as an adaptation response to ATP demise in OXPHOS diseases. Our study revealed also the activation of a compensatory attempt to restore total ATP level through the activation of anaerobic glycolysis in a Ca^{2+}-dependent manner (M'Baya et al., 2010). This study revealed a double hint of Ca^{2+} signalling deregulation in complex II deficiency. On the one hand Ca^{2+} overload may favour the activation of glycolytic ATP production and on the other hand favoured Ca^{2+}-mediated mitochondrial pathology (M'Baya et al., 2010).

5.4 Calcium deregulation in OXPHOS diseases linked to defects in OXPHOS assembly and iron homeostasis: COX and frataxin deficiencies

Leigh's syndrome associated with COX deficiency is usually caused by mutations of SURF1, a gene coding a putative COX assembly factor. Fibroblasts isolated from patients harboring SURF1 mutation displayed a low Ca^{2+} influx through SOC (store operated Ca^{2+} channels) as

compared to control fibroblast (Wasniewska et al., 2001). The energy state of the mitochondrial membrane in mutated cells is naturally decreased. Accordingly, it was demonstrated that mitochondria can control SOC in a numerous cell types and that the collapse of mitochondrial membrane potential, either by an uncoupler or an inhibitor of the respiratory chain, greatly reduces the SOC (Makowska et al., 2000). In an earlier study, Handran and collaborators failed to document either mitochondrial morphology alteration or intracellular Ca^{2+} deregulation in COX-deficient human fibroblasts (Handran et al., 1997). This discrepancy between these results may be accounted on the partial recovery of COX enzyme activity in COX deficient fibroblasts. Fibroblasts are not a robust system for the study of mitochondrial dysfunction and cultured cells relays less on mitochondria for ATP production. It was thus concluded that this deficiency is not detrimental to fibroblast or that anaerobic respiration rescues the phenotype. In a strange manner, SURF1-/- KO mouse displayed mild reduction of COX activity in all tissues and did not show encephalopathy. These mice show a complete protection from in vivo neurodegeneration induced by exposure to high doses of kainic acid (a glutamatergic epiloptogenic agonist). Thus the ablation of SURF1 drastically reduces the glutamate-induced increase of Ca^{2+} both in the cytosol and the mitochondria. Authors postulate that reduced buffering capacity by SURF1-/- mitochondria in the contact sites between mitochondria and plasma membrane or the ER may promote the feedback closure of the Ca^{2+} channels thus inhibiting the cytosolic Ca^{2+} transient rise (Dell'agnello et al., 2007).

As introduced in chapter 2-2-2, Friedreich's ataxia (FA) is an autosomal recessive disease caused by decreased expression of the mitochondrial protein frataxin. The biological function of frataxin is unclear. The homologue of frataxin in yeast, YFH1, is required for cellular respiration and was suggested to regulate mitochondrial iron homeostasis. Patients suffering from FA exhibit decreased ATP production in skeletal muscle. Accordingly, overexpression of frataxin in mammalian cells causes a Ca^{2+}-induced up-regulation of tricarboxylic acid cycle flux and respiration, which, in turn, leads to an increased mitochondrial membrane potential and results in an elevated cellular ATP content. Thus, frataxin appears to be a key activator of mitochondrial energy conversion and oxidative phosphorylation (Ristow et al., 2000).

It was reported that mean mitochondrial iron content was increased in FA fibroblasts harboring expansion of intronic GAA repeat in frataxin leading to its reduced expression, and that staurosporine-induced caspase 3 activity was higher in FA fibroblasts than controls. Treatment of cells with BAPTA, AM rescued FA from oxidant-induced death. These data indirectly demonstrate that FA fibroblasts displayed an increased cytosolic Ca^{2+} content leading to increased sensitivity to oxidative stress (Wong & Cortopassi, 1997).

5.5 Calcium deregulation linked to mitochondrial DNA polymorphism

mtDNA is highly polymorphic and its variation in humans may contribute to individual differences in function as well as susceptibility to various diseases such as neurodegenerative diseases. Kazuno and collaborators searched for mtDNA polymorphisms that have mitochondrial functional significance using cybrid cells. Increased mitochondrial basal Ca^{2+} levels and increased agonist evoked cytosolic Ca^{2+} signals were observed in two closely linked nonsynonymous polymorphisms. Interestingly, these data highlight the role

of mitochondrial polymorphisms in the pathology of neurodegenerative diseases by affecting Ca^{2+} dynamics (Kazuno et al., 2006).

5.6 Calcium deregulation in Pyruvate Dehydrogenase deficiency

Aerobic metabolism may also affect mitochondrial Ca^{2+} homeostasis. Thus, deregulation of Ca^{2+} handling was also reported in human fibroblasts from a patient with an inherited defect in pyruvate dehydrogenase (PDH). Indeed, these cells show a decrease ability to sequester cytosolic Ca^{2+} into mitochondria without affecting basal cytosolic and mitochondrial Ca^{2+} levels. It was postulated that reduced mitochondrial uptake is linked to decreased mitochondrial potential (Padua et al., 1998).

6. OXPHOS therapies: The place for Ca^{2+} modulating drugs

OXPHOS disorders are complex and heterogeneous group of multisystem diseases. The fact that they can result from mutations in hundreds of genes distributed across all of the chromosomes as well as the mtDNA, render the understanding of causative factors and the identification of common disease-related factors difficult. Accordingly effective therapeutic interventions are still not readily available. There are two main approaches to mitochondrial disease therapy: genetic and metabolic pharmacological (for recent review see (Roestenberg et al., 2011) and (Wallace et al., 2010)).

New approaches for genetic therapies for nDNA-encoded mitochondrial diseases as well as for mtDNA diseases are beginning to offer alternatives for individuals suffering from these devastating disorders. For mtDNA, these approaches include: (*a*) import of normal mtDNA polypeptides into the mitochondrion to complement the mtDNA defect, (*b*) reduction of the proportion of mutant mtDNAs (heteroplasmy shifting), and (*c*) direct medication of the mtDNA. Researchers are focusing also on the possible use of stem cell as a medication of OXPHOS disorders. However, these approaches are not as likely to relieve the devastating symptoms suffered by individuals with bioenergetic diseases.

The pharmacological approach includes the use of: (a) cofactors that increase the production of ATP (coQ, Idebenone, and succinate), (b) vitamins and metabolic supplements (thiamine, riboflavine, carnitine and L-arginine), (c) reactive oxygen species scavengers and mitochondrial antioxidants (CoQ/Idebenone, Vitamin E and Vitamin C), (d) modulators of PTP (cyclosporin A), and (e) regulators of mitochondrial biogenesis (bezafibrate and sirtuin analogs).

Current interventions based on metabolic correction include the use of mitochondrial-targeted drugs (compounds and peptides targeted to the mitochondrial matrix) such as mitoquinone "MitoQ", a derivative of coenzyme Q10, and SS-peptides, Szesto-Schiller peptides, a novel class of small cell permeable peptide antioxidants.

Another alternative to rescue mitochondrial bioenergetics defects is the use the mitochondrial Na^{+}/Ca^{2+} exchanger inhibitor benzothiazepine CGP37157 (Cox & Matlib, 1993). CGP37157 normalized aberrant mitochondrial Ca^{2+} handling during hormone stimulation of cybrid cells carrying the tRNALys mutation associated with MERRF syndrom (Brini et al., 1999). Short-term pre-treatment with CGP37157 (1 µM, 2 min) fully normalized the amplitude of the hormone-induced mitochondrial Ca^{2+} signal in fibroblasts from patients with isolated complex I deficiency (Visch et al., 2004), without altering this

parameter in healthy fibroblasts. Similar result was obtained recently in a study including a large number of patient fibroblasts with complex I deficiency (Willems et al., 2009). Also the reduced maximal [ATP] in the mitochondrial matrix and cytosol were fully normalized by CGP37157 treatment. The effect of CGP37157 was independent of the presence of extracellular Ca^{2+}, excluding a stimulatory effect on Ca^{2+} entry across the plasma membrane (Willems et al., 2009).

It is worth to mention that CGP37157 may also stimulate the IP_3-induced release of Ca^{2+} from intracellular stores. In addition to these effects, CGP37157 was demonstrated to inhibit capacitative store refilling (Malli et al., 2005; Poburko et al., 2007). As far as its specificity is concerned, recent studies suggest that CGP37157 can also directly act on L-type Ca^{2+} channels (Thu le et al., 2006). Thus the use of this drug will hamper Ca^{2+}-stimulated processes that depend on Ca^{2+} entry across the plasma membrane (Luciani et al., 2007).

All over, these findings suggest that the mitochondrial Na^+/Ca^{2+} exchanger is a potential target for drugs aiming to restore or improve Ca^{2+}-stimulated mitochondrial ATP synthesis in OXPHOS deficiencies and highlight the role of Ca^{2+} deregulation in the development of mitochondrial and cellular pathology in OXPHOS diseases.

7. Conclusion

This literature analysis highlights the broad Ca^{2+} deregulation in different models of OXPHOS diseases and demonstrates the cross regulation between Ca^{2+} and bioenergetics in the development of mitochondrial and cellular pathologies. Some studies revealed also the potential use of Ca^{2+} modulating drugs to reveres mitochondrial pathology. These studies may encourage researcher to investigate systematically Ca^{2+} deregulation in OXPHOS and help to reveal new targets for the development of new or combined therapies to rescue mitochondrial pathology in these diseases.

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9. Abbreviations

ANT, adenine nucleotide translocator; ATP, adenosine triphosphate; $[Ca^{2+}]_{cyt}$, cytosolic calcium-concentration; $[Ca^{2+}]_{er}$, endoplasmic reticulum calcium-concentration; $[Ca^{2+}]_{mt}$, mitochondrial calcium-concentration; Ca^{2+}, calcium; DNA, Deoxyribonucleic acid, ETC, electron transport chain; ER, endoplasmic reticulum; $\Delta\psi$, electrical potential; IMM, inner mitochondrial membrane; IP_3, inositol 1,4,5-triphosphate; IP_3R, inositol triphosphate receptor; MCU, mitochondrial Ca^{2+} uniporter; NCX/HCX, Na^+/Ca^{2+} exchanger and H^+/Ca^{2+} exchanger; OMM, outer mitochondrial membrane; OXPHOS, oxidative phosphorylation; PMCA, plasma membrane Ca^{2+}-ATPase; SERCA, sarco-Endoplasmic reticulum Ca^{2+}-ATPase; RYR, ryanodine receptor; SERCA, sarco-Endoplasmic reticulum Ca^{2+}-ATPase; SOC, store operated channel.

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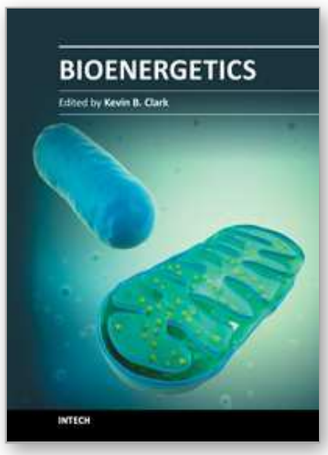

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Cellular life depends upon energy storage, transformation, utilization, and exchange in order to optimally function and to stay-off death. The over 200-year-old study of how cells transform biological fuels into usable energy, a process broadly known as bioenergetics, has produced celebrated traditions in explaining origins of life, metabolism, ecological adaptation, homeostasis, biosynthesis, aging, disease, and numerous other life processes. InTech's edited volume, Bioenergetics, brings together some of these traditions for readers through a collection of chapters written by international authorities. Novice and expert will find this book bridges scientific revolutions in organismic biology, membrane physiology, and molecular biology to advance the discipline of bioenergetics toward solving contemporary and future problems in metabolic diseases, life transitions and longevity, and performance optimization.

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