

Review

Respiratory chain defects: what do we know for sure about their consequences in vivo?

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Received 2 June 2004; received in revised form 6 July 2004; accepted 7 July 2004
Available online 30 July 2004

Abstract

The function and the structure of mitochondria have been the subject of intensive research since the discovery of these organelles. Yet, the investigation of patients with mitochondrial disease reveals that we do not understand a large part of the underlying pathogenic processes. This has disastrous consequences in terms of the therapy possibly proposed to the patients and their family. An attempt is made in this short review to question our present ideas on the potential consequences of mitochondrial dysfunctions and to enlighten new observations which might be valuable in the understanding of the physiopathology of these diseases.

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Keywords: Mitochondrial disease; Metabolism; Respiratory chain; ATP; Succinate; Superoxide

1. Introduction

The identification of disease causing genes in mitochondrial (mt) disorders has made considerable progress in the last decade [1]. Indeed, numerous mutations in both the mitochondrial DNA and a number of nuclear genes have been reported in association with a striking diversity of clinical presentations [2]. Yet, therapy is essentially supportive and prenatal diagnosis is often the only offer that can be made to affected families [3]. After a burst of hope triggered by reports on the successful use of peptide nucleic acids to change mitochondrial heteroplasmy in cell models [4], further attempts have shown the major limitation of such an approach [5]. The idea to change the level of heteroplasmy in the particular case of mutant mtDNA is however still there, mostly based on the observation that muscle satellite cells, and possibly other cell types, could harbour less (or eliminate more) mitochondria with mutant

mtDNA when stimulated to grow both in vivo or in vitro [6]. But so far, the only achievement in the field of therapy of mt disorders has been obtained thanks to our understanding of the functional impairment associated with the pathogenesis. In 2000, depletion of ubiquinone in the respiratory chain, despite unknown molecular basis, has been shown to be possibly counteracted by oral supplementation with the lacking cofactor [7]. A second example is constituted by Friedreich ataxia, the most common recessive ataxia, resulting from a lack of function of frataxin (a mitochondrial protein involved in handling of the iron necessary to the synthesis of iron–sulfur cluster in the mitochondria) [8]. The life-threatening cardiomyopathy associated with this condition has been shown to involve a severe oxidative stress which can be partially counteracted by idebenone, a potent antioxidant derivative of ubiquinone [9]. Beside these two conditions, our understanding of the pathogenesis of most mitochondrial diseases is so poor that new rationales for therapy are badly missing. The purpose of this paper is to briefly review a few facts and some hypotheses on the actual or potential consequences of respiratory chain defects and their clinical expression.

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2. ATP depletion, but to which extent?

It is often a good idea to identify a unifying factor accounting for the consequence(s) of the impairment of one given cellular function. In the case of mitochondrial function in a cell, ATP production is an obvious and excellent candidate. Its central role in almost every cellular process could easily account for the involvement of any cell/tissue/organ in mitochondrial diseases. In the particular case of mutations affecting mtDNA, the heteroplasmy phenomenon provided an additional key to explain specific organ involvement. For a long time, this provided a kind of rationale according to which variable energy demand and variable load of mutant mtDNA concur to explain the different clinical presentation of mitochondrial diseases [10]. Noticeably, mutations in nuclear genes encoding respiratory chain or Krebs cycle key proteins were then considered in textbooks as being lethal, and thus, as highly improbable [11]. That was the time when organs were classified using a simple scale, according to their low or high energy demand. Accordingly, the optic nerve, the unique one being affected in Leber hereditary optic neuropathy (LHON), was considered to be the Achilles' heel of the human body in term of susceptibility to mitochondrial dysfunction, due to a specifically high requirement in ATP [10]. However, since the beginning of the LHON story, there was this annoying observation that cells harbouring homoplasmic load of LHON-causing mtDNA mutation only had a very slight (if any) decrease in complex I activity. This decrease would predictably hardly affect ATP production by mitochondria (see, however Ref. [12]). A second irritating point was that neither mtDNA mutations/deletions affecting RC function (ATP synthesis), nor severe complex I deficiency (with unknown molecular bases at that time) necessarily affected the optic nerve. But we could live with that, since a differential load in mutant mtDNA could always be advocated as a complementary explanation to justify sparing of the optic nerve.

The discovery in early 1995 that deleterious mutations in typical housekeeping nuclear genes encoding Krebs cycle [13] or respiratory chain [14] key components do exist in humans raised the immediate question: how affected patients with such mutations could possibly see, and have no optic nerve problem? The question is evidently reinforced nowadays by the report of a number of mutations in CI-subunit encoding genes not resulting in blindness [15,16]. Thus ATP production was not “the” unifying factor accounting for optic nerve involvement (or various clinical involvements as well). But hopefully, the superoxide-triggered apoptosis would come to our help.

3. Superoxide overproduction?

Mitochondrion is known to work similarly to an atomic powerhouse in a cell by providing ATP through a dangerous oxygen-dependent system. Indeed since Fridovich's [16]

pioneer work, it is known that superoxides are produced *in vivo* in cells. They mostly escape the respiratory chain with a number of potential deleterious consequences, if not kept under tight control. A number of specialized enzymes are required to process these dangerous molecules. Among these consequences, triggering of apoptosis provides an alternative (concurrent) element to ATP depletion to account for tissue-specific involvement in OXPHOS diseases. Indeed, it has been shown that (i) impaired respiratory chain can produce more superoxides, depending on the type of deficiency, (ii) superoxide overproduction can override superoxide dismutase activity, and (iii) superoxides or their derivatives can readily trigger apoptosis [17]. In keeping with this, these processes can be controlled *in vitro* by a superoxide-reactive spin-trap [17]. According to this view, the differential ability of cells to handle oxygen and get rid of dangerous oxygen species would be a determining factor and apoptotic features a hallmark for superoxide-triggered insult. Armed with this conviction, Tfam-knocked out mice with severe mtDNA depletion in specific organs targeted thanks to the Cre-Lox technology were analyzed for apoptotic features. In both heart and brain, massive apoptotic features were noticed [18,19]. A similar type of investigation was carried out in Frataxin-knocked out mice with severe respiratory chain deficiency as well, but, to our surprise, no sign of significant apoptotic process could be observed in the brains of these mice [20]. Again, superoxide-triggered apoptosis was not “the” general unifying mechanism.

If ATP depletion, and if superoxide overproduction, are not providing the magic key, what could be the other factors? The answer is that we simply do not know. We can only mention intriguing observations which might—or not—be relevant but deserve our full attention.

4. Metabolic imbalance

Mitochondrion is often presented as the energy-providing organelle of the cell, but it is also a fantastic factory where hundreds of compounds are processed for further use in the cell. The role of a secondary blockade of mitochondrial metabolic pathways has often been postulated in mitochondrial pathology but indeed seldom demonstrated. The demonstration by L. Colleaux and collaborators in this meeting that mutant GC1 causes neonatal myoclonic epilepsy is in this view quite interesting. GC1 is a mitochondrial glutamate-proton symporter recently characterized in human [21]. Its activity tested *in vitro* in human cells, or in isolated mitochondria from mouse brain, heart and liver, appears rather negligible (low oxidation rate) in terms of mitochondrial ATP-providing substrate. Yet, its impairment presumably causes mishandling of glutamate, possibly affecting the ability to manage excitotoxicity in brain. This illustrates that impairing a “minor”, yet unsuspected, mitochondrial pathway can have devastating

consequences in some specific cells. To which extent such mechanism occurs in relation with respiratory chain impairment in the brain is yet unknown, but this surely deserves our attention.

5. Disturbed signalling pathways

Succinate dehydrogenase deficiency is known to cause either severe encephalomyopathy [14] or tumor formation [22]. To reconcile these observations, a primary role of improper superoxide handling has been postulated by us and others [23]. This was mainly based on the idea that superoxide signalling is actually able to trigger both cell death and proliferation, and that SDH defect affects superoxide production by the respiratory chain [24]. However, an alternative view is now emerging that possibly discloses a new mitochondrial signalling pathway. Investigations presented in this meeting by Brière et al. show extensive succinate accumulation in both succinate dehydrogenase-lacking tumors and in succinate dehydrogenase defective

cultured cells. The predictable consequence of this huge succinate accumulation is the feedback inhibition of the hydroxyprolyl oxidase enzyme which produces succinate (see Selak et al., this meeting), with the stabilisation of the HIF transcription factor as a direct consequence and with a dysregulation of the cell proliferation (Fig. 1). Although it is still too early to choose between these two mechanisms, these observations again point to a major impact of disturbed signalling through metabolic mishandling in mitochondrial diseases.

Even if with somewhat unsuspected consequences, metabolic blockade is still a “known territory”, but there is predictably much more to come based on the many questions which remain open in our field of research.

6. How is the whole thing organised?

Although the discussion on the organisation and structure of mitochondria has been going on for years and years, our view progressively changes to include new information. The

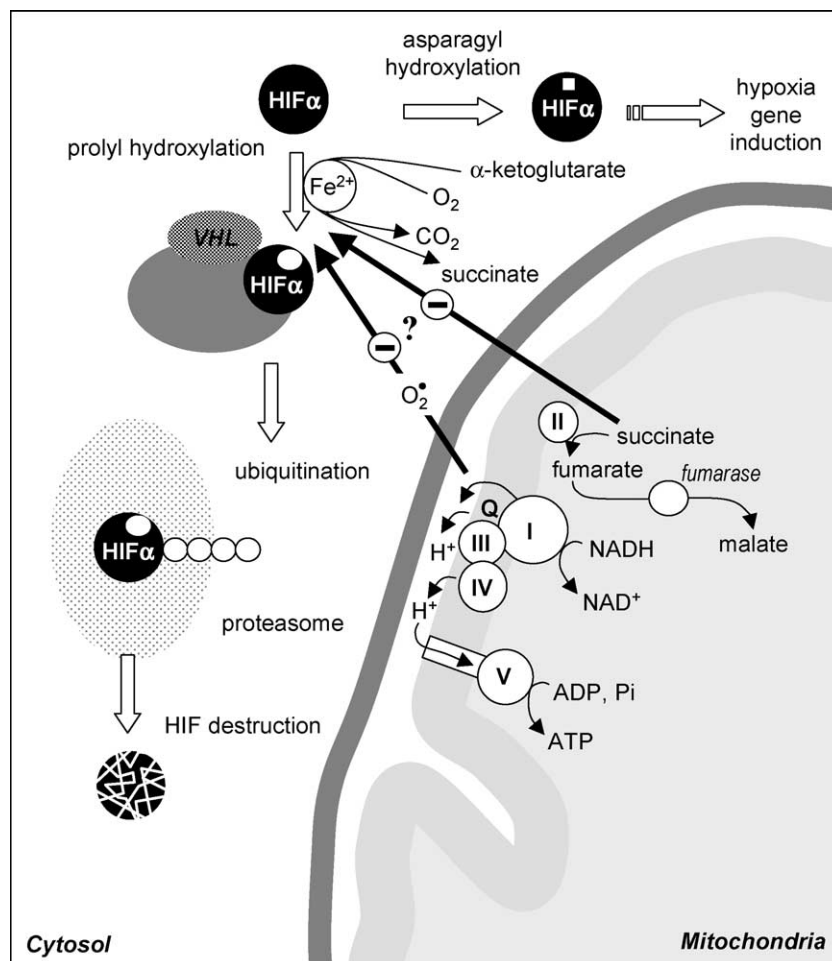


Fig. 1. The succinate connection in tumor formation. The schema features the potential consequences of mitochondrial succinate dehydrogenase (complex II) loss of activity on the cytosolic prolyl hydroxylase governing the stability of the HIF-1 α factor which in turn controls the expression of a number of hypoxia-induced genes. Noticeably, both succinate and superoxides are susceptible to inhibit the prolyl hydroxylase. HIF: hypoxia-inducing factor; VHL: von Hippel-Lindau; I, II, III, IV, V: the various complexes of the respiratory chain.

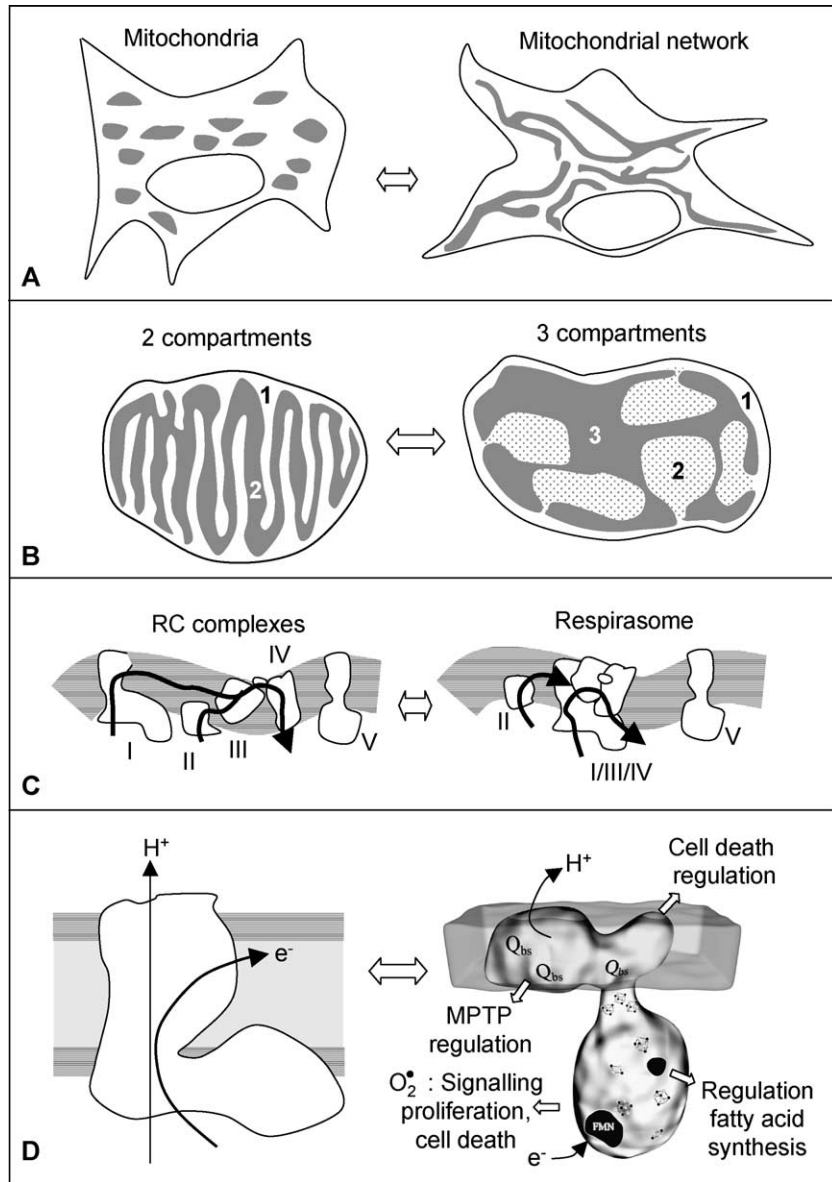


Fig. 2. Our changing view on mitochondrial structure. (A) The mitochondria in the cells, from punctuated to a network organisation. (B) The varying number of intra-mitochondrial compartments depending on cristae isolation from outer membrane. (C) The respiratory chain from a pseudo-linear organisation to an organisation as supercomplexes. (D) Respiratory chain complex I, a simple electron/proton carrier or a multifunctional complex.

overall structure of the mitochondrial network, the definition of the compartments defined by the inner membrane [25], the organisation of the respiratory chain itself into super-complexes forming a respirasome [25,26], all concur to add

more difficulty in predicting the actual in vivo consequences of primary anomalies (Fig. 2). Finally, while the organisation of the mitochondrial network is known to continuously change, with fusion/fission being frequently observed,

Table 1
Examples of recognized mitochondrial bifunctional proteins

Protein	Function 1	Function 2	Reference
Cytochrome <i>c</i>	electron transfer	apoptosis-inducing factor	[32]
AIF protein	apoptosis-inducing factor	CI assembly/maintenance	this meeting
GRIM-19 protein	apoptosis-inducing factor	CI component	[33]
Dihydrolipoamide succinyltransferase (DLST)	TCA cycle enzyme	biogenesis of respiratory chain	[30]
Succinate dehydrogenase	TCA cycle enzyme	tumor suppressor	[34]
Fumarase	TCA enzyme cycle	tumor suppressor	[35]
NADH dehydrogenase subunit 2 (ND2)	CI activity	control of Src signalling	[36]

a static organisation of the RC is also questionable. As photosystems upon light-stimulation of thylakoids [27], it might associate differently upon solicitation of its function by respiratory substrates.

7. What codes for what?

Since the recognition of the occurrence of DNA in the mitochondria, we know that respiratory chain build-up requires the concerted action of both the nuclear and the mitochondrial genomes [28]. Now we also know that dysfunction of a cytosolic protein can also affect the build-up of the RC for example by perturbing nucleotide precursors provided to the mitochondrion [29]. Even more recently, it has been reported that a mitochondrial gene (ND2) encodes a protein addressed and recruited at the cell membrane where it plays a role as a Src unique domain-interacting protein [30]. Src is known as a protein tyrosine kinase critical for controlling diverse cellular functions, regulating the synaptic NMDA receptor activity.

We therefore face a situation that can hardly be more complex: both genomes potentially encoding proteins targeted in or out of the mitochondria, affecting—or not— intra-mitochondrial processes. . . but yes, it can be even more complex! Indeed, it progressively appears that a number of long-known mitochondrial proteins are indeed bifunctional (Table 1). The difficulty lies here in the fact that the “second” (?) alternative function is just unpredictable. Such is the case for the recent discovery reported during this meeting that apoptosis-inducing factor (AIF), previously known as a mitochondrial cell death-promoter protein, is also involved in the building/maintenance of an active complex I.

8. Concluding remarks

The picture that emerges from this plethora of information makes it very important to have an integrated view on mitochondrial structure and function. Once considered as largely over with the detailed description of the respiratory chain and the major metabolic pathways, mitochondrial science has more and more questions in front of it. We urgently need to reinvestigate all these aspects and their interrelation in order to have a better chance to understand the phenotypic complexity of mitochondrial diseases and to identify new targets for therapy. In keeping with this, the growing number of animal models covering more and more aspects of mitochondrial diseases or physiology constitutes an invaluable help [31].

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