FEBS Letters

journal homepage: www.FEBSLetters.org

Review

Respiratory chain complex I, a main regulatory target of the cAMP/PKA pathway is defective in different human diseases

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ARTICLE INFO

ABSTRACT

In mammals, complex I (NADH-ubiquinone oxidoreductase) of the mitochondrial respiratory chain has 31 supernumerary subunits in addition to the 14 conserved from prokaryotes to humans. Multiplicity of structural protein components, as well as of biogenesis factors, makes complex I a sensible pace-maker of mitochondrial respiration. The work reviewed here shows that the cAMP/PKA pathway regulates the biogenesis, assembly and catalytic activity of complex I and mitochondrial oxygen superoxide production. The structural, functional and regulatory complexity of complex I, renders it particularly vulnerable to genetic and sporadic pathological factors. Complex I dysfunction has, indeed, been found, to be associated with several human diseases. Knowledge of the pathogenetic mechanisms of these diseases can help to develop new therapeutic strategies.

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1. Introduction

Complex I (NADH-ubiquinone oxidoreductase, E.C. 1.6.5.3) is the first enzyme of the mitochondrial respiratory chain, being the entry port in the chain of the reducing equivalents of NADH, produced by mitochondrial dehydrogenases of respiratory substrates [1–4]. The enzyme catalyzes the transfer of electrons from NADH to ubiquinone in the inner mitochondrial membrane and conserves the free energy, so made available, as a transmembrane electrochemical proton gradient ($\Delta\mu H^+$) according to reaction (1).

$$NADH + UQ + 5H_{in}^+ \leftrightarrow NAD^+ + UQH_2 + 4H_{out}^+ \qquad (1)$$
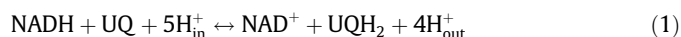
The complex thus contributes by 40% to the generation of the respiratory chain $\Delta\mu H^+$, which is utilized to make ATP from ADP in the mitochondrial process of oxidative phosphorylation (OXPHOS) [4,5]. In human cells the ATP produced by OXPHOS covers, under physiological conditions, more than 80% of the ATP that cells need. This requirement is particularly stringent in organs like, for example, the human brain, which even though it only represents 2% of the body weight, utilizes around 20% of all the oxygen we consume [6].

Mammalian complex I, with a molecular mass of $\approx 1 \times 10^6$ Da, is the largest enzyme of the mitochondrial OXPHOS system. The complex contains multiple redox centers: one FMN, nine iron–sulfur (FeS)-centers and two protein bound semiquinones [1–4,7,8]. A series of these provides the sequential, stepwise transfer of electrons from NADH to the ubiquinone of the pool, coupled to proton

pumping [4,7–9]. During this process direct electron leakage to oxygen may, however, occur at different redox sites in the enzyme [4,10–12].

In humans and mammals mitochondrial complex I has, in addition to the 14 subunits of the catalytic core, conserved from prokaryotes to humans, at least 31 supernumerary subunits [4,13]. Seven subunits of the complex are encoded by the mitochondrial DNA, 38 by nuclear genes located in different chromosomes [1,4,12,13]. What is the hidden code of these numerous supernumerary subunits? Some of them appear to be involved in the assembly of the complex [4,14–17], its regulation [4,18] and ancillary functions [4,19,20]. The GRIM-19 (B16.6) subunit, for example, belongs to the group of genes associated with retinoid–interferon induced mortality [19]. Moreover, a plethora of post-translational modifications of complex I subunits have been detected. In particular, various subunits of the complex, NDUFS4 [21,22], NDUFB11 [23], NDUFA1 [23], NDUFA10 [24], NDUFA7 (B14.5a) [25] have been found to be phosphorylated. Other post-translational modifications have also been observed [26]. In addition, several proteins have been identified which assure the proper assembly and stability of the complex [27–29].

Complex I is a regulable pacemaker of the mitochondrial respiratory function [15,30–32], is a major site of cellular oxygen superoxide production [10–12,33], is involved in apoptosis [34,35] and age-related functional decline [36–38]. Various hormones, neurotransmitters and cell growth factors regulate, through the activation of cellular signal transduction networks, the expression and functional activity of complex I [30,39–42].

Genetic dysfunctions of complex I represent, on the other hand, the largest of primary mitochondrial diseases [43–46] and dysfunctions of the complex have been found in sporadic and familial forms of Parkinson [47], cancer [48,49] and other disorders [46,50,51].

Understanding the mechanisms of the biogenesis, energy transduction, reactive oxygen species (ROS) production and regulation of complex I is crucial to comprehend the pathophysiological role of the complex in human diseases and to develop of therapeutical strategies.

The present paper reviews features of the regulation by the cAMP/PKA pathway of the biogenesis, functional capacity, ROS interaction in complex I and the pathophysiological implication of dysfunctions of the complex.

2. Complex I subunits are phosphorylated by the cAMP-dependent protein kinase

Various mitochondrial proteins have been found in the phosphorylated form [52–54]. In the nineties our group discovered that among the phosphorylated proteins of the inner mitochondrial membrane, proteins of 18 and 6–10 kDa are structural subunits of complex I. We showed that phosphorylation of these subunits is catalyzed by the cAMP-dependent protein kinase (PKA) [55,56]. Phosphorylation of complex I subunits can, in fact, be mediated by the PKA present in both the cytosol [57] and mitochondria [58].

Edman sequencing of the electrophoretic protein band of ≈18 kDa of the purified bovine heart complex I showed the presence in this band of two subunits of the complex, namely the GDHG and the AQDQ subunits (NDUFS4 gene) [21,22]. The position of the latter corresponded to the band labeled by ^{32}P in the presence of PKA and [γ-^{32}P]ATP [21]. Chen et al. [23] confirmed PKA-dependent phosphorylation of protein(s) in bovine heart mitochondria, and showed, in the 18 kDa ^{32}P-labeled protein band of complex I, an additional serine phosphorylated, previously undetected [59], subunit (ESSS subunit) [23]. Two-dimensional IEF/

SDS–PAGE electrophoresis of purified bovine heart complex I, ^{32}P labeling and immunoblotting with an antibody specific for the serine-173 phosphorylated C-terminus of the NDUFS4 protein, which is conserved in animal species [22], showed that this site is phosphorylated by PKA. It was also shown that precursor and mature human NDUFS4 protein produced by "in vitro" heterologous expression, were both ^{32}P-labeled when incubated with [γ-^{32}P]ATP and PKA [22]. Thin layer chromatography amino-acid mapping of radioactive proteins showed the presence of phosphothreonine and phosphoserine both in the precursor and mature form of the NDUFS4 protein [22]. S173A replacement in the conserved phosphorylation site resulted in disappearance of phosphoserine from the mature form [22]. It should be mentioned that mass spectrometry of the trypsin digest of the heterologous human NDUFS4 protein, phosphorylated by PKA, identified peptide ions of the protein with 82% coverage of the sequence, but the in silico predicted carboxy terminal peptide ions of the last C-terminal 4–7 residues were, however, not detected [22] see also [23]. It is worth noting that this can be due to general problems in detection of this and other phosphoproteins, like the low dynamic state of phosphorylation of the protein, loss of the C-terminus in the preparation/treatment of the mass-spectrometer sample and the small size (217.63 m/z) of the terminal peptide ion generated by tryptic proteolysis (see also [60]).

3. The cAMP/PKA pathway regulates complex I activity and ROS balance in cell cultures

In mammalian cells there are subcellular pools of cAMP and PKA [57,58,61–63]. In response to activation, by extracellular effectors, the plasma membrane adenylyl cyclase (tmAC) produces and releases cAMP in the cytosol, where its level is determined by the opposite activities of the cyclase and phosphodiesterase(s) (Fig. 1) [57,62].

Cytosolic cAMP cannot apparently move into the internal space of subcellular organelles. There is, however, in the mitochondrial matrix, and other organelles, a soluble bicarbonate-activable adenylyl cyclase (sAC), which produces cAMP inside the organelles (Fig. 1) [64–66].

All the members of the complex, involved in reversible cAMP-dependent protein phosphorylation, are associated with the outer [70] and the inner mitochondrial membrane [58,69,71]. The complex consists of A-kinase anchor protein(s) (AKAPs) [58,67,68], catalytic and regulatory subunits of PKA, phosphodiesterase(s) and serine phosphatase(s) [58,67–71] (Fig. 1).

A strong depression of the NADH-ubiquinone oxidoreductase activity of complex I, increase in the mitochondrial level of oxygen superoxide and in the cellular level of hydrogen superoxide have been observed in cells brought in the G0 phase by serum deprivation [40,72], or impaired by genetic disorder [73,74] as well as in K-ras oncogenic transformed cells [75]. It has to be recalled that complex I, besides being the major mitochondrial producer of ROS [4,10–12], is by itself particularly vulnerable to oxidative stress [10,76]. The question remains open whether, under the conditions described, it is a dysfunction in complex I that causes ROS overproduction, or if it is the latter to cause inhibitory, oxidative damage of the complex. The two processes can, in fact, set up a detrimental cycle.

In all the cases described, addition to the cells of a permeant derivative of cAMP, or activation of tmAC by cholera toxin [40] or the β-adrenoceptor agonist isoproterenol [42], reversed the inhibition of complex I activity and the accumulation of ROS, without having, however, any effect on ROS scavengers [40,42,72]. The rescue effect exerted by cAMP production in response to activation of tmAC, shows that, under these conditions at least, it is the
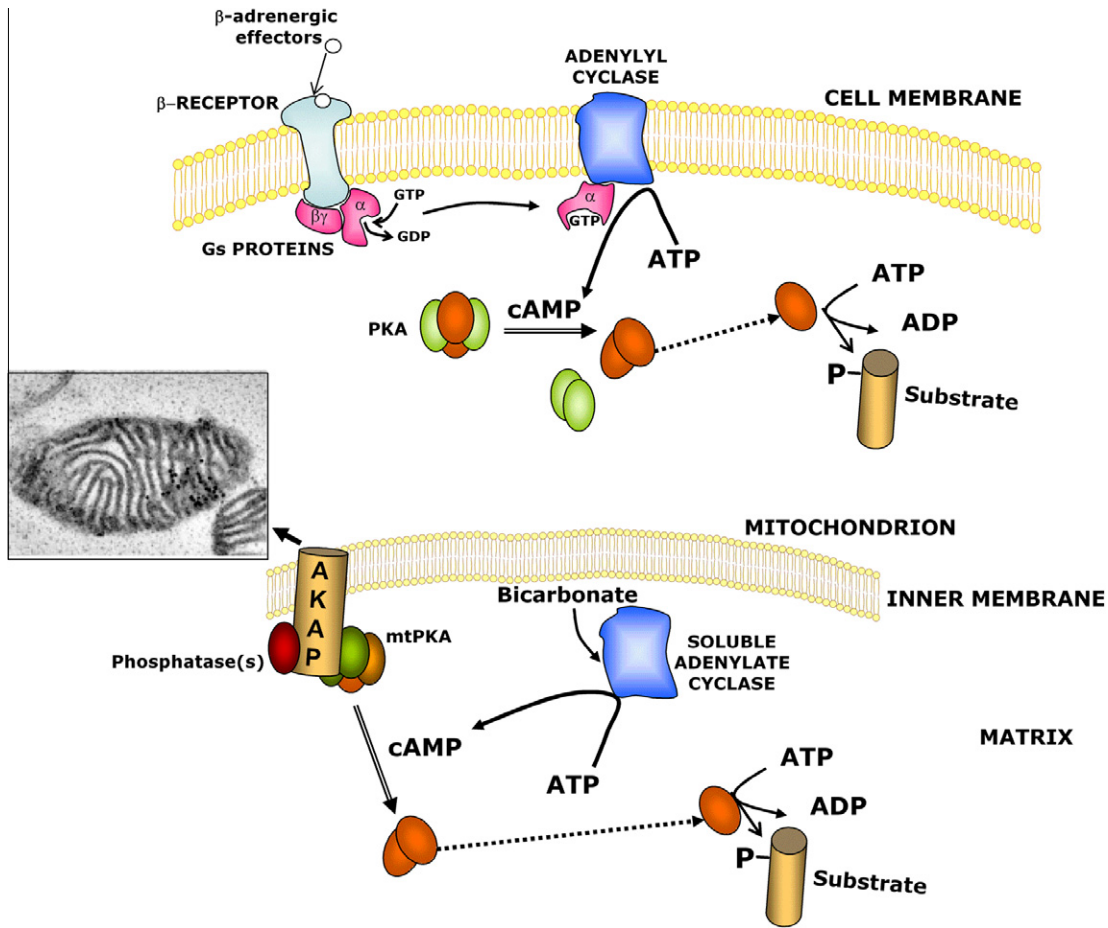
Fig. 1. Subcellular compartmentalization of the cAMP/PKA system. cAMP is produced by the plasma membrane adenylyl cyclase, in response to extracellular signals [40,42,57] and by the bicarbonate-activated adenylyl cyclase localized in mitochondria and other subcellular structures [64–66]. Different subcellular pools of cAMP [63] can thus be generated, with activation of PKA and other cAMP effectors. PKA, consisting of two cAMP-binding regulatory subunits (in green) and two catalytic subunits (in red), dissociates upon binding of cAMP to the regulatory subunits (R-PKA). Specific AKAP proteins, associated with various subcellular membranes, bind R-PKA and in this way tether the PKA tetramer to membranes together with specific protein phosphatases [58,62,67,68]. These complexes accomplish phosphorylation/dephosphorylation of proteins bound or close to the complexes [67–69]. PKA has been found to be associated with both the outer [70] and the inner membrane/matrix fraction of mammalian mitochondria [58] where it can phosphorylate various mitochondrial proteins. In the left inset a transmission electron microscopy picture of mitochondria isolated from rat heart exposed to the AKAP121 antibody, conjugated with colloidal-gold secondary antibody is shown. Numerous gold particles of AKAP protein appear to be associated with the mitochondrial cristae (from [58]).

elevation of cytosolic cAMP concentration to promote the activity of complex I and to lower the ROS level, although also mitochondrial cAMP can provide an additional contribution.

Immunodetection with specific antibodies showed that in cell cultures cAMP promoted, under the conditions in which it activated complex I and reversed ROS accumulation, phosphorylation of serine [15,40,69] in the RVS site in the carboxy terminus of the NDUFS4 protein [22]. Evidence that PKA-mediated phosphorylation of the NDUFS4 subunit is, indeed, responsible for the cAMP activation of complex I, was clearly provided by the finding that cAMP-dependent serine phosphorylation of complex I subunit(s) and complex I activation were missing in patient's fibroblasts with an NDUFS4 mutation, which caused disappearance of the protein product of the gene from the cells [15,18]. It has also been found that addition to cell cultures of phosphatase inhibitors also promotes complex I activity [42].

The mechanism by which PKA dependent phosphorylation of the NDUFS4 subunit, and possibly others, can result in activation of complex I and lowering of ROS level is presented in the last part of Section 4. In any case, the activation of complex I by cAMP/PKA system shows that the functional capacity of the complex can respond, in the cell, to a series of regulatory factors, which include

ligands of plasma membrane receptors of the cAMP cascade, networks of cell signal transduction pathways, as well as metabolic processes which contribute to cellular bicarbonate homeostasis (see Fig. 1).

4. The cAMP/PKA system regulates the expression and the assembly of complex I

In addition to the regulatory effects due to post-translational protein phosphorylation, the cAMP/PKA system regulates also the biogenesis of the mitochondrial OXPHOS system [41,77,78]. A central role in this regulatory function is played by the cAMP/Ca^{2+} response element (CRE) binding protein (CREB) [79]. CREB binds to the DNA consensus sequence TGACGTCA in the promoters of target nuclear genes [79]. The transcriptional activity of CREB is induced by serine phosphorylation in its conserved kinase inducible domain by PKA, Ca^{2+}/calmodulin protein kinases or other kinases [80,81].

A role of CREB in mitochondrial biogenesis was highlighted by the discovery that the CREB transcriptional complex activates the expression of the master gene PGC-1α (the transcriptional coactivator PPARc coactivator 1α) [93,94]. PGC-1α is also activated by
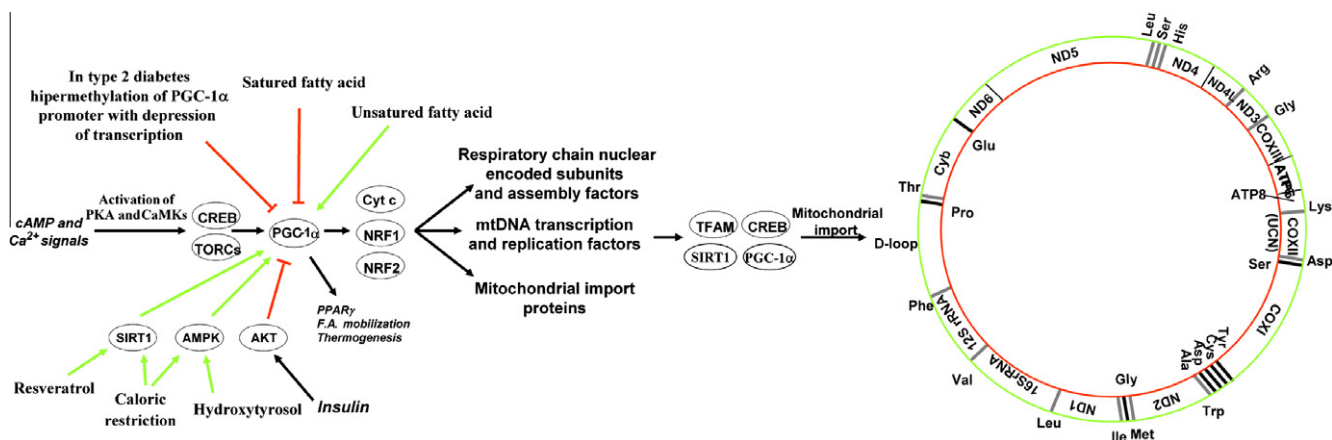
Fig. 2. Schematic representation of PKA and CREB mediated pathways, which control mitochondrial biogenesis. Activation of protein kinases by cAMP and/or Ca2+ results in phosphorylation of CREB and TORCs proteins. Phosphorylated CREB and TORCs proteins bind to the CREB complex on gene promoters and exert their transcriptional coactivator action [82]. CREB and TORC promote the transcription of PGC-1α and cytochrome c genes. PGC-1α is also activated by post-translational modifications, like deacetylation by SIRT1 [83], phosphorylation by AMPK [84] and inhibited by phosphorylation by the insulin activated AKT [85]. The expression of PGC-1α can be regulated at transcriptional level by methylation of the promoter, as found in type II diabetes in which hypermethylation of the promoter inhibits transcription [86]. The diet also influences the expression of PGC-1α. Caloric restriction, and thus the AMP/ATP and NAD⁺/NADH ratios, regulate the activities of AMPK and SIRT1 respectively [87,88]. A diet rich in polyunsaturated fatty acids apparently promotes, one rich in saturated fatty acids down-regulates PGC-1α expression [89]. Several compounds present in the diet like hydroxytyrosol of olive oil [90], and resveratrol of red wine [91], regulate the expression of PGC-1α. PGC-1α, in combination with other factors, activates the expression of the nuclear respiratory transcription factors, NRF1 and NRF2. NRF1 and NRF2 activate the expression of the mitochondrial transcription and replication factors (TFAM, TFB1M, TFB2M and MRP RNA) and nuclear genes coding for structural proteins and assembly factors of OXPHOS complexes, mitochondrial import, ion channel and shuttle proteins. The CREB protein, after mitochondrial import, promotes the expression of mtDNA [78]. Recently PGC-1α and SIRT1 have been found also in mitochondria, where they physically interact with mTFA [92].

post-translational modification, such as deacetylation by the NAD-dependent deacetylase sirtuin-1 (SIRT1) [83], whose expression is promoted by fasting [88]. Compounds present in the diet like hydroxytyrosol of olive oil [90], and resveratrol of red wine [91], regulate the expression of PGC-1α, through the activation of AMP kinase, and/or SIRT1 (Fig. 2).

Induced expression of PGC-1α activates a transcriptional regulatory cascade, which amplifies the impact of CREB-mediated signal transduction on mitochondrial biogenesis [94]. PGC-1α promotes the expression and activity of the nuclear respiratory transcription factors 1 and 2 (NRF1 and NRF2) [95]. NRF1 and NRF2 control the expression of nuclear genes coding for structural proteins of mitochondrial respiratory chain and the F_oF_1-ATP synthase, enzymes of heme biosynthesis and proteins involved in mitochondrial import of nuclear encoded subunits of OXPHOS complexes and complex assembly (Fig. 2) [96].

NRF1 and NRF2 also promote replication and transcription of the mitochondrial genome (see Fig. 2) [96]. The transcriptional activity of both the heavy and light mtDNA strands is controlled by the mitochondrial transcriptional factor A (TFAM). This interacts with the mitochondrial RNA polymerase [97] together with two additional transcription factors (mitochondrial transcription factor TFB1M and TFB2M) and a fraction of PGC-1α found in mitochondria [92]. In this way, a transcriptional cascade (Fig. 2) confers to mammalian cells the capacity to upregulate in a concerted process the expression of nuclear and mitochondrial genes encoding subunits of OXPHOS complexes.

It has been discovered that, in addition to the nucleus, CREB is also localized in the inner mitochondrial compartment [98]. In neuronal cell cultures CREB protein was found to bind to the mtDNA D-loop [99]. In these cells, the overexpression of mito-tagged CREB increased the transcript levels of the ND2, ND4, and ND5 mitochondrial genes of complex I. We have found [78] that "in vitro" expressed [³⁵S] methionine-labeled CREB is imported in isolated mitochondria by the membrane potential-dependent mechanism [100]. Both mitochondrial surface binding and import into the inner mitochondrial compartment of radioactive CREB was inhibited by an antibody against Tom20 (outer membrane

translocator subunit 20) [78]. CREB protein, once imported in mitochondria, produced a strong stimulation of the synthesis of subunits of OXPHOS complexes encoded by mitochondrial genes, an effect that required CREB phosphorylation by PKA [78]. The stimulatory effect was particularly potent in the case of ND1, ND6, and cox III/ATP6 proteins, whose synthesis was two- to three-fold enhanced by the combination of CREB and cAMP [78].

The assembly of a mature, functional complex in the inner mitochondrial membrane is achieved in a multi-step process which, in addition to the quantitatively coordinated expression of the 38 nuclear-encoded and the seven mitochondrial-encoded subunits, involves: (i) chaperon-assisted folding changes of the newly synthesized proteins; (ii) mitochondrial import of the nuclear-encoded precursor proteins through the outer membrane (TOM) and inner membrane (TIM) translocator systems; (iii) proteolytic maturation of the precursor proteins; (iv) stepwise incorporation in the complex, in which one copy of each subunit has to be incorporated in the proper near-neighbor functional position [27–29,46]. In addition, the synthesis and assembly of the protein subunits has to be coordinated with the production and the correct binding at the catalytic centers of FMN and non-heme iron–sulfur centers [101].

It has been found that, in addition to de novo synthesis of an entire complex from all the individual subunits, an exchange of individual subunits, in particular those more superficially located in the complex, with newly synthesized subunits can also take place [102].

Work in our laboratory showed that the $\Delta\psi$ dependent import in isolated mitochondria of [³⁵S]-methionine labeled NDUFS4 precursor protein, produced "in vitro" by expression of the full-length NDUFS4 cDNA, is promoted when it is phosphorylated by added PKA and inhibited by phosphatase (Fig. 3) [103].

The import/maturation in mitochondria of the NDUFS4 protein, completed in the same time-span in which cAMP stimulated complex I activity in cell cultures, was completely suppressed by site-specific substitution of serine 173 with alanine in the C-terminal RVS phosphorylation site. These observations, showing that the functional capacity of complex I depends on phosphorylation and
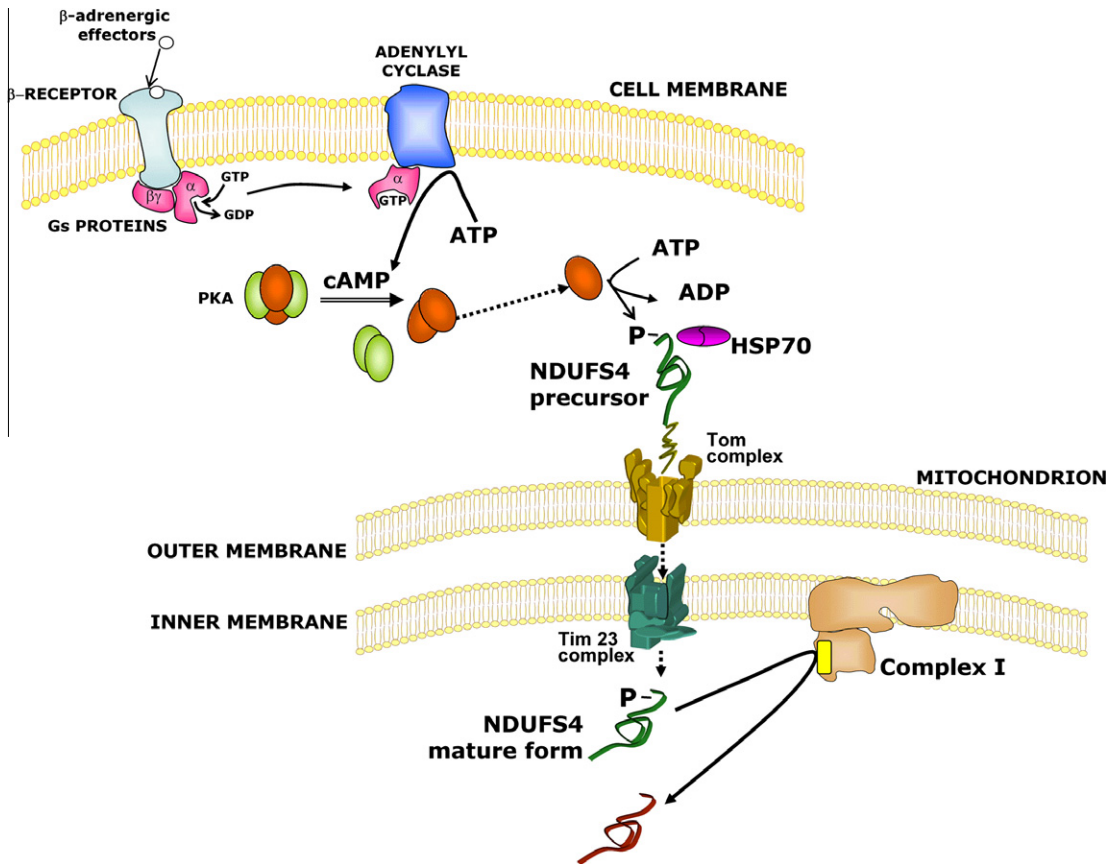
Fig. 3. Scheme describing the impact of the cAMP/PKA system on the mitochondrial import of the NDUFS4 subunit and its assembly in complex I. Elevation of the cytosolic level of cAMP by stimulation of plasma membrane adenylyl cyclase results in PKA-dependent phosphorylation of the NDUFS4 C-terminus. This promotes the binding of the NDUFS4 precursor to the cytosolic heat shock protein 70 (Hsp70). Phosphorylated NDUFS4 precursor is imported into mitochondria where it undergoes to cleavage of the presequence [103]. Newly imported NDUFS4 protein (in green) is assembled in complex I during de novo synthesis of the complex or in exchange with the pre-existing (damaged, in red) subunit in the mature complex.

import of newly synthesized NDUFS4 protein, support the proposed mechanism of exchange of subunits in the assembled complex with newly imported ones to maintain complex I homeostasis (Fig. 3) [102]. This process can, in fact, play a proof-checking role for subunits, superficially exposed to the matrix space where they can be oxidatively-damaged by ROS, with depression of the catalytic activity of the complex. Substitution of the oxidatively damaged subunits with newly synthesized ones can play a role in the functional rescue of complex I. Oxidation of complex I subunits, accompanied by reduced functional capacity of the complex, have been observed in aging [37,38]. Results from the author's laboratory has shown that oxidation of complex I subunits, by the addition to fibroblast cell cultures of ROS generating tert-butyl hydroperoxide, depressed the functional capacity of complex I. Activation of the cAMP/PKA system promoted the import of newly synthesized NDUFS4 subunit into mitochondria and restored its dynamic assembly (unpublished observations).

5. Genetic dysfunctions of complex I are associated with different human diseases

Isolated complex I deficiency is the most frequent case of primary mitochondrial defects [43–46]. Complex I pathological mutations have been initially found in mitochondrial-DNA encoded subunits [104]. The first fatal mutation in a nuclear gene of complex I, detected in a newborn [105], was a base duplication in exon 5 of the NDUFS4 gene encoding the 18 kDa AQDQ subunit of the complex, previously found to be phosphorylated by PKA [21]. Later,

other mutations in the NDUFS4 gene were detected in different encephalopathic patients [46]. In all these cases the NDUFS4 mutations resulted in: (i) disappearance of the protein from the patient's cells; (ii) suppression of the last step in complex I assembly; (iii) disappearance of cAMP dependent serine phosphorylation of complex I subunits and of the stimulatory effect of cAMP on mitochondrial respiration; (iv) stabilization of alternative splice products of the gene [46]. In the NDUFS4 mutations, which resulted in complete suppression of the NADH-ubiquinone oxidoreductase activity of complex I, no ROS accumulation was observed [73].

A pathological C1564A mutation was also detected in the nuclear NDUFS1 gene coding for the 75 kDa iron–sulfur protein subunit of complex I [73]. This is the largest conserved subunit of the complex and contains the N1b, N4, N5 Fe–S clusters of the electron transfer pathway in the enzyme [7–9].

In the fibroblasts of the patient harboring the C1564A mutation (Q522K substitution in the protein), the mitochondrial content of the normally assembled complex decreased as compared to normal cells and an additional lower molecular mass of the complex appeared [73]. The mutation resulted also in a marked, but not complete, suppression of the NADH-ubiquinone oxidoreductase activity. In the patient's fibroblasts large accumulation of H_2O_2 and intramitochondrial O_2^- was detected. The Q522K mutation in the NDUFS1 subunit alters, apparently, the redox function of the Fe–S center(s) bound to this protein, with inhibition of the normal catalytic activity of the complex and increased production of oxygen superoxide. The enhanced production of ROS can in turn cause further oxidative damage in a "vicious cycle" leading to
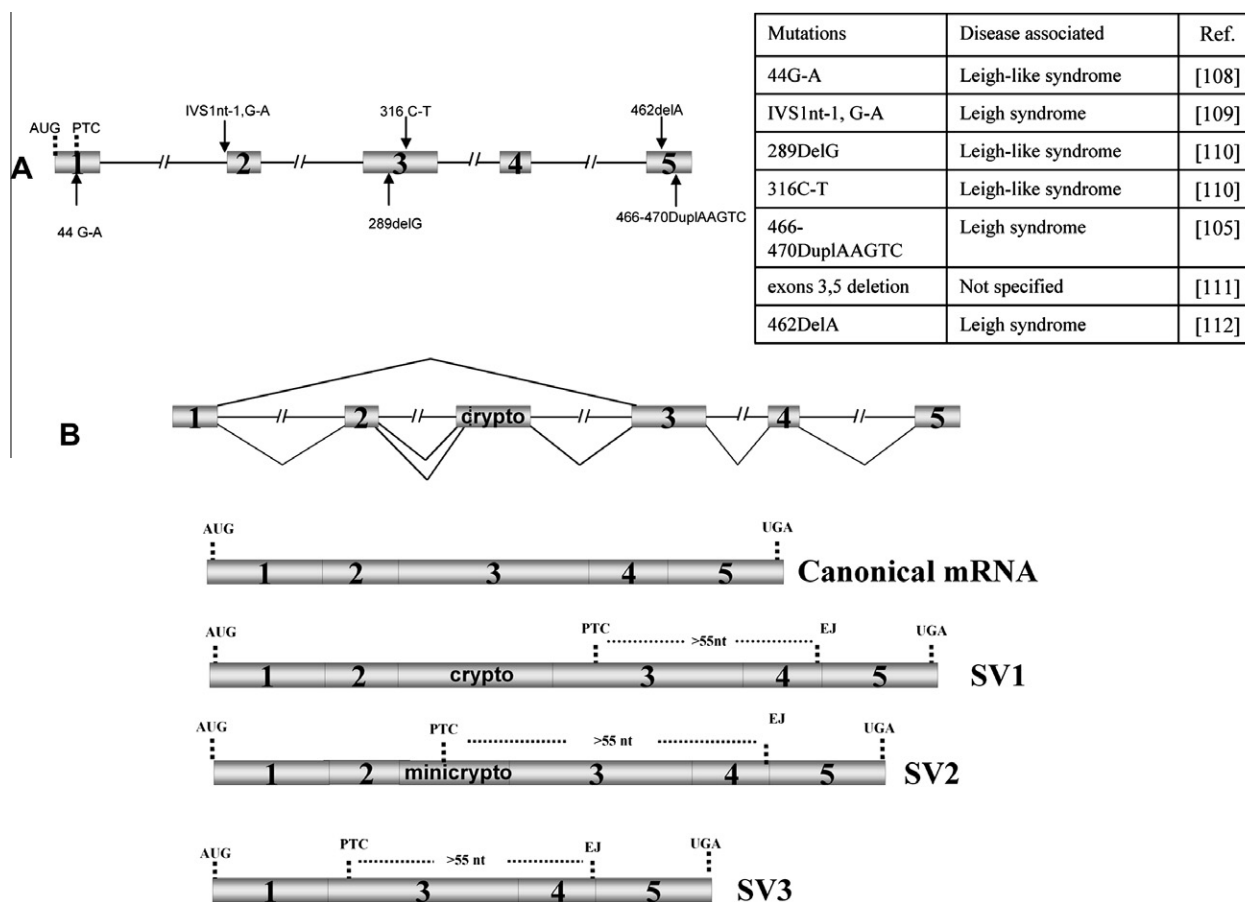
Fig. 4. (Panel A) Structure of the *NDUFS4* human gene of complex I with location of pathological mutations found in neurological patients with complex I deficiency. In the Table five exonic mutations, one intronic mutation shown in the structure of the gene and a sixth mutation with deletion of a region including exons 3 and 5, with the associated diseases are listed. For details see text and the references in the table. (Panel B), alternative splicing products of the NDUFS4 gene detected in human fibroblasts. The scheme shows the mechanism of production of the alternative splicing products normally produced by the NDUFS4 gene but up-regulated in the exonic 44G–A mutation as well as in the intronic IVS1nt-1, G–A mutation. For details see text and refs. [105,108–112].

amplification of the biochemical damage and disease progression. The depression of the activity of complex I was counteracted by the addition to the fibroblast culture of dibutyryl-cAMP, which also prevented the accumulation of oxygen free radicals [73]. Selected antioxidants and β-agonists could offer a rational therapeutical strategy in patients carrying mutations in *NDUFS1* or in other complex I genes, which result in a dysfunction centered on excess of ROS production and oxidative stress.

A more recent example of how a dysfunction in the cAMP/PKA system can result in a detrimental impairment of the catalytic activity of complex I and overproduction of ROS was provided by a study of the mitochondrial bioenergetic function in human skin fibroblasts of Down Syndromes patients [74]. Down Syndrome is a multifactorial disorder caused by trisomy of human chromosome 21, resulting in mental retardation, premature aging and neurodegeneration. A strong decrease in the cellular level of cAMP and depression of PKA activity were found in the patient's fibroblasts [74]. The serine phosphorylation of the NDUFS4 subunit and the catalytic activity of complex I were markedly reduced and the level of mitochondrial oxygen superoxide and cellular H_2O_2 increased in the patient's fibroblasts. All these changes were reversed by the addition to the fibroblasts of dibutyryl-cAMP. These observations, besides contributing to understand the pathogenetic mechanism of the Down Syndrome, may help to develop therapeutical approaches based on the use of β-adrenoceptor agonists which, as previously reported, rescue complex I deficiency and decrease ROS levels in human cell cultures.

It can also be mentioned that work in progress along other lines in the author's laboratory, in collaboration with the Department of Biotechnology and Biosciences, University of Milano-Bicocca, showed that K-ras oncogenic expression in "in vitro" transformed cells and in human tumors was associated with a dysregulation of the cAMP/PKA pathway. This appeared to result in fragmentation of mitochondria and depression of complex I activity. These defects also were all rescued by promoting formation of cAMP with forskolin (unpublished observations).

6. Pathological mutations of the NDUFS4 subunit of complex I stabilize alternative splice transcripts of the gene

A study of the NDUFS4 gene transcription in patients with pathological mutations of the gene revealed new features of surveillance mechanisms regulating the levels of alternative transcripts [106,107]. The five exons of the gene result in a canonical mRNA coding for the 175 residues precursor protein of the 18 kDa (AQDQ) subunit of complex I [22] (Fig. 4).

In a patient with a 44G → A, nonsense mutation, in the first exon caused, as expected, disappearance of the mature protein. This mutation which introduced a premature termination codon (PTC) in the proximity of the canonical AUG start codon, rather than eliciting nonsense mediated decay (NMD) degradation of the canonical mRNA, upregulated three PTC containing alternative transcripts (splice variants 1, 2 and 3) (Fig. 4) [106,107]. The splice variants 1 and 2 (SV1 and SV2) result from insertion between

exons 2 and 3 of a crypto exon which uses two alternative acceptor sites (Fig. 4). The splice variant 3 (SV3) derives from exon 2 skipping. These three alternative transcripts are also produced in normal cells, but their level is kept low by different RNA surveillance mechanisms. SV1 and SV2 were found to be degraded in the cytosol by NMD, the level of SV3 was down-regulated directly in the nucleus by a process different from NMD, which affected the production of the transcript rather than its stability [107]. In the patient fibroblasts the 44G → A nonsense mutation inactivated NMD degradation of SV1 and SV2 and nuclear down regulation of SV3 (Fig. 4). In a neurological patient with a homozygous splice acceptor site mutation in intron 1 (IVS1nt-1, G → A) of the *NDUFS4* gene (Fig. 4) only a mRNA transcript, in which exon 2 was skipped, was detected [111]. Amplification of this transcript and sequencing showed that it corresponded to the PTC containing SV3 isoform detected in the patient with 44 G → A nonsense exonic mutation in the *NDUFS4* gene (Fig. 4) [107]. The accumulation of the aberrant alternative transcripts, caused by exonic or intronic mutation in the *NDUFS4* gene, able to escape the mRNA surveillance mechanisms, can represent another deleterious event contributing to the pathogenetic mechanism of the NDUFS4 mutations in neurological patients. In vitro translation experiment showed, in fact, that the aberrant alternative transcripts were able to produce potential toxic proteins [107].

7. Selective degradation of the NDUFS3 subunit of complex I. An involvement of granzyme A mediated NDUFS3 cleavage in type I diabetes?

Type I diabetes is an autoimmune disease in which, killer lymphocytes (natural killer and cytotoxic T lymphocytes) destroy the insulin producing β-cells of the pancreas [113]. The destruction of β-cells by lymphocytes involves: formation of immune synapse through lymphocyte-binding to class I hystocompatibility complex proteins on the surface of β-cells [113] and perforin mediated injection into β-cells of members of granzyme serine proteases, where they initiate a cascade of events leading to cell death [113]. A mechanism of cell death induced by granzyme B has been proposed that involves caspase and Bid-dependent apoptosis [113].

Recent work has provided evidence showing that killer lymphocytes can induce a caspase independent cell death by delivery of granzyme A into mitochondria of the target cells [114]. Granzyme A once entered into the inner mitochondrial compartment digests, selectively, the NDUFS3 subunit of complex I [114]. Degradation of this subunit results in inhibition of the normal catalytic activity of the complex and promotion of its capacity to produce ROS. Elevation of the cellular ROS level is proposed to trigger a series of downstream events, which include translocation of the endoplasmic reticulum-associated SET complex into the nucleus, where its cleavage by granzyme A induces the release of proteins, responsible for DNA fragmentation and ultimately cell death [114].

Mitochondrial proteomic analysis, carried out in our laboratory, has revealed a selective decrease of the content of the NDUFS3 subunit of complex I in brain mitochondria of rats in which insulin-deficient type I diabetes was induced by streptozotocin treatment. The decreased content of the NDUFS3 subunit, which was essentially due to post-transcriptional events, resulted in a severe impairment of the enzymatic activity of complex I (unpublished observations).

How insulin deficiency, hyperglycemia and/or other secondary alterations do result in the selective degradation of the NDUFS3 subunit of complex I in the brain of diabetic rats remains to be clarified. It is possible that the streptozoticin-induced diabetes results in activation of granzyme A or of different endogenous protease(s) in neuronal mitochondria (see also Ref. [114]). Is it possible that selective NDUFS3 degradation by granzyme A is critically involved in a mechanism of destruction of pancreatic β-cells in human diabetes? (see also Ref. [115]).

8. Conclusions

The work presented provides an appraisal of the regulation by the cAMP/PKA pathway of the expression, assembly and catalytic activity of complex I of the mitochondrial respiratory chain in humans and mammals. The control of complex I functional capacity and of ROS production/level by the cAMP/PKA pathway has far-reaching implications in human pathophysiology. Complex I is a pace-maker of the overall activity of mitochondrial oxidative phosphorylation. In organs like brain, heart, kidney, skeletal muscle, and others, which undergo changes from rest to various degrees of activity, the ATP production by OXPHOS has to respond to the continually changing energy demand. Complex I with its abundance of structural and ancillary proteins offers the prerequisites to respond to a variety of regulatory stimuli. Regulation of complex I by the cAMP/PKA pathway, which is at the cross-road of signal transduction networks, amplifies the signals to which mitochondrial respiratory metabolism can respond. The same complexity of the regulatory network of complex I makes it particularly vulnerable to gene mutations, as well as to sporadic, endogenous and exogenous factors. The deleterious consequence of this, is, in fact, underlined by the discoveries of the different human diseases in which complex I dysfunction was, so far, found to play a determinant role. The possibility emerges, from what reported, of new therapeutical strategies for diseases in which combined dysregulation of the cAMP/PKA pathway and of complex I are involved.

Acknowledgements

References

[1] Walker, J.E. (1992) The NADH: ubiquinone oxidoreductase (complex I) of respiratory chains. Q. Rev. Biophys. 25, 232–253.
[2] Yagi, T. and Matsuno-Yagi, A. (2003) The proton-translocating NADH-quinone oxidoreductase in the respiratory chain: the secret unlocked. Biochemistry 42, 2266–2274.
[3] Brandt, U. (2006) Energy converting NADH:quinone oxidoreductase (complex I). Annual Review of Biochemistry 75, 69–92.
[4] Papa, S., Petruzzella, V. and Scacco, S. (2007) Electron transport, Structure, redox-coupled protonmotive activity and pathological disorders of respiratory chain complexes in: Handbook of Neurochemistry and Molecular Neurobiology (Lajtha, A., Dienel, G. and Gibson, G., Eds.), pp. 93–118, Springer Verlag, Berlin Heidelberg.
[5] Boyer, P.D., Chance, B., Ernster, L., Mitchel, I.P., Racker, E. and Slater, E.C. (1977) Oxidative phosphorylation and photophosphorylation. Annu. Rev. Biochem. 46, 955–966.
[6] Erecińska, M. and Silver, I.A. (1989) ATP and brain function. Cereb. Blood Flow Metab. 9, 2–19.
[7] Ohnishi, T. (1998) Iron-sulfur clusters/semiquinones in complex I. Biochim. Biophys. Acta 1363, 186–206.
[8] Hirst, J., Carrol, J., Fearnley, I.M., Shannnon, R.J. and Walker, J.E. (2003) The nuclear encoded subunits of complex I from bovine heart mitochondria. Biochim. Biophys. Acta 1604, 135–150.
[9] Efremov, R.G., Baradaran, R. and Sazanov, L.A. (2010) The architecture of respiratory complex I. Nature 465, 441–445.
[10] Cadenas, E. and Davies, K.J. (2000) Mitochondrial free radical generation, oxidative stress, and aging. Free Radic. Biol. Med. 29, 222–230.
[11] Grivennikova, V.G. and Vinogradov, A.D. (2006) Generation of superoxide by the mitochondrial complex I. Biochim. Biophys. Acta 1757, 553–561.
[12] Hirst, J. (2010) Towards the molecular mechanism of respiratory complex I. Biochem. J. 425, 327–339.

[13] Carroll, J., Fearnley, I.M., Skehel 1, J.M., Shannon, R.J., Hirst, J. and Walker, J.E. (2006) Bovine complex i is a complex of 45 different subunits. J. Biol. Chem 281, 32724–32727.

[14] Antonicka, H., Ogilvie, I., Taivassalo, T., Anitori, R.P., Haller, R.G., Vissing, J., Kennaway, N.G. and Shoubridge, E.A. (2003) Identification and characterization of a common set of complex I assembly intermediates in mitochondria from patients with complex I deficiency. J. Biol. Chem. 278, 43081–43088.

[15] Scacco, S., Petruzzella, V., Budde, S., Vergari, R., Tamborra, R., Panelli, D., van den Heuvel, L.P., Smeitink, J.A. and Papa, S. (2003) Pathological mutations of the humanNDUFS4 gene of the 18-kDa (AQDQ) subunit of complex I affect the expression of the protein and the assembly and function of the complex. J. Biol. Chem. 278, 44161–44167.

[16] Scheffler, I.E., Yadava, N. and Potluri, P. (2004) Molecular genetics of complex I-deficient Chinese hamster cell lines. Biochim. Biophys. Acta 1659, 160–171.

[17] Potluri, P., Yadava, N. and Scheffler, I.E. (2004) The role of the ESSS protein in the assembly of a functional and stable mammalian mitochondrial complex I (NADH-ubiquinone oxidoreductase). Eur. J. Biochem. 271, 3265–3273.

[18] Papa, S., Scacco, S., Sardanelli, A.M., Vergari, R., Papa, F., Budde, S., van den Heuvel, L. and Smeitink, J. (2001) Mutation in the NDUFS4 gene of complex I abolishes cAMP dependent activation of the complex in a child with fatal neurological syndrome. FEBS Lett. 489, 259–262.

[19] Fearnley, I.M., Carroll, J., Shannon, R.J., Runswick, M.J., Walker, J.E. and Hirst, J. (2001) GRIM-19, a cell death regulatory gene product, is a subunit of bovine mitochondrial NADH: ubiquinone oxidoreductase (complex I). J. Biol. Chem. 276, 38345–38348.

[20] Carrol, J., Fearnley, I.M., Shannon, R.J., Hirst, J. and Walker, J.E. (2003) Analysis of the subunit composition of complex I from bovine heart mitochondria. Mol. Cell. Proteomics 2, 117–126.

[21] Papa, S., Sardanelli, A.M., Cocco, T., Speranza, F., Scacco, S. and Technikova-Dobrova, Z. (1996) The nuclear-encoded 18 kDa (IP) AQDQ subunit of bovine heart complex I is phosphorylated by the mitochondrial cAMP-dependent protein kinase. FEBS Lett. 379, 299–301.

[22] De Rasmo, D., Palmisano, G., Scacco, S., Technikova-Dobrova, Z., Panelli, D., Cocco, T., Sardanelli, A.M., Gnoni, A., Micelli, L., Trani, A., Di Luccia, A. and Papa, S. (2010) Phosphorylation pattern of the NDUFS4 subunit of complex I of the mammalian respiratory chain. Mitochondrion 10, 464–471.

[23] Chen, R., Fearnley, I.M., Peak-Chew, S.Y. and Walker, J.E. (2004) The phosphorylation of subunits of complex I from bovine heart mitochondria. J. Biol. Chem. 279, 26036–26045.

[24] Schilling, B., Aggeler, R., Schulenberg, B., Murray, J., Row, R.H., Capaldi, R.A. and Gibson, B.W. (2005) Mass spectrometric identification of a novel phosphorylation site in subunit NDUFA10 of bovine mitochondrial complex I. FEBS Lett. 579, 2485–2490.

[25] Pocsfalvi, G., Cuccurullo, M., Schlosser, G., Scacco, S., Papa, S. and Malori, A. (2007) Phosphorylation of B14.5a subunit from bovine heart complex I identified by titanium dioxide selective enrichment and shotgun proteomics. Mol. Cell Proteomics 6, 231–237.

[26] Ahn, B.H., Kim, H.S., Song, S., Lee, I.H., Liu, J., Vassilopoulos, A., Deng, C.X. and Finkel, T. (2008) A role for the mitochondrial deacetylase Sirt3 in regulating energy homeostasis. Proc. Natl. Acad. Sci. USA 105, 14447–14452.

[27] Mckenzie, M. and Ryan, M.T. (2010) Assembly factors of human mitochondrial complex I and their defects in disease. IUBMB Life 62, 497–502.

[28] Fernández-Vizarra, E., Tiranti, V. and Zeviani, M. (2009) Assembly of the oxidative phosphorylation system in humans: what we have learned by studying its defects. Biochim. Biophys. Acta 1793, 200–211.

[29] Koopman, W.J., Nijtmans, L.G., Dieteren, C.E., Roestenberg, P., Valsecchi, F., Smeitink, J.A. and Willems, P.H. (2010) Mammalian mitochondrial complex I: biogenesis, regulation, and reactive oxygen species generation. Antioxid. Redox Signal 12, 1431–1470.

[30] Hüttemann, M., Lee, I., Samavati, L., Yu, H. and Doan, J.W. (2007) Regulation of mitochondrial oxidative phosphorylation through cell signalling. Biochim. Biophys. Acta 1773, 1701–1720.

[31] Remacle, C., Barbieri, M.R., Cardol, P. and Hamel, P.P. (2008) Eukaryotic complex I: functional diversity and experimental systems to unravel the assembly process. Mol. Genet. Genomics 280, 93–110.

[32] Yadava, N., Potluri, P. and Scheffler, I.E. (2008) Investigations of the potential effects of phosphorylation of the MWFE and ESSS subunits on complex I activity and assembly. Int. J. Biochem. Cell Biol. 40, 447–460.

[33] Fato, R., Bergamini, C., Leoni, S., Strocchi, P. and Lenaz, G. (2008) Generation of reactive oxygen species by mitochondrial complex I: implications in neurodegeneration. Neurochem. Res. 33, 2487–2501.

[34] Angell, R., Lindner, D.J., Shapiro, P.S., Hofmann, E.R. and Kalvakolanu, D.V. (2000) Identification of GRIM-19, a novel cell death-regulatory gene induced by the interferon and retinoic acid combination, using a genetic approach. J. Biol. Chem. 275, 33416–33426.

[35] Fontaine, E. and Bernardi, P. (1999) Progress on the mitochondrial permeability transition pore: regulation by complex I and ubiquinone analogs. J. Bioenerg. Biomembr. 31, 335–345.

[36] Papa, S. (1996) Mitochondrial oxidative phosphorylation changes in the life span. Molecular aspects and physiopathological implications. Biochim. Biophys. Acta 1276, 87–105.

[37] Ventura, B., Genova, M.L., Bovina, C., Formiggini, G. and Lenaz, G. (2002) Control of oxidative phosphorylation by Complex I in rat liver mitochondria: implications for aging. Biochim. Biophys. Acta 1553, 249–260.

[38] Gadaleta, M.N., Cormio, A., Pesce, V., Lezza, A.M. and Cantatore, P. (1998) Aging and mitochondria. Biochimie 80, 863–870.

[39] Robinson, B.H. (1998) Human complex I deficiency: clinical spectrum and involvement of oxygen free radicals in the pathogenicity of the defect. Biochim. Biophys. Acta 1364, 271–286.

[40] Scacco, S., Vergari, R., Scarpulla, R.C., Technikova-Dobrova, Z., Sardanelli, A.M., Lambo, R., Lorusso, V. and Papa, S. (2000) CAMP-dependent phosphorylation of the nuclear encoded 18-kDa (IP) subunit of respiratory complex I and activation of the complex in serum-starved mouse fibroblast cultures. J. Biol. Chem. 275, 17578–17582.

[41] Franko, A., Mayer, S., Thiel, G., Mercy, L., Arnould, T., Hornig-Do, H.T., Wiesner, R.J. and Goffart, S. (2008) CREB-1alpha is recruited to and mediates upregulation of the cytochrome c promoter during enhanced mitochondrial biogenesis accompanying skeletal muscle differentiation. Mol. Cell Biol. 28, 2446–2459.

[42] De Rasmo, D., Gattoni, G., Papa, F., Santeramo, A., Pacelli, C., Cocco, T., Micelli, L., Sardaro, N., Larizza, M., Scivetti, M., Milano, S. and Signorile, A. (2011) The β-adrenoceptor agonist isoproterenol promotes the activity of respiratory chain complex I and lowers cellular reactive oxygen species in fibroblasts and heart myoblasts. Eur. J. Pharmacol. 652, 15–22.

[43] Smeitink, J., van den Heuvel, L. and DiMauro, S. (2001) The genetics and pathology of oxidative phosphorylation. Nat. Rev. Genet. 2, 342–352.

[44] Petruzzella, V. and Papa, S. (2002) Mutations in human nuclear genes encoding for subunits of human mitochondrial respiratory complex I: the NDUFS4 gene. Gene 286, 149–154.

[45] Bugiani, M., Invernizzi, F., Alberio, S., Briem, E., Lamantea, E., Carrara, F., Moroni, I., Farina, L., Spada, M., Donati, M.A., Uziel, G. and Zeviani, M. (2004) Clinical and molecular findings in children with complex I deficiency. Biochim. Biophys. Acta 1659, 136–147.

[46] Papa, S., Petruzzella, V., Scacco, S., Sardanelli, A.M., Iuso, A., Panelli, D., Vitale, R., Trentadue, R., De Rasmo, D., Capitanio, N., Piccoli, C., Papa, F., Scivetti, M., Bestini, E., Rizza, T. and De Michele, G. (2009) Pathogenetic mechanisms in hereditary dysfunctions of complex I of the respiratory chain in neurological diseases. Biochim. Biophys. Acta 1787, 502–517.

[47] Papa, S., Sardanelli, A.M., Capitanio, N. and Piccoli, C. (2009) Mitochondrial respiratory disfuction and mutations in mitochondrial DNA in PINK1 familial Parkinsonism. J. Bioenerg. Biomembr. 41, 509–516.

[48] Chatterjee, A., Mambo, E. and Sidransky, D. (2006) Mitochondrial DNA mutations in human cancer. Oncogene 25, 4663–4674.

[49] Ishikawa, K., Takenaga, K., Akimot, O.M., Koshikawa, N., Yamaguchi, A., Imanishi, H., Nakada, K., Honma, Y. and Hayashi, J. (2008) ROS-generating mitochondrial DNA mutations can regulate tumor cell metastasis. Science 320, 661–664.

[50] Pagliarini, D.J., Calvo, S.E., Chang, B., Sheth, S.A., Vafai, S.B., Ong, S.E., Walford, G.A., Sugiana, C., Boneh, A., Chen, W.K., Hill, D.E., Vidal, M., Evans, J.G., Thorburn, D.R., Carr, S.A. and Mootha, V.K. (2008) A mitochondrial protein compendium elucidates complex I disease biology. Cell 134, 112–123.

[51] Atorino, L., Silvestri, L., Koppen, M., Cassina, L., Ballabio, A., Marconi, R., Langer, T. and Casari, G. (2003) Loss of m-AAA protease in mitochondria causes complex I deficiency and increased sensitivity to oxidative stress in hereditary spastic paraplegia. J. Cell. Biol. 163, 777–787.

[52] Verdanis, A. (1977) Protein kinase activity at the inner membrane of mammalian mitochondria. J. Biol. Chem. 252, 807–813.

[53] Technikova-Dobrova, Z., Sardanelli, A.M. and Papa, S. (1993) Phosphorylation of mitochondrial proteins in bovine heart. Characterization of kinases and substrates. FEBS Lett. 322, 51–55.

[54] Technikova-Dobrova, Z., Sardanelli, A.M., Stanca, M.R. and Papa, S. (1994) CAMP-dependent protein phosphorylation in mitochondria of bovine heart. FEBS Lett. 350, 187–191.

[55] Sardanelli, A.M., Technikova-Dobrova, Z., Scacco, S., Speranza, F. and Papa, S. (1995) Characterization of proteins phosphorylated by the cAMP-dependent protein kinase of bovine heart mitochondria. FEBS Lett. 377, 470–474.

[56] Sardanelli, A.M., Technikova-Dobrova, Z., Speranza, F., Mazzocca, A., Scacco, S. and Papa, S. (1996) Topology of the mitochondrial cAMP-dependent protein kinase and its substrates. FEBS Lett. 396, 276–278.

[57] Taylor, S.S., Buechler, J.A. and Yonemoto, W. (1990) CAMP-dependent protein kinase: framework for a diverse family of regulatory enzymes. Annu. Rev. Biochem. 59, 971–1005.

[58] Sardanelli, A.M., Signorile, A., Nuzzi, R., De Rasmo, D., Technikova-Dobrova, Z., Drahota, Z., Occhiello, A., Pica, A. and Papa, S. (2006) Occurrence of A-kinase anchor protein and associated cAMP-dependent protein kinase in the inner compartment of mammalian mitochondria. FEBS Lett. 580, 5690–5696.

[59] Carroll, J., Shannon, R.J., Fearnley, I.M., Walker, J.E. and Hirst, J. (2002) Definition of the nuclear encoded protein composition of bovine heart mitochondrial complex I. Identification of two new subunits. J. Biol. Chem. 277, 50311–50317.

[60] Steen, H., Jebanathirajah, J.A., Rush, J., Morrice, N. and Kirschner, M.W. (2006) Phosphorylation analysis by mass spectrometry: myths, facts, and the consequences for qualitative and quantitative measurements. Mol. Cell Proteomics 5, 172–181.

[61] Beavo, J.A. and Brunton, L.L. (2002) Cyclic nucleotide research still expanding after half a century. Nat. Rev. Mol. Cell Biol. 3, 710–718.

[62] Baillie, G.S., Scott, J.D. and Houslay, M.D. (2005) Compartmentalisation of phosphodiesterases and protein kinase A: opposites attract. FEBS Lett. 579, 3264–3270.

[63] Zaccolo, M. and Pozzan, T. (2002) Discrete microdomains with high concentration of cAMP in stimulated rat neonatal cardiac myocytes. Science 295, 1711–1715.

[64] Zippin, J.H., Chen, Y., Nahirney, P., Kamenetsky, M., Wuttke, M.S., Fischman, D.A., Levin, L.R. and Buck, J. (2003) Compartmentalization of bicarbonate-sensitive adenylyl cyclase in distinct signaling microdomains. FASEB J. 17, 82–84.

[65] Buck, J., Sinclair, M.L., Schapal, L., Cann, M.J. and Levin, L.R. (1999) Cytosolic adenylyl cyclase defines a unique signaling molecule in mammals. Proc. Natl. Acad. Sci. USA 96, 79–84.

[66] Chen, Y., Cann, M.J., Litvin, T.N., Iourgenko, V., Sinclair, M.L., Levin, L.R. and Buck, J. (2000) Soluble adenylyl cyclase as an evolutionarily conserved bicarbonate sensor. Science 289, 625–628.

[67] Wong, W. and Scott, J.D. (2004) AKAP Signalling complexes: focal points in space and time. Nat. Rev. Mol. Cell Biol. 5, 959–970.

[68] Feliciello, A., Gottesman, M.E. and Avvedimento, E.V. (2001) The biological functions of A-kinase anchor proteins. J. Mol. Biol. 308, 99–114.

[69] Technikova-Dobrova, Z., Sardanelli, A.M., Speranza, F., Scacco, S., Signorile, A., Lorusso, V. and Papa, S. (2001) Cyclic adenosine monophosphate-dependent phosphorylation of mammalian mitochondrial proteins: enzyme and substrate characterization and functional role. Biochemistry 40, 13941–13947.

[70] Chen, Q., Lin, R. and Rubin, C. (1997) Organelle-specific targeting of protein kinase AII (PKAII). Molecular and in situ characterization of murine A kinase anchor proteins that recruit regulatory subunits of PKAII to the cytoplasmic surface of mitochondria. J. Biol. Chem. 272, 15247–15257.

[71] Signorile, A., Sardanelli, A.M., Nuzzi, R. and Papa, S. (2002) Serine (threonine) phosphatase(s) acting on cAMP-dependent phosphoproteins in mammalian mitochondria. FEBS Lett. 512, 91–94.

[72] Piccoli, C., Scacco, S., Bellomo, F., Signorile, A., Iuso, A., Boffoli, D., Scrima, R., Capitanio, N. and Papa, S. (2006) CAMP controls oxygen metabolism in mammalian cells. FEBS Lett. 580, 4539–4553.

[73] Iuso, A., Scacco, S., Piccoli, C., Bellomo, F., Petruzzella, V., Trentadue, R., Minuto, M., Ripoli, M., Capitanio, N., Zeviani, M. and Papa, S. (2006) Dysfunctions of cellular oxidative metabolism in patients with mutations in the NDUFS1 and NDUFS4 genes of complex I. J. Biol. Chem. 281, 10374–10380.

[74] Valenti, D., Manente, G.A., Moro, L., Marra, E. and Vacca, R.A. (2011) Deficit of complex I activity in human skin fibroblasts with chromosome 21 trisomy and overproduction of reactive oxygen species by mitochondria: involvement of the cAMP/PKA signalling pathway. Biochem. J. 435, 679–688.

[75] Baracca, A., Chiaradonna, F., Sgarbi, G., Solaini, G., Alberghina, L. and Lenaz, G. (2010) Mitochondrial Complex I decrease is responsible for bioenergetic dysfunction in K-ras transformed cells. Biochim. Biophys. Acta 1797, 314–323.

[76] Hurd, T.R., Requejo, R., Filipovska, A., Brown, S., Prime, T.A., Robinson, A.J., Fearnley, I.M. and Murphy, M.P. (2008) Complex I within oxidatively stressed bovine heart mitochondria is glutathionylated on Cys-531 and Cys-704 of the 75-kDa subunit: potential role of CYS residues in decreasing oxidative damage. J. Biol. Chem. 283, 24801–24815.

[77] Scarpulla, R.C. (2008) Transcriptional paradigms in mammalian mitochondrial biogenesis and function. Physiol. Rev. 88, 611–638.

[78] De Rasmo, D., Signorile, A., Roca, E. and Papa, S. (2009) CAMP response element-binding protein (CREB) is imported into mitochondria and promotes protein synthesis. FEBS J. 276, 4325–4333.

[79] Mayr, B. and Montminy, M. (2001) Transcriptional regulation by the phosphorylation- dependent factor CREB. Nat. Rev. Mol. Cell Biol. 2, 599–609.

[80] Shaywitz, A.J. and Greenberg, M.E. (1999) CREB: a stimulus induced transcription factor activated by a diverse array of extracellular signals. Annu. Rev. Biochem. 68, 821–861.

[81] Zhang, X., Odom, D.T., Koo, S.H., Conkright, M.D., Canettieri, G., Best, J., Chen, H., Jenner, R., Herbolsheimer, E., Jacobsen, E., Kadam, S., Ecker, J.R., Emerson, B., Hogenesch, J.B., Unterman, T., Young, R.A. and Montminy, M. (2005) Genome-wide analysis of cAMP response element binding protein occupancy, phosphorylation, and target gene activation in human tissues. Proc. Natl. Acad. Sci. USA 102, 4459–4464.

[82] Screaton, R.A., Conkright, M.D., Katoh, Y., Best, J.L., Canettieri, G., Jeffries, S., Guzman, E., Niessen, S., Yates III, J.R., Takemori, H., Okamoto, M. and Montminy, M. (2004) The CREB coactivator TORC2 functions as a calcium- and cAMP-sensitive coincidence detector. Cell 119, 61–74.

[83] Gerhart-Hines, Z., Rodgers, J.T., Bare, O., Lerin, C., Kim, S.H., Mostoslavsky, R., Alt, F.W., Wu, Z. and Puigserver, P. (2007) Metabolic control of muscle mitochondrial function and fatty acid oxidation through SIRT1/PGC-1alpha. EMBO J. 26, 1913–1923.

[84] Zong, H., Ren, J.M., Young, L.H., Pypaert, M., Mu, J., Birnbaum, M.J. and Shulman, G.I. (2002) AMP kinase is required for mitochondrial biogenesis in skeletal muscle in response to chronic energy deprivation. Proc. Natl. Acad. Sci. USA 99, 15983–15987.

[85] Li, X., Monks, B., Ge, Q. and Birnbaum, M.J. (2007) Akt/PKB regulates hepatic metabolism by directly inhibiting PGC-1alpha transcription coactivator. Nature 447, 1012–1016.

[86] Barrès, R., Osler, M.E., Yan, J., Rune, A., Fritz, T., Caidahl, K., Krook, A. and Zierath, J.R. (2009) Non-CpG methylation of the PGC-1alpha promoter through DNMT3B controls mitochondrial density. Cell Metab. 10, 189–198.

[87] Hardie, D.G. (2011) Sensing of energy and nutrients by AMP-activated protein kinase. Am. J. Clin. Nutr. 93, 891S–896S.

[88] Rodgers, J.T., Lerin, C., Haas, W., Gygi, S.P., Spiegelman, B.M. and Puigserver, P. (2005) Nutrient control of glucose homeostasis through a complex of PGC-1alpha and SIRT1. Nature 434, 113–118.

[89] Yuzefovych, L., Wilson, G. and Rachek, L. (2010) Different effects of oleate vs. palmitate on mitochondrial function, apoptosis, and insulin signaling in L6 skeletal muscle cells: role of oxidative stress. Am. J. Physiol. Endocrinol. Metab. 299, E1096–E10105.

[90] Hao, J., Shen, W., Yu, G., Jia, H., Li, X., Feng, Z., Wang, Y., Weber, P., Wertz, K., Sharman, E. and Liu, J. (2010) Hydroxytyrosol promotes mitochondrial biogenesis and mitochondrial function in 3T3-L1 adipocytes. J. Nutr. Biochem. 21, 634–644.

[91] Lagouge, M., Argmann, C., Gerhart-Hines, Z., Meziane, H., Lerin, C., Daussin, F., Messadeq, N., Milne, J., Lambert, P., Elliott, P., Geny, B., Laakso, M., Puigserver, P. and Auwerx, J. (2006) Resveratrol improves mitochondrial function and protects against metabolic disease by activating SIRT1 and PGC-1alpha. Cell 127, 1109–1122.

[92] Aquilano, K., Vigilanza, P., Balzelli, S., Pagliei, B., Rotilio, G. and Circolo, M.R. (2010) Peroxisome proliferator-activated receptor gamma co-activator 1alpha (PGC-1alpha) and sirtuin 1 (SIRT1) reside in mitochondria: possible direct function in mitochondrial biogenesis. J. Biol. Chem. 285, 21590–21599.

[93] Wu, Z., Huang, X., Feng, Y., Handschin, C., Feng, Y., Gulicksen, P.S., Bare, O., Labow, M., Spiegelman, B. and Stevenson, S.C. (2006) Transducer of regulated CREB-binding proteins (TORCs) induce PGC-1alpha transcription and mitochondrial biogenesis in muscle cells. Proc. Natl. Acad. Sci. USA 103, 14379–14384.

[94] Lin, J., Handschin, C. and Spiegelman, B.M. (2005) Metabolic control through the PGC-1 family of transcription coactivators. Cell Metab. 1, 361–370.

[95] Wu, Z., Puigserver, P., Andersson, U., Zhang, C., Adelmant, G., Mootha, V., Troy, A., Cinti, S., Lowell, B., Scarpulla, R.C. and Spiegelman, B.M. (1999) Mechanisms controlling mitochondrial biogenesis and respiration through the thermogenic coactivator PGC-1. Cell 98, 115–124.

[96] Kelly, D.P. and Scarpulla, R.C. (2004) Transcriptional regulatory circuits controlling mitochondrial biogenesis and function. Genes Dev. 18, 357–368.

[97] Falkenberg, M., Gaspari, M., Rantanen, A., Trifunovic, A., Larsson, N.-G. and Gustafsson, C.M. (2002) Mitochondrial transcription factors B1 and B2 activate transcription of human mtDNA. Nat. Genet. 31, 289–294.

[98] Cammarota, M., Paratcha, G., Bevilaqua, L.R., Levi de Stein, M., Lopez, M., Pellegrino de Iraldi, A., Izquierdo, I. and Medina, J.H. (1999) Cyclic AMP-responsive element binding protein in brain mitochondria. J. Neurochem. 72, 2272–2277.

[99] Ryu, H., Lee, J., Impey, S., Ratan, R.R. and Ferrante, R.J. (2005) Antioxidants modulate mitochondrial PKA and increase CREB binding to D-loop DNA of the mitochondrial genome in neurons. Proc. Natl. Acad. Sci. USA 102, 13915–13920.

[100] Neupert, W. and Brunner, M. (2002) The protein import motor of mitochondria. Nat. Rev. Mol. Cell Biol. 3, 555–565.

[101] Lill, R. and Mühlenhoff, U. (2008) Maturation of iron-sulfur proteins in eukaryotes: mechanisms, connected processes, and diseases. Annu. Rev. Biochem. 77, 669–700.

[102] Lazarou, M., McKenzie, M., Ohtake, A., Thorburn, D.R. and Ryan, M.T. (2007) Analysis of the assembly profiles for mitochondrial and nuclear-DNA-encoded subunits into Complex I. Mol. Cell Biol. 27, 4228–4237.

[103] De Rasmo, D., Panelli, D., Sardanelli, A.M. and Papa, S. (2008) CAMP-dependent protein kinase regulates the mitochondrial import of the nuclear encoded NDUFS4 subunit of complex I. Cell Signal. 20, 989–997.

[104] Schon, E.A., Bonilla, E. and DiMauro, S. (1997) Mitochondrial DNA mutations and pathogenesis. J. Bioenerg. Biomembr. 29, 131–149.

[105] van den Heuvel, L., Ruitenbeek, W., Smeets, R., Gelman-Kohan, Z., Elpeleg, O., Loeffen, J., Trijbels, F., Mariman, E., de Bruijn, D. and Smeitink, J. (1998) Demonstration of a new pathogenic mutation in human complex I deficiency: a 5-bp duplication in the nuclear gene encoding the 18-kD (AQDQ) subunit. Am. J. Hum. Genet. 62, 262–268.

[106] Petruzzella, V., Panelli, D., Torraco, A., Stella, A. and Papa, S. (2005) Mutations in the NDUFS4 gene of mitochondrial complex I alter stability of the splice variants. FEBS Lett. 579, 3770–3776.

[107] Panelli, D., Petruzzella, V., Vitale, R., De Rasmo, D., Munnich, A., Rötig, A. and Papa, S. (2008) The regulation of PTC containing transcripts of the human NDUFS4 gene of complex I of respiratory chain and the impact of pathological mutations. Biochimie 90, 1452–1460.

[108] Petruzzella, V., Vergari, R., Puzziferri, I., Boffoli, D., Lamantea, E., Zeviani, M. and Papa, S. (2001) A nonsense mutation in the NDUFS4 gene encoding the 18 kDa (AQDQ) subunit of complex I abolishes assembly and activity of the complex in a patient with Leigh-like syndrome. Hum. Mol. Genet. 10, 529–535.

[109] Benit, P., Steffann, J., Lebon, S., Chretien, D., Kadhom, N., de Lonlay, P., Goldenberg, A., Dumez, Y., Dommergues, M., Rustin, P., Munnich, A. and Rotig, A. (2003) Genotyping microsatellite DNA markers at putative disease loci in inbred/multiplex families with respiratory chain complex I deficiency allows rapid identification of a novel nonsense mutation (IVS1nt-1) in the NDUFS4 gene in Leigh syndrome. Hum. Genet. 112, 563–566.

[110] Budde, S.M., van den Heuvel, L.P., Janssen, A.J., Smeets, R.J., Buskens, C.A., DeMeirlenk, L., Van Coster, R., Baethmann, M., Voit, T., Trijbels, J.M. and Smeitink, J.A. (2000) Combined enzymatic complex I and III deficiency associated with mutations in the nuclear encoded NDUFS4 gene. Biochem. Biophys. Res. Commun. 18, 63–68.

[111] Rötig, A., Lebon, S., Zinovieva, E., Mollet, J., Sarzi, E., Bonnefont, J.P. and Munnich, A. (2004) Molecular diagnostics of mitochondrial disorders. Biochim. Biophys. Acta 1659, 129–135.

[112] Anderson, S.L., Chung, W.K., Frezzo, J., Papp, J.C., Ekstein, J., Di Mauro, S., Rubin, B.Y. (2008) A novel mutation in NDUFS4 causes Leigh syndrome in an Ashkenazi Jewish family. J. Inherit. Metab. Dis. Dec 26.

[113] Thomas, H.E., Trapani, J.A. and Kay, T.W. (2010) The role of perforin and granzymes in diabetes. Cell Death Differ. 17, 577–585.

[114] Lieberman, J. (2010) Granzyme A activates another way to die. Immunol. Rev. 235, 93–104.

[115] Held, W., MacDonald, H.R., Weissman, I.L., Hess, M.W. and Mueller, C. (1990) Genes encoding tumor necrosis factor alpha and granzyme A are expressed during development of autoimmune diabetes. Proc. Natl. Acad. Sci. USA 87, 2239–2243.