Complex I deficiencies in neurological disorders

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Complex I is the point of entry in the mitochondrial electron transport chain for NADH reducing equivalents, and it behaves as a regulatable pacemaker of respiratory ATP production in human cells. Defects in complex I are associated with several human neurological disorders, including primary mitochondrial diseases, Parkinson disease (PD), and Down syndrome, and understanding the activity and regulation of complex I may reveal aspects of the underlying pathogenic mechanisms. Complex I is regulated by cyclic AMP (cAMP) and the protein kinase A (PKA) signal transduction pathway, and elucidating the role of the cAMP/PKA system in regulating complex I and oxygen free radical production provides new perspectives for devising therapeutic strategies for neurological diseases.

Complex I: an adaptable pacemaker of oxidative phosphorylation (OXPHOS) in human cells

Complex I of the mitochondrial respiratory chain has recently been identified as a cAMP-regulated enzyme complex defective in several neurological diseases. These findings reveal novel molecular mechanisms underlying the pathogenesis of these disorders and open new perspectives for treating these diseases.

In human cells, mitochondrial OXPHOS produces sufficient amounts of ATP for most cellular requirements [1]. This is particularly true in neuronal cells, which have a great need for aerobic ATP production. In addition, ATP production has to be continually adapted to cover current requirements, with marked changes in production levels in tissues alternating between different states [1,2].

Complex I of the respiratory chain (NADH-ubiquinone oxidoreductase, EC 1.6.5.3) [1,3] is the point in the respiratory chain where reducing equivalents, conveyed by NAD-linked dehydrogenases, are introduced. This complex contributes 40% of the electrochemical proton gradient (\(\Delta \mu^H^+\)), set up across the inner mitochondrial membrane by electron flow down the respiratory chain, and utilized by the ATP synthase, or complex V, to make ATP from ADP (Figure 1) [1]. However, complex I, as well as complex III [4,5], of the respiratory chain can also transfer single electrons to oxygen, which forms oxygen superoxide.

\begin{itemize}
  \item \textbf{Glossary}
  \begin{itemize}
    \item \textit{\(\Delta \mu^H^+\)}: transmembrane electrochemical proton gradient setup across the inner mitochondrial membrane by electron flow down the respiratory chain. It is utilized by ATP synthase to make ATP.
    \item Cardiolipin: is a type of diphosphatidylglycerol lipid that is found primarily in the inner membrane of mitochondria. The presence of cardiolipin in this membrane is important for the proper structure and activity of many proteins involved in mitochondrial metabolism and function.
    \item Complex I: a multi-subunit proton-motive NADH-ubiquinone oxidoreductase of the mitochondrial respiratory chain.
    \item CREB: cAMP response element-binding protein is a protein that belongs to the basic leucine zipper superfamily of transcription factors. It binds to DNA sequences called cAMP response elements (CRE) to modulate gene transcription. Its transcriptional activity is regulated by cAMP/CRE\textsuperscript{beta}-dependent phosphorylation of the protein to create phospho-CREB.
    \item Cyclic AMP: 3’-5’-cyclic adenosine monophosphate is an important second messenger in intracellular signal transduction and is involved in many biological processes. It is synthesized from ATP by adenylyl cyclase and can be degraded to AMP by phosphodiesterases.
    \item D-Loop: noncoding region of approximately 1200 base pairs found in mitochondrial DNA. It is the regulatory region for replication and transcription of mitochondrial DNA.
    \item DS: Down syndrome is a genetic disorder caused by trisomy of chromosome 21. Patients with DS show early aging associated with decline in conventional intellectual abilities and a high tendency to develop neuropathological features.
    \item FMN: flavin mononucleotide, a prosthetic group of various oxidoreductases including NADH dehydrogenase.
    \item GRIM-19: gene associated with retinoid and interferon-induced mortality. The GRIM19 protein, is an accessory subunit of the mitochondrial respiratory chain complex I.
    \item Leigh disease: is a rare neurometabolic disorder that affects the central nervous system. It is an inherited disorder that affects infants between the age of 3 months and 2 years and, in rare cases, teenagers and adults.
    \item NDUFS1: NADH dehydrogenase (ubiquinone) Fe–S protein 1, 75 kDa subunit. This protein is the largest subunit of complex I and contains four Fe–S clusters. Mutations in this gene are associated with complex I deficiency.
    \item NDUFS4: NADH dehydrogenase supernumerary subunit, 18 kDa A0D0 subunit of complex I. This protein does not contain Fe–S clusters. Mutations in the gene are associated with complex I deficiency.
    \item NRF1 and NRF2: nuclear respiratory factors 1 and 2 are transcription factors that activate the expression of nuclear genes of OXPHOS complexes, heme biosynthesis, and mitochondrial DNA transcription and replication.
    \item PD: Parkinson disease is the most common human neurodegenerative movement disorder. PD is a sporadic condition the vast majority of the time, but 5–10% of the cases are caused by gene mutations in several different genes. Mutations in more than ten genes have, so far, been identified as responsible for hereditary forms of Parkinsonism, including PARK6, which encodes for PTEN-induced putative kinase 1 (PINK1).
    \item PGC-1\textalpha: peroxisome proliferator-activated receptor gamma coactivator 1-alpha is the master transcriptional coactivator that regulates expression of genes of the OXPHOS system.
    \item PKA: cAMP-dependent protein kinase.
    \item ROS: reactive oxygen species include oxygen superoxide anion, hydrogen peroxide, and the hydroxyl radical. These compounds are chemically reactive and their presence can damage DNA, RNA, or proteins. Although the cell has enzymes whose purpose is to convert ROS molecules to oxygen and water,
  \end{itemize}
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excessive production can overwhelm these protective mechanisms and lead to
the accumulation of damaged cellular components.
sAC: soluble adenylyl cyclase is an enzyme that converts ATP to cAMP. It is
insensitive to G protein regulation, is localized in organelles such as the
mitochondria and nucleus, and is regulated by bicarbonate.
SIRT1: Sir2p1, also known as NAD-dependent deacetylase sirtuin-1, is a
member of the sirtuin family. It is involved in protein deacetylation, chromatin
silencing, and the regulation of transcription.
SIRT3: mitochondrial NAD-dependent deacetylase sirtuin-3 is a member
of the sirtuin family. SIRT3 exhibits NAD⁺-dependent deacetylase activity
and has been implicated in the regulation of metabolic mitochondrial processes.
STAT3: signal transducer and transcription activator mediates cellular
responses to interleukins and other growth factors.
TFAM: mitochondrial transcription factor A is a key protein activator of
mitochondrial DNA replication and transcription.
Threshold of respiratory enzymes: the percent inhibition of enzyme that results in
a depression of the overall respiratory rate.
TIM: translocase of the inner membrane, a complex of proteins in the inner
mitochondrial membrane that facilitates the translocation of proteins across
the membrane.
tmAC: plasma membrane adenylyl cyclase, activated by G-proteins.
TOM: translocase of the outer membrane, a complex of proteins in the outer
mitochondrial membrane that facilitates the translocation of proteins across
the membrane.

\([\text{O}_2^-]\) in the mitochondrial matrix; consequently, mito-
ochondrial DNA and subunits of the OXPHOS complexes
exposed to this space experience oxidative damage from
the superoxide radical (Figure 1 and Box 1).

In mammals, complex I has a total of 44 or 45 subunits
[1,6,7]. Fourteen of these are conserved from prokaryotes
to humans, the others are supernumerary [1,6]. Given that
the 14 conserved subunits are sufficient for the redox and
proton-motive activity of the complex, the function of the
large number of supernumerary subunits, which bring
with them increased genetic complexity and vulnerability
as well as phenotypic variability, is an open question [1].
The supernumerary subunits may be an evolutionary
attribute that in mammalian cells makes complex I a regu-
lated pacemaker of OXPHOS.

In mammals, some of the supernumerary subunits are
essential for assembly of the complex (Figure 2) [8–10].
Complex I activity is affected by post-translational modifi-
cations to the constituent subunits, including phosphoryla-
tion [9,11,12], acetylation [13], glutathionylation [14], and
proteolytic maturation or degradation [15], that occur in
response to a variety of factors and events [2,11]. The
discovery that the cAMP/PKA system plays a role in regu-
lating the functional capacity of complex I and oxygen free
radical balance in mitochondria was a major breakthrough
in understanding the role of these post-translational modi-
fications [11,16]. Complex I plays a role in the functional
assembly of the other respiratory chain complexes [17] and
is involved in apoptosis [18], mitochondrial structural dy-
namics [19], and age-related functional decline [20].

The biogenesis of complex I involves the coordinated
expression of mitochondrial and nuclear structural genes
and depends on protein trafficking among the nucleus,
cytosol, and mitochondria to achieve the stepwise assembly
of the mature oligomer [10] (Figures 1–3). In addition to the
de novo assembly of complex I, the exchange of individual
subunits into an established complex can also occur [10].

Complex I is clearly a multi-faceted component of the
respiratory chain, critically involved in the overall func-
tioning of mitochondrial respiratory metabolism. Here, we
explore the role of the cAMP/PKA system in regulating

Figure 1. The mitochondrial extension of the cAMP/PKA system – a view of a
mitochondrial section with confining cytosol. Mitochondrial section with
surrounding cytosol showing a cristae with complex I (NADH-ubiquinone
oxidoreductase, CxI), complex III (bc1, ubiquinone-cytochrome c oxidoreductase,
CxIII) and complex IV (cytochrome c oxidase, CxIV) assembled in the respira-
tory chain, cytochrome c molecules, and ATP synthase dimer (CxV, FoF1) at the cristae
curvature [1,83]. For the X-ray atomic structures, see the following PDB codes: CxI,
3MBS; CxIII, 3OXH; CxIV, 10CC; CxV, 10O1. The black lines show the proton flow in
OXPHOS. The blue continuous lines show electron flow in the respiratory chain and
ROS production. Whereas complex I releases ROS only at the matrix side, complex III
releases ROS at both the inner and the outer sides of the inner mitochondrial
membrane [5]. The arrowed-blue lines indicate oxidative ROS attack to
mitochondrial DNA, CxI, and CxIV moieties exposed to the matrix. Blue blocks
represent the masses of plasma membrane β-receptors, G proteins, adenylyl cyclase,
soluble adenylyl cyclase, cytosolic and mitochondrial PDEs, PKA, catalytic (C) and
regulatory (R) subunits of PKA, and membrane AKAP (A). The TOM complex is
shown in yellow, and the TIM complex is shown in green. Red arrows show the
regulatory impact of the cAMP/PKA pathway on the mitochondrial import of
the NDUF54 subunit of complex I, activation of the complex, and prevention of
ROS production. Abbreviations: AKAP, PKA anchor protein; cAMP/PKA, cyclic AMP/
protein kinase A; IMM, inner mitochondrial membrane; OMM, outer mitochondrial
membrane; OXPHOS, oxidative phosphorylation; PDE, phosphodiesterase; ROS,
reactive oxygen species; TIM, translocase of the inner membrane; TOM, translocase
of the outer membrane.

complex I and its involvement in neurological diseases.
This understanding allows us to explore the therapeutic
potential provided by developments in this field.

cAMP regulation of complex I and ROS balance
The cellular content of complex I is determined by ‘de novo’
biogenesis of its subunits and their exchange with the
‘aged’ subunits committed to proteolytic degradation
[21]. The cAMP/PKA system exerts a positive effect on
the renewal of the complex, both at transcriptional and post-translational level [11,15].

Several experiments have shown that cAMP has a positive post-translational regulatory effect on complex I and ROS balance. The application of cAMP rescues the effects of complex I inhibition and increased cellular ROS levels that occur in G0 phase human and murine cells [16,22], in cells of patients with gene alterations [15,23], and in oxidatively damaged cells [24]. In the cases described, adding a permeable derivative of cAMP to the cell cultures, or activating the plasma membrane adenyl cyclase (tmAC), reverse the inhibition of complex I and the accumulation of ROS in a few minutes [16,22–24], without having an effect on ROS scavengers [16,22]. cAMP produced in the cytosol by tmAC, and in the mitochondrial matrix by the bicarbonate-activated soluble adenyl cyclase (sAC), can contribute to these effects (Figure 1). All of the factors responsible for reversible cAMP-dependent protein phosphorylation – such as AKAP, PKA [25], protein phosphatase(s) [26], sAC [27], and cAMP-phosphodiesterase [28] – are present in the inner mitochondrial compartment (Figure 1). It is worth mentioning, in this context, that cannabinoid binding to the brain mitochondrial CB1 receptor decreases the mitochondrial content of cAMP with specific depression of complex I activity [29].

Under cell culture conditions in which cAMP activates complex I and reverses ROS accumulation, it promotes serine phosphorylation in the conserved Arg-Val-Ser (RVS) site in the carboxy terminus of the subunit encoded by the NDUF54 gene [11,12]. The mitochondrial import of NDUF54 and its assembly into complex I are stimulated by its PKA-mediated phosphorylation and inhibited by phosphatases [30,31] (Figure 1). PKA-mediated phosphorylation of the NDUF54 protein promotes its mitochondrial translocation by the translocase of the outer membrane (TOM/translocase of the inner membrane (TIM) system, assisted by the cytosolic HSP70 chaperone [30,31]. The import and maturation in mitochondria of NDUF54, completed in the same time span in which cAMP stimulates complex I activity in cell cultures, is suppressed by the site-specific substitution of serine 173 with alanine in the C-terminal phosphorylation site [12,30]. By contrast, stimulation of complex I activity by cAMP in cell cultures is abolished by CCCP or valinomycin, which suppress the membrane potential (∆ψ)-dependent import of the NDUF54 protein into mitochondria [24]. Activation of the cAMP/PKA system rescues oxidatively damaged complex I by promoting mitochondrial import of the NDUF54 subunit and its assembly in the complex in exchange for the endogenous carboxylated subunit [24]. Substituting newly synthesized subunits for damaged subunits can play a role in functionally rescuing complex I, under those pathophysiological conditions in which the subunits are oxidatively damaged to the point of functional impairment.

The biogenesis of OXPHOS complexes is mediated by the peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) transcriptional cascade [32], and the expression of complex I appears to be more profoundly affected than other complexes by factors influencing this transcriptional pathway [11,33]. Upon phosphorylation by PKA, the protein cAMP response element (CREB) activates PGC-1α expression [34], which in turn induces the expression of downstream nuclear and mitochondrial transcription factors [35]. In addition to the nucleus, CREB has also been found inside mitochondria, where it binds to the mitochondrial DNA D-loop [36] and induces the expression of the mitochondria encoded
Box 1. Pathophysiology of complex I

- Complex I is the point of entry for NADH reducing equivalents into the respiratory chain and behaves as a regulatable pacemaker of respiratory ATP production in mitochondria (OXPHOS). This function is particularly critical in neuronal cells, which require large amounts of aerobic ATP for their excitability.
- In addition to sequential electron transfer from NADH to the mitochondrial ubiquinone pool, single electron transfer from redox centers to oxygen can also take place in complex I with oxidative damage of subunits of the same and other OXPHOS complexes, mitochondrial DNA, and phospholipids (cardiolipin). ROS production by complex I can be induced by enhanced redox pressure and/or complex I defects.
- The cAMP/PKA system regulates the functional capacity of complex I and the oxygen free radical balance in mitochondria.
- PKA-mediated phosphorylation of the NDUFS4 subunit promotes its mitochondrial import and assembly into complex I. This allows exchange of ‘aged’ subunits in the complex with newly synthesized replacements, thus rescuing the activity of the damaged complex and preventing further ROS production.
- cAMP/PKA-mediated CREB phosphorylation primes the PGC-1α biogenetic cascade of OXPHOS complexes, in particular of complex I, from both nuclear and mitochondrial DNA.
- In addition to phosphorylation, which exerts a beneficial effect on complex I, subunits of the complex can undergo other post-translational modifications, such as acetylation, glutathionylation, carbonylation, and proteolytic degradation. These, in response to a variety of factors/events, modulate the functional capacity of the complex.

PKA-mediated phosphorylation, independently of changes in NAD⁺ [40]. Both SIRT1 and PGC-1α have also been found inside mitochondria [41], and as in the nucleus, SIRT1-mediated deacetylation of PGC-1α can activate the transcription of mtDNA genes encoding subunits of the OXPHOS complexes [41].

The transcription cascade upstream of OXPHOS complexes can be stimulated by cAMP produced by tmA. For example, cAMP levels are raised in response to increased glucagon and catecholamine secretion induced by nutrient deprivation (caloric restriction, CR) [42] or by polyphenol-induced inhibition of cAMP degradation by phosphodiesterases, as occurs with the red-wine polyphenol resveratrol [43] (Figure 3). Olive oil hydroxytyrosol promotes PGC-1α transcriptional activity and both the expression and activity of complex I [44]. Conversely, dysfunction in the cAMP/PKA system can impair the transcriptional cascade. In both post-mortem samples of brain hippocampal tissues from Alzheimer patients and the APPsw M17 cell line, defective expression of phospho-CREB, PGC-1α, NRF1, NRF2, and mitochondrial transcription factor A (TFAM) were observed; in the APPsw M17 cells, these defects could be rescued by the CAMP-induced activation of PKA [45].

In addition to SIRT1, there are other members of the sirtuin family in mitochondria (SIRT3, 4, and 5) [38]. SIRT3 deacetylates and activates complex I [13], as well as NADP/NADP+ glutamate dehydrogenase and NADP-isocitrate dehydrogenase [38]. Increases in the NADPH level promote glutathione reduction by glutathione reductase and ROS scavenging by glutathione peroxidase [38]. These SIRT3-dependent reactions can promote complex I activity by preventing oxidative damage to mtDNA and matrix exposed subunits of the complex, as well as preventing damage to cardiolipin, which is required for complex I activity [46].

Figure 3. cAMP-modulated cascade of biogenesis of the mitochondrial OXPHOS system. The proteins and their interactions are represented in yellow, mRNAs in white. CREB phosphorylation by PKA primes the PGC-1α transcription cascade, which controls the expression of nuclear and mitochondrial genes of OXPHOS complex. CREB also controls the expression of SIRT1, which, in turn, promotes CREB-dependent expression of PGC-1α [34]. The expression of PGC-1α is affected by nutritional fatty acids [84] and hydroxytyrosol (HT) [44]. Increased cellular levels of cAMP, consequent to enhanced production induced by caloric restriction or depressed degradation caused by a polyphenol such as resveratrol, promote the cAMP-promoted transcription cascade of OXPHOS complexes. The PGC-1α and SIRT1 proteins, produced by the mRNAs exported in the cytosol, can reach the nucleus or be imported into mitochondria; TFAM and SIRT3 are imported into mitochondria. The PGC-1α protein is activated by NAD⁺-dependent deacetylation, catalyzed by SIRT1 [32,38] and by AMPK-mediated phosphorylation [39]. Resveratrol indirectly activates SIRT1 induction, in sequence, inhibition of PDE4, increase of cAMP level, and the activation of EPAC–CamKK–AMPK—increase of NAD⁺ level [43]. AMPK is activated by hydroxytyrosol [44]. A set of G proteins regulate mitochondrial dynamics, and among these the Drp1 protein promotes mitochondrial fission. Inactivation of Drp1 by PKA-dependent phosphorylation results in promotion of fusion [19,85]. The activity of the respiratory chain complexes, in particular complex I, is affected by the mitochondrial fusion/fission process. Conversely, specific inhibition of complex I results in mitochondrial fission [19].

Abbreviations: AMPK, AMP-activated protein kinase; Camkk; calcium/calmodulin-dependent protein kinase; Cam; cyclic AMP; CREB; AMP response element binding protein; DRP1, dynamin-related protein 1; EPAC, exchange proteins activated by cAMP; NRF1 and NRF2, nuclear respiratory factor 1 and 2; OXPHOS, oxidative phosphorylation; PDE, phosphodiesterase; PGC-1α, peroxisome proliferator-activated receptor gamma coactivator 1-alpha; PKA, protein kinase A; SIRT, sirtuin; TFAM, mitochondrial transcription factor A.

structural OXPHOS components, in particular the seven genes of complex I [37].

The transcriptional activity of PGC-1α is switched on upon its deacetylation by NAD⁺-dependent sirtuin1 (SIRT1) [38] and phosphorylation by AMP-activated protein kinase (AMPK) [39]. SIRT1 can also be activated by
Table 1. Neurological diseases with complex I dysfunction tested for cAMP effect

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Genes</th>
<th>Mitochondrial alterations</th>
<th>ROS level</th>
<th>Effect of cAMP</th>
<th>Refs</th>
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<tr>
<td>Leigh neuropathy</td>
<td>NDUF5</td>
<td>Disappearance of the NDUF5 protein from cells</td>
<td>Depressed</td>
<td>Loss of cAMP effect</td>
<td>[15]</td>
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<td>Suppression of complex I activity</td>
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<td>Defective complex I assembly</td>
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<td>Down syndrome</td>
<td>Trisomy 21</td>
<td>Defective NDUF5 phosphorylation</td>
<td>Increased</td>
<td>Rescue action</td>
<td>[15]</td>
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<tr>
<td>PINK1 hereditary PD</td>
<td>PINK1</td>
<td>Degradation of mitochondrial respiration</td>
<td>Increased</td>
<td>Rescue action</td>
<td>[23]</td>
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<td>ND5</td>
<td>Fragmentation of mitochondrial network</td>
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<td>ND6</td>
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*Cellular phenotype rescued by activation of cAMP cascade (Box 2).

Hereditary mitochondrial neurological diseases

Genetic dysfunction of complex I is the most prevalent of the ‘primary’ mitochondrial OXPHOS disorders (≥1,500 births) [47], and pathological mutations have been found in all seven mitochondrial DNA-encoded genes of the complex, which are maternally inherited, as well as in 13 of the nuclear genes. These disorders have recently been reviewed elsewhere [47,48]. Pathological mutations in the NDUF54 gene were the first to be detected in a nuclear-encoded complex I gene [49], and so far the most mutations have been found in this gene [15,50,51]. The impact on complex I structure and function of fatal homozygous mutations in different exons or introns of NDUF54 have been examined in detail in the fibroblasts of several patients [15,51]. All of the mutations resulted in the disappearance of the fully assembled complex I and the appearance of an 830 kDa subcomplex in which no NDUF54 protein was detectable [15,52]. In addition, the mutations resulted in the disappearance of cAMP-dependent serine phosphorylation of complex I subunit(s) and the stimulatory effect of cAMP on mitochondrial respiration [15]. The lack of a completely assembled complex, with loss of the regulatory effect of cAMP, being common to the NDUF54 pathological mutations underlines the primary pathogenic impact of the gene defects [15,51] (Table 1). In some cases, residual complex I activity and NAD-linked respiration was detected [8]. The NDUF54 subunit, which does not contain an Fe–S cluster, is incorporated in the last step of complex I together with peripheral subunits holding redox centers of the NADH dehydrogenase module of the complex [10] (Figure 2). The PKA-mediated incorporation of the NDUF54 subunit determines correct and stable juxtaposition of the subunits of the NADH dehydrogenase module and its functional connection to the quinone reductase module in the complex [10], preventing the direct reduction of O2 to O2* [15,16,23]. In NDUF54 KO mice, a 200 kDa fragment was detected containing catalytic subunits of the NADH dehydrogenase module detached from the complex [53,54]. This fragment could apparently bind to the 830 kDa subcomplex, or directly to complex III, resulting, however, in an altered respiratory activity that was not regulatable by cAMP [8,15]. In addition, mutations in NDUF54 have the detrimental consequence of causing the cellular accumulation of alternatively spliced forms, which can interfere with canonical gene expression [15].

The 830 kDa intermediate complex I, easily detected by polyacrylamide gel electrophoresis in all NDUF54 patients described, can be used as a hallmark to search for pathological NDUF54 mutations [51]. Notably, random samples of 500 healthy Ashkenazi Jewish subjects showed a one in 1000 carrier frequency of a c.462delA mutation in the NDUF54 gene that eliminates the PKA phosphorylation site in the protein, suggesting that NDUF54 mutations should be considered in all patients of Ashkenazi descent with neurological disorders [50].

The contribution of genetic defects in the NDUF54 subunit of complex I to disease pathogenesis has been verified in animal models. Inactivating the NDUF54 gene in mice by homozygous deletion of exon 2 led to the development of a fatal encephalomyopathy, prevented synthesis of the protein, and resulted in defective and/or unstable assembly and catalytic deficiency of complex I in different tissues [53–56]. A fatal encephalopathy, resembling the human disease, also developed in mice when inactivation of the NDUF54 gene was restricted to neurons and glia [57]. The residual complex I activity detected in KO mice was measurable in intact tissues and cells, but was not present in inside-out vesicles of the inner mitochondrial membrane devoid of the matrix content (SMP) [55], which are known to retain the full complement of normally assembled respiratory complexes [1]. This confirms that suppression of the NDUF54 protein loosens or destabilizes the normal attachment of the NADH dehydrogenase fragment to the complex. In intact mitochondria, this fragment, or what remains of it, can still bind to the residual respiratory chain, restoring limited, functionally impaired respiratory activity [53–56].

Mutations in other nuclear complex I structural and assembly factor genes in neurological patients have been reviewed previously [47,48]. For example, the C1564A mutation (a Glu222Lys substitution in the protein) in the NDUF51 gene (encoding the 75 kDa subunit with four Fe–S centers) resulted in decreased levels of the complex, depressed NADH-ubiquinone oxidoreductase activity, and ROS accumulation in patient fibroblasts [15] (Table 1 and Box 2). The decreased complex I activity could be counteracted by cAMP, which also prevented ROS accumulation [15]. Patient fibroblasts with NDUF51 mutations show fibrotic activation with increased mobility and invasiveness that can, potentially, contribute to tumor spread [58]. It is interesting to note that caspase-cleavage of the NDUF51-encoded protein is involved in disrupting mitochondrial function in apoptosis [18].
Parkinson disease
In sporadic PD, the most common human neurodegenerative movement disorder, decreased complex I activity and oxidative damage to its subunits have been found in post-mortem patient samples of the substantia nigra, including dopaminergic neurons [59]. The general view that complex I deficiency is involved in dopaminergic neuron death and the pathogenesis of PD is supported by findings that a Parkinsonism disorder is induced in drug abusers exposed to 1-methyl-4-phenylpyridinium (MPP+), a metabolite of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) that inhibits complex I, and that treatment of animals with the complex I inhibitor rotenone produces characteristic PD features [59]. This view can now be revised in light of the finding that suppressing a functional NDUFS4 gene in KO mice, which results in the loss of complex I activity in primary mesencephalic neuron cultures, did not promote the death of dopaminergic neurons in culture and did not prevent, but rather increased, their sensitivity to rotenone-induced cell death [60]. These observations indicate that defective complex I can be a risk that predisposes an individual to PD, or a disease development factor for PD originating from dysfunction of other factors/processes that are critically involved in the death of dopaminergic neurons [60,61].

Mutations in more than 10 PARK genes have been shown to be responsible for hereditary forms of PD, which all together account for 10% of cases [62]. Many of these genes impinge, directly or indirectly, on mitochondrial dynamics and function [62,63]. PARK6, encoding the PINK1 serine threonine protein kinase [64], regulates the protease HtrA2 whose anti-apoptotic activity is enhanced by the protein product of the GRIM-19 gene, a subunit of complex I involved in the assembly of the complex [6,63]. Fibroblasts from an early onset female PD patient bearing a homozygous Trp437X PINK1 mutation showed the coexistence of missense mutations in the ND5 and ND6 genes of complex I [65], and fibroblasts from the patient showed depressed levels of OXPHOS and increased ROS production [66]. The patient’s mother, heterozygous for the PINK1 mutation, harbored the ND5 and ND6 mutations and developed late-onset PD. The father, also heterozygous for the PINK1 mutation, but with normal ND5 and ND6 genes, did not develop PD. In fruit fly and mouse models reproducing pathological PINK1 mutations, decreased complex I activity has been observed [67], and it has been shown that neuroblastoma cell lines with reduced PINK1 expression display mitochondrial fragmentation and reduced OXPHOS capacity [68]. These dysfunctions were rescued by activating the cAMP/PKA system [68].

A possible therapeutic approach for PD has been reported, based on a chimeric derivative of a noncoding p137 RNA transcript of human cytomegalovirus (HCMV) attached to the rabies virus glycoprotein peptide (RVG) [69]. When the chimera was injected intravenously into rats exposed to the neurotoxin 6-hydroxydopamine (6-OHDA), which kills dopaminergic neurons, it crossed the blood–brain barrier and delivered the p137 RNA to the brain. The p137 RNA was detected in the nigral tissue and found to be physically attached to complex I, it protected the activity of the complex and mitochondrial respiration against the inhibition caused by 6-OHDA, and attenuated the loss of dopaminergic neurons and the functional deficit induced by the lesions [69]. It has been shown that in HCMV-infected neuronal cell cultures, p137 RNA interacts with the GRIM-19 protein in the assembled complex I and prevents its nuclear relocalization and apoptosis induced by rotenone [70]. GRIM-19, a protein of the retinoid-interferon induced mortality genes, initially detected in the nucleus, is also found as a component of complex I [6,71]. Nuclear localization of GRIM-19 induces cell death and suppresses neoplastic growth by inhibiting the gene stimulatory function of STAT3 [71]. Thus, preventing GRIM-19 nuclear relocalization by p137 RNA may represent the molecular mechanism of the protective effect of noncoding RNA on neuron viability [70]. GRIM-19 is phosphorylated at a threonine residue in the carboxy terminus [72], and deletion of the GRIM-19 carboxy terminus abrogates the death promoting activity of GRIM-19 [73]. The activity of GRIM-19 is controlled by its reversible phosphorylation. Clearly these findings warrant further studies to identify the kinase(s) and phosphatase(s) determining the phosphorylation state of GRIM-19 and its role in PD and related neurological disorders.

Down syndrome
Down syndrome is caused by trisomy of chromosome 21, with altered expression of genes located in this chromosome [74]. It is characterized by mental deficiency, premature aging, and neurodegeneration. The altered expression and/or function of chromosome 21 genes coding for systems controlling the cellular oxygen free radical balance have been observed in the context of this disease [74]. Studies of mitochondrial function in skin fibroblasts from a Down syndrome patient provide another example of how a dysfunction in the cAMP/PKA system can result in impaired activity of complex I and overproduction of ROS [23]. A decrease in cAMP levels and depressed PKA activity were found in the fibroblasts of patients [23], and both phosphorylation of the NDUFS4 subunit and the catalytic activity of complex I were reduced, whereas the ROS levels were

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**Box 2. Complex I deficiency in neurological disorders**

- Complex I genetic deficiency is the most prevalent of the so-called primary neurological mitochondrial diseases.
- Mutations in NDUFS4 are one of the largest groups of pathologic mutations in nuclear genes of complex I, and the pathogenic impact of NDUFS4 mutations has been verified in knockout mice. The NDUFS4 mutations result in disappearance of the protein from the patient’s complex I and abrogate the cAMP stimulatory effect on complex I-dependent respiration.
- Complex I deficiency and oxidative damage are pathogenic factors in PD. New findings from studies of complex I knockout mice and hereditary forms of PD show that genetic dysfunction of complex I can be a risk factor, predisposing an individual to disease development that can originate from different causes of dopaminergic neuron death. Cell lines with defective expression of the PARK6/PINK1 gene, one of the genes mutated in hereditary PD, show mitochondrial dysfunction that is rescued by the addition of cAMP.
- Down syndrome patient cells may display defective cAMP/PKA-dependent phosphorylation of the NDUFS4 subunit, depressed complex I activity, and increased ROS levels, all of which can be rescued by the addition of cAMP.
increased as compared with control fibroblasts (Table 1). All of these changes were reversed by the addition of dibutylryl-cAMP to the cells. Apart from contributing to an understanding of the pathogenetic mechanisms underlying Down syndrome, these contributions may help to develop therapies based, for example, on the use of β-adrenoceptor agonists that rescue complex I deficiency and decrease ROS levels in human cells [22].

**Concluding remarks and future perspectives**

The abundance of supernumerary structural subunits and assembly factors for complex I confers a plasticity that makes the complex an adaptable regulator of the overall OXPHOS system in mammalian cells. Control of complex I by the cAMP system, which functions at the crossroads of several signal transduction networks, amplifies the signals to which complex I, and with it the OXPHOS system, respond. This may explain why the expression and functional activity of complex I are more profoundly affected than those of other respiratory complexes by environmental factors and intrinsic physiological states. At the same time, all of the above can make complex I particularly vulnerable to detrimental factors acting at epigenetic, genetic, and protein/cellular levels. The susceptibility of brain neurons to deficiencies in OXPHOS, and complex I in particular, is greater than other cell types [75]. This is due to a combination of factors that includes the following: high demand of respiratory ATP for neuronal electrical excitability, higher production of ROS, and a relative insufficiency of ROS scavenger systems [76]. Complex I also exhibits a lower threshold in brain for the effects of mutations than other tissues [77]. There are, however, additional specific factors that contribute to the impact of complex I and OXPHOS deficiencies in different brain regions and neuron subpopulations (reviewed in [75]).

The reported complex I defects in neurological diseases result either from primary gene alterations of its structural and ancillary proteins or, secondarily, from dysfunctions in signaling and expression cascades. The critical pathophysiological role of cAMP signaling in regulating the expression and functional capacity of complex I and preventing ROS production provides an important step forward in devising therapeutic strategies for diseases associated with its dysfunction.

Lifestyle habits and nutritional measures can contribute to maintaining an ‘active’ cAMP cascade, such as controlling caloric intake and the quality of dietary fats, with a preference for vegetable oils, olive oil in particular with its monounsaturated oleic acid and polyphenol content. This promotes the renewal of OXPHOS complexes, in particular complex I, and prevents increases in cellular ROS level. Both of the primary cAMP actions will exert beneficial effects on mitochondrial function. The recent finding that cAMP rescues the depression of complex I activity and the increased ROS level in primary cell cultures of Down syndrome patients [23] provides an interesting perspective for treating this disorder.

The use and further development of compounds that can effect controlled, possibly tissue-specific, increases in the cellular level of cAMP should be implemented, either by activating its production by adenyl cyclase (β-adrenoceptor molecules for example) or inhibiting phosphodies- terases (resveratrol, rolipram, epigallocatechin gallate, etc). Investigations using animal models into the effect of chronic administration of antidepressant drugs, such as catecholamine and serotonin reuptake inhibitors, on the plasticity of the cAMP/CREB-mediated transcription cascade of OXPHOS complexes in neuronal cells [78,79] should provide information relevant to the therapeutic use of long-term activation of the cAMP cascade in neurological diseases and mental disorders [80–82]. The therapeutic potential and safety of HCMV RNA in PD and related neurodegenerative disorders calls for further study. Better elucidation and molecular implementation of cellular pathways in the quality control and clearing of mutated and/or oxidatively damaged complex I subunits and misfolded proteins, accumulating in brain degenerative disorders [21], will contribute further to therapeutic strategies for neurological disorders with complex I deficiencies (Box 3).

**Box 3. Outstanding questions**

- What are the functions of the large number of nuclear-encoded supernumerary subunits of human complex I? Can gene polymorphisms, and/or the production of alternative transcripts, confer additional tissue specificity to the activities of the supernumerary subunits?
- Disease-causing mutations have been detected in all the seven mitochondrial DNA-encoded genes of complex I. These are maternally inherited and present different clinical patterns depending on the relative abundance of the mutated DNA, which can vary throughout a lifetime. Can these clinical patterns be affected by the positive regulatory effect of cAMP on mitochondrial ROS balance and the expression of mitochondrial genes?
- Are pathological mutations in the nuclear genes of complex I confined only to the 13 genes identified so far?
- PKA-mediated phosphorylation of the NDUFS4 subunit promotes its mitochondrial import and assembly into complex I, replacing aged subunits and restoring the activity of the complex. Does this mechanism also work for other subunits of complex I and other OXPHOS complexes?
- Like other cellular systems, the functional capacity of OXPHOS complexes depends on the balance between biogenesis and degradation of their protein constituents. In aging and neurodegenerative diseases, increased damage or degradation of protein constituents contributes to the decline in the functional capacity of OXPHOS complexes, complex I in particular. Can positive effectors of the cAMP/PKA system help prevent such a decline by promoting replacement of damaged subunits of OXPHOS complexes?
- Aside from the cases reported, can complex I deficiency play a pathogenic role in other neurological diseases or in mental disorders?

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