View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by Elsevier - Publisher Connector

Available online at www.sciencedirect.com

ScienceDirect

Biochimica et Biophysica Acta 1773 (2007) 1701–1720

Biochimica et Biophysica Acta BBA

www.elsevier.com/locate/bbamcr

Review

Regulation of mitochondrial oxidative phosphorylation through cell signaling

Maik Hüttemann [a,*], Icksoo Lee [a], Lobelia Samavati [b], Hong Yu [a], Jeffrey W. Doan [a]

[a] *Center for Molecular Medicine and Genetics, Wayne State University School of Medicine, Detroit, MI 48201, USA*

[b] *Department of Medicine, Division of Pulmonary/Critical Care and Sleep Medicine, Wayne State University School of Medicine, Detroit, MI 48201, USA*

Received 14 August 2007; received in revised form 1 October 2007; accepted 2 October 2007
Available online 9 October 2007

Abstract

The mitochondrial oxidative phosphorylation (OxPhos) system plays a key role in energy production, the generation of free radicals, and apoptosis. A lack of cellular energy, excessive radical production, and dysregulated apoptosis are found alone or in combination in most human diseases, including neurodegenerative diseases, stroke, cardiovascular disorders, ischemia/reperfusion, and cancer. In the context of its relevance to human disease, this article reviews current knowledge about the regulation of OxPhos with a focus on cell signaling and discusses identified phosphorylation sites with the aid of crystal structures of OxPhos complexes. Several recent studies have shown that all OxPhos components can be phosphorylated; even the small electron carrier cytochrome *c* is tyrosine phosphorylated in vivo. We propose that in higher organisms, in contrast to bacteria, cell signaling pathways are the main regulator of energy production, triggered for example by hormones. Pathways that have been identified to act on OxPhos include protein kinases A and C and growth factor activated receptor tyrosine kinase signaling. Present knowledge about kinases and phosphatases that execute signals at the level of the mitochondrial OxPhos system, and newly emerging concepts, such as the translocation of kinases to the mitochondria upon stimulation of a signaling pathway, are discussed.
© 2007 Elsevier B.V. All rights reserved.

Keywords: NADH-dehydrogenase; bc_1 complex; Cytochrome *c* oxidase; ATP synthase; Cancer; Neurodegenerative disease

1. Introduction

Energy homeostasis is a main determinant of survival in all higher organisms. It has been estimated that an average person consumes 100 kcal/h at rest, roughly corresponding to the energy output of a 100 W light bulb or the production of about 65 kg of ATP/day [1]. During exercise these values can increase many fold. The initial pathway for breaking down carbohydrates from food to pyruvate via glycolysis in the cytosol produces only a small amount of ATP, whereas the oxidation of pyruvate in the mitochondria provides more than 15 times the ATP produced by anaerobic glycolysis [2]. In contrast to many unicellular organisms including yeast, which have evolved mechanisms allowing them to grow and survive in the absence of oxygen, animals fully rely on cell respiration. This is so because the roughly 10^{14} cells present in a human are not only highly specialized and energy expensive but cell density is also high, requiring efficient delivery of food and oxygen and removal of carbon dioxide through the body's circulation. Thus, provision of a constant energy supply through glycolysis alone is not feasible in animals under healthy conditions (theoretically, one would have to eat constantly), but there are conditions in which energy is primarily derived from glycolysis, such as seen in the fetus during the first half of the pregnancy [3] and in cancer, where it is known as the Warburg effect [4,5]. In both examples, the rest of the body provides glucose and disposes of the end product of the glycolytic pathway, lactate, to sustain growth as long as possible. For its own survival and that of the mother, the fetus later must switch to aerobic energy production, whereas cancer if not stopped would eventually starve its host to death.

Cell respiration takes place in the mitochondria, organelles the size of bacteria (2–4 μm). The number of mitochondria varies significantly among cell types. For example it was estimated that mammalian hepatocytes and oocytes contain 800 and 100,000 mitochondria, respectively [6]. Mitochondria have two membranes and contain their own DNA encoding 13

* Corresponding author. Tel.: +1 313 577 9150; fax: +1 313 577 5218.
E-mail address: mhuttema@med.wayne.edu (M. Hüttemann).

0167-4889/$ - see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbamcr.2007.10.001

proteins, all of which are located in the inner mitochondrial membrane and are part of the oxidative phosphorylation (OxPhos) machinery.

OxPhos is a key functional unit in the mitochondria, and combines electron transport with cell respiration and ATP synthesis (Fig. 1). It not only produces the vast majority of cellular energy, but is also involved in radical production and apoptosis. OxPhos consists of the electron transport chain (ETC), which comprises NADH-dehydrogenase (complex I), succinate dehydrogenase (complex II), ubiquinone, bc_1 complex (complex III), cytochrome *c* (Cyt *c*), and cytochrome *c* oxidase (CcO; complex IV). The majority of electrons transferred by the ETC are derived from NADH and enter the chain via complex I. In addition, complex II feeds electrons directly into the ubiquinone/ubiquinol pool, and further input sources include the electron-transferring flavoprotein (ETF), ubiquinone oxidoreductase, and glycerol-3-phosphate dehydrogenase. These electrons are transferred through the ETC to oxygen, while the mitochondrial membrane potential is generated by pumping protons across the inner mitochondrial membrane. Finally, the proton gradient is utilized by ATP synthase (complex V), which couples the backflow of those protons previously pumped by the ETC to the production of ATP (Fig. 1).

Cellular energy demand ranges widely depending on the function and activity of the cell. Thus adjustment of energy production to physiological demand is essential to all higher organisms. The first mechanism identified by which OxPhos is controlled is called respiratory control as was shown with isolated mitochondria [7]. Here, OxPhos activity is limited by the availability of the substrates for ATP synthesis, ADP and phosphate. Thus, OxPhos activity increases when ATP utilization generates ADP and phosphate. In addition to this basic substrate limitation mechanism there are several further levels of control, and nature has cultivated three major regulatory means, which are found alone or in combination (Fig. 1). There are 1) tissue-specific and/or developmental expression of isozymes, which equip a cell with variants that perform the same chemistry but are adapted to tissue-specific requirements, 2) allosteric control, mediated through small molecules that bind to the enzyme, changing its kinetic properties, and 3) cell signaling. The latter two mechanisms can be viewed as a hierarchy: allosteric control represents an intracellular response, for example triggered by changes in the concentration of substrate or product molecules. In contrast, cell signaling primarily serves the purpose of higher-order communication between cells or organs. For example, glucagon and insulin, released by the pancreatic alpha and beta cells, respectively, reach their target organs such as liver and muscle through the blood stream. There, they bind to specific receptors and trigger an amplification cascade, which eventually adjusts cell function to the physiological circumstances the whole organism faces at the time of hormone release. In the example given glucagon, a starvation signal, is present after extended periods of food deprivation, e.g., after overnight rest, and triggers a major metabolic response through the cAMP-dependent pathway in liver. The liver, as an altruistic organ, then provides food in the form of glucose for other organs such as the brain. After ingestion of food insulin is released, signaling that metabolic building blocks have become available and have to be taken up by the cells. The effect of insulin is mediated through the phosphoinositol-3 kinase pathway.

Within the cell there is a sequence of kinases and phosphatases, which add or remove phosphate groups from downstream kinases and/or phosphatases or endpoint targets. It is now clear that the traditional view of linear, i.e., hierarchical, signaling cascades is an oversimplification because there is considerable crosstalk between different signaling pathways. This crosstalk sometimes complicates the interpretation of experimental results and, when overlooked, may also account for some discrepancies reported in the literature.

Several recent review articles have summarized data on phosphorylated proteins identified within the mitochondria and on signaling pathways that act on mitochondria [8–11]. The goal

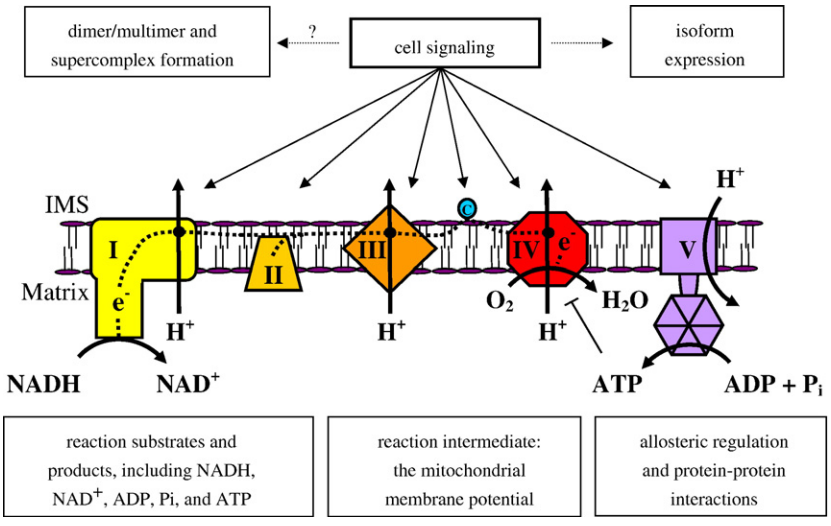


Fig. 1. Regulation of mammalian oxidative phosphorylation (OxPhos). At the functional level OxPhos is regulated through a hierarchy of regulatory mechanisms: organs express tissue-specific isozymes, hormones mediate signals from the outside of the cell to the mitochondria through signaling cascades, and allosteric regulation allows fine-tuning of energy production within the cell IMS: intermembrane space.

of this article is to provide a focused view of current knowledge about regulation of the mitochondrial OxPhos system as an endpoint of cell signaling due to its emerging recognition as an important factor in human pathophysiology.

2. Cell signaling targets all mitochondrial OxPhos components in mammals

Partial or complete crystal structures from mammals and/or homologous structures from bacteria are now available from all components of the OxPhos system (Fig. 2). They are not only essential for a better understanding of the catalytic mechanisms of the individual enzymes but also for the interpretation of regulatory events. For example, after a phosphorylation site has been mapped the structural information can be used to interpret changes in enzymatic properties. Several different types of data have been acquired in recent years, such as proteomic studies analyzing protein phosphorylation on a larger scale. Often, however, the information obtained represents just one piece of the puzzle. In order to understand the complex regulation of aerobic energy metabolism the approach must be integrative, linking a molecular event, such as the attachment of a phosphate group to a specific amino acid, to functional change, such as changes in enzyme activity.

We here discuss current knowledge of the regulation of OxPhos complexes and the physiological relevance of such regulation. Although many open questions remain, CcO is the best-studied complex with respect to regulation through isoform expression, allosteric control, and reversible phosphorylation. Thus, CcO serves as an example of what can be expected to be revealed for the entire OxPhos system in the future.

2.1. NADH-dehydrogenase (NADH-DH) (complex I)

With 46 subunits and a molecular weight close to 1 MDa [12], NADH-DH is the most complex OxPhos member and also the least understood. Recently, a partial crystal structure containing eight subunits from the hydrophilic arm of complex I from *Thermus thermophilus* was resolved [13] (Fig. 2A). This bacterial enzyme has a smaller number of only 15 subunits, 14 of which have homologs in mammals. Bacterial complex I presumably lacks several regulatory mechanisms that are yet to be discovered in mammals, including additional regulatory subunits, similar to CcO as discussed below.

2.1.1. cAMP-dependent signaling to the mitochondria

The majority of studies of cell signaling targeting ETC complexes investigated the effect of the cAMP-dependent pathway. Most effects of increased intracellular cAMP are mediated through phosphorylation reactions catalyzed by protein kinase A (PKA), a serine/threonine-specific kinase [14]. However, it remains to be clarified whether cytoplasmic PKA can localize to the mitochondrial matrix and/or intermembrane space. Several studies have concluded that there was PKA activity in the mitochondria. For example, incubation of cow heart mitochondria with cAMP in the presence of [γ-^{32}P] ATP showed labeling of four proteins [15]. Other studies also suggested the presence of PKA in mitochondria of yeast, mammalian cell lines, and a variety of mammalian tissues [16–24]. Other studies have provided a different model of action of cAMP-dependent signaling in the mitochondria. For example, A-kinase anchoring proteins (AKAPs) have recently gained much attention because they localize PKA to cellular compartments in close proximity to its targets [25]. Several recent studies have indicated that AKAPs localize PKA to the outer side of the outer mitochondrial membrane. In germ cells AKAP84 has been shown to control the migration of mitochondria from the cytoplasm in round spermatids to the cytoskeleton in elongating spermatids [26], and AKAP1-mediated cellular localization of PKA to the mitochondria is required during oocyte maturation in mice, which starts 1.5 h after initiation of the maturation process [27]. Similarly, stimulation of the cAMP pathway in the human embryonic kidney cell line HEK293 leads to a sub-mitochondrial localization of PKA to the outer side of the outer mitochondrial membrane, mediated by AKAP84 and AKAP121 [28,29]. At the same sub-cellular location PKA phosphorylates the proapoptotic protein BAD on serine 112, a process that is AKAP-mediated [30,31]. In contrast, a recent study on rat heart mitochondria concluded that AKAP121 was localized within the mitochondria [32].

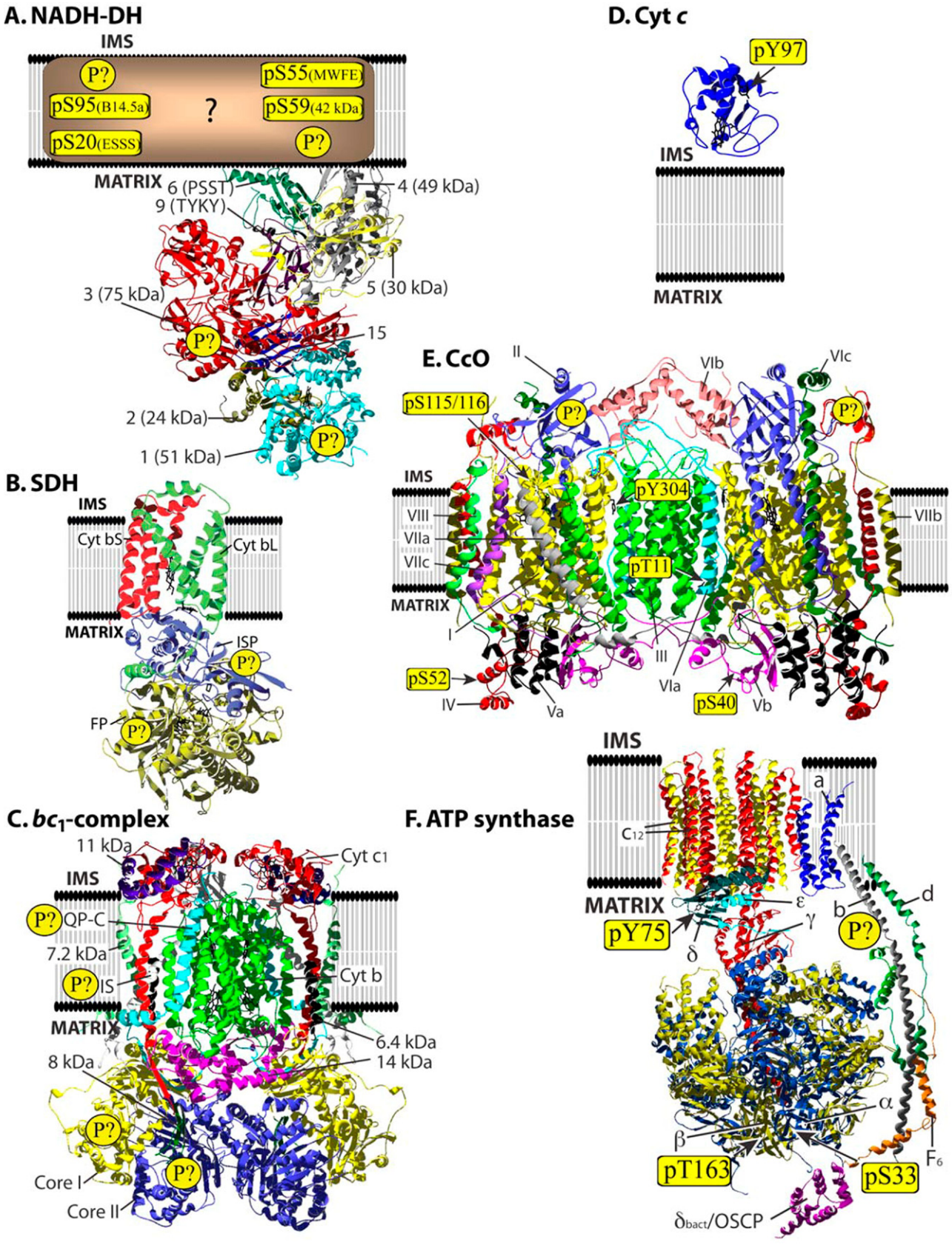
Possibly, both models co-exist and PKA can modify other signaling compounds on the outer side of the mitochondria, but it can also translocate into the mitochondria and directly phosphorylate mitochondrial proteins (Fig. 3). In addition, tissue-specific differences may favor one mode of action over the other.

The action and localization of certain kinases, such as PKA, and the importance of scaffolding proteins, such as AKAPs, for the convergence and specific targeting of phosphorylation events to recipient proteins, should always be addressed by in vivo studies. For example, the combination of a purified OxPhos complex with a commercially available kinase should be interpreted cautiously and should be validated in the physiological context in vivo (see also section ‘Cytochrome *c* oxidase’).

cAMP-dependent phosphorylation of complex I was first studied by the Papa group. They suggested that PKA directly phosphorylates complex I in heart tissue, leading to enzyme activation [33,34]. A radioactively labeled band was obtained in the 18 kDa size range and it was proposed that the N-terminal sequence containing the AQDQ-18 kDa subunit was targeted for phosphorylation. cAMP-dependent phosphorylation of an 18 kDa band was also shown in rat C6 glioma cells in cell culture after treatment with forskolin or dibutyryl-cAMP [35]. However, mapping of phospho-epitopes by mass spectroscopy was not performed in those studies. Later, using heart submitochondrial particles and isolated complex I with an in vitro PKA phosphorylation approach, the Walker lab showed via mass spectroscopy that under similar conditions, instead of the AQDQ subunit the N-terminal sequence containing ESSS-18 kDa subunit of complex I was phosphorylated on serine 20 in addition to serine 55 of the 10 kDa-MWFE subunit [36] (Fig. 2A). Although the membrane-containing arm of complex I that contains the ESSS and MWFE subunits has not yet been crystallized, the phosphorylation sites are likely to be located on

the matrix and intermembrane space sides, respectively [36]. No experiments were performed to address functional consequences of the dual phosphorylation of complex I.

In order to assess in vivo phosphorylation of complex I the Gibson and Capaldi labs isolated the cow heart enzyme, separated the sample by SDS-PAGE, and stained the gel with the



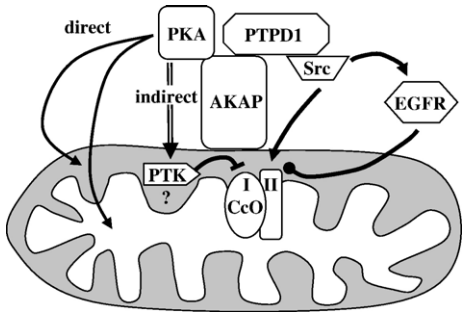


Fig. 3. Protein kinase A (PKA), Src, and EGFR signaling to the mitochondria. Upon stimulation of the cAMP-dependent pathway PKA translocates to the outer site of the outer mitochondrial membrane, which is mediated by A-kinase anchoring proteins (AKAPs) that provide a signaling hub for additional proteins including Src kinase. Two models have been suggested for cAMP signaling to the mitochondria that may co-exist. Catalytic and/or regulatory subunits of PKA translocate into the mitochondria and are present in the intermembrane and matrix space (direct model). Alternatively, PKA can activate a downstream kinase (PTK, protein tyrosine kinase), which then phosphorylates target proteins, such as CcO (indirect model). PKA and Src inhibit and activate mitochondrial respiration via phosphorylation of CcO subunits I and II, respectively. Another player appears to be EGFR, which is phosphorylated by Src, and translocates from the plasma membrane to the mitochondria where it interacts with but does not phosphorylate CcO subunit II. It remains to be seen in what compartment phosphorylation of EGFR occurs, on the outside of the mitochondria perhaps at the AKAP signaling hub, or separately on the inside.

phosphate-specific fluorescent probe Pro-Q-Diamond [37]. Numerous bands were stained even after alkaline phosphatase treatment, a commonly used control, which nonspecifically removes phosphate groups; thus the specificity of the probe has to be further evaluated in future studies. The authors tried to identify phosphorylated epitopes by tandem mass spectrometry (MS/MS). Although the ultimate proof through MS/MS sequencing was difficult to achieve, they concluded through indirect controls that the NDUFA10 (42 kDa) subunit was phosphorylated on serine 59. This residue is not evolutionarily conserved in mammals and functional implications of its phosphorylation have not been addressed yet. Recently, serine 59 phosphorylation in cow heart was confirmed by another proteomic study using isolated complex I followed by mass spectrometry [38]. In addition these authors detected a second phosphorylation site on serine 95 in the B14.5a subunit (Fig. 2A and Table 1).

Recent advances in technology have made possible phosphoproteomic studies, which can be applied in a high throughput fashion. Often 2D gel electrophoresis followed by the identification of phosphorylated protein spots is combined with MS/MS to identify phosphorylated peptides and, if possible, to map phosphorylated amino acids. Identification of phosphorylated amino acids using mass spectrometry is not routine due to system-immanent limits, including loss of the unstable phosphate moiety after ionization and, often-encountered, low sensitivity. In addition, proteins usually are only partially phosphorylated and the unphosphorylated form often represents the main protein fraction, as was shown with mitochondrial proteins from pig [39] and potato [40]. Therefore, the identification of enriched phosphorylated proteins is feasible whereas low-abundance fragments are often missed. Phospho-proteomic studies with increased sensitivity will play a major role in the future because they will allow a global assessment of protein phosphorylation after induction or inhibition of a signaling pathway. Ideally, those experiments will be accompanied by functional studies with the ultimate goal of drawing structure–function relationships.

A recent phospho-proteomic study on porcine mitochondria using 2D gel electrophoresis, staining of phosphorylated proteins with Pro-Q-Diamond, and identification of phosphorylated proteins through mass spectrometry suggested phosphorylation of all OxPhos complexes [39] (see also below). For complex I seven subunits showed Pro-Q-Diamond staining: the 75 kDa Fe–S protein 1, flavoprotein 1, 39 kDa 1α subcomplex, 24 kDa subunit, 23 kDa subunit (mitochondrial precursor), 22 kDa 1β subcomplex, and the 15 kDa subunit. Although no phosphate groups were assigned to specific amino acids and no functional studies were undertaken for any of the OxPhos complexes, this study provides strong evidence that the input point of the OxPhos system is targeted for regulation via cell signaling cascades, which would be expected for a key metabolic enzyme. Phosphorylation of the 39 kDa subunit on a yet-to-be-identified tyrosine residue was also inferred based on studies on isolated rat brain mitochondria [41].

An interesting finding was that complex I subunit ND2 seems to have a second role that, at first sight, appears not to be related to electron transfer and does not take place in the mitochondria. In rat ND2 was shown to interact with Src tyrosine kinase at excitatory synapses, localizing Src to *N*-methyl-D-aspartate receptors [42]. The authors found that ND2 was not targeted for phosphorylation and thus proposed that it functions as an adaptor protein. It has been shown that Src also localizes to the mitochondria, in particular to the intermembrane space [43], and phosphorylates CcO subunit II [44] (Fig. 3). It remains elusive whether ND2 can also function as a scaffolding protein in the mitochondria, in which case one would expect that a subunit near ND2 should be targeted for phosphorylation. A regulatory action mediated by Src kinase at complexes I and IV would be physiologically sensible, because it would allow a concerted change of enzyme activities both at the input point and the endpoint of the electron transport chain.

Fig. 2. Reversible phosphorylation targets all enzymes of the OxPhos system. Crystal structure data of the hydrophilic arm of complex I from *Thermus thermophilus* [13], of complex II from porcine heart [197], of cow heart complex III [48], of horse heart cytochrome *c* [198], and of cow heart complex IV [76] were used. A complete structure for mammalian ATP synthase is not available yet. In order to provide a preliminary model partial structures for complex V were modeled together from the following parts: F_O unit from *Escherichia coli* [199], F_1 and the central stalk from cow heart [200], and parts of the peripheral stalk from cow heart [201]. In addition. the bacterial δ subunit from *E. coli*, which is homologous to mammalian OSCP, was modeled onto the α subunit based on a protein NMR spectroscopy study revealing this interaction [202]. Crystallographic data were processed with the program Swiss-PDBViewer 3.7 and rendered using the program POV-Ray 3.6. Identified mammalian phosphorylated amino acids are highlighted in sticks (see also Table 1). Subunits that have been reported as being phosphorylated in vivo but where the sites remain unknown are indicated (P?).

Table 1
Identified phospho-epitopes in mammalian OxPhos complexes

OxPhos complex	Species and tissue	Phosphorylated amino acid	Phospho-epitope	Reference	Method and comments	Kinase prediction [a]
NADH-DH	Cow heart	Ser95, B14.5a subunit	ASEKKAV**S**PAPPIKR	[38]	MS [b]	Cdk5, Cdc2, Erk1, low confidence
NADH-DH	Cow heart	Ser59, 42 kDa subunit	GNICSGK**S**KLAKEIA	[37,38]	MS	No known motif
NADH-DH	Cow heart	Ser20, ESSS (18 kDa) subunit	TLAGKRP**S**EPTLRWQ	[36]	Edman degradation/MS	PKA, medium confidence
NADH-DH	Cow heart	Ser55, MWFE (10 kDa) subunit	MERDRRV**S**GVNRSYVS	[36]	Edman degradation/MS	PKA, medium confidence
Cyt *c*	Cow heart	Tyr97	EREDLIA**Y**LKKATNE	[62]	MS	No known motif
CcO	Cow liver	Tyr304, subunit I	MDVDTRA**Y**FTSATMI	[83]	MS, cAMP-dependent	No known motif
CcO	Rabbit heart	Ser115 and Ser116, subunit I	SLHLAGV**SS**ILGAINF	[118]	MS, after ischemia/reperfusion	No known motif
CcO	Rabbit heart	Thr52, subunit IV	KAPWGSL**T**RDEKVEL	[118]	MS, after ischemia/reperfusion	DNA PK, low confidence
CcO	Rabbit heart	Ser40, subunit Vb	MLPPKAA**S**GTKEDPN	[118]	MS, after ischemia/reperfusion	No known motif
CcO	Cow heart	Thr11, subunit VIa	AKGDHGG**T**GARTWRF	[120]	Crystal structure	No known motif
ATP synthase	Human skeletal muscle	Thr213(163) [c] β subunit	GGAGVGK**T**VLIMELI	[137]	Indirect	No known motif
ATP synthase	Mouse brain	Ser76(33) [c] α subunit	EETGRVL**S**IGDGIAR	[134]	BEMAD [d]/MS	Akt, Medium confidence
ATP synthase	Human NIH3T3 cells	Tyr75, δ subunit	EDGTTSK**Y**FVSSGSV	[133]	Indirect, PDGF-dependent	PDGFR kinase, low confidence

[a] Based on the Scansite Motif Scan web tool (http://scansite.mit.edu/motifscan_seq.phtml). The predictions are purely speculative and have to be viewed with extreme caution.
[b] Mass spectrometry sequencing.
[c] Numbering according to mature peptide based on cow.
[d] β-Elimination/Michael addition with DTT labeling.

Taking a side glance at plant chloroplasts, enzyme activity of the analogous plastid NADH-DH can be strongly increased upon stimulation with H_2O_2 or Ca^{2+}. This is mediated through threonine phosphorylation of the NDH-F subunit and maximal enzyme activity correlates with full phosphorylation [45].

So far, no tissue-specific subunit isoforms of complex I have been identified, although there is evidence of functional tissue-specific differences. Recently, the Villani group has shown variability of the P/O ratio (moles of ATP synthesized from ADP per 2 electrons flowing through the ETC), a measure of the efficiency of OxPhos. Comparison of rat brain, heart, and liver mitochondria revealed P/O values of 2.0, 2.8., and 2.6, respectively with complex I substrates but no differences were observed with complex II substrates suggesting that the site responsible for P/O variability is complex I [46]. The authors hypothesized that the low P/O ratio found in the brain might convey neuro-protection, because a higher rate but lower yield would accommodate the high energy demand of the brain and at the same time lower intracellular oxygen concentrations, the substrate for ROS production. Although the underlying mechanisms for P/O variability are unclear, differences in complex I phosphorylation in addition to tissue-specific subunit isoforms might account for those observations.

2.2. Succinate dehydrogenase (SDH, complex II)

The four-subunit SDH complex is part of the Krebs cycle and oxidizes succinate to fumarate, feeding the released electrons into the ETC. In contrast to the other ETC complexes, SDH does not pump protons across the inner mitochondrial membrane and therefore does not contribute to the generation of the mitochondrial membrane potential. Very little is known about the regulation of complex II, which is surprising since it integrates Krebs cycle metabolism into the ETC. Indeed, complex II seems to be regulated through cell signaling, as was recently indirectly demonstrated in plant and animal mitochondria by assigning complex II subunits that can be phosphorylated. 2D gel electrophoresis of isolated mitochondrial porcine proteins, followed by Pro-Q-Diamond staining, and identification of phosphorylated proteins via mass spectrometry, suggested phosphorylation of the flavoprotein precursor subunit and the iron sulfur cluster containing B subunit [39] (Fig. 2B). In potato tubers 14 mitochondrial phospho-proteins were identified after incubating intact isolated mitochondria with [γ-^{32}P]ATP, followed by 2D gel electrophoresis and MS/MS analysis of labeled spots [40]. Among those proteins identified were no subunits of complex I whereas the flavoprotein (A subunit) of complex II was radioactively labeled (Fig. 2B). A third study with the goal of identifying posttranslational modifications on complexes II and III reported several modifications, including N-terminal acetylation and oxidation of methionines [47]. However, the study failed to detect a single phosphorylated peptide, which may be explained by the omission of the calcium chelator EGTA and phosphatase inhibitors during mitochondria isolation. Under such conditions calcium activated phosphatases appear to prevail, leading to dephosphorylation of most mitochondrial proteins [39].

2.3. Cytochrome c reductase (complex III; bc_1 complex)

Complex III catalyzes the transfer of electrons from reduced ubiquinone to oxidized Cyt *c* via a Q-cycle mechanism, which leads to the translocation of protons across the inner mitochondrial membrane. The mammalian enzyme contains 11 subunits per monomer but functions as a dimer, which was confirmed by the X-ray crystal structure [48]. Hardly anything is known about regulatory mechanisms acting on this enzyme. A phospho-proteomic study already discussed in the previous section indicated phosphorylation of the β-mitochondrial processing peptidase (β-MPP) subunit in potato [40], and the porcine heart core protein I precursor, core protein II, Rieske iron sulfur protein precursor, and ubiquinone binding protein [39] (Fig. 2C). Phosphorylated amino acids, relevant signaling pathways, and effects of those phosphorylations remain elusive.

2.4. Cytochrome c (Cyt c)

The one-electron carrier Cyt *c* is located in the mitochondrial intermembrane space and shuttles electrons from bc_1 complex to cytochrome *c* oxidase. Cyt *c* from human, cow, and mouse contains 104 amino acids and a covalently linked heme group. The heme iron is further coordinated by histidine 18 and methionine 80. Cyt *c* is highly positively charged with a pI of 9.6. Cyt *c* is essential for aerobic energy production and Cyt *c* knockout mice die around midgestation [49]. This is plausible because at that developmental stage energy production that relies only 5% on aerobic metabolism before gestation day 9 switches to aerobic energy metabolism relying 95% on OxPhos after gestation day 11, as was demonstrated with cultured rat embryos [3].

In addition to its essential role in aerobic energy metabolism, Cyt *c* also participates in type II apoptosis that involves the release of Cyt *c* into the cytosol, which binds to Apaf-1, leading to activation of procaspase-9 to caspase-9 [50]. Although not understood in detail, the release of Cyt *c* into the cytosol is considered a crucial proapoptotic signal [51,52]. Interestingly, Cyt *c* has a third function as a cardiolipin peroxidase at early stages during apoptosis, where it selectively oxidizes cardiolipin [53], a lipid of the mitochondrial inner membrane that binds to Cyt *c*. This mechanism may play an important role in the release of Cyt *c* after the induction of apoptosis, which requires dissociation from the inner mitochondrial membrane. In contrast, a fourth function as a reactive oxygen species scavenger has been proposed for Cyt *c* under normal (non-apoptotic) conditions [54].

Cyt *c* has a testes-specific isoform in rodents [55], which is mainly expressed in spermatocytes and cells in the later stages of spermatogenesis, whereas Sertoli cells, spermatogonia, and interstitial cells express the otherwise ubiquitously expressed, so-called somatic Cyt *c* isoform [56], and both isoforms are gradually expressed in intermediate cells during sperm maturation [57]. Testes-specific Cyt *c* has an interesting feature in that it shows a threefold increased activity to reduce hydrogen peroxide compared with the somatic isoform, but also shows a three to fivefold activity to induce apoptosis [58]. The authors concluded that the first effect protects sperm from radical damage whereas the second effect is part of a suicide mechanism of male germ cells, which selects for functional and undamaged sperm. However, the syntenic region of testes Cyt *c* in the human genome contains a non-transcribed pseudogene [59], and it remains elusive whether humans converge the functions of the somatic and sperm-specific Cyt *c* variants found in rodents into a single protein expressed in all human tissues.

One means of Cyt *c* regulation has been known for three decades: ATP binds to Cyt *c* at physiological concentrations, which affects the binding of Cyt *c* to CcO, leading to an inhibition of the reaction between Cyt *c* and CcO, and the elimination of the low K_m phase of the otherwise biphasic kinetics with CcO [60]. An ATP binding site was later mapped to the conventional "left side" of the molecule [61]. A similar regulatory mechanism is present in CcO as discussed below that also leads to an inhibition of respiration.

A novel regulatory means mediated by phosphorylation was recently discovered by our group [62]: Cyt *c* isolated from cow heart tissue under conditions that preserved the physiological phosphorylation state revealed phosphorylation on tyrosine 97, as shown by tandem-MS. Clear differences were observed in respiration with CcO, which was inhibited in the presence of tyrosine 97-phosphorylation and showed pronounced sigmoidal kinetics [62]. The inhibitory effect of tyrosine 97-phosphorylation on respiration with CcO was surprising because tyrosine 97 does not directly participate in Cyt *c*–CcO binding [63]. In addition, there are many studies where basic residues of Cyt *c* were modified into neutral or acidic moieties [64–66], with the most dramatic changes in enzyme kinetics being observed when amino acids were altered that directly participate in binding. However, the localization of tyrosine 97 in the midst of the only relatively hydrophobic region on Cyt *c* [67] might explain far-reaching structural disturbances ranging to the other side of the molecule, as was deduced from a spectral shift of the heme iron–methionine 80 absorption band from 695 to 687 nm [62].

Several questions related to Cyt *c* phosphorylation remain open due to its other functions. We have proposed that tyrosine 97 phosphorylation may affect apoptosis [62] because an essential residue for the interaction with Apaf-1, lysine 7, is spatially located next to tyrosine 97 [68]. Based on structural considerations the formation of a salt bridge is possible that would neutralize the positive charge of lysine 7, which might prevent apoptosome formation. In addition, a conformational change in Cyt *c* was earlier observed in apoptotic cells [69], which could be explained with a change of the phosphorylation status.

Tyrosine 97 phosphorylation might also affect the cardiolipin peroxidase activity of Cyt *c*. The Kagan lab has shown that this mechanism involves a tyrosine radical [53]. In addition, lipid binding capability would be required that could be provided by this relatively hydrophobic area [67,70]. However, there are three additional conserved tyrosine residues in mammalian Cyt *c* as potential candidates for the chemistry involved, and more experiments are needed to decide this issue.

2.5. Cytochrome c oxidase (complex IV; CcO)

CcO is the terminal enzyme of the electron transport chain. It accepts electrons from reduced Cyt *c* and transfers them to

molecular oxygen, which is reduced to water. This reaction requires protons that, taken up from the matrix, contribute to the generation of the mitochondrial membrane potential. In addition to those chemical protons, CcO pumps protons across the inner mitochondrial membrane, a process that is still not fully understood and is a matter of intense debate [71–75]. Mammalian CcO has been crystallized as a dimer, which is composed of 13 subunits per monomer [76]. In addition to the 3 largest mitochondrial encoded subunits, which contain the 4 catalytic redox centers, the mammalian enzyme contains 10 nuclear encoded subunits. The function of the nuclear encoded subunits and their isoforms remains largely unknown. However, several studies have started to shed light on some of those, and thus regulation of CcO has been studied in more detail compared to the other OxPhos complexes. These studies provide a preliminary taste of what may be revealed in the future for the entire OxPhos system. CcO shows all three regulatory features found in key metabolic enzymes: isoform expression, allosteric control, and phosphorylation (Fig. 1).

2.5.1. Isoforms

There are three liver- and heart-type isoform pairs of subunits VIa, VIIa, and VIII, which have been known for several decades (reviewed in [77]). Liver-type CcO is expressed in organs such as liver, kidney, and brain, whereas the heart-type isozyme is expressed in heart and skeletal muscle. In addition, three more isoforms were discovered recently. These are a lung-specific isoform of CcO subunit IV, a testes-specific isoform of subunit VIb, and a third isoform of subunit VIII [59,78,79]. There is only one published knockout mouse model, which lacks the subunit VIa heart-type isoform. The mice are viable under no-stress conditions, but showed only 23% CcO activity in heart tissue compared to the wild-type, which correlated with reduced CcO amount (23%) [80].

Functional data including enzyme kinetics have so far been reported only for the better-studied liver- and heart-type CcO isozymes. Older studies tried to analyze differences between the two major isozymes (e.g., [81,82]) but no consistent results were obtained. The Avadhani lab used sub-mitochondrial particles and reported a K_m for Cyt *c* of 26 and 8 μM and maximal turnover values of 495 and 750 s^{-1} at high ionic strength for heart and liver CcO, respectively. These data are in agreement with the idea that heart-type CcO is found in tissues with a high aerobic capacity and a large number of mitochondria, whereas other tissues including the brain cannot afford such a high mitochondrial mass due to their specialized functions, but still fully depend on aerobic energy production, which requires a more active enzyme. However, CcO kinetics may have to be re-evaluated for two reasons: 1) as discussed below, phosphorylation of CcO that decisively regulates enzyme activity has to be considered; this requires isolation conditions that preserve the physiological phosphorylation status [83], and 2) some components commonly used during enzyme isolation or during activity measurements have to be carefully considered. Examples include the detergent cholate that strongly binds to and activates CcO, which has to be removed by dialysis [76]; the detergent dodecylmaltoside that was suggested to monomerize CcO [84]; and *N*,*N*,*N*′,*N*′-tetramethyl-*p*-phenylendiamine (TMPD), which facilitates electron transfer between molecules and thus alters enzyme kinetics because Cyt *c* does not have to dissociate from CcO in order to be re-reduced [60]. Therefore, it would be desirable to develop a generally accepted and applied protocol.

2.5.2. Allosteric control

The largest nuclear encoded subunit, CcO IV, has been shown to bind ATP at the matrix side, leading to an allosteric inhibition of enzyme activity at high intra-mitochondrial ATP/ADP ratios, adjusting energy production to the physiological demand [85,86]. Computer modeling of an ATP molecule into a pocket comprised of subunit IV and the catalytic subunits I and II supported these findings [78]. It was then postulated that CcO IV is a pivotal regulator of CcO activity in higher organisms and yeast in contrast to bacteria, which lack this subunit [87].

Subunit Va, which has no transmembrane helix and localizes to the matrix site, was shown to bind the thyroid hormone T2, releasing the allosteric ATP inhibition, and allowing a high turnover even at a high ATP/ADP ratio [88].

The muscle specific isoform of subunit VIa was suggested to decrease the pumped proton to electron stoichiometry at high ATP/ADP ratios [89]. In contrast, CcO containing the VIa liver-type isoform pumps protons at a high ratio in the presence of cardiolipin independently of the ATP/ADP ratio, whereas the addition of palmitate leads to a decreased proton/electron stoichiometry [90].

There are several studies using protein interaction techniques that suggest additional regulatory means, which need further investigation. CcO subunit II was shown to bind to the epidermal growth factor receptor (EGFR) by phage display screening and co-immunoprecipitation [91]. EGFR signaling is involved in important cellular processes, such as survival, proliferation, and migration. Binding of EGFR to CcO subunit II requires tyrosine 845 phosphorylation on EGFR, which is mediated by c-Src, suggesting that EGFR and c-Src act synergistically (Fig. 3). This idea is further supported by the fact that CcO subunit II is a target of c-Src [44], as detailed below.

Another physical partner of CcO subunit II is cyclin G1, which was demonstrated by yeast two-hybrid work and confirmed by co-immunoprecipitation in an osteosarcoma cell line that overexpresses cyclin G1 [92]. The authors proposed that cyclin G1, which is induced by tumor suppressor p53, might participate in the induction of apoptosis through its interaction with CcO.

Subunit Vb, which similarly to subunit Va localizes to the mitochondrial matrix side, was shown by a yeast two-hybrid screen to interact with the cytosolic human androgen receptor [93]. In addition, it was reported for the RIα regulatory subunit of PKA that it can also bind to CcO subunit Vb [94]. The authors concluded that activation of the cAMP pathway using adenylyl cyclase activator forskolin leads to CcO inhibition through this direct interaction. As discussed below, it is now evident that the cAMP-dependent pathway leads to phosphorylation of CcO, which strongly inhibits enzyme activity [83].

2.5.3. Competitive inhibition of CcO by nitric oxide (NO)

Over the last two decades the work of many labs has established the importance of NO action on mitochondrial function, which cannot be addressed here in detail (for reviews see references [95–97]). NO is synthesized by three types of NO synthases (NOS), a neuronal (nNOS) and an endothelial NOS (eNOS) isoform that are constitutively expressed, and an inducible form (iNOS). Interestingly, a subtype of nNOS localizes to the mitochondria and was therefore denoted mitochondrial NOS (mtNOS) [98]. mtNOS can directly interact with CcO on the matrix side by binding to subunit Va [99], which increases local concentrations of NO at its target enzyme. Mechanistically, NO competes with oxygen at the binuclear heme a_3-Cu_B binding site, which leads to reversible CcO inhibition [100,101]. Increased cellular NO levels coincide with many human diseases, including arthritis [102], sepsis and septic shock [103], trauma [104], cancer [105], obesity, and diabetes [106]. Despite other actions of NO in the cell its inhibitory action on CcO might at least in part explain some aspects of NO-related pathological conditions, because inhibition of oxidative energy production would decrease cellular energy levels. The inhibitory effect on cell respiration has also been proposed as a general mechanism underlying neurodegenerative diseases mediated by an over-production of NO [107].

2.5.4. Calcium and zinc binding sites

Mammalian CcO contains a calcium–sodium exchange site in subunit I [108], whereas in bacteria this site contains a strongly bound calcium [109]. The function of reversible calcium binding is unknown. There have been reports indicating that CcO can bind at least two zinc ions at two different sites, which leads to enzyme inhibition and particularly to inhibition of the proton pumping process [110–112]. It is unclear where variation of zinc concentrations may affect CcO activity, and whether zinc really constitutes a physiological regulator. Perhaps it might play a role for the regulation of sperm motility before and after ejaculation in testes, which have higher zinc levels compared to other tissues. That would be sensible because CcO activity would be down-regulated during periods of quiescence whereas zinc ions would be diluted after ejaculation and allow aerobic energy production when needed. Interestingly, reduction of zinc concentration clearly improves sperm motility, and it was concluded that this effect was related to improved mitochondrial performance [113].

2.5.5. Phosphorylation of CcO

Only during the last few years has it become evident that CcO can be phosphorylated at several subunits and it is likely that this mechanism plays the pre-eminent role in regulating enzyme activity.

The first report indicating CcO phosphorylation was published by Steenaart and Shore [114]. The authors labeled mitochondrial proteins phosphorylated by endogenous kinases using [γ-^{32}P]ATP and concluded that CcO subunit IV can be phosphorylated. However, the identification of a phosphorylated residue and possible functional effects still remain elusive.

The Kadenbach lab isolated CcO from cow heart and incubated the solubilized enzyme with commercially available PKA, cAMP, and [γ-^{32}P]ATP [115]. After SDS-PAGE and autoradiography they observed a signal for subunit Vb and a faint signal for subunits II or III. Later, applying the same in vitro approach they presented data suggesting that subunit I was also phosphorylated [116]. These experiments demonstrate the modification of heart CcO by PKA in vitro both on the intermembrane and matrix side of the enzyme. Functional studies showed that the in vitro phosphorylated CcO was inhibited [115]. The Avadhani lab recently reported phosphorylation of CcO subunits I, IV, and Vb after ischemia in rabbit hearts, and they proposed that PKA directly phosphorylates CcO under those conditions because phosphorylation was abolished in the presence of PKA inhibitor H89 [117]. They later assigned the phosphorylated amino acids by mass spectrometry to serines 115 and 116 of subunit I, threonine 52 of subunit IV, and serine 40 of subunit Vb [118]. The sites identified (Table 1) are not PKA consensus sites, suggesting that PKA acts upstream or, more likely, that the inhibitor H89 also targets one or more other kinases that mediate those phosphorylations.

Since in vivo phosphorylation sometimes requires auxiliary proteins for specific targeting of the signal, such as AKAPs in case of PKA, other labs have started to evaluate cAMP-dependent signaling in a more physiological context. To address this point, our group initially focused on liver instead of heart tissue. The starvation hormone glucagon is the strongest physiological signal for increasing intracellular cAMP levels in liver without affecting other signaling pathways [119]. In heart there is no such exquisite trigger, and G-protein linked receptor-mediated increase of cAMP often parallels an increase in calcium. Therefore, liver CcO was isolated in the presence or absence of theophylline, a phosphodiesterase inhibitor that creates high cAMP levels, and the phosphorylation status was preserved by the addition of phosphatase inhibitors [83]. High cAMP produced an enzyme that was tyrosine phosphorylated on subunit I in vivo whereas the control enzyme did not show a signal. Subunits II or III and IV were also tyrosine phosphorylated, but there was no change after theophylline treatment. The site of phosphorylation was identified by mass spectrometry to be CcO subunit I tyrosine 304 (Fig. 2D). Phosphorylated CcO was fully or strongly inhibited up to 10 μM Cyt *c* substrate concentrations, even in the presence of allosteric activator ADP [83]. Treatment of liver tissue or human liver cancer HepG2 cells with the physiological hormone glucagon or the adenylyl cyclase activator forskolin, which both increase intracellular cAMP, produced a similar response. Interestingly, theophylline, which is one of the oldest anti-asthma drugs, also led to CcO inhibition and lowered ATP levels in the lung at concentrations used in therapy. It was then proposed that this mechanism might explain the effect of theophylline on asthma relief because airway constriction during asthma requires energy [83].

Although the above studies clearly show that cAMP signaling leads to CcO inhibition, in vivo tyrosine 304 phosphorylation of liver CcO contrasts with in vitro phosphorylation of heart CcO using PKA, which is a serine/threonine kinase and

thus cannot phosphorylate tyrosine 304. Probably, PKA activates a downstream tyrosine kinase, which then phosphorylates CcO subunit I. In addition there might be tissue-specific differences in PKA signaling to the mitochondria, and PKA might localize to the inner compartments of the mitochondria in some tissues, such as heart as was proposed for complex I phosphorylation in that tissue (see Section 2.1). There is clear evidence that signaling pathways can operate differently in different tissues. The presence or absence of kinases and phosphatases, and tissue-specific expression of isoforms that may in one case but not in the other be targeted by cell signaling pathways, can account for tissue-specific differences. Examples are tyrosine phosphatase PTP 1B, which localizes to the mitochondria, and is only found in brain but is absent in muscle, heart, and liver tissue [41], and CcO subunit VIa, which occurs as a pair of liver- and heart-type isoforms: based on a high resolution crystal structure Yoshikawa and coworkers were able to show that the VIa heart isoform is threonine 11 phosphorylated [120] (Fig. 2E), which was confirmed by mass spectrometry (S. Yoshikawa, personal communication). The role of tissue-specific CcO subunit isoforms is still under debate. The presence or absence of a kinase motif as observed for the CcO VIa isoform pair may be revealed as a common theme allowing tissue-specific regulation through cell signaling.

In addition to cAMP-dependent signaling, Miyazaki and coworkers have shown that CcO subunit II can be phosphorylated by non-receptor tyrosine kinase c-Src in osteoblasts, a process that appears to be important for the bone-resorbing activity of these cells, which might be altered in rheumatoid arthritis [44,121]. The authors found a positive correlation between CcO activity and c-Src kinase activity but the phosphorylated tyrosine residue remains unknown. Src is involved in several cellular processes including cell adhesion, movement, and proliferation.

Another interesting regulator of CcO is protein kinase Cε (PKCε), which emerges as a target for hypoxic preconditioning. Studies on rat neonatal cardiac myocytes using the PKC activators diacylglycerol or 4β-PMA resulted in the phosphorylation of an 18 kDa protein in vitro [122,123]. These studies revealed that PKCε co-immunoprecipitated with CcO and that the radiolabeled 18 kDa band contains subunit IV, as was shown by mass spectrometry. The phosphorylation site was not assigned but stimulation of PKCε resulted in about two- to fourfold activation of CcO. Src kinase is also phosphorylated by PKCε, which leads to activation [124,125]. However, no tyrosine phosphorylation of CcO subunit II was observed after PKCε stimulation [123]. Perhaps this can be explained with tissue-specific differences in cell signaling involving additional auxiliary components in some tissues.

2.6. ATP synthase (complex V)

ATP synthase utilizes the proton gradient generated by the ETC in order to produce ATP from ADP and phosphate. The enzyme is a nano-motor consisting of a soluble component, F_1, which is in the matrix and contains the catalytic ATP/ADP/P_i binding sites, and a membrane bound F_O part that couples proton backflow to ATP synthesis through a peripheral and a rotating central stalk [126,127]. The F_1 part consists of five subunits with the stoichiometry $\alpha_3\beta_3\gamma\delta\varepsilon$ and the core F_O part contains subunits ab_2c_{10-14}, the so called oligomycin sensitivity-conferring protein (OSCP), and subunits d, e, f, g, F_6, and A6L. Several partial crystal structures including the F_1 component from mammals and parts of F_O from bacteria have been resolved and are presented as a combined structure in Fig. 2F.

The activity of ATP synthase is dependent on $\Delta\Psi_m$ and maximal rates of ATP synthesis in bacteria occur at $\Delta\Psi_m$=100–120 mV, as has been demonstrated for ATP synthases from *E. coli* and *P. modestum* [128].

In mammals there is so far no evidence for tissue-specific or developmentally regulated isoforms. However, three isoforms of the β subunit have been identified in tobacco plants, two of which seem to be ubiquitously expressed and the third one exclusively expressed in pollen [129]. Since tissue-specific isozymes of ATP synthase have hardly been studied, and considering the numerous isoforms of CcO subunits, the presence of isoforms in plants probably just represents the tip of the iceberg and much more remains to be learned about tissue-specific energy requirements at the level of this central enzyme.

In order to prevent wasteful ATP hydrolysis a small 10 kDa inhibitory protein IF_1 binds to the soluble F_1 component [130]. In addition to the transmembraneous subunits e and g, IF_1 also mediates ATP synthase dimer formation [131].

In 1995 the first study was published showing that ATP synthase can be phosphorylated on the δ subunit in cultured mouse cortical neurons, triggered by platelet-derived growth factor (PDGF) [132]. The δ subunit is part of the central rotating stalk, and phosphorylation can be expected to lead to changes in enzyme activity, but additional experiments are needed. The Pedersen lab later extended those initial data and demonstrated that PDGF-mediated phosphorylation of the δ subunit also occurs in NIH3T3 and kidney cells, and the authors provided indirect evidence that this phosphorylation occurs on the only tyrosine residue present in this subunit, tyrosine 75 [133] (Fig. 2F). Growth hormone receptor-triggered signaling to the mitochondria may also affect other OxPhos complexes, because it leads to proliferation and cell survival, and Cyt *c* might be a first target to look at due to its participation in apoptosis and the fact that it can be phosphorylated.

Using mouse brain tissue, a recent phospho-proteomic study applying an improved protocol called BEMAD (β-Elimination/Michael addition with DTT labeling) for the detection of phospho-peptides identified Ser76 of the alpha subunit as being phosphorylated [134] (Fig. 2F).

There is plenty of evidence that phosphorylation can occur on additional sites of ATP synthase in mammals, yeast, and plants. After in vitro incubation of potato tuber inside-out sub-mitochondrial particles with [γ-^{32}P]ATP the δ subunit was labeled in addition to the b subunit, which is part of the peripheral stalk [135]. A similar approach using intact potato tuber mitochondria revealed phosphorylation of the α and β subunits [40], which comprise the catalytic F_1 part. A recent study of thylakoid ATP synthase from barley resolved six variants of the β subunit on 2D gels caused by differences in serine/threonine phosphorylation

[136]. In humans the β subunit can also be phosphorylated, and four phosphorylated variants were identified after incubation of cultured skeletal myoblasts with [γ-^{32}P]ATP, followed by 2D gel analysis [137]. The authors provided some evidence that threonine 213, which is located in the catalytic nucleotide-binding site, is one phosphorylation site (Fig. 2F). The study also showed that, at least under cell culture conditions, the unphosphorylated β subunit was undetectable.

Other studies using Western blot analysis and anti-phospho tyrosine antibodies on rat brain mitochondria indicated phosphorylation of the α and γ subunits [41], whereas Pro-Q-Diamond staining of porcine mitochondrial proteins pointed to phosphorylation of the mature peptides of the α and OSCP (oligomycin sensitivity-conferring protein) subunits [39].

Interestingly, even for yeast, which was long considered a "zero background" organism for tyrosine phosphorylation studies, it was recently shown to contain phosphorylated tyrosine residues on the α and β subunits (yeast nomenclature: ATP1p and ATP2p) in addition to phosphorylated serine and or threonine residues [138]. Using MALDI-FTICR-MS (matrix-assisted laser desorption/ionization Fourier transform ion cyclotron resonance mass spectrometry), the authors were able to map specific phosphorylation sites to tyrosine 434 and serines 413 and 426 of the α subunit, and tyrosine 7 of the β subunit. However, there was some ambiguity because anti-phospho antibodies did not show a signal for the α subunit [138]. Such results might be explainable because mass spectrometry and immunological approaches show varying sensitivity to differentiating phosphorylated and unphosphorylated peptide species. For example, ionization during mass spectrometry often leads to loss of a phosphate group and antibodies may require adjacent amino acids in addition to the phosphorylated amino acid in order to bind, a problem especially encountered with anti-phospho-serine and -threonine antibodies, whereas the phospho-tyrosine group appears to be a big enough epitope for specific recognition.

Clearly, comprehensive mapping of phosphorylation sites and functional studies are necessary to understand regulation of ATP synthase in light of cell signaling.

3. OxPhos supercomplexes

There is now ample evidence that the OxPhos machinery is organized into higher-order complexes. ATP synthase from rat liver has recently been shown to co-purify with the phosphate and the adenine nucleotide carriers, which provide ATP synthase with its substrates ADP and phosphate [139,140]. Another interesting aspect of ATP synthase is its occurrence in dimeric and oligomeric states, which was demonstrated for several organisms including yeast and mammals [141–144], and it was proposed that cristae morphology is dependent on these states. Yeast mutants of the e and g subunits, in which the dimeric and oligomeric states of ATP synthase are destabilized, showed reduced levels of mitochondrial membrane potential, which could not be explained through uncoupling of the membrane or activity changes of OxPhos complexes [145]. However, the phosphorylation state of ATP synthase and of the other OxPhos complexes was not preserved during mitochondria isolation, which might explain those findings. The Lippe group recently speculated that under physiological conditions monomeric and oligomeric states might be regulated through phosphorylation and they performed 2D gel analysis of monomeric and dimeric ATP synthase followed by Western analysis with anti-phospho tyrosine antibodies [146]. The authors found that the γ subunit was tyrosine phosphorylated only in the monomeric but not in the dimeric state. Although the phosphorylated tyrosine residue remains unknown this work suggests that not only oligomeric states of ATP synthase but also aggregation of the other members of the OxPhos machinery into supercomplexes may be regulated through cell signaling. Interestingly, tyrosine 304 phosphorylation of CcO subunit I resulted in enhanced sigmoidal kinetics and resulted in a Hill coefficient of about 4 in contrast to a value of 2 for the tyrosine 304 unphosphorylated enzyme [83]. One interpretation of an increased Hill coefficient would be an increase in monomer–monomer interaction in the CcO dimer, and it will therefore be interesting to see if such phosphorylation affects the stoichiometry of CcO in OxPhos supercomplexes.

Many studies on larger aggregates of OxPhos complexes employed methods developed by Schägger and coworkers, blue native and more recently clear-native gel electrophoresis [147,148], which allows the separation of higher-order supercomplexes under comparably gentle conditions using mild detergents, such as digitonin. Various stoichiometries of OxPhos components in supercomplexes have been observed in yeast, plants, and mammals, including the following variants: I–III_2, I–III_2–IV, I–III_2–IV_2, I–III_2–IV_3, I–III_2–IV_4 (for current reviews see [149–151]). The arrangement of OxPhos complexes into assembly lines would make possible a more efficient electron transfer due to substrate channeling. This would be especially beneficial to speed up energy production in conditions that require increased ATP production, such as during exercise. Not only electron transfer might be affected but also the backflow of protons through the OxPhos complexes (intrinsic uncoupling) and the production of reactive oxygen species (ROS) as a byproduct of the OxPhos machinery might be altered. It has been postulated that intrinsic uncoupling is a mechanism involved in processes such as thermogenesis [152], and ROS are important in cell signaling including the induction of apoptosis [153]. Thus, changes in supercomplex stoichiometry, probably regulated by cell signaling, and their functional consequences seem to be an interesting new avenue for future research.

Cardiolipin is an important mitochondrial lipid, which constitutes about 20% of lipids in the inner mitochondrial membrane. It was shown for bc_1 complex and CcO that cardiolipin stabilizes supercomplexes in yeast [154,155]. In humans there is a rare recessive X-linked disease, Barth syndrome, with variable symptoms including motor delays, hypotonia, delayed growth, and cardiomyopathy, which is caused by mutations in the cardiolipin synthesis pathway [156]. A recent study on lymphoblasts from patients with Barth syndrome carrying mutations in the *TAZ1* gene revealed labile supercomplexes of complexes I, II, and IV [157].

4. Mitochondrial kinases and phosphatases that may act on OxPhos complexes

Cell signaling affects mitochondria through direct phosphorylation and dephosphorylation of mitochondrial proteins, or through changes in regulation of gene expression of proteins that localize to the organelle. An example of the latter case is contractile activity in skeletal muscle, which is paralleled by increased calcium fluxes, and leads to activation of a series of kinases including PKC, extracellular signal-regulated kinases ERK-1 and ERK-2, and ribosomal S6 kinase (reviewed in [158]). The important role of calcium in gene regulation was demonstrated in myotubes using calcium ionophore A23187, which induced the expression of Cyt *c* and CcO subunit Vb genes and was mediated by a PKC-dependent pathway [159]. Contractile activity also leads to the induction of peroxisome proliferator activated receptor co-activator 1α (PGC-1α), a key nuclear factor that coordinates the expression of nuclear encoded genes that function in the mitochondria [160]. Another independent mechanism altering mitochondrial biogenesis after chronic contractile activity is an acceleration of protein import into the mitochondria [161].

However, little is known about mitochondrial kinases and phosphatases, their OxPhos substrates, and the transduction of the signal from the cytosol to the mitochondria. Based on selected examples we will outline emerging principles of such signaling relevant to OxPhos. For recent comprehensive reviews of kinases and phosphatases that have been associated with mitochondria see references [8–10].

4.1. Translocation of signaling molecules to the mitochondria

Apparently the static picture of kinases and phosphatases with a fixed localization to cellular compartments has to be at least in part modified to a more dynamic model. There are increasing numbers of signaling components that change their sub-cellular localization upon alterations in the signaling state: ubiquitously expressed tyrosine kinase c-Abl, which localizes to the nucleus and cytoplasm, translocates from the endoplasmic reticulum (ER) to the mitochondria, where it participates in the induction of apoptosis; this was demonstrated after cells were subjected to ER stress triggered with the calcium ionophore A23187, brefedin A, or tunicamycin [162]. Epidermal growth factor receptor translocates from the plasma membrane to the mitochondria within 20 min after stimulation with epidermal growth factor, where it interacts with CcO subunit II [91]. As discussed in the Complex I section, PKA signaling goes along with a translocation of AKAPs to the outer side of the outer mitochondrial membrane. AKAPs not only provide a platform for PKA signaling, but they also appear to be a hub for other signaling pathways, converging signals to the mitochondria. Livigni and coworkers [163] have recently shown that protein tyrosine phosphatase PTPD1, and c-Src share the same scaffold with PKA (Fig. 3). This model raises an interesting question related to other studies discussed above that inferred localization of PKA and c-Src to the inner compartments of the mitochondria. A co-existence of both models is possible, particularly in a tissue-specific context. For example, c-Src localization to the mitochondrial intermembrane space was demonstrated in cells involved in bone formation that overexpress this kinase. In other cell types with lower expression, c-Src might instead be part of the signaling hub and stay on the outer side of the mitochondria.

Akt (protein kinase B) localization to all compartments in the mitochondria upon stimulation of the phosphoinositol-3-kinase pathway was shown to be dynamically regulated [164]. Stimulation of cells with insulin-like growth factor-1, insulin, and stress resulted in phosphorylation and activation of Akt, followed by a fast accumulation in the mitochondria. The study concluded that the β subunit of ATP synthase and glycogen synthase kinase-3β were targets for phosphorylation by Akt.

Protein kinase Cδ (PKCδ) is a stress-activated kinase involved in triggering the production of free radicals and the induction of apoptosis. Translocation of PKCδ to the mitochondria in mouse keratinocytes was shown after stimulation with 2-*O*-tetradecanoylphorbol-13-acetate (TPA), which induced cell death [165]. Translocation of PKCδ to the mitochondria does not occur during ischemia, but was observed with 5 min of reperfusion applied after 30 min ischemia in rat hearts, followed by a decrease in mitochondrial respiration and an increase in superoxide radical production [166].

Tyrosine phosphorylation in the mitochondria has recently gained attention, in part because tyrosine kinases and phosphatases known to play pivotal functions in the cytosol or nucleus also localize to the mitochondria [9]. Based on studies with rat brain, Src and other Src family kinases Lyn, Fyn, Csk, and Fgr were shown to localize to the mitochondrial intermembrane space [9,43].

The presence of those tyrosine kinases not only raises the question of their involvement in OxPhos regulation but also implies the presence of tyrosine phosphatases. Protein phosphatase Shp-2 was the first identified tyrosine phosphatase that localizes to the mitochondrial intermembrane space and the outer mitochondrial membrane [167]. It represents another example of dual localization because it is also a component of the signaling scaffold located on the inner side of the plasma membrane as part of the Ras pathway. Interestingly, mutations in the *PTPN11* gene, which encodes Shp-2, account for about 50% of cases with Noonan syndrome [168], an autosomal dominant disorder characterized by dysmorphic facial features, congenital heart disease, short stature, webbed neck, chest deformity, and mental retardation. In Noonan patients Shp-2 is constitutively active [169], and it remains to be seen if disease pathology has a mitochondrial component. Mitochondrial targets of Shp-2 are currently unknown but due to their sub-mitochondrial co-localization CcO tyrosine 304 [83] and Cyt *c* tyrosine 97 [62] are possible candidate epitopes with known function, both leading to activation of respiration in the unphosphorylated state. Interestingly, calcium is believed to be a major activator of mitochondria, including OxPhos, and Shp-2 mutations were recently shown to enhance calcium oscillations in cardiomyocytes [170]. Direct dephosphorylation of OxPhos components by Shp-2 or indirect activation of OxPhos through calcium would lead to increased respiration and an increase of the mitochondrial

membrane potential followed by an increase in reactive oxygen species (ROS), which are produced at elevated mitochondrial membrane potential levels [153,171,172]. ROS play an important role in organ development but the amount has to be well controlled because fetal antioxidant activities such as superoxide dismutase are depressed until just prior to parturition [173], which might account for the increased ROS in fetal tissue in certain pathological conditions [174]. The importance of a narrow window of ROS production for proper heart development and function was shown in a recent study using knockout mice lacking the mitochondrial thioredoxin reductase, another protective weapon against ROS [175]. The ubiquitous knockout of the gene caused embryonic death after mid-gestation and organ maldevelopment, including the heart, whereas cardiac tissue-restricted inactivation of the gene resulted in fatal dilated cardiomyopathy. Another relevant example is diabetes, where oxidative damage caused by ROS was found in fetuses of diabetic rats, leading to congenital abnormalities, including abnormalities of the heart and great vessels, and neuronal damage [176].

5. Cancer, apoptosis, free radicals, and neurodegenerative diseases

Mitochondria seem to play an essential role in cancer proliferation. Most solid tumors show a reduction in mitochondrial mass of 25–60% compared to normal proliferating tissue [177]. This disequilibrium appears to signal the cell that it is in an undifferentiated state, comparable to embryonic cells, which show similarly low mitochondrial amounts [177]. Compared to differentiated cells tumors rely less on respiration and more on glycolysis, which is known as the Warburg effect [4,5].

The reasons cancer cells utilize glycolysis even in the presence of oxygen – what is known as aerobic glycolysis – are currently being elucidated. One obvious factor appears to be an enormous demand for energy in rapidly growing cells. Under normal conditions mitochondria produce most cellular ATP. However, respiration and in particular the shuttling of NADH from the cytosol into the mitochondria are slow processes (Fig. 4). Therefore, as long as glucose supply is unlimited, glycolysis allows considerably faster ATP production compared to OxPhos [178].

Another key aspect of increased glycolysis results from its linkage to the pentose phosphate pathway, which provides essential building blocks for biosynthesis, such as the five-carbon sugar ribose 5-phosphate and NADPH. Ribose 5-phosphate and its derivatized forms are components of DNA, RNA, and the cofactors ATP, NADH, FAD, and coenzyme A. NADPH is required for biosynthetic pathways, including fatty acid, cholesterol, and nucleotide synthesis. The cell can adjust the amount and ratio of pentose and NADPH production according to its needs, because the pentose phosphate pathway is linked to glycolysis through transketolase and transaldolase reactions, which as a result allows the interconversion between five- and six-carbon sugars. The increased utilization of the pentose phosphate pathway in cancer cells compared to normal cells has been elegantly demonstrated through metabolic studies using

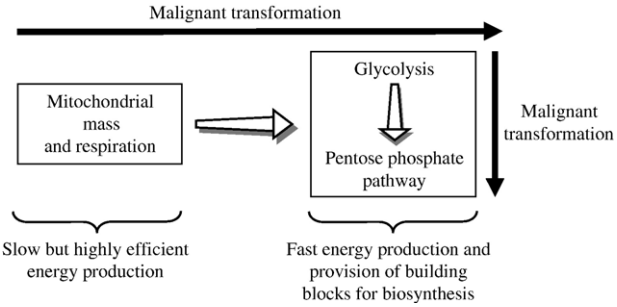


Fig. 4. Toward a better understanding of the Warburg effect. During malignant transformation cells switch from aerobic to glycolytic energy production. Cancer cells require the supply of high levels of glucose, which is not only used for fast energy production through the glycolytic pathway but is also essential for the generation of building blocks for the rapidly dividing cell through the pentose 5-phosphate pathway. It appears that the more aggressive and advanced the cancer the less reliant it is on mitochondrial respiration. In addition the balance between glycolysis and the pentose 5-phosphate pathway shifts toward the latter, satisfying the increased demand for NADPH and five-carbon sugars for biosynthesis.

[1-^{14}C]glucose and [6-^{14}C]glucose followed by the determination of [^{14}C]CO_2 production [179]. The authors showed that utilization of the pentose phosphate pathway was increased fourfold in prostate tumors compared to normal cells, and more than 12 times elevated in a poorly differentiated urothelial tumor compared to normal urothelium, whereas a well-differentiated urothelial tumor showed an intermediate, more than threefold increase. One could speculate that the ratio of the two linked pathways is a marker for cancer differentiation, staging, and aggressiveness (Fig. 4).

Cancer cells require these major metabolic changes to develop a rapid and unrestricted proliferation, and the reduced amount of mitochondria and the reduced capacity of the remaining mitochondria to respire might be interpreted as markers for de-differentiation in cancers: the less differentiated the more aggressive and the less reliant on mitochondrial respiration. Interestingly, cancer therapy approaches, including ionizing radiation and many of the chemical differentiation enhancing agents, may act at least in part by increasing the mitochondrial complement, and thereby returning the cell to the differentiated, respiring, and non-proliferating state. This is so because such agents aim at a transient nuclear cell cycle inhibition, which suppresses mitochondrial DNA synthesis much less efficiently [180].

Mutations in mitochondrial DNA (mtDNA) have been identified in many tumors (reviewed in [181]), and have been proposed as a possible tool for cancer diagnosis [182]. Once present, such mutations may enhance tumorigenesis as was recently shown with cybrid cells containing the same nuclear DNA in combination with or without mutant mtDNA [183]. However, in most cases mtDNA mutations are probably a secondary event and not the cause of cancer.

It is now well established that cell signaling pathways are dysregulated in cancers. Classic examples, which pointed to the importance of signal transduction, are cancers caused by viruses, such as the Rous sarcoma virus that infects chickens and expresses a kinase, v-Src, homologous to the cellular kinase

c-Src but lacking a control region that inactivates the kinase. Another example is the Abelson murine leukemia virus encoding the active v-Abl kinase that is homologous to the cellular kinase c-Abl. Interestingly, as discussed above both Src and Abl localize to the mitochondria, where they might directly mediate the Warburg phenotype at the mitochondrial level after viral infection. A further example is tumor suppressor and transcriptional activator p53. p53 is regulated through cell signaling and is very often mutated in cancers. Recently it was shown that p53 regulates the expression of SCO2, a CcO assembly factor, and that deletion of either p53 or SCO2 represses mitochondrial respiration [184], perhaps through decreased amounts of fully assembled CcO.

Mitochondria are emerging as a target for cancer therapy for another reason: they are involved in apoptosis, a process that cancer cells manage to evade. Mitochondrial or type II apoptosis involves several proteins that are mostly localized on the outside of the mitochondria such as anti-apoptotic proteins Bcl-2, Bcl-xL, Mcl-1, and proapoptotic proteins Bax and Bak. Bax and Bak are involved in pore formation after the induction of apoptosis, which leads to the release of proteins located in the mitochondrial intermembrane space including apoptosis inducing factor (AIF) and OxPhos component Cyt *c*. Several studies are underway testing chemical compounds that enhance apoptosis (reviewed in [185]). For example Genasense™ is an antisense oligonucleotide that targets and down-regulates anti-apoptotic protein Bcl-2. Cells are then more susceptible to apoptosis, which can be exploited by co-administration of a standard chemotherapeutic drug.

A number of other anti-neoplastic compounds currently in use or in (pre-)clinical testing including topoisomerase II inhibitor etoposide, anti-microtubule agent taxol, and steroid mimetic CD437 also appear to directly target mitochondria [186]. The converging principle seems to be the release of Cyt *c* and the induction of apoptosis, although the exact mechanisms remain elusive in most cases.

Another mechanism by which the mitochondrial OxPhos system appears to be involved in the carcinogenesis process is increased free radical production. Free radicals are a byproduct of the ETC, and increased levels lead to enhanced damage of DNA, proteins, and lipids. Free radicals were thus proposed to contribute to genomic instability and cancer progression. Increased free radical production can derive from dysfunctional ETC complexes. This was recently shown for mutations in genes encoding complex II subunits B, C, and D, which have been associated with human paragangliomas that include head, neck, and adrenal cancers [187]. It was shown that the introduction of such a mutation of the C subunit into cultured B6 cells results in significantly increased superoxide and hydrogen peroxide levels and a fivefold increase in glucose uptake, another hallmark of cancer metabolism [188].

The capability of complex II to produce free radicals may also contribute to the pathology of Friedreich ataxia, an autosomal recessive neurodegenerative disease caused by mutations in the frataxin gene. The frataxin protein is associated with the inner mitochondrial membrane and was shown to directly interact with complex II [189]. Dysfunctional frataxin might induce free radical production, perhaps by interfering with complex II assembly or by inducing structural changes in complex II. Increased amounts of free radicals produced by the mitochondrial ETC are emerging as common factor in neurodegenerative disease, including Parkinson's disease, Alzheimer's disease, Friedreich ataxia, and multiple sclerosis. This can be at least in part explained by the fact that neuronal tissue is highly aerobic but at the same time very sensitive to oxidative damage due to relatively low levels of antioxidants and antioxidant enzymes (reviewed in [190]).

Dysfunctional mitochondria play an important role in various neurodegenerative diseases. Among OxPhos complexes, significantly decreased activity of CcO above all was found in Alzheimer disease (AD) patients [191,192]. It was recently shown that amyloid precursor protein accumulates in mitochondria of AD patients, clogging the protein import machinery of the mitochondria [193]. This mechanism can also explain decreased protein levels of the core protein 1 of bc_1 complex and the β-chain of ATP synthase in AD patients [194], a pattern which is also observed in patients with Parkinson's disease (PD). PD is the most common neurodegenerative movement disorder. Several PD mutations have been identified in genes that encode proteins involved in mitochondrial function and regulation, including α-synuclein, parkin, the mitochondrial kinase PINK1, and the mitochondrial protease OMI (reviewed in [195]). Interestingly, α-synuclein, the major component of Lewy bodies found in PD patients, was shown to interact with CcO by yeast two-hybrid and co-immunoprecipitation studies [196]. A general mechanism underlying dopaminergic cell loss as found in PD patients might be increased cellular stress and ROS production leading to cell death over time.

6. Concluding remarks

A large and still increasing number of human diseases are associated with a dysfunctional OxPhos machinery. The two distinct key factors involved are a lack of energy and increased free radical production. Because of the central role of OxPhos in cellular function the activity of its components has to be tightly regulated and fine-tuned, as is clear from considering developmental and tissue-specific energy requirements, and as is also seen in the expression of subunit isoforms in CcO. Moreover, higher organisms have to adapt to a constantly changing metabolic environment, as seen in distinct states such as during starvation, feeding, and exercising. Adaptation to those conditions requires communication between cells and organs and is mediated through hormones and electrical signals that activate cell signaling cascades. Since the first reports on the phosphorylation of OxPhos components more than a decade ago the importance of this new field is increasingly recognized because it is the link between physiology at the organismal level and molecular changes within the mitochondria. Only 14 phosphorylation sites have been mapped to date (Table 1) and the functional consequences of most of these events have yet to be studied. One would expect co-regulation of OxPhos components under certain conditions, and we recently proposed that this may be the case for Cyt *c* and CcO, which share 5 out of 10 amino

acids in their phospho-epitopes (Table 1). To map further phosphorylation sites and to study mitochondrial function in a physiologically meaningful context, standard protocols to isolate mitochondria have to be modified in order to prevent dephosphorylation during the isolation of mitochondria and OxPhos enzymes. Such protocols are available [62,83], although additional optimization may further increase the yield of phosphorylated protein species, which is desirable for both mapping of phosphorylation sites and functional studies. As a next step, discovery of kinases and phosphatases that act on OxPhos is necessary to reconstruct the intracellular signaling pathways. Those signaling molecules could then become future targets for therapy, in particular for the non-traditional mitochondrial diseases, such as neurodegenerative diseases, diabetes, and cancer. Current knowledge of cell signaling targeting OXphos constitutes the tip of the iceberg, and major discoveries can be expected that will revolutionize our understanding of the regulation of energy production, free radical generation, and apoptosis.

Acknowledgements

We gratefully thank Dr. Lawrence I. Grossman for suggestions on the manuscript and Philip Morris USA Inc. and Philip Morris International, the National Multiple Sclerosis Society, the Ralph Wilson Medical Research Foundation, and the Center for Molecular Medicine and Genetics of Wayne State University for supporting our research. We apologize to authors whose names were not cited here due to space limitations and to authors whose work on non-mammalian OxPhos complexes was not included.

References

[1] P. Rich, Chemiosmotic coupling: the cost of living, Nature 421 (2003) 583.
[2] B. Alberts, A. Johnson, J. Lewis, M. Raff, K. Roberts, P. Walter, The Molecular Biology of the Cell, 4th ed.Garland Science, New York, 2002.
[3] G.M. Morriss, D.A. New, Effect of oxygen concentration on morphogenesis of cranial neural folds and neural crest in cultured rat embryos, J. Embryol. Exp. Morphol. 54 (1979) 17–35.
[4] O. Warburg, K. Posener, E. Negelein, Uber den Stoffwechsel der Carcinomzelle, Biochem. Z. 152 (1924) 309–344.
[5] O. Warburg, On the origin of cancer cells, Science 123 (1956) 309–314.
[6] I.E. Scheffler, Mitochondria, John Wiley & Sons, New York, 1999.
[7] B. Chance, G.R. Williams, Respiratory enzymes in oxidative phosphorylation. I. Kinetics of oxygen utilization, J. Biol. Chem. 217 (1955) 383–393.
[8] D.J. Pagliarini, J.E. Dixon, Mitochondrial modulation: reversible phosphorylation takes center stage? Trends Biochem. Sci. 31 (2006) 26–34.
[9] M. Salvi, A.M. Brunati, A. Toninello, Tyrosine phosphorylation in mitochondria: a new frontier in mitochondrial signaling, Free Radic. Biol. Med. 38 (2005) 1267–1277.
[10] C. Horbinski, C.T. Chu, Kinase signaling cascades in the mitochondrion: a matter of life or death, Free Radic. Biol. Med. 38 (2005) 2–11.
[11] S. Vogt, A. Rhiel, V. Koch, B. Kadenbach, Regulation of oxidative phosphorylation by inhibition of its enzyme complexes via reversible phosphorylation, Curr Enz Inhibit 3 (2007) 189–206.
[12] J. Carroll, I.M. Fearnley, J.M. Skehel, M.J. Runswick, R.J. Shannon, J. Hirst, J.E. Walker, The post-translational modifications of the nuclear encoded subunits of complex I from bovine heart mitochondria, Mol. Cell Proteomics 4 (2005) 693–699.
[13] L.A. Sazanov, P. Hinchliffe, Structure of the hydrophilic domain of respiratory complex I from *Thermus thermophilus*, Science 311 (2006) 1430–1436.
[14] B.D. Gomperts, I.M. Kramer, P.E.R. Tatham, Signal Transduction, Elsevier Academic Press, London, UK, 2003.
[15] Z. Technikova-Dobrova, A.M. Sardanelli, S. Papa, Phosphorylation of mitochondrial proteins in bovine heart. Characterization of kinases and substrates, FEBS Lett. 322 (1993) 51–55.
[16] G. Muller, W. Bandlow, Protein phosphorylation in yeast mitochondria: cAMP-dependence, submitochondrial localization and substrates of mitochondrial protein kinases, Yeast 3 (1987) 161–174.
[17] A.A. Kondrashin, M.V. Nesterova, Y.S. Cho-Chung, Subcellular distribution of the R-subunits of cAMP-dependent protein kinase in LS-174T human colon carcinoma cells, Biochem. Mol. Biol. Int. 45 (1998) 237–244.
[18] B. Kleitke, H. Sydow, A. Wollenberger, Evidence for cyclic AMP-dependent protein kinase activity in isolated guinea pig and rat liver mitochondria, Acta Biol. Med. Ger. 35 (1976) K9–K17.
[19] M.J. Dimino, R.R. Bieszczad, M.J. Rowe, Cyclic AMP-dependent protein kinase in mitochondria and cytosol from different-sized follicles and corpora lutea of porcine ovaries, J. Biol. Chem. 256 (1981) 10876–10882.
[20] J.W. Burgess, E.W. Yamada, cAMP-dependent protein kinase isozymes with preference for histone H2B as substrate in mitochondria of bovine heart, Biochem. Cell Biol. 65 (1987) 137–143.
[21] C. Pariset, S. Weinman, Differential localization of two isoforms of the regulatory subunit RII alpha of cAMP-dependent protein kinase in human sperm: biochemical and cytochemical study, Mol. Reprod. Dev. 39 (1994) 415–422.
[22] M. Corso, M. Thomson, Protein phosphorylation in mitochondria from human placenta, Placenta 22 (2001) 432–439.
[23] Z. Technikova-Dobrova, A.M. Sardanelli, F. Speranza, S. Scacco, A. Signorile, V. Lorusso, S. Papa, Cyclic adenosine monophosphate-dependent phosphorylation of mammalian mitochondrial proteins: enzyme and substrate characterization and functional role, Biochemistry 40 (2001) 13941–13947.
[24] G. Schwoch, B. Trinczek, C. Bode, Localization of catalytic and regulatory subunits of cyclic AMP-dependent protein kinases in mitochondria from various rat tissues, Biochem. J. 270 (1990) 181–188.
[25] G. Griffioen, J.M. Thevelein, Molecular mechanisms controlling the localisation of protein kinase A, Curr. Genet. 41 (2002) 199–207.
[26] R.Y. Lin, S.B. Moss, C.S. Rubin, Characterization of S-AKAP84, a novel developmentally regulated A kinase anchor protein of male germ cells, J. Biol. Chem. 270 (1995) 27804–27811.
[27] K.J. Newhall, A.R. Criniti, C.S. Cheah, K.C. Smith, K.E. Kafer, A.D. Burkart, G.S. McKnight, Dynamic anchoring of PKA is essential during oocyte maturation, Curr. Biol. 16 (2006) 321–327.
[28] Q. Chen, R.Y. Lin, C.S. Rubin, 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 (1997) 15247–15257.
[29] L. Cardone, A. Carlucci, A. Affaitati, A. Livigni, T. DeCristofaro, C. Garbi, S. Varrone, A. Ullrich, M.E. Gottesman, E.V. Avvedimento, A. Feliciello, Mitochondrial AKAP121 binds and targets protein tyrosine phosphatase D1, a novel positive regulator of src signaling, Mol. Cell. Biol. 24 (2004) 4613–4626.
[30] H. Harada, B. Becknell, M. Wilm, M. Mann, L.J. Huang, S.S. Taylor, J.D. Scott, S.J. Korsmeyer, Phosphorylation and inactivation of BAD by mitochondria-anchored protein kinase A, Mol. Cell 3 (1999) 413–422.
[31] A. Affaitati, L. Cardone, T. de Cristofaro, A. Carlucci, M.D. Ginsberg, S. Varrone, M.E. Gottesman, E.V. Avvedimento, A. Feliciello, Essential role of A-kinase anchor protein 121 for cAMP signaling to mitochondria, J. Biol. Chem. 278 (2003) 4286–4294.
[32] A.M. Sardanelli, A. Signorile, R. Nuzzi, D.D. Rasmo, Z. Technikova-Dobrova, Z. Drahota, A. Occhiello, A. Pica, S. Papa, Occurrence of A-kinase anchor protein and associated cAMP-dependent protein kinase in

the inner compartment of mammalian mitochondria, FEBS Lett. 580 (2006) 5690–5696.
[33] S. Papa, A.M. Sardanelli, T. Cocco, F. Speranza, S.C. Scacco, Z. Technikova-Dobrova, 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 (1996) 299–301.
[34] S. Papa, A.M. Sardanelli, S. Scacco, Z. Technikova-Dobrova, cAMP-dependent protein kinase and phosphoproteins in mammalian mitochondria. An extension of the cAMP-mediated intracellular signal transduction, FEBS Lett. 444 (1999) 245–249.
[35] P. Pasdois, C. Deveaud, P. Voisin, V. Bouchaud, M. Rigoulet, B. Beauvoit, Contribution of the phosphorylable complex I in the growth phase-dependent respiration of C6 glioma cells in vitro, J. Bioenerg. Biomembranes 35 (2003) 439–450.
[36] R. Chen, I.M. Fearnley, S.Y. Peak-Chew, J.E. Walker, The phosphorylation of subunits of complex I from bovine heart mitochondria, J. Biol. Chem. 279 (2004) 26036–26045.
[37] B. Schilling, R. Aggeler, B. Schulenberg, J. Murray, R.H. Row, R.A. Capaldi, B.W. Gibson, Mass spectrometric identification of a novel phosphorylation site in subunit NDUFA10 of bovine mitochondrial complex I, FEBS Lett. 579 (2005) 2485–2490.
[38] G. Pocsfalvi, M. Cuccurullo, G. Schlosser, S. Scacco, S. Papa, A. Malorni, Phosphorylation of B14.5a subunit from bovine heart complex I identified by titanium dioxide selective enrichment and shotgun proteomics, Mol. Cell Proteomics 6 (2006) 231–237.
[39] R.K. Hopper, S. Carroll, A.M. Aponte, D.T. Johnson, S. French, R.F. Shen, F.A. Witzmann, R.A. Harris, R.S. Balaban, Mitochondrial matrix phosphoproteome: effect of extra mitochondrial calcium, Biochemistry 45 (2006) 2524–2536.
[40] N.V. Bykova, H. Egsgaard, I.M. Moller, Identification of 14 new phosphoproteins involved in important plant mitochondrial processes, FEBS Lett. 540 (2003) 141–146.
[41] O. Augereau, S. Claverol, N. Boudes, M.J. Basurko, M. Bonneu, R. Rossignol, J.P. Mazat, T. Letellier, J. Dachary-Prigent, Identification of tyrosine-phosphorylated proteins of the mitochondrial oxidative phosphorylation machinery, Cell. Mol. Life Sci. 62 (2005) 1478–1488.
[42] J.R. Gingrich, K.A. Pelkey, S.R. Fam, Y. Huang, R.S. Petralia, R.J. Wenthold, M.W. Salter, Unique domain anchoring of Src to synaptic NMDA receptors via the mitochondrial protein NADH dehydrogenase subunit 2, Proc. Natl. Acad. Sci. U. S. A. 101 (2004) 6237–6242.
[43] M. Salvi, A.M. Brunati, L. Bordin, N. La Rocca, G. Clari, A. Toninello, Characterization and location of Src-dependent tyrosine phosphorylation in rat brain mitochondria, Biochim. Biophys. Acta 1589 (2002) 181–195.
[44] T. Miyazaki, L. Neff, S. Tanaka, W.C. Horne, R. Baron, Regulation of cytochrome *c* oxidase activity by c-Src in osteoclasts, J. Cell Biol. 160 (2003) 709–718.
[45] H.R. Lascano, L.M. Casano, M. Martin, B. Sabater, The activity of the chloroplastic Ndh complex is regulated by phosphorylation of the NDH-F subunit, Plant Physiol. 132 (2003) 256–262.
[46] T. Cocco, C. Pacelli, P. Sgobbo, G. Villani, Control of OXPHOS efficiency by complex I in brain mitochondria, Neurobiol. Aging (in press).
[47] B. Schilling, J. Murray, C.B. Yoo, R.H. Row, M.P. Cusack, R.A. Capaldi, B.W. Gibson, Proteomic analysis of succinate dehydrogenase and ubiquinol-cytochrome *c* reductase (Complex II and III) isolated by immunoprecipitation from bovine and mouse heart mitochondria, Biochim. Biophys. Acta 1762 (2006) 213–222.
[48] S. Iwata, J.W. Lee, K. Okada, J.K. Lee, M. Iwata, B. Rasmussen, T.A. Link, S. Ramaswamy, B.K. Jap, Complete structure of the 11-subunit bovine mitochondrial cytochrome bc_1 complex, Science 281 (1998) 64–71.
[49] K. Li, Y. Li, J.M. Shelton, J.A. Richardson, E. Spencer, Z.J. Chen, X. Wang, R.S. Williams, Cytochrome *c* deficiency causes embryonic lethality and attenuates stress-induced apoptosis, Cell 101 (2000) 389–399.
[50] D.R. Green, Apoptotic pathways: paper wraps stone blunts scissors, Cell 102 (2000) 1–4.
[51] G. Kroemer, B. Dallaporta, M. Resche-Rigon, The mitochondrial death/life regulator in apoptosis and necrosis, Annu. Rev. Physiol. 60 (1998) 619–642.
[52] V.P. Skulachev, Cytochrome *c* in the apoptotic and antioxidant cascades, FEBS Lett. 423 (1998) 275–280.
[53] V.E. Kagan, V.A. Tyurin, J. Jiang, Y.Y. Tyurina, V.B. Ritow, A.A. Amoscato, A.N. Osipov, N.A. Belikova, A.A. Kapralov, V. Kini, I.I. Vlasova, Q. Zhao, M. Zou, P. Di, D.A. Svistunenko, I.V. Kurnikov, G.G. Borisenko, Cytochrome *c* acts as a cardiolipin oxygenase required for release of proapoptotic factors, Nat. Chem. Biol. 1 (2005) 223–232.
[54] M.O. Pereverzev, T.V. Vygodina, A.A. Konstantinov, V.P. Skulachev, Cytochrome *c*, an ideal antioxidant, Biochem. Soc. Trans. 31 (2003) 1312–1315.
[55] B. Hennig, Change of cytochrome *c* structure during development of the mouse, Eur. J. Biochem. 55 (1975) 167–183.
[56] E. Goldberg, D. Sberna, T.E. Wheat, G.J. Urbanski, E. Margoliash, Cytochrome *c*: immunofluorescent localization of the testis-specific form, Science 196 (1977) 1010–1012.
[57] R.A. Hess, L.A. Miller, J.D. Kirby, E. Margoliash, E. Goldberg, Immunoelectron microscopic localization of testicular and somatic cytochromes *c* in the seminiferous epithelium of the rat, Biol. Reprod. 48 (1993) 1299–1308.
[58] Z. Liu, H. Lin, S. Ye, Q.Y. Liu, Z. Meng, C.M. Zhang, Y. Xia, E. Margoliash, Z. Rao, X.J. Liu, Remarkably high activities of testicular cytochrome *c* in destroying reactive oxygen species and in triggering apoptosis, Proc. Natl. Acad. Sci. U. S. A. 103 (2006) 8965–8970.
[59] M. Hüttemann, S. Jaradat, L.I. Grossman, Cytochrome *c* oxidase of mammals contains a testes-specific isoform of subunit VIb—the counterpart to testes-specific cytochrome *c*? Mol. Reprod. Dev. 66 (2003) 8–16.
[60] S. Ferguson-Miller, D.L. Brautigan, E. Margoliash, Correlation of the kinetics of electron transfer activity of various eukaryotic cytochromes *c* with binding to mitochondrial cytochrome *c* oxidase, J. Biol. Chem. 251 (1976) 1104–1115.
[61] D.B. McIntosh, J.C. Parrish, C.J. Wallace, Definition of a nucleotide binding site on cytochrome *c* by photoaffinity labeling, J. Biol. Chem. 271 (1996) 18379–18386.
[62] I. Lee, A.R. Salomon, K. Yu, J.W. Doan, L.I. Grossman, M. Hüttemann, New prospects for an old enzyme: mammalian cytochrome *c* is tyrosine-phosphorylated in vivo, Biochemistry 45 (2006) 9121–9128.
[63] V.A. Roberts, M.E. Pique, Definition of the interaction domain for cytochrome *c* on cytochrome *c* oxidase. III. Prediction of the docked complex by a complete, systematic search, J. Biol. Chem. 274 (1999) 38051–38060.
[64] N. Staudenmayer, S. Ng, M.B. Smith, F. Millett, Effect of specific trifluoroacetylation of individual cytochrome *c* lysines on the reaction with cytochrome oxidase, Biochemistry 16 (1977) 600–604.
[65] H.T. Smith, N. Staudenmayer, F. Millett, Use of specific lysine modifications to locate the reaction site of cytochrome *c* with cytochrome oxidase, Biochemistry 16 (1977) 4971–4974.
[66] S. Ferguson-Miller, D.L. Brautigan, E. Margoliash, Definition of cytochrome *c* binding domains by chemical modification. III. Kinetics of reaction of carboxydinitrophenyl cytochromes *c* with cytochrome *c* oxidase, J. Biol. Chem. 253 (1978) 149–159.
[67] R.E. Dickerson, T. Takano, D. Eisenberg, O.B. Kallai, L. Samson, A. Cooper, E. Margoliash, Ferricytochrome *c*. I. General features of the horse and bonito proteins at 2.8 A resolution, J. Biol. Chem. 246 (1971) 1511–1535.
[68] T. Yu, X. Wang, C. Purring-Koch, Y. Wei, G.L. McLendon, A mutational epitope for cytochrome *c* binding to the apoptosis protease activation factor-1, J. Biol. Chem. 276 (2001) 13034–13038.
[69] R. Jemmerson, J. Liu, D. Hausauer, K.P. Lam, A. Mondino, R.D. Nelson, A conformational change in cytochrome *c* of apoptotic and necrotic cells is detected by monoclonal antibody binding and mimicked by association of the native antigen with synthetic phospholipid vesicles, Biochemistry 38 (1999) 3599–3609.
[70] M. Rytomaa, P.K. Kinnunen, Evidence for two distinct acidic phospholipid-binding sites in cytochrome *c*, J. Biol. Chem. 269 (1994) 1770–1774.

[71] M. Ruitenberg, A. Kannt, E. Bamberg, K. Fendler, H. Michel, Reduction of cytochrome *c* oxidase by a second electron leads to proton translocation, Nature 417 (2002) 99–102.
[72] S. Yoshikawa, K. Muramoto, K. Shinzawa-Itoh, H. Aoyama, T. Tsukihara, K. Shimokata, Y. Katayama, H. Shimada, Proton pumping mechanism of bovine heart cytochrome *c* oxidase, Biochim. Biophys. Acta 1757 (2006) 1110–1116.
[73] J.P. Hosler, S. Ferguson-Miller, D.A. Mills, Energy transduction: proton transfer through the respiratory complexes, Annu. Rev. Biochem. 75 (2006) 165–187.
[74] I. Belevich, M.I. Verkhovsky, M. Wikstrom, Proton-coupled electron transfer drives the proton pump of cytochrome *c* oxidase, Nature 440 (2006) 829–832.
[75] K. Faxen, G. Gilderson, P. Adelroth, P. Brzezinski, A mechanistic principle for proton pumping by cytochrome *c* oxidase, Nature 437 (2005) 286–289.
[76] T. Tsukihara, H. Aoyama, E. Yamashita, T. Tomizaki, H. Yamaguchi, K. Shinzawa-Itoh, R. Nakashima, R. Yaono, S. Yoshikawa, The whole structure of the 13-subunit oxidized cytochrome *c* oxidase at 2.8 A, Science 272 (1996) 1136–1144.
[77] L.I. Grossman, M.I. Lomax, Nuclear genes for cytochrome *c* oxidase, Biochim. Biophys. Acta 1352 (1997) 174–192.
[78] M. Hüttemann, B. Kadenbach, L.I. Grossman, Mammalian subunit IV isoforms of cytochrome *c* oxidase, Gene 267 (2001) 111–123.
[79] M. Hüttemann, T.R. Schmidt, L.I. Grossman, A third isoform of cytochrome *c* oxidase subunit VIII is present in mammals, Gene 312 (2003) 95–102.
[80] N.B. Radford, B. Wan, A. Richman, L.S. Szczepaniak, J.L. Li, K. Li, K. Pfeiffer, H. Schägger, D.J. Garry, R.W. Moreadith, Cardiac dysfunction in mice lacking cytochrome-*c* oxidase subunit VIaH, Am. J. Physiol. Heart Circ. Physiol. 282 (2002) H726–H733.
[81] P. Merle, B. Kadenbach, Kinetic and structural differences between cytochrome *c* oxidases from beef liver and heart, Eur. J. Biochem. 125 (1982) 239–244.
[82] U. Buge, B. Kadenbach, Influence of buffer composition, membrane lipids and proteases on the kinetics of reconstituted cytochrome-*c* oxidase from bovine liver and heart, Eur. J. Biochem. 161 (1986) 383–390.
[83] I. Lee, A.R. Salomon, S. Ficarro, I. Mathes, F. Lottspeich, L.I. Grossman, M. Hüttemann, cAMP-dependent tyrosine phosphorylation of subunit I inhibits cytochrome *c* oxidase activity, J. Biol. Chem. 280 (2005) 6094–6100.
[84] M.D. Suarez, A. Revzin, R. Narlock, E.S. Kempner, D.A. Thompson, S. Ferguson-Miller, The functional and physical form of mammalian cytochrome *c* oxidase determined by gel filtration, radiation inactivation, and sedimentation equilibrium analysis, J. Biol. Chem. 259 (1984) 13791–13799.
[85] J. Napiwotzki, K. Shinzawa-Itoh, S. Yoshikawa, B. Kadenbach, ATP and ADP bind to cytochrome *c* oxidase and regulate its activity, Biol. Chem. 378 (1997) 1013–1021.
[86] S. Arnold, B. Kadenbach, The intramitochondrial ATP/ADP-ratio controls cytochrome *c* oxidase activity allosterically, FEBS Lett. 443 (1999) 105–108.
[87] B. Ludwig, E. Bender, S. Arnold, M. Hüttemann, I. Lee, B. Kadenbach, Cytochrome *c* oxidase and the regulation of oxidative phosphorylation, Chem. Bio. Chem. 2 (2001) 392–403.
[88] S. Arnold, F. Goglia, B. Kadenbach, 3,5-Diiodothyronine binds to subunit Va of cytochrome-*c* oxidase and abolishes the allosteric inhibition of respiration by ATP, Eur. J. Biochem. 252 (1998) 325–330.
[89] V. Frank, B. Kadenbach, Regulation of the H+/e− stoichiometry of cytochrome *c* oxidase from bovine heart by intramitochondrial ATP/ADP ratios, FEBS Lett. 382 (1996) 121–124.
[90] I. Lee, B. Kadenbach, Palmitate decreases proton pumping of liver-type cytochrome *c* oxidase, Eur. J. Biochem. 268 (2001) 6329–6334.
[91] J.L. Boerner, M.L. Demory, C. Silva, S.J. Parsons, Phosphorylation of Y845 on the epidermal growth factor receptor mediates binding to the mitochondrial protein cytochrome *c* oxidase subunit II, Mol. Cell. Biol. 24 (2004) 7059–7071.
[92] M.V. Kato, The mechanisms of death of an erythroleukemic cell line by p53: involvement of the microtubule and mitochondria, Leuk. Lymphoma 33 (1999) 181–186.
[93] A.M. Beauchemin, B. Gottlieb, L.K. Beitel, Y.A. Elhaji, L. Pinsky, M.A. Trifiro, Cytochrome *c* oxidase subunit Vb interacts with human androgen receptor: a potential mechanism for neurotoxicity in spinobulbar muscular atrophy, Brain Res. Bull. 56 (2001) 285–297.
[94] W.L. Yang, L. Iacono, W.M. Tang, K.V. Chin, Novel function of the regulatory subunit of protein kinase A: regulation of cytochrome *c* oxidase activity and cytochrome *c* release, Biochemistry 37 (1998) 14175–14180.
[95] G.C. Brown, Regulation of mitochondrial respiration by nitric oxide inhibition of cytochrome *c* oxidase, Biochim. Biophys. Acta 1504 (2001) 46–57.
[96] P.S. Brookes, Mitochondrial nitric oxide synthase, Mitochondrion 3 (2004) 187–204.
[97] A.C. Gorren, B. Mayer, Nitric-oxide synthase: a cytochrome P450 family foster child, Biochim. Biophys. Acta 1770 (2006) 432–445.
[98] A. Tatoyan, C. Giulivi, Purification and characterization of a nitric-oxide synthase from rat liver mitochondria, J. Biol. Chem. 273 (1998) 11044–11048.
[99] T. Persichini, V. Mazzone, F. Polticelli, S. Moreno, G. Venturini, E. Clementi, M. Colasanti, Mitochondrial type I nitric oxide synthase physically interacts with cytochrome *c* oxidase, Neurosci. Lett. 384 (2005) 254–259.
[100] G.C. Brown, C.E. Cooper, Nanomolar concentrations of nitric oxide reversibly inhibit synaptosomal respiration by competing with oxygen at cytochrome oxidase, FEBS Lett. 356 (1994) 295–298.
[101] C. Giulivi, Functional implications of nitric oxide produced by mitochondria in mitochondrial metabolism, Biochem. J. 332 (Pt 3) (1998) 673–679.
[102] S. Cuzzocrea, Role of nitric oxide and reactive oxygen species in arthritis, Curr. Pharm. Des. 12 (2006) 3551–3570.
[103] J. Assreuy, Nitric oxide and cardiovascular dysfunction in sepsis, Endocr. Metab. Immune Disord Drug Targets 6 (2006) 165–173.
[104] J. Steiner, D. Rafols, H.K. Park, M.S. Katar, J.A. Rafols, T. Petrov, Attenuation of iNOS mRNA exacerbates hypoperfusion and upregulates endothelin-1 expression in hippocampus and cortex after brain trauma, Nitric Oxide 10 (2004) 162–169.
[105] D. Fukumura, S. Kashiwagi, R.K. Jain, The role of nitric oxide in tumour progression, Nat. Rev. Cancer 6 (2006) 521–534.
[106] D.W. Stepp, Impact of obesity and insulin resistance on vasomotor tone: nitric oxide and beyond, Clin. Exp. Pharmacol. Physiol. 33 (2006) 407–414.
[107] S. Moncada, J.P. Bolanos, Nitric oxide, cell bioenergetics and neurodegeneration, J. Neurochem. 97 (2006) 1676–1689.
[108] A. Kirichenko, T. Vygodina, H.M. Mkrtchyan, A. Konstantinov, Specific cation binding site in mammalian cytochrome oxidase, FEBS Lett. 423 (1998) 329–333.
[109] C. Ostermeier, A. Harrenga, U. Ermler, H. Michel, Structure at 2.7 A resolution of the *Paracoccus denitrificans* two-subunit cytochrome *c* oxidase complexed with an antibody FV fragment, Proc. Natl. Acad. Sci. U. S. A. 94 (1997) 10547–10553.
[110] D.A. Mills, B. Schmidt, C. Hiser, E. Westley, S. Ferguson-Miller, Membrane potential-controlled inhibition of cytochrome *c* oxidase by zinc, J. Biol. Chem. 277 (2002) 14894–14901.
[111] S.S. Kuznetsova, N.V. Azarkina, T.V. Vygodina, S.A. Siletsky, A.A. Konstantinov, Zinc ions as cytochrome *c* oxidase inhibitors: two sites of action, Biochemistry (Mosc) 70 (2005) 128–136.
[112] K. Faxen, L. Salomonsson, P. Adelroth, P. Brzezinski, Inhibition of proton pumping by zinc ions during specific reaction steps in cytochrome *c* oxidase, Biochim. Biophys. Acta 1757 (2006) 388–394.
[113] M.B. Sorensen, M. Stoltenberg, G. Danscher, E. Ernst, Chelation of intracellular zinc ions affects human sperm cell motility, Mol. Hum. Reprod. 5 (1999) 338–341.
[114] N.A. Steenaart, G.C. Shore, Mitochondrial cytochrome *c* oxidase subunit IV is phosphorylated by an endogenous kinase, FEBS Lett. 415 (1997) 294–298.

[115] E. Bender, B. Kadenbach, The allosteric ATP-inhibition of cytochrome *c* oxidase activity is reversibly switched on by cAMP-dependent phosphorylation, FEBS Lett. 466 (2000) 130–134.
[116] I. Lee, E. Bender, B. Kadenbach, Control of mitochondrial membrane potential and ROS formation by reversible phosphorylation of cytochrome *c* oxidase, Mol. Cell. Biochem. 234–235 (2002) 63–70.
[117] S.K. Prabu, H.K. Anandatheerthavarada, H. Raza, S. Srinivasan, J.F. Spear, N.G. Avadhani, Protein kinase A-mediated phosphorylation modulates cytochrome *c* oxidase function and augments hypoxia and myocardial ischemia-related injury, J. Biol. Chem. 281 (2006) 2061–2070.
[118] J.K. Fang, S.K. Prabu, N.B. Sepuri, H. Raza, H.K. Anandatheerthavarada, D. Galati, J. Spear, N.G. Avadhani, Site specific phosphorylation of cytochrome *c* oxidase subunits I, IVi1 and Vb in rabbit hearts subjected to ischemia/reperfusion, FEBS Lett 581 (2007) 1302–1310.
[119] E.K. Ainscow, M.D. Brand, The responses of rat hepatocytes to glucagon and adrenaline. Application of quantified elasticity analysis, Eur. J. Biochem. 265 (1999) 1043–1055.
[120] T. Tsukihara, K. Shimokata, Y. Katayama, H. Shimada, K. Muramoto, H. Aoyama, M. Mochizuki, K. Shinzawa-Itoh, E. Yamashita, M. Yao, Y. Ishimura, S. Yoshikawa, The low-spin heme of cytochrome *c* oxidase as the driving element of the proton-pumping process, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 15304–15309.
[121] T. Miyazaki, S. Tanaka, A. Sanjay, R. Baron, The role of c-Src kinase in the regulation of osteoclast function, Mod. Rheumatol. 16 (2006) 68–74.
[122] M. Ogbi, J.A. Johnson, Protein kinase Cepsilon interacts with cytochrome *c* oxidase subunit IV and enhances cytochrome *c* oxidase activity in neonatal cardiac myocyte preconditioning, Biochem. J. 393 (2006) 191–199.
[123] M. Ogbi, C.S. Chew, J. Pohl, O. Stuchlik, S. Ogbi, J.A. Johnson, Cytochrome *c* oxidase subunit IV as a marker of protein kinase Cepsilon function in neonatal cardiac myocytes: implications for cytochrome *c* oxidase activity, Biochem. J. 382 (2004) 923–932.
[124] P. Ping, J. Zhang, Y.T. Zheng, R.C. Li, B. Dawn, X.L. Tang, H. Takano, Z. Balafanova, R. Bolli, Demonstration of selective protein kinase C-dependent activation of Src and Lck tyrosine kinases during ischemic preconditioning in conscious rabbits, Circ. Res. 85 (1999) 542–550.
[125] C. Song, T.M. Vondriska, G.W. Wang, J.B. Klein, X. Cao, J. Zhang, Y.J. Kang, S. D'Souza, P. Ping, Molecular conformation dictates signaling module formation: example of PKCepsilon and Src tyrosine kinase, Am. J. Physiol. Heart Circ. Physiol. 282 (2002) H1166–H1171.
[126] H. Noji, R. Yasuda, M. Yoshida, K. Kinosita Jr., Direct observation of the rotation of F1-ATPase, Nature 386 (1997) 299–302.
[127] Y. Sambongi, Y. Iko, M. Tanabe, H. Omote, A. Iwamoto-Kihara, I. Ueda, T. Yanagida, Y. Wada, M. Futai, Mechanical rotation of the c subunit oligomer in ATP synthase (F0F1): direct observation, Science 286 (1999) 1722–1724.
[128] G. Kaim, P. Dimroth, ATP synthesis by F-type ATP synthase is obligatorily dependent on the transmembrane voltage, Embo J. 18 (1999) 4118–4127.
[129] E. Lalanne, C. Mathieu, F. Vedel, R. De Paepe, Tissue-specific expression of genes encoding isoforms of the mitochondrial ATPase beta subunit in *Nicotiana sylvestris*, Plant Mol. Biol. 38 (1998) 885–888.
[130] W. Rouslin, Regulation of the mitochondrial ATPase in situ in cardiac muscle: role of the inhibitor subunit, J. Bioenerg. Biomembranes 23 (1991) 873–888.
[131] J.J. Garcia, E. Morales-Rios, P. Cortes-Hernandez, J.S. Rodriguez-Zavala, The inhibitor protein (IF1) promotes dimerization of the mitochondrial F1F0-ATP synthase, Biochemistry 45 (2006) 12695–12703.
[132] F.X. Zhang, W. Pan, J.B. Hutchins, Phosphorylation of F_1F_0 ATPase delta-subunit is regulated by platelet-derived growth factor in mouse cortical neurons in vitro, J. Neurochem. 65 (1995) 2812–2815.
[133] Y.H. Ko, W. Pan, C. Inoue, P.L. Pedersen, Signal transduction to mitochondrial ATP synthase: evidence that PDGF-dependent phosphorylation of the delta-subunit occurs in several cell lines, involves tyrosine, and is modulated by lysophosphatidic acid, Mitochondrion 1 (2002) 339–348.
[134] K. Vosseller, K.C. Hansen, R.J. Chalkley, J.C. Trinidad, L. Wells, G.W. Hart, A.L. Burlingame, Quantitative analysis of both protein expression and serine/threonine post-translational modifications through stable isotope labeling with dithiothreitol, Proteomics 5 (2005) 388–398.
[135] A. Struglics, K.M. Fredlund, I.M. Moller, J.F. Allen, Two subunits of the F0F1-ATPase are phosphorylated in the inner mitochondrial membrane, Biochem. Biophys. Res. Commun. 243 (1998) 664–668.
[136] G. Del Riego, L.M. Casano, M. Martin, B. Sabater, Multiple phosphorylation sites in the beta subunit of thylakoid ATP synthase, Photosynth. Res. 89 (2006) 11–18.
[137] K. Hojlund, K. Wrzesinski, P.M. Larsen, S.J. Fey, P. Roepstorff, A. Handberg, F. Dela, J. Vinten, J.G. McCormack, C. Reynet, H. Beck-Nielsen, Proteome analysis reveals phosphorylation of ATP synthase beta-subunit in human skeletal muscle and proteins with potential roles in type 2 diabetes, J. Biol. Chem. 278 (2003) 10436–10442.
[138] U. Krause-Buchholz, J.S. Becker, M. Zoriy, C. Pickhardt, M. Przybylski, G. Rödel, J.S. Becker, Detection of phosphorylated subunits by combined LA-ICP-MS and MALDI-FTICR-MS analysis in yeast mitochondrial membrane complexes separated by blue native/SDS-PAGE, Int. J. Mass Spectr. 248 (2006) 56–60.
[139] C. Chen, Y. Ko, M. Delannoy, S.J. Ludtke, W. Chiu, P.L. Pedersen, Mitochondrial ATP synthasome: three-dimensional structure by electron microscopy of the ATP synthase in complex formation with carriers for Pi and ADP/ATP, J. Biol. Chem. 279 (2004) 31761–31768.
[140] Y.H. Ko, M. Delannoy, J. Hullihen, W. Chiu, P.L. Pedersen, Mitochondrial ATP synthasome. Cristae-enriched membranes and a multiwell detergent screening assay yield dispersed single complexes containing the ATP synthase and carriers for Pi and ADP/ATP, J. Biol. Chem. 278 (2003) 12305–12309.
[141] I. Arnold, K. Pfeiffer, W. Neupert, R.A. Stuart, H. Schägger, Yeast mitochondrial F1F0-ATP synthase exists as a dimer: identification of three dimer-specific subunits, Embo J. 17 (1998) 7170–7178.
[142] H. Schägger, K. Pfeiffer, Supercomplexes in the respiratory chains of yeast and mammalian mitochondria, Embo J. 19 (2000) 1777–1783.
[143] P. Paumard, J. Vaillier, B. Coulary, J. Schaeffer, V. Soubannier, D.M. Mueller, D. Brethes, J.P. di Rago, J. Velours, The ATP synthase is involved in generating mitochondrial cristae morphology, Embo J. 21 (2002) 221–230.
[144] G. Arselin, J. Vaillier, B. Salin, J. Schaeffer, M.F. Giraud, A. Dautant, D. Brethes, J. Velours, The modulation in subunits e and g amounts of yeast ATP synthase modifies mitochondrial cristae morphology, J. Biol. Chem. 279 (2004) 40392–40399.
[145] C. Bornhovd, F. Vogel, W. Neupert, A.S. Reichert, Mitochondrial membrane potential is dependent on the oligomeric state of F1F0-ATP synthase supracomplexes, J. Biol. Chem. 281 (2006) 13990–13998.
[146] F. Di Pancrazio, E. Bisetto, V. Alverdi, I. Mavelli, G. Esposito, G. Lippe, Differential steady-state tyrosine phosphorylation of two oligomeric forms of mitochondrial F0F1ATPsynthase: a structural proteomic analysis, Proteomics 6 (2006) 921–926.
[147] H. Schägger, G. von Jagow, Blue native electrophoresis for isolation of membrane protein complexes in enzymatically active form, Anal. Biochem. 199 (1991) 223–231.
[148] I. Wittig, R. Carrozzo, F.M. Santorelli, H. Schägger, Supercomplexes and subcomplexes of mitochondrial oxidative phosphorylation, Biochim. Biophys. Acta 1757 (2006) 1066–1072.
[149] H. Schägger, Respiratory chain supercomplexes of mitochondria and bacteria, Biochim. Biophys. Acta 1555 (2002) 154–159.
[150] M.L. Genova, C. Bianchi, G. Lenaz, Supercomplex organization of the mitochondrial respiratory chain and the role of the Coenzyme Q pool: pathophysiological implications, Biofactors 25 (2005) 5–20.
[151] G. Lenaz, M.L. Genova, Kinetics of integrated electron transfer in the mitochondrial respiratory chain: random collisions versus solid state electron channeling, Am. J. Physiol. Cell Physiol. 292 (2006) C1221–C1239.
[152] B. Kadenbach, Intrinsic and extrinsic uncoupling of oxidative phosphorylation, Biochim. Biophys. Acta 1604 (2006) 77–94.
[153] B. Kadenbach, S. Arnold, I. Lee, M. Hüttemann, The possible role of cytochrome *c* oxidase in stress-induced apoptosis and degenerative diseases, Biochim. Biophys. Acta 1655 (2004) 400–408.
[154] K. Pfeiffer, V. Gohil, R.A. Stuart, C. Hunte, U. Brandt, M.L. Greenberg,

H. Schägger, Cardiolipin stabilizes respiratory chain supercomplexes, J. Biol. Chem. 278 (2003) 52873–52880.
[155] K. Brandner, D.U. Mick, A.E. Frazier, R.D. Taylor, C. Meisinger, P. Rehling, Taz1, an outer mitochondrial membrane protein, affects stability and assembly of inner membrane protein complexes: implications for Barth Syndrome, Mol. Biol. Cell 16 (2005) 5202–5214.
[156] G. Li, S. Chen, M.N. Thompson, M.L. Greenberg, New insights into the regulation of cardiolipin biosynthesis in yeast: implications for Barth syndrome, Biochim. Biophys. Acta 1771 (2006) 432–441.
[157] M. McKenzie, M. Lazarou, D.R. Thorburn, M.T. Ryan, Mitochondrial respiratory chain supercomplexes are destabilized in Barth Syndrome patients, J. Mol. Biol. 361 (2006) 462–469.
[158] D.A. Hood, Invited Review: contractile activity-induced mitochondrial biogenesis in skeletal muscle, J. Appl. Physiol. 90 (2001) 1137–1157.
[159] D. Freyssenet, M. Di Carlo, D.A. Hood, Calcium-dependent regulation of cytochrome c gene expression in skeletal muscle cells. Identification of a protein kinase c-dependent pathway, J. Biol. Chem. 274 (1999) 9305–9311.
[160] H. Pilegaard, B. Saltin, P.D. Neufer, Exercise induces transient transcriptional activation of the PGC-1alpha gene in human skeletal muscle, J. Physiol. 546 (2003) 851–858.
[161] M. Takahashi, A. Chesley, D. Freyssenet, D.A. Hood, Contractile activity-induced adaptations in the mitochondrial protein import system, Am. J. Physiol. 274 (1998) C1380–C1387.
[162] Y. Ito, P. Pandey, N. Mishra, S. Kumar, N. Narula, S. Kharbanda, S. Saxena, D. Kufe, Targeting of the c-Abl tyrosine kinase to mitochondria in endoplasmic reticulum stress-induced apoptosis, Mol. Cell. Biol. 21 (2001) 6233–6242.
[163] A. Livigni, A. Scorziello, S. Agnese, A. Adornetto, A. Carlucci, C. Garbi, I. Castaldo, L. Annunziato, E.V. Avvedimento, A. Feliciello, Mitochondrial AKAP121 links cAMP and src signaling to oxidative metabolism, Mol. Biol. Cell 17 (2006) 263–271.
[164] G.N. Bijur, R.S. Jope, Rapid accumulation of Akt in mitochondria following phosphatidylinositol 3-kinase activation, J. Neurochem. 87 (2003) 1427–1435.
[165] L. Li, P.S. Lorenzo, K. Bogi, P.M. Blumberg, S.H. Yuspa, Protein kinase Cdelta targets mitochondria, alters mitochondrial membrane potential, and induces apoptosis in normal and neoplastic keratinocytes when overexpressed by an adenoviral vector, Mol. Cell. Biol. 19 (1999) 8547–8558.
[166] E.N. Churchill, L.I. Szweda, Translocation of deltaPKC to mitochondria during cardiac reperfusion enhances superoxide anion production and induces loss in mitochondrial function, Arch. Biochem. Biophys. 439 (2005) 194–199.
[167] M. Salvi, A. Stringaro, A.M. Brunati, E. Agostinelli, G. Arancia, G. Clari, A. Toninello, Tyrosine phosphatase activity in mitochondria: presence of Shp-2 phosphatase in mitochondria, Cell. Mol. Life Sci. 61 (2004) 2393–2404.
[168] M. Tartaglia, E.L. Mehler, R. Goldberg, G. Zampino, H.G. Brunner, H. Kremer, I. van der Burgt, A.H. Crosby, A. Ion, S. Jeffery, K. Kalidas, M.A. Patton, R.S. Kucherlapati, B.D. Gelb, Mutations in PTPN11, encoding the protein tyrosine phosphatase SHP-2, cause Noonan syndrome, Nat. Genet. 29 (2001) 465–468.
[169] B.G. Neel, H. Gu, L. Pao, The 'Shp'ing news: SH2 domain-containing tyrosine phosphatases in cell signaling, Trends Biochem. Sci. 28 (2003) 284–293.
[170] P. Uhlen, P.M. Burch, C.I. Zito, M. Estrada, B.E. Ehrlich, A.M. Bennett, Gain-of-function/Noonan syndrome SHP-2/Ptpn11 mutants enhance calcium oscillations and impair NFAT signaling, Proc. Natl. Acad. Sci. U. S. A. 103 (2006) 2160–2165.
[171] S.S. Liu, Cooperation of a "reactive oxygen cycle" with the Q cycle and the proton cycle in the respiratory chain-superoxide generating and cycling mechanisms in mitochondria, J. Bioenerg. Biomembranes 31 (1999) 367–376.
[172] S.S. Korshunov, V.P. Skulachev, A.A. Starkov, High protonic potential actuates a mechanism of production of reactive oxygen species in mitochondria, FEBS Lett. 416 (1997) 15–18.
[173] B. Mackler, R.E. Person, T.D. Nguyen, A.G. Fantel, Studies of the cellular distribution of superoxide dismutases in adult and fetal rat tissues, Free Radic. Res. 28 (1998) 125–129.
[174] A.G. Fantel, R.E. Person, Involvement of mitochondria and other free radical sources in normal and abnormal fetal development, Ann. N. Y. Acad. Sci. 959 (2002) 424–433.
[175] M. Conrad, C. Jakupoglu, S.G. Moreno, S. Lippl, A. Banjac, M. Schneider, H. Beck, A.K. Hatzopoulos, U. Just, F. Sinowatz, W. Schmahl, K.R. Chien, W. Wurst, G.W. Bornkamm, M. Brielmeier, Essential role for mitochondrial thioredoxin reductase in hematopoiesis, heart development, and heart function, Mol. Cell. Biol. 24 (2004) 9414–9423.
[176] M. Siman, Congenital malformations in experimental diabetic pregnancy: aetiology and antoxidative treatment. Minireview based on a doctoral thesis, Ups J Med Sci 102 (1997) 61–98.
[177] P.L. Pedersen, Tumor mitochondria and the bioenergetics of cancer cells, Prog. Exp. Tumor Res. 22 (1978) 190–274.
[178] T. Bui, C.B. Thompson, Cancer's sweet tooth, Cancer Cells 9 (2006) 419–420.
[179] R.C. Krieg, R. Knuechel, E. Schiffmann, L.A. Liotta, E.F. Petricoin III, P. C. Herrmann, Mitochondrial proteome: cancer-altered metabolism associated with cytochrome *c* oxidase subunit level variation, Proteomics 4 (2004) 2789–2795.
[180] K.H. von Wangenheim, H.P. Peterson, Control of cell proliferation by progress in differentiation: clues to mechanisms of aging, cancer causation and therapy, J. Theor. Biol. 193 (1998) 663–678.
[181] A. Chatterjee, E. Mambo, D. Sidransky, Mitochondrial DNA mutations in human cancer, Oncogene 25 (2006) 4663–4674.
[182] J.P. Jakupciak, G.D. Dakubo, S. Maragh, R.L. Parr, Analysis of potential cancer biomarkers in mitochondrial DNA, Curr. Opin. Mol. Ther. 8 (2006) 500–506.
[183] S. Ohta, Contribution of somatic mutations in the mitochondrial genome to the development of cancer and tolerance against anticancer drugs, Oncogene 25 (2006) 4768–4776.
[184] S. Matoba, J.G. Kang, W.D. Patino, A. Wragg, M. Boehm, O. Gavrilova, P.J. Hurley, F. Bunz, P.M. Hwang, p53 regulates mitochondrial respiration, Science 312 (2006) 1650–1653.
[185] V.R. Fantin, P. Leder, Mitochondriotoxic compounds for cancer therapy, Oncogene 25 (2006) 4787–4797.
[186] J.M. Grad, E. Cepero, L.H. Boise, Mitochondria as targets for established and novel anti-cancer agents, Drug Resist. Updat. 4 (2001) 85–91.
[187] F. Schiavi, C.C. Boedeker, B. Bausch, M. Peczkowska, C.F. Gomez, T. Strassburg, C. Pawlu, M. Buchta, M. Salzmann, M.M. Hoffmann, A. Berlis, I. Brink, M. Cybulla, M. Muresan, M.A. Walter, F. Forrer, M. Valimaki, A. Kawecki, Z. Szutkowski, J. Schipper, M.K. Walz, P. Pigny, C. Bauters, J.E. Willet-Brozick, B.E. Baysal, A. Januszewicz, C. Eng, G. Opocher, H.P. Neumann, Predictors and prevalence of paraganglioma syndrome associated with mutations of the SDHC gene, Jama 294 (2005) 2057–2063.
[188] B.G. Slane, N. Aykin-Burns, B.J. Smith, A.L. Kalen, P.C. Goswami, F.E. Domann, D.R. Spitz, Mutation of succinate dehydrogenase subunit C results in increased O2.−, oxidative stress, and genomic instability, Cancer Res. 66 (2006) 7615–7620.
[189] P. Gonzalez-Cabo, R.P. Vazquez-Manrique, M.A. Garcia-Gimeno, P. Sanz, F. Palau, Frataxin interacts functionally with mitochondrial electron transport chain proteins, Hum. Mol. Genet. 14 (2005) 2091–2098.
[190] V. Calabrese, R. Lodi, C. Tonon, V. D'Agata, M. Sapienza, G. Scapagnini, A. Mangiameli, G. Pennisi, A.M. Stella, D.A. Butterfield, Oxidative stress, mitochondrial dysfunction and cellular stress response in Friedreich's ataxia, J. Neurol. Sci. 233 (2005) 145–162.
[191] W.D. Parker Jr., J. Parks, C.M. Filley, B.K. Kleinschmidt-DeMasters, Electron transport chain defects in Alzheimer's disease brain, Neurology 44 (1994) 1090–1096.
[192] F. Bosetti, F. Brizzi, S. Barogi, M. Mancuso, G. Siciliano, E.A. Tendi, L. Murri, S.I. Rapoport, G. Solaini, Cytochrome *c* oxidase and mitochondrial F1F0-ATPase (ATP synthase) activities in platelets and brain from patients with Alzheimer's disease, Neurobiol. Aging 23 (2002) 371–376.
[193] L. Devi, B.M. Prabhu, D.F. Galati, N.G. Avadhani, H.K. Anandatheertha-

varada, Accumulation of amyloid precursor protein in the mitochondrial import channels of human Alzheimer's disease brain is associated with mitochondrial dysfunction, J. Neurosci. 26 (2006) 9057–9068.
[194] S.H. Kim, R. Vlkolinsky, N. Cairns, G. Lubec, Decreased levels of complex III core protein 1 and complex V beta chain in brains from patients with Alzheimer's disease and Down syndrome, Cell. Mol. Life Sci. 57 (2000) 1810–1816.
[195] P.M. Abou-Sleiman, M.M. Muqit, N.W. Wood, Expanding insights of mitochondrial dysfunction in Parkinson's disease, Nat. Rev. Neurosci. 7 (2006) 207–219.
[196] H. Elkon, J. Don, E. Melamed, I. Ziv, A. Shirvan, D. Offen, Mutant and wild-type alpha-synuclein interact with mitochondrial cytochrome *c* oxidase, J. Mol. Neurosci. 18 (2002) 229–238.
[197] F. Sun, X. Huo, Y. Zhai, A. Wang, J. Xu, D. Su, M. Bartlam, Z. Rao, Crystal structure of mitochondrial respiratory membrane protein complex II, Cell 121 (2005) 1043–1057.
[198] R. Sanishvili, K.W. Volz, E.M. Westbrook, E. Margoliash, The low ionic strength crystal structure of horse cytochrome *c* at 2.1 A resolution and comparison with its high ionic strength counterpart, Structure 3 (1995) 707–716.
[199] V.K. Rastogi, M.E. Girvin, Structural changes linked to proton translocation by subunit c of the ATP synthase, Nature 402 (1999) 263–268.
[200] C. Gibbons, M.G. Montgomery, A.G. Leslie, J.E. Walker, The structure of the central stalk in bovine F(1)-ATPase at 2.4 A resolution, Nat. Struct. Biol. 7 (2000) 1055–1061.
[201] V.K. Dickson, J.A. Silvester, I.M. Fearnley, A.G. Leslie, J.E. Walker, On the structure of the stator of the mitochondrial ATP synthase, Embo J. 25 (2006) 2911–2918.
[202] S. Wilkens, D. Borchardt, J. Weber, A.E. Senior, Structural characterization of the interaction of the delta and alpha subunits of the *Escherichia coli* F1F0-ATP synthase by NMR spectroscopy, Biochemistry 44 (2005) 11786–11794.