Cell. Mol. Life Sci. 63 (2006) 1236–1248
1420-682X/06/111236-13
DOI 10.1007/s00018-005-5585-2

Cellular and Molecular Life Sciences

Review

Regulation of mitochondrial oxidative phosphorylation by second messenger-mediated signal transduction mechanisms

A. Boneh

Department of Paediatrics, University of Melbourne, Metabolic Service, Genetic Health Services Victoria, Murdoch Children's Research Institute, Royal Children's Hospital, Flemington Road, Parkville Victoria 3052 (Australia), Fax: +61 3 8341 6390, e-mail: avihu.boneh@ghsv.org.au

Received 11 December 2005; received after revision 14 January 2006; accepted 6 February 2006
Online First 29 March 2006

Abstract. The mitochondrial oxidative phosphorylation system is responsible for providing the bulk of cellular ATP molecules. There is a growing body of information regarding the regulation of this process by a number of second messenger-mediated signal transduction mechanisms, although direct studies aimed at elucidating this regulation are limited. The main second messengers affecting mitochondrial signal transduction are cAMP and calcium. Other second messengers include ceramide and reactive oxygen species as well as nitric oxide and reactive nitrogen species. This review focuses on available data on the regulation of the mitochondrial oxidative phosphorylation system by signal transduction mechanisms and is organised according to the second messengers involved, because of their pivotal role in mitochondrial function. Future perspectives for further investigations regarding these mechanisms in the regulation of the oxidative phosphorylation system are formulated.

Keywords. Oxidative phosphorylation, mitochondrial respiratory chain, signal transduction, calcium, reactive oxygen species, reactive nitrogen species, protein kinase A, protein kinase C, calcineurin, calmodulin.

Introduction

Mitochondria are considered the 'power houses' of cells and are responsible for providing the bulk of cellular ATP molecules through a series of electron transfer processes linked with oxidative phosphorylation. This process is up- or down-regulated according to the needs of the tissue involved. Metabolic processes do not occur independently but, rather, are regulated in response to intracellular or extracellular signals, with tight topographic (i.e. localisation within specific intracellular compartments) and temporal (short-term, repetitive, long-term) regulatory mechanisms. Cellular response to extracellular signals is mediated by specific mechanisms that are collectively termed signal transduction pathways. In general, these pathways consist of the following structure (Fig. 1a): a receptor, located on the cell membrane, leads to the production or release of a second messenger, via the modulation of a G protein. The second messenger(s) activates a kinase (or phosphatase) that, in turn, phosphorylates (or dephosphorylates, respectively) the target protein (e.g. enzyme, channel). Research in recent years has shown that kinases involved in signal transduction also phosphorylate and, hence, activate or inactivate nuclear transcription factors that enhance or inhibit, respectively, expression of genes. Thus, it has been argued that: 'The reversible phosphorylation of proteins regulates nearly every aspect of cell life' [1]. In addition, 'cross-talk' mechanisms between the signalling pathways provide a biochemical basis for the counteracting and/or additive or synergistic roles of these signalling pathways on cellular functions.

In view of its importance in providing ATP molecules to the cell under basal conditions and following temporary needs (such as muscle contraction), the dearth of direct studies to elucidate the regulatory role of signal transduction mechanisms on the various complexes comprising the mitochon-

drial oxidative phosphorylation (OXPHOS) system in response to energy requirements is intriguing. Nevertheless, evidence is growing that indicates that these complexes are indeed regulated by signal transduction mechanisms. This information is largely derived from indirect measurements of OXPHOS system activity, or specific inhibition of particular complexes in studies on the mitochondrial role in cell growth, malignant transformation and apoptosis.

This review focuses specifically on available data on the regulation of the OXPHOS system complexes by second messenger-mediated signal transduction mechanisms. It is organised according to the second messengers involved, because of their pivotal role in mitochondrial function. The main second messengers affecting mitochondrial signal transduction are cAMP and calcium. Other second messengers include ceramide, reactive oxygen species, nitric oxide and reactive nitrogen species. The data are summarised in Table 1.

The OXPHOS system

The mitochondrial OXPHOS system comprises five multiprotein complexes located at the inner mitochondrial mem-

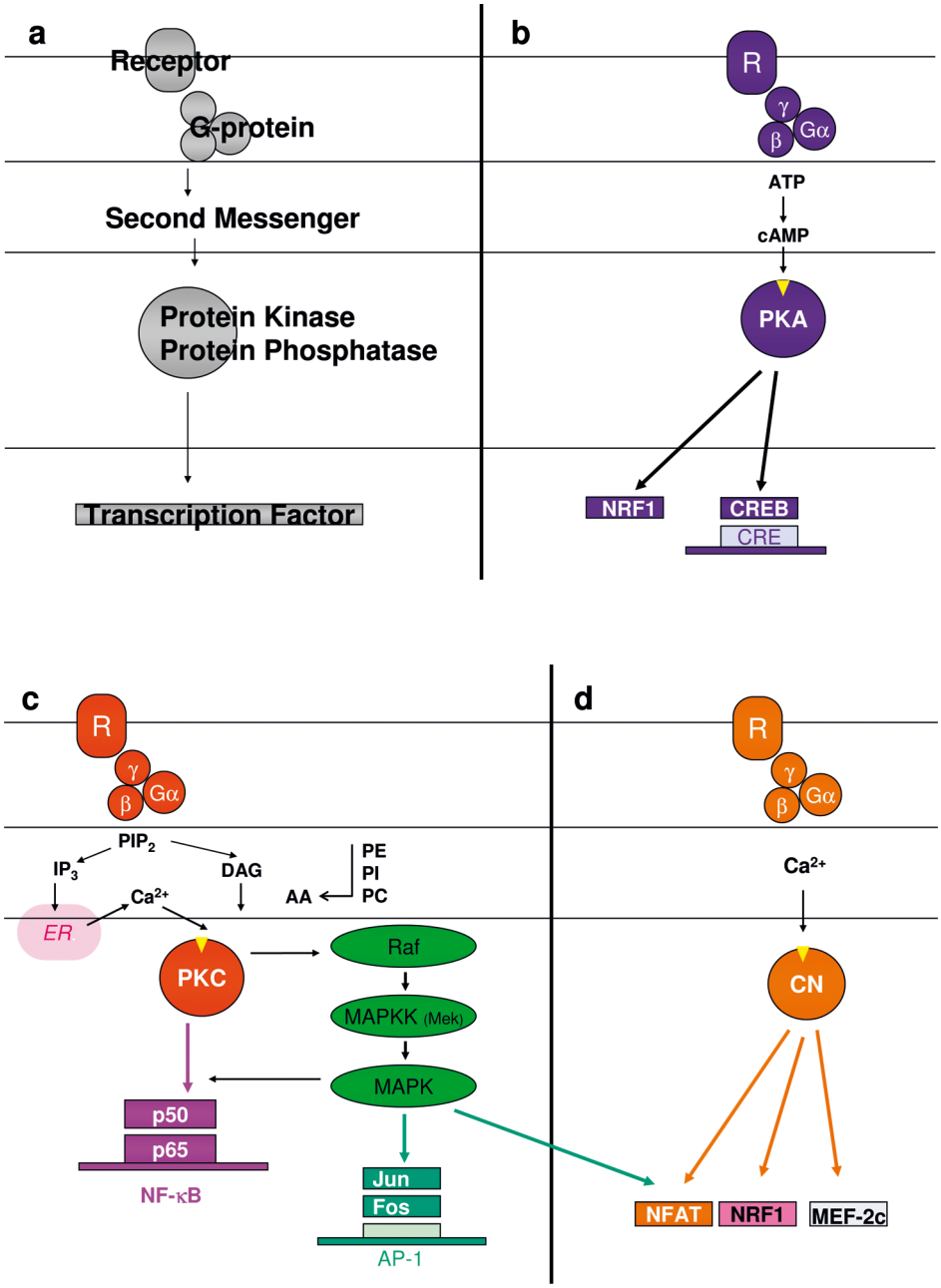

Figure 1. Signal transduction pathways. (*a*) general structure of signalling pathways. (*b*) cAMP and protein kinase A (PKA) signalling pathway. (*c*) Calcium-phospholipid and PKC signalling pathway. (*d*) Calcium-calcineurin signalling pathway.

Table 1. Second messengers, protein kinases and phosphatases, transcription factors and their effect on the various components of the mitochondrial respiratory chain.

Second-messenger	Tissue	Protein kinase	Transcription factor	Complex (subunit)	References
cAMP	bovine heart, BALB/3T3 cells	PKA	CRE, CREB, NRF1	complex I (NDUFS4 NDUFA1)[1] ↑ complex IV ↓ '18 kDa', ESSS, B17, SDAP, B9, MWFE, MNLL, subunits of complex III and ATP synthase; '42 kDa' associated with complex I (NDUFA10[3]), III, IV, V cytochrome c ↑	9, 11–13, 16, 18, 24, 25, 29, 30, 88
Calcium	cultured rabbit renal proximal tubule cells	PKCα, ERK1/2	?	F(0)F(1)-ATPase (β-subunit)[2] ↓	40, 41
	HeLa cells rat myocardium	PKCδ	?	{ATP production ↑; ROS production ↓}	34, 35, 66, 67
	rat myocardium	PKCε	?	?	67
	cardiac myocytes of a transgenic mouse model, neonatal rat cardiac myocytes	calcineurin MAPK	NFAT-3, AP-1, NRF-1, MEF-2c	complex IV (subunit IV) ↓ complex IV (subunit I) ↓ complex I (ND_3) ↓	44–45
	bovine heart mitochondria	mitochondrial phosphatase (?)	?	complex IV ↑	25
	transgenic mouse skeletal muscle	calcium/calmodulin-dependent protein kinase IV	mtTFA NRF-1, NRF-2		50
	rat embryonic hippocampal cells	calmodulin-kinase II PKCα PKA	?	?	89
ROS (H_2O_2)	cultured proximal tubular cells Human U-937 cells Rat parotic gland cells	PKCδ[2]	NFκB	complex I ↓, V ↓	66, 68, 69, 90
	jurkat T cells	MAPK	?	?	65
	human fibroblasts	?	?	complex III (cytochrome b & c1 mRNA) ↑	91
	osteoclasts rat brain mitochondria	tyrosine kinase (Src) tyrosine phosphatase Shp-2, PTP 1B	?	complex I (39 kDa), ?other complexes subunits	71–73
NO	rat brain synaptosomes	PKC[2]	?	complex IV↓, V↓	74, 77, 78, 84
RNS	murine macrophage J774 cells rat heart cells	PKC[2] PKA[2]	NFκB, AP-1, CREB	complex I ↓, II ↓, V ↓	74, 80–82
DAG	*Saccharommyces cerevisiae*	PKC	?	complex III ↑	43
Ceramide (short chain)	rat heart, cerebellar granule cells, hepatocytes	PKC (ζ[2])	?	complex I ↑, complex IV ↑, complex III[2] ↓	54–55
	human umbilical- vein endothelial cells	ceramide-dependent kinase	?	complex III [2]	57
?	cultured rat neonatal cardiac myocytes	PKCε	?	complex IV (18 kDa/subunit IV) ↑	42

NFAT, nuclear factor of activated T cells; AP-1, activating protein 1; NRF-1, nuclear respiratory factor 1; mtTFA, mitochondrial transcription factor A; ?, identity unknown.
[1] through direct phosphorylation of the subunit. [2] probably. [3] The identity of the kinase responsible for NDUFA10 phosphorylation is not unequivocally clear.

brane. Complexes I–IV are responsible for electron transfer and are referred to as the mitochondrial respiratory chain (MRC). Complex V, ATPase, is responsible for the eventual production of ATP. In addition, two dissociable electron carriers, coenzyme Q (ubiquinone) and cytochrome c, play an important role in the function of this system. Over a hundred genes are responsible for coding the components of the OXPHOS system, their co-localisation within the mitochondria and their assembly into five functional complexes, as well as the two electron carriers. Of these, 13 genes are located on the mitochondrial DNA. Complex I (NADH;ubiquinone oxidoreductase), the entry point of electrons from NADH into the MRC, is the largest and the most complex of all the components of the system. This complex comprises over 47 proteins, 7 of which are encoded by the mitochondrial DNA, and has an L-shaped structure with one arm within the inner mitochondrial membrane and the other protruding into the mitochondrial matrix. Complex II [succinate-coenzyme Q (ubiquinone) oxidoreductase], the entry point of electrons from succinate into the MRC, contains the enzyme succinate dehydrogenase and is encoded exclusively by nuclear DNA. Complex III (ubiquinone-cytochrome C oxidoreductase), the electron acceptor for coenzyme Q, comprises 10 nuclear-encoded subunits and 1 mitochondrially encoded unit, cytochrome b. Complex IV (cytochrome oxidase; COX), is a haem-copper respiratory oxidase which catalyses the coupled acceptance of electrons from cytochrome c to the reduction of O_2 to water. Human complex IV contains three mitochondrial (subunits I–III) and ten nuclear-encoded subunits, which are partly expressed in tissue-specific isoforms. Complex V, F_0-F_1 ATP synthetase, is composed of 12 nuclear and 2 mitochondrially encoded subunits. The F_0 component spans the inner mitochondrial membrane. The F_1 component protrudes into the matrix. The two components are connected by a 'stalk'. Complex V catalyses the synthesis of ATP from ADP and phosphate.

The mitochondrial cAMP and protein kinase A signal transduction pathway

The cAMP and cAMP-dependent protein kinase (PKA) signal transduction pathway regulates a wide spectrum of cellular processes, most notably those in response to hormonal stimulation (e.g. glucagon, thyroid hormone, catecholamines). cAMP, the second messenger in this pathway, is produced by adenylate cyclase following stimulation of a receptor located on the cell membrane, via the activation of a membranous G protein. cAMP affects cellular function by activating PKA, a tetrameric complex comprising two regulatory subunits (R subunits) and two catalytic subunits (C subunits) (Fig. 1b).

Several observations provide the basis for a specific mitochondrial cAMP and PKA signalling pathway, in addition to the cytosolic pathway: (i) there are gradients of intracellular cAMP concentrations [2], with higher concentrations in specific locations, including the mitochondria, (ii) cAMP can rapidly enter mitochondria and accumulate in the matrix [3], and (iii) PKA is 'anchored' to specific intracellular sites by specific proteins (AKAP). The gene products of AKAP-1 (primarily AKAP121) and AKAP-2 have been identified as critical to the function of PKA in mitochondria [4–6 and reviewed in refs. 7, 8]). Recent findings indicate that these AKAPs play a role in the nucleation of additional kinases and phosphatases that are involved in signalling, including PKC, cAMP-phosphodiesterases and tyrosine kinase [reviewed in ref. 8].

PKA localised to the inner mitochondrial membrane has been shown to phosphorylate three inner membrane proteins of 29, 18 and 6.5 kDa, as determined by SDS-PAGE [9, 10]. The authors suggested that the 18-kDa protein phosphorylated by PKA is the '18-kDa subunit' of complex I, a component of the matrix arm of the complex [11–13]. In a patient with Leigh's-like syndrome and duplication in the cDNA NDUFS-4 gene encoding the 18-kDa subunit, cAMP-mediated phosphorylation of the 18-kDa protein was abolished [14]. It was suggested that this may be the first description of a patient with a mutation in a gene encoding a component of the OXPHOS system that leads to abnormal signalling [15].

However, this notion has been recently disputed. Studying bovine heart mitochondria, Chen et al. [16] have demonstrated cAMP-associated *in vitro* phosphorylation of two complex I proteins located in the inner membrane arm of the complex. They have shown that the phosphorylated band migrating at 18 kDa on SDS-PAGE represents, in fact, three proteins: the 18-kDa subunit, subunit ESSS and the N-α acetylated subunit, B17. Only the ESSS subunit was phosphorylated, at serine 20, a site preceding the transmembrane region [16]. This small protein, which is encoded by a gene located on the X chromosome, has recently been shown to be essential for the assembly of mammalian complex I [17], making its phosphorylation an attractive putative regulatory mechanism. However, phosphorylation of this subunit could not be demonstrated in resting mitochondria using multiplexed proteomics technology, suggesting that it is not phosphorylated significantly *in vivo* [18]. In addition, the site of this phosphorylation is not conserved in mammals [16]. Thus, the significance of this phosphorylation in the regulation of complex I assembly is not clear at present.

Chen et al. [16] have also demonstrated the phosphorylation of a 10-kDa protein (at serine 55), identified as complex I subunit MWFE (in rat heart mitochondria, this protein migrated at 6 kDa [19]). This subunit, found in bovine cardiac and skeletal muscle, has 80% homology with the human NDUFA-1 gene product. Zhuchenko et al. [20] have reported the isolation, mapping, and genomic organisation of this gene, which is located on the

X chromosome. The promoter region of the gene contains a cAMP response element (CRE; see Fig. 1), suggesting that the expression of this subunit is linked to cAMP signalling pathways. The phosphorylation site of MWFE is thought to be located in the mitochondrial intermembrane space. Originally, this gene was thought to encode an 'accessory' protein in complex I but studies have confirmed that MWFE is, in fact, essential for the assembly of the mitochondrially encoded subunits of complex I, thus making is essential for complex I activity [21–23].

Taken together, current data suggest that two proteins in complex I, namely ESSS and the NDUFA-1 gene product MWFE which are essential for its assembly and function, may be phosphorylated and potentially regulated by the cAMP signalling pathway. Since the phosphorylation site of ESSS is thought to be located on the matrix side and that of MWFE is in the intermembrane space, it is theoretically plausible that two different cAMP-dependent kinases, possibly anchored to the mitochondria by different AKAPs are responsible for this phosphorylation. However, direct evidence for their *in vivo* cAMP-dependent phosphorylation and regulation is not available yet and further studies will be needed to elucidate the signalling mechanism regulating these proteins.

Recent studies suggest a role for cAMP-dependent phosphorylation in the regulation of complex IV activity [24]. There are three consensus sequences for cAMP-dependent phosphorylation in the bovine heart enzyme (in subunits I, III and Vb) but studies on isolated mitochondria from bovine heart indicated that the phosphorylation site in complex IV is only at Ser441 in subunit I [25]. cAMP-dependent phosphorylation of isolated cytochrome c oxidase from bovine kidney and heart, and of the reconstituted heart enzyme, has been shown to switch on the allosteric inhibition of this complex by ATP at high intramitochondrial ATP/ADP ratios [24]. By contrast, ATP-mediated inhibition of complex IV is switched off by calcium-induced dephosphorylation of the enzyme (see below). These observations have led to the proposal that the allosteric ATP inhibition of complex IV activity at high ATP/ADP ratios acts as a 'second' regulatory mechanism of the function of the OXPHOS system in addition to the 'first' mechanism of proton gradient across the inner mitochondrial membrane (i.e. membrane potential) [26, 27]. From a signalling perspective, the opposing effects of the two processes are an example of 'cross-talk' between the two signalling pathways in the physiological regulation of complex IV activity.

A novel cAMP-mediated signalling pathway that involves tyrosine phosphorylation in mitochondria has recently been proposed by Lee et al. [28]. Tyr304 in subunit I of complex IV, a site that has been shown to be conserved through evolution, was phosphorylated in the presence of the phosphodiesterase inhibitor theophylline in mitochondria isolated from bovine liver. Concomitantly, complex IV activity was inhibited through modification of its kinetic characteristics [28]. Glucagon, which leads to physiological activation of cAMP production through a receptor/G protein-mediated pathway, and foreskolin, which activates adenylate cyclase leading to elevated intracellular cAMP concentrations, had a similar effect on the catalytic activity of the complex. Taken together, the investigators concluded that this phosphorylation is thus physiologically significant and proposed that PKA affects cytochrome c oxidase activity indirectly by activating a tyrosine kinase located at the inter-membrane space [28]. From a signalling perspective, the physiological effects of such putative 'cross-talk' between pathways lead-

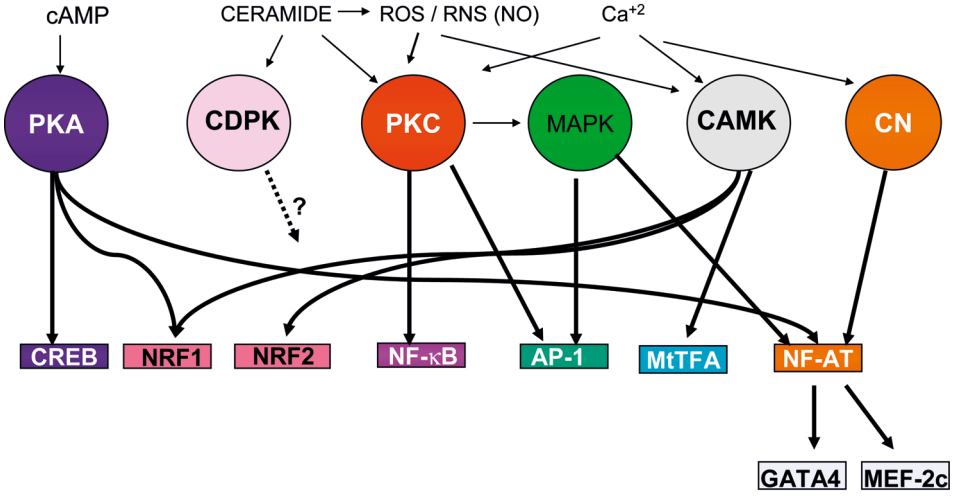

Figure 2. Mitochondrial signalling pathways: second messengers, protein kinases, protein phosphatase and transcription factors involved in the regulation of the OXPHOS system. PKA down-regulates NFAT. All other arrows indicate up-regulation.
CDPK, Ceramide-dependent protein kinase; CAMK, calmodulin kinase; CN, calcineurin.

ing to tyrosine phosphorylation and those leading to serine/threonine phosphorylation could be of prime importance and should be further explored.

In contrast to the mechanism of the putative cAMP and PKA-mediated regulation of complex I and complex IV, which involves phosphorylation of subunits of the complexes, the regulation of cytochrome c by this signalling pathway is mediated by the activation of transcription factors that regulate the expression of the cytochrome c gene. Several recognition sites are present on the promoter of the cytochrome c gene, including the cAMP response element binding-protein (CREB) and nuclear respiratory factor 1 (NRF-1). Both these transcription factors are activated by phosphorylation of serine residues (Figs. 1b, 2). In BALB/3T3 cells, cAMP leads to activation of PKA, which, in turn, activates the transcription factor CREB and up-regulates the cytochrome c gene [29]. Further studies established that activation of CREB and NRF-1 in response to serum stimulation of BALB/3T3 cells is sequential, with a rapid activation of CREB, followed by a slower activation of NRF-1 [30]. Thus, elevated cAMP levels are hypothesized to lead to rapid phosphorylation and induction of the CREB site. This, in turn, leads to a rapid up-regulation of the cytochrome c gene, in keeping with the role of the cytochrome as a regulator of cellular respiration [30]. It should be noted, however, that serum-dependent activation of NRF-1 is not exclusively cAMP dependent and may be through casein kinase II (CKII), underscoring, again, possible cross-talk between signalling pathways in the regulation of the OXPHOS system. The observation that subunit IV of complex IV (COX-IV) remained unaffected under these stimulatory conditions indicates that different control mechanisms activate cytochrome c and COX-IV [29, 30]. Moreover, enhanced respiratory activity, as shown in these studies by respirometry, resulted from the specific transcriptional induction of cytochrome c rather than through enhancement of the whole MRC [30].

NRF-1 and NRF-2 are involved in the activation of many of the known nuclear genes that encode components of the OXPHOS system. NRF-1 activates genes encoding cytochrome c, at least one subunit of complexes III, IV and V, as well as components of the mitochondrial transcription and replication machinery. NRF-2 activates genes encoding subunit IV and Vb of complex IV. The observation that NRF-1 is activated by cAMP suggests that cAMP signalling could regulate other components of the OXPHOS system. To date, there is no experimental evidence for such regulation.

The role of calcium as a mitochondrial second messenger

The role of calcium in cellular signal transduction has been in the focus of research in recent years. Calcium concentrations vary in different subcellular organelles under quiescent conditions and change following cellular stimulation. Upon physiological stimulation (e.g. hormones, ATP consumption), cytosolic calcium concentrations increase following its release from the endoplasmic reticulum by opening inositol tris-phosphate-gated channels. This spatio-temporal regulation of intracellular calcium concentrations enables the modulation of various calcium-dependent signalling cascades, resulting in different cellular responses. Mitochondria have been shown to rapidly accumulate and release calcium and to respond to calcium-mediated cell stimulations. Thus, mitochondrial calcium concentrations increase in parallel with cytosolic calcium, following cellular stimulation [reviewed in refs. 31, 32]. Topographic studies in living HeLa cells using three-dimensional images with green fluorescent proteins have indicated that mitochondria form a large network that is in close apposition and largely linked with the endoplasmic reticulum [33]. Microdomains of high calcium concentrations were shown in these contact areas and the outer surface of the inner mitochondrial membrane was exposed to calcium concentrations that were higher than those in the cytosol [33]. These findings provide a putative mechanism for the rapid accumulation of calcium by mitochondria.

The increase in mitochondrial calcium concentrations is followed by increased NADH production in various cell types [reviewed in ref. 32]. Rizzuto's group has shown that mitochondrial Ca^{2+} accumulation triggers the activation of the OXPHOS system leading to increased ATP synthesis. This activation is dependent on both cell metabolism and substrate supply to mitochondria. They have demonstrated a so-called 'cellular memory' that allows long-term activation of ATP synthesis, up to 30 min after the removal of the agonist [34]. They have also shown that the mitochondrial calcium signals are modulated by PKC subspecies β, δ, ζ (see below) [35]. On the other hand, mitochondrial ability to accumulate calcium is abolished when the OXPHOS system is disrupted, leading to secondary perturbation of other calcium-dependent mitochondrial enzymes that play a role in intermediary metabolism [32]. These observations underlie the reciprocal relationship between modulation of the OXPHOS system by calcium and modulation of calcium concentrations by the OXPHOS system.

The main calcium-dependent signalling pathways operative in mitochondria are those of calcium-phospholipid-dependent protein kinase, calcium-calmodulin kinase and the calcineurin phosphatase (Fig. 2). However, most of the current information about the role of these signalling pathways in regulating the OXPHOS system is derived from studies on apoptosis and malignant transformation and therefore is indirect and might be speculative. Detailed experimental elucidation of these signalling cas-

cades leading to the regulation of the OXPHOS system awaits further studies.

The calcium-phospholipid and PKC signal transduction pathway

The calcium-phospholipid signalling pathway was unravelled following the discovery and characterisation PKC and has been shown to mediate short- and long-term physiological functions as well as cell transformation and apoptosis [36, 37]. The PKC family comprises 11 ubiquitous subspecies of serine-threonine kinases and 2 (or possibly 3) PKC-related kinases [38]. PKC subspecies are grouped into classical, novel and atypical PKCs, depending on their calcium and lipid dependence and protein interactions. Activation of the 'classical PKCs' (α, β I, β II, γ) is Ca^{2+}-dependent, whereas that of the 'novel PKCs' (δ, ε, η, θ, μ) and the 'atypical PKCs' (ζ, ι) is Ca^{2+}-independent [38].

In this signalling pathway, the cell membrane receptor leads to a G protein-mediated activation of membrane phospholipase(s) thereby generating second messengers: diacylglycerol (DAG) and phosphatidylinositol tris-phosphate (PIP_3). DAG increases the affinity of calcium-dependent PKC subspecies to the second messenger, rendering it active at lower calcium concentrations. PIP_3 leads to an elevated cytosolic Ca^{2+} concentration, released from intracellular stores in the endoplasmic reticulum. Under quiescent conditions, PKC subspecies are mostly cytosolic, but following an extracellular signal they translocate to the membranous compartment of the cell. Trafficking of PKC subspecies to intracellular membranous organelles is regulated by a group of adaptor proteins, which are collectively called receptors for activated C kinase (RACKs) [reviewed in ref. 39]. PKC activates two families of transcription factors, nuclear factor kappa-B (NFκB) and activating protein-1 (AP-1), through different pathways (Fig. 1c). One pathway involves translocation of NFκB to the nucleus and its activation following PKC-mediated phosphorylation of IκB. The other pathway involves PKC-mediated activation of mitogen activated protein kinase (MAPK) through activation of its kinase (MAPKK). The MAPK signalling pathway comprises three main branches: c-Jun N-terminal kinases (JNKs), extracellular signal-regulated kinases (ERKs) and p38 kinases. Activation of these branches follows phosphorylation of MAPK kinases: MEKK1, leading to activation of JNK proteins, Ras/Raf and MEK1/MEK2 leading to phosphorylation of ERK1 and ERK2 kinases, and MKK3 and MKK6 that phosphorylate the p38 kinases. PKC-mediated activation of MAPK leads to upregulation of the expression of *c-jun* and *c-fos* oncogenes, which constitute the AP-1 transcription factor. MAPK-mediated activation of NFκB through phosphorylation of IκB provides a link between the two pathways of PKC-mediated transcription factor(s) activation.

Evidence for the role of PKC in the regulation of the OXPHOS system complexes can be indirectly derived from studies on the cytotoxic effects of anti-tumour compounds. The anti-neoplastic compound cisplatin was shown to cause decreases in oxidative phosphorylation and electron transport rate, along with hyperpolarisation of the mitochondrial membrane of renal proximal tubules [40]. Activation of PKC-α mediated the sustained decrease in mitochondrial membrane hyperpolarisation and oxidative phosphorylation but not the decrease in electron transfer rate. Sustained mitochondrial dysfunction was also affected by ERK1/2, through a PKC-independent pathway. These findings suggest that activation of PKC-α and/or ERK1/2 lead to decreased activity of ATP synthase, rather than affecting OXPHOS system complexes [40]. Indeed, the α, β, and ε subunits of F(1)F(0)-ATPase have PKC consensus motifs [41]. PKC-α phosphorylated the β subunit of F(1)F(0)-ATPase on a serine residue and decreased F(0)F(1)-ATPase activity, oxidative phosphorylation and ATP production [41].

PKCε has been shown to mediate a twofold enhancement of complex IV activity in cultured neonatal cardiac myocytes by the phosphorylation of an 18-kDa protein, the subunit IV of complex IV [42]. The authors suggested that the binding of PKCε to complex IV causes conformational changes in the complex and enhances its activity. In yeast cells, a PKC-dependent mechanism inhibiting complex III activity and reactive oxygen species (ROS) generation is thought to function via interference with the phosphatidylinositol signal [43].

In addition to calcium, several other second messengers and regulators of PKC subspecies are also known to regulate the OXPHOS system. These include sphingosine, ROS, species, nitric oxide (NO) and reactive nitrogen species (RNS) (see below). This makes this kinase family, in theory, a potential key regulator of OXPHOS system function. However, current direct evidence for such a role is very limited.

The calcium-calcineurin and calmodulin signal transduction pathways

Calcineurin is a calcium-activated serine/threonine phosphatase. It is comprised of a 59- to 63-kDa catalytic subunit (calcineurin A), a 19-kDa calcium-binding protein (calcineurin B), and the calcium-binding protein (calmodulin). The latter is associated with specific kinases, which are dependent on its activation.

Activated calcineurin dephosphorylates cytoplasmic nuclear factor of activated T cells (NFAT), leading to its translocation to the nucleus and to transcriptional induc-

tion of various genes, in concert with other signalling pathways (Fig. 1d). Kinases such as JNKs, casein kinase I and II (CKI and CKII), PKA and others phosphorylate specific NFAT family members inhibiting their activation and nuclear translocation. These kinases likely also participate in rephosphorylating NFAT within the nucleus.

The mitochondrial calcium-calcineurin signalling pathway has been associated with cardiac hypertrophy [for an extensive review see ref. 44]. Sayen et al. [45] hypothesised that the pathogenetic mechanism linking this signalling pathway and cardiac hypertrophy was through perturbation of mitochondrial function. This was based on several previous findings: (i) Electrical pacing of neonatal rat cardiac myocytes (which may be considered a 'first messenger' in this pathway) leads to 'physiological hypertrophy'. This is preceded by induction of the transcription factors AP-1 and NRF-1, which enhance the expression of genes encoding specific OXPHOS system proteins, as indicated above [46]. (ii) NFAT interacts with the transcription factors GATA4 and MEF2c, which have also been shown to regulate the expression of mitochondrial proteins [46]. (iii) All four NFAT transcription factors are expressed in the myocardium. Studying cardiac mitochondria of transgenic mice with cardiac-specific expression of an active calcineurin cDNA and measuring OXPHOS system activity by polarography, Sayen et al. [45] have shown decreased activity and protein content of the ND_3 subunit of complex I and subunit I of complex IV (both encoded by mitochondrial DNA), as well as subunit IV of complex IV oxidase (encoded by genomic DNA), and increased superoxide production. However, it has become apparent that the pathogenic cascade leading to cardiac hypertrophy involves a cross-talk mechanism between several signalling pathways as well as several transcription factors [reviewed in ref. 47]. One of the significant pathways in this pathogenic cascade is the MAPK pathway, which has been shown to both affect calcineurin-NFAT signalling as well as being affected by it. It should be noted that although the two pathways have been shown to play a pivotal role in the pathogenesis of cardiac hypertrophy, no experimental evidence has been presented thus far to suggest a regulatory role for MAPK on OXPHOS system activity.

Increased cytosolic Ca^{2+} concentrations and activation of Ca^{2+}/calmodulin-dependent protein kinase (CAMK) mediate the adaptive response to decreased intracellular ATP concentrations following contractile activity of muscle during endurance exercise. This response involves increased glucose transport and fatty acid oxidation, as sources of energy, along with an increase in OXPHOS system activity. This process is also mediated by activation of AMP-kinase [48]. As a result, several transcription factors are activated: myocyte-enhancing factor 2A (regulating GLUT4 expression), PPAR-α (up-regulating levels of enzymes of fatty acid β-oxidation), mitochondrial transcription factor A (mtTFA; activating the expression of the mitochondrial genome) and NRF 1 and 2 (up-regulating OXPHOS system complexes) (Fig. 2). The well-orchestrated expression of the multitude of proteins involved in these adaptations is probably mediated by the rapid activation of the peroxisome proliferator-activated receptor (PPAR)-γ co-activator (PGC)-1 [48; and reviewed in ref. 49].

Evidence for this mechanism has been provided by Wu et al. [50], who have shown that calcium/calmodulin-dependent protein kinase IV induces the expression of PGC-1 in transgenic mice that selectively express a constitutively active form of the kinase in skeletal muscle. PGC-1 binds to various transcription factors to maximize transcriptional activity, and leads to up-regulation of mitochondrial enzymes involved in fatty acid metabolism and electron transport, as well as mitochondrial DNA replication. Finally, calcium-mediated dephosphorylation turns off the mechanism of allosteric inhibition of complex IV activity by ATP at high ATP/ADP ratios, as mentioned above [24, 25]. The identity of the cytosolic phosphatase responsible for this reaction is not clear at present.

Ceramide-mediated signal transduction

Ceramides are the core structures of naturally occurring sphingolipids, and are produced de novo by the condensation of serine and palmitoyl-CoA or from hydrolysis of sphingomyelin. Ceramides of various chain length have long been shown to act as second messengers, mediating cellular response, particularly in the signal transduction pathways of cytokines [reviewed in ref. 51]. They have been shown to have different effects on different PKC subtypes and are generally thought to cause apoptosis [52, 53]. They lead to inhibition of the classical PKC subtypes as well as inhibition of NFκB activity [52], and to activation of the atypical PKC subtype ζ [53].

Given their role in apoptosis, several investigators have studied the effect of ceramides on mitochondrial function, the OXPHOS system and the production of ROS. Inasmuch as mitochondrial membrane potential may represent the overall function of the OXPHOS system, short-chain (C_2)-ceramide led to its dramatic decrease in rat heart mitochondria and in cerebellar granule cells [54, 55], most likely through targeting complex III [54]. C_2-ceramide enhanced whereas C_{16}-ceramide inhibited the activity of complex IV [54]. Garcia-Ruiz et al. [56] have shown that treatment of liver cells with tumour necrosis factor (TNF) resulted in an increase in mitochondrial C_2-ceramide concentration, interruption of the electron flow at the ubiquinone pool of complex III, and an increase in hydrogen peroxide production. The authors suggested that the observed dual response to ceramide concentrations could be explained by PKCζ activation. PKCζ is ac-

tivated by ceramide independently of DAG and has been shown to have a dual, or bifunctional, regulatory role in various cells. A further study suggested that this process could be mediated by the ceramide-dependent signalling pathway, which involves ceramide-activated protein kinase [57].

Thus, short-chain ceramide is hypothesized to mediate signalling pathways directly, leading to alteration of complex III activity, but its precise pathway is not clear. In addition, short-chain ceramide may affect the OXPHOS system activity indirectly, by increasing ROS production (see below).

ROS-mediated signal transduction

ROS are by-products of the MRC, generated primarily in complexes I and III but possibly also in complex II [58–60]. Under stress, ROS production is mediated by several factors and second messengers, including ceramide, as indicated above. ROS generation is augmented during hypoxia, a process that can be attenuated by site-specific inhibition of the mitochondrial electron transport [61]. ROS mediate apoptosis at high intracellular concentrations but recent studies indicate that at lower concentrations, ROS act as intracellular signalling agents. Although the signalling cascade(s) responsible for the mitochondrial response to non-toxic concentrations of ROS is not fully identified, studies focussing on the adaptation of cellular respiration to hypoxia/ischaemia and to reoxygenation have shed light on the possible steps constituting these cascades. There is a large body of literature regarding the effects of ROS on mitochondrial function. For reasons of brevity, the following discussion is restricted to the possible role ROS play in the regulation of the aforementioned signalling pathways in relation to the regulation of the OXPHOS system. ROS have been the focus of attention in the research on vascular endothelial damage and the following section is based largely on literature regarding this pathophysiological process.

H_2O_2 has been shown to activate PKC by tyrosine phosphorylation in a mechanism that seems to be independent of receptor-coupled generation of lipid second messengers [62]. Further studies indicated that phosphorylation of PKCδ at Tyr311 was critical for activation of the kinase by H_2O_2 [63]. In addition, H_2O_2 also activates phospholipase C [64], leading to PKC activation. Furthermore, Waypa et al. [59] have shown that a hypoxic insult to rat pulmonary arterial myocytes leads to the generation of ROS which, in turn, lead to increases in intracellular calcium concentration. Taken together, these findings suggest that H_2O_2 can activate PKC in both a second messenger-dependent and independent processes. In addition, it has also been suggested that activation of MAPK by H_2O_2 is through (at least in part) activation of PKC [65].

More specifically, PKCδ [66, 67] and PKCε [67] have been shown to translocate to the mitochondria following H_2O_2 stimulation in a variety of cells. This response was dose dependent in human U-937cells [66]. A possible role for PKCδ in regulating OXPHOS system activity is suggested by the observation that Rottlerin, which has been widely used as a specific PKCδ inhibitor, uncouples mitochondrial oxidative phosphorylation and decreases cellular ATP production [68]. It should be noted, however, that the specificity of Rottlerin as a PKCδ inhibitor may be in doubt [68]. Finally, Mayr et al. [69] have shown that ROS generated during ischaemic cardiac preconditioning led to profound changes in the activities of complexes I and V, with partial fragmentation of these mitochondrial enzymes and of the E_2 component of the pyruvate dehydrogenase complex. Using a PKCδ null mouse model, they have shown that this PKC subspecies mediates this response.

Recent findings suggest a role for ROS-induced tyrosine phosphorylation in the regulation of complex IV [reviewed in ref. 70]. ROS were found to activate tyrosine kinases of the Src family in mitochondrial preparations from osteoclasts [71], placenta and brain [discussed in ref. 72], leading to up-regulation of complex IV [71]. Potential tyrosine-phosphorylated proteins in several OXPHOS complexes (including the 39-kDa subunit of complex I) were identified [72]. The phosphorylation of these proteins is counteracted by their dephosphorylation by tyrosine phosphatases such as Shp-2 [73] and PTP 1B [72]. Important to note is that these studies were done in mitochondria-rich preparations and their physiological significance is not clear at this stage.

NO and RNS

NO has been the focus of numerous studies indicating that this second messenger is involved in maintaining homeostasis as well as mediating cytotoxic effects [reviewed in refs. 74, 75]. NO is synthesised both in the cytosol and mitochondria from arginine by a group of synthases. Constitutive NO synthase (cNOS) includes all calcium-dependent NOs species (mainly in endothelial and neuronal cells) and generates low concentrations of NO. Inducible NOS (iNOS) is calcium independent, is constantly activated, is expressed following pro-inflammatory signalling, and produces larger amounts of NO, for longer time periods. NO binds to iron-sulphur loci in proteins and forms stable nitrosyl complexes through its reaction with haem-containing proteins. One such haemprotein is soluble guanylyl cyclase (sGC), which is activated by NO leading to the conversion of guanosine trisphosphate (GTP) into cyclic guanosine monophosphate (cGMP). A downstream signalling pathway, including cGMP-dependent kinases and phosphodiesterases, leads, in turn, to degradation of cAMP [reviewed in ref. 74].

NO binds to the haem-copper complexes of cytochrome oxidase, leading to enhanced production of cGMP and the reversible inhibition of the MRC [reviewed in ref. 76, 77]. Brown and Cooper have shown that low, physiological concentrations of NO inhibit oxygen consumption in nerve synaptosomes from rat brain. NO reversibly inhibited complex IV activity by binding to the oxygen-binding site in competition with oxygen in mitochondria purified from beef heart, which could explain the higher apparent K_m for oxygen in NO-containing tissues [78]. An additional important observation was reported by Nisoli et al. [79], who have shown that NO generated by endothelial nitric oxide synthase (eNOS, one of the cNOS enzymes), plays a role in mitochondrial biogenesis by upregulating the production of PGC-1α in a cGMP-dependent manner, thus stimulating expression of NRF-1 and mTFA. As mentioned above, these transcription factors initiate the expression of nuclear and mitochondrial genes that encode mitochondrial proteins. Studying a range of cell lines, they have shown that overexpression of NO, cGMP or eNOS dramatically increased the numbers of mitochondria in these cells [79].

Thus, the direct effect of NO on the regulation of oxidative phosphorylation is bi-phasic, with low NO concentrations leading to a reversible inhibition of oxygen consumption and complex IV activity, but an increased number of mitochondria and thus increased capacity of the OXPHOS system complexes. The indirect effects of NO on the OXPHOS system are mediated via the production of RNS.

RNS are generated through the oxidation of NO or through its reaction with ROS [reviewed in refs. 74, 75], usually when cellular NO concentrations are high. The most widely studied RNS compound is peroxynitrite, a potent oxidative species produced by the reaction between NO and superoxide. Peroxynitrite has been shown to inhibit the activities of complex I, complex II and complex V [80–84].

RNS affect OXPHOS system complexes through several direct toxic biochemical reactions such as interaction with glutathione and thiol modifications, interaction with iron-sulphur sites, nitrosylation of secondary amines, nitration of tyrosine residues and interaction with lipids and phospholipids [reviewed in refs. 74, 77]. However, several lines of evidence suggest that s-nitrosylation could, at least in theory, alter OXPHOS system function through its effect on signal transduction. First, s-nitrosylation reactions modify G protein-related function, and signalling through tyrosine kinase, tyrosine phosphatase, PKC and adenylate cyclase [reviewed in ref. 74]. In addition, non-toxic levels of peroxynitrite have been shown to activate phospholipase C [85], thereby modulating PKC activity. Moreover, s-nitrosylation has been shown to regulate several transcription factors, including NFκB, AP-1 and CREB [reviewed in ref. 74]. Taken together, in addition to a direct toxic effect on the components of the OXPHOS system, RNS-induced s-nitrosylation could potentially alter OXPHOS system function through perturbation of signal transduction cascades. At present, however, direct experimental evidence for this hypothetical mechanism is not available.

Summary and future perspectives

The role of signal transduction mechanisms in the regulation of the OXPHOS system is slowly unfolding. Experimental evidence suggests that cAMP, calcium, ceramide, ROS, NO and RNS are mediating both intracellular and extracellular signals through activation of PKA, PKC and other kinases as well as calcineurin and other phosphatases. These kinases and phosphatases, in turn, lead to activation of transcription factors and to the regulation of the expression of both nuclear and mitochondrial genes that encode OXPHOS system proteins (Fig. 2). Alternatively, these kinases and phosphatases phosphorylate or dephosphorylate specific subunits of the OXPHOS system complexes, thereby activating or inhibiting them. Taken together, the diversity of the possibilities of regulatory mechanisms within the similarity of the signalling pathways is enormous (Table 1), and deserves further study. The data presented in this review are based, by and large, on studies designed to elucidate the role of mitochondrial function in processes leading to cell growth, differentiation and death (apoptosis). Thus, studies specifically designed to characterise the signalling pathways that modulate the OXPHOS system will enable a better understanding of the regulation of oxidative phosphorylation. These studies could focus on several aspects.

First, the possible regulation of each complex by signal transduction mechanisms has not been fully explored. Indeed, several mitochondrial phosphoproteins, in addition to those discussed above, have been found in bovine heart, using a fluorescent phosphosensor dye [18, 86] and tryptic mass mapping of radiolabelled bands from mitochondrial membranes [16]. These findings raise the possibility that signal transduction mechanisms regulate additional proteins, which are components of the OXPHOS system. The identification of the regulatory mechanisms leading to the phosphorylation of these proteins, and the elucidation of the biological significance of this phosphorylation await further study.

Most of the phosphoproteins that have been associated with regulation of the OXPHOS system are encoded by nuclear genes. Future studies could identify proteins that are mitochondrially encoded and regulated by signal transduction mechanisms. Moreover, Gopalakrishnan and Scarpulla [29] suggested that genes encoding proteins of the OXPHOS system are not uniformly regulated under all physiological conditions. They surmised that there

might be a difference between the regulation of nuclear and mitochondrial genes. For example, whilst proteins encoded and synthetized within the mitochondria are presumably phosphorylated by mitochondrial PKA, those that are encoded by the genomic DNA and synthetized in ribosomes may be phosphorylated by the cytosolic PKA. Of interest would be to examine whether this phosphorylation plays a role in the regulation of complex synthesis and assembly. Moreover, do other signalling cascades behave in the same manner? Are their effects on transcription factors regulating the expression of nuclear and mitochondrial genes similar or different?

The integration of signalling pathways in a 'cross talk' leading to the regulation of the OXPHOS system under quiescent conditions as well as following increased needs (e.g. muscle contraction) or under stress conditions is far from being elucidated. It is conceivable that different complex subunits are regulated by different signalling pathways and that there may be 'cross-talk' between these pathways, leading to additive, synergistic or inhibitory effects on complex activity (Fig. 2). What are the effectors of these signalling pathways? Understanding the integration of the effects of these pathways will enable a better understanding of the oxidative phosphorylation process in general and of the function of specific complexes.

Another avenue of research could be the elucidation of the tissue-specific roles of second messengers, kinases and transcription factors. Are the mechanisms described above similar in all tissues? What are the physiological consequences of these mechanisms with regards to the function of these tissues?

Finally, studying patient cell lines and tissues will enable a better understanding of the possible role of the signalling pathways in the pathogenesis of the phenotypes of defects in the OXPHOS system, as suggested for other inborn errors of metabolism [87]. More importantly, it will provide a better understanding of the normal physiological role of the signalling pathways in the regulation of oxidative phosphorylation.

Acknowledgement. This review is dedicated to the memory of Prof. Y. Nishizuka.

1 Cohen P. (2002) The origins of protein phosphorylation. Nat. Cell Biol. **4:** E127–E130
2 Cooper D. M., Mons N. and Karpen J. W. (1995) Adenylyl cyclases and the interaction between calcium and cAMP signalling. Nature **374:** 421–424
3 DiPilato L. M., Cheng X. and Zhang J. (2004) Fluorescent indicators of cAMP and Epac activation reveal differential dynamics of cAMP signaling within discrete subcellular compartments. Proc. Natl. Acad. Sci. USA **101:** 16513–1651815
4 Huang L. J., Wang L., Ma Y., Durick K., Perkins G., Deerinck T. J. et al. (1999) NH2-terminal targeting motifs direct dual specificity A-kinase-anchoring protein 1 (D-AKAP1) to either mitochondria or endoplasmic reticulum. J. Cell Biol. **145:** 951–959
5 Huang L. J., Durick K., Weiner J. A., Chun J. and Taylor S. S. (1997) Identification of a novel protein kinase A anchoring protein that binds both type I and type II regulatory subunits. J. Biol. Chem. **272:** 8057–8064
6 Wang L., Sunahara R. K., Krumins A., Perkins G., Crochiere M. L., Mackey M. et al. (2001) Cloning and mitochondrial localization of full-length D-AKAP2, a protein kinase A anchoring protein. Proc. Natl. Acad. Sci. USA **98:** 3220–3225
7 Horbinski C. and Chu C. T. (2005) Kinase signaling cascades in the mitochondrion: a matter of life or death. Free. Radic. Biol. Med. **38:** 2–11
8 Feliciello A., Gottesman M. E. and Avvedimento E. V. (2005) cAMP-PKA signaling to the mitochondria: protein scaffolds, mRNA and phosphatases. Cell Signal. **17:** 279–287
9 Sardanelli A. M., Technikova-Dobrova Z., Speranza F., Mazzocca A., Scacco S. and Papa S. (1996) Topology of the mitochondrial cAMP-dependent protein kinase and its substrates. FEBS Lett. **396:** 276–278
10 Papa S., Sardanelli A. M., Scacco S. and Technikova-Dobrova Z. (1999) cAMP-dependent protein kinase and phosphoproteins in mammalian mitochondria: an extension of the cAMP-mediated intracellular signal transduction. FEBS Lett. **444:** 245–249
11 Sardanelli A. M., Technikova-Dobrova Z., Scacco S. C., Speranza F. and Papa S. (1995) Characterization of proteins phosphorylated by the cAMP-dependent protein kinase of bovine heart mitochondria. FEBS Lett. **377:** 470–474
12 Papa S., Sardanelli A. M., Cocco T., Speranza F., Scacco S. C. and Technikova-Dobrova Z. (1996) The nuclear-encoded 18 kDa (IP) AQDQ subunit of bovine heart complex I is phosphorylated by the mitochondrial cAMP-dependent protein kinase. FEBS Lett. **379:** 299–301
13 Scacco S., Vergari R., Scarpulla R. C., Technikova-Dobrova Z., Sardanelli A., Lambo R. et al. (2000) cAMP-dependent phosphorylation of the nuclear encoded 18-kDa (IP) subunit of respiratory complex I and activation of the complex in serum-starved mouse fibroblast cultures. J. Biol. Chem. **275:** 17578–17582
14 Papa S., Scacco S., Sardanelli A. M., Vergari R., Papa F., Budde S. et al. (2001) Mutation in the NDUFS4 gene of complex I abolishes cAMP-dependent activation of the complex in a child with fatal neurological syndrome. FEBS Lett. **489:** 259–262
15 Scacco S., Petruzzella V., Budde S., Vergari R., Tamborra R., Panelli D. et al. (2003) Pathological mutations of the human NDUFS4 gene of the 18-kDa (AQDQ) subunit of complex I affect the expression of the protein and the assembly and function of the complex. J. Biol. Chem. **278:** 44161–44167
16 Chen R., Fearnley I. M., Peak-Chew S. Y. and Walker J. E. (2004) The phosphorylation of subunits of complex I from bovine heart mitochondria. J. Biol. Chem. **279:** 26036–26045
17 Potluri P., Yadava N. and Scheffler I. E. (2004) The role of the ESSS protein in the assembly of a functional and stable mammalian mitochondrial complex I (NADH-ubiquinone oxidoreductase). Eur. J. Biochem. **271:** 3265–3273
18 Schulenberg B., Aggeler R., Beechem J. M., Capaldi R. A. and Patton W. F. (2003) Analysis of steady-state protein phosphorylation in mitochondria using a novel fluorescent phosphosensor dye. J. Biol. Chem. **278:** 27251–27255
19 Raha S., Myint A. T., Johnstone L. and Robinson B. H. (2002) Control of oxygen free radical formation from mitochondrial complex I: roles for protein kinase A and pyruvate dehydrogenase kinase. Free Radic. Biol. Med. **32:** 421–430
20 Zhuchenko O., Wehnert M., Bailey J., Sun Z. S. and Lee C. C. (1996) Isolation, mapping and genomic structure of an X-linked gene for a subunit of human mitochondrial complex I. Genomics **37:** 281–288
21 Au H. C., Seo B. B., Matsuno-Yagi A., Yagi T. and Scheffler I. E. (1999) The NDUFA1 gene product (MWFE protein) is essential for activity of complex I in mammalian mitochondria. Proc. Natl. Acad. Sci. USA **96:** 4354–4359

22 Yadava N., Potluri P., Smith E. N., Bisevac A. and Scheffler I. E. (2002) Species-specific and mutant MWFE proteins: their effect on the assembly of a functional mammalian mitochondrial complex I. J. Biol. Chem. **277:** 21221–21230

23 Yadava N., Houchens T., Potluri P. and Scheffler I. E. (2004) Development and characterization of a conditional mitochondrial complex I assembly system. J. Biol. Chem. **279:** 12406–12413

24 Bender E. and Kadenbach B. (2000) The allosteric ATP-inhibition of cytochrome c oxidase activity is reversibly switched on by cAMP-dependent phosphorylation. FEBS Lett. **466:** 130–134

25 Lee I., Bender E. and Kadenbach B. (2002) Control of mitochondrial membrane potential and ROS formation by reversible phosphorylation of cytochrome c oxidase. Mol. Cell. Biochem. **234–235:** 63–70

26 Kadenbach B., Huttemann M., Arnold S., Lee I. and Bender E. (2000) Mitochondrial energy metabolism is regulated via nuclear-coded subunits of cytochrome c oxidase. Free. Radic. Biol. Med. **29:** 211–221

27 Ludwig B., Bender E., Arnold S., Huttemann M., Lee I. and Kadenbach B. (2001) Cytochrome C oxidase and the regulation of oxidative phosphorylation. ChemBioChem **2:** 392–403

28 Lee I., Salomon A. R., Ficarro S., Mathes I., Lottspeich F., Grossman L. I. et al. (2005) cAMP-dependent tyrosine phosphorylation of subunit I inhibits cytochrome c oxidase activity. J. Biol. Chem. **280:** 6094–6100

29 Gopalakrishnan L. and Scarpulla R. C. (1994) Differential regulation of respiratory chain subunits by a CREB-dependent signal transduction pathway: role of cyclic AMP in cytochrome c and COXIV gene expression. J. Biol. Chem. **269:** 105–113

30 Herzig R. P., Scacco S. and Scarpulla R. C. (2000) Sequential serum-dependent activation of CREB and NRF-1 leads to enhanced mitochondrial respiration through the induction of cytochrome c. J. Biol. Chem. **275:** 13134–13141

31 Campanella M., Pinton P. and Rizzuto R. (2004) Mitochondrial Ca^{2+} homeostasis in health and disease. Biol. Res. **37:** 653–660

32 Bianchi K., Rimessi A., Prandini A., Szabadkai G. and Rizzuto R. (2004) Calcium and mitochondria: mechanisms and functions of a troubled relationship. Biochim. Biophys. Acta **1742:** 119–131

33 Rizzuto R., Pinton P., Carrington W., Fay F. S., Fogarty K. E., Lifshitz L. M. et al. (1998) Close contacts with the endoplasmic reticulum as determinants of mitochondrial Ca^{2+} responses. Science **280:** 1763–1766

34 Jouaville L. S., Pinton P., Bastianutto C., Rutter G. A. and Rizzuto R. (1999) Regulation of mitochondrial ATP synthesis by calcium: evidence for a long-term metabolic priming. Proc. Natl. Acad. Sci. USA **96:** 13807–13812

35 Pinton P., Leo S., Wieckowski M. R., Di Benedetto G. and Rizzuto R. (2004) Long-term modulation of mitochondrial Ca^{2+} signals by protein kinase C isozymes. J. Cell Biol. **165:** 223–232

36 Nishizuka Y. (1992) Intracellular signaling by hydrolysis of phospholipids and activation of protein kinase C. Science **258:** 607–614

37 Nishizuka Y. (1995) Protein kinase C and lipid signaling for sustained cellular responses. FASEB J. **9:** 484–496

38 Mellor H. and Parker P. J. (1998) The extended protein kinase C superfamily. Biochem. J. **332:** 281–292

39 Schechtman D. and Mochly-Rosen D. (2001) Adaptor proteins in protein kinase C-mediated signal transduction. Oncogene **20:** 6339–6347

40 Nowak G. (2002) Protein kinase C-alpha and ERK1/2 mediate mitochondrial dysfunction, decreases in active Na^+ transport and cisplatin-induced apoptosis in renal cells. J. Biol. Chem. **277:** 43377–43388

41 Liu X., Godwin M. L. and Nowak G. (2004) Protein kinase C-alpha inhibits the repair of oxidative phosphorylation after S-(1,2-dichlorovinyl)-L-cysteine injury in renal cells. Am. J. Physiol. Renal. Physiol. **287:** F64–F73

42 Ogbi M., Chew C. S., Pohl J., Stuchlik O., Ogbi S. and Johnson J. A. (2004) 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:** 923–932

43 Machida K., Tanaka T., Fujita K. and Taniguchi M. (1998) Farnesol-induced generation of reactive oxygen species via indirect inhibition of the mitochondrial electron transport chain in the yeast *Saccharomyces cerevisiae*. J. Bacteriol. **180:** 4460–4465

44 Molkentin J. D. (2004) Calcineurin-NFAT signaling regulates the cardiac hypertrophic response in coordination with the MAPKs. Cardiovasc. Res. **63:** 467–475

45 Sayen M. R., Gustafsson A. B., Sussman M. A., Molkentin J. D. and Gottlieb R. A. (2003) Calcineurin transgenic mice have mitochondrial dysfunction and elevated superoxide production. Am. J. Physiol. Cell Physiol. **284:** C562–C570

46 Xia Y., McMillin J. B., Lewis A., Moore M., Zhu W. G., Williams R. S. et al. (2000) Electrical stimulation of neonatal cardiac myocytes activates the NFAT3 and GATA4 pathways and up-regulates the adenylosuccinate synthetase 1 gene. J. Biol. Chem. **275:** 1855–1863

47 Wilkins B. J. and Molkentin J. D. (2004) Calcium-calcineurin signaling in the regulation of cardiac hypertrophy. Biochem. Biophys. Res. Commun. **322:** 1178–1191

48 Ojuka E. O. (2004) Role of calcium and AMP kinase in the regulation of mitochondrial biogenesis and GLUT4 levels in muscle. Proc. Nutr. Soc. **63:** 275–278

49 Baar K. (2004) Involvement of PPAR gamma co-activator-1, nuclear respiratory factors 1 and 2 and PPAR alpha in the adaptive response to endurance exercise. Proc. Nutr. Soc. **63:** 269–273

50 Wu H., Kanatous S. B., Thurmond F. A., Gallardo T., Isotani E., Bassel-Duby R. et al. (2002) Regulation of mitochondrial biogenesis in skeletal muscle by CaMK. Science **296:** 349–352

51 Hannun Y. A. (1994) The sphingomyelin cycle and the second messenger function of ceramide. J. Biol. Chem. **269:** 3125–3128

52 Pyne S. and Pyne N. J. (2000) Sphingosine 1-phosphate signalling in mammalian cells. Biochem. J. **349:** 385–402

53 Wang Y. M., Seibenhener M. L., Vandenplas M. L. and Wooten M. W. (1999) Atypical PKC zeta is activated by ceramide, resulting in coactivation of NFkappaB/JNK kinase and cell survival. J. Neurosci. Res. **55:** 293–302

54 Di Paola M., Cocco T. and Lorusso M. (2000) Ceramide interaction with the respiratory chain of heart mitochondria. Biochemistry **39:** 6660–6668

55 Falluel-Morel A., Aubert N., Vaudry D., Basille M., Fontaine M., Fournier A. et al. (2004) Opposite regulation of the mitochondrial apoptotic pathway by C2-ceramide and PACAP through a MAP-kinase-dependent mechanism in cerebellar granule cells. J. Neurochem. **91:** 1231–1243

56 Garcia-Ruiz C., Colell A., Mari M., Morales A. and Fernandez-Checa J. C. (1997) Direct effect of ceramide on the mitochondrial electron transport chain leads to generation of reactive oxygen species: role of mitochondrial glutathione. J. Biol. Chem. **272:** 11369–11377

57 Corda S., Laplace C., Vicaut E. and Duranteau J. (2001) Rapid reactive oxygen species production by mitochondria in endothelial cells exposed to tumor necrosis factor-alpha is mediated by ceramide. Am. J. Respir. Cell. Mol. Biol. **24:** 762–768

58 Turrens J. F. and Boveris A. (1980) Generation of superoxide anion by the NADH dehydrogenase of bovine heart mitochondria. Biochem. J. **191:** 421–427

59 Waypa G. B., Marks J. D., Mack M. M., Boriboun C., Mungai P. T. and Schumacker P. T. (2002) Mitochondrial reactive oxygen species trigger calcium increases during hypoxia in pulmonary arterial myocytes. Circ. Res. **91:** 719–726

60 McLennan H. R. and Degli Esposti M. (2000) The contribution of mitochondrial respiratory complexes to the production of reactive oxygen species. J. Bioenerg. Biomembr. **32:** 153–162
61 Duranteau J., Chandel N. S., Kulisz A., Shao Z. and Schumacker P. T. (1998) Intracellular signaling by reactive oxygen species during hypoxia in cardiomyocytes. J. Biol. Chem. **273:** 11619–11624
62 Konishi H., Tanaka M., Takemura Y., Matsuzaki H., Ono Y., Kikkawa U. et al. (1997) Activation of protein kinase C by tyrosine phosphorylation in response to H_2O_2. Proc. Natl. Acad. Sci. USA **94:** 11233–11237
63 Konishi H., Yamauchi E., Taniguchi H., Yamamoto T., Matsuzaki H., Takemura Y. et al. (2001) Phosphorylation sites of protein kinase C delta in H_2O_2-treated cells and its activation by tyrosine kinase *in vitro*. Proc. Natl. Acad. Sci. USA **98:** 6587–6592
64 Wang X. T., McCullough K. D., Wang X. J., Carpenter G. and Holbrook N. J. (2001) Oxidative stress-induced phospholipase C-gamma 1 activation enhances cell survival. J. Biol. Chem. **276:** 28364–28371
65 Whisler R. L., Goyette M. A., Grants I. S. and Newhouse Y. G. (1995) Sublethal levels of oxidant stress stimulate multiple serine/threonine kinases and suppress protein phosphatases in Jurkat T cells. Arch. Biochem. Biophys. **319:** 23–35
66 Majumder P. K., Mishra N. C., Sun X., Bharti A., Kharbanda S., Saxena S. et al. (2001) Targeting of protein kinase C delta to mitochondria in the oxidative stress response. Cell Growth Differ. **12:** 465–470
67 Fryer R. M., Wang Y., Hsu A. K. and Gross G. J. (2001) Essential activation of PKC-delta in opioid-initiated cardioprotection. Am. J. Physiol. Heart Circ. Physiol. **280:** H1346–H1353
68 Soltoff S. P. (2001) Rottlerin is a mitochondrial uncoupler that decreases cellular ATP levels and indirectly blocks protein kinase Cdelta tyrosine phosphorylation. J. Biol. Chem. **276:** 37986–37992
69 Mayr M., Metzler B., Chung Y. L., McGregor E., Mayr U., Troy H. et al. (2004) Ischemic preconditioning exaggerates cardiac damage in PKC-delta null mice. Am. J. Physiol. Heart Circ. Physiol. **287:** H946–H956
70 Salvi M., Brunati A. M. and Toninello A. (2005) Tyrosine phosphorylation in mitochondria: a new frontier in mitochondrial signaling. Free Radic. Biol. Med. **38:** 1267–1277
71 Miyazaki T., Neff L., Tanaka S., Horne W. C. and Baron R. (2003) Regulation of cytochrome c oxidase activity by c-Src in osteoclasts. J. Cell Biol. **160:** 709–718
72 Augereau O., Claverol S., Boudes N., Basurko M. J., Bonneu M., Rossignol R. et al. (2005) Identification of tyrosine-phosphorylated proteins of the mitochondrial oxidative phosphorylation machinery. Cell. Mol. Life Sci. **62:** 1478–1488
73 Salvi M., Stringaro A., Brunati A. M., Agostinelli E., Arancia G., Clari G. et al. (2004) Tyrosine phosphatase activity in mitochondria: presence of Shp-2 phosphatase in mitochondria. Cell. Mol. Life Sci. **61:** 2393–2404
74 Liaudet L., Soriano F. G. and Szabo C. (2000) Biology of nitric oxide signaling. Crit. Care Med. **28:** N37–N52
75 Wolin M. S. (2000) Interactions of oxidants with vascular signaling systems. Arterioscler. Thromb. Vasc. Biol. **20:** 1430–1442
76 Brown G. C. (2003) Cell biology: NO says yes to mitochondria. Science **299:** 838–839
77 Brown G. C. and Borutaite V. (2004) Inhibition of mitochondrial respiratory complex I by nitric oxide, peroxynitrite and S-nitrosothiols. Biochim. Biophys. Acta **1658:** 44–49
78 Brown G. C. and Cooper C. E. (1994) Nanomolar concentrations of nitric oxide reversibly inhibit synaptosomal respiration by competing with oxygen at cytochrome oxidase. FEBS Lett. **356:** 295–298
79 Nisoli E., Clementi E., Paolucci C., Cozzi V., Tonello C., Sciorati C. et al. (2003) Mitochondrial biogenesis in mammals: the role of endogenous nitric oxide. Science **299:** 896–899
80 Clementi E., Brown G. C., Foxwell N. and Moncada S. (1999) On the mechanism by which vascular endothelial cells regulate their oxygen consumption. Proc. Natl. Acad. Sci. USA **96:** 1559–1562
81 Jekabsone A., Ivanoviene L., Brown G. C. and Borutaite V. (2003) Nitric oxide and calcium together inactivate mitochondrial complex I and induce cytochrome c release. J. Mol. Cell. Cardiol. **35:** 803–809
82 Cassina A. M., Hodara R., Souza J. M., Thomson L., Castro L., Ischiropoulos H. et al. (2000) Cytochrome c nitration by peroxynitrite. J. Biol. Chem. **275:** 21409–21415
83 Stadler J., Billiar T. R., Curran R. D., Stuehr D. J., Ochoa J. B. and Simmons R. L. (1991) Effect of exogenous and endogenous nitric oxide on mitochondrial respiration of rat hepatocytes. Am. J. Physiol. **260:** C910–C916
84 Brookes P. S., Bolanos J. P. and Heales S. J. (1999) The assumption that nitric oxide inhibits mitochondrial ATP synthesis is correct. FEBS Lett. **446:** 261–263
85 Nagasawa K., Nishida K., Nagai K., Shimohama S. and Fujimoto S. (2004) Differential expression profiles of PLC-beta1 and -delta1 in primary cultured rat cortical neurons treated with N-methyl-D-aspartate and peroxynitrite. Neurosci. Lett. **367:** 246–249
86 Schulenberg B., Goodman T. N., Aggeler R., Capaldi R. A. and Patton W. F. (2004) Characterization of dynamic and steady-state protein phosphorylation using a fluorescent phosphoprotein gel stain and mass spectrometry. Electrophoresis **25:** 2526–2532
87 Boneh A. (2002) A model for PKC involvement in the pathogenesis of inborn errors of metabolism. Trends Mol. Med. **8:** 524–531
88 Papa S. (2002) The NDUFS4 nuclear gene of complex I of mitochondria and the cAMP cascade. Biochim. Biophys. Acta **1555:** 147–153
89 Hongpaisan J., Winters C. A. and Andrews S. B. (2004) Strong calcium entry activates mitochondrial superoxide generation, upregulating kinase signaling in hippocampal neurons. J. Neurosci. **24:** 10878–10887
90 Morigi M., Macconi D., Zoja C., Donadelli R., Buelli S., Zanchi C. et al. (2002) Protein overload-induced NFkappaB activation in proximal tubular cells requires H(2)O(2) through a PKC-dependent pathway. J. Am. Soc. Nephrol. **13:** 1179–1189
91 Suzuki H., Kumagai T., Goto A. and Sugiura T. (1998) Increase in intracellular hydrogen peroxide and upregulation of a nuclear respiratory gene evoked by impairment of mitochondrial electron transfer in human cells. Biochem. Biophys. Res. Commun. **249:** 542–545