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Review

Evidence of undiscovered cell regulatory mechanisms: phosphoproteins and protein kinases in mitochondria

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Abstract. The finding that mitochondria contain substrates for protein kinases lead to the discovery that protein kinases are located in the mitochondria of certain tissues and species. These include pyruvate dyhydrogenase kinase, branched-chain α-ketoacid dehydrogenase kinase, protein kinase A, protein kinase Cδ, stress-activated kinase and A-Raf as well as unidentified kinases. Recent evidence suggests that mitochondrial protein kinases may be involved in physiological processes such as apoptosis and steroidogenesis. Additionally, the novel finding of low-molecular-weight GTP-binding proteins in mitochondria suggests the possibility that these may interact with mitochondrial protein kinases to regulate the activity of mitochondrial effector proteins. The fact that there are components of cellular regulatory systems in mitochondria indicates the exciting possibility of undiscovered systems regulating mitochondrial physiology.

Key words. Phosphoprotein; mitochondria; protein kinase; apoptosis; steroidogenesis; placenta; GTP-binding proteins.

Introduction

Protein kinases play a vital role in cellular signalling and can phosphorylate key substrates such as receptors, ion channels, cytoskeletal elements, nucleic acid regulatory factors and enzymes [1]. Once reversibly phosphorylated, kinase substrates can affect various regions of the cell and have profound effects on cellular functions such as cellular metabolism, growth and differentiation [2], apoptosis [1] and steroidogenesis [3, 4].

Protein kinases can be targeted to various parts of the cell by specific binding proteins and in this way the cell can ensure that protein phosphorylation occurs at precise cellular locations [5]. Studies conducted over three decades ago indicated that protein kinase substrates were present in mitochondria [6]. The finding of protein kinases in mitochondria suggested that protein-kinase-binding proteins were anchoring protein kinases to the organelle. Data on the types of mitochondrial protein kinase as well as their substrates and binding proteins in different tissues and species are starting to accumulate. There is still, however, a paucity of information on the physiological roles of some mitochondrial protein kinases.

Phosphorylation of mitochondrial proteins

Phosphorylated mitochondrial proteins were first reported in the late 1960s when Linn and colleagues [6] investigated the effects of phosphorylation on the activity of the pyruvate dehydrogenase complex. Evidence that mitochondrial proteins could be phosphorylated by kinases that resided in the mitochondria was obtained when investigators incubated isolated mitochondria with γ^{32}P-labelled ATP and detected phosphorylation of protein substrates [7]. When isolated rat liver mitochondria were incubated with γ^{32}P-ATP, phosphoproteins of 32 and 42 kDa were detected and identified as two different

forms of succinyl-CoA synthetase [7]. Additionally, a 35-kDa mitochondrial protein was shown to be phosphorylated in rat hepatocytes in response to glucagon; the identity of the mitochondrial protein kinase responsible for the phosphorylation of the 35-kDa protein, however, was not known [8]. These studies showed that mitochondria contained enzymes capable of catalysing protein phosphorylation.

Protein kinases associated with metabolism and respiration

Two of the better characterized mitochondrial protein kinases have been studied due to an interest in the metabolism of certain amino acids and carbohydrates. These are pyruvate deyhydrogenase (PD) kinase (PDK) and branched-chain α-ketoacid dehydrogenase (BCKD) kinase (BCKDK). The PD complex contains E1 components comprised of α and β subunits which catalyse the production of acetyl-CoA from pyruvate [9]. This oxidative decarboxylation reaction also results in the formation of NADH from NAD^+. The activity of the complex is inhibited by phosphorylation of E1 on three serine sites by up to four isoenzymes of PDK which are components of the PD complex [10]. Activity can be restored by PD phosphatase, a dimer consisting of catalytic and regulatory subunits [11].

The BCKD complex in mammalian mitochondria catalyses the oxidative decarboxylation of branched-chain α-ketoacids formed from leucine, isoleucine and valine [12]. The complex contains a thiamine pyrophosphate-dependent branched-chain α-ketoacid decarboxylase (E1) that contains α and β subunits of 45.5 and 37.5 kDa, respectively. Like the PD complex, BCKD activity is negatively controlled by phosphorylation, and BCKDK phosphorylates E1 on serine 293 [13]. This mechanism appears to regulate metabolism in response to physiological requirements. In rats starved of protein, the BCKDK protein content in the complex increases, levels of BCKD complex phosphorylation are higher and the activity of the complex decreases [14].

Interestingly, the amino acid sequences of the four PDK isoenzymes and BCKDK do not contain a common sequence found in other mammalian serine/threonine kinases, but have highly conserved C-terminal regions that share homology to prokaryotic histidine protein kinases [15, 16]. These findings led to the proposal that PDK and BCKDK have evolved from bacterial proteins [10]. If so, these mammalian mitochondrial protein kinases have evolved a distinct structural and functional characteristic. Prokaryotic histidine kinases use ATP to autophosphorylate histidine residues and the target histidine residue is thought to interact with the γ-phosphate on ATP [17]. This model is dissimilar to that used by PDK and BCKDK which phosphorylate serine residues on their target proteins. PDK can to catalyse the transfer of the γ-phosphate to the side chain of serine on its substrate E1 [18, 19]. Glutamate 243 of PDK appears essential in the mechanism, by activating the hydroxyl group of the target serine and thus priming it for interaction with the γ-phosphate [19, 20]. The unique characteristics of PDK and BCKDK have led to them being described as belonging to a novel mitochondrial protein kinase family [12] and investigations for their inclusion in the ATPase/kinase superfamily are ongoing [21].

Protein kinase A activity in mitochondria

Early studies identified cAMP-dependent kinase activity in purified preparations of mitochondria from guinea pig and rat liver suggesting the presence of mitochondrial protein kinase A (PKA) [22]. PKA consists of two regulatory subunits (R subunits) which bind cAMP and two catalytic subunits (C subunits) [2, 23]. The complete holoenzyme is not active, although when cAMP binds to the R subunits, the C subunits are able to disassociate as free and active units [24] and can migrate to different areas of the cell [25, 26].

From the efforts of a number of laboratories, we now know that in a range of species and tissues, PKA is targeted to the mitochondria. For example, protein kinases dependent on cAMP have also been detected in mitochondria from porcine ovaries [27], bovine heart [28], yeast [29], human sperm [30], a crustacean [31], human colon carcinoma [32] and human placenta [33]. The presence of mitochondrial PKA suggests the possibility of a PKA-mediated signalling system that is operating inside the organelle. A logical step in investigating the possibility of such a signalling system is to look for the substrate of a mitochondrial PKA. Some substrates of mitochondrial PKAs have already been characterized. In bovine heart mitochondria, a mitochondrial PKA is responsible for the phosphorylation of an 18-kDa protein [34]. Furthermore, mitochondrial PKA in yeast has been shown to phosphorylate and retard in vitro the ability of mitochondrial telomere-binding protein to bind a telomeric oligonucleotide [35]. These important findings herald the coming of many more reports on novel mitochondrial physiological mechanisms. The challenge for the future is to determine how mitochondrial PKAs are stimulated and what cellular processes they modulate.

PKA anchoring proteins

In the whole cell, the discovery of a family of proteins that bind PKA and target it to various regions of the cell led to an understanding of how PKA could have such var-

ied effects on cellular physiology. A kinase-anchoring proteins (AKAPs) use amphipathic helix regions to bind to specific polypeptide regions in the dimerisation domain of the R subunits of PKA and localise PKA to various regions of the cell [36]. By targeting PKA to specific cellular regions, AKAPs can regulate the specificity of a cellular signal in many signal transduction systems [5, 26, 36]. PKA RIα and RIβ are usually found in the cytoplasm, whereas RIIα and RIIβ are usually associated with organelles. AKAPs can display specificity for RI or RII subunits or can have a dual specificity (D-AKAP). The two D-AKAPs (I and II) known to date display in vitro affinity for RIα, RIIα as well as RIIβ, but not RIβ [37].

After the finding of PKA activity in mitochondria from human placenta, it was proposed that these mitochondria use an AKAP to localize the protein kinase to the organelle [33]. In rat and mouse brown adipose tissue analysed by Western blot, D-AKAP immunoreactivity has been identified in mitochondrial fractions as well as cytosolic and cell debris fractions [38]. There are two N-terminal splice variants of D-AKAP2, N0 and N1. In mouse fibroblasts, the N0 spliced variant is localised in mitochondria whereas N1 is localised in the endoplasmic reticulum. Residues 1–30 at the N-terminal end of the protein are vital for targeting to the mitochondria [37]. Several groups are also at the stage where they are able to identify the subtype of mitochondrial AKAPs. S-AKAP84 [39, 40], D-AKAP1 [37] and AKAP149 [41] have also been localised to mitochondria and are splice variants of a single gene [26]. These mitochondrial AKAPs have been proposed to link PKA to the outer mitochondrial membrane in a way that allows phosphorylation of mitochondrial proteins including ATP synthase [26].

D-AKAP2 has a putative regulator-of-G-protein-signalling sequence, although to date, no interaction between G proteins and D-AKAP2 has been demonstrated [38]. Therefore, whether the mitochondrial D-AKAP2 interacts with a mitochondrial G protein is still unknown. Interestingly, there is evidence to suggest that heterotrimeric G proteins are present in mitochondria. Whether these are similar or identical to G-proteins found in other parts of the cell is not yet established. $G_{\alpha i}$ and $G_{\alpha s}$ immunoreactivity is present in isolated mitochondria from human placenta [42]. The purification of these mitochondrial G proteins is an important task for the future and will allow studies on their interaction with signal transduction molecules in the mitochondria such as D-AKAP2.

cAMP independent protein kinases

As well as finding cAMP-dependent mitochondrial protein phosphoprotein, Neymark and colleagues [43] also reported the cAMP-independent phosphorylation of a 55-kDa protein. This finding suggested the presence of an additional mitochondrial kinase other than PKA. Novel mitochondrial protein kinases other than PKA were also discovered in a species of yeast which has four protein kinases, only one of which is cAMP dependent [44]. In beef heart mitochondria, several studies have demonstrated the presence of mitochondrial PKA which phosphorylates mitochondrial proteins of 42, 29, 18 and 6 kDa [34, 45]. Nonetheless, in bovine heart mitochondria, there are also 44-, 39- and 31-kDa, proteins phosphorylated by a cAMP-independent mitochondrial protein kinase [46]. In isolated mitochondria from human placenta the phosphorylation of a 20-kDa protein is inhibited by herbimycin, suggesting that these mitochondria contain a tyrosine kinase [33].

The increase in commercially available antibodies to many of the known protein kinases has allowed the identification of several novel species of protein kinase found in mitochondria. Indeed, the list of protein kinases other than PDK, BCKDK and PKA in mitochondria has expanded in the last 3 years (see table 1) to include A-Raf [47], protein kinase Cδ (PKCδ) [48] and stress-activated protein kinases (SAPKs), also known as c-Jun amino-terminal kinases (JNKs) [49]. Investigators will soon be using antibodies to known protein kinases to determine the identity of various cAMP-independent kinases that are known to interact with substrate mitochondrial proteins in a range of species.

Table 1. Mammalian mitochondrial protein kinases.

Protein kinase	Function
A-Raf	may be involved in apoptotic mechanisms [47]
BCKDK	well-characterized control of decarboxylation of α-ketoacids formed from leucine, isoleucine and valine [14]
PDK	well-characterized control of acetyl-CoA formation [10]
PKA	Appears to phosphorylate 6-, 18-, 29-, 42-kDa mitochondrial proteins in bovine heart [34, 45]; may phosphorylate BAD and down regulate apoptosis [58]; appears capable of phosphorylating the StAR protein in steroidogenic tissue
PKCδ	may be involved in regulation of membrane potential [48]
SAPK	may phosphorylate Bcl-xL and down-regulate the inhibitory role of Bcl-xL on apoptosis [49]
Unidentified cAMP-independent protein kinase	appears to phosphorylate 31-, 39- and 44-kDa mitochondrial proteins in bovine heart [46]
Unidentified tyrosine kinase	may phosphorylate a 20-kDa mitochondrial protein in human placenta [33]

The role of mitochondrial kinases in apoptosis

Mitochondria play an important role in apoptosis or pre-programmed cell death. What is not clear, however, is the extent to which mitochondrial protein kinases are used by the organelle in the modulation of apoptotic mechanisms. The process of apoptosis relies on aspartate-specific cysteine proteases (caspases) which are synthesised constituatively as inactive pro-enzymes. Once activated by the removal of the N-terminal sequence, caspases can activate more downstream caspases in a cascade sequence [50]. Downstream caspases are responsible for a number of attacks on cellular proteins that bring about the death of the cell. Mitochondria accelerate the caspase-mediated destruction by releasing cytochrome c [51, 52] which recruits cytosolic apoptotic protein adaptor Apaf-1 [53]. Once activated by cytochrome c, Apaf-1 is able to complex with pro-caspases leading to their activation [52].

Bcl-2 and homologues that make up the Bcl-2-like family of proteins have important regulatory effects on apoptosis [54]. Recent evidence indicates that mitochondrial protein kinases are involved in the regulatory process exerted on apoptosis by Bcl-2-like family members. Bcl-2 itself has a protective effect against apoptosis in cells made susceptible by stimuli including radiation [55], tumour necrosis factor [56] and Epstein-Barr virus infection [57].

The Bcl-2-related, but pro-apoptotic protein BAD is associated with the outer mitochondrial membrane. Phosphorylation of BAD on serine 112 can be inhibited by a peptide that inhibits AKAP binding to the R subunit of PKA [58]. This indicates that BAD is phosphorylated by a mitochondrial AKAP/PKA complex and a cellular survival system has been suggested that could use this phosphorylation mechanism to inactivate BAD [58].

The protein kinase A-Raf has been located in mitochondria and proposed to phosphorylate proteins which mediate the apoptotic pathway [47]. Additionally, stimuli such as damage to DNA or heat shock can activate an SAPK, JNK1 [59], and p38 MAPK [60]. The human monocyte-like cell line U937 responds to ionizing γ-radiation by targeting SAPK to the mitochondria where it phosphorylates a Bcl-2-like protein, Bcl-x_L on threonine residues 47 and 115 [49]. A mutant Bcl-x_L in which these two SAPK-sensitive threonine residues have been replaced with alanine residues, Bcl-x_L (A-47, -115) is a more potent inhibitor of ionising-radiation-induced apoptosis than native Bcl-x_L. As a consequence, Kharbanda et al. [49] proposed that phosphorylation of Bcl-x_L by mitochondrial SAPK modulates apoptosis by down-regulating the inhibitory effect of Bcl-x_L on apoptosis (see fig. 1). Thus, this mitochondrial protein kinase apparently plays an important part in the apoptotic mechanism. In the future, experiments that fractionate mitochondrial membranes into inner membrane, outer membrane and contact point vesicles [61, 62] could be used to determine the mitochondrial location of SAPK. Also important will be the characterisation of the cellular mechanism that targets SAPK to the mitochondria.

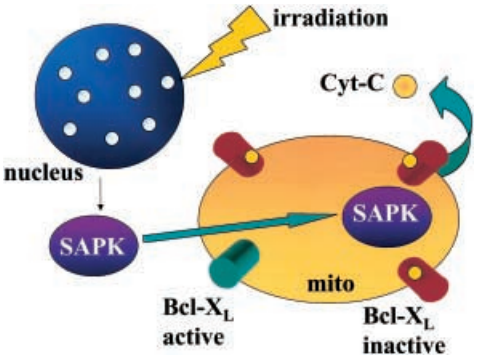

Figure 1. Proposed model of the role of mitochondrial SAPK in apoptosis. Radiation causes damage to nuclear material which triggers an unknown mechanism resulting in translocation of SAPK to the mitochondria. SAPK phosphorylates Bcl-x_L, attenuating its ability to prevent release of caspase-activating molecules such as cytochrome c.

Possible roles of mitochondrial kinases in steroidogenesis

The first and rate-limiting step of steroidogenesis involves the transport of cholesterol from the outer mitochondrial membrane to the inner membrane where it is converted to pregnenolone by cytochrome P450 side chain cleavage (P450scc) [3, 63]. In adrenal and gonadal tissue, this step is stimulated by the steroidogenic acute regulatory (StAR) protein, the expression of which is increased by hormonal stimulation of steroidogenic tissue. StAR was discovered by Orme-Johnson and colleagues who showed the appearance of a set of proteins in the mitochondria of adrenal cells [64, 65] and corpus luteum cells [66] of the rat. Two-dimensional electrophoresis demonstrated that these proteins resided in the 30–32 kDa molecular-weight range and were phosphorylated after the tissue was stimulated with a hormone. Clark and colleagues purified and named the StAR protein in 1994 [4, 67].

Very little is known about the mechanism by which the StAR protein stimulates cholesterol flux to the inner membrane. Most of the authors who have speculated on the mechanism of StAR agree, however, that StAR likely facilitates the flow of cholesterol through points of contact between the inner and outer membrane [3, 4, 68, 69]. These contact points contain the voltage-dependent anion channel, the ATP translocator [70], small GTP-binding proteins [3, 61, 71] and the peripheral benzodiazepine receptor (PBr) [68]. These proteins may form a bridge that

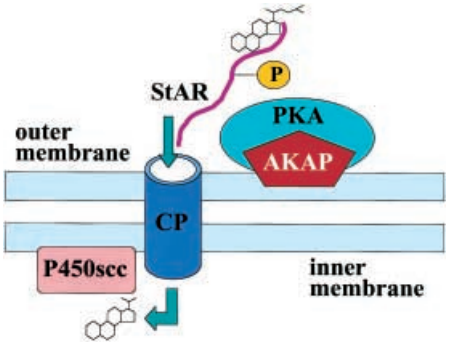

Figure 2. Proposed model of steroidogenic modulation by mitochondrial PKA. As a result of hormonal stimulation at the cell surface, StAR is synthesised and moves towards the mitochondria. PKA anchored in the mitochondria by AKAP phosphorylates StAR, increasing its ability to increase cholesterol passage through the contact point to reach P450 scc.

links the outer and inner membrane and may form the contact point. Furthermore, PBr has been proposed to form a tunnel for cholesterol [68]. The discovery that the phosphorylation status of StAR affects its bioactivity raises the possibility that mitochondrial kinases regulate steroidogenesis (see fig. 2).

The StAR protein can be phosphorylated by PKA at serines 57 and 195 [72]. A mutation which prevents phosphorylation of serine 195 results in an attenuation of StAR activity suggesting that phosphorylation of this residue is part of a cellular signalling system which can modulate steroidogenesis. PKA is present in the mitochondria of adrenal tissue [73], so future experiments must determine whether PKA is held in the mitochondria of steroidogenic tissue by an AKAP. Additionally, future experiments should determine whether mitochondrial PKA can phosphorylate StAR once it has reached the mitochondria. Also interesting will be to determine the extent to which this mechanism can regulate steroidogenesis.

The placenta does not contain StAR and does not have an as acute steroidogenic response to hormonal stimulation as the adrenal and gonads [3]. There is, however, evidence to suggest that phosphorylation of mitochondrial proteins may regulate the development of steroidogenic placental cells. In the developing placenta which invades the uterus, only the syncytiotrophoblast develops cellular machinery to perform steroidogenesis [74]. Interestingly, the cytotrophoblast mitochondria contain significantly higher levels of a 16- and 20-kDa tyrosine-phosphoprotein which has been proposed to indicate a signal determining the fate of the trophoblast [33]. As mentioned, placental mitochondria contain a tyrosine kinase [33] and it will be important to determine whether this kinase regulates the development of steroidogenic and non-steroidogenic cells in the placenta.

An evolutionary perspective

Mitochondria are believed to have evolved 1.5 billion years ago from oxidative bacteria taking up residence in glycolytic proto-eukaryotic cells, and genetic information from the genome of the bacterial ancestors of modern mitochondria is thought to have been lost to the nucleus of the cells that engulfed them [31]. Control of the mitochondrial protein import including products of these ex-mitochondrial genes is tightly controlled by the outer and inner membranes which contain translocases [75] and channels [70]. Therefore, while the integration of the mitochondrion into the eukaryotic cell has reached an advanced level due to its evolutionary history, the mitochondrion retains some level of autonomy and control over its discrete physiology. For the mitochondrion to be symbiotically receptive to the metabolic needs of the host cell, however, one would expect mechanisms which allow messages to be transmitted from the cytoplasm to the mitochondrion. Because the mitochondrion can contain kinase activity and protein substrates, messages may be conveyed to the mitochondrion via protein kinase/phosphorylation systems [76]. To allow such communication, the mitochondrion utilises protein kinases that may have distinct evolutionary histories. These mitochondrial protein kinases may have evolved from primitive bacterial proteins and more recent proto-eukaryotic cell proteins.

‘Orphan’ cellular molecules, novel regulators of cell physiology?

A number of low-molecular-weight GTP-binding proteins have recently been located in mitochondria [3, 61, 62, 71], one of which has been identified as the 28-kDa protein Ran [42]. These mitochondrial GTP-binding proteins appear to be concentrated in the outer mitochondrial membrane and the contact sites [42, 62]. The flow of cholesterol through the contact point into the steroidogenic pathway may be regulated by these GTP-binding proteins [3, 61]. The challenge for the future is to determine how the mitochondrial GTP-binding proteins are regulated. In other parts of the cell, low-molecular-weight GTP-binding proteins interact with protein kinases as part of cellular signalling systems and in cellular functions [77]. Future studies examining whether mitochondrial, GTP-binding proteins and protein kinases can interact would be desirable.

Furthermore, many protein kinases in mitochondria from numerous tissues and species have not been characterised, and undoubtedly, a large number of mitochondrial protein kinases have yet to be discovered. Very little is known about the physiological functions of these mitochondrial protein kinases, and there are many ‘orphan’ kinases whose function has still to be discovered. As the

mitochondria have vital functions in an array of physiological processes including steroidogenesis and apoptosis, much work still needs to be done and many benefits gained from elucidating the functions of mitochondrial protein kinases.

1 Graves J. D. and Krebs E. G. (1999) Protein phosphorylation and signal transduction. Clin. Pharmacol. Ther. **82:** 111–121
2 Greengard P. (1976) Phosphorylated proteins as physiological effectors. Science **199:** 146–152
3 Thomson M. (1998) Molecular and cellular mechanisms used in the acute phase of stimulated steroidogenesis. Horm. Metab. Res. **30:** 16–28
4 Stocco D. M. (2001) StAR protein and the regulation of steroid hormone biosynthesis. Annu. Rev. Physiol. **63:** 193–213
5 Pawson T. and Scott J. D. (1997) Signalling through scaffold, anchoring, and adaptor proteins. Science **278:** 2075–2080
6 Linn T. C., Pettit F. H. and Reed L. J. (1969) Alpha-keto acid dehydrogenase complexes. X. Regulation of the activity of the pyruvate dehydrogenase complex from beef kidney mitochondria by phosphorylation and dephosphorylation. Proc. Natl. Acad. Sci. USA **62:** 234–241
7 Steiner A. W. and Smith R. A. (1981) Endogenous protein phosphorylation in rat brain mitochondria: occurrence of a novel ATP-dependent form of the autophosphorylated enzyme succinyl-CoA synthetase. J. Neurochem. **37:** 582–593
8 Vargas A. M., Halestrap A. P. and Denton R. M. (1982) The effects of glucagon, phenylephrine and insulin on the phosphorylation of cytoplasmic, mitochondrial and membrane-bound proteins of intact liver cells from starved rats. Biochem. J. **208:** 221–229
9 Heigenhauser G. J. and Parolin M. L. (1999) Role of pyruvate dehydrogenase in lactate production in exercising human skeletal muscle. Adv. Exp. Med. Biol. **474:** 205–218
10 Harris R. A., Huang B. and Wu P. (2001) Control of pyruvate dehydrogenase kinase gene expression. Adv. Enzyme Regul. **41:** 269–288
11 Huang B., Gudi R., Wu P., Harris R. A., Hamilton J. and Popov K. M. (1998) Isoenzymes of pyruvate dehydrogenase phosphatase: DNA-derived amino acid sequences, expression, and regulation. J. Biol. Chem. **273:** 17680–17688
12 Harris R. A., Popov K. M., Zhao Y., Kedishvili N. Y., Shimomura Y. and Crabb D. W. (1995) A new family of protein kinases – the mitochondrial protein kinases. Adv. Enzyme Regul. **35:** 14–162
13 Harris R. A., Hawes J. W., Popov K. M., Zhao Y., Shimomura Y., Sato J. et al. (1997) Studies on the regulation of the mitochondrial alpha-ketoacid dehydrogenase complexes and their kinases. Adv. Enzyme Regul. **37:** 271–279
14 Harris R. A., Paxton R. and Jenkins P. (1985) Nutritional control of branched chain alpha-ketoacid dehydrogenase in rat hepatocytes. Fed. Proc. **44:** 2463–2468
15 Popov K. M., Kedishvili N. Y., Zhao Y., Shimomura Y., Crabb D. W. and Harris R. A. (1993) Primary structure of pyruvate dehydrogenase kinase establishes a new family of eukaryotic protein kinases. J. Biol. Chem. **268:** 26602–22606
16 Popov K. M., Zhao Y., Shimomura Y., Kuntz M. J. and Harris R. A. (1992) Branched-chain alpha-ketoacid dehydrogenase kinase: molecular cloning, expression, and sequence similarity with histidine protein kinases. J. Biol. Chem. **267:** 13127–13130
17 Tanaka T., Saha S. K., Tomomori C., Ishima R., Liu D., Tong K. I. et al. (1998) NMR structure of the histidine kinase domain of the *E. coli* osmosensor EnvZ. Nature **396:** 88–92
18 Thelen J. J., Miernyk J. A. and Randall D. D. (2000) Pyruvate dehydrogenase kinase from *Arabidopsis thaliana*: a protein histidine kinase that phosphorylates serine residues. Biochem. J. **349:** 195–201
19 Tuganova A., Yoder M. D. and Popov K. M. (2001) An essential role of Glu-243 and His-239 in the phosphotransfer reaction catalyzed by pyruvate dehydrogenase kinase. J. Biol. Chem. **276:** 17994–17999
20 Bowker-Kinley and M. Popov K. M. (1999) Evidence that pyruvate dehydrogenase kinase belongs to the ATPase/kinase superfamily. Biochem. J. **344:** 47–53
21 Wynn R. M., Chuang J. L., Cote C. D. and Chuang D. T. (2000) Tetrameric assembly and conservation in the ATP-binding domain of rat branched-chain alpha-ketoacid dehydrogenase kinase. J. Biol. Chem. **275:** 30512–30519
22 Kleitke B., Sydow H. and Wollenberger A. (1976) Evidence for cyclic AMP-dependent protein kinase activity in isolated guinea pig and rat liver mitochondria. Acta Biol. Med. Ger. **35:** K9–K17
23 Beebe S. J. (1994) The cAMP-dependent protein kinases and cAMP signal transduction. Semin. Cancer Biol. **5:** 285–294
24 Brostrom M. A., Reimann E. M., Walsh D. A. and Krebs E. G. (1970) A cyclic 3′, 5′-AMP-stimulated protein kinase from cardiac muscle. Adv. Enzyme Regul. **8:** 191–203
25 Meinkoth J. L., Ji Y., Taylor S. S. and Feramisco J. R. (1990) Dynamics of the distribution of cyclic AMP-dependent protein kinase in living cells. Proc. Natl. Acad. Sci. USA **87:** 9595–9599
26 Feliciello A., Gottesman M. E. and Avvedimento E. V. (2001) The biological functions of A-kinase anchor proteins. J. Mol. Biol. **308:** 99–114
27 Dimino M. J., Bieszczad R. R. and Rowe M. J. (1981) Cyclic AMP-dependent protein kinase in mitochondria and cytosol from different-sized follicles and corpora lutea of porcine ovaries. J. Biol. Chem. **256:** 10876–10882
28 Burgess J. W. and Yamada E. W. (1987) cAMP-dependent protein kinase isozymes with preference for histone H2B as substrate in mitochondria of bovine heart. Biochem. Cell Biol. **65:** 137–143
29 Muller G. and Bandlow W. (1987) cAMP-dependent protein kinase activity in yeast mitochondria. Z. Naturforsch. C**42:** 1291–1302
30 Pariset C. and Weinman S. (1994) 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:** 415–422
31 Vellai T. and Vida G. (1999) The origin of eukaryotes: the difference between prokaryotic and eukaryotic cells. Proc. R. Soc. Lond. B. **266:** 1571–1577
32 Kondrashin A. A., Nesterova M. V. and Cho-Chung Y. S. (1998) Subcellular distribution of the R-subunits of cAMP-dependent protein kinase in LS-174T human colon carcinoma. Biochem. Mol. Biol. Int. **45:** 237–244
33 Corso M. and Thomson M. (2001) Protein phosphorylation in mitochondria from human placenta. Placenta **22:** 432–439
34 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
35 Tomaska L. (1998) Phosphorylation of mitochondrial telomere binding protein of *Candida parapsilosis* by cAMP-dependent protein kinase. Biochem. Biophys. Res. Commun. **242:** 457–460
36 Colledge M. and Scott J. D. (1999) AKAPs: from structure to function. Trends Cell Biol. **9:** 216–221
37 Huang L. J., Durick K., Weiner J. A., Chun J. and Taylor S. S. (1997) D-AKAP2, a novel protein kinase A anchoring protein with a putative RGS domain. Proc. Natl. Acad. Sci. USA **94:** 11184–11189
38 Wang L., Sunahara R. K., Krumins A., Perkins G., Crochiere M. L., Mackey M. et al. (2000) Cloning and mitochondrial localization of full-length D-AKAP2, a protein kinase A anchoring protein. Proc. Natl. Acad. Sci. USA **98:** 3220–3225

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

40 Lin R. Y., Moss S. B. and Rubin C. S. (1995) Characterization of S-AKAP84, a novel developmentally regulated A kinase anchor protein of male germ cells. J. Biol. Chem. **270:** 27804–27811

41 Trendelenburg G., Hummel M., Riecken E. O. and Hanski C. (1996) Molecular characterization of AKAP149, a novel A kinase anchor protein with a KH domain. Biochem. Biophys. Res. Commun. **225:** 313–319

42 Kuyznierewicz I. and Thomson M. (2000) GTP-binding proteins in mitochondria of human placenta identified as G alpha i and Ran. In: Molecular Steroidogenesis, pp. 287–288, Okamoto M., Ishimura Y. and Nawata H. (eds), Academy Press, Tokyo

43 Neymark M. A., Bieszczad R. R. and Dimino M. J. (1984) Phosphorylation of mitochondrial proteins in isolated porcine ovarian follicles after treatment with luteinizing hormone. FEBS Lett. **157:** 124–128

44 Muller G. and Bandlow W. (1987) Protein phosphorylation in yeast mitochondria: cAMP-dependence, submitochondrial localization and substrates of mitochondrial protein kinases. Yeast **3:** 161–174

45 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

46 Technikova-Dobrova Z., Sardanelli A. M. and Papa S. (1993) Phosphorylation of mitochondrial proteins in bovine heart: characterization of kinases and substrates. FEBS Lett. **322:** 51–55

47 Yuryev A., Ono M., Goff S. A., Macaluso F. and Wennogle L. P. (2000) Isoform-specific localization of A-RAF in mitochondria. Mol. Cell. Biol. **20:** 4870–4878

48 Li L., Lorenzo P. S., Bogi K., Blumberg P. M. and Yuspa S. H. (1999) Protein kinase Cδ targets mitochondria, alters mitochondrial membrane potential, and induces apoptosis in normal and neoplastic keratinocytes when overexpressed by an adenoviral vector. Mol. Cell. Biol. **19:** 8547–8558

49 Kharbanda S., Saxena S., Yoshida K., Pandey P., Kaneki M., Wang Q. H. et al. (2000) Translocation of SAPK/JNK to mitochondria and interaction with Bcl-x(L) in response to DNA damage. J. Biol. Chem. **275:** 19433–19433

50 Robertson J. D. and Orrenius S. (2000) Molecular mechanisms of apoptosis induced by cytotoxic chemicals. Crit. Rev. Toxicol. **30:** 609–627

51 Liu X., Kim C. N., Yang J., Jemmerson R. and Wang X. (1996) Induction of the apoptotic program in cell-free extracts: requirement for dATP and cytochrome c. Cell **86:** 147–157

52 Zou H., Henzel W. J., Liu X., Lutschg A. and Wang X. (1997) Apaf-1, a human protein homologous to *C. elegans* CED-4, participates in cytochrome c-dependent activation of caspase-3. Cell **90:** 405–413

53 Denecker G., Vercammen D., Declercq W. and Vandenabeele P. (2001) Apoptotic and necrotic cell death induced by death domain receptors. Cell. Mol. Life Sci. **58:** 356–370

54 Adams J. M. and Cory S. (1998) The Bcl-2 protein family: arbiters of cell survival. Science **281:** 1322–1326

55 Sentman C. L., Shutter J. R., Hockenbery D., Kanagawa O. and Korsmeyer S. J. (1991) Bcl-2 inhibits multiple forms of apoptosis but not negative selection in thymocytes. Cell **67:** 879–888

56 Sun S. Y., Yue P., Zhou J. Y., Wang Y. H., Kim H. R. C., Lotan R. et al. (2001) Overexpression of Bc12 blocks TNF-related apoptosis-inducing ligand (TRAIL)-induced apoptosis in human lung cancer cells. Biochem. Biophys. Res. Commun. **280:** 788–797

57 Henderson S., Rowe M., Gregory C., Croomcarter D., Wang, F., Longnecker R. et al. (1991) Induction of bcl-2 expression by Epstein-Barr-virus latent membrane protein-1 protects infected B-cells from programmed cell-death. Cell **65:** 1107–1115

58 Harada H., Becknell B., Wilm M., Mann M., Huang L. J., Taylor S. S. et al. (1999) Phosphorylation and inactivation of BAD by mitochondria-anchored protein kinase A. Mol. Cell **3:** 413–422

59 Derijard B., Hibi M., Wu I. H., Barrett T., Su B., Deng T. et al. (1994) JNK1: a protein kinase stimulated by UV light and Ha-Ras that binds and phosphorylates the c-Jun activation domain. Cell **76:** 1025–1037

60 Shimizu T., Kato,T. J., Tachibana A. and Sasaki M. S. (1999) Coordinated regulation of radioadaptive response by protein kinase C and p38 mitogen-activated protein kinase. Exp. Cell Res. **251:** 424–432

61 Thomson M., Korn M. and Hall P. F. (1995) GTP-binding proteins in adrenocortical mitochondria. Biochim. Biophys. Acta **1248:** 159–169

62 Sleer L. S. and Hall P. F. (2000) Partial characterization of mitochondrial G proteins in adrenal cells. Biochim. Biophys. Acta **1463:** 99–106

63 Stone, H. and Hechtor, O. (1954) Studies on ACTH action on perfused bovine adrenals: site of action of ACTH corticosteroidogenesis. Arch. Biochem. Biophys. **51:** 457–469

64 Krueger R. J. and Orme-Johnson N. R. (1983) Acute adrenocorticotropic hormone stimulation of adrenal corticosteroidogenesis. J. Biol. Chem. **258:** 10159–10167

65 Epstein L. F. and Orme-Johnson N. R. (1991) Regulation of steroid hormone biosynthesis: identification of precursors of a phosphoprotein targeted to the mitochondrion in stimulated rat adrenal cortex cells. J. Biol. Chem. **266:** 19739–19734

66 Pon L. A. and Orme-Johnson N. R. (1986) Acute stimulation of steroidogenesis in corpus luteum and adrenal cortex by peptide hormones. J. Biol. Chem. **261:** 6594–6599

67 Clark B. J., Wells J., King S. R. and Stocco D. M. (1994) The purification cloning and expression of a novel luteneinizing hormone-induced mitochondrial protein in mouse Leydig MA-10 cells. J. Biol. Chem. **269:** 28314–28322

68 Papadopoulos V., Amri H., Li H., Boujrad N., Vidic B. and Garnier M. (1997) Targeted disruption of the peripheral-type benzodiazepine receptor gene inhibits steroidogenesis in the R2C Leydig tumor cell line. J. Biol. Chem. **272:** 32129–32135

69 Kallen C. B., Arakane F., Christenson L. K., Watari H., Devoto L. and Strauss J. F. R. (1998) Unveiling the mechanism of action and regulation of the steroidogenic acute regulatory protein. Mol. Cell. Endocrinol. **145:** 39–45

70 Brdiczka D., Beutner G., Ruck A., Dolder M. and Wallimann T. (1998) The molecular structure of mitochondrial contact sites. Their role in regulation of energy metabolism and permeability transition. Biofactors **8:** 235–242

71 Thomson M. (1998) What are GTP-binding proteins doing in mitochondria? Biochim. Biophys. Acta **1403:** 211–218

72 Arakane F., King S. R., Du Y., Kallen C. B., Walsh L. P., Watari H. et al. (1997) Phosphorylation of steroidogenic acute regulatory protein (StAR) modulates its steroidogenic activity. J. Biol. Chem. **272:** 32656–32662

73 Hasegawa K. (1977) Cyclic AMP-dependent protein kinase in rat adrenal mitochondrial fraction – studies on the mechanism of ACTH action. Nippon Naibunpi Gakkai Zasshi **53:** 1094–1105

74 Albrecht E. D. and Pepe G. J. (1990) Placental steroid biosynthesis in primate pregnancy. Endocr. Rev. **11:** 124–150

75 Rehling P., Wiedemann N., Pfanner N. and Truscott K. N. (2001) The mitochondrial import machinery for preproteins. Crit. Rev. Biochem. Mol. Biol. **36:** 291–336

76 Tomaska L. (2000) Mitochondrial protein phosphorylation: lessons from yeasts. Gene **255:** 59–64

77 Takai Y., Sasaki T. and Matozaki T. (2001) Small GTP-binding proteins. Physiol. Rev. **81:** 153–208