Mitochondrial AKAP121 Links cAMP and src Signaling to Oxidative Metabolism

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AKAP121 focuses distinct signaling events from membrane to mitochondria by binding and targeting cAMP-dependent protein kinase (PKA), protein tyrosine phosphatase (PTPD1), and mRNA. We find that AKAP121 also targets src tyrosine kinase to mitochondria via PTPD1. AKAP121 increased src-dependent phosphorylation of mitochondrial substrates and enhanced the activity of cytochrome c oxidase, a component of the mitochondrial respiratory chain. Mitochondrial membrane potential and ATP oxidative synthesis were enhanced by AKAP121 in an src- and PKA-dependent manner. Finally, siRNA-mediated silencing of endogenous AKAP121 drastically impaired synthesis and accumulation of mitochondrial ATP. These findings indicate that AKAP121, through its role in enhancing cAMP and tyrosine kinase signaling to distal organelles, is an important regulator in mitochondrial metabolism.

INTRODUCTION

Protein kinase A (PKA) is an essential mediator in most cAMP-dependent signaling pathways. A family of proteins named A-kinase anchor proteins (AKAPs) has been identified that enhance cAMP-dependent PKA signaling pathways (Rubin, 1994; Gray et al., 1998; McKnight et al., 1998; Feliciello et al., 2001; Houslay and Adams, 2003; Tasken and Aandahl, 2004; Taylor et al., 2004; Wong and Sott, 2004). AKAP121 (also called D-AKAP1), AKAP149, and AKAP84 arise from a single gene by alternative pre-mRNA splicing (Lin et al., 1995; Trendelenburg et al., 1996; Chen et al., 1997; Huang et al., 1999; Furusawa et al., 2002). AKAP121 and AKAP84 tether PKA to the mitochondrial outer surface. This localization is mediated by the interaction of AKAP121 and AKAP84 with β tubulin, an integral component of mitochondrial outer membrane (Cardone et al., 2002). AKAP121 is widely expressed in several tissues and its accumulation is regulated at the transcriptional level by the cAMP/PKA pathway (Feliciello et al., 1998). Anchoring of PKA to mitochondria supports cAMP signaling and suppresses apoptosis (Harada et al., 1999; Affaitati et al., 2003). AKAP121, via a KH domain at its COOH-terminus, binds at least two mRNAs that encode mitochondrial proteins (Ginsberg et al., 2003; Ranganathan et al., 2005). This multicomponent system, reminiscent of other AKAP complexes at cell membranes, ensures efficient translation and import of nuclear-encoded mitochondrial proteins. It is suggested that PKA may phosphorylate some of these proteins cotranslationally, as well as acting on AKAP121 itself to regulate the stability of the RNA-AKAP121 complex (Ginsberg et al., 2003; Feliciello et al., 2005).

In addition, AKAP121 and AKAP84 bind the central core of PTPD1, a classical nonreceptor protein tyrosine phosphatase (Moller et al., 1994). PTPD1 binds to and activates src, enhancing EGF-dependent mitogenic signaling (Cardone et al., 2004). By translocating PTPD1 to the outer membrane of mitochondria, AKAP121 inhibits PTPD1-dependent EGF signaling to the nucleus. These data suggest a model whereby AKAP121, by targeting PTPD1/src complex to mitochondria, may shift the focus of tyrosine kinase signaling from membrane to specific distal organelles, such as mitochondria (Feliciello et al., 2005).

We tested this hypothesis and found that AKAP121, indeed, targets src tyrosine kinase to mitochondria. By manipulating the localization and expression of AKAP121, we were able to modulate cAMP- and src-dependent signaling to mitochondria, affecting phosphorylation of mitochondrial substrates, activity of components of the respiratory chain, mitochondrial membrane potential (ΔΨm) and oxidative synthesis of ATP.

MATERIALS AND METHODS

Cell Lines
The human embryonic kidney cell line HEK293 was cultured in Dulbecco’s modified Eagle’s medium (DMEM) containing 10% fetal calf serum (FCS) in an atmosphere of 5% CO2. Where indicated, HEK293 cells were stably transfected with a CMV-G418S vector expressing AKAP121 and selected for 4 wk in medium containing G418 (800 μg/ml). Resistant clones were isolated, screened for expression of the transgene, and pooled (10 and 5 independent positive clones for PTPD1 and CMV, respectively). Pools were expanded and grown in medium supplemented with 400 μg/ml G418. GC2 cells were derived from primary mouse preleptotene spermatocytes by stable cotransfection with transgenes encoding SV40 large T-antigen and a temperature-sensitive variant of the p53 transcriptional regulator protein (Wolkolwicz et al., 1996). GC2 cells were grown at 37°C in DMEM supplemented with 10% FCS. Primary cultures of human fibroblasts were obtained from cutaneous...
Plasmids and Transfection

Mouse pCEP4-AKAP121 cDNA was a gift of Dr. C. Rubin (Albert Einstein College of Medicine, New York). An AKAP84 mutant lacking the first 30 amino acids was generated by PCR using specific oligonucleotides. The PCR product was subcloned in the CMV vector. cDNA coding the kinase-inactive form of Src (Lys259 changed to methionine) was cloned into pCt5G (Barone and Courtneidge, 1996) and was kindly provided by Prof. A. Migliaccio (Second University of Naples, Italy). The vector encoding for human PTPD1 (Moller et al., 1994) was purchased from Clontech (Piscataway, NJ). The vector containing a short hairpin RNA targeting mouse AKAP121 (nucleotides 301–321, ATG + 1) was subcloned in pRNA-H1/neo vector (Genscript, Piscataway, NJ). Blast search confirmed that this sequence specifically recognizes mouse. A scrambled sequence subcloned in the same vector was used as negative control.

Immunoprecipitation and Immunoblot Analysis

Cells were homogenized in lysis buffer (20 mM Tris-HCl, pH 7.4, 0.15 M NaCl, 10 mM EDTA, 0.25% Triton X-100, 0.05% Tween-20, 0.02% sodium azide) containing aprotonin (5 μg/ml), leupeptin (10 μg/ml) pepstatin (2 μg/ml), and 0.5 mM phenylmethylsulfonyl fluoride. The lysates were cleared by centrifugation at 10,000 × g for 15 min. Cell lysates (2 mg) were immunoprecipitated with the indicated antibodies. An aliquot of cell lysate (100 μg) or immunoprecipitates were resolved by SDS-PAGE gel and transferred to Immobilon P membrane. The immunoblot analysis was performed as previously described (Hovius et al., 1990).

Immunofluorescence Analysis

Cells were rinsed with phosphate-buffered saline (PBS) and fixed in 3.7% formaldehyde for 20 min. After permeabilization with 0.1% Triton X-100 in PBS for 5 min, the cells were incubated with PBS 1/10,000 Tween-20, 0.02% sodium azide containing aprotonin (5 μg/ml), leupeptin (10 μg/ml), pepstatin (2 μg/ml), and 0.5 mM phenylmethylsulfonyl fluoride. The lysates were cleared by centrifugation at 10,000 × g for 15 min. Cell lysates (2 mg) were immunoprecipitated with the indicated antibodies. An aliquot of cell lysate (100 μg) or immunoprecipitates were resolved by SDS-PAGE gel and transferred to Immobilon P membrane. The immunoblot analysis was performed as previously described (Cardone et al., 2004).

Assays for Cytochrome c Oxidase Activity and Mitochondrial ATP Synthesis

The activity of cytochrome c oxidation on purified mitochondria was determined by spectrophotometric measurement of the rate of reduced cytochrome c oxidation at 550 nm (Couperstein and Lazarov, 1951). Cytochrome c (type VI, Sigma) was reduced by dithiorthreitol (DTT) at a final concentration of 0.5 mM. Cytochrome c reduction was assessed measuring A550/A565 ratio. In our conditions, this ratio was between 15 and 20. Mitochondrial fractions were suspended in 60 mM of phosphate buffer (pH 7.4) containing 0.6% lauryl maltoside and centrifuged for 10 min at 10,000 × g. The assay was performed in a total volume of 1 ml of phosphate buffer (60 mM) containing 0.1 M of mitochondrial proteins, 30 μM final concentration of reduced cytochrome c. The decrease in absorbance at 550 nm was measured for 1 min with 15 seconds integration time (Stiegerova et al., 2000).

Assay for Oxidative ATP Synthesis

Cells were harvested by trypsinization 48 h after transfection, washed twice in PBS, and counted in a hemo-cytometer. A replicate for each sample was prepared that had been treated for 1 h with 4 μg/ml rotenone (Sigma). The emission recorded from samples treated with rotenone was defined as baseline luminescence corresponding to a nonmitochondrial source of ATP. As-says were performed using the ATP luminescence assay kit HS II (Roche, Nutley, NJ) according to manufacturer's instructions, using 3000 cells per sample. Light emission was recorded in a single measure of 2 s using a Lumat LB 9507 lumimeter (Berthold Technologies, Bad Wildbad, Germany).

Quantitative Analysis of Mitochondrial DNA

Mitochondrial DNA content relative to nuclear genome from control (CMV) and AKAP121 expressing cells was evaluated by PCR using oligonucleotide primers specific for nuclear (β globin) and mitochondrial genes (NADH dehydrogenaseI and cytochrome c). The primer sequences used were: cytochrome c: FW: 5′-CCTAGGGGACCCAGACATATT-3′; rev: 5′-TCTAGATGTGTCCTTCTGATT-3′; β globin: FW: 5′-CAGCTCCCTTCTCATCAAACC-3′; rev: 5′-ATTATGATGCTGCTAGTG-3′; NADHd: FW: 5′-CGACCTCCTTGAATGCTGG-3′; chromeb: FW: 5′-GGATGTCGGGATACATATTGGAC-3′.

Imaging Mitochondrial Membrane Potential

The Δψm was assessed using the fluorescent dye tetra-methyl rhodamine ethyl ester (TMRM) in the “redistribution mode.” Cells transfected with CMV and AKAP121 were loaded with TMRE, 20 nM, for 30 min in a medium containing 156 mM NaCl, 3 mM KCl, 2 mM MgSO4, 1.25 mM KH2PO4, 2 mM CaCl2, 10 mM glucose, and 10 mM HEPES. The pH was adjusted to 7.35 with NaOH (Abramov et al., 2004). At the end of the incubation, cells were washed in the same medium containing 20 mM dye and allowed to equilibrate. A decline of mitochondria-localized intensity of fluorescence was indicative of mitochondrial membrane depolarization. Confocal images were obtained using a Zeiss inverted S10 confocal laser scanning microscope (Thornwood, NY) and a 63× oil immersion objective. The illumination intensity of 543 Xenon lamp, used to excite TMRE fluorescence, was kept to a minimum of 0.5% of laser output to avoid phototoxicity.

RESULTS

AKAP121 Assembles a Tyrosine Kinase/Phosphatase Signaling Complex on Mitochondria

We previously showed that AKAP121 binds and targets PTPD1 to subcellular organelles. Because PTPD1 associates with src, we hypothesized that AKAP121 may form a scaffold complex that includes not only PTPD1 and PKA, but src as well. In this context, PTPD1 may act as molecular bridge between the anchor protein and src. To test this notion, we performed immunoprecipitation experiments using total lysates prepared from HEK293 cells. This cell line expresses endogenous PTPD1 and low levels of AKAP121 (Figure 1A). Cells were transiently transfected with AKAP121 vector with or without PTPD1 vector. Forty-eight hours after transfection, total lysates were prepared and subjected to immunoprecipitation. Figure 1A shows that AKAP121 was precipitated significant amounts of PTPD1 and src (Figure 1B).

To determine if AKAP121 targets src tyrosine kinase on mitochondria in intact cells, we performed immunostaining analysis using specific antibodies directed against src, PTPD1 and AKAP121. Primary mouse pre-leptotene spermatocytes (GC2) express high levels of endogenous AKAP121 and PTPD1. Both proteins partly colocalize on mitochondria (Cardone et al., 2004). We analyzed src localization in GC2 cells by double immunostaining with anti-AKAP121 and anti-src antibody. The signals were collected.
AKAP121 were detectable. We confirmed this finding using perinuclear and nuclear regions, where no mitochondria or mitochondrial matrix. However, src staining was also evident (Figure 2, d–f), a protein that selectively localizes to the matrix. Similarly, src immuno-staining partly overlapped that of AKAP121 (Figure 2, a–c), suggesting that both proteins colocalize on mitochondria. We then monitored the activity of COX. HEK293 cells were transiently transfected with expression vectors encoding PTPD1 and AKAP121. Forty-eight hours after transfection, mitochondrial and supernatant fractions were isolated, size-fractionated on SDS-PAGE, and immunoblotted with the indicated antibodies. As shown in Figure 3, AKAP121 copurified with the mitochondria-enriched fraction, as did the mitochondrial voltage-dependent anion channel (VDAC), whereas tubulin and MAPK were found exclusively in supernatant fractions. In untransfected control cells most of endogenous c-src protein was found in the supernatant. Expression of AKAP121, PTPD1, or both significantly increased the amount of c-src recovered in the mitochondrial fraction. Note that coexpression of AKAP121 and PTPD1 translocates an amount of src to mitochondria roughly equivalent to AKAP121 or PTPD1 alone. PTPD1 and src are not only localized to mitochondria. Significant amounts of these enzymes have been found associated with other organelles and cell structures. This suggests that interaction with specific targeting sites is critically dependent on the absolute levels and binding affinity of AKAP121, src, and PTPD1. Src activity was not required for mitochondrial localization promoted by AKAP121. Thus, cotransfection of HEK293 with AKAP121 and an src kinase dead (src K–) increased src levels in the mitochondrial fraction. A similar increase was seen when AKAP121-transfected cells were treated with the src inhibitor, PP2. We conclude that AKAP121 recruits c-src to mitochondria independently on its kinase activity.

AKAP121 Enhances src-dependent Phosphorylation and Activation of Mitochondrial Substrates

Some src is normally found in the mitochondrial matrix, where it phosphorylates and stimulates the activity of cytochrome c oxidase (COX), a component of the mitochondrial respiratory chain (Miyazaki et al., 2003). We wanted to determine if src localization by AKAP121/PTPD1 on mitochondria correlated with enhanced phosphorylation of mitochondrial src substrates. HEK293 cells were transiently transfected with AKAP121 and PTPD1 vectors for 24 h and subsequently harvested and lysed. Mitochondrial fractions were prepared and subjected to immunoblot analysis with anti-phosphotyrosine antibody. As shown in Figure 4A, expression of AKAP121 or PTPD1, to a lesser degree, markedly enhanced tyrosine phosphorylation of mitochondrial proteins. In contrast to src localization, stimulation of tyrosine phosphorylation by AKAP121 required src activity. Thus, treatment with PP2 or expression of src K– reduced phosphorylation to control levels. The phosphorylation of some mitochondrial substrates in cells transfected with AKAP121 and PTPD1 was reduced, compared with cells expressing AKAP121 alone. We have evidence that PTPD1 is not only localized to mitochondria, but in the absence of AKAP121, a significant fraction is linked to the actin cytoskeleton. In this compartment, PTPD1 regulates EGF-dependent src-FAK signaling (Carlucci, Gedressi, Avvedimento, Garbi, and Feliciello, unpublished results).

We then monitored the activity of COX. HEK293 cells were transiently transfected with the indicated expression vectors. Forty-eight hours after transfection, cells were harvested, lysed, and mitochondrial COX activity was assayed. Figure 4B shows that AKAP121 increased COX activity by ca. 30%. HEK293 cells transiently expressing AKAP84, the
smaller splice variant of AKAP121, also showed increased COX activity compared with their control HEK293 cells (our unpublished data). To prove that AKAP-mediated targeting of src to mitochondria was required for COX activity, we tested an AKAP84 mutant carrying a deletion of the mitochondrial targeting (MT) domain (H90041–30). This mutant retained the ability to bind PTPD1/src and PKA, but failed to target these proteins to mitochondria (Cardone et al., 2004). AKAP84/H90041–30 was transiently transfected in HEK293 cells, and COX activity was assayed. The expression of mutant protein was comparable to that of wild-type AKAP121 (our unpublished data). Figure 4B shows that AKAP84/H90041–30 acted as dominant negative, reducing COX activity by ca. 40% relative to controls. Stimulation of COX activity by AKAP121 depended on active src kinase. Treatment of the transfected cells with PP2 reduced COX activity below control levels. The amount of COX protein, as shown by Western blot, was unaffected by transfection and treatment with PP2 (Figure 4B, inset). This indicates that the number of mitochondria per cell remained constant during these treatments.

Elevated oxidative respiratory chain activity is associated with increased mitochondrial DNA content (Deveau et al., 2004). We therefore asked whether AKAP121 promoted mitochondrial DNA accumulation. Using semiquantitative PCR, we monitored the accumulation of two mitochondrial genes, NADH dehydrogenase and cytochrome B. The nuclear β globin gene was used as an internal control. Figure 4C shows that AKAP121 increased the levels of NADH dehydrogenase and cytochrome B by ca. 50%, compared with control cells transfected with the CMV vector (CMV). To confirm this finding, we performed Southern blot analysis on total cellular DNA using as probes mitochondrial and β globin cDNAs. Control cells and cells expressing...
AKAP121 were serum-deprived overnight, transferred to 10% FCS, and harvested at the indicated times. As shown in Figure 4D, AKAP121 increased both basal and serum-stimulated mitochondrial DNA levels by ~2–3-fold.

**AKAP121 Regulates ΔΨm and Oxidative ATP Synthesis**

Electron flux through the respiratory chain is used to reduce free oxygen at the level of complex IV. Hydrogen then moves from the mitochondrial matrix to the intermembrane space. This generates an electrochemical gradient, expressed as ΔΨm (Saris and Carafoli, 2005). The ΔΨm is fundamental for the efficient production of ATP and requires the coordinated activity of several enzyme complexes, including COX. Because AKAP121-src stimulated COX activity, we asked if this anchor protein enhanced the ΔΨm under basal or stress conditions using a fluorescence assay (Abramov et al., 2004). Indeed, mitochondria in HEK293 transiently transfected with AKAP121 were hyperpolarized in comparison to control cells (Figure 5A, a and d). Hyperpolarization was inhibited by 30-min exposure to H89 (10 μM), an inhibitor of PKA and other kinases (Figure 5Ae). Similarly, treatment with PP2 (10 μM) for 30 min reduced polarization (Figure 5Af). Treatment with H89 or PP2 also decreased the intensity of mitochondrial localized fluorescence in control cells (Figure 5, A and B). The localized fluorescence intensity of cells expressing AKAP121 was significantly higher than in control cells.

AKAP121, by facilitating the electrochemical gradient along the inner mitochondrial membrane, should enhance ATP synthesis. Figure 7A shows that cells transfected with AKAP121 had higher ATP levels than controls. H89 abrogated this increase. Treatment with CPT-cAMP, a cAMP analog, increased the ATP levels in control cells and, to a lesser extent, in AKAP121 expressing cells, compared with untreated cells. This suggests that AKAP121 increased mitochondrial PKA signaling close to maximum levels. In fact, in serum-deprived AKAP121 cells, the effects of cAMP on mitochondrial PKA signaling were more evident (Affaitati et al., 2003; Ginsberg et al., 2003). Taken together, these data imply that PKA up-regulates ATP synthesis and this effect is enhanced by AKAP121. ATP accumulation was also dependent on src activity. Thus, ATP concentrations were lowered by PP2 in both control and, more dramatically, in AKAP121-transfected cells. Western blot analysis showed that neither
PP2 nor H89 reduced expression of AKAP121 (our unpublished data).

We then examined the effects of serum deprivation on ATP accumulation. Twenty-four hours after transfection, cells were serum-deprived for 18 h and their mitochondrial ATP concentration was determined. Serum-deprivation significantly reduced ATP levels in control cells and in cells expressing AKAP121. These findings, along with the data reported in Figure 6B, suggest that growth factors stimulate oxidative phosphorylation and that AKAP121 increases mitochondrial robustness and resistance to oxidative stresses.

To confirm that AKAP121 regulates the mitochondrial respiratory chain, we silenced endogenous AKAP121 by DNA vector-based siRNA and measured ATP levels. As control, we used the same vector carrying a scrambled RNA sequence. Mouse fibroblasts (NIH3T3), which express AKAP121, were transiently transfected and the efficiency of silencing was evaluated by immunoblot analysis (Figure 7B). Expression of siRNA_AKAP121 decreased endogenous AKAP121 levels ca. 2.5-fold. The control vector, which expresses siRNA_scrambled, did not reduce AKAP121 concentrations. Consistent with our hypothesis, the synthesis of mitochondrial ATP was suppressed by siRNA_AKAP121 but not siRNA_scrambled. The extent of inhibition by siRNA was comparable to that provoked by src inhibition with PP2 (Figure 7C).

DISCUSSION

Our data shows that mitochondrial AKAP121 forms a signaling complex that includes, in addition to PKA, PTPD1 and src. AKAP121 increases cAMP and src signaling to mitochondria. Thus, tyrosine phosphorylation of some mitochondrial substrates, activity of COX, ΔΨm, and ATP synthesis were enhanced by AKAP121 in an src- and PKA-dependent manner.
AKAPs, by colocalizing signaling enzymes and their substrates, are proposed to ensure efficient propagation of transduction events generated at distal sites to specific intracellular compartments (Rubin, 1994; Feliciello et al., 1997; Gray et al., 1998; McKnight et al., 1998; Feliciello et al., 2001; Houslay and Adams, 2003; Tasken and Aandahl, 2004; Taylor et al., 2004; Wong and Scott, 2004). AKAP121 clearly plays this role in mitochondria. By localizing PKA at the outer membrane of mitochondria, AKAP121 increases PKA-dependent phosphorylation/inactivation of proapoptotic

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**Figure 7.** AKAP121 controls oxidative ATP synthesis. (A) HEK293 cells were transiently transfected with AKAP121 or control vector (CMV). Twenty-four hours after transfection, cells were treated with PP2 (10 μM), H89 (10 μM), or CPT-cAMP (250 μM) for 30 min, or serum deprived (SD) for 18 h and harvested. ATP accumulation was evaluated as described in the Materials and Methods. The data are expressed as fold increase over the control (CMV) that was set as 1 and represent the mean ± SEM of four independent experiments. (B) HEK293 cells were transiently transfected with siRNA control vector, or with the vector targeting AKAP121 (siRNA-AKAP121) or a scrambled sequence (siRNA-scrambled). Cells were harvested 24 and 48 h after transfection. Cell lysates were sequentially immunoblotted with anti-AKAP121 and anti-tubulin antibodies. (C) Assay of oxidative ATP synthesis in cells from B harvested at 48 h from transfection. The data represent fold increase over basal value from control cells (siRNA vector) that was set as 1 and are expressed as SEM of three independent experiments. (D) Model of AKAP121 effects on oxidative phosphorylation and mitochondrial membrane potential.
protein BAD and enhances cell survival (Harada et al., 1999; Affaitati et al., 2003). AKAP121 also facilitates PKA-dependent phosphorylation and activation of StAR, a mitochondrial steroidogenic factor that localizes in mitochondria of adrenal and testicular Leydig cells. In doing so, AKAP121 increases the biosynthesis of steroid hormones in a PKA-dependent manner (Stocco, Dyson, Jones, and Gottesman, unpublished results).

Signaling enzymes other than PKA are also bound and targeted by AKAP121. The amino-terminus of AKAP121 interacts with the central core of PTPD1, localizing the phosphatase on mitochondria (Cardone et al., 2004). PTPD1 is an effector for EGF signal transduction from the membrane to the nucleus. AKAP121 binding diverts PTPD1 to mitochondria and down-regulates this transduction pathway. We report here that AKAP121, via PTPD1, targets src to mitochondria. AKAP121 enhances src-dependent tyrosine phosphorylation of some mitochondrial substrates, facilitating the mitochondrial respiratory chain and increasing ATP synthesis. This conclusion is based both on AKAP121 overproduction and AKAP121 knockdown by siRNA.

The importance of tyrosine kinase signaling in mitochondrial function is supported by several lines of evidence (Abram and Courtneidge, 1999; Ko et al., 2002; Boerner et al., 2004; Augereau et al., 2005; Salvi et al., 2005). Tyrosine phosphorylation of mitochondrial proteins is stimulated in vitro by ATP and H2O2 (Augereau et al., 2005). ATP production at state 3 likewise enhances phosphorylation; this stimulation is ablated by PT2. Phosphorylation and activation of COX by mitochondrial src is postulated to play an important role in osteoclast function and bone remodeling (Miyazaki et al., 2003). The 39-kDa subunit of complex I is tyrosine-phosphorylated, and subunits of complexes II, III, and IV may also be tyrosine kinase substrates (Augereau et al., 2005). Platelet-derived growth factor (PDGF) signaling is linked to tyrosine phosphorylation of the c and δ subunits of the mitochondrial ATP synthase complex. This accounts for the enhanced activity of ATP synthase seen in a variety of PDGF-treated cells, including cortical neurons, mouse fibroblasts, and kidney cells (Evtodienko et al., 2000; Ko et al., 2002; Boerner et al., 2004). Moreover, serum deprivation has been linked to loss of mitochondrial respiratory control (Gottlieb et al., 2002).

Components of the respiratory chain can also be phosphorylated and regulated by PKA (Yang et al., 1998; Ludwig et al., 2001, Papa et al., 2002). A functional interplay between cAMP, tyrosine kinase, and mitochondrial COX has been recently described (Lee et al., 2005). These authors found that high cAMP levels induced phosphorylation of COX subunit I at tyrosine304 and inhibited COX activity. The responsible tyrosine kinase has not been identified.

We propose that AKAP121 is a nodal point where PKA and src signaling integrate, increasing the rate and magnitude of signaling to mitochondria. The mechanism by which src bound to AKAP121-PTPD1 phosphorylates substrates located within the mitochondrial matrix is still unknown. One possibility is that src may translocate inside mitochondria through the outer/inner mitochondrial transport system (Endo et al., 2003). AKAP121 increases the absolute levels of src anchored at the outer membrane of mitochondria. This facilitates transport of src, as well as PKA, inside the organelle, where both kinases normally reside (Yang et al., 1998; Miyazaki et al., 2003; Papa et al., 2003). This regulation is critical for mitochondrial physiology and explains the essential role of AKAP121 in cell survival, steroidogenesis, and oxidative phosphorylation. Identification of the critical mitochondrial substrates of PKA and src, and the functional relationship between these two signaling enzymes on mitochondria will, of course, require further study.

In this article we also present evidence that mitochondrial DNA content is increased by AKAP121. In this regard, our preliminary data suggest that cAMP and PKA are functionally linked to this process. In Saccharomyces cerevisiae, mitochondrial activity (citric acid cycle and oxidative respiration) and mtDNA content are coregulated. Mitochondrial activities and mtDNA are down-regulated by growth in glucose, whereas growth in a nonfermentable carbon source stimulates oxidative phosphorylation and increases mtDNA content. The cAMP/PHA signal transduction pathway positively regulates both reactions (Robertson et al., 2000; Cho et al., 2001; Griffioen and Thevelein, 2002). We have shown that AKAP121 concomitantly regulates mtDNA content and oxidative ATP synthesis. We cannot, however, ascertain which of the two events is the primary regulator of mammalian mitochondrial metabolism.

In summary, we demonstrate for the first time that AKAP121 regulates src events on mitochondria and highlight a unique role of this protein in the regulation of oxidative metabolism (Figure 7D). This mechanism increases the complexity of the symbiotic relationship developed million years ago between primordial eukaryotic cells and the aerobic bacteria that are thought to be mitochondrial ancestors. In view of the ubiquitous expression of AKAP121, our findings reveal an efficient mechanism that may be used in most or all mammalian cells to adapt physiologically to rapid changes in carbon source availability.

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