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Organelles in focus

# Mitochondria: A kinase anchoring protein 1, a signaling platform for mitochondrial form and function

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ABSTRACT

Mitochondria are best known for their role as cellular power plants, but they also serve as signaling hubs, regulating cellular proliferation, differentiation, and survival. A kinase anchoring protein 1 (AKAP1) is a scaffold protein that recruits protein kinase A (PKA) and other signaling proteins, as well as RNA, to the outer mitochondrial membrane. AKAP1 thereby integrates several second messenger cascades to modulate mitochondrial function and associated physiological and pathophysiological outcomes. Here, we review what is currently known about AKAP1's macromolecular interactions in health and disease states, including obesity. We also discuss dynamin-related protein 1 (Drp1), the enzyme that catalyzes mitochondrial fission, as one of the key substrates of the PKA/AKAP1 signaling complex in neurons. Recent evidence suggests that AKAP1 has critical roles in neuronal development and survival, which are mediated by inhibitory phosphorylation of Drp1 and maintenance of mitochondrial integrity.



**Organelle facts**

- Mitochondria carry out many essential processes including ATP production.
- Mitochondria are shaped by fission and fusion reactions, which influence function.
- The scaffold protein AKAP1 assembles a "signalasome" at the mitochondrial surface, including kinases, phosphatases, and mRNA.
- AKAP1 maintains mitochondrial and cellular health.
- AKAP1 is highly expressed in adipocytes and may play a role in obesity.
- PKA in the AKAP1 complex inhibits mitochondrial fission and apoptosis, but promotes dendrite development by phosphorylating the mitochondrial fission enzyme Drp1.

## 1. Introduction

The second messenger cyclic AMP (cAMP) signals predominantly via cAMP-dependent activation of protein kinase A (PKA). In the absence of cAMP, PKA is a tetramer of two regulatory subunits (either type RI or RII) bound to two catalytic subunits. Upon binding of cAMP to the regulatory subunits, the catalytic subunits are released in an active state to phosphorylate serine/threonine residues. In addition to mediating the cAMP response, the regulatory subunits target the PKA catalytic subunits to various subcellular locations by binding to a group of proteins termed A kinase anchoring proteins (AKAPs). The AKAPs are an unrelated group of proteins that are defined by the presence of an amphipathic helix that binds the PKA regulatory subunit. The first AKAP to be identified, AKAP1 (aka, D-AKAP1, AKAP121, AKAP149, S-AKAP84) localizes predominantly type II PKA holoenzymes to the outer mitochondrial membrane (OMM). In this review we will discuss the role of AKAP1, outer mitochondrial PKA, and other AKAP1 interactors in mitochondrial function under both normal

*Abbreviations:* AKAP, A kinase anchoring protein; BAD, Bcl-2-associated death promoter; cAMP, cyclic AMP; CaN, calcineurin; Casp, caspase; Drp1, dynamin-related protein 1; Fo-f, ATP synthase Fo complex; HIF-1a, hypoxia induced factor 1a; KH, K homology; LPL, lipoprotein lipase; Mff, mitochondrial fission factor; MnSOD, manganese superoxide dismutase; OMM, outer mitochondrial membrane; PDE4a, phosphodiesterase type 4a; PKA, protein kinase A; PP1, protein phosphatase 1; PTEN, phosphatase and tensin homolog; PPARγ, coactivator peroxisome proliferator-activated receptor γ; PTPD1, protein tyrosine phosphatase D1; Siah2, seven in absentia homolog; StAR, steroidogenic acute regulatory; TOM, transporter outer membrane; Ub, ubiquitin; ΔΨm, mitochondrial membrane potential.

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and pathological states, focusing in particular on regulation of mitochondrial fission by AKAP1.

## 2. Organelle function

### 2.1. AKAP1 isoforms

AKAP1 transcripts are broadly expressed in tissues including in heart, liver, kidney, skeletal muscle and brain, and they can be subject to alternative splicing (Huang et al., 1999). A common N-terminal exon (amino acids, aa 1–572 in human) encodes both the mitochondrial targeting sequence (aa 1–30) and the PKA binding helix (aa 347–360 in human). Full-length AKAP1 (AKAP121 in mouse; AKAP149 in human) is produced by splicing of this common exon to a series of highly conserved C-terminal exons that encode an RNA binding K homology (KH) domain and a Tudor domain. A short AKAP1 variant (S-AKAP84) is generated by alternative splicing of the first coding exon to a different C-terminal exon that encodes 22 residues, with low sequence conservation, followed by a stop codon. S-AKAP84 expression is largely restricted to testis (Lin et al., 1995). Additional AKAP1 splice variants have been described, but the majority are non-coding and their significance is unclear. The N1 variant was identified in the mouse and includes 33 additional N-terminal residues (Ma and Taylor, 2002). The N1 N-terminal exon was reported to target AKAP1 to the endoplasmic reticulum (Ma and Taylor, 2008). However, this exon is not represented in EST databases, is poorly conserved, and lacks a start codon in species other than mouse. The most abundant and best-characterized product of the AKAP1 gene is full length AKAP1, which is the focus of this review.

### 2.2. AKAP1 interactions

The N-terminal mitochondrial targeting sequence for AKAP1 initiates import through the translocase of the outer membrane (TOM) complex, but it is then laterally released into the OMM to act as a transmembrane anchor, with the remainder of the protein facing the cytosol (Ma and Taylor, 2008).

In neuronal PC12 cells, overexpression of AKAP1 attenuated serum starvation-induced apoptosis and resulted in enhanced phosphorylation and inhibition of the proapoptotic BAD protein. Expression of an AKAP1 point mutant incapable of binding PKA (AKAP1ΔPKA) increased the sensitivity of PC12 cells to apoptotic challenges (Affaitati et al., 2003). Treatment with a peptide derived from the AKAP1 N-terminus to delocalize endogenous AKAP1 reduced oxidative ATP synthesis and mitochondrial membrane potential ($\Delta\Psi m$) and increased oxidative stress resulting in cardiomyocyte death, highlighting the critical role of mitochondrial-localized PKA in cell survival (Perrino et al., 2010). Recently, AKAP1 was shown to associate with the $Na^+/Ca^{2+}$ exchanger Ncx3 at the OMM to facilitate $Ca^{2+}$ efflux from mitochondria and confer neuroprotection from hypoxia (Scorziello et al., 2013).

Besides PKA, AKAP1 recruits various other macromolecules to mitochondria, including a whole host of signaling proteins (summarized in Table 1, Fig. 1). For instance, AKAP1 has been shown to interact with a complex of protein tyrosine phosphatase D1 (PTPD1) (Cardone et al., 2004) and the non-receptor tyrosine kinase Src (Livigni et al., 2006). Overexpression of wild-type AKAP1 increased mitochondrial $\Delta\Psi m$, while expression of either AKAP1ΔPKA or an AKAP1 deletion mutant that cannot bind PTPD1/Src reduced $\Delta\Psi m$ below control levels (Cardone et al., 2004; Livigni et al., 2006). An additional layer of complexity is added by the reported association of AKAP1 with type 4 phosphodiesterases (PDE4), which is predicted to limit the availability of cAMP for PKA activation (Asirvatham et al., 2004). In cardiomyocytes, AKAP1 recruits the calcium-responsive phosphatase calcineurin (CaN, aka PP2B) and prevents cardiac hypertrophy (Abrenica et al., 2009).

Interestingly, human AKAP1 (AKAP149) has also been localized to the nuclear envelope (NE), although this was not replicated by other laboratories. AKAP1 was proposed to target protein phosphatase 1 (PP1) to facilitate reassembly of the NE at the end of mitosis (Steen et al., 2003). PP1 is known to interact with numerous regulatory proteins through an RVxF motif (Roy and Cyert, 2009), and the AKAP1 interaction site was initially postulated to be KGVLF (aa 155–159 in humans) (Steen et al., 2000). However, a subsequent report identified a C-terminal sequence (RYVSF, aa 637–641) as responsible for the PP1 interaction with AKAP1 (Rogne et al., 2009).

Through its C-terminal KH domain, AKAP1 was reported to bind to the 3′-UTR of specific mRNAs. KH domains interact with 4 bases of single-stranded RNA or DNA via a binding cleft composed of two α-helices, a connecting GXXG loop, and a β-sheet (Valverde et al., 2008). AKAP1 associated mRNAs include those encoding the F0-f subunit of mitochondrial ATP synthase, manganese superoxide dismutase (MnSOD, SOD2), and steroidogenic acute regulator (STAR), a cholesterol-binding protein important for rapid steroid synthesis (Ginsberg et al., 2003; Grozdanov and Stocco, 2012). By localizing mRNAs close to the OMM, AKAP1 is believed to foster local translation of mitochondrial proteins; indeed, AKAP1 overexpression increased mitochondrial MnSOD levels (Ginsberg et al., 2003; Grozdanov and Stocco, 2012). In contrast, AKAP1 associates with the 3′-UTR of the lipoprotein lipase (LPL) mRNA to inhibit LPL translation by a PKA-dependent mechanism (Ranganathan et al., 2002). Finally, AKAP1 can self-associate via its C-terminal KH and Tudor domains. RNA binding to the KH domain is required for AKAP1 self-association; however, the physiological role of AKAP1 self-association has yet to be determined (Rogne et al., 2006).

## 3. Cell physiology

### 3.1. AKAP1 in fat metabolism and bone mineralization

AKAP1 is the most abundant AKAP in adipose tissue (Bridges et al., 2006), and recent evidence links AKAP1 to fat metabolism, cardiovascular function, and obesity. LPL hydrolyzes circulating triglycerides to promote fatty acid and monoacylglycerol uptake into adipose tissue, skeletal muscle, and heart tissue. LPL S447X is a common gain-of-function allele which provides modest cardiovascular protection (Rip et al., 2006). A recent report suggests that the S447X allele may enhance LPL mRNA translation because of reduced binding by the KH domain of AKAP1 (Ranganathan et al., 2012). Furthermore, AKAP1 transcription appears to be differentially regulated in lean and obese patients. Microarray and qPCR analyses of subcutaneous abdominal adipose tissue from an obese group of individuals showed a four-fold reduction in AKAP1 expression when compared to expression levels in a lean group, despite similar calorie and fat intake and exercise levels (Marrades et al., 2010). Perhaps reduced AKAP1 expression in obese individuals contributes to increased fat uptake into adipocytes by disinhibiting LPL translation. In another study, AKAP1 mRNA levels in bone tissue were positively correlated with bone mineral density in growing rats from several strains, indicating AKAP1 may promote bone growth and mineralization (Alam et al., 2010).

### 3.2. AKAP1 signaling in mitochondrial dynamics and neuronal development and survival

Mitochondria are highly dynamic organelles that undergo fission and fusion to change their morphology in a tightly regulated