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PAP7, a PBR/PKA-RIα-associated protein: a new element in the relay of the hormonal induction of steroidogenesis☆

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Abstract

The precise mechanism by which the hormone-induced minimal cAMP levels act at the mitochondria to activate cholesterol transport and steroid synthesis is unknown. We propose that this mechanism involves a macromolecular signaling complex where a newly identified peripheral-type benzodiazepine receptor (PBR)-associated protein (PAP7) binds the regulatory subunit RIα of the cAMP-dependent protein kinase A (PKA), thus allowing for local efficient catalytic activation and phosphorylation of the substrate steroidogenesis acute regulatory protein (StAR), leading to cholesterol transfer from the low affinity StAR to the high affinity PBR cholesterol binding protein. The mouse and human PAP7 proteins were cloned, their genomic organization and chromosomal localization characterized, their tissue distribution evaluated and subcellular localization defined. PAP7 is highly expressed in steroidogenic tissues, where it follows the pattern of PKA-RIα expression and data from a human adrenal disease suggest that it participates in PKA-RIα-mediated tumorigenesis and hormone-independent hypercortisolism. PAP7 is localized in the Golgi and mitochondria and inhibition of PAP7 expression results in reduced hormone-induced cholesterol transport into mitochondria and decreased steroid formation. Taken together, these data suggest that PAP7 functions as an A-kinase anchoring protein (AKAP) critical in the cAMP-dependent steroid formation.

Keywords: Steroidogenesis; Gonads; Adrenal; Carney complex; Mitochondria; Cholesterol transport

1. Introduction

Steroidogenesis is regulated by trophic hormones such as adrenocorticotropic hormone (ACTH) in adrenocortical cells and luteinizing hormone (LH) in testicular Leydig and ovarian cells [1–3]. The primary point of control of the acute stimulation of steroidogenesis by peptide hormones and cAMP involves the first step in this biosynthetic pathway where cholesterol is converted to pregnenolone by the C27 cholesterol side chain cleavage cytochrome P450 enzyme (P450scc) and auxiliary electron transferring proteins, localized on inner mitochondrial membranes (IMM) [1–3]. Pregnenolone then leaves the mitochondrion to undergo enzymatic transformation in the endoplasmic reticulum that will give rise to the final steroid products. Detailed studies have shown that the rate-determining step in the pathway of hormone-stimulated steroid biosynthesis is the transport of the precursor, cholesterol, from intracellular sources into the mitochondria [1–3]. Despite our extensive knowledge on the signal transduction mechanism mediating the action of gonadotropins and ACTH on steroidogenesis, there are still many questions on the mechanisms responsible to transduce the signal received from a small number of the second messenger cAMP molecules to a maximally stimulated cholesterol transfer into mitochondria and steroid production by the cells within minutes. Indeed, the concentrations of hCG needed to induce maximal cAMP synthesis are about 15 times larger than those needed to maximally stimulate testosterone secretion [1]. Thus, it is still unclear how these minimal cAMP levels could result in a cAMP-dependent protein kinase A (PKA)-mediated activation of cholesterol transport and maximal steroid formation.

2. Peripheral-type benzodiazepine receptor (PBR): a mitochondrial high affinity cholesterol binding protein

PBR was originally discovered because it binds the benzodiazepine diazepam with relatively high affinity [4]. PBR, although present in all tissues examined, was found

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to be particularly high in steroid producing tissues, where it was localized primarily in the outer mitochondrial membrane (OMM) [4–6]. In various cell systems, it has been observed that PBR drug ligands stimulate the formation of steroids [4]. With isolated mitochondria, PBR ligands also stimulate the production of pregnenolone. To identify the exact step in mitochondrial pregnenolone formation activated by PBR ligands, the amount of cholesterol present in the OMM and IMM before and after treatment with PBR ligands was quantified. The results obtained demonstrated that PBR ligands stimulated pregnenolone formation by inducing PBR-mediated translocation of cholesterol from the OMM to the IMM. Studies by different laboratories corroborated these observations in ovarian granulosa, adrenal cortical and brain glia steroidogenic cells [5]. Further studies demonstrated that targeted disruption of the PBR gene in Leydig cells resulted in the arrest of cholesterol transport into mitochondria and steroid formation; transfection of the PBR-disrupted cells with a PBR cDNA rescued steroidogenesis [7]. These studies were recently further confirmed using a PBR 7-mer peptide antagonist that we developed [8]. This peptide, when transduced into Leydig cells, inhibited the benzodiazepine-stimulated steroid formation, by directly inhibiting the drug binding as well as the hormone-stimulated steroidogenesis probably by inhibiting the binding of the endogenous PBR ligand.

The role of PBR in cholesterol transport was further clarified by studies employing site-directed mutagenesis of PBR and in vitro expression [9]. From these studies, a region of the cytosolic carboxyl-terminus of the receptor was identified as the cholesterol binding site with the tyrosine residues in this segment as the most critical amino acid conferring the cholesterol binding activity [9,10]. Further in vitro reconstitution experiments unveiled that PBR binds cholesterol with low nanomolar affinity [10,11]. In vivo studies, in which adrenal and ovarian PBR levels were pharmacologically [12,13] or developmentally [14] modulated, further demonstrated that the levels of PBR correlated with the ability of the steroidogenic tissues to form steroids.

Analyzing the cDNA sequence from different mammals have shown that the 18 kDa PBR protein contained 169 amino acids with a high degree of identity (71%) and homology (80%) between the species [4,6]. Hydropathy profile analysis of the amino acid sequences suggested a putative five transmembrane structure that has been experimentally confirmed [15]. By using molecular dynamics simulations based on the amino acid sequences, three-dimensional (3D) models of human and mouse PBR were developed and showed the five transmembrane segments as five α-helices spanning the OMM [16,17]. The models suggested that PBR might function as a channel since it can accommodate a cholesterol molecule in the space delineated by the five helices without serious geometrical modifications. According to these models, PBR would present a hydrophilic, but uncharged, molecular surface on its inner face allowing the amphiphilic cholesterol molecules to cross the OMM within this PBR channel and reach the IMM. Further studies on the structure of the receptor indicated that the 18 kDa mitochondrial PBR protein is organized in clusters of four to six molecules. Addition of hCG to Leydig cells induces a rapid increase in PBR ligand binding [18] and redistribution of PBR molecules in large clusters [19]. These hCG-induced changes and steroid formation were inhibited by a PKA inhibitor [18,19], suggesting the presence of a cAMP-inducible element regulating the PBR structure and function. In subsequent studies, using the R2C Leydig cell line, which produces high levels of steroids in a constitutive manner, we also observed that a cytosolic proteinaceous component regulated the ligand binding ability and function of the mitochondrial PBR [20].

In search of endogenous PBR ligands, porphyrins [21] and a 10 kDa polypeptide, the diazepam binding inhibitor (DBI) [4] were identified. DBI was originally purified from brain by monitoring its ability to displace diazepam from the $GABA_A$ receptors [22]. DBI was also independently purified and characterized for its ability to bind long chain acyl-CoA-esters [23] and it is also known as acyl-CoA binding protein (ACBP). DBI is highly expressed in steroidogenic cells, displaces radiolabeled benzodiazepines in competition studies and can be crosslinked to PBR [5]. Isolated rat and bovine DBI stimulates mitochondrial pregnenolone formation [4,5]. The role of DBI in the acute hormone-stimulated steroidogenesis was conclusively shown by suppressing DBI levels in the hormone-responsive MA-10 [24] and the constitutively steroid producing R2C Leydig cells [20], using a cholesterol-linked phosphorothioate oligodeoxynucleotide antisense to DBI. DBI-depleted cells did not produce steroids, suggesting that the presence of DBI is crucial for the acute stimulation of steroidogenesis by trophic hormones and the constitutive steroid synthesis.

Few PBR-associated proteins (PAPs) were described, located either in the outer and inner membrane of the mitochondria or in the cytosol. McEnery et al. [25] presented evidence indicating that the photolabeled 18 kDa PBR was associated with two proteins of 32 and 30 kDa. They were, respectively, identified as VDAC and the adenine nucleotide transporter (ANT). From these proteins, the 18 kDa PBR is the isoquinoline binding component of the complex [11], whereas the presence of VDAC increases the ability of the 18 kDa protein to bind benzodiazepines [26]. The role of ANT in this complex remains unclear. Recently, a transient PBR expression in hepatic stellate cells has been observed and immunoreactive PBR showed colocalization not only with mitochondrial manganese-dependent superoxide dismutase (MnSOD) but also with ANT1 [27]. However, how these mitochondrial membrane proteins are organized and contribute to cholesterol transport from cytosol to IMM during steroidogenesis still remain unclear. Thus, there must exist certain cytosolic proteins that might interact and regulate these mitochondrial membrane elements.

3. StAR, a unique hormone-induced mitochondrial cholesterol transporter

Initially, steroidogenesis acute regulatory protein (StAR) was described as a rapidly induced 30 kDa phosphoprotein in ACTH-treated rat and mouse adrenocortical cells, and in LH-treated rat corpus luteum and mouse Leydig cells [3,28]. Subsequently, a 37 kDa form of this protein was described, which contains a N-terminal mitochondrial signal sequence [3,28]. StAR protein plays an important role in the process of steroidogenesis by regulating the transport of cholesterol [3,28]. This action of StAR protein on the metabolism of cholesterol was demonstrated in non-steroidogenic (kidney monkey COS-1) cells, where cotransfection of StAR protein, P450scc and adrenotoxin led to a six-fold increase in formation of 3β-hydroxy-5-cholestenoic acid [29]. In gonadal and adrenal cells, StAR protein is newly synthesized in response to trophic hormones and this synthesis parallels the maximal steroid synthesis in response to hormones [30]. The mechanism of hormone action on StAR protein expression is a cAMP-induced process on StAR gene transcription requiring on-going protein synthesis [31]. Expression of 37 kDa StAR precursor in the absence of hormones induced a two- to three-fold increase in progesterone production by MA-10 cells and isolated mitochondria. The cytosolic precursor of StAR protein is cleaved to produce the 30 kDa mitochondrial "mature" StAR protein and its phosphorylated counterparts. This protein processing is believed to occur at the level of the mitochondrial membrane contact sites and to be responsible for cholesterol transport across the mitochondrial membranes [3,32,33]. In addition to mitochondrial import, the role of phosphorylation was indicated to be important for StAR function [34].

Acute stimulation of steroidogenesis is observed both in vitro and in vivo within 5–15 min upon hormone addition [3,35], a time frame close to the half-life of the newly synthesized StAR [3] but not total StAR mRNA and protein [3,30]. Moreover, as noted above it has been shown that StAR protein does not need to enter into mitochondria to stimulate steroidogenesis and may function by activating a mitochondrial receptor or transport mechanism [3,32,33]. The importance of OMM in mediating StAR activity was clearly demonstrated recently in a series of experiments where StAR was linked to proteins part of the mitochondrial protein import system [33]. Disruption of StAR gene in knock-out mice showed that serum testosterone (in the male) and progesterone (in the female) levels as well as GnRH and FSH levels in $StAR^{(-/-)}$ mice did not differ from the wild-type mice, suggesting that gonadal steroidogenesis and development were not suppressed by the absence of StAR and that enough steroids were made to support critical steroid-dependent processes [36]. These data were in contrast to the profound effects observed in adrenal function [36]. These observations, as well as several other data, questioned the role of StAR protein as a unique mitochondrial cholesterol transporter. Indeed, steroidogenesis ceased in the PBR null cells despite the presence of high levels of StAR protein [7] and PBR knock-out mice were embryonic lethal [5]. In addition, hormone-stimulated steroidogenesis was maintained in the presence of nigericin, a K+/H+ exchanger, which greatly inhibits (90%) the hormone-induced accumulation of StAR protein [37]. The recent prediction that StAR is a low affinity (high micromolar) cholesterol binding protein [38] suggests that although it might bind and release fast cholesterol, it may not be able to target cholesterol by itself to the right place in the mitochondria. Taken together, these studies indicate that although the presence of hormone-induced StAR protein in critical in inducing and maintaining continuous steroid formation it might not be limiting and that other OMM components participate in this process to make it limiting. Actually, the close association of StAR protein with PBR in a cell system was recently demonstrated to occur using fluorescence energy transfer procedures [39].

4. Identification of a PBR and PKA-RIα-associated protein, PAP7

Considering the observations of the rapidity of the hormone-induced phenomena, it seemed likely that other, probably cytoplasmic, proteins may participate in or induce the formation of a macromolecular signaling complex at OMM, responsible for transducing the hormonal signal. In search of such a molecule, we applied the yeast two-hybrid technique and screened a mouse testis cDNA library using PBR as bait [40]. Five positive clones, named as PBR-associated proteins, were isolated based on their ability to interact with PBR. PAP3 nucleotide sequence coded for a product identical to the preciously isolated meg1 protein [41]. The other four PAPs, PAP7, PAP8, PAP17 and PAP20, could not been found in Genbank database. PAP7 and PAP17 are different clones of the same protein product. PAP8 and PAP20 are novel genes. When we looked for specialized motifs in the protein sequence all PAPs have fatty acylation (myristoylation) sites and PKC phosphorylation sites. The nucleotide sequences of PAP7 and PAP20 have been deposited in Genbank (accession numbers: AF022770 and AF020338).

Interestingly, when screening a human lymphocyte library with the regulatory subunit RIα of PKA as bait, PAP7 was also isolated as a protein with in vivo selectivity for PKA-RIα [40]. This finding suggested that, perhaps, a PKA-related signal transduction mechanism may target PAP7 to mitochondria rich in PBR. Furthermore, these data provided some new hypotheses about the pathway by which PKA activity regulates steroidogenic proteins, such as StAR, causing changes in PBR function, leading to cholesterol uptake and transport into the mitochondria. Recombinant glutathione-*S*-transferase-PAP7 interacted with both the mitochondrial PBR and cytosolic PKA-RIα in MA-10 Leydig cells, which further confirmed the association between PAP7 and PBR or PKA-RIα [40].

Mouse PAP7 cDNA encodes for a 525 amino acid protein with a calculated molecular mass of approximately 60.2 kDa and an acidic isoelectric point (pI) of 5.02 [41]. In subsequent studies, we identified and characterized a 3572 bp full-length human PAP7 cDNA (GenBank accession number: AY150218; 42) which encodes for a 528 amino acid, 60 kDa protein with pI of 5.05. Alignment analysis showed 87% identity in nucleotide and 85% identity in amino acid sequence between human and mouse PAP7. Sequence motif analysis using Swiss-Prot Prosite profile scan indicated that both human and mouse PAP7 have an ACBP signature motif. As noted above, the endogenous PBR ligand is DBI also known as ACBP. Further analysis demonstrated that both the human and mouse PAP7 have a 30% amino acid identity with human DBI/ACBP [42,43]. Since DBI/ACBP is the only known endogenous PBR ligand, these data further supports the finding that PAP7 interacts with PBR and suggests that PAP7 may act as an endogenous PBR ligand.

Both mouse and human PAP7 gene contained eight exons and seven introns. Almost all of the exons in human and mouse are of the same size. However, the mouse and human introns are completely different. Searching the corresponding contigs from draft human genome database in NCBI GenBank, PAP7 cDNA was found to be highly homologous with human genomic super contig NT_004525, which showed that human PAP7 gene was approximately 48 kb in length. The total mouse 29 kb PAP7 gene sequence was obtained [42] (GenBank accession number: AF501319), whereas the genomic sequence of human PAP7 is much longer [43]. The chromosome localization of human and mouse PAP7 genes were analyzed separately by fluorescence in situ hybridization (FISH) with the BAC clone containing the PAP7 sequence, as probe. The mouse PAP7 gene was mapped to chromosome 1H3–5 and the human was mapped to chromosome 1q32–41 [42,43].

Originally, we reported a 1543 bp mouse PAP7 cDNA encoding for a 445 amino acid protein [40]. However, despite the identification of the full-length PAP7 cDNA encoding for a predicted 60 kDa protein, SDS-PAGE followed by immunoblot analysis revealed in the mouse a major 52–54 kDa [40] and in the human a 56–60 kDa immunoreactive proteins [43], suggesting that the 52–54 kDa mouse protein might be due to an alternative splicing that removes the first 93 amino acids. In support of this hypothesis, we reported that there is an additional shorter PAP7 mRNA transcript in mouse testis [40].

Nearly all of the human tissues and cell lines expressed PAP7 by mRNA dot blot analysis [40,43]. However, in some steroidogenic tissues, such as testis, the expression of PAP7 was high compared to other tissues. In placenta, stomach, heart and the leukemia cell line K-562, PAP7 mRNA was also found to be present at high levels. In agreement with the mRNA data, human PAP7 was found in most tissues examined by immunohistochemistry. PAP7 immunoreactivity was very strong in adrenal cortex, atrium, colon epithelial cells, esophagus, jejunum, renal cortex, testicular Leydig cells, vena cava and ventricular muscle. Liver expressed low level of PAP7 protein, which is consistent with the results of the immunoblot analyses. Central and peripheral nervous system tissues, such as autonomic nerve, caudate nucleus, cerebella cortex, dura mater, peripheral nerve, spinal cord and thalamus, did not show any immunoreactivity for PAP7. Immunoblot also showed that PAP7 is highly expressed in adrenal, ovary, skin and testis [43]. Similar results were observed in the mouse [40]. Mouse PAP7 mRNA was found by Northern blot analysis in adrenal, brain, heart, liver, testis, and ovarian tissues. Interestingly, mouse PAP7 has a 3 kb major mRNA transcript in these tissues and an additional 1.7 kb transcript found only in testis, as discussed above. PAP7 mRNA was also abundant in three cell lines, C6 glioma, MA-10 Leydig, and Y1 adrenocortical, which have been widely used for studying the mechanisms regulating steroid biosynthesis. PAP7 expression level in these cell lines was proportionally correlated with their steroidogenic ability. The levels of PAP7 message in these cell lines parallel the PBR mRNA expression pattern [40]. Immunohistochemistry results showed mouse PAP7 was present in both Leydig and germ cells of the testis, in fasciculata reticularis and glomerulosa cells of the adrenal gland, and theca and granulosa cells of the ovary. In brain, mouse PAP7 immunoreactivity was very strong in the hippocampus and specific neuronal and glial cells of the cortex. Strong immunoreactivity was also found in the paraventricular and superoptic nuclei regions of the hypothalamus. Liver and kidney expressed low levels of PAP7 protein, which showed same expressing pattern to human PAP7. Moreover, in situ hybridization studies indicated that PAP7 mRNA is also highly expressed in brain, adrenal, ovary, and testis [40].

5. PAP7 and GOCAP1

Immunofluorescence confocal microscopy demonstrated that the main subcellular locations of PAP7 are the trans-Golgi apparatus and mitochondria, although its presence at the endoplasmic reticulum cannot be excluded [42]. Interestingly, in a recent search, PAP7 was found to show 89% (in mouse) and 96% (in human) amino acid identity to the recently cloned human Golgi complex associated protein 1 GOCAP1 (accession number: AB043587), a protein involved in the maintenance of Golgi structure and function through its interaction with the integral Golgi protein giantin [44], suggesting that PAP7 and GOCAP1 are the same protein. Our results of immunofluorescence microscopy confirmed the localization of PAP7/GOCAP1 in the trans-Golgi apparatus [42]. However, our result also showed that PAP7/GOCAP1 is also present in mitochondria and possibly in the endoplasmic reticulum as well. The mitochondrial localization of PAP7 is in agreement with the previous data showing an association of PAP7 with the mitochondrial PBR protein [40]. Treatment of steroidogenic cells with a Golgi perturbing drug increased

the amount of PAP7 present in mitochondria and potentiated the hormone-induced steroidogenesis suggesting that cholesterol transport is a dynamic process involving protein-protein interactions and vesicular transport [42,45].

6. PAP7 and Carney complex

Carney complex (CNC) is a multiple neoplasia syndrome caused by germline inactivating PKA-RIα mutations associated with primary pigmented nodular adrenocortical disease (PPNAD) and increased steroid synthesis [46–48]. The complex was first described as an association of lentigines, primary pigmented nodular adrenocortical disease and a variety of other endocrine and non-endocrine tumors. PPNAD is characterized by excessive cortisol production from otherwise small or normal-sized adrenal glands and is associated with a "paradoxical" rise in glucocorticoid urinary excretion in response to the graded administration of dexamethasone during Liddle's test [47]. To date there has been no explanation of this unusual steroidogenic behavior of PPNAD.

Three observations brought us to look closer in the relationship of PAP7 to human adrenal pathology. First, there was the high level of PAP7 protein expression in human adrenal cortex. Second, the recent finding that the tumor suppressor gene encoding PKA-RIα, mapped to chromosome 17q22–24, is mutated in CNC [49,50]. Third, the localization of PAP7 in several tissues targets of CNC, such as pituitary, thyroid, skin, heart and steroidogenic tissues, including both the gonads and adrenal cortex. The latter is affected in CNC by PPNAD, a peculiar adrenal disorder that is characterized by ACTH-independent hypercortisolism that is stimulated by exogenous steroids [47].

PAP7 was found to be tightly linked in its expression profile with PKA-RIα in PPNAD tissues. PAP7 and PKA-RIα protein levels were decreased in PPNAD nodules. However, high levels of both PKA-RIα and PAP7 proteins were seen in the adrenal tissue surrounding the nodules [43]. These studies showed that PAP7 in steroidogenic tissues follows the pattern of PKA-RIα expression, suggesting that it might participate in PKA-RIα-mediated tumorigenesis and hormone-independent hypercortisolism. These findings correspond well with the data suggesting that CNC tumors carrying PKA-RIα mutations respond to cAMP stimulation with an increase in PKA activity compared to adrenal steroidogenic tissues that do not have PKA-RIα mutations [50].

The coordinated regulation of PKA-RIα and PAP7 expression seen in PPNAD tissues was further confirmed in lymphoblastoid cell lines derived from patients with inactivating PKA-RIα mutations [43]. Moreover, the specificity of this type of regulation in adrenal tumors with PKA-RIα mutations is supported by the observations that there was no difference in PAP7 protein expression in cases of adrenal hyperplasia and there was no significant difference between normal and brain, breast and colon tumor PAP7 mRNA levels [43].

We believe that these findings might offer an insight in the understanding, on one hand, of the cause of tumor formation in PPNAD and, on the other hand, the paradoxical responses of its hypercortisolism. Considering that PKA-RIα functions as a tumor suppressor [49], the presence of PKA-RIα mutations in PPNAD would lead to tumor formation, which over time will expand within the tissue. In the mean time, in the adrenal tissue surrounding the nodule, the presence of even relatively low cAMP levels would result in an amplified activation of the PKA, as previously shown [50]. Targeting of the residual PKA-RIα by increased amounts of PAP7 in the appropriate subcellular compartment, i.e. mitochondria, would induce and sustain steroid formation, although in a dysregulated manner with regards to cAMP and other stimuli, leading perhaps to "paradoxical" responses.

7. Role of PAP7 in steroidogenesis

The critical role of PAP7 and the macromolecular complex formed by PAP7 in the mitochondria was shown in a number of key experiments. Overexpression of the full-length PAP7 protein increased the hCG-stimulated steroid synthesis by MA-10 Leydig cells. However, overexpression of the partial PAP7 fragment, which includes the PBR and PKA-RIα binding domains, inhibited hCG-stimulated progesterone formation in MA-10 Leydig cells, indicating that overexpressed partial PAP7 fragment acts as a competitor of endogenous PAP7 having a dominant-negative effect [40]. Since this partial PAP7 fragment has a PBR and PKA-RIα binding domain, it may prevent PBR and/or PKA-RIα from interacting with endogenous PAP7, thus inhibiting cholesterol accumulation into mitochondria and subsequent steroid formation. This also suggests that the partial fragment of PAP7 lacks, or has an ineffective functional domain present as compared to the wild-type protein. In addition, overexpression of the partial PAP7 fragment did not alter the hCG-stimulated cAMP accumulation, indicating that the dominant-negative effect seen is downstream of cAMP synthesis. This effect was subsequently localized at the level of cholesterol transport to P450scc. The role of PAP7 in the hormone-induced steroid formation was further demonstrated using oligonucleotides antisense to PAP7, which specifically inhibited the hCG-stimulated progesterone formation by MA-10 cells [50].

8. Is PAP7 an AKAP?

It has been suggested that PKA-RIα is compartmentalized in Leydig cells so that it has preferential access to endogenously produced cAMP [51]. The compartmentalization of PKA, mediated through the specific binding of R subunits to various organelles, has been proposed as a

mechanism to target the response to cAMP [52]. In this regard, PAP7 is a PKA-RIα anchoring protein targeting the kinase to mitochondria. Anchoring to the mitochondria could be accomplished via PAP7 myristoylation and PAP7 has myristoylation sites [40]. There, PKA could phosphorylate specific protein substrates, such as StAR. Phosphorylation of StAR has been shown to be responsible, in part, for the regulation of steroidogenesis by hormones [34].

Recently a family of specific anchor proteins, named A-kinase anchor proteins (AKAPs), has been described to recruit the PKA holoenzyme close to it substrate proteins or destination organelle. This compartmentalization of PKA may amplify the cAMP mediated hormonal response. More than 50 functionally related but structurally unrelated proteins belong to AKAP family and most of them can bind to PKA-RII [53]. Usually, an AKAP has two conserved domains: a tethering domain for association with PKA and a targeting domain for subcellular location [54]. The structure of PKA has been thoroughly investigated. There are two different forms, the type I with the regulating unit dimer of RIα and RIβ and type II with those of RIIα and RIIβ.

By comparison with AKAP, we found that the function of PAP7 in transducing the cAMP signal is close to that of AKAPs in various signal transduction mechanisms. PAP7's role in the cAMP-PKA pathway is supported by the findings that (1) it was isolated using PKA-RIα as bait in a two-yeast

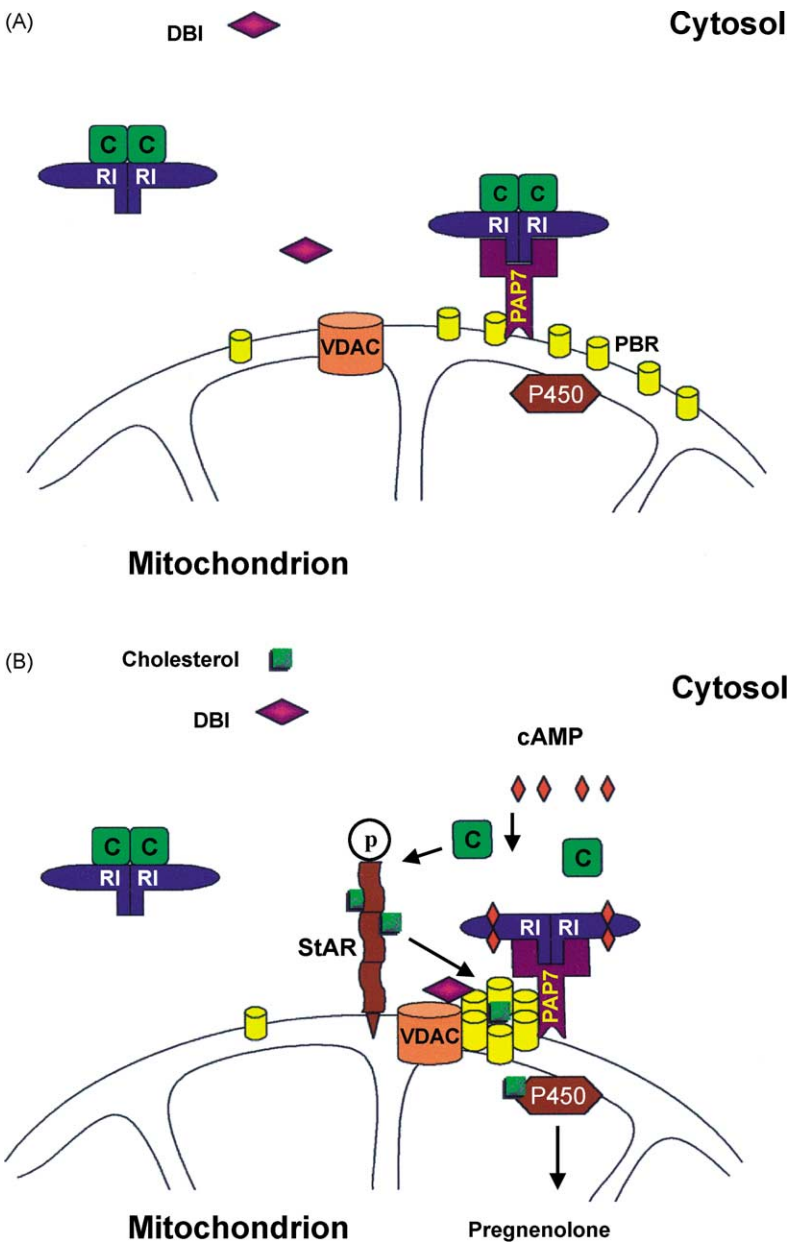

Fig. 1. Schematic representation of the role of PAP7 in the regulation of cholesterol transport into the mitochondria; (A) basal state; (B) hormone-induced state.

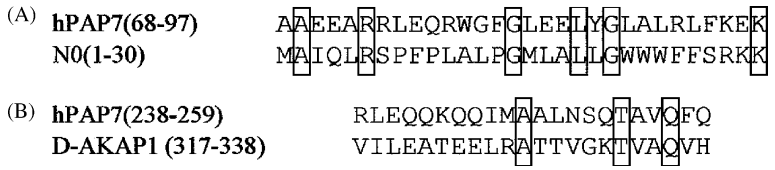

Fig. 2. Alignment of the amino acid sequences of the N0 isoform of D-AKAP1 targeting domain 1–30 and human PAP7 peptide residue 68–97 (A) and the dual type I and type II domain (317–338) of D-AKAP1 and hPAP7 238–259 (B). The boxes show identical residues.

hybridization system, (2) over expression of the partial PAP7 fragment, which includes the PBR and PKA-RIα binding domains inhibited the hCG-stimulated progesterone formation by Leydig cells, and (3) oligonucleotides antisense to PAP7 inhibited the hormone-induced steroid synthesis [40]. In addition, we observed that PAP7 is recruited to the rich in PBR OMM from the trans-Golgi apparatus in a manner associated with increased steroid formation ([45] and unpublished data). Thus, although we have not yet directly proven that PKA-RIα is guided by PAP7 to mitochondria, the data presented suggest that such movement may trigger StAR phosphorylation at the OMM and cholesterol transport. Fig. 1 shows a schematic representation of the role of PAP7 in transducing the cAMP signal to activate cholesterol transport into mitochondria. In the absence of hormonal stimuli, PAP7 is present in the Golgi and mitochondria (Fig. 1A). Hormone-induced cAMP (Fig. 1B) binds to the PKA-RIα associated with PAP7 at sites rich in PBR and where PBR polymers are formed in response to hormone treatment [55] causing the release of the active catalytic domain, which phosphorylates the newly synthesized StAR protein. Phosphorylation modulates the steroidogenic activity of StAR [34]. Cholesterol then is transferred from the low affinity StAR to the high affinity PBR, probably functioning as a cholesterol exchanger against steroid product(s) arising from cytochrome P450 action [55].

Although, most AKAPs have been identified as PKA-RII binding proteins, Taylors' group reported the existence of dual-AKAP1 (D-AKAP1) that can interact with both RI and RII PKA subunits [56–58]. D-AKAP1 contains several different isoforms generated from splicing at the extreme 5′ and 3′ ends [54,58]. These isoforms might contribute to its distinct subcellular distribution. The targeting motif lies at NH2 terminal of each D-AKAP1 protein. Two isoforms, N0 and N1, found to be localized at the mitochondria and endoplasmic reticulum, respectively [54]. The first 30 amino acid residues of N0 define a mitochondria targeting motif [54,58]. N1 carries 33 additional amino acid residues at the amino terminus of N0, and it is believed that these 33 amino acid residues suppress the mitochondrial targeting and expose the ER targeting motif. No homology was found between PAP7 and D-AKAP1. However, a comparison of the 30 amino acids of N0 to PAP7 shows certain homology at residue 68–97 (Fig. 2A), suggesting that PAP7 may be part of the AKAP family of proteins. Interestingly, it has been shown that both type I and type II PKA bind to D-AKAP1 amino acids 317–338, which form the R-binding domain [54,58]. Fig. 2B shows alignment results with D-AKAP R-binding domain and PAP 7, indicating that there are some conserved amino acids present between these AKAPs and PAP7. These findings suggest that PAP7 might belong to the AKAP family of proteins, a suggestion that has to be further proven.

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