Molecular and Cellular Endocrinology 300 (2009) 37–42

Contents lists available at ScienceDirect

Molecular and Cellular Endocrinology

journal homepage: www.elsevier.com/locate/mce

Review

Hormonal activation of a kinase cascade localized at the mitochondria is required for StAR protein activity

Cecilia Poderoso, Paula Maloberti, Alejandra Duarte, Isabel Neuman, Cristina Paz, Fabiana Cornejo Maciel, Ernesto J. Podesta*

Instituto de Investigaciones Moleculares de Enfermedades Hormonales, Neurodegenerativas y Oncológicas (IIMHNO), Department of Biochemistry, School of Medicine, University of Buenos Aires, Paraguay 2155, 5th, C1121ABG Buenos Aires, Argentina

ARTICLE INFO

Article history:
Received 22 August 2008
Received in revised form 7 October 2008
Accepted 10 October 2008

Keywords:
Cholesterol transport
Protein phosphorylation
ERK1/2
MEK1/2
PKA

ABSTRACT

It is known that ERK1/2 and MEK1/2 participate in the regulation of Star gene transcription. However, their role in StAR protein post-transcriptional regulation is not described yet. In this study we analyzed the relationship between the MAPK cascade and StAR protein phosphorylation and function. We have demonstrated that (a) steroidogenesis in MA-10 Leydig cells depends on the specific of ERK1/2 activation at the mitochondria; (b) ERK1/2 phosphorylation is driven by mitochondrial PKA and constitutive MEK1/2 in this organelle; (c) active ERK1/2 interacts with StAR protein, leads to StAR protein phosphorylation at Ser^{232} only in the presence of cholesterol; (d) directed mutagenesis of Ser^{232} (S232A) inhibited *in vitro* StAR protein phosphorylation by ERK1; (e) transient transfection of MA-10 cells with StAR S232A cDNA markedly reduced the yield of progesterone production. We show that StAR protein is a substrate of ERK1/2, and that mitochondrial ERK1/2 is part of a multimeric complex that regulates cholesterol transport.

Contents

1. Introduction

Many human mitochondrial disorders are related to abnormalities in mitochondrial proteins. In steroidogenic tissues, truncation of the Steroidogenic Acute regulatory protein (StAR protein) is associated with the steroid deficiency disease, congenital lipoid adrenal hyperplasia (Lin et al., 1995). StAR protein is a component of a protein complex that functions in the rate limiting step of steroidogenesis (Stocco, 2000), by transporting cholesterol from the outer to the inner mitochondrial membranes. In all steroidogenic tissues, phosphorylation-dependent events are required for the acute stimulation of steroid biosynthesis through the activation of protein kinases (Dufau et al., 1977; Le and Schimmer, 2001; Pezzi et al., 1996; Podesta et al., 1978, 1979; Sala et al., 1979; Schimmer et al., 1977). Among those are the cAMP dependent protein kinase (PKA), the protein kinase C (PKC), the calcium/calmodulin-dependent protein kinase and the mitogen activated protein kinases (MAPKs). Thus hormones, ions or growth factors modulate steroid biosynthesis by the post-translational phosphorylation of proteins. The question still remains as to how phosphorylation events can transmit a specific signal to its mitochondrial site of action.

Although it is known that StAR is a phosphoprotein and that it is involved in the mechanism of action of steroidogenic hormones, the role of StAR protein phosphorylation is not completely understood. The transcription of the Star gene increases in a cAMP (cAMP)-PKA-dependent manner (Stocco, 2000). In addition, the non-genomic

* Corresponding author. Tel.: +54 11 45083672x36; fax: +54 11 4508 3672x31.
E-mail address: biohrdc@fmed.uba.ar (E.J. Podesta).

0303-7207/$ – see front matter © 2008 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.mce.2008.10.009

post-translational effects of PKA have been reported in relationship to StAR protein (Arakane et al., 1997). PKA phosphorylates murine and human StAR proteins at specific motifs like Ser 56/57 and Ser 194/195, respectively (Arakane et al., 1997; Fleury et al., 2004).

In addition to the well-documented role of PKA activation in trophic hormone-stimulated steroid biosynthesis, ERK1/2 and its upstream activator MEK1/2 also participate in the regulation of steroidogenesis through genomic and non-genomic actions (Gyles et al., 2001; Manna et al., 2007; Martinat et al., 2005; Martinelle et al., 2004).

In this paper we will discuss the role of ERK in the regulation of steroid biosynthesis, the phosphorylation of StAR protein by ERK1/2 at the mitochondria and the role of this phosphorylation in StAR protein function and activity.

2. Role of MAPKs in the regulation of steroid biosynthesis

There are several reports studying the role of the members of the MAPKs in the regulation of steroid synthesis both at the levels of genomic and not genomic regulation.

One of the first reports (Gyles et al., 2001) indicated that cAMP-induced steroid synthesis is dependent upon the phosphorylation and activation of ERKs. ERK activation results in enhanced phosphorylation of SF-1 and increased steroid production through increased transcription of the Star gene. They also showed that adenylate cyclase activation with forskolin caused a time-dependent increase in ERK activity and translocation from cytoplasm to nucleus. These findings correlate with an increase in StAR mRNA levels, StAR protein accumulation, and steroidogenesis. Similarly, ERK inhibition led to a reduction in the levels of forskolin-stimulated StAR mRNA, StAR protein, and steroid secretion.

In agreement with Gyles et al., Martinelle et al. (2004) described the involvement of the ERK cascade in human chorionic gonadotropin (hCG)-induced steroidogenesis by primary cultures of immature rat Leydig cell. Their findings indicate that PKA and PKC function as upstream kinases in connection with transduction of the signal from the gonadotropin receptor to the ERK cascade. These MAPKs enhance the stimulatory effects of hCG on the novo synthesis of StAR protein and the activity of protein phosphatase 2A, which are associated with increased androgen production by Leydig cells. Inhibition of MEK1/2 by U0126 suppressed all of these cellular responses to hCG. Martinat et al. (2005) showed also that preincubation of rat primary Leydig cells and the mouse tumoral Leydig cell line MLTC-1 with the MEK1/2 inhibitors U0126 and PD98059 reduced LH-induced steroidogenesis. However they showed that these inhibitors tonically enhance the expression of the StAR protein. In a recent paper Manna et al. (2007) also showed that inhibition of ERK1/2 activity by U0126 increased Star gene expression and decreased progesterone production in MA-10 Leydig cells stimulated with dibutyryl cAMP.

Otis et al. (2007) showed that the inhibition of proliferation and stimulation of hypertrophy induced by Angiotensin II (AII) involve both ERK1/2 and p38 MAPK activation. The increase in cell protein content induced by AII entails formation of cortical actin ring and Rac-dependent activation of ERK1/2 and p38. They also showed that AII-induced activation of ERK1/2 and p38 are implicated in aldosterone secretion by enhancing expression of Star gene and 3β-hydroxysteroid dehydrogenase (Otis et al., 2007). According to Casal et al. (2007), AII activated ERK1/2 within 10 min in bovine adrenocortical glomerulosa cells, a maximal activation was achieved within 30 min and ERK1/2 phosphorylation levels decreased thereafter.

In adrenal zona fasciculata cells, using the Y1 cell line as a system, Le and Schimmer (2001), showed that ACTH increases MEK phosphorylation and MEK activity in wild-type Y-1 cells and in the protein kinase deficient mutant Kin-8. The effect of ACTH on MEK and ERK (5 min incubation) is evidenced in growth arrested cells but not in logarithmically growing cells. In agreement with these results, Lotfi et al. (2000) showed that FGF2 elicits a strong mitogenic response in G_0/G_1-arrested cells with a rapid and transient activation of ERK. Using rats exposed to different ACTH dosage as well as variable duration, Ferreira et al. (2007) showed that ACTH increased adrenal weight and corticosterone levels when compared with control or dexamethasone-treated animals. They also showed that ACTH increases ERKs activation in a time and dose dependent manner. They conclude that chronic ACTH induces ERKs activation and that this plays an important role in the induction of cell proliferation as well as in steroidogenesis (Ferreira et al., 2007).

It is interesting that treatment of MA-10 Leydig cells with phorbol-12-myristate-13-acetate (PMA) to activate PKC enhances Star gene expression (associated with a slight increase in progesterone synthesis) but not its phosphorylation, detected by the use of an antibody that recognizes a PKA-dependent phosphorylation. Inhibition of ERK1/2 activity by U0126 decreased PMA-stimulated Star gene expression (Manna et al., 2007).

It appears that the activation of the MEK1/2–ERK1/2 cascade enhances steroid synthesis. In contrast, whether the MEK1/2, ERK1/2 cascade is necessary for the induction of Star gene expression is less obvious. In addition, although it is known that the activity of StAR protein can be regulated by post-translational phosphorylation, it was not clear until recently that StAR protein is a target for the MEK1/2, ERK1/2 cascade.

In this regard, we recently described the role of MEK1/2–ERK1/2 cascade in the hCG/LH stimulation of StAR protein activity and steroid synthesis (Poderoso et al., 2008). As described by Manna et al. (2007) and Martinelle et al. (2004), we showed that PKA is an upstream kinase in the stimulation of MEK and ERK activities. The effect observed with the inhibitors of MEK1/2 on progesterone synthesis induced by hCG or cAMP were not mediated by inhibition of PKA, since this enzyme remained fully active in the presence of both inhibitors U0126 and PD98095 (Poderoso et al., 2008). These inhibitors had not effect on the activity of the P450scc, since 22R-OH-cholesterol, a freely diffusible analogue of cholesterol, initiated steroid production even when the inhibitors were in the culture medium (Martinelle et al., 2004; Poderoso et al., 2008).

We also showed that active ERK1/2 is necessary for steroidogenesis using a different approach. The overexpression of a wild-type form of ERK2 in MA-10 Leydig cells produced an increase in steroid production stimulated by submaximal concentration of cAMP (Poderoso et al., 2008). An inactive form of ERK2, the H230R variant, which fails to interact with MEK1, but retains the ability to interact with MEK2 in a weakened fashion, did not produce the effect of wild-type ERK2 (Poderoso et al., 2008).

3. MEK1/2 and ERK1/2 at the mitochondria

It is interesting that the inhibitors appear to act at a site in the regulatory pathway after PKA activation and before the transport of cholesterol. Thus, one of the targets may be located at the mitochondria site. Gyles et al. (2001) have observed that activation of adenylate cyclase by forskolin caused a time-dependent increase in ERK activity and translocation from cytoplasm to nucleus. We demonstrated by western blot analysis and confocal studies that in addition to a time-dependent increase in ERK activity and translocation from cytoplasm to nucleus there is a temporal mitochondrial ERK1/2 activation which is obligatory for PKA-mediated steroidogenesis in Leydig cells (Poderoso et al., 2008). It is important to note that the phosphorylation of mitochondrial ERK precedes the

increase in steroid production and that the dose of hormone that elicits ERK activation at the mitochondria is in the order of that needed to elicit steroid synthesis. After stimulation with cAMP, pERK1/2 was located in the cytosol and mitochondria, and to a much lesser extent in the nuclear fractions. In both, the mitochondria and the cytosol, an early peak of ERK1/2 phosphorylation was followed by a slow progressive decrease of the signal during the first hour of cAMP action. hCG stimulation led to pERK1/2 activation with a similar profile to that induced by cAMP. On the contrary, after EGF stimulation, pERK1/2 is mainly localized in the cytosol and nucleus. In mitochondria, activation peaked early (5 min) and then decayed. We also found two different pools of MEK1/2 and pMEK1/2 constitutively present in the cytosol and mitochondria. Interestingly, MEK1/2 responded differently to stimulation depending on distribution (Poderoso et al., 2008).

cAMP clearly induced prolonged MEK1/2 phosphorylation in mitochondria, but had a less significant effect on the cytosolic kinases. Conversely, EGF induced a sustained and robust MEK1/2 activation in the cytosol but only a modest phosphorylation in mitochondria. Although both EGF and cAMP increased total cytosolic MEK1/2, only EGF promoted its phosphorylation in this subcellular fraction.

The increase of mitochondrial pMEK1/2 and pERK1/2 due to cAMP action was abolished by treatment of the cells with the PKA inhibitor H89 and by PKA knockdown experiments (Poderoso et al., 2008). Accordingly, PKA activity in mitochondria showed a clear increase after 5 min of cAMP action in parallel with the appearance of the phosphorylated forms of MEK1/2 and ERK1/2 in the organelles.

It is known that a family of proteins named A-kinase anchor proteins (AKAPs) enhances cAMP-dependent pathways (Feliciello et al., 2001; Rubin, 1994). AKAPs enhance cAMP signal by anchoring PKA near its cellular substrate. AKAP121 derived from mouse tethers PKA to the mitochondrial outer surface (Angelo and Rubin, 2000; Diviani and Scott, 2001). Purified AKAP121 KH domain binds the 3′ untranslated regions of transcripts encoding the Fo-f subunit of mitochondrial ATP synthase and manganese superoxide dismutase (Ginsberg et al., 2003).

In agreement with these results, Li et al. (2001) using the yeast two-hybrid system and PBR/TSPO as bait have previously identified

Fig. 1. (Panel A) Mouse StAR protein aminoacidic sequence: comparison with ERK1/2 consensus docking and phosphorylation sites. (Panel B) StAR protein aminoacidic sequence: comparison of the region 191–281 between species. Ser-Pro residues is marked in bold inside a square; underlined residues belong to the D-domain.

a protein that interacts with PBR/TSPO, the PBR-associated protein PAP7. Using the regulatory subunit R1α of PKA as bait, they also isolated PAP7. It was proposed that PAP7 may function by targeting the PKA isoenzyme to organelles rich in PBR/TSPO i.e. the mitochondria, where phosphorylation of specific substrates may induce the reorganization of PBR/TSPO topography and function. Therefore the possibility of StAR protein being phosphorylated at the mitochondria by the activation of a cascade of kinases, including ERK is quite high.

4. StAR protein as substrate of ERK1/2

Upon examination of the StAR protein structure we found a consensus sequence that would allow the docking of StAR protein to ERK1/2 and a consensus site for ERK1/2 phosphorylation. A typical docking site known as the D domain (**KTK**LTWL**LSI**) was found between amino acids 235 and 244. This site is conserved among ERK1/2 upstream kinase, MAPK phosphatase and ERK substrate (Zhou et al., 2006). Regarding the ERK1/2 phosphorylation site in StAR protein, only two Serine-Proline motifs, targets for ERK1/2 phosphorylation are detectable in the mature form of the murine StAR protein at Ser232 and Ser277 (Fig. 1, panel A). According to the database Expasy Prosite (http://expasy.org/prosite/), Ser232 has a 90% probability of being phosphorylated, whereas the probability of Ser277 is only 5%. Moreover Ser277 is relatively less conserved among species (Fig. 1, panel B). Ser232 (PLAGS232PS) is adjacent to the docking D domain (−2). In support of a predicted StAR–ERK interaction, it was possible to show an interaction of ERK1/2 with StAR protein by treatment of subcellular fractions with pERK-GST. This experiment showed that StAR protein interacts with pERK1 only in the mitochondrial fraction since in the cytosol where both proteins are present in the soluble forms; the pull down experiment does not render a positive result (Fig. 2). In addition, we also showed that MEK phosphorylation via PKA, together with StAR protein and pERK1/2 increase cholesterol transport and mitochondrial synthesis of progesterone in a cell free assay (Poderoso et al., 2008).

To study the phosphorylation of StAR protein by ERK1/2, we performed an *in vitro* phosphorylation assay using the mature pure recombinant StAR protein (30 kDa) and two forms of ERK1, wild type and the inactive mutant K71A. StAR protein was indeed phosphorylated by the wild-type ERK1 and not by the K71A mutant. Interestingly, phosphorylation of StAR protein by wild-type ERK1

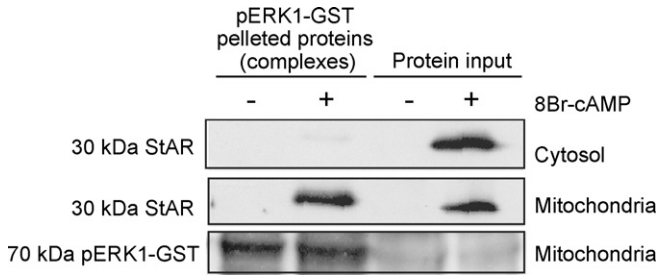

Fig. 2. Interaction between ERK1/2 and StAR protein in mitochondria of MA-10 Leydig cells. MA-10 Leydig cells were treated with or without 8Br-cAMP (0.5 mM) for 3 h. Cytosolic and mitochondrial subcellular fractions were obtained and analyzed with the corresponding markers. The pull down experiment was performed by incubation of each subcellular fraction with or without human pERK1-GST bound to agarose beads. Input and pelleted proteins bound to pERK1-GST (complexes) were analyzed by SDS-PAGE and Western blot. The immunoblots show the bands corresponding to StAR protein and pERK1/2, as loading control. Detailed description of the procedures is published in Materials and methods of Poderoso et al. (2008), where data were originally published as Fig. 4C (Poderoso et al., 2008).

requires the presence of cholesterol (Fig. 3). In the absence of cholesterol, the phosphorylation was notably low. In contrast, StAR protein phosphorylation by PKA is dependent neither on previous ERK phosphorylation nor on the presence of cholesterol.

When a mutated form of StAR protein is used, S232A in which Ser232 was changed to Ala, a non-phosphorylable aminoacid, the mutation impeded the phosphorylation of StAR protein by active ERK, confirming that this residue is indeed the target of the kinase.

Transient transfection of MA-10 Leydig cells with the mutated form of Star cDNA partially blocked the stimulation in progesterone production enhanced by cAMP treatment. Instead, the transfection with a mutant of Star cDNA in which the Ser was replaced to a Glutamic acid (S232E) did not produce such effect, suggesting that the negative charged aminoacid partially mimics the negative charge of the phosphate group present in the phospho-Ser (Poderoso et al., 2008).

In summary, PKA phosphorylates StAR protein and in addition activates the constitutive mitochondrial MEK1/2 pool. Interestingly, constitutive mitochondrial PKA-phosphorylated MEK1/2 preferentially activates a non-phosphorylated mitochondrial pool of ERK1/2 when the three kinases interact at the outer mitochondrial membrane, a crucial site of the steroidogenic process. ERK1/2 at the mitochondria has a functional interaction with StAR pro-

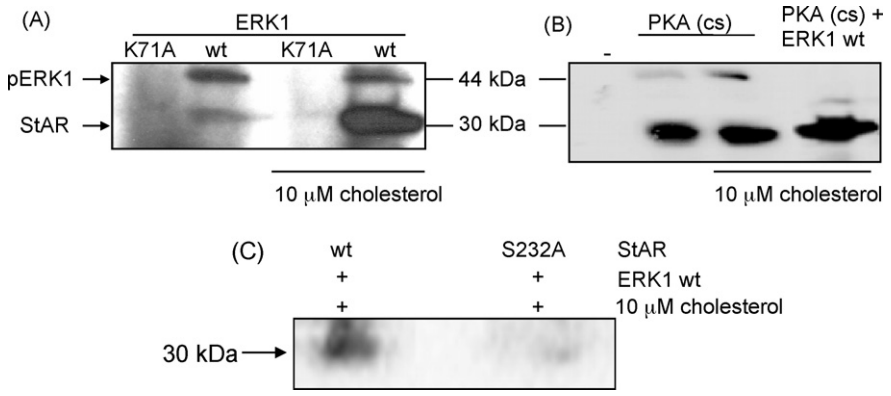

Fig. 3. *In vitro* phosphorylation of StAR protein. The *in vitro* phosphorylation assay was performed using ^{32}P-γ-ATP as phosphate donor; wild type or S232A mutated StAR protein as substrate; ERK1 and/or PKA as kinases. Phosphorylated protein levels were analyzed by SDS-PAGE and autoradiography. (Panel A) The phosphorylation assay was performed in the presence of two variants of ERK1: constitutively active His tagged wild-type ERK1 and the mutated inactive form of ERK1 (K71A), in the presence and absence of cholesterol. (Panel B) The phosphorylation assay was performed using PKA alone or together with constitutively active His tagged wild-type ERK1, in the presence and absence of cholesterol. (Panel C) The phosphorylation assay was performed in the presence of 10 μM of cholesterol using wild type and the mutated form (S232A) of StAR protein as substrate, constitutively active His tagged wild-type ERK1 as kinase. Detailed description of the procedures is published in Materials and methods of Poderoso et al. (2008), where data were originally published as Figs. 5A, B and D (Poderoso et al., 2008).

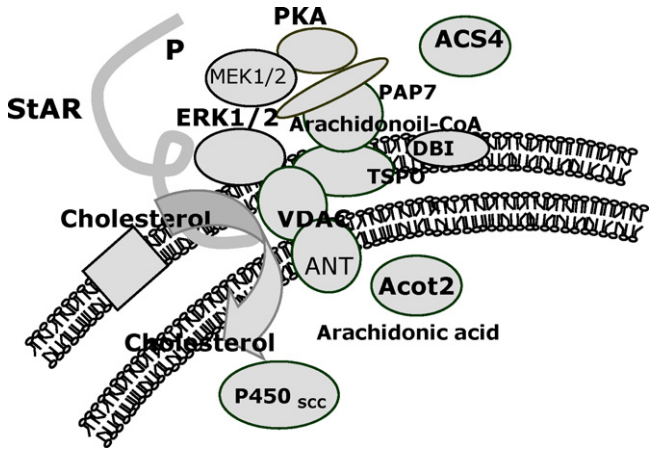

Fig. 4. Scheme of the organization of several proteins involved in the regulation of steroidogenesis.

tein, MEK1/2 and PKA, thus forming a mitochondrial multi-complex (Fig. 4).

The presence of a multi-protein complex in the outer mitochondrial membrane has functional repercussion for steroidogenesis. In this complex, phosphorylation of StAR protein by ERK1/2 is a key process for cholesterol transport. It is known that StAR protein works together with TSPO which in turn interacts with these proteins. It will be very interesting to further analyze the role of Ser232-StAR protein phosphorylation in the assembly of this multi-protein complex and its functions.

StAR protein phosphorylation by ERK1/2 is not dependent on previous StAR protein phosphorylation by PKA but requires the presence of cholesterol. It has been suggested that StAR protein is a molten globule that changes its carboxi-terminal helix when cholesterol approaches the hydrophobic surface (Miller, 2007; Petrescu et al., 2001). Cholesterol has been shown to act as an allosteric modulator, facilitating further binding of StAR protein to the ligand (Petrescu et al., 2001). However at a certain moment when cholesterol needs to reach the P450scc, it will be necessary to release cholesterol from the StAR protein–TSPO interaction. This will allow the transfer of cholesterol from the outer to the inner mitochondrial membrane, to reach the high rate of pregnenolone synthesis. Since ERK phosphorylation of StAR protein requires cholesterol, it can be suggested that StAR protein phosphorylation at Ser232 occurs after cholesterol–StAR interaction. Therefore it may be possible that the negative charge at the Ser232 site will produce a conformational change in StAR protein reducing its affinity for cholesterol. This will allow cholesterol transport across the mitochondrial membrane to produce the high rate of pregnenolone synthesis. It will be very interesting to study this hypothesis in the near future.

5. Concluding remarks

It is clear that StAR protein is a physiological substrate of PKA, and that this phosphorylation is required for its function. It is now, also clear that the activation of the PKA, MEK, ERK cascade at the mitochondria, which results in StAR protein phosphorylation by ERK at Ser232, is also very important for StAR protein function and activity. However, the question that still remains to be answered is whether StAR protein is also phosphorylated in steroidogenic tissues that are stimulated by a cAMP-independent activation. This became a very important issue in steroidogenesis, due to the importance of StAR protein phosphorylation for its function.

StAR protein is one of the components of a multiprotein complex located at the mitochondria, composed also by ACS4, Acot2, PKA, MEK1/2, ERK1/2, VDAC and TSPO (Fig. 4). ACS4 and Acot2 are involved in the release of arachidonic acid, a key step for the activation of Star DNA transcription. In turn, PKA, MEK1/2 and ERK1/2 are responsible for the double phosphorylation of StAR that directly interacts with VDAC, an annion channel (data not shown and Bose et al., 2008).

It would very interesting to analyze whether and how this double phosphorylation affects StAR protein structure in the multimeric complex and cholesterol transport.

Acknowledgements

This work was supported by grants of CONICET (Consejo Nacional de Investigaciones Cientificas y Técnicas, National Research Council), UBA (Universidad de Buenos Aires, University of Buenos Aires), ANPCyT (Agencia National de Promoción Científica y Técnica, National Agency of Scientific and Technical Promotion).

Thanks are due to Drs. Cecilia Poderoso, Daniela P. Converso, Paula Maloberti, Alejandra Duarte, Isabel Neuman, Soledad Galli, Fabiana Cornejo Maciel, Cristina Paz, María C. Carreras, Juan J. Poderoso, Ernesto J. Podestá for allowing the inclusion of data of their publication entitled “A Mitochondrial Kinase Complex Is Essential to Mediate an ERK1/2-Dependent Phosphorylation of a Key Regulatory Protein in Steroid Biosynthesis”.

References

Angelo, R.G., Rubin, C.S., 2000. Characterization of structural features that mediate the tethering of caenorhabditis elegans protein kinase A to a Novel A kinase anchor protein. Insights into the anchoring of PKAI isoforms. J. Biol. Chem. 275, 4351–4362.

Arakane, F., King, S.R., Du, Y., Kallen, C.B., Walsh, L.P., Watari, H., Stocco, D.M., Strauss III, J.F., 1997. Phosphorylation of steroidogenic acute regulatory protein (StAR) modulates its steroidogenic activity. J. Biol. Chem. 272, 32656–32662.

Bose, M., Whittal, R.M., Miller, W.L., Bose, H.S., 2008. Steroidogenic activity of StAR requires contact with mitochondrial VDAC1 and phosphate carrier protein. J. Biol. Chem. 283, 8837–8845.

Casal, A.J., Ryser, S., Capponi, A.M., Wang-Buholzer, C.F., 2007. Angiotensin II-induced mitogen-activated protein kinase phosphatase-1 expression in bovine adrenal glomerulosa cells: implications in mineralocorticoid biosynthesis. Endocrinology 148, 5573–5581.

Diviani, D., Scott, J.D., 2001. AKAP signaling complexes at the cytoskeleton. J. Cell Sci. 114, 1431–1437.

Dufau, M.L., Tsuruhara, T., Horner, K.A., Podesta, E., Catt, K.J., 1977. Intermediate role of adenosine 3′:5′-cyclic monophosphate and protein kinase during gonadotropin-induced steroidogenesis in testicular interstitial cells. Proc. Natl. Acad. Sci. U.S.A. 74, 3419–3423.

Feliciello, A., Gottesman, M.E., Avvedimento, E.V., 2001. The biological functions of A-kinase anchor proteins. J. Mol. Biol. 308, 99–114.

Ferreira, J.G., Cruz, C.D., Neves, D., Pignatelli, D., 2007. Increased extracellular signal regulated kinases phosphorylation in the adrenal gland in response to chronic ACTH treatment. J. Endocrinol. 192, 647–658.

Fleury, A., Mathieu, A.P., Ducharme, L., Hales, D.B., LeHoux, J.G., 2004. Phosphorylation and function of the hamster adrenal steroidogenic acute regulatory protein (StAR). J. Steroid Biochem. Mol. Biol. 91, 259–271.

Ginsberg, M.D., Feliciello, A., Jones, J.K., Avvedimento, E.V., Gottesman, M.E., 2003. PKA-dependent binding of mRNA to the mitochondrial AKAP121 protein. J. Mol. Biol. 327, 885–897.

Gyles, S.L., Burns, C.J., Whitehouse, B.J., Sugden, D., Marsh, P.J., Persaud, S.J., Jones, P.M., 2001. ERKs regulate cyclic AMP-induced steroid synthesis through transcription of the steroidogenic acute regulatory (StAR) gene. J. Biol. Chem. 276, 34888–34895.

Le, T., Schimmer, B.P., 2001. The regulation of MAPKs in Y1 mouse adrenocortical tumor cells. Endocrinology 142, 4282–4287.

Li, H., Degenhardt, B., Tobin, D., Yao, Z.-x., Tasken, K., Papadopoulos, V., 2001. Identification, localization, and function in steroidogenesis of PAP7: a peripheral-type benzodiazepine receptor- and PKA (RI{alpha})-associated protein. Mol. Endocrinol. 15, 2211–2228.

Lin, D., Sugawara, T., Strauss III, J.F., Clark, B.J., Stocco, D.M., Saenger, P., Rogol, A., Miller, W.L., 1995. Role of steroidogenic acute regulatory protein in adrenal and gonadal steroidogenesis. Science 267, 1828–1831.

Lotfi, C.F., Lepique, A.P., Forti, F.L., Schwindt, T.T., Eichler, C.B., Santos, M.O., Rebustini, I.T., Hajj, G.N., Juliano, L., Armelin, H.A., 2000. Proliferative signaling initiated in ACTH receptors. Braz. J. Med. Biol. Res. 33, 1133–1140.

Manna, P.R., Jo, Y., Stocco, D.M., 2007. Regulation of Leydig cell steroidogenesis by extracellular signal-regulated kinase 1/2: role of protein kinase A and protein kinase C signaling. J. Endocrinol. 193, 53–63.

Martinat, N., Crepieux, P., Reiter, E., Guillou, F., 2005. Extracellular signal-regulated kinases (ERK) 1, 2 are required for luteinizing hormone (LH)-induced steroidogenesis in primary Leydig cells and control steroidogenic acute regulatory (StAR) expression. Reprod. Nutr. Dev. 45, 101–108.

Martinelle, N., Holst, M., Soder, O., Svechnikov, K., 2004. Extracellular signal-regulated kinases are involved in the acute activation of steroidogenesis in immature rat Leydig cells by human chorionic gonadotropin. Endocrinology 145, 4629–4634.

Miller, W.L., 2007. StAR search—what we know about how the steroidogenic acute regulatory protein mediates mitochondrial cholesterol import. Mol. Endocrinol. 21, 589–601.

Otis, M., Campbell, S., Payet, M.D., Gallo-Payet, N., 2007. The growth-promoting effects of angiotensin II in adrenal glomerulosa cells: an interactive tale. Mol. Cell. Endocrinol. 273, 1–5.

Petrescu, A.D., Gallegos, A.M., Okamura, Y., Strauss III, J.F., Schroeder, F., 2001. Steroidogenic acute regulatory protein binds cholesterol and modulates mitochondrial membrane sterol domain dynamics. J. Biol. Chem. 276, 36970–36982.

Pezzi, V., Clark, B.J., Ando, S., Stocco, D.M., Rainey, W.E., 1996. Role of calmodulin-dependent protein kinase II in the acute stimulation of aldosterone production. J. Steroid Biochem. Mol. Biol. 58, 417–424.

Poderoso, C., Converso, D.P., Maloberti, P., Duarte, A., Neuman, I., Galli, S., Maciel, F.C., Paz, C., Carreras, M.a.C., Poderoso, J.J., PodestÃ¡, E.J., 2008. A mitochondrial kinase complex is essential to mediate an ERK1/2-dependent phosphorylation of a key regulatory protein in steroid biosynthesis. PLoS ONE 3, e1443.

Podesta, E.J., Dufau, M.L., Solano, A.R., Catt, K.J., 1978. Hormonal activation of protein kinase in isolated Leydig cells. Electrophoretic analysis of cyclic AMP receptors. J. Biol. Chem. 253, 8994–9001.

Podesta, E.J., Milani, A., Steffen, H., Neher, R., 1979. Steroidogenesis in isolated adrenocortical cells. Correlation with receptor-bound adenosine e 3′:5′-cyclic monophosphate. Biochem. J. 180, 355–363.

Rubin, C.S., 1994. A kinase anchor proteins and the intracellular targeting of signals carried by cyclic AMP. Biochim. Biophys. Acta 1224, 467–479.

Sala, G.B., Hayashi, K., Catt, K.J., Dufau, M.L., 1979. Adrenocorticotropin action in isolated adrenal cells. The intermediate role of cyclic AMP in stimulation of corticosterone synthesis. J. Biol. Chem. 254, 3861–3865.

Schimmer, B.P., Tsao, J., Knapp, M., 1977. Isolation of mutant adrenocortical tumor cells resistant to cyclic nucleotides. Mol. Cell. Endocrinol. 8, 135–145.

Stocco, D.M., 2000. The role of the StAR protein in steroidogenesis: challenges for the future. J. Endocrinol. 164, 247–253.

Zhou, T., Sun, L., Humphreys, J., Goldsmith, E.J., 2006. Docking interactions induce exposure of activation loop in the MAP kinase ERK2. Structure 14, 1011–1019.