Molecular and Cellular Endocrinology ■■ (2015) ■■–■■

Contents lists available at ScienceDirect

Molecular and Cellular Endocrinology

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

Review

The role of mitochondrial fusion and StAR phosphorylation in the regulation of StAR activity and steroidogenesis

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ARTICLE INFO

Article history:
Received 10 October 2014
Received in revised form 12 December 2014
Accepted 13 December 2014
Available online

Keywords:
ERK-dependent StAR phosphorylation
Mitochondrial dynamics
Mitochondrial phosphatases

ABSTRACT

The steroidogenic acute regulatory (StAR) protein regulates the rate-limiting step in steroidogenesis, *i.e.* the delivery of cholesterol from the outer (OMM) to the inner (IMM) mitochondrial membrane. StAR is a 37-kDa protein with an N-terminal mitochondrial targeting sequence that is cleaved off during mitochondrial import to yield 30-kDa intramitochondrial StAR. StAR acts exclusively on the OMM and its activity is proportional to how long it remains on the OMM. However, the precise fashion and the molecular mechanism in which StAR remains on the OMM have not been elucidated yet. In this work we will discuss the role of mitochondrial fusion and StAR phosphorylation by the extracellular signal-regulated kinases 1/2 (ERK1/2) as part of the mechanism that regulates StAR retention on the OMM and activity.

Contents

Abbreviations: StAR, steroidogenic acute regulatory; OMM, outer mitochondrial membrane; IMM, inner mitochondrial membrane; ERK1/2, extracellular signal-regulated kinases 1/2; LH, luteinizing hormone; FSH, follicle-stimulating hormone; ACTH, adrenocorticotropin hormone; Ang II, angiotensin II; hCG, human chorionic gonadotropin; CAH, congenital adrenal hyperplasia; PKA, cAMP-dependent protein kinase A; PKC, protein kinase C; IMS, mitochondrial intramembranous space; VDAC1, voltage dependent anion channel; ATAD3a, ATPase family AAA domain containing 3A; MAPKs, mitogen activated protein kinases; AKAPs, A-kinase anchor proteins; 8Br-cAMP, 8-bromoadenosine 3′,5′-cyclic monophosphate; MKP, mitogen-activated protein kinase phosphatase; pERK, phospho-ERK; GST, glutathione S-transferase; MEK1/2, mitogen-activated protein kinase kinase 1/2; SHP2, src homology 2-containing phosphotyrosine phosphatase 2; ACSL4, Acyl-CoA synthetase 4; AA, arachidonic acid; AA-CoA, arachidonoyl-CoA; PP2A, serine/threonine 2A; Mfn, mitofusin; ER, endoplasmic reticulum; OPA1, optic atrophy 1; MAM, mitochondria associated membrane; Drp1, dynamin related protein 1.

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http://dx.doi.org/10.1016/j.mce.2014.12.011
0303-7207/

1. Introduction

The regulation of steroidogenesis is predominantly controlled by pituitary-synthesized trophic hormones, the luteinizing (LH) (Dufau, 1998), the follicule-stimulating (FSH) and adrenocorticotropin hormones (ACTH), angiotensin II (Ang II) (Gallo-Payet and Battista, 2014; Manna and Stocco, 2005) and many other factors, such as cytokines (Bornstein et al., 2004; Haidan et al., 2000) and growth factors (Manna and Stocco, 2005). Normal function of both ovary and testis is long recognized to be dependent on LH and FSH. Primarily, LH acts on internal theca in the ovary and in Leydig cells in the testis, while FSH regulates the function of granulosa cells in the ovary and Sertoli in the testis. Ang II is considered to be the main hormonal stimulus of the zona glomerulosa, whereas ACTH is the main stimulus of both zona fasciculata and reticularis of the adrenal cortex.

Please cite this article in press as: Ana F. Castillo, Ulises Orlando, Katia E. Helfenberger, Cecilia Poderoso, Ernesto J. Podesta, The role of mitochondrial fusion and StAR phosphorylation in the regulation of StAR activity and steroidogenesis, Molecular and Cellular Endocrinology (2015), doi: 10.1016/j.mce.2014.12.011

The classical mechanism utilized in trophic hormone-responsive steroid synthesis consists of an increase in the activation of a second messenger, which in turn, triggers a regulatory cascade resulting in the mobilization and delivery of cholesterol from the outer (OMM) to the inner (IMM) mitochondrial membrane (Crivello and Jefcoate, 1980; Privalle et al., 1983). Indeed, intramitochondrial cholesterol transport is the rate-limiting and regulating step in steroidogenesis, and is dependent on *de novo* protein synthesis, as inhibitors of protein synthesis have been shown to decrease the steroidogenic response (Davis and Garren, 1968; Ferguson, 1963).

This review will focus on the current level of understanding concerning the regulation of cholesterol transport and the role of protein phosphorylation with a view to prospective research. It will discuss viewpoints that may help to unravel the mechanisms governing Steroidogenic Acute Regulatory protein (StAR) retention in the OMM and steroidogenesis.

2. Steroidogenic Acute Regulatory protein (StAR)

StAR protein was discovered in search of the factor that triggers the rate-limiting and regulating step in acute steroidogenesis (Alberta et al., 1989; Krueger and Orme-Johnson, 1983; Pon et al., 1986; Stocco and Sodeman, 1991). The purification of the protein and the cloning and sequencing of the murine cDNA were accomplished in 1994 (Clark et al., 1994). In the following years, both the mouse and human structural genes for StAR and this amino acidic sequence (284 residues in mouse, 285 residues in human) were isolated and characterized (Clark et al., 1994; Sugawara et al., 1995). These characterizations allowed for the study of transcription regulation and structural analysis of StAR.

An analysis of several publications on the matter reveals a close correlation between StAR gene tissue-specific expression and tissue capacity to produce steroids, which indicates StAR specific role in steroidogenesis. Transient transfection experiments demonstrated that StAR cDNA-derived protein expression in MA-10 cells increased steroid synthesis in the absence of LH or human chorionic gonadotropin (hCG). In addition, transient transfection of COS-1 cells with StAR cDNA increased the conversion of cholesterol to pregnenolone (Lin et al., 1995; Stocco and Clark, 1996; Sugawara et al., 1995).

The crucial role of StAR in the regulation of steroidogenesis has been inferred from patients suffering from lipoid congenital adrenal hyperplasia (lipoid CAH), an autosomal recessive disorder in which both adrenal and gonadal steroid biosynthesis is severely impaired due to mutations in the StAR gene (Bose et al., 1996; Lin et al., 1995). The targeted disruption of the StAR gene in mice resulted in a phenotype that is essentially identical to that found in lipoid CAH in humans (Caron et al., 1997; Hasegawa et al., 2000). StAR expression, activation and extinction are regulated by protein kinase A (PKA), protein kinase C (PKC), as well as a host of other signaling pathways (Manna and Stocco, 2005; Stocco and Clark, 1996).

3. The role of StAR in acute steroidogenic response: retention in the OMM and steroidogenesis

StAR mechanism of action has been extensively studied, although its complete understanding remains elusive. StAR is synthesized as a 37-kDa protein, imported into mitochondria via a typical mitochondrial leader sequence and cleaved to a 30-kDa intramitochondrial form (Allen et al., 2006; Granot et al., 2007a; Jefcoate, 2002; Miller, 2013). Upon reaching the matrix, the 30-kDa StAR is degraded, with a half-life of 4–5 hours (Granot et al., 2007a; Midzak et al., 2011). Inside the matrix, StAR protein levels are controlled by the ATP-dependent Lon protease (Granot et al., 2007b). It has been recently pointed out that acute accumulation of StAR protein in the matrix provokes a "mitochondria to nucleus" signaling which, in turn, activates selected transcription of genes encoding mitochondrial proteases relevant for enhanced clearance of StAR (Bahat et al., 2014). Moreover, we have observed that the presence of mitochondrial extracellular signal-regulated kinases (ERK) is strictly necessary for protecting StAR from unknown proteases to avoid further degradation, which constitutes one of the mechanisms involved in the regulation of mitochondrial StAR levels (Duarte et al., 2014).

It was initially suggested that 37-kDa StAR is a "precursor" and that 30-kDa StAR is the biologically active "mature form". Crystallography has indicated that 30-kDa StAR acts in the intramembranous space (IMS) to shuttle cholesterol from the OMM to the IMM (Tsujishita and Hurley, 2000). Additional data have also proposed that IMM proteolysis in adrenal cells is essential in cholesterol fluxes, with a role of StAR in the IMM (Artemenko et al., 2001; Jefcoate, 2002). In addition, it has been shown that disrupting mitochondrial membrane potential ($\Delta\Psi m$) prevents the appearance of 30-kDa StAR in the mitochondria and inhibits steroidogenesis, which suggests that StAR import into the mitochondrial matrix and its subsequent processing are necessary for normal steroidogenesis (Allen et al., 2006; Artemenko et al., 2001; Granot et al., 2007a).

However, deletion of 62 N-terminal amino acids results in a cytoplasmic form of StAR (N-62 StAR) which retains complete activity and appears to interact with the OMM (Black et al., 1994; Miller, 2007). Moreover, when expressed in cytoplasm or added to mitochondria *in vitro*, both the 37- and 30-kDa StAR forms are equally active (Arakane et al., 1996).

It was suggested that StAR mitochondrial leader serves to target the C-terminus of StAR to unidentified receptors or effectors on the OMM, *i.e.* that the mitochondrial import machinery is important in increasing the local effective concentration of StAR on the OMM, thus transiently augmenting StAR action before it is terminated by StAR import into the mitochondria (Arakane et al., 1998; Miller and Strauss, 1999). This model suggests that the active form of StAR is a partially unfolded form, where an N-terminal domain enters the mitochondria, whereas the partially unfolded molten globule form of the C-terminus interacts with the OMM. Direct evidence has been presented that StAR exists as such a molten globule. Limited proteolysis has identified an N-terminal domain that retains a significant degree of structure, while the C-terminal domain is less tightly folded at the low pH that StAR may experience at the mitochondrial membrane. These data suggest that this tightly folded N-terminal domain makes StAR pause as it enters the mitochondria, increasing the opportunity for the C-terminus to exert its activity.

StAR is constitutively active when immobilized on the OMM, but it is inactive when localized to the IMS or to the matrix (Bose et al., 2002). Cells expressing StAR immobilized on the outside of the OMM by fusion to the C-terminus of Tom20 achieve maximal constitutive steroidogenesis, while StAR bound to Tim9 in the IMS is inactive. The Tim9/StAR protein was found to be active with steroidogenic mitochondria *in vitro*, which shows that this protein was inactive in transfected cells because of its IMM location, not because of its structure. Similarly, StAR was also inactive when localized in the mitochondrial matrix (Bose et al., 2002).

Mitochondrial protein-import assays of StAR with a modified leader peptide confirmed that StAR acts exclusively on the OMM: slowing down StAR mitochondrial entry produces an increase in activity, whereas speeding its entry up generates a decrease. Again, StAR activity was determined by its occupancy time on the OMM.

These data further demonstrate that StAR acts exclusively on the OMM (Arakane et al., 1996; Bose et al., 2002), and its activity in promoting steroidogenesis is proportional to its residency time in such location (Bose et al., 2002). Thus, it is StAR cellular localization, not its cleavage, that determines whether it is active or not. StAR has a sterol-binding pocket that accommodates a single molecule of

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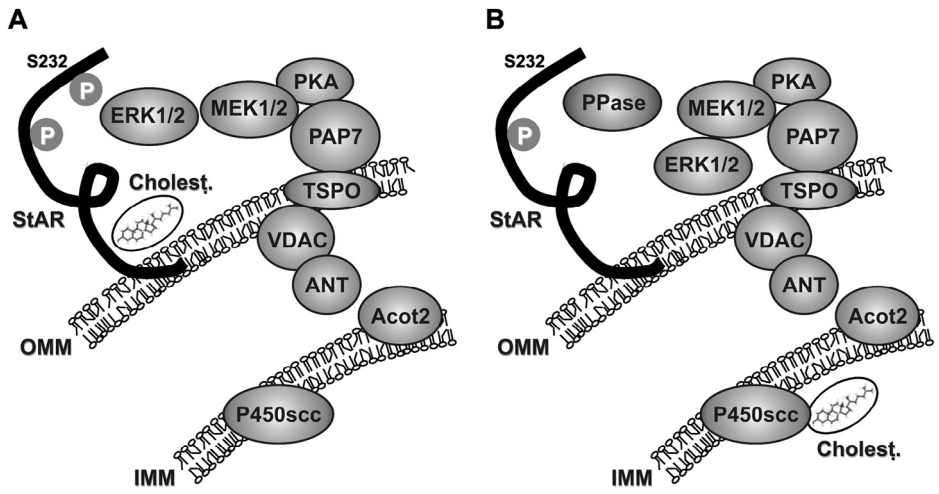

Fig. 1. Summary of the proposed mechanism for the StAR phospho-dephosphorylation cycle and cholesterol delivery to the IMM. (A) After hormone stimulation, StAR is translocated to the OMM in a mitochondrial fusion dependent-manner, phosphorylated by PKA and subsequently binds a molecule of cholesterol in its hydrophobic pocket. Then, cholesterol-bound StAR is available for ERK mitochondrial phosphorylation in Ser232, increasing StAR activity and cholesterol transport. This mechanism may be facilitated by conformational changes in StAR molecule that could allow cholesterol release in P450scc proximities and its further conversion to pregnenolone. (B) Then, StAR may be dephosphorylated by mitochondrial phosphatases and probably become available to bind another cholesterol molecule and to be phosphorylated in Ser232 by ERK again. After the temporal frame of ERK activity, StAR might be translocated to the mitochondrial matrix and degraded by proteases. This StAR activity cycle could enable the metabolism of many molecules of cholesterol *per unit* of StAR protein. Abbreviations: S232 (Ser232, StAR residue phosphorylated by ERK), Cholest. (cholesterol), TSPO (translocator protein 18 kDa), PAP7 (TSPO-associated protein 7), VDAC (voltage-dependent anion channel), ANT (adenine nucleotide translocase), Acot2 (acyl-CoA thioesterase 2), PPase (phosphatase), P450scc (cytochrome P450 side chain cleavage).

cholesterol (Tsujishita and Hurley, 2000), and the interaction of StAR with the OMM involves conformational changes (Baker et al., 2005; Bose et al., 1999) necessary for StAR to accept and discharge cholesterol molecules.

Although N-62 StAR does not enter the mitochondria, immunoelectron microscopy has shown that relatively few StAR molecules are associated with the OMM (Arakane et al., 1996, 1998). These few molecules would be thus required to transport several cholesterol molecules, while StAR only binds one. This suggests that StAR should transport more than one cholesterol molecule before entering the mitochondria and be processed.

To mediate cholesterol transport to the IMM, StAR interacts with the voltage dependent anion channel (VDAC1) in the OMM and also interacts with the ATPase family AAA domain containing 3A (ATAD3a) protein in the IMM, forming a core of a larger multiprotein complex that mediates cholesterol import (Bose et al., 2008; Martin et al., 2014; Papadopoulos and Miller, 2012; Rone et al., 2012) (Fig. 1). These data are in agreement with mitochondrial membrane contact sites formation to facilitate StAR cholesterol delivery to the IMM (Stocco and Clark, 1996). Nevertheless it remains unclear which role each protein plays in the mitochondrial import of cholesterol. In this regard, the role of the translocator protein 18 kDa (TSPO), included in the mitochondrial protein complex, has been questioned and even discarded from the cholesterol transport mechanism (Morohaku et al., 2013).

Despite the numerous studies that have further established that StAR acts on the OMM, the mechanisms governing StAR retention on the OMM and steroidogenesis remain unclear.

4. Functional phosphorylation of StAR by PKA and ERK

In all steroidogenic tissues, phosphorylation-dependent events are required for the acute stimulation of steroid biosynthesis through the activation of protein kinases. Among those are PKA, PKC, the calcium/calmodulin-dependent protein kinase and the mitogen activated protein kinases (MAPKs). In this way, hormones, ions or growth factors modulate steroid biosynthesis by the post-translational phosphorylation of proteins.

Protein phosphorylation is an integral component of signal transduction pathways within eukaryotic cells and is regulated by the fine interplay of protein kinases and phosphatases. In turn, numerous cellular responses are regulated by the reversible phosphorylation of serine (Ser), threonine (Thr) and tyrosine (Tyr) residues. Although StAR has been recognized as a phosphoprotein for many years now, the regulation of StAR phosphorylation by different kinases and the function of this modification in StAR mechanisms of action, import and processing remain unclear.

PKA is associated with the mitochondria and it is known that a family of proteins named A-kinase anchor proteins (AKAPs) enhances cAMP-dependent pathways (Feliciello et al., 2001; Rubin, 1994) by anchoring PKA near its cellular substrate. Particularly, AKAP121 tethers PKA to the mitochondrial outer surface (Angelo and Rubin, 2000; Diviani and Scott, 2001). StAR protein contains two consensus sites for phosphorylation by PKA which are conserved across all species and in which the amino acid sequence of StAR has been determined, namely Ser55/56/57 and Ser194/195. The phosphorylation of Ser194/195 (murine and human respectively) by PKA has been identified as an obligatory event in the regulation of StAR function (Arakane et al., 1997; Fleury et al., 2004). Interestingly, a recent work shows that KO mice for StAR Ser194 completely abrogated StAR function in adrenal gland and testis, clearly indicating that this residue is essential for normal StAR activity (Sasaki et al., 2014). In this regard, Dyson et al. (2008) have established that a mitochondrial AKAP121 binds type II PKA and enhances StAR-mediated steroidogenesis in MA-10 mouse Leydig tumor cells.

The overexpression of ERK2 wild type (WT) has resulted in an increase in progesterone production stimulated by submaximal concentrations of 8-bromoadenosine 3′,5′-cyclic monophosphate (8Br-cAMP) in MA-10 cells (Poderoso et al., 2008). On the other hand, a dominant negative of ERK2 has failed to produce the effect exerted by the WT, which suggests the involvement of this cascade in steroid production in Leydig cells (Poderoso et al., 2008). These results are in agreement with those performed with ERK activity inhibitors (Hirakawa and Ascoli, 2003).

Regarding ERK-dependent StAR phosphorylation, a consensus sequence was found on StAR structure that might allow the docking

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of StAR to ERK1/2, as well as a consensus site for ERK1/2 phosphorylation (Poderoso et al., 2008). A typical docking site known as the D domain (**KTK**LTWL**LSI**) was found between amino acids 235 and 244, and it is conserved among ERK1/2 upstream kinase, MAPK phosphatase (MKP) and ERK substrates (Zhou et al., 2006). *In silico* studies and the direct analysis of murine StAR sequence have led to determine the existence of only two serine-proline motifs, targets for ERK1/2 phosphorylation, *i.e.* Ser232 and Ser277. According to the Expasy Prosite database (http://expasy.org/prosite/), Ser232 has a 90% probability of being phosphorylated, whereas Ser277 has only 5%. Moreover, Ser277 is relatively less conserved among species, while Ser232 (PLAGS232PS) is adjacent to the docking D domain. In support of a predicted StAR–ERK interaction, an interplay has been shown between ERK1/2 and StAR through the treatment of subcellular fractions with a recombinant protein where pERK is coupled to the glutathione S-transferase (GST). This experiment showed that StAR protein interacts with pERK only in the mitochondrial fraction from stimulated cells, while in the cytosol, where both proteins are present in soluble forms, the pull-down experiment rendered negative results. In addition, MEK-phosphorylation via PKA, together with StAR and pERK, have increased cholesterol transport and mitochondrial synthesis of progesterone in a cell free assay (Poderoso et al., 2008).

Several groups, including ours, have shown that ERK1/2 and MEK1/2 are targeted to the mitochondria in different tissues, particularly on the OMM (Alonso et al., 2004; Baines et al., 2002; Poderoso et al., 2008); and it is probably this submitochondrial domain that ERK interacts with StAR through StAR's docking domains. Then, mitochondrial ERK1/2 is a cholesterol transport regulator modulating StAR phosphorylation (Poderoso et al., 2008).

The presence of pERK, together with PKA catalytic subunit, has been proven absolutely essential to achieve maximal steroidogenesis by isolated mitochondria from MA-10 cells (Poderoso et al., 2008). Thus, the process of protein phospho-dephosphorylation is a common and crucial event in the mechanism of hormone action upon the regulation of steroid synthesis.

It has also been shown that active ERK1 prompts a slight increase in StAR phosphorylation, which notably increases in the presence of cholesterol *in vitro* (Poderoso et al., 2008). StAR phosphorylation by PKA is neither necessary nor dependent on the presence of cholesterol in the reaction media (Baker et al., 2007), in agreement with our observations (Poderoso et al., 2008). This result indicates that StAR phosphorylation by kinases other than PKA is modulated by the endogenous ligand of the protein, in agreement with recent work showing that cholesterol is a potent activator of StAR metabolism (Rajapaksha et al., 2013).

Moreover, in non-dependent PKA steroidogenic tissues such as glomerulosa cells, where Ang II acts mainly through Ca^{2+} increase and PKC activity, ERK is also involved in steroid synthesis (Lehoux and Lefebvre, 2007; McNeill et al., 1998; Natarajan et al., 2002; Watanabe et al., 1996). Nevertheless, a direct relationship between ERK and StAR in this system has not been elucidated yet and it is a subject of study in our laboratory (unpublished data).

5. Phosphatases in StAR function

While the phosphorylation of StAR has been extensively described, much less is known about phosphatases involved in StAR activity or metabolism. Indeed, the role of putative mitochondrial phosphatases upon StAR is merely speculative up to date. Our laboratory has published a large amount of data concerning the action of phosphatases in the acute regulation of steroidogenesis (Cano et al., 2006; Cornejo Maciel et al., 2001; Gorostizaga et al., 2007; Paz et al., 2002; Poderoso et al., 2002). Particularly, a tyrosine phosphatase SHP2 (src homology 2-containing phosphotyrosine phosphatase 2) has been indicated as a key protein in regulating steroid synthesis. In this regard, SHP2 participates in the transcriptional regulation of the Acyl-CoA synthetase 4 (ACSL4), which in turn participates in the regulation of intramitochondrial arachidonic acid (AA) levels (Castillo et al., 2006; Duarte et al., 2007; Maloberti et al., 2005). Then, AA lipoxygenated or epoxygenated products induce the expression of the StAR gene (Stocco et al., 2001).

Through the action on ACSL4 protein levels, SHP2 affects arachidonoyl-CoA (AA-CoA) production and metabolism and, finally, the steroidogenic capacity of MA-10 cells: overexpression (or knockdown) of SHP2 leads to increased (or decreased) steroid production (Cooke et al., 2011). Moreover, SHP2 is needed for the correct association of ERK with mitochondria, as an indirect way of controlling StAR phosphorylation by ERK activity (Duarte et al., 2012).

Although SHP2 activity exerts a regulatory effect on StAR function in a transcriptional-dependent manner, direct dephosphorylation of StAR protein by Ser/Thr phosphatases in mitochondria has not been established. A plausible candidate is PP2A (serine/threonine 2A), localized in mitochondria of several tissues including steroidogenic cells (Merrill et al., 2013; Poderoso et al., 2002). Our group has determined that MKP-1 is localized in mitochondria from MA-10 Leydig cells (Brion et al., 2011); therefore MKP-1 could be a good candidate to modulate mitochondrial ERK activity and, concomitantly, StAR phosphorylation and function.

6. Mitochondrial dynamics in steroid synthesis

Mitochondrial fusion/fission events, a mechanism also referred to as "mitochondrial dynamics" is important for maintaining the integrity of these organelles. Mitochondrial dynamics allows mitochondrial replication, repair of defective mitochondria, selective elimination of depolarized mitochondria via mitophagy and propagation of intra-mitochondrial calcium waves (Martin, 2011). It has been established that mitochondrial plasticity facilitates the movement of these organelles within the cell, and that mitochondrial rearrangements are important for the normal function of the cell, and for protection against aging-related changes. Two dynamin-like GTPases involved in mitochondrial fusion, mitofusin (Mfn) 1 and 2, are implicated in the modulation of mitochondria–mitochondria and endoplasmic reticulum (ER)–mitochondria interactions. Mfn 1 and 2 are located on the OMM, mediating mitochondrial fusion in concert with another GTPase, OPA1 (optic atrophy 1), on the IMM. Mfn 1 and 2 are extensively expressed in tissues, as demonstrated in brain (mainly Mfn2), liver, adrenal glands and testis and it has been proven that Mfn2 is enriched at contact sites between ER and mitochondria (mitochondria associated membrane, MAM) (de Brito and Scorrano, 2009). Interestingly, ACSL4, already mentioned in this review as a key enzyme in steroidogenesis, is a protein localized in the MAM, where it is active (Lewin et al., 2002).

Mitochondrial fission requires dynamin-related protein 1 (Drp1), a cytosolic protein which is recruited to the OMM by a poorly characterized multiprotein complex. Recent work has shown that PKA recruitment to the mitochondria results in mitochondrial elongation by Drp1 phosphorylation and inactivation in neurons (Merrill et al., 2011).

Despite the importance of mitochondrial dynamics in several metabolic and hormonal conditions, very little is known about its role in steroidogenic tissues. As a first approach, a paper published almost 30 years ago showed that in adrenal cells, mitochondria displayed a change in morphology and intracellular localization after hormone stimulation by cAMP (Soto et al., 1986). More recently, it was observed that in H295R adrenocortical cells, mitochondria move across the cell after ACTH stimulation, in a PKA-dependent manner. The authors investigated the role of ACTH/cAMP-stimulated mitochondrial trafficking in regulating cortisol production and observed that this microtubule-dependent movement could be involved in

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the transport of substrates between the ER and mitochondria (Li and Sewer, 2010).

We recently demonstrated that steroid synthesis depends on changes in mitochondrial fusion that can be regulated in a hormone-dependent manner. Mfn2 is promptly up-regulated after the steroidogenic stimuli, thus suggesting that mitochondrial dynamics might be central for steroidogenesis. Furthermore, blocking mitochondrial fusion by knocking down Mfn2 expression has a negative impact on steroid synthesis (Duarte et al., 2012). The changes observed in mitochondrial fusion might also be central for the formation of the mitochondrial multiprotein complex that delivers cholesterol to the P450scc system, as hormone-stimulated mitochondrial rearrangement is required for the re-localization of the ERK1/2 protein to mitochondria. Moreover, the abrogation of mitochondrial fusion prevents the association of ACSL4 with the mitochondria, showing clearly that MAM formation depends on mitochondrial fusion (Duarte et al., 2012). As previously mentioned in this review, SHP2 modulates both mitochondrial fusion and ERK1/2 localization in mitochondria (Duarte et al., 2012). In agreement with Li and Sewer (2010), mitochondrial fusion might represent a limiting step in the onset of processes that require transport of intermediate products, *e.g.* liposoluble steroid hormones between organelles, probably mediated by MAM. Then, these products might reach the plasma membrane without moving across the hydrophilic cytoplasm. More recently, we have shown that mitochondrial fusion is an essential process in regulating StAR mRNA levels and in driving StAR protein to the mitochondrial context, probably to the OMM (Duarte et al., 2014). This point will be discussed later in this paper.

7. Role of StAR phosphorylation and mitochondrial fusion on the localization of StAR on the OMM and steroidogenesis

In silico molecular modeling showed a possible conformational change in StAR C-terminus due to cholesterol binding, which could increase the exposure of StAR Ser232 and the docking domain for ERK, thus making StAR a substrate for ERK binding and phosphorylation (Poderoso et al., 2008). This is supported by the fact that there is a loss of StAR helical structure upon cholesterol binding (Petrescu et al., 2001). These modifications and interactions between StAR and ERK are probably taking place in the OMM, since this submitochondrial domain is the region that anchors both, StAR and ERK, as previously described. In turn, the use of S232A, a mutated form of StAR in which Ser232 was changed to Ala, a non phosphorylable amino acid, prevented StAR phosphorylation by active ERK, confirming that this residue is indeed the kinase target. Transient transfection of MA-10 Leydig cells with StAR S232A cDNA partially blocked the stimulation of progesterone production enhanced by cAMP treatment. In contrast, the transfection with other StAR mutant, in which Ser232 was replaced to a glutamic acid (S232E), did not produce such effect, suggesting that the negatively charged amino acid partially mimics the negative charge of the phosphate group present in the phospho-Ser232 (Poderoso et al., 2008).

Cholesterol has been shown to act as an allosteric modulator, facilitating further binding of StAR to the ligand (Petrescu et al., 2001) and strongly stabilizing the partially unfolded state in the StAR molecule (Mathieu et al., 2002). However, at a certain point when cholesterol needs to reach the P450scc, cholesterol release from StAR interaction becomes necessary. As ERK phosphorylation of StAR requires cholesterol, it can be suggested that StAR phosphorylation at Ser232 occurs after cholesterol–StAR interaction. Therefore, the negative charge at the Ser232 site might produce a conformational change in StAR, reducing its affinity for cholesterol and favoring its release. This might in turn allow cholesterol transport across the mitochondrial membrane to produce high rates of pregnenolone synthesis (Fig. 1). It is hard to elucidate a putative model to describe the effect of the Ser232 phosphorylation on StAR affinity for cholesterol. StAR molecular structure has been partially studied (Mathieu et al., 2002; Petrescu et al., 2001; Tsujishita and Hurley, 2000; Yaworsky et al., 2005) and it can be inferred that the Ser232 residue is localized in one of the last β barrels of the START domain (Reitz et al., 2008; Yaworsky et al., 2005). It is well known that amino acidic phosphorylation involves changes in protein stability and regulates affinity with other components. After protein phosphorylation, acidic loops formation has been described for other proteins (Papaleo et al., 2011). Cholesterol release could result from a pH-dependent transition to a molten globule structure, involving the loss of association between the C-terminal α-helix and lipid molecules in the OMM. Under acidic pH conditions, the cholesterol affinity for START domain is significantly decreased (Reitz et al., 2008). Thus, ERK phosphorylation of StAR could determine a local decrease in pH, leading to a putative conformational change on StAR, rendering the protein slightly more capable of releasing its bound cholesterol.

We have shown that the over-expression of StAR S232A significantly abolishes the presence of mitochondrial StAR after hCG or cAMP stimulation. ERK phosphorylation affects mitochondrial StAR levels post-transcriptionally, as the expression of transfected StAR S232A is independent of cellular endogenous regulation (Duarte et al., 2014). In MA-10 cells, a mitochondrial module includes MEK, ERK and cholesterol with a direct physical association between StAR and ERK (Poderoso et al., 2008). Their interaction favors StAR phosphorylation by ERK and hence could promote phospho-StAR retention in the mitochondria, particularly on the OMM where ERK resides (Alonso et al., 2004).

Again, it is the OMM localization of StAR, and not its cleavage from the 37-kDa to the 30 kDa form, that determines its activity (Bose et al., 2002). Then, the longest StAR retention time at the OMM might render the maximal StAR activity in cholesterol transport, in agreement with previous data (Miller, 2013). ERK is transiently activated after hormone stimulation in MA-10 cells and is dephosphorylated as a normal regulation pathway, mainly by MKPs (Bey et al., 2003; Cobb et al., 1991; Poderoso et al., 2008). Interaction between phospho-StAR and ERK in mitochondria could protect ERK from dephosphorylation and inactivation. The temporal frame of ERK activity (1–2 h) in mitochondria correlates with the period of StAR major activity and cholesterol transport after hormone stimulation.

These results are entirely in line with MKP-1 down-regulation and the concomitant increase in progesterone levels after 2 h of stimulation, probably due to a long-lasting effect of active ERK in mitochondria (Brion et al., 2011).

The presence of ERK and StAR in the OMM and the regulation of steroidogenesis depends also on mitochondrial fusion, which is a hormone-regulated step in which the induction of Mfn2 plays an essential role (Duarte et al., 2012; Poderoso et al., 2013). Our recent work clearly shows that mitochondrial fusion through the increase of Mfn2 levels in mitochondria is strictly required for StAR protein synthesis and mitochondrial localization after hormone stimulation. Mitochondrial fusion reduction, by knocking-down Mfn2, correlates with a decrease in mitochondrial StAR protein and mRNA levels. The direct effect of mitochondrial fusion or Mfn2 on StAR gene expression is unknown to date. In this regard, we have established several hypotheses such as: (1) mitochondrial fusion allows the interaction of ACSL4 with mitochondria and forms MAM structures, thus enabling AA proper metabolism and increasing StAR expression; (2) Mfn2 could modulate StAR mRNA levels directly, as described for other mitochondrial proteins (Papanicolaou et al., 2012; Xu et al., 2010) or (3) there is a stabilizing effect of mitochondrial fusion on StAR mRNA, as different variants of StAR mRNA levels are regulated by stabilization mediated by cAMP in MA-10 cells (Castillo et al., 2011; Zhao et al., 2005). Then, mitochondrial fusion could mediate the approach between StAR mRNA and a specific AKAP1

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in mitochondria that binds StAR mRNA (Grozdanov and Stocco, 2012), thus stabilizing and increasing StAR mRNA levels.

8. Conclusion

Taken together, these results give a new insight into StAR as a substrate of kinases and phosphatases involved in steroid biosynthesis and the retention of StAR on the OMM. It could be concluded that the interaction of StAR with a multiprotein complex may contribute to the retention of StAR on the OMM.

The phospho-dephosphorylation of StAR may contribute to binding and releasing cholesterol and, in turn, allow cholesterol transport across the mitochondrial membrane to produce high rates of pregnenolone synthesis with a few molecules of StAR protein. In this work, we reviewed StAR mechanisms of action in the cholesterol transport to the P450scc, to achieve maximal steroid production. We also established the relevance of mitochondrial phosphorylation events and mitochondrial fusion in the correct localization of this key protein to exert its action in specialized cells, as novel participants in StAR activity and steroidogenesis.

Acknowledgements

We thank María T. Rancez for providing language help and writing assistance.

This work was supported by CONICET (PIP 2012–2014 – COD 11220110100485) http://www.conicet.gov.ar, UBA (UBACYT 2011–2014 – 2002 0100100 849; 2014–2017 – 20020130100271BA) http://www.uba.ar/secyt/, INC (Resolución Ministerial No. 493/14) to EJP http://www.msal.gov.ar/inc/novedades-proy-invest.php. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

References

Alberta, J.A., Epstein, L.F., Pon, L.A., Orme-Johnson, N.R., 1989. Mitochondrial localization of a phosphoprotein that rapidly accumulates in adrenal cortex cells exposed to adrenocorticotropic hormone or to cAMP. J. Biol. Chem. 264, 2368–2372.

Allen, J.A., Shankara, T., Janus, P., Buck, S., Diemer, T., Hales, K.H., et al., 2006. Energized, polarized, and actively respiring mitochondria are required for acute Leydig cell steroidogenesis. Endocrinology 147, 3924–3935.

Alonso, M., Melani, M., Converso, D., Jaitovich, A., Paz, C., Carreras, M.C., et al., 2004. Mitochondrial extracellular signal-regulated kinases 1/2 (ERK1/2) are modulated during brain development. J. Neurochem. 89, 248–256.

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., Sugawara, T., Nishino, H., Liu, Z., Holt, J.A., Pain, D., et al., 1996. Steroidogenic acute regulatory protein (StAR) retains activity in the absence of its mitochondrial import sequence: implications for the mechanism of StAR action. Proc. Natl. Acad. Sci. U.S.A. 93, 13731–13736.

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

Arakane, F., Kallen, C.B., Watari, H., Foster, J.A., Sepuri, N.B., Pain, D., et al., 1998. The mechanism of action of steroidogenic acute regulatory protein (StAR). StAR acts on the outside of mitochondria to stimulate steroidogenesis. J. Biol. Chem. 273, 16339–16345.

Artemenko, I.P., Zhao, D., Hales, D.B., Hales, K.H., Jefcoate, C.R., 2001. Mitochondrial processing of newly synthesized steroidogenic acute regulatory protein (StAR), but not total StAR, mediates cholesterol transfer to cytochrome P450 side chain cleavage enzyme in adrenal cells. J. Biol. Chem. 276, 46583–46596.

Bahat, A., Perlberg, S., Melamed-Book, N., Lauria, I., Langer, T., Orly, J., 2014. StAR enhances transcription of genes encoding the mitochondrial proteases involved in its own degradation. Mol. Endocrinol. 28, 208–224.

Baines, C.P., Zhang, J., Wang, G.W., Zheng, Y.T., Xiu, J.X., Cardwell, E.M., et al., 2002. Mitochondrial PKCepsilon and MAPK form signaling modules in the murine heart: enhanced mitochondrial PKCepsilon-MAPK interactions and differential MAPK activation in PKCepsilon-induced cardioprotection. Circ. Res. 90, 390–397.

Baker, B.Y., Yaworsky, D.C., Miller, W.L., 2005. A pH-dependent molten globule transition is required for activity of the steroidogenic acute regulatory protein, StAR. J. Biol. Chem. 280, 41753–41760.

Baker, B.Y., Epand, R.F., Epand, R.M., Miller, W.L., 2007. Cholesterol binding does not predict activity of the steroidogenic acute regulatory protein, StAR. J. Biol. Chem. 282, 10223–10232.

Bey, P., Gorostizaga, A.B., Maloberti, P.M., Lozano, R.C., Poderoso, C., Cornejo Maciel, F., et al., 2003. Adrenocorticotropin induces mitogen-activated protein kinase phosphatase 1 in Y1 mouse adrenocortical tumor cells. Endocrinology 144, 1399–1406.

Black, S.M., Harikrishna, J.A., Szklarz, G.D., Miller, W.L., 1994. The mitochondrial environment is required for activity of the cholesterol side-chain cleavage enzyme, cytochrome P450scc. Proc. Natl. Acad. Sci. U.S.A. 91, 7247–7251.

Bornstein, S.R., Rutkowski, H., Vrezas, I., 2004. Cytokines and steroidogenesis. Mol. Cell. Endocrinol. 215, 135–141.

Bose, H.S., Sugawara, T., Strauss, J.F., 3rd, Miller, W.L., 1996. The pathophysiology and genetics of congenital lipoid adrenal hyperplasia. International Congenital Lipoid Adrenal Hyperplasia Consortium. N. Engl. J. Med. 335, 1870–1878.

Bose, H.S., Whittal, R.M., Baldwin, M.A., Miller, W.L., 1999. The active form of the steroidogenic acute regulatory protein, StAR, appears to be a molten globule. Proc. Natl. Acad. Sci. U.S.A. 96, 7250–7255.

Bose, H.S., Lingappa, V.R., Miller, W.L., 2002. Rapid regulation of steroidogenesis by mitochondrial protein import. Nature 417, 87–91.

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.

Brion, L., Maloberti, P.M., Gomez, N.V., Poderoso, C., Gorostizaga, A.B., Mori Sequeiros Garcia, M.M., et al., 2011. MAPK phosphatase-1 (MKP-1) expression is up-regulated by hCG/cAMP and modulates steroidogenesis in MA-10 Leydig cells. Endocrinology 152, 2665–2677.

Cano, F., Poderoso, C., Cornejo Maciel, F., Castilla, R., Maloberti, P., Castillo, F., et al., 2006. Protein tyrosine phosphatases regulate arachidonic acid release, StAR induction and steroidogenesis acting on a hormone-dependent arachidonic acid-preferring acyl-CoA synthetase. J. Steroid Biochem. Mol. Biol. 99, 197–202.

Caron, K.M., Soo, S.C., Wetsel, W.C., Stocco, D.M., Clark, B.J., Parker, K.L., 1997. Targeted disruption of the mouse gene encoding steroidogenic acute regulatory protein provides insights into congenital lipoid adrenal hyperplasia. Proc. Natl. Acad. Sci. U.S.A. 94, 11540–11545.

Castillo, A.F., Cornejo Maciel, F., Castilla, R., Duarte, A., Maloberti, P., Paz, C., et al., 2006. cAMP increases mitochondrial cholesterol transport through the induction of arachidonic acid release inside this organelle in Leydig cells. FEBS J. 273, 5011–5021.

Castillo, A.F., Fan, J., Papadopoulos, V., Podesta, E.J., 2011. Hormone-dependent expression of a steroidogenic acute regulatory protein natural antisense transcript in MA-10 mouse tumor Leydig cells. PLoS ONE 6, e22822.

Clark, B.J., Wells, J., King, S.R., Stocco, D.M., 1994. The purification, cloning, and expression of a novel luteinizing hormone-induced mitochondrial protein in MA-10 mouse Leydig tumor cells. Characterization of the steroidogenic acute regulatory protein (StAR). J. Biol. Chem. 269, 28314–28322.

Cobb, M.H., Boulton, T.G., Robbins, D.J., 1991. Extracellular signal-regulated kinases: ERKs in progress. Cell Regul. 2, 965–978.

Cooke, M., Orlando, U., Maloberti, P., Podesta, E.J., Cornejo Maciel, F., 2011. Tyrosine phosphatase SHP2 regulates the expression of acyl-CoA synthetase ACSL4. J. Lipid Res. 52, 1936–1948.

Cornejo Maciel, F., Poderoso, C., Gorostizaga, A., Paz, C., Podesta, E.J., 2001. LH/chorionic gonadotropin signaling pathway involves protein tyrosine phosphatase activity downstream of protein kinase A activation: evidence of an obligatory step in steroid production by Leydig cells. J. Endocrinol. 170, 403–411.

Crivello, J.F., Jefcoate, C.R., 1980. Intracellular movement of cholesterol in rat adrenal cells. Kinetics and effects of inhibitors. J. Biol. Chem. 255, 8144–8151.

de Brito, O.M., Scorrano, L., 2009. Mitofusin-2 regulates mitochondrial and endoplasmic reticulum morphology and tethering: the role of Ras. Mitochondrion 9, 222–226.

Davis, W.W., Garren, L.D., 1968. On the mechanism of action of adrenocorticotropic hormone. The inhibitory site of cycloheximide in the pathway of steroid biosynthesis. J. Biol. Chem. 243, 5153–5157.

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

Duarte, A., Castillo, A.F., Castilla, R., Maloberti, P., Paz, C., Podesta, E.J., et al., 2007. An arachidonic acid generation/export system involved in the regulation of cholesterol transport in mitochondria of steroidogenic cells. FEBS Lett. 581, 4023–4028.

Duarte, A., Poderoso, C., Cooke, M., Soria, G., Cornejo Maciel, F., Gottifredi, V., et al., 2012. Mitochondrial fusion is essential for steroid biosynthesis. PLoS ONE 7, e45829.

Duarte, A., Castillo, A.F., Podesta, E.J., Poderoso, C., 2014. Mitochondrial fusion and ERK activity regulate steroidogenic acute regulatory protein localization in mitochondria. PLoS ONE 9, e100387.

Dufau, M.L., 1998. The luteinizing hormone receptor. Annu. Rev. Physiol. 60, 461–496.

Dyson, M.T., Jones, J.K., Kowalewski, M.P., Manna, P.R., Alonso, M., Gottesman, M.E., et al., 2008. Mitochondrial A-kinase anchoring protein 121 binds type II protein kinase A and enhances steroidogenic acute regulatory protein-mediated steroidogenesis in MA-10 mouse Leydig tumor cells. Biol. Reprod. 78, 267–277.

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

Ferguson, J.J., Jr., 1963. Protein synthesis and adrenocorticotropin responsiveness. J. Biol. Chem. 238, 2754–2759.

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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.
Gallo-Payet, N., Battista, M.C., 2014. Steroidogenesis-adrenal cell signal transduction. Compr. Physiol. 4, 889–964.
Gorostizaga, A., Cornejo Maciel, F., Brion, L., Maloberti, P., Podesta, E.J., Paz, C., 2007. Tyrosine phosphatases in steroidogenic cells: regulation and function. Mol. Cell. Endocrinol. 265–266, 131–137.
Granot, Z., Melamed-Book, N., Bahat, A., Orly, J., 2007a. Turnover of StAR protein: roles for the proteasome and mitochondrial proteases. Mol. Cell. Endocrinol. 265–266, 51–58.
Granot, Z., Kobiler, O., Melamed-Book, N., Eimerl, S., Bahat, A., Lu, B., et al., 2007b. Turnover of mitochondrial steroidogenic acute regulatory (StAR) protein by Lon protease: the unexpected effect of proteasome inhibitors. Mol. Endocrinol. 21, 2164–2177.
Grozdanov, P.N., Stocco, D.M., 2012. Short RNA molecules with high binding affinity to the KH motif of A-kinase anchoring protein 1 (AKAP1): implications for the regulation of steroidogenesis. Mol. Endocrinol. 26, 2104–2117.
Haidan, A., Bornstein, S.R., Liu, Z., Walsh, L.P., Stocco, D.M., Ehrhart-Bornstein, M., 2000. Expression of adrenocortical steroidogenic acute regulatory (StAR) protein is influenced by chromaffin cells. Mol. Cell. Endocrinol. 165, 25–32.
Hasegawa, T., Zhao, L., Caron, K.M., Majdic, G., Suzuki, T., Shizawa, S., et al., 2000. Developmental roles of the steroidogenic acute regulatory protein (StAR) as revealed by StAR knockout mice. Mol. Endocrinol. 14, 1462–1471.
Hirakawa, T., Ascoli, M., 2003. The lutropin/choriogonadotropin receptor-induced phosphorylation of the extracellular signal-regulated kinases in Leydig cells is mediated by a protein kinase a-dependent activation of ras. Mol. Endocrinol. 17, 2189–2200.
Jefcoate, C., 2002. High-flux mitochondrial cholesterol trafficking, a specialized function of the adrenal cortex. J. Clin. Invest. 110, 881–890.
Krueger, R.J., Orme-Johnson, N.R., 1983. Acute adrenocorticotropic hormone stimulation of adrenal corticosteroidogenesis. Discovery of a rapidly induced protein. J. Biol. Chem. 258, 10159–10167.
Lehoux, J.G., Lefebvre, A., 2007. Angiotensin II activates p44/42 MAP kinase partly through PKCepsilon in H295R cells. Mol. Cell. Endocrinol. 265–266, 121–125.
Lewin, T.M., Van Horn, C.G., Krisans, S.K., Coleman, R.A., 2002. Rat liver acyl-CoA synthetase 4 is a peripheral-membrane protein located in two distinct subcellular organelles, peroxisomes, and mitochondrial-associated membrane. Arch. Biochem. Biophys. 404, 263–270.
Li, D., Sewer, M.B., 2010. RhoA and DIAPH1 mediate adrenocorticotropin-stimulated cortisol biosynthesis by regulating mitochondrial trafficking. Endocrinology 151, 4313–4323.
Lin, D., Sugawara, T., Strauss, J.F., 3rd, Clark, B.J., Stocco, D.M., Saenger, P., et al., 1995. Role of steroidogenic acute regulatory protein in adrenal and gonadal steroidogenesis. Science 267, 1828–1831.
Maloberti, P., Castilla, R., Castillo, F., Cornejo Maciel, F., Mendez, C.F., Paz, C., et al., 2005. Silencing the expression of mitochondrial acyl-CoA thioesterase I and acyl-CoA synthetase 4 inhibits hormone-induced steroidogenesis. FEBS J. 272, 1804–1814.
Manna, P.R., Stocco, D.M., 2005. Regulation of the steroidogenic acute regulatory protein expression: functional and physiological consequences. Curr. Drug Targets Immune Endocr. Metabol. Disord. 5, 93–108.
Martin, L.A., Kennedy, B.E., Karten, B., 2014. Mitochondrial cholesterol: mechanisms of import and effects on mitochondrial function. J. Bioenerg. Biomembr (Epub ahead of print).
Martin, S.J., 2011. Mitochondrial fusion: bax to the fussure. Dev. Cell 20, 142–143.
Mathieu, A.P., Fleury, A., Ducharme, L., Lavigne, P., LeHoux, J.G., 2002. Insights into steroidogenic acute regulatory protein (StAR)-dependent cholesterol transfer in mitochondria: evidence from molecular modeling and structure-based thermodynamics supporting the existence of partially unfolded states of StAR. J. Mol. Endocrinol. 29, 327–345.
McNeill, H., Puddefoot, J.R., Vinson, G.P., 1998. MAP kinase in the rat adrenal gland. Endocr. Res. 24, 373–380.
Merrill, R.A., Dagda, R.K., Dickey, A.S., Cribbs, J.T., Green, S.H., Usachev, Y.M., et al., 2011. Mechanism of neuroprotective mitochondrial remodeling by PKA/AKAP1. PLoS Biol. 9, e1000612.
Merrill, R.A., Slupe, A.M., Strack, S., 2013. N-terminal phosphorylation of protein phosphatase 2A/Bbeta2 regulates translocation to mitochondria, dynamin-related protein 1 dephosphorylation, and neuronal survival. FEBS J. 280, 662–673.
Midzak, A., Rone, M., Aghazadeh, Y., Culty, M., Papadopoulos, V., 2011. Mitochondrial protein import and the genesis of steroidogenic mitochondria. Mol. Cell. Endocrinol. 336, 70–79.
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.
Miller, W.L., 2013. Steroid hormone synthesis in mitochondria. Mol. Cell. Endocrinol. 379, 62–73.
Miller, W.L., Strauss, J.F., 3rd, 1999. Molecular pathology and mechanism of action of the steroidogenic acute regulatory protein, StAR. J. Steroid Biochem. Mol. Biol. 69, 131–141.
Morohaku, K., Pelton, S.H., Daugherty, D.J., Butler, W.R., Deng, W., Selvaraj, V., 2013. Translocator protein/peripheral benzodiazepine receptor is not required for steroid hormone biosynthesis. Endocrinology 155, 89–97.
Natarajan, R., Yang, D.C., Lanting, L., Nadler, J.L., 2002. Key role of P38 mitogen-activated protein kinase and the lipoxygenase pathway in angiotensin II actions in H295R adrenocortical cells. Endocrine 18, 295–301.
Papadopoulos, V., Miller, W.L., 2012. Role of mitochondria in steroidogenesis. Best Pract. Res. Clin. Endocrinol. Metab. 26, 771–790.
Papaleo, E., Ranzani, V., Tripodi, F., Vitriolo, A., Cirulli, C., Fantucci, P., et al., 2011. An acidic loop and cognate phosphorylation sites define a molecular switch that modulates ubiquitin charging activity in Cdc34-like enzymes. PLoS Comput. Biol. 7, e1002056.
Papanicolaou, K.N., Phillippo, M.M., Walsh, K., 2012. Mitofusins and the mitochondrial permeability transition: the potential downside of mitochondrial fusion. Am. J. Physiol. Heart Circ. Physiol. 303, H243–H255.
Paz, C., Cornejo Maciel, F., Maloberti, P., Walsh, L.P., Stocco, D.M., Podesta, E.J., 2002. Protein tyrosine phosphatases are involved in LH/chorionic gonadotropin and 8Br-cAMP regulation of steroidogenesis and StAR protein levels in MA-10 Leydig cells. J. Endocrinol. 175, 793–801.
Petrescu, A.D., Gallegos, A.M., Okamura, Y., Strauss, J.F., 3rd, Schroeder, F., 2001. Steroidogenic acute regulatory protein binds cholesterol and modulates mitochondrial membrane sterol domain dynamics. J. Biol. Chem. 276, 36970–36982.
Poderoso, C., Cornejo Maciel, F., Gorostizaga, A., Bey, P., Paz, C., Podesta, E.J., 2002. The obligatory action of protein tyrosine phosphatases in ACTH-stimulated steroidogenesis is exerted at the level of StAR protein. Endocr. Res. 28, 413–417.
Poderoso, C., Converso, D.P., Maloberti, P., Duarte, A., Neuman, I., Galli, S., et al., 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.
Poderoso, C., Duarte, A., Cooke, M., Orlando, U., Gottifredi, V., Solano, A.R., et al., 2013. The spatial and temporal regulation of the hormonal signal. Role of mitochondria in the formation of a protein complex required for the activation of cholesterol transport and steroids synthesis. Mol. Cell. Endocrinol. 371, 26–33.
Pon, L.A., Hartigan, J.A., Orme-Johnson, N.R., 1986. Acute ACTH regulation of adrenal corticosteroid biosynthesis. Rapid accumulation of a phosphoprotein. J. Biol. Chem. 261, 13309–13316.
Privalle, C.T., Crivello, J.F., Jefcoate, C.R., 1983. Regulation of intramitochondrial cholesterol transfer to side-chain cleavage cytochrome P-450 in rat adrenal gland. Proc. Natl. Acad. Sci. U.S.A. 80, 702–706.
Rajapaksha, M., Kaur, J., Bose, M., Whittal, R.M., Bose, H.S., 2013. Cholesterol-mediated conformational changes in the steroidogenic acute regulatory protein are essential for steroidogenesis. Biochemistry 52, 7242–7253.
Reitz, J., Gehrig-Burger, K., Strauss, J.F., 3rd, Gimpl, G., 2008. Cholesterol interaction with the related steroidogenic acute regulatory lipid-transfer (START) domains of StAR (STARD1) and MLN64 (STARD3). FEBS J. 275, 1790–1802.
Rone, M.B., Midzak, A.S., Issop, L., Rammouz, G., Jagannathan, S., Fan, J., et al., 2012. Identification of a dynamic mitochondrial protein complex driving cholesterol import, trafficking, and metabolism to steroid hormones. Mol. Endocrinol. 26, 1868–1882.
Rubin, C.S., 1994. A kinase anchor proteins and the intracellular targeting of signals carried by cyclic AMP. Biochim. Biophys. Acta 1224, 467–479.
Sasaki, G., Zubair, M., Ishii, T., Mitsui, T., Hasegawa, T., Auchus, R.J., 2014. The contribution of serine 194 phosphorylation to steroidogenic acute regulatory protein function. Mol. Endocrinol. 28, 1088–1096.
Soto, E.A., Kliman, H.J., Strauss, J.F., 3rd, Paavola, L.G., 1986. Gonadotropins and cyclic adenosine 3′,5′-monophosphate (cAMP) alter the morphology of cultured human granulosa cells. Biol. Reprod. 34, 559–569.
Stocco, D.M., Clark, B.J., 1996. Regulation of the acute production of steroids in steroidogenic cells. Endocr. Rev. 17, 221–244.
Stocco, D.M., Sodeman, T.C., 1991. The 30-kDa mitochondrial proteins induced by hormone stimulation in MA-10 mouse Leydig tumor cells are processed from larger precursors. J. Biol. Chem. 266, 19731–19738.
Stocco, D.M., Clark, B.J., Reinhart, A.J., Williams, S.C., Dyson, M., Dassi, B., et al., 2001. Elements involved in the regulation of the StAR gene. Mol. Cell. Endocrinol. 177, 55–59.
Sugawara, T., Lin, D., Holt, J.A., Martin, K.O., Javitt, N.B., Miller, W.L., et al., 1995. Structure of the human steroidogenic acute regulatory protein (StAR) gene: StAR stimulates mitochondrial cholesterol 27-hydroxylase activity. Biochemistry 34, 12506–12512.
Tsujishita, Y., Hurley, J.H., 2000. Structure and lipid transport mechanism of a StAR-related domain. Nat. Struct. Biol. 7, 408–414.
Watanabe, G., Lee, R.J., Albanese, C., Rainey, W.E., Batlle, D., Pestell, R.G., 1996. Angiotensin II activation of cyclin D1-dependent kinase activity. J. Biol. Chem. 271, 22570–22577.
Xu, F.Y., McBride, H., Acehan, D., Vaz, F.M., Houtkooper, R.H., Lee, R.M., et al., 2010. The dynamics of cardiolipin synthesis post-mitochondrial fusion. Biochim. Biophys. Acta 1798, 1577–1585.
Yaworsky, D.C., Baker, B.Y., Bose, H.S., Best, K.B., Jensen, L.B., Bell, J.D., et al., 2005. pH-dependent interactions of the carboxyl-terminal helix of steroidogenic acute regulatory protein with synthetic membranes. J. Biol. Chem. 280, 2045–2054.
Zhao, D., Duan, H., Kim, Y.C., Jefcoate, C.R., 2005. Rodent StAR mRNA is substantially regulated by control of mRNA stability through sites in the 3′-untranslated region and through coupling to ongoing transcription. J. Steroid Biochem. Mol. Biol. 96, 155–173.
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.

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