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COMMENTARY

Role of the Steroidogenic Acute Regulatory Protein (StAR) in Steroidogenesis

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ABSTRACT. The rate-limiting, hormone-regulated, *enzymatic* step in steroidogenesis is the conversion of cholesterol to pregnenolone by the cholesterol side-chain cleavage enzyme system (CSCC), which is located on the matrix side of the inner mitochondrial membrane. However, it has long been observed that hydrophilic cholesterol-like substrates capable of traversing the mitochondrial membranes are cleaved to pregnenolone by the CSCC in the absence of any hormone stimulation. Therefore, the true regulated step in the acute response of steroidogenic cells to hormone stimulation is the delivery of cholesterol to the inner mitochondrial membrane and the CSCC. It has been known for greater than three decades that transfer of cholesterol requires *de novo* protein synthesis; however, prior to this time the regulatory protein(s) had yet to be identified conclusively. It is the purpose of this commentary to briefly review a number of the candidates that have been proposed as the acute regulatory protein. As such, we have summarized the available information that describes the roles of transcription, translation, and phosphorylation in this regulation, and have also reviewed the supporting cases that have been made for several of the proteins put forth as the acute regulator. We close with a comprehensive description of the Steroidogenic Acute Regulatory protein (StAR) that we and others have identified and characterized as a family of proteins that are synthesized and imported into the mitochondria in response to hormone stimulation, and for which strong evidence exists indicating that it is the long sought acute regulatory protein. BIOCHEM PHARMACOL 51;3:197–205, 1996.

KEY WORDS. acute steroidogenesis; mitochondria; cholesterol transfer; StAR

The cellular events that rapidly occur in response to the trophic hormone stimulation of steroidogenic tissues result in the synthesis and secretion of the steroid hormones. These rapid or acute effects of hormone stimulation can be distinguished from the slower chronic effects in that acute effects occur within minutes, whereas chronic effects are those that occur on the order of many hours, and usually involve increased gene transcription and translation of the protein enzymes involved in the biosynthesis of these steroids. While both areas have been studied intensely, this commentary will focus only on those studies designed to elucidate the factors and mechanisms involved in the *acute* regulation of steroid production in response to hormone stimulation.

The biosynthesis of steroids in all steroidogenic tissues studied to date begins with the enzymatic conversion of the substrate cholesterol to form pregnenolone. This reaction is catalyzed by the CSCC†, which is located on the matrix side of the inner mitochondrial membrane. Therefore, in order to initiate and sustain steroidogenesis, it is necessary to provide a constant supply of cholesterol to the CSCC. Although the rate-limiting *enzymatic* step in steroidogenesis is the conversion of cholesterol to pregnenolone by the CSCC [1–3], the *true* rate limiting step in this process is the delivery of the substrate to the inner mitochondrial membrane and the CSCC [4–8]. The major barrier to be overcome in the delivery of cholesterol to the CSCC is the aqueous space between the outer and inner mitochondrial membranes through which this relatively hydrophobic compound must pass. Since the aqueous diffusion of cholesterol is extremely slow [9–11] and could not provide sufficient substrate to account for the rapid and large increase in steroid production observed in steroidogenic cells, it follows that the successful stimulation of steroidogenesis would require the activation of a mechanism that rapidly transports cholesterol across this barrier. Thus, the regulation of steroidogenesis is controlled by factors that facilitate the translocation of cholesterol from cellular stores across the aqueous intermembrane space of the mitochondria to the inner membrane.

Requirement of de Novo Protein Synthesis for Acute Steroidogenesis

Early studies demonstrated that steroid production in response to hormone stimulation had an absolute requirement for the

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† *Abbreviations:* CSCC, cholesterol side-chain cleavage enzyme system; SCP_2, sterol carrier protein 2; $P450_{scc}$, cytochrome P450 side-chain cleavage; SAP, steroidogenesis activator polypeptide; PBR, peripheral benzodiazepine receptor; DBI, diazepam binding inhibitor; cAMP, cyclic AMP; PCR, polymerase chain reaction; LCAH, lipoid congenital adrenal hyperplasia; and StAR, Steroidogenic Acute Regulatory protein.

synthesis of new proteins [12, 13]. These studies also demonstrated that when inhibitors of protein synthesis were added to hormone-stimulated cells, the loss of steroid biosynthetic capacity was very rapid, having a half-life on the order of minutes. Later, it was determined that the inhibitor-sensitive step in this process was located in the mitochondria, and that these inhibitors had no effect on the activity of the CSCC complex itself [14]. The observation that *de novo* protein synthesis was indispensable for the acute production of steroids in response to hormone stimulation has also been made more recently in several different steroidogenic tissues [15–20]. Observations in mouse adrenal and Leydig tumor cells demonstrated that while cholesterol could be delivered to a "pre-steroidogenic pool" in the presence of cycloheximide, pregnenolone production did not take place until the inhibitor was removed and the cells were subsequently stimulated with hormone [21]. This action ostensibly mobilized cholesterol from the "pre-steroidogenic pool" to the "steroidogenic pool," which was in the inner mitochondrial membrane. As a result of studies of this kind, the hypothesis arose that the acute production of steroids was dependent on a rapidly synthesized, cycloheximide sensitive, and highly labile protein that appeared in response to trophic hormone treatment and that functioned to transfer cholesterol to the inner mitochondrial membrane.

Is Transcription Required for Acute Steroidogenesis?

Investigations were performed to determine if, in a manner similar to that observed for translation, *de novo* transcription was also required for the acute production of steroids. One study [22] indicated that new transcription was required for the acute production of steroids in rat testicular cells in response to hormone treatment; however, the majority of such studies have indicated that acute steroidogenesis could occur independently of new mRNA synthesis [23–27]. The rapidity with which steroid synthesis begins in response to hormone stimulation would seem to make it most difficult to account for both the transcription and translation of a new protein in this time frame [28]. Thus, mechanisms were proposed in which either an existing, inactive protein required for steroidogenesis was converted rapidly to an active protein by the action of trophic hormone or a new protein was synthesized rapidly using a pre-existing and stable mRNA [28]. We will discuss recent observations in our laboratory which support the idea that transcription is not required for the initial phases of the acute production of steroids in response to hormone stimulation. Since it is clear, [12, 13, 28–30] that a newly synthesized protein is required for cholesterol transfer, then any mechanism proposed for the activation of a pre-existing mRNA with trophic hormone and resulting in the synthesis of a new protein would be consistent with these data. In summary, it appears that while *de novo* protein synthesis is required for the acute regulation of steroid production in response to hormone stimulation, as we shall discuss later, *de novo* transcription is not.

Role of Phosphorylation in Steroidogenesis

The steroidogenic response is mediated through the interaction of the trophic hormones with specific receptors on the cell surface of steroidogenic cells, which in turn activates the cAMP second messenger system [27, 31–34]. The resulting increase in intracellular cAMP activates cAMP-dependent protein kinase and results in the phosphorylation of proteins on either threonine or serine residues [35]. Thus, steroidogenesis would appear to be dependent on the phosphorylation of a protein(s) substrate in response to hormone stimulation. Incubation of steroidogenic cells with amino acid analogs incapable of being phosphorylated has demonstrated that phosphorylation of a protein on a threonine residue is required for steroidogenesis [36, 37]. The presence of newly synthesized phosphoproteins has been observed in stimulated adrenal cells [18, 38–40], in primary cultures of mouse Leydig cells [17], as well as in stimulated MA-10 cells [41], but a cause-and-effect relationship between the phosphorylation of these proteins and steroid production has not been established as yet. This is a most important area that requires significant attention in the immediate future and, as such, our laboratory is actively pursuing this area.

Role of Cellular Architecture in Steroidogenesis

Several studies have indicated that cellular architecture plays an important role in the delivery of cholesterol to the mitochondria for subsequent steroidogenesis. The transfer of cholesterol to the outer mitochondrial membrane in response to trophic hormone stimulation was found to occur in the absence of *de novo* protein synthesis [4, 6, 7], but was inhibited by compounds that disrupted microtubules and microfilaments [4, 29, 42–47]. Thus, cytoskeletal elements appear to have an important function in steroidogenesis [44, 45, 48–50]. Trophic hormone stimulation of human granulosa cells results in a morphological rounding of the cells, which causes a clustering of steroidogenic organelles potentially bringing mitochondria in closer contact to pools of cholesterol such as those found in the lipid droplets of steroidogenic cells [51]. More recently, studies have demonstrated the importance of intermediate filaments in the movement of cholesterol within Y-1 mouse adrenal tumor cells and primary cultures of bovine adrenal and rat Leydig cells [52–55]. These studies demonstrated that both lipid droplets and mitochondria are co-localized on intermediate filaments and hypothesized that this co-localization was the means by which cholesterol was delivered to the mitochondria from the lipid droplet. Thus, it appears that subcellular structures such as microtubules, microfilaments, and intermediate filaments are instrumental in the delivery of cholesterol to the mitochondria. However, there is no convincing evidence that any of these structures play a role in the rate-limiting step, the translocation of cholesterol from the outer mitochondrial membrane to the inner mitochondrial membrane where the cleavage of cholesterol to pregnenolone takes place.

Proposed Candidates for the Acute Regulatory Protein

Since the observation that a newly synthesized protein(s) was absolutely required in the acute regulation of steroidogenesis was first made more than three decades ago, several putative

protein candidates for this role have been proposed. Those proteins that have received the most attention are as follows:

Sterol Carrier Protein 2 SCP_2. SCP_2 is a 13 kDa protein that has been found to be present most abundantly in liver, but has also been found in high levels in steroidogenic tissues. SCP_2 was shown to transfer cholesterol from lipid droplets to mitochondria in a 1:1 ratio and was shown to be capable of stimulating steroid production in isolated adrenal mitochondria [56–59]. Also, co-expression of SCP_2 with cholesterol side-chain cleavage enzyme and adrenodoxin in COS-7 cells resulted in a 2.5-fold increase in steroid production over that seen with expression of the steroidogenic enzyme system alone [60]. In addition, treatment of adrenal cells with anti-SCP_2 antibody resulted in an inhibition of steroid production [61]. However, while the synthesis of SCP_2 has been shown to be under the regulation of ACTH [62], this regulation only occurred after many hours and, in fact, acute stimulation of adrenal cells with ACTH had no effect on SCP_2 levels [63]. Furthermore, while SCP_2 has been demonstrated to be regulated by AMP analogs in the ovary, it is also regulated in a similar manner in a non-steroidogenic granulosa cell line, indicating that its regulation is not obligatorily coupled to steroidogenesis [64]. Thus, the evidence that SCP_2 is able to effectively transfer cholesterol to the inner mitochondrial membrane and the $P450_{scc}$ in amounts adequate to support steroidogenesis in response to hormone stimulation is not convincing at this time. It is possible, however, that SCP_2 may function to maintain sterol movement within the cell in support of steroidogenesis, perhaps by affecting the utilization of peroxisome-derived cholesterol [65].

SAP. Originally purified as a 2.2 kDa peptide from rat adrenal cells [66, 67], SAP was later determined to be a 30 amino acid (3.2 kDa) peptide when it was purified from rat Leydig tumor cells [68]. This peptide was found to be present only in steroidogenic cells, its levels could be acutely increased by trophic hormone stimulation, and this increase was prevented by cycloheximide [69–71]. Notably, addition of SAP to isolated mitochondria was able to increase steroid production by 4- to 5-fold in a concentration-dependent manner, indicating that SAP may play a role in cholesterol transfer within this organelle [66, 67]. Therefore, because of these reported characteristics, this small peptide must still be considered when discussing intramitochondrial cholesterol transport, even though a mechanism whereby SAP can transport cholesterol to the inner mitochondrial membrane has not yet, been demonstrated satisfactorily.

PBR, DBI, and the 8.2 kDa Protein. PBR is a 18 kDa protein present in high concentrations in the outer mitochondrial membranes of most cell types including steroidogenic tissues [72–77]. The endogenous ligand for PBR is a 10 kDa peptide, the DBI, also known as endozepine [78]. PBR agonists have been shown to increase steroid production in mouse Leydig or adrenal tumor cells, rat adrenal primary cultures, and in isolated mitochondria, while a PBR antagonist inhibited trophic hormone-stimulated steroid production in Leydig tumor cells [76, 77, 79–84]. Furthermore, addition of DBI to a cholesterol side-chain cleavage reconstituted enzyme system was able to stimulate the conversion of cholesterol to pregnenolone [85]. In support of the antagonist effect, depletion of DBI from Leydig tumor cells using cholesterol-linked phosphorothioate antisense oligonucleotides to DBI resulted in a loss of trophic hormone-stimulated steroid production in these cells [86]. Although the stimulating effects of PBR agonists are well documented, the mechanism whereby the DBI/PBR system can effect the transfer of cholesterol to the inner mitochondrial membrane is not clear. Recent studies have indicated that hormone stimulation of MA-10 mouse Leydig tumor cells resulted in the induction of a higher affinity DBI binding site on the PBR, which, in turn, resulted in the increased transfer of cholesterol to the inner membrane [87]. These higher affinity DBI sites were shown to be induced continuously in the constitutive steroid producing rat R2C Leydig tumor cell line [88]. Hence, it was suggested that a hormone-induced structural change in PBR results in a steady supply of cholesterol to the CSCC. Thus, a model has been proposed in which the association of PBR and DBI with several additional proteins formed a pore complex through which cholesterol and possibly other molecules could theoretically pass to the inner mitochondrial membrane [89].

However, many inconsistencies arise in studies dealing with the role of DBI/PBR in the transfer of cholesterol to the inner mitochondrial membrane during the acute regulation of steroidogenesis. It was reported [90] that acute synthesis of des-(Gly-Ile)-DBI was increased rapidly by ACTH in adrenocortical cells and the half-life of this protein was very short, both characteristics for a potential steroidogenic regulatory protein [12, 13, 21]. However, more recent studies have shown that DBI is not acutely regulated by trophic hormone treatment and that its half-life is greater than 3 hr [91]; thus, it is unlikely that hormonal regulation of DBI could be responsible for acute steroidogenesis. It was also demonstrated that rat adrenal DBI and PBR levels were reduced dramatically following 9 days of hypophysectomy. ACTH administration to these animals resulted in an increase in steroidogenesis that peaked within 1 hr while both PBR and DBI mRNA and protein levels showed no increase for approximately 12 hr, leading the authors to conclude that steroidogenesis and the presence of PBR/DBI were not temporally related [92]. Lastly, while many investigations using ligands of the PBR have shown that such treatment is stimulatory, a number of investigations have demonstrated that treatment of steroidogenic tissues with PBR ligands is inhibitory to steroidogenesis [93–96].

StAR. Another group of proteins that have been speculated to be involved in the acute regulation of steroid production in steroidogenic tissues is a family of mitochondrial proteins and phosphoproteins that have been described and characterized during the past decade. The earliest studies on this group of proteins were elegantly performed in Orme-Johnson's laboratory using radiolabeling and two-dimensional PAGE. In these studies, they demonstrated that these proteins consisted of a family of approximately 30 kDa proteins that rapidly appeared in response to corticotropic hormone stimulation of rat adrenal cells and were cycloheximide sensitive [16]. Later

studies indicated that similar proteins were also synthesized in response to trophic hormone treatment in the corpus luteum [97] as well as in primary cultures of mouse testicular Leydig cells [17, 98]. These proteins were then determined to be phosphoproteins in both adrenal and corpus luteum cells [18, 38]. This group then used amino acid analogs to demonstrate that steroidogenesis could be inhibited under conditions only partially inhibitory to protein synthesis, further indicating the necessity for the production of a labile protein in the acute regulation of steroidogenesis [30]. Cell fractionation illustrated that these proteins were localized to the mitochondria of hormone-stimulated adrenal cells [39, 99]. Recent observations indicated that in ACTH-stimulated rat adrenal cortex cells, the 30 kDa mitochondrial proteins arise as a result of the processing of two larger precursor forms having molecular weights of 37 and 32 kDa, respectively [40]. Mitochondrial proteins similar or identical in molecular weights and isoelectric points have also been described in hormone-stimulated luteal and adrenal glomerulosa cells [100, 101]. Thus, the studies from Orme-Johnson's laboratory provided the first more than reasonable indications that these proteins may be involved in the acute regulation of steroidogenesis.

Previous observations from our laboratory indicated that MA-10 mouse Leydig tumor cells also synthesize a family of 37 kDa, 32 kDa and 30 kDa mitochondrial proteins in response to trophic hormone and cAMP analog treatment and that the appearance and quantity of these proteins are highly correlative with the temporal appearance and quantitative levels of steroids produced [19, 20, 37, 41, 102–105]. While many of these observations were compelling for a role of these proteins in the regulation of steroidogenesis, they remained correlative and it became increasingly necessary that we establish a direct cause-and-effect relationship between the synthesis of these proteins and the production of steroids. With this goal in mind, we embarked on the purification, cloning, and expression of the 30 kDa mitochondrial proteins. Within the past 2 years we have purified the 30 kDa protein to homogeneity, obtained amino acid sequence for several tryptic peptides, and used this sequence information to design degenerative oligonucleotides for cloning the cDNA. A cDNA library was generated using mRNA isolated from hormone-stimulated MA-10 cells and a 400 bp PCR product was amplified from the cDNA library using the degenerative oligonucleotides. The PCR product was then used to probe the library, and a 1500 bp full-length cDNA clone was isolated. Comparison of the nucleic acid and deduced amino acid sequences to the GenEMBL and SWISS-PROT data bases revealed that the cDNA encoded a novel protein. *In vitro* transcription, translation, and mitochondrial processing of the protein products were used to confirm that the cloned cDNA encodes all forms of the 30 kDa mitochondrial protein family previously reported [106, 107]. Most importantly, however, was our observation that transient transfection and expression of this protein in MA-10 cells resulted in a significant increase in steroid production in the absence of hormone stimulation of any kind [106]. We have since extended these studies to include observations that the transient transfection of the 30 kDa protein can account for a rate and capacity of steroid production equal to that produced by hormone stimulation.* We have also generated stable transfected MA-10 cell lines that constitutively synthesize the 30 kDa protein and that produce steroid constitutively at a level several times higher than basal parental MA-10 cells. Lastly, we have transiently transfected non-steroidogenic monkey COS 1 kidney cells for expression of the 30 kDa protein (in conjunction with the terminal enzyme in the CSCC, cytochrome $P450_{scc}$ and adrenodoxin) and shown that these cells become highly steroidogenic only in the presence of the 30 kDa protein.* Therefore, for the first time in studies dealing with the acute regulation of steroid hormone production in response to the synthesis of a putative protein factor, we have demonstrated a direct cause-and-effect relationship between the expression of a specific protein and increased steroid production. As such, we have named this protein the Steroidogenic Acute Regulatory protein or StAR [106].

We have also performed studies designed to determine if regulation of the StAR gene is instrumental in the regulation of steroid production in MA-10 cells. These studies indicated that in response to hormone treatment three RNA transcripts of 3.4, 2.7 and 1.6 kb are induced in MA-10 cells and that the synthesis of StAR parallels the increase in RNA in a time frame that is concomitant with steroid production. Consistent with a steroidogenic function, the distribution of StAR is highly specific, localized by immunoblot analysis only to murine ovaries, adrenals, and testis. In addition, *in situ* hybridization analysis was used to examine the developmental regulation of StAR gene expression. These studies demonstrated that StAR transcripts first appear at embryonic day 10.5 specifically in the urogenital ridge and subsequent expression is restricted to the interstitial regions of the testis and the cortical cells of the adrenal gland. In contrast, embryonic ovarian tissue is devoid of StAR transcripts, which is consistent with the lack of a need for embryonic estrogen. However, copious amounts of StAR mRNA are expressed in the adult ovary. This specific localization of StAR to steroidogenically active cells further suggests a direct link between StAR expression and steroidogenesis [108].

Preliminary studies from our laboratory on the role of transcription in the acute regulation of steroidogenesis suggest that transcription is not required in the initial production of steroids in MA-10 cells (unpublished observations). Similar levels of steroid production accumulated for the first 60–90 min when MA-10 cells were treated with dibutyryl-cAMP in the presence or absence of the transcription inhibitor actinomycin D. Following this time period, steroid production in the inhibitor-treated cells plateaued, while control cells continued to make large amounts of steroid. While StAR mRNA was not detected in unstimulated MA-10 cells by Northern analysis, low levels were detected when reverse transcriptase-PCR was used. Although the effect of actinomycin D on StAR mRNA levels has yet to be determined, it is possible that synthesis of StAR could occur from pre-existing mRNA that is present in

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control cells. These observations support earlier studies which indicated that the initiation of steroidogenesis, while requiring *de novo* translation, does not require *de novo* transcription. However, it is clear from our studies that longer term steroid production is ultimately dependent on transcription of the StAR gene.

In addition to the studies on StAR in MA-10 cells and in embryonic and adult mouse tissue, we have also isolated a cDNA for StAR from a human adrenal library and demonstrated that the deduced amino acid sequence from the human StAR cDNA is 87% identical to the mouse sequence [109]. In this same study, it was also shown that the tissue distribution of its mRNA is restricted to adrenal, gonadal, and possibly kidney in the human. We also demonstrated that the co-expression of the human StAR cDNA with $P450_{scc}$ and adrenodoxin in COS cells results in a significant increase in steroid production. Lastly, fluorescence *in situ* hybridization was used to demonstrate that StAR resides on chromosome 8, region p11.2 in the human, and that a StAR pseudogene resides on chromosome 13.

Mutations in StAR and Lipoid Congenital Adrenal Hyperplasia

Perhaps the most striking evidence for the importance of StAR in cholesterol transfer and the acute regulation of steroidogenesis is found in the results recently obtained from studies on the disease LCAH. LCAH is a lethal condition that results from a complete inability of the newborn infant to synthesize steroids. The lack of mineralocorticoids and glucocorticoids results in death within days to weeks of birth if not detected and treated with adequate replacement therapy. This condition is manifested by the presence of large adrenals containing very high levels of cholesterol and cholesterol esters and also by an increased amount of lipid accumulation in testicular Leydig cells, though the level in the testis is somewhat lower than that seen in adrenals. Since isolated mitochondria from adrenals and gonads of affected patients could not convert cholesterol to pregnenolone [110–113] this disease was understandably thought to be due to an abnormality of $P450_{scc}$ enzyme activity, which converts cholesterol to pregnenolone [114]. Recently, however, this enzyme has been shown to be normal in patients who suffered from this disease [115]. Additionally, and most importantly, the genes and/or mRNA and/or protein for several other candidates which either play a role or which have been purported to play a role in the regulation of steroidogenesis, such as adrenodoxin, adrenodoxin reductase, SAP, SCP_2, HSP-78, and PBR and its ligand, DBI, have also been shown to be present at apparently normal levels of abundance in these patients [115, 116]. Recent clinical studies surprisingly showed that placental steroidogenesis persists in LCAH [117]. StAR provided a new candidate to test in this disease. Testicular tissue of two patients and genomic DNA of a third patient with LCAH have mutations in the gene for StAR [118]. These mutations were found to consist of C to T transitions in the gene sequences, which resulted in the premature insertion of stop codons. This resulted in the truncation of StAR protein by 28 amino acids in two of the patients and 93 amino acids in another. These truncations were confirmed by Western analysis following expression of the mutated cDNAs in COS 1 cells [118]. In addition, virtually none of the precursor form of StAR expressed from the cDNAs of these patients was converted to the mature mitochondrial form. Most importantly, expression of the StAR cDNA from these patients in COS 1 cells indicated that the protein produced was completely inactive in its ability to promote steroidogenesis, whereas the normal human protein resulted in an 8-fold increase in steroid production when expressed. Finally, the need for StAR activity could be circumvented by using freely diffusable 20α-hydroxycholesterol as a substrate for steroidogenesis [118]. Therefore, in a most dramatic manner the indispensable role of StAR in the production of steroids has been demonstrated.

Proposed Model for Cholesterol Transfer by StAR

Because of our observations on the expression of StAR precursor followed by its processing to the mature mitochondrial forms [106], and because of the observations made during the studies on LCAH [118], we have formulated a model whereby StAR may act in the acute regulation of cholesterol transfer to the CSCC. We propose that in response to hormone stimulation the 37 kDa precursor protein is synthesized rapidly in the cytosol, targeted to the mitochondria via its signal sequence, and interacts with a specific receptor on the mitochondrial outer membrane. As the transfer of this protein to the inner mitochondrial compartment begins, "contact sites" between the inner and outer membranes are formed and the signal sequence is removed by a matrix processing protease forming the 32 kDa form of the protein. Further processing of the protein removes the targeting sequence, and the mature 30 kDa protein remains associated with either the inner mitochondrial or inter-membrane compartment. We propose that it is during the processing of the protein with the accompanying formation of "contact sites" during protein import that cholesterol is able to transfer from the outer to the inner mitochondrial membrane [119] and, hence, be available to the CSCC. Following processing of the 37 kDa protein to the 30 kDa proteins, the membranes once again separate, and no further cholesterol transfer can occur. Thus, it is the continued synthesis and import of additional precursor proteins that allow for the continued transport of cholesterol to the inner membrane. Since the half-lives of the 37 and 32 kDa precursors of the 30 kDa mitochondrial proteins have been shown to be very short [40], this would explain the observation that steroidogenesis decays very quickly in the absence of new protein synthesis. In support of this model, we and others have demonstrated the presence of such precursor proteins [19, 40] and have shown that the 30 kDa proteins are found associated with the inner mitochondrial compartment [19, 106]. These observations, coupled to those indicating that the transport of mitochondrial proteins across the membranes occurs at contact sites [120–125], make this a viable model at this point in time. We should point out, however, that we have no infor-

mation as to whether this transfer of cholesterol occurs in a passive manner when contact sites are formed or whether StAR has the capacity to act as a cholesterol binding protein and, hence, carry cholesterol to the inner mitochondrial membrane.

In summary, we report at this time that the long sought protein factor responsible for the transfer of cholesterol from cellular stores to the inner mitochondrial membrane, and as such is necessary for regulating the acute production of steroids, has been identified. The key future studies concerning StAR will be to determine the elements responsible for the regulation of this most important gene and, of course, to determine the mechanism whereby StAR is able to effect the transfer of cholesterol to the inner mitochondrial membrane and the CSCC. To this end, additional information on the requirements for the processing of the StAR, as well as the role of phosphorylation, will be most valuable.

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