A Review of the Characteristics of the Protein Required for the Acute Regulation of Steroid Hormone Biosynthesis: The Case for the Steroidogenic Acute Regulatory (StAR) Protein (44214)

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Abstract. **The acute regulation of steroid hormone biosynthesis requires the *de novo* synthesis of a protein whose function is to effect the transfer of cholesterol from the outer to the inner mitochondrial membrane where it is cleaved to pregnenolone. This review attempts to summarize the list of this protein's characteristics, which have emerged as a result of experimental observations, and to make the case that the Steroidogenic Acute Regulatory (StAR) Protein is the acute regulator of steroid hormone synthesis.** [P.S.E.B.M. 1998, Vol 217]

In order for steroid hormones to be synthesized by most steroidogenic tissues in response to trophic hormone stimulation, it is generally accepted that new protein synthesis must occur. This observation was first made more than three decades ago (1), and has been repeated many times since (2–22). More specifically, the role of the newly-synthesized protein has been placed precisely at the step in which the substrate for all steroid hormones, cholesterol, is transferred from the outer to the inner mitochondrial membrane for its subsequent conversion to pregnenolone (23–32). This conversion is the first enzymatic step in the steroidogenic cascade and occurs as a result of the action of the cytochrome P-450scc enzyme, which resides on the matrix side of the inner mitochondrial membrane (33–36). Due to the hydrophobic nature of cholesterol, such assisted transfer through the aqueous mitochondrial inter-membrane space is required. Much has been written in the past as well as in more recent times concerning both the components involved in cholesterol transfer and the possible mechanisms by which its transfer to the P-450scc occurs. These subjects have been summarized in greater detail in a number of review articles (37–48).

During the long search for the putative regulatory protein, a number of observations were made that gave rise to several consensus characteristics for this protein. In addition to the early characteristics, which were agreed upon by most investigators in the field, one might also surmize that there would be yet additional traits that such a protein might have based on its specialized function. The purpose of this review is, therefore, to revisit many of the established characteristics of the regulatory protein, discuss additional characteristics that would seem to apply to such a protein, and then attempt to make the case that the Steroidogenic Acute Regulatory (StAR) protein fulfills all of these requirements. As such, it appears that StAR is the regulatory protein. This does not preclude the probability that other proteins are also necessary for cholesterol transfer and thus, steroid hormone biosynthesis to occur. However, for the acute stimulation of steroid hormone synthesis, there is no strong evidence that other proteins are regulatory in nature. It is also now clear that steroid hormone biosynthesis may occur in the absence of StAR in selected steroidogenic tissues such as the brain (49) and the human placenta (50). Lastly, there also exists the probability that whereas the vast majority of steroid biosynthesis occurs as a result of trophic hormone stimulation and StAR action, a lesser portion of steroid production may occur in a StAR independent manner (51).

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Early Characteristics of the Regulatory Protein

Cycloheximide Sensitivity. The studies of Stone and Hechter placed the rate-limiting step in the production of steroid hormones at the conversion of cholesterol to pregnenolone (52). Later investigations by Ferguson and Garren indicated that this conversion was sensitive to the protein synthesis inhibitors puromycin and cycloheximide (1–8). Thus, one of the first observed characteristics of the regulatory protein was that it was protein synthesis inhibitor sensitive. This has most often been referred to as the "cycloheximide sensitive" characteristic in the literature. StAR has also been shown to be sensitive to inhibitors of protein synthesis by several different laboratories. The first to demonstrate this sensitivity was the work of Orme-Johnson who showed that trophic hormone–stimulated, newly-synthesized 30-kDa proteins in rat adrenal glands (53), rat corpora lutea (54) and rat Leydig cells (55) were sensitive to cycloheximide. These proteins are almost certainly identical to the StAR protein. In addition, cycloheximide sensitivity of a family of 30-kDa mitochondrial proteins, now known to be StAR, was also demonstrated in MA-10 mouse Leydig tumor cells (56, 57). Therefore, the StAR protein satisfies one of the original criteria for the regulatory protein: sensitivity to protein synthesis inhibitors.

Trophic Hormone Inducibility. Under the conditions in which the above experiments were performed, the characteristic of cycloheximide sensitivity clearly implies that the regulatory protein is newly synthesized in response to trophic hormone stimulation. There are now many examples in a number of different species using several different steroidogenic tissues in which increased synthesis of the StAR protein has been observed in response to trophic hormone stimulation (58–77). These studies have indicated that in adrenal, ovarian and testicular tissue, StAR is synthesized by the appropriate trophic hormone and that its synthesis is seen within a time frame that is consistent with the production of steroids. Lastly, in most steroidogenic tissues, the trophic hormone stimulatory signal is transduced through the cAMP second messenger pathway. In the majority of the studies referred to in this section, StAR synthesis was also demonstrated to be increased by treatment with cAMP analog. Thus, StAR fulfills a second characteristic of the putative regulatory protein: trophic hormone inducibility.

Rapidly Synthesized. A third characteristic that evolved from the studies of Ferguson and Garren was that the regulatory protein was very rapidly synthesized in response to hormone stimulation (2, 3). This "rapidly synthesized" characteristic arose as a result of the observation that steroid hormone production occurred very quickly in response to stimulation and that this rapid production could be blocked by cycloheximide (2, 3). Once again, the laboratory of Orme-Johnson was the first to demonstrate that the 30-kDa proteins were synthesized within minutes of hormone stimulation and their synthesis paralleled steroid hormone biosynthesis (53). Since that time, a number of studies have corroborated the finding that the synthesis of the StAR protein occurs within a time frame that is tightly coupled to steroid hormone biosynthesis (61, 62, 65, 66, 74, 78, 79). Thus, another characteristic of the regulatory protein is satisfied by known properties of the StAR protein.

High Lability. Studies in which protein synthesis inhibitors were placed on pre-stimulated adrenal cortex cells indicated that the rate of steroid hormone biosynthesis decreased very rapidly following the inhibition of *de novo* protein synthesis (3). This observation brought forth yet another of the early characteristics of the regulatory protein, namely, that it was "rapidly turned over" or "highly labile" (3). While the synthesis of the 30-kDa protein was indeed very rapid, its turnover rate or half-life was, in fact, much longer than would be expected for a highly labile protein. This apparent discrepancy was resolved when it was found that the StAR protein is a mitochondrial protein that is first synthesized in the cytosol as a larger, 37-kDa precursor protein and then quickly imported into and processed by the mitochondria into the 30-kDa form (57, 80). The significance of this finding is that it is now believed that the active form of StAR is the precursor form and once imported into the mitochondria, has no further action in cholesterol transfer (42, 57, 80). While it was suggested earlier that it was during import of StAR and the concomitant formation of contact sites between the outer and inner mitochondrial membranes that StAR was able to transfer cholesterol (42, 57), this theory may not be completely correct in light of more recent findings (81). However, it appears clear that under normal circumstances in which StAR is imported into the mitochondria and processed, the transfer of StAR to the inner mitochondrial compartments renders it inactive in cholesterol transfer. This somewhat unorthodox mechanism allows the StAR protein to fulfill yet another characteristic of the regulatory protein, that of being "highly labile," when the half-life of its active form is considered.

Specificity to Steroidogenic Tissue. Another of the regulatory protein's characteristics, which has not been as widely studied and accepted as the classic criteria, but perhaps just as important, is that such a protein would be confined to steroidogenic tissues, given the highly specific nature of its proposed role. Although this point will be re-visited in greater detail later, it should be noted that once again Orme-Johnson's laboratory determined that the 30-kDa protein they studied was found in adrenal (53), ovary (54) and testis (55), but not in adipocytes (82) or liver cells (55). Thus, an early indication that the StAR protein was found exclusively in steroidogenic tissues was obtained.

Characteristics Consistent With the Acute Regulatory Protein

The above criteria have been regarded as the classical characteristics that many investigators have used in describing the putative regulatory protein over the years. These

observations are still considered accurate. The number of characteristics were limited by the fact that the protein had not yet been unequivocally identified. With the recent purification, cloning, sequencing and expression of the StAR protein, additional information and, more importantly, biological reagents, became available (58). The availability of reagents such as cDNAs and antisera have, in a more complete manner, allowed for the demonstration that StAR fulfills the characteristics of the acute regulatory protein. These additional studies will be described below, and the case for StAR in acutely regulating steroidogenesis using these observations will be made.

The Protein Increases Steroidogenesis. It is reasonable that the expression of the regulatory protein should result in an increase in steroid production in the absence of trophic hormone stimulation. When the cDNA for the StAR protein was expressed in either unstimulated MA-10 mouse Leydig tumor cells (58), or in COS-1 cells co-transfected with P-450scc and adrenodoxin (42, 59, 83), steroid production was increased several-fold. Also, addition of COS-1 cell lysate containing expressed StAR protein to mitochondria isolated from unstimulated MA-10 cells resulted in a dose- and time-dependent increase in steroid production (78). Thus, the expression of StAR within cells and the addition of StAR protein to isolated mitochondria were capable of increasing steroid production, a characteristic expected for such a regulatory protein.

Specificity to Steroidogenic Tissues. As mentioned briefly above, given the role proposed for the regulatory protein one might expect such a specific protein to be confined to steroidogenic tissues. Using an antipeptide antisera to the StAR protein, it was shown that StAR appeared to be highly specific to the steroidogenic tissues in the mouse, appearing in the adrenal, ovary, and testis and not in any of the other tissues screened (79). Also, Northern analysis indicated that StAR mRNA was essentially confined to the adrenal, testis, and ovary in human tissues (83). In this study, a small amount of StAR mRNA was found in the human kidney (83). The reason for StAR mRNA being present in the kidney is not known, but in the case of the mouse, the StAR protein does not appear to be expressed in the kidney (79). Lastly, while StAR appears to be absent in one steroidogenic tissue, namely, the human placenta (83), this tissue appears to regulate steroidogenesis in a different but unknown manner (50). However, StAR protein has been demonstrated in the placenta of at least one other animal, the cow (65, 77). Thus, StAR is highly specific for steroidogenic tissues, a criterion that is, once again, logical for the regulatory protein.

Developmental Profile. It can be reasoned further that the regulatory protein should appear during the course of embryonic development within a specific time frame and within specific cells in the embryo corresponding with steroid production. To this end, Clark *et al.* studied the expression of the StAR mRNA in the developing mouse embryo (79). They determined that in the developing adrenal cortex and testis, StAR mRNA was expressed specifically within the steroidogenic cells of those organs, and its temporal appearance coincided with steroid production. In contrast, the developing ovary, which does not make steroids *in utero,* did not show expression of StAR. Also, during the fetal development in the cow, it was observed that StAR expression occurred at Weeks 12 and 13 of gestation in the fetal testes, a steroidogenic organ, but not in the fetal ovary, which does not produce steroids *in utero* (77).

Interaction with the Mitochondria. Since the first enzymatic step in the biosynthesis of steroids occurs in the inner mitochondrial membrane, a logical assumption might be that in order to deliver cholesterol to this site, some interaction between the regulatory protein and the mitochondria should take place. StAR has been shown to be a mitochondrial protein, which, while nuclear encoded and synthesized in the cytosol, is quickly imported into the mitochondria. The interaction between the StAR protein and the mitochondria has been demonstrated biochemically using intact cells in culture (55–57, 64, 84–87), biochemically using a completely *in vitro* system (78), and immunocytologically using immunogold electron microscopy (78). Indeed, when StAR is transcribed from a cDNA and then translated *in vitro,* addition of mitochondria to the incubation results in the import and processing of the precursor StAR to all of its mature forms (78). The mitochondrial location of StAR and studies implicating that its action occurs as a result of interaction with the outer mitochondrial membrane have also been reported recently (81). Therefore, consistent with this characteristic, StAR has been shown to be associated with the mitochondria, the site of the first and regulated step in steroid hormone biosynthesis.

Up-Regulation by Additional Steroidogenic Stimuli. As stated above, the majority of steroidogenic tissues employ trophic hormone activation of the cAMP second messenger system in regulating steroidogenesis. However, there are other examples of tissues that can produce steroids with substances that do not appear to act through cAMP. The question then becomes one of whether StAR is also involved in steroid hormone production in these instances. In the rabbit, the luteotrophic agent is estrogen, not LH (88). Townson *et al.* were able to show clearly that in the rabbit corpora lutea, both progesterone production and StAR synthesis were regulated by estrogen (66). In adrenal glomerulosa cells, production of aldosterone can be regulated by compounds other than ACTH, such as angiotensin II and those that result in Ca^{++}-mobilization and do not act through the cAMP second messenger pathway. These agents have been shown to result in increased aldosterone production as well as a concomitant increase in the level of StAR protein in H295R human adrenocortical cells (61). In the sheep corpora lutea, in addition to LH, progesterone and StAR mRNA expression were both up-regulated by growth hormone, the former probably acting through the tyrosine kinase pathway (62). Thus, in addition to trophic hormone stimulation, StAR can be induced by

compounds that stimulate steroid hormone production through alternate signalling pathways, a characteristic also consistent with its purported action.

Downregulation by Antisteroidogenic Agents. Whereas a number of studies have demonstrated that steroidogenesis and StAR protein expression can be increased with various steroidogenic stimuli, several studies have also shown that when steroid production was inhibited, the mechanism appeared to be through the disruption of StAR synthesis. Bosmann *et al.* found that treatment of mice with lipopolysaccharide for as little as 2 hr resulted in an almost complete inhibition of testosterone biosynthesis and a concomitant decrease in StAR protein content, whereas other proteins involved in steroidogenesis were unaffected at this time (67). In the sheep, treatment with prostaglandin F2α (PGF2α), a luteolytic agent resulting in degradation of the corpus luteum and cessation of progesterone synthesis, resulted in a decrease in StAR mRNA expression (62). In a similar study, treatment with PGF2α was also shown to decrease StAR mRNA expression in the rat ovary (73). Lastly, a recent study has demonstrated that the organophosphate cholesterol ester hydrolase (CEH) inhibitor, diethylumbelliferyl phosphate, which at low concentrations inhibits steroidogenesis by blocking the transfer of cholesterol into the mitochondria and not by inhibiting CEH, also inhibited the synthesis of the StAR protein (89). Therefore, some agents that decrease steroid production also decrease mRNA and/or protein levels of StAR, indicating that expression of this protein may represent a key control point for the downregulation of steroid hormone synthesis in addition to its role as a stimulator of steroidogenesis.

Mutations Result in Loss of Steroidogenesis. One might also predict that if the regulatory protein were inactived through mutation or some other such event, steroid hormone biosynthesis would be severely affected. Such a condition exists and has demonstrated that several different mutations in the StAR gene which render the protein either entirely or largely inactive, result in greatly impaired steroidogenesis. These mutations cause a condition in humans known as congenital lipoid adrenal hyperplasia, a potentially fatal disease if not detected and treated (51, 59, 90). To date, mutations in the StAR gene are the only known causes of this disease, an observation that underscores the importance of StAR in regulated steroid hormone production and fulfills another expected characteristic of the regulatory protein.

Expression in Steroidogenic Tissues of Different Species and Highly Conserved. It was observed earlier that StAR protein was not found in adipocytes or liver (55, 82). It is now known that StAR is essentially confined to specific steroidogenic cell types both during development and in adult tissues (79, 83). It could be argued that such an integral protein should be widespread among different species and located exclusively in their steroidogenic cells. Also, it is reasonable to expect that such a protein would be highly conserved among different species. As such, the StAR protein and/or StAR mRNA have been shown to be present in the steroidogenic tissues of the mouse (67, 90), rat (68, 73), human (59, 61, 64, 69, 72, 83), sheep (62), cow (63, 65, 70, 75, 77), pig (74), and rabbit (66). In species in which StAR cDNA has been cloned and sequenced and the amino acid sequence deduced, StAR is highly conserved. For example, the mouse and rat StAR amino acid sequences are 96% identical (68), the human and mouse sequences are 87% identical and 92% similar (64), and the bovine and mouse amino acid sequences are 84% homologous (63). In addition, a partial cDNA, isolated from the sheep, has 80% identity with the corresponding regions of the other species (62). Also, a 280-bp partial cDNA in the pig was shown to be 89%, 91%, and 87% identical to the same regions in the mouse, sheep and human StAR cDNAs respectively (74). In general, an 85%–90% identity and greater than 90% similarity in StAR have been seen in the various species studied to date. This indicates a high degree of conservation of this protein, a common feature of important proteins.

Summary

Using the classical characteristics determined from early experiments and through the addition of more recent observations, the Steroidogenic Acute Regulatory (StAR) protein appears to be the only candidate that fulfills all of the requirements for the putative regulatory protein. However, it should be clearly noted that whereas the StAR protein appears to be the acute regulatory protein as described by Ferguson, Garren and others, it is very likely not the only protein involved in the transfer of cholesterol to the P-450scc enzyme. Thus, it is important to distinguish between the role of StAR as the rapidly synthesized regulatory protein responsible for initiating a series of events that culminate in cholesterol transfer and the roles of other proteins that may also be indispensable for this transfer. We can only make the case that StAR represents the "trophic hormone stimulated, rapidly synthesized, cycloheximide sensitive, highly labile protein," which has been sought for over three decades, not that it is the only protein involved in cholesterol transfer. The roles of other proteins, such as the peripheral benzodiazepine receptor (39), its ligand the diazepine binding inhibitor (39), the steroidogenesis activator polypeptide (91–93), and perhaps even the role of the first two enzymes in the steroidogenic pathway, P-450scc and 3β hydroxysteroid dehydrogenase (75, 94, 95) in assisted cholesterol transfer must also be evaluated. However, given the characteristics of these proteins with regard to the time required for hormone induction and cycloheximide sensitivity, it is highly unlikely that any of them will prove to be regulatory in nature within an acute time frame. Therefore, assuming that StAR is, in fact, the regulatory protein, the identity and role of other components in the transfer of cholesterol to the inner mitochondrial membrane remain the most interesting aspects of the field of steroidogenesis.

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