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THE STEROIDOGENIC ACUTE REGULATORY (StAR) PROTEIN

DOUGLAS M. STOCCO

Department of Cell Biology and Biochemistry, Texas Tech University Health Sciences Center, Lubbock, Texas USA.

The biosynthesis of steroid hormones is a fundamental process without which life itself and continuation of the species would be impossible. For example, the adrenal gland synthesizes mineralocorticoids which are responsible for the maintenance of salt balance and hence blood pressure in the body and glucocorticoids which function in carbohydrate metabolism and stress management. In addition, the male gonads synthesize the steroid hormone testosterone and the female gonads synthesize estrogen and progesterone, hormones which are absolutely indispensable for the maintenance of reproductive capacity. Thus, the production of steroid hormones represent an essential metabolic pathway in the body.

Synthesis of steroid hormones by steroidogenic tissues are under the control of pituitary peptides which interact with highly specific receptor proteins on the surface of the steroidogenic cells in question. In the case of the gonads, the synthesis of steroids are under the control of the pituitary peptides Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH). These peptide hormones interact with their specific receptors and through the cAMP second messenger pathway, result in the very rapid production of the appropriate steroid hormone. For over three decades this process has been known to require the de novo synthesis of a protein(s) which acts to acutely regulate steroid production. Summarizing a great deal of experimental work, the role of this protein factor has been shown to be the rapid translocation of the common substrate used in all steroid hormone biosynthesis, namely cholesterol, from cellular stores to the inner mitochondrial membrane. The matrix facing side of the inner mitochondrial membrane is the site of the cholesterol side-chain cleavage enzyme which performs the first enzymatic step in steroid hormone biosynthesis. Therefore, the transfer of cholesterol is absolutely required for the formation of steroids and poses a difficult problem in that cholesterol, which is very hydrophobic, must cross the aqueous intermembrane space quickly. Since cholesterol would not be able to traverse this barrier unassisted, the role of the putative regulatory protein was thought to be the expedition of this transfer. The identity of this protein factor had remained a mystery for over three decades despite an intense search for it. A number of years ago investigations were initiated in an effort to find and characterize this protein factor. Studies in our laboratory as well as in the laboratories of others using a combination of hormone stimulation and radiolabeling techniques demonstrated the presence of several newly synthesized mitochondrial proteins in response to acute hormone stimulation in adrenal and testicular Leydig cells. Further characterization of these proteins indicated a series of very strong correlations between their appearance and the appearance of steroid hormone biosynthesis. Despite the correlations, it became apparent that an additional approach would be required to provide the unequivocal proof necessary to demonstrate the role of this protein in steroidogenesis. Therefore, we purified the protein to homogeneity, obtained amino acid sequence data for several tryptic peptides and used these sequences to design degenerate oligonucleotides. These oligos and PCR were used to prepare a 400 bp oligonucleotide which was successful as a probe in isolating a full length cDNA from a library. This cDNA was then sequenced and was found to be a unique protein. Importantly, expression of the cDNA for this protein in several different systems have all resulted in an increased production of steroids in the absence of hormone stimulation of the cells. Thus, we have been successful in finding and characterizing the long sought acute regulator of steroidogenesis. We have named this protein StAR for Steroidogenic Acute Regulatory protein.

Soon after the initial work on StAR, data was obtained in collaboration with other laboratories which served to most dramatically underscore the importance of StAR in normal cellular function. The congenitally lethal condition known as lipoid Congenital Adrenal Hyperplasia (lipoid CAH) is characterized by death within weeks of birth if undetected. The clinical manifestations are a severe depression of steroids of any kind in the newborn and thus death can result from either a lack of glucocorticoids which are necessary for normal lung de-

velopment or from a lack of mineralocorticoids which are essential for blood pressure maintenance. The adrenal gland and the testes of these individuals are, however, replete with extremely high levels of cholesterol and cholesterol esters, indicating an inability to convert cholesterol to the first steroid formed, pregnenolone. This disease was formerly thought to be due to a defect in the enzyme CSCC which converts cholesterol to pregnenolone. However, later studies have shown that not only is the gene for CSCC normal in these patients, but so were several additional proteins which are involved in the production of steroids and also thought to be involved in the mobilization of cholesterol. We and our collaborators were able to unequivocally demonstrate that the condition lipoid CAH was due to mutations in the StAR gene. To date mutations in the StAR gene are the only known cause of lipoid CAH and a condition which was once thought to be extremely rare is more common than previously estimated and in countries like Japan as many as 1 in 200 persons may be genetic carriers for this disease. Thus, in a dramatic fashion the cause of this disease and the role of the StAR protein were unequivocally established with this human StAR gene knockout.

Since our initial description of the characteristics of the StAR protein, we have continued studies on this fundamentally important protein. The StAR cDNA and gene has been cloned from many cDNA and genomic libraries respectively. In different steroidogenic tissues and in several different species, StAR has been shown to be highly homologous, having greater than 85% identity at both the nucleotide and amino acid level in most species studied to date. Also, studies have been performed which indicate that the StAR gene is regulated in a time and tissue specific manner in response to trophic hormone stimulation and during the course of development. It has also been determined that this regulation occurs at least in part through the action of the orphan nuclear transcription factor SF-1. In addition, phosphorylation of the StAR protein on the serine residue at position 194 has been shown to be required for full steroidogenic activity. Recently, mice containing a knockout of the StAR gene have been developed and initial characterization of the animals performed. Their phenotype is very similar to that observed in the human disease, and the availability of such animals will allow for experimentation not possible with the human condition.

In addition to the positive regulation of the StAR gene by SF-1, it has also been demonstrated that StAR expression can be strongly inhibited by another transcription factor, DAX-1. Overexpression of DAX-1 in steroidogenic cells resulted in a complete inhibition of stimulated steroid hormone biosynthesis and also in a complete inhibition of StAR expression. Closer investigation of this observation led to the finding that DAX-1 was able to inhibit StAR gene expression by binding to hairpin structures in single stranded DNA which were near the SF-1 regulatory site in the StAR promotor. This finding may have implications in the condition known as Dosage Sensitive Sex Reversal, since its phenotype is a lowered level of circulating steroids and appears to be caused by a duplication of the X chromosome region housing the DAX-1 gene.

We have also performed studies on the mechanism of action whereby the StAR protein can result in the transfer of the hormone substrate cholesterol to the inner mitochondrial membrane. As such we have constructed cDNAs which give rise to proteins which have truncations of both the N-terminal and C-terminal ends of the StAR protein. When expressed, it was observed that the C-terminal portion of the protein is responsible for cholesterol transfer, an observation in keeping with the fact that all mutations in StAR resulting in lipoid CAH are found in the C-terminus of the protein. We have also recently been able to demonstrate that direct interaction of StAR protein with the mitochondrial membrane surface results in cholesterol transfer. Also, a recent study indicated that three proteins intimately involved in steroid hormone biosynthesis, namely StAR and the first two enzymes in the steroidogenic cascade, P450 side chain cleavage and 3β-hydroxysteroid dehydrogenase, all were found to reside in mitochondrial contact sites. Thus, it may be possible that not only does StAR deliver cholesterol to the inner mitochondrial membrane, but it does so at sites where the substrate can be quickly converted into progesterone, an extremely important steroid. (Supported by NIH grant HD17481).