Discuss the role of StAR protein in steroid hormone synthesis

This essay will determine the exact role of StAR, its structure, regulation, and problems associated with mutations, and thereby review recent research that has been carried out in relation to the role of StAR in steroid hormone synthesis.

Steroidogenesis

The synthesis of steriod hormones is essential for many functions of the body. The adrenal gland synthesises glucocorticoids and mineralocorticoids which regulate metabolism and water balance, and also small amounts of sex hormones such as androgens (Yadav, 2004). The main site of androgen synthesis is the Leydig cells in the testes, that of oestradiol in the ovary. The testes and ovaries are collectively called gonads and gonadal steroids are essential for reproductive function (Yadav, 2004). Steroids also have an important role in the brain, and steriods found here are called neurosteroids. Progesterone is involved in myelination and dehydroepiandrosterone in plasticity (Sierra, 2004). All these hormones require strict regulation to ensure normal bodily function.

An Introduction to StAR

The steroidogenic acute regulatory protein (StAR) was first identified by Orme-Johnson *et al.* in the mid 1980's. They discovered that this protein with molecular weight 28,000 was expressed in rat corpus luteum and adrenal cortex cells, both steroidogenic tissues, and that its synthesis was stimulated by human chorionic gonadotrophin and cyclic AMP (Orme-Johnson & Pon, 1986). It was later described by Clark *et al.* (1994). They were the first to clone StAR and therefore identify it as a novel protein. Their study used MA-10 cells in mouse Leydig tumour cells, and they found StAR to be synthesized in response to stimulation by luteinizing hormone.

StAR is important in transferring cholesterol, the precursor for all steroid hormones, from the outer to inner mitochondrial membrane where P450scc is located (Stocco, 2007).
P450scc is a side chain cleavage enzyme which converts cholesterol to pregnenolone, and is the rate-limiting enzyme in steroidogenesis (Hill *et al.*, 2010). Figure 1 represents the steps that cholesterol undertakes before reaching StAR.

Before StAR was given its name, Stocco & Sodeman (1991) discovered that the 30-kDa protein found in the mitochondria of MA-10 mouse tumour Leydig cells, which was in fact StAR, was formed from two precursors with masses of 32-kDa and 37-kDa.

The essentiality of StAR was demonstrated by studying patients with congenital lipoid adrenal hyperplasia. Mutations in StAR were found in these patients, showing that this protein is necessary for gonadal and adrenal steroidogenesis (Lin *et al.*, 1995). This disease will be discussed further later in the essay.

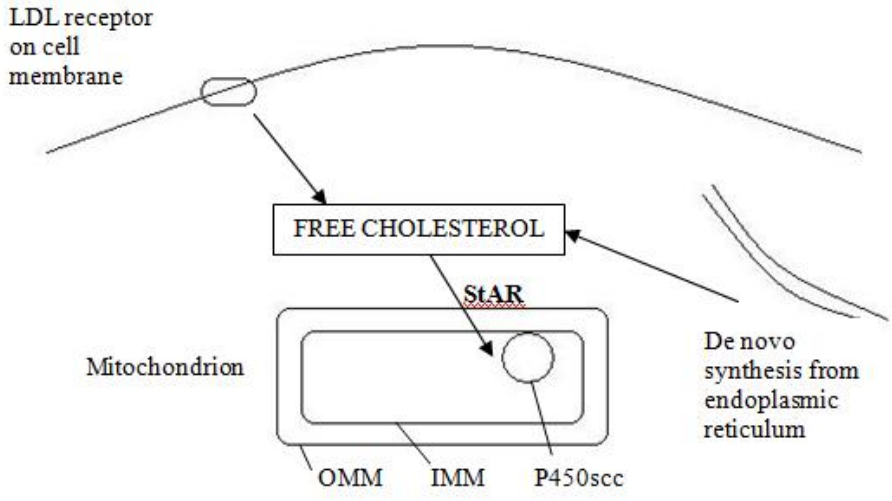

Figure 1: Simplified diagram showing the movement, synthesis and destination of cholesterol within a cell (Source: Redrawn from Miller, 2007). OMM – outer mitochondrial membrane, IMM – inner mitochondrial membrane, LDL receptor – low density lipoprotein receptor, P450scc – cholesterol side chain cleavage enzyme.

The Structure of StAR

The structure of StAR has been very recently revised in relation to mutations, as seen in Figure 2.

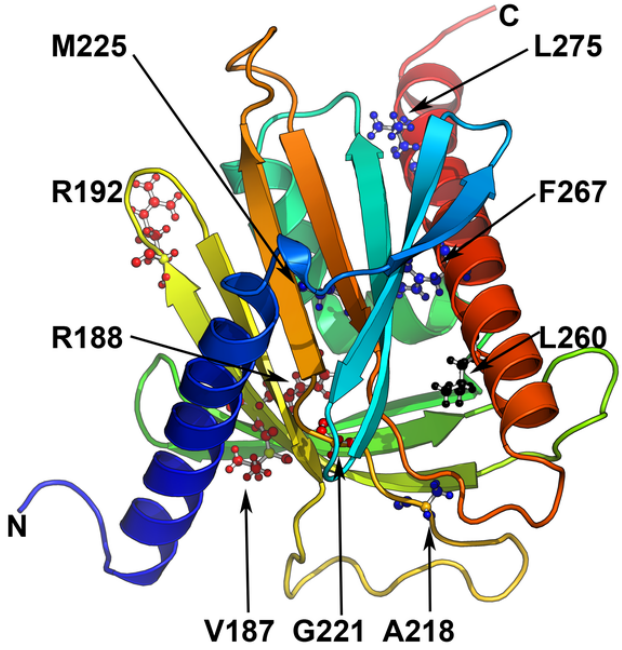

Figure 2: A representation of StAR protein from the N terminal to C terminal. Each labelled point is a potential mutation site. (Source: Flück *et al.*, 2011)

StAR acts exclusively on the outer mitochondrial membrane to bind cholesterol, and is inactivated when imported into the mitochondria (Bose *et al.*, 2002). This process is important in the regulation of steroidogenesis. Figure 3 shows the binding of cholesterol in both the wild type and a mutated StAR molecule.

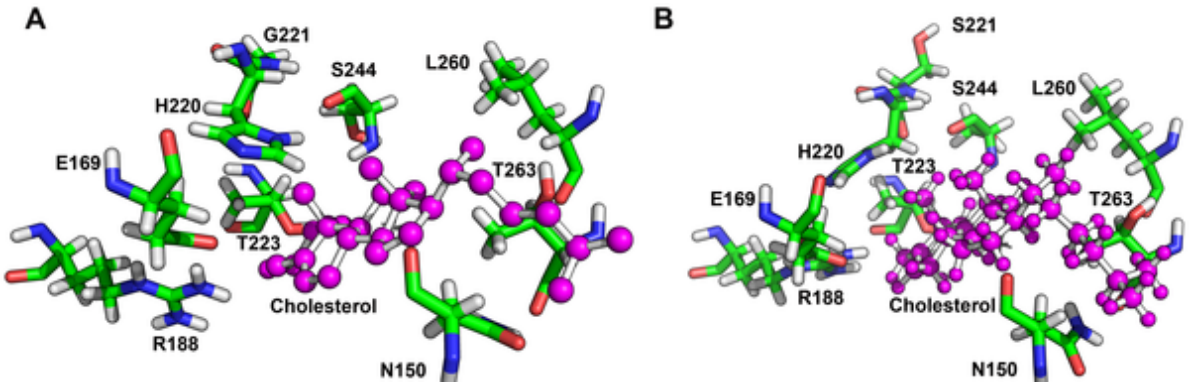

Figure 3: The binding pocket of StAR. Cholesterol is represented in pink, and the interacting amino acids are also shown. A – wild type StAR binding cholesterol. B – a mutation (S221) disrupts the binding process. (Source: Flück *et al.*, 2011)

The Regulation of StAR

Many signalling pathways have been identified to be involved in regulating the transcription of StAR. Figure 4 shows how the signalling molecules interact with each other. cAMP is the main regulator, promoting both expression and phosphorylation. Phosphorylation of serine 195 increases activity (Arakane *et al.*, 1997).

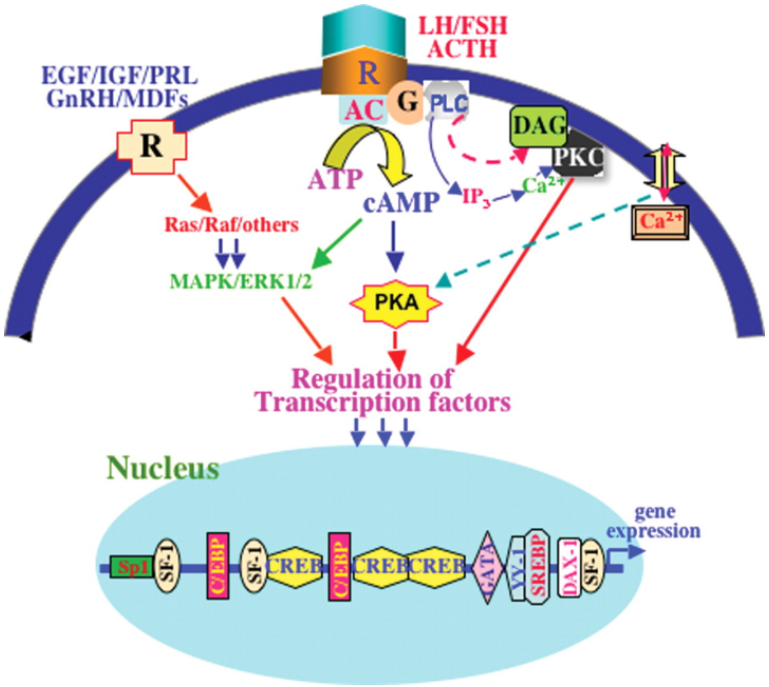

Figure 4: Schematic showing the different signalling pathways that regulate the transcription of StAR (Source: Manna *et al*., 2009)

One recent insight has been into the role of adiponectin, an adipokine, in the expression of StAR and consequently levels of steroid hormone, specifically cortisol (Ramanjaneya *et al.*, 2011). The study demonstrated that adiponectin increases StAR expression in H295R cells, which are adrenal cells that express all the key enzymes involved in steroidogenesis. The change in expression can be seen in Figure 5. The study also showed that adiponectin activated AMPK, AKT and ERK1/2 MAPK signalling pathways. This study is significant for obese people as an increased level of adiponectin would result in increased levels of cortisol.

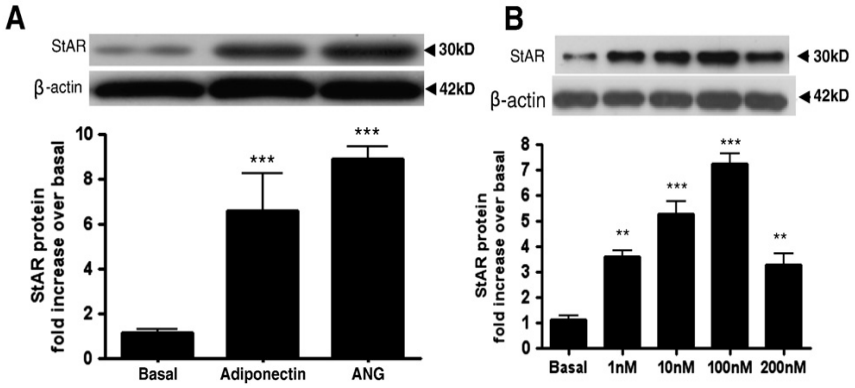

Figure 5: Results from Ramanjaneya *et al.* showing how StAR expression is significantly increased in the prescence of adiponectin. Shown using a Western blot normalized to β-actin. Angiotensin acted as a positive control. (Source: Ramanjaneya *et al.*, 2011)

A similar study by Ramanjaneya *et al.* (2008) looked into the effects of two neuropeptide orexins, A and B, on the expression of StAR. They found that these molecules work through G-protein signalling pathways to upregulate StAR in the adrenal H295R cells in a dose dependent manner.

Cdk5 is another protein that has been found to be involved in regulating StAR levels in mouse Leydig cells. The researchers (Lin *et al.*, 2009) found that levels of StAR were directly correlated with the amount and activity of Cdk5, and therefore the amount of androgen produced. However, even though p35 is usually a coregulator of Cdk5 in its neuronal function, there was no correlation of p35 levels when related to

StAR. The researchers suggest that this is because p35 and StAR comptete for a binding domain on Cdk5, but stress that further research is required to confirm this.

As stated in a review by Sierra (2004), StAR is widely distributed in the brain. Meethal *et al.* (2009) have found that it is part of regulatory feedback loop. They found that StAR is processed differently depending on the amount of peripheral hormones present, such as GnRH or sex steriods. Therefore the amount of steroids produced in the brain is regulated by gonadal steroidogenesis.

Mutations, Congenital Lipoid Hyperplasia, and Other Diseases

A recent study by Sahakitrungruang *et al.* (2010) has identified the effects of a mutation in StAR which results in the partial loss of its function. They found that nonclassic congenital lipoid adrenal hyperplasia, where steroid hormone synthesis is impaired, can result from different mutations in the StAR gene, many of which are located in the C-terminal helix where cholesterol binds. This type of mutation was first identified by Baker *et al.* (2006). Congenital lipoid hyperplasia affects the production of oestrogen, a steroid essential for pregnancy. The first successful pregnancy in a StAR deficient woman was reported by Khoury *et al.* (2009). Another successful case has been reported by Sertedaki *et al.* (2009). This particular patient had a mutation in StAR and could not conceive, but when oestrogens were administered she was able to become pregnant. Oestrogens were administered until the placenta could fully function, as other research has shown that steroidogenesis in the placenta is independent of StAR (Strauss *et al.*, 1996). However, these results should not be generalized as only a small number of cases have been reported.

The effects of StAR mutations are very different between males and females. Females with mutations still experience menstrual bleeding and develop normal secondary sexual characteristics. This is because enough oestrogens can be produced using StAR independent mechanisms of transporting cholesterol across the mitochondrial membrane, and also because the ovaries are not stimulated early in development (Fujieda *et al.*, 1997). Males are affected much more because the gonads produce androgens from foetal life, and if this is impaired then male external genitalia fail to develop and there is also a build up of lipid droplets in the Leydig cells. This is due to accumulation of cholesterol and can cause mechanical damage (Sertedaki *et al.*, 2009). Figure 6 shows how the build up of lipids can occur, using adrenal cells as an example.

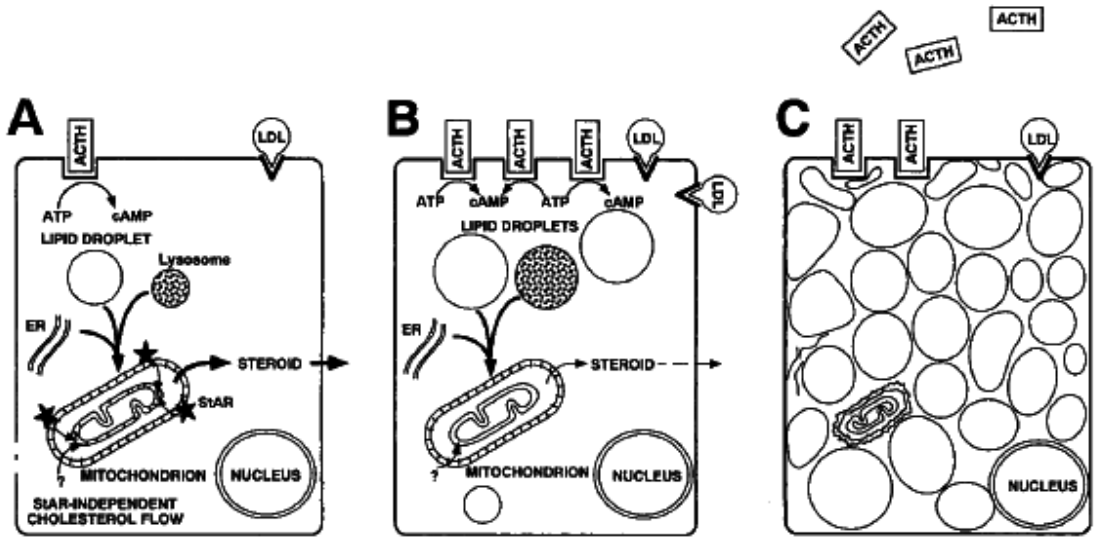

Figure 6: A – a normal adrenal cell stimulated by ACTH produces steriods. B – this cells has no StAR but some steroid is still produced by independent mechanisms. This would be a placental cell or in early congenital lipoid hyperplasia. C – lipid droplets accumulate and damage the cell structure. Stimulation continues as there is no negative feedback.

Source: Miller, W.L. (1997) Congenital lipoid adrenal hyperplasia: the human gene knockout for the steroidogenic acute regulatory protein. *Journal of Molecular Endocrinology* 19: 227-240 © Society for Endocrinology 1997. Reproduced by permission.

This two stage model of lipoid CAH was first described by Bose *et al.* (1996).

A perhaps less obvious place where StAR is essential for steroidogenesis is in the skin. Hannen *et al.* (2011) have recently researched into steroid hormone synthesis in keratinocytes. They concluded that in the condition of eczema, StAR was not found in the basal layer of the skin as it would be normally, and was also abnormally expressed in skin conditions such as psoriasis which are treated with cortisol. However, the absence of StAR could be secondary to these skin conditions as patients with StAR mutations have not been reported to have eczema or psoriasis.

The role of StAR in the brain remains unclear as neurological symptoms are not common to patients with severe mutations, although some cases of neurological damage in patients with lipoid CAH have been reported (Bhangoo *et al.,* 2005). However, this damage could be secondary and more research is required into this area.

Conclusions

The steroidogenic acute regulatory protein mediates the rate-limiting step in steroidogenesis: the transfer of cholesterol across the mitochondrial membrane to the side chain cleavage enzyme. Cholesterol binds to the molecule on the outer mitochondrial membranes for transfer. StAR is regulated by many different signalling pathways, and new regulatory signals are being discovered. Its importance in steroidogenic tissues can be demonstrated by looking at diseases associated with mutations. Congenital lipoid hyperplasia is the biggest health issue linked to mutations in the StAR protein due to the inability to synthesize essential steriods from cholesterol, and results in the build up of lipid droplets in cells. Males and females are affected differently by mutations due to sex steroids being needed for foetal development of sexual characteristics in males.

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