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Molecular pathology and mechanism of action of the steroidogenic acute regulatory protein, StAR☆

Walter L. Miller[a,*], Jerome F. Strauss III[b]

[a]Department of Pediatrics and the Metabolic Research Unit, University of California, Room 209, Building MR IV, San Francisco, CA 94121, USA
[b]Center for Research on Reproduction and Women's Health and the Department of Obstetrics and Gynecology, University of Pennsylvania Medical Center, Philadelphia, PA 19104, USA

Abstract

The first and rate-limiting step in the synthesis of all steroid hormones is the conversion of cholesterol to pregnenolone by the mitochondrial enzyme, P450scc. Tropic hormones such ACTH and gonadotropins induce steroidogenesis via cAMP by elaborating intracellular cAMP which stimulates P450scc activity in two distinct ways. Chronic stimulation (h to days) occurs through the induction of P450scc gene transcription leading to increased P450scc protein and consequent increased steroidogenic capacity. Acute regulation, over minutes, occurs through the phosphorylation of preexisting StAR and the rapid synthesis of new StAR protein. StAR, the steroidogenic acute regulatory protein, increases the flow of cholesterol into mitochondria, thus regulating substrate availability to whatever amount of P450scc is available. In the absence of StAR, up to 14% of maximal StAR-induced level of steroidogenesis persists as StAR-independent steroidogenesis. Congenital lipoid adrenal hyperplasia, an autosomal recessive disorder in which conversion of cholesterol to pregnenolone is severely impaired, results in female genitalia in 46,XY genetic males, variable onset of a severe salt-losing crisis in the first months of life, but normal feminization and cyclical vaginal bleeding in 46,XX females. Lipoid CAH was once thought to be due to P450scc mutations, but in fact homozygous P450scc mutations cannot exist in human beings as they would prohibit placental progesterone production, causing spontaneous abortion of the affected fetus. Lipoid CAH is caused by StAR mutations, which result in tropic hormone-induced intracellular accumulation of cholesterol in the adrenals and gonads. Our two-hit model, which considers the persistence of StAR-independent steroidogenesis and the differences in the fetal and postnatal ages at which the testis, adrenal zona glomerulosa, adrenal zona fasciculata and ovary are stimulated, predicts and explains all of the various clinical manifestations of lipoid CAH.

Structure–function studies of StAR show that the critical domains for biological activity reside in the protein's carboxy-terminus. When the N-terminal mitochondrial targeting sequences are deleted and the resulting N-62 StAR remains in the cytoplasm, it retains the ability to stimulate steroidogenesis both in intact cells or when added to isolated mitochondria *in vitro*. These observations suggest that StAR acts on the outer mitochondrial membrane to promote sterol translocation to P450scc, and that the importation of StAR into mitochondria terminates its action. Data from circular dichroism and Fourier-transform infrared spectroscopy show that the mutant StAR proteins in lipoid CAH are misfolded, suggesting disrupted interaction with another protein. Preliminary data suggest that StAR facilitates cholesterol desorption from membranes, stimulating transfer from the outer mitochondrial (donor) membrane to the inner mitochondrial (acceptor) membrane.

1. Introduction

The first, rate-limiting and hormonally regulated step in the biosynthesis of all steroid hormones is the conversion of cholesterol to pregnenolone. Because steroidogenic cells do not have a mechanism to store significant quantities of steroids, hormone secretion is directly related to the rate of steroid synthesis. Steroid synthesis is regulated both chronically and acutely. The chronic response takes hours to weeks, such as in ACTH-induced Cushing Disease or gonadotropin-induced gonadal development at puberty, and is

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* Corresponding author.

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mediated by increased transcription of the various genes encoding the steroidogenic enzymes largely through the intermediary of cAMP (for review see Ref. [1, 2]). The acute stimulation of steroidogenesis occurs within minutes, such as in the rapid rise in serum cortisol in response to intravenously administered ACTH. This acute response is mediated by the steroidogenic acute regulatory protein, StAR, which facilitates the movement of cholesterol into mitochondria for conversion to pregnenolone (for review see [3, 4]). Mutations in StAR cause congenital lipoid adrenal hyperplasia [5]. The history of the study of lipoid CAH, the demonstration that StAR mutations cause this disease, and the mechanisms by which the cell biology of StAR results in the lipoid CAH phenotype were reviewed recently [6, 7]. This article will summarize these prior findings and incorporate newer findings, with a specific emphasis on lipoid CAH.

2. Mitochondria and cytochrome P450scc

The conversion of cholesterol to pregnenolone is catalyzed by mitochondrial P450scc, where scc designates side chain cleavage. This single enzyme is encoded by a single gene [8, 9] on chromosome 15q23–q24 [10], and catalyzes three distinct sequential reactions on a single active site. Cholesterol sequentially undergoes 20-hydroxylation, 22-hydroxylation and scission of the 20,22 carbon–carbon bond to yield pregnenolone and isocaproaldehyde (for review see Ref. [11]). The catalysis of each of these three reactions requires a pair of electrons. NADPH donates electrons to a flavoprotein termed adrenodoxin reductase (or ferredoxin reductase), which then passes them to an iron/sulfur protein termed adrenodoxin (or ferredoxin), which in turn passes through to any mitochondrial form of P450 such as P450scc. There is only one type of ferredoxin reductase and ferredoxin, each encoded by unique-copy genes [12–15], hence, these are generic electron transfer proteins that donate electrons to all of the various mitochondrial P450 enzymes. Thus the same electron-transport proteins are used by the two isozymes of P450c11 (P450c11β and P450c11AS) [16] and by the enzymes involved in the activation and inactivation of vitamin D [17–19]. P450scc is active only when it is bound to the inner mitochondrial membrane [20], hence, delivery of cholesterol from the outer to the inner mitochondrial membrane is a crucial step. The exact mechanism of this cholesterol movement is unclear; recent data show that StAR is not mandatory for this step, but StAR does accelerate it substantially.

It has long been known that ACTH can induce adrenal steroidogenesis very rapidly, but that this induction could be inhibited by inhibitors of protein synthesis, such as cycloheximide, suggesting that a short-lived protein was essential for the acute response [21–24]. That factor was eventually identified [25–27] and then cloned and named StAR [28]. Strong evidence for StAR's role came from transfecting a rat StAR cDNA expression vector into mouse Leydig MA-10 cells and finding a six-fold increase in steroidogenesis [28]. The definitive proof of StAR's crucial role then came from finding mutations resulting in an absence of functional protein in congenital lipoid adrenal hyperplasia (lipoid CAH), thus providing the same kind of powerful genetic data normally derived from 'knockout' mice [5].

3. Congenital lipoid adrenal hyperplasia and the biology of StAR

Lipoid CAH severely disrupts the synthesis of all adrenal and gonadal steroids. Affected 46,XY genetic males are born with normal female external genitalia, reflecting an absence of fetal testicular testosterone synthesis between 6 and 12 weeks of gestation, when normal male sexual differentiation requires testosterone. At birth the adrenals are massively enlarged and engorged with the cholesterol and cholesterol esters that have been termed 'lipoid' deposits. Affected newborns generally have low but measurable levels of steroid hormones due to StAR-independent steroidogenesis (see below). If appropriate steroid hormone replacement therapy is not provided, these infants will soon die from glucocorticoid and mineralocorticoid deficiency. However, with proper hormonal replacement, these patients can survive to adulthood [29, 30]. Because cholesterol accumulates in the adrenals and because virtually no steroids were detected in the patients' blood or produced from patient tissues incubated *in vitro*, it was previously thought that lipoid CAH was due to a mutation in the enzyme that converts cholesterol to pregnenolone; formerly termed '20,22 desmolase' and now known to be P450scc [29–34]. However, the P450scc gene has a normal size and RFLP pattern in patients with lipoid CAH [34], and in 1991 Lin et al. showed that P450scc mRNA of normal sequence was expressed in normal abundance in affected lipoid CAH tissues, definitively ruling out P450scc mutations as the potential cause of lipoid CAH [35]. Although this initial report was disbelieved by some, it was later confirmed by three independent groups [36–38]. Furthermore, the placenta, a fetal rather than maternal tissue, continues to produce progesterone in affected lipoid CAH fetuses, demonstrating that the entire P450scc system is biologically normal in lipoid CAH [39]. It is now clear that mutations in P450scc, ferredoxin, ferredoxin reductase or 3βHSD-I will never be found in a liveborn human being, as mutation in any of these genes would prevent placental production of progesterone, which is needed

to maintain the second and third trimesters of human pregnancy [40]. Thus, mutations in any of these genes would result in spontaneous abortion at the end of the first trimester, when the pregnancy normally undergoes the 'luteo-placental shift' of progesterone production away from the corpus luteum to the placenta.

A clear understanding of the biology of StAR, but not of its mechanism of action, has emerged from clinical and genetic studies of lipoid CAH and basic studies of StAR biochemistry. Water-soluble hydroxysterols such as 20-hydroxycholesterol or 22*R*-cholesterol traverse the mitochondrial membranes readily, thus their conversion to pregnenolone can be used to measure maximal P450scc activity in a cell [41]. In the presence of StAR, sufficient cholesterol reaches P450scc to approach this maximal capacity, but in the absence of StAR, low levels of StAR-independent steroidogenesis persist. The realization that adrenal and gonadal cells had both StAR-dependent and StAR-independent modes of steroidogenesis permitted us to explain all of the clinical findings in lipoid CAH [42]. When nonsteroidogenic COS-1 cells are transfected with vectors expressing P450scc, ferredoxin and ferredoxin reductase, or a vector for a fusion protein of all three components, the cells make small amounts of pregnenolone [5, 20, 43], even though COS-1 cells lack StAR. The level of this StAR-independent steroidogenesis in COS-1 cells is about 14% of what is seen in the presence of StAR [5, 42, 44–47]. The brain [48] and placenta both make steroid hormones, but neither of these tissues expresses StAR [44, 49], thus StAR-independent steroidogenesis is of substantial physiologic importance. It is not known whether this physiologic StAR-independent steroidogenesis proceeds by the same mechanism as the StAR-independent steroidogenesis seen in transfected COS-1 cells. One potential mechanism is that small amounts of cholesterol reach the inner mitochondrial membrane without any special effector molecule. Alternatively, other molecules such as MLN-64, which is structurally related to StAR and is expressed in the placenta and brain, may exert StAR-like actions [50]. This latter mechanism is attractive, as it provides an additional level of control of steroidogenesis in these tissues. The regulation of mitochondrial cholesterol transport is crucial, as the spontaneous P450scc gene knockout in the rabbit proves that P450scc is the *only* enzyme that can convert cholesterol to pregnenolone [51], and cell transfection experiments prove that P450scc can function only when it is bound to the inner mitochondrial membrane [20] (for review see [52]). The rabbits lacking the P450scc gene survive to term because in the rabbit, unlike in human females, the mother's corpus luteum of pregnancy continues to secrete large amounts of progesterone until the time of delivery [40].

4. Clinical findings in lipoid CAH

The clinical features of lipoid CAH are summarized in Table 1. The first review of the clinical findings in lipoid CAH was by Hauffa in 1985, summarizing the 32 cases reported in the world's literature at that time [30]. Hauffa found that all the patients had phenotypically normal female external genitalia irrespective of chromosomal sex (indicating a severe defect in fetal testicular synthesis of testosterone), hyponatremia, hyperkalemia, low urinary 17-hydroxycorticosteroids and 17-ketosteroids (indicating a profound lesion in adrenal steroid biosynthesis), hyperpigmentation (due to overproduction of ACTH), and an excellent response to glucocorticoid and mineralocorticoid replacement. Fourteen of 28 patients were genetic males, identified by karyotype, buccal smear, gonadal appearance or gonadal histology, as expected for an autosomal recessive disease. Although 21 of these patients had died, two reports on long-term survival [29, 30] showed that patients with lipoid CAH could do well if diagnosed early in infancy and treated with appropriate glucocorticoid and mineralocorticoid replacement therapy. Hauffa *et al.* were also the first to point out the genetic clustering of lipoid CAH, with 18 of the initial 32 patients being of Japanese heritage. Although Hauffa *et al.* did not question the then-prevailing, incorrect idea that lipoid CAH was caused by a mutation in '20,22 desmolase' (P450scc), they did point out certain inconsistencies in the clinical presentations and hormonal findings. Only 15 of 28 patients had clinical signs of adrenal insufficiency at 2 weeks of age, when patients with severe 21-hydroxylase deficiency are usually having severe salt-wasting episodes; furthermore, several patients did not become clinically ill

Table 1
Clinical features in lipoid CAH

Normal placental steroidogenesis
Normal term gestation and parturition
Normal development at birth (except genitalia in 46,XY genetic males)
Female external genitalia in 46,XY genetic males
Hypergonadotropic hypogonadism
Low serum cortisol and other steroids in newborns, absent later on
Low to absent urinary steroid excretion
High ACTH; hyperpigmentation in most neonates
Variable onset of hyponatremia and hyperkalemia (1 day to 6 months)
High plasma renin activity
Adrenal hyperplasia with 'foamy' appearing cells containing 'lipoid' (cholesterol ester) deposits
Minimal histologic changes in Leydig cells
Spontaneous feminization in 46,XX genetic females at appropriate age of puberty
Anovulatory monthly vaginal bleeding in postpubertal 46,XX genetic females

until after 3 months of age. Also, many patients had low, but measurable levels of adrenal steroids in the newborn period, but these steroids became unmeasurable after a few years.

These clinical inconsistencies with a presumed lesion in P450scc were ignored or explained away in a variety of ways. However, the demonstration that P450scc was wholly normal in lipoid CAH [35] initiated a reexamination of lipoid CAH clinically and conceptually. Tanae *et al*. [53] and Matsuo *et al*. [54] reported that 46,XX genetic females with lipoid CAH underwent spontaneous breast development and vaginal bleeding at the normal age of puberty associated with serum estradiol levels ranging from 22 to 85 pg/ml. These preliminary reports raised important questions, but were largely overlooked until our demonstration that StAR mutations caused lipoid CAH triggered intensive study of the disease. In a comprehensive worldwide study of the clinical and genetic findings in lipoid CAH, Bose *et al*. [42] found that all infants were born at term gestation and that none were postmature, ruling out a role of fetal adrenal steroids in the timing of the onset of parturition. These 20 patients were of normal size, only five had mild neonatal hypoglycemia and none had true hyaline membrane disease due to diminished production of pulmonary surfactant. These data provide powerful evidence that feto-placental glucocorticoids and estrogens are not required for the maturation of hepatic gluconeogenic enzymes or the expression of surfactant apoproteins, even though this is widely concluded from the findings in mice with glucocorticoid receptor knockouts [55]. The onset of hyponatremia and hyperkalemia varied considerably, from 1 day to 2 months of age, and most infants who were tested had measurable levels of cortisol; furthermore, some 46,XY males had measurable testosterone, which failed to rise following tropic stimulation (Table 1). This picture of an incomplete lesion in steroidogenesis, which was inconsistent with the traditional view of lipoid CAH, was confirmed in a subsequent study of 19 patients by Nakae *et al*. [47]. Most remarkably, many infants survived for extended periods of time without hormonal replacement therapy: among our 20 cases, treatment was not begun until after age 2 months in seven infants, and one survived for 6 months without glucocorticoid or mineralocorticoid replacement; similarly, although only two of the 19 patients reported by Nakae *et al*. [47] received initial treatment at or after 2 months of age, one of these survived to 10 months before experiencing a salt-wasting crisis (see Section 5). Thus, these clinical studies showed that although the adrenal lesion in lipoid

Table 2
StAR mutations causing lipoid CAH (51 patients 90 alleles)

Mutation No.	Nucleotide change	Amino acid change	Affected alleles	Activity[a] (% of wild-type StAR)
1	C898T	Q258X	39	12–17
2	G671T	R182L	7	11
3	A632G	E169G	1	13
4	593delTT	Frame	3	9
5	940del3bp	*Δ*R272	2	11
6	947insA	Frame	2	10
7	650delC	Frame	2	10
8	548insTT	Frame	2	20
9	C703T	R193X	2	14
10	T → A@-11 I4/E5	Frame	2	20
11	C779T	A218V	5	14–20
12	T950C	L275P	1	24
13	G631A	E169K	1	14
14	246insG	Frame	6	–
15	DNA insertion	Frame	1	–
16	261delT	Frame	2	–
17	838delA	Frame	2	–
18	189delG	Frame	1	–
19	T800C	M225T	1	44
20	C759T	Q212X	1	–
21	564del13bp	Frame	1	–
22	InsT@ + 3 E2/I2	Frame	2	–
23	none found[b]	3	–	
	control vector			13–15
				–

[a] Primarily from the studies of Bose *et al*. [42] with some data from [5, 45, 47].[b]StAR alleles in which no mutation was found, even though the patient's other allele had an identified mutation, thus these must represent unidentified mutant StAR alleles. The three alleles indicated do not include patient 14 in Bose *et al*. [42] in which no mutation was found on either allele.

CAH eventually becomes more severe than in any other form of CAH, some steroid synthesis persists in infancy so that the onset of clinically apparent glucocorticoid and mineralocorticoid deficiency may be delayed for quite some time. By contrast, except for Nakae's patient who became ill at age 10 months, all 46,XY patients with lipoid CAH have had wholly normal female external genitalia, even though the age of onset of glucocorticoid and mineralocorticoid deficiencies varies substantially. Thus the testicular lesion appears to be more severe than the adrenal lesion, and manifests at a much earlier stage of development.

5. Mutations in the StAR gene and genotype/phenotype correlations

The molecular lesions in at least 51 patients with lipoid CAH have been identified and described [5, 38, 42, 45–47, 56–58]; these mutations and the activities of the corresponding mutant StAR proteins are summarized in Table 2. Of these 51 patients, 36 are of Japanese or Korean ancestry, among whom the Q258X mutation accounts for 39 of 58 affected alleles (67%). Numerous other patients have been reported in abstracts and oral presentations, but the identified mutations duplicate those in Table 2. We have devised rapid PCR-based RFLP diagnostic tactics for ten of these mutations, including the common Q258X mutation found in the Japanese/Korean populations, and the R182L mutation found in Palestinian Arabs [42]. When the mutations in Table 2 are compared to the structure of the StAR gene, it is apparent that mutations can be found anywhere in the gene (Fig. 1). Mutations causing premature translational termination or altering of the StAR reading frame are common, and, as they substantially alter the structure of the StAR protein, it is not surprising that these mutations have no StAR activity, as they induce no greater than the ~14% background conversion of cholesterol to pregnenolone (Table 2). However, amino acid replacement (missense) mutations are found only in carboxy-terminal 40% of the 285 amino acid StAR protein. Most of these amino acid changes (E169G, E169K, R182L, A218V, and ΔR272) eliminate all StAR activity, but L275P and M225T respectively converted 24 and 44% as much cholesterol to pregnenolone as the wild-type StAR. After subtracting the StAR-independent background of 14%, the L275P mutant retained 10% of activity and the M225T mutant retained 30% of activity. Four of these mutant proteins have been characterized biophysically by sedimentation equilibrium ultracentrifugation, near- and far-ultraviolet circular dicroism (CD) spectroscopy and Fourier transform infra-red (FTIR) spectroscopy [59]. Both the wild-type and mutant proteins exist as monomers in solution, and are composed primarily of α-helical structures; however, all four mutant proteins are misfolded. Of particular note, the partially active L275P mutant has an FTIR spectrum that resembles the wild-type, while the wholly inactive E169G, R182L and ΔR272 mutants have FTIR spectra that resemble one another, but are substantially different from the wild-type [59]. The patient carrying the L275P mutant (patient 13 in [42] and [6]) did not begin to receive steroidal replacement therapy until the age of 4 months, at which time the clinical picture was comparatively mild (Na 123 mEq/L, K 6.9 mEq/L). The M225T mutant was found in a patient also carrying the common Japanese mutant Q258X [47]. This is the only established case of lipoid CAH in which a 46,XY patient had mild virilization of the external genitalia, consisting of mild cliteromegaly, minimal posterior labial fusion and mild rugation of the labia. Because of these findings, this infant was followed closely and had normal basal and ACTH-stimulated cortisol values at 1 and 3 months of age, but eventually experienced a salt-losing crisis at 10 months, associated with hyponatremia, hyperkalemia and grossly elevated ACTH and plasma renin values [47]. Thus, there is an excellent correlation between the severity of the StAR mutation as assessed *in vitro* with the age of onset of a clinical salt-wasting crisis, and possibly also with genital virilization.

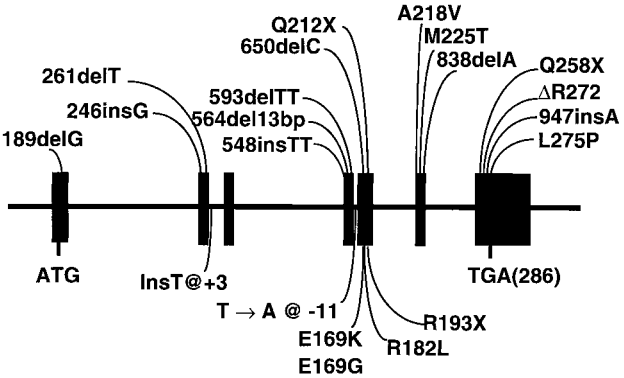

Fig. 1. Diagram of the human StAR gene. The sizes of the introns and exons and the base numbering system are as described by Sugawara *et al.* [70]. The StAR mutations published as of mid-1998 are shown; all of the amino acid replacement mutations shown have been studied in transfected cells by Bose *et al.* [42] or Nakae *et al.* [47] using the F2 P450scc fusion protein of Harikrishna *et al.* [43]; the quantitative results of these experiments are shown in Table 1.

An initially unexpected phenotypic manifestation of lipoid CAH is that even the most severely affected 46,XX female patients undergo spontaneous feminization, breast development and cyclical vaginal bleeding at the usual age of puberty [46, 53, 54, 56]. This is not correlated with the molecular severity of the mutation, but is explained by our two-hit model of the cellular pathophysiology of lipoid CAH [42].

6. The two-hit model of lipoid CAH

The conceptualization of StAR-independent steroidogenesis led directly to our formulation of the two-hit model of lipoid CAH [42]. The first hit is mutation of the StAR gene and the elimination of StAR-dependent steroidogenesis (Fig. 2). This substantially reduces the cell's total steroidogenic capacity and eliminates the acute response, but permits StAR-independent steroidogenesis to continue. The preservation of StAR-independent steroidogenesis permits normal placental steroidogenesis and term gestation; StAR-independent steroidogenesis also explains the low, but detectable levels of steroid hormones seen in the sera of affected patients in the first month of life [30, 42] and their initial survival without treatment for 1–2 months [30, 42], whereas untreated patients with other forms of salt-wasting CAH do not survive that long. However, StAR-independent production of adrenal steroids is subnormal, and concentrations of these steroid hormones are low. Consequently, there is increased secretion of the corresponding tropic hormones: ACTH, the gonadotropins and angiotensin II. These tropic hormones stimulate increased cellular uptake of LDL cholesterol and increased *de novo* cellular synthesis of cholesterol from acetate. This tropic hormone stimulation results in the accumulation of cholesterol esters constituting the second hit of the two-hit model. The accumulated cholesterol and cholesterol esters eventually disrupt the cell, either via physical engorgement of the cell with lipid droplets or by a chemical action of cholesterol oxidation products, or both. This second hit destroys the cell's low levels of StAR-independent steroidogenesis, accounting for the wholly unmeasurable levels of steroid in the serum of older children with lipoid CAH and for the total absence of circulating testosterone in 46,XY fetuses.

The two-hit model also explains why affected 46,XX patients undergo spontaneous feminization and cyclical vaginal bleeding at the usual age of puberty. Normal fetal ovaries do not express the genes for the steroidogenic enzymes and hence do not make steroids [60]. Unlike the testes and adrenals, the ovaries only start to make steroid hormones when they are first stimulated by gonadotropins at the onset of puberty. Human preovulatory granulosa cells do not even express StAR [49, 61]. Consequently, the ovaries of 46,XX female infants with lipoid CAH have received only the first hit: they lack the capacity to mount an acute steroidogenic response (e.g. the pulse of pro-

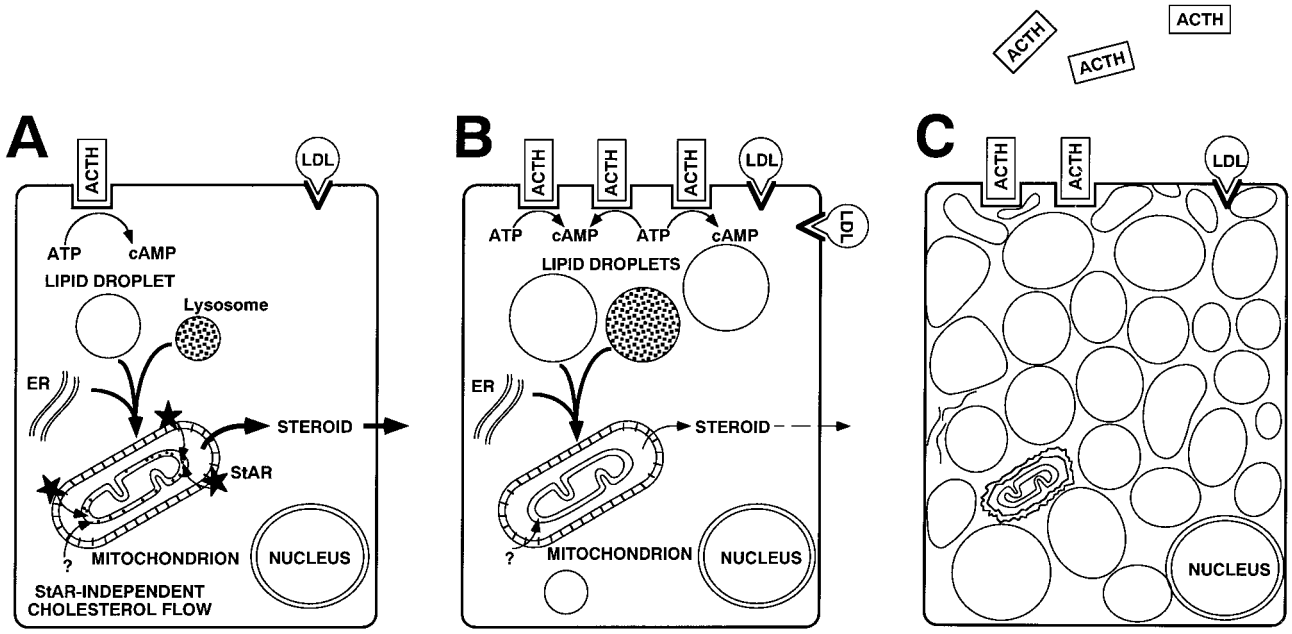

Fig. 2. Model of lipoid CAH in an adrenal cell. (a) Under normal conditions, cholesterol is derived from endogenous synthesis in the endoplasmic reticulum (ER), from cholesterol esters in lipid droplets and from LDL cholesterol which is taken up by receptor-mediated endocytosis and processed in lysosomes. Cholesterol is transported to the outer mitochondrial membrane by as-yet undefined processes. StAR promotes the movement of cholesterol from the outer to the inner mitochondrial membrane, but may also be mediated by StAR-independent mechanisms. (b) In the absence of StAR in early lipoid CAH, StAR-independent mechanisms can still move some cholesterol into the mitochondria, resulting in a low level of steroidogenesis. Decreased adrenal steroidogenesis results in increased corticotropin secretion, stimulating further production of cholesterol and its accumulation as cholesterol esters in lipid droplets. (c) As lipid droplets accumulate they engorge the cell, damaging its cytoarchitecture both through physical displacement and by the chemical action of cholesterol autooxidation products. Steroidogenic capacity is destroyed, although tropic stimulation continues. In the ovary, follicular cells remain unstimulated and undamaged until they are recruited at the beginning of each cycle. Small amounts of estradiol are produced by StAR-independent steroidogenesis, as in panel B, resulting in feminization and withdrawal bleeding, but the cycles are anovulatory, resulting in infetility and progressive hyperogonadotropic hypogonadism.

gesterone made in mid-cycle in response to the LH surge) but these ovaries retain a capacity for low-level StAR-independent steroidogenesis. Thus, when puberty begins, the affected ovary can produce some estrogen, which then produces spontaneous and temporally appropriate breast development. However in the ovary, unlike the adrenal or testis, only a small number of cells, a single follicle, is 'recruited' and stimulated by gonadotropins in each monthly cycle, while the remaining follicles remain unstimulated. This gonadotropin stimulation quickly results in cholesterol accumulation in these cells (the second hit in lipoid CAH) so that the secretion of large amounts of progesterone in the later phases of the ovarian cycle does not occur [56]. Meanwhile, the follicles that were not recruited remain unstimulated and have not been forced to accumulate cholesterol esters and hence constitute a reservoir of steroidogenic cells undamaged by the second hit of lipoid CAH. With each monthly cycle, a new undamaged follicle is recruited, producing estrogen in temporally normal monthly cycles, as the timing is hypothalamic and largely independent of ovarian events. This leads to cyclic uterine estrogen withdrawal bleeding that resembles normal menses but, as there is no progesterone [56], these cycles are probably anovulatory. All 46,XX lipoid CAH females who have reached the age of puberty have experienced this spontaneous feminization and cycling [42, 46, 47, 54, 56], as predicted by our two-hit model.

Thus, lipoid CAH is the StAR gene knockout of nature [6], and study of lipoid CAH has revealed how the StAR protein functions in physiology. It is of interest that recently-described StAR gene knockout mice have precisely the same phenotypic findings as those found in lipoid CAH patients, serving to confirm our two-hit model [62]. This mouse model of lipoid CAH may clarify the one remaining question concerning the pathophysiology of lipoid CAH, the highly variable age of onset of the mineralocorticoid-deficient salt-wasting crisis. We have previously suggested that the level of intrauterine stimulation of the mineralocorticoid pathway may depend on the mother's salt balance and state of hydration [6, 42]. The patients with the earliest age of salt-loss tended to come from hot dry climates, while those with the later ages of onset tended to come from cool moist climates, suggesting that in hot climates maternal chronically compensated hypovolemia stimulated the fetal renin/angiotensin system *in utero*, provoking early development of the second hit in cells destined to become the zona glomerulosa. Knockout mice can be bred nearly to genetic homogeneity, eliminating potentially confounding variables in the maternal background. Prospectively manipulating the thermal environment and the availablity of salt and water to congenic animals harboring StAR-knockout fetuses may provide a test for this hypothesis, although it must be remembered that the human and mouse adrenals exhibit important differences.

7. Mechanism of StAR action

7.1. Structure and function: C-terminal domains are essential for StAR's steroidogenic activity

Although it is clear that StAR plays a key role in regulating steroid hormone synthesis at the cholesterol side-chain cleavage step, the exact mechanism of StAR's participation in this process remains unknown. It was initially suggested that StAR must be imported into mitochondria to promote steroidogenesis and that during the import process contact sites formed between the outer and inner mitochondrial membranes allowing cholesterol to flow down a chemical gradient to reach P450scc [3, 28]. This notion was inconsistent with the observation that deletion of only 27 carboxy-terminal amino acids in the Q258X mutant could cause lipoid CAH, and was refuted by the discovery that StAR lacking the mitochondrial targeting sequence (N-62 StAR), and thus incapable of being imported into mitochondria, was as effective as wild-type StAR in stimulating pregnenolone production by COS-1 cells transfected with the side-chain cleavage system [63, 64]. Immuno-electron microscopy showed that N-62 StAR was distributed throughout the cytoplasm in transfected COS-1 cells, while the processed 30 kDa product of full-length StAR was almost exclusively located inside mitochondria. This result could be duplicated *in vitro*, where N-62 StAR was also incapable of being imported into isolated mitochondria, whereas full-length StAR was imported and processed to the mature 30 kDa protein [63, 64].

In contrast, truncations of the C-terminus StAR resulted in biologically inactive protein, demonstrating that C-terminal domains of StAR are critical to the protein's steroidogenic activity. These observations are in agreement with the StAR gene mutations found in patients with lipoid CAH, which to date have revealed amino acid replacement clustered in exons V–VII of the gene encoding the C-terminus, but not in exons encoding the N-terminus (Table 2). Moreover, the amino acid sequence similarity of the C-terminal domains of MLN64 and the C-terminal domains of StAR reinforces the concept that the C-terminus is essential for biological activity. Amino acid replacements causing lipoid CAH affect conserved residues in both proteins [50] and site-directed mutagenesis of other residues conserved in StAR and MLN64 ablate or significantly reduce StAR's steroidogenic activity.

7.2. StAR acts on the outer mitochondrial membrane

The ability of StAR to stimulate steroidogenesis when it is primarily localized to the cytoplasmic compartment raised the possibility of two different mechanisms of action: StAR could act on the outer surface of the mitochondria or it could act in the cytoplasm on some other molecule which then affected the mitochondria. To explore these two options, recombinant human N-62 StAR protein was produced in *E. coli* and tested for steroidogenic activity on isolated bovine corpus luteum mitochondria [64]. A mutant recombinant protein was also produced as a control in which the A218V mutation (mutation 11 in Table 2) was introduced into the N-62 StAR protein. The recombinant wild-type N-62 StAR stimulated pregnenolone production by the isolated mitochondria in a dose and time-dependent fashion: significant increases in steroid production were observed within minutes at concentrations of recombinant protein in the nanomolar range. In contrast, the mutant recombinant protein was completely inactive. Recombinant N-62 StAR protein, but not the mutant recombinant protein, also increased the conversion of exogenous ^{3}H cholesterol into ^{3}H-pregnenolone. These observations are most consistent with a direct effect of StAR on the outer mitochondrial membrane.

Recent experiments indicated that StAR can function as a sterol transfer protein (Kallen, Billheimer and Strauss, unpublished observations). Recombinant N-62 StAR, but not the recombinant mutant StAR, promotes transfer of cholesterol from unilamellar vesicles to an acceptor at nanomolar concentrations. The sterol transfer does not involve fusion of liposomes with the acceptor, suggesting that StAR acts by stimulating desorption of cholesterol from the donor vesicle. In contrast to sterol carrier protein 2, StAR's activation of lipid transfer is restricted to cholesterol; phospholipid transfer is not stimulated. These new findings raise the possibility that StAR acts on the relatively cholesterol-rich outer mitochondrial membrane to eject cholesterol which gravitates to the cholesterol-poor inner membrane.

The nature of the interaction between StAR and the outer mitochondrial membrane remains to be clarified. Studies by Papadopolous *et al.* [65–67] suggest an essential function for the peripheral benzodiazepine receptor (PBR), an abundant protein in the outer mitochondrial membranes, in the cholesterol translocation process. Work from this group suggests that StAR is incapable of stimulating steroidogenesis in the absence of PBR [68]. These data could be interpreted as placing StAR upstream from PBR and raise the possibility that StAR is a PBR ligand. However, the immunohistochemical and electron microscopic demonstration that N-62 StAR is not selectively accumulated by mitochondria suggests that mitochondria lack abundant high affinity receptors for the C-terminus of StAR [64]. Hence, either very few high affinity sites or transient interactions are sufficient to promote cholesterol movement. Preliminary studies demonstrating that StAR can transfer cholesterol from unilamellar liposomes to trypsin-treated, heat-inactivated mitochondria suggest that StAR may be acting through a nonprotein (or at least a trypsin-insensitive) molecule on the mitochondrial outer membrane (Kallen, Bilheimer and Strauss, unpublished observations). The rather modest degree of misfolding of the partially active L275P and the wholly inactive E169G mutants [59] would seem to favor the model of transient interactions rather than the presence of a small number of high-affinity binding sites on the outer mitochondrial membrane.

While the experiments summarized above seem to suggest that the StAR N-terminus is not a critical part of the protein, it must be remembered that the structure/function studies have been carried out in transfected COS-1 cells in which large amounts of StAR protein are proceed, greatly exceeding the amount of StAR produced in steroidogenic cells under physiological conditions. The N-terminus is probably critical to ensure that StAR can act efficiently at low concentrations since it directs the protein to mitochondria, where the C-terminus can exert biological effects (Fig. 3).

7.3. StAR import and StAR inactivation

If StAR acts on the outer surface of the mitochondria, then import of the protein into the mitochondria removes it from its putative site of action. Thus, instead of being a critical step in stimulation of steroidogenesis, import is now thought to be the mechanism by which StAR's action is rapidly terminated. Consistent with this idea are pulse-chase studies examining the half-life of StAR in transfected COS-1 cells [64]. Wild-type StAR is rapidly imported into mitochondria and processed to mature protein with a half-life of 10 to 15 min. The mature intramitochondrial protein has a much longer half-life. Thus, the rapid fall in steroidogenesis following treatment of cells with protein synthesis inhibitors probably results from cessation of new translation and the rapid import of StAR into mitochondria where it so no longer active.

7.4. The role of phosphorylation

StAR was originally identified as a phosphoprotein [25] and initial experiments demonstrated a correlation between phosphorylated forms of StAR and steroidogenesis [26, 27]. The phosphoryl-

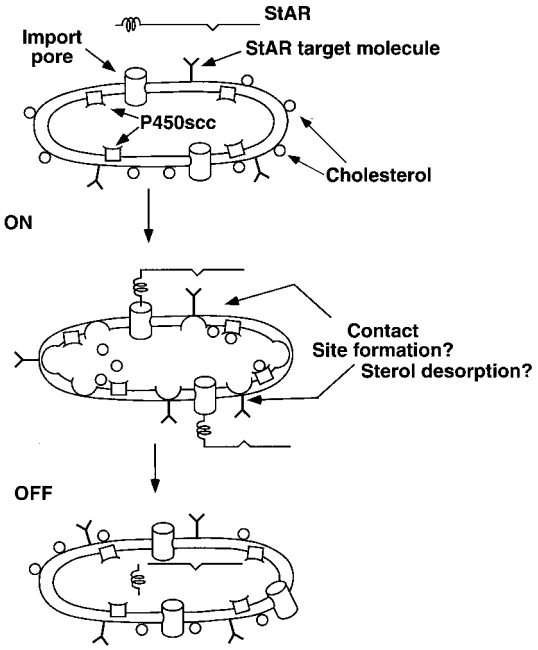

Fig. 3. Potential mechanisms of StAR function. Preprocessed StAR protein interacts with outer mitochondrial membrane and transiently activates cholesterol transport to the inner membrane. Cholesterol transport may be mediated via the formation of intermembrane contact sites, pore formation, or cholesterol desorption. Cholesterol then serves as the substrate for P450scc, which catalyzes pregnenolone formation. The StAR protein is rapidly imported, and therefore rendered inactive for cholesterol transport; the import process involves cleavage of the StAR mitochondrial signal sequence, forming the mature protein.

ation of StAR was induced by cAMP, suggesting that it was catalyzed by protein kinase A. All of the known StAR protein sequences contain two conserved consensus protein kinase A phosphorylation motifs which in human StAR are located on serine 57 and serine 195 [69] Human StAR is rapidly phosphorylated on both of these serine residues in response to 8-bromo-cAMP, but only serine 195 appears to be functionally significant. Mutation of this residue to a nonphosphorylatable alanine residue reduces the steroidogenic activity of the mutant protein by more than 50% compared to wild-type StAR in COS-1 cells transfected with cholesterol side-chain cleavage enzyme. In contrast, mutation of serine 195 to an aspartic acid residue, which mimics the charge effect of phosphorylation, modestly increases the activity of the protein in COS-1 cells. These observations suggest that post- or cotranslational modification of StAR can increase the activity of existing or newly made StAR protein, providing a mechanism by which tropic hormones acting through the intermediacy of cAMP can rapidly increase pregnenolone synthesis.

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References

[1] C.C.D. Moore, W.L. Miller, The role of transcriptional regulation in steroid hormone biosynthesis, J. Steroid. Biochem. Mol. Biol. 40 (1991) 517–525.
[2] D.W. Hum, W.L. Miller, Transcriptional regulation of human genes for steroidogenic enzymes, Clin. Chem. 39 (1993) 333–340.
[3] D.M. Stocco, B.J. Clark, Regulation of the acute production of steroids in steroidogenic cells, Endocr. Rev. 17 (1996) 221–244.
[4] B.J. Clark, D.M. Stocco, Steroidogenic acute regulatory protein: the StAR still shines brightly, Mol. Cell Endocr. 134 (1997) 1–8.
[5] D. Lin, T. Sugawara, J.F. Strauss III., B.J. Clark, D.M. Stocco, P. Saenger, A. Rogol, W.L. Miller, Role of steroidogenic acute regulatory protein in adrenal and gonadal steroidogenesis, Science 267 (1995) 1828–1831.
[6] W.L. Miller, Congenital lipoid adrenal hyperplasia: the human gene knockout of the steroidogenic acute regulatory protein, J. Mol. Endocrinol. 17 (1997) 227–240.
[7] W.L. Miller, Lessons from congenital lipoid adrenal hyperplasia, Curr. Opin. Endocr. Diab. 5 (1998) 155–161.
[8] B. Chung, K.J. Matteson, R. Voutilainen, T.K. Mohandas, W.L. Miller, Human cholesterol side-chain cleavage enzyme, P450scc: cDNA cloning, assignment of the gene to chromosome 15, and expression in the placenta, Proc. Natl. Acad. Sci. U.S.A. 83 (1986) 8962–8966.
[9] K. Morohashi, K. Sogawa, T. Omura, Y. Fujii-Kuriyama, Gene structure of human cytochrome P-450(scc), cholesterol desmolase, J. Biochem. 101 (1987) 879–887.
[10] R.S. Sparkes, I. Klisak, W.L. Miller, Regional mapping of genes encoding human steroidogenic enzymes: P450scc to 15q23–q24, adrenodoxin to 11q22; adrenodoxin reductase to 17q24–q25; and P450c17 to 10q24–q25, DNA Cell Biol. 10 (1991) 359–365.
[11] W.L. Miller, Molecular biology of steroid hormone synthesis, Endocr. Rev. 9 (1988) 295–318.
[12] J. Picado-Leonard, R. Voutilainen, L. Kao, B. Chung, J.F. Strauss III., W.L. Miller, Human adrenodoxin: cloning of three cDNAs and cycloheximide enhancement in JEG-3 cells, J. Biol. Chem. 263 (1988) 3240–3244 corrected 11016.
[13] S.B. Solish, J. Picado-Leonard, Y. Morel, R.W. Kuhn, T.K. Mohandas, I. Hanukoglu, W.L. Miller, Human adrenodoxin reductase: two mRNAs encoded by a single gene of chromosome 17cen → q25 are expressed in steroidogenic tissues, Proc. Natl. Acad. Sci. U.S.A. 71 (1988) 7104–7108.
[14] C.-Y. Chang, D.-A. Wu, C.-C. Lai, W.L. Miller, B. Chung, Cloning and structure of the human adrenodoxin gene, DNA 7 (1988) 609–615.
[15] D. Lin, Y. Shi, W.L. Miller, Cloning and sequence of the human adrenodoxin reductase gene, Proc. Natl. Acad. Sci. U.S.A. 87 (1990) 8516–8520.
[16] C.E. Fardella, W.L. Miller, Molecular biology of mineralocorticoid metabolism, Ann. Rev. Nutrition 16 (1996) 443–470.
[17] F.J. Dilworth, S.M. Black, Y.D. Guo, W.L. Miller, G. Jones, Construction of a P450c27 fusion enzyme: a useful tool for analysis of vitamin D_3-25-hydroxylase activity, Biochem. J. 320 (1996) 267–271.
[18] G.K. Fu, D. Lin, M.Y.H. Zhang, D.D. Bikle, C.H.L. Shackleton, W.L. Miller, A.A. Portale, Cloning of human 25-hydroxy vitamin

D-1α-hydroxylase and mutations causing vitamin D-dependant rickets type I, Mol. Endocrinol. 11 (1997) 1961–1970.
[19] G.K. Fu, A.A. Portale, W.L. Miller, Complete structure of the human gene for the vitamin D 1α-hydroxyase, P450c1α, DNA Cell Biol. 16 (1997) 1499–1507.
[20] S.M. Black, J.A. Harikrishna, G.D. Szklarz, W.L. Miller, The mitochondrial environment is required for activity of the cholesterol side-chain cleavage enzyme, cytochrome P450scc, Proc. Natl. Acad. Sci. U.S.A. 91 (1994) 7247–7251.
[21] D. Stone, O. Hechter, Studies on ACTH action in perfused bovine adrenals: aspects of progesterone as an intermediary in corticosteroidogenesis, Arch. Biochem. Biophys. 54 (1955) 121–128.
[22] J.J. Ferguson, Protein synthesis and adrenocorticotropin responsiveness, J. Biol. Chem. 238 (1963) 2754–2759.
[23] L.D. Garren, R.L. Ney, W.W. Davis, Studies on the role of protein synthesis in the regulation of corticosterone production by adrenocorticotropic hormone *in vivo*, Proc. Natl. Acad. Sci. U.S.A. 53 (1965) 1443–1450.
[24] L.D. Garren, W.W. Davis, R.M. Crocco, Puromycin analogs: action of adrenocorticotropic hormone and the role of glycogen, Science 152 (1966) 1386–1388.
[25] L.A. Pon, N.R. Orme-Johnson, Acute stimulation of steroidogenesis in corpus luteum and adrenal cortex by peptide hormones, J. Biol. Chem. 261 (1986) 6594–6599.
[26] L.F. Epstein, N.R. Orme-Johnson, Regulation of steroid hormone biosynthesis: identification of precursors of a phosphoprotein targeted to the mitochondrion in stimulated rat adrenal cortex cells, J. Biol. Chem. 266 (1991) 19739–19745.
[27] D.M. Stocco, T.C. Sodeman, The 30 kDa mitochondrial protein induced by hormone stimulation in MA-10 mouse Leydig tumor cells are processed from larger precursors, J. Biol. Chem. 266 (1991) 19731–19738.
[28] B.J. Clark, J. Wells, S.R. King, D.M. Stocco, The purification, cloning and expression of a novel luteinizing hormone-induced mitochondrial protein in MA-10 cells mouse Leydig tumor cells: characterization of the steroidogenic acute regulatory protein (StAR), J. Biol. Chem. 269 (1994) 28314–28322.
[29] R.T. Kirkland, J.L. Kirkland, C.M. Johnson, M.G. Horning, L. Librik, G.W. Clayton, Congenital lipoid adrenal hyperplasia in an 8-year old phenotypic female, J. Clin. Endocrinol. Metab. 36 (1973) 488–496.
[30] B.P. Hauffa, W.L. Miller, M.M. Grumbach, F.A. Conte, S.L. Kaplan, Congenital adrenal hyperplasia due to deficient cholesterol side-chain cleavage activity (20,22 desmolase) in a patient treated for 18 years, Clin. Endocrinol. 23 (1985) 481–493.
[31] A.M. Camacho, A. Kowarski, C.J. Migeon, A. Brough, Congenital adrenal hyperplasia due to a deficiency of one of the enzymes involved in the biosynthesis of pregnenolone, J. Clin. Endocrinol. Metab. 28 (1968) 153–161.
[32] H.J. Degenhart, K.H.A. Visser, H. Boon, N.J.D. O'Doherty, Evidence for deficiency of 20α cholesterol hydroxylase activity in adrenal tissue of a patient with lipoid adrenal hyperplasia, Acta Endocrinologia 71 (1972) 512–518.
[33] S. Koizumi, S. Kyoya, T. Miyawaki, H. Kidani, T. Funabashi, H. Nakashima, Y. Nakanuma, G. Ohta, E. Itagaki, M. Katagiri, Cholesterol side-chain cleavage enzyme activity and cytochrome P450 content in adrenal mitochondria of a patient with congenital lipoid adrenal hyperplasia (Prader disease), Clin. Chim. Acta 77 (1977) 301–306.
[34] K.J. Matteson, B. Chung, M.S. Urdea, W.L. Miller, Study of cholesterol side chain cleavage (20,22 desmolase) deficiency causing congenital lipoid adrenal hyperplasia using bovine-sequence P450scc oligodeoxyribonucleotide probes, Endocrinology 118 (1986) 1296–1305.
[35] D. Lin, S.E. Gitelman, P. Saenger, W.L. Miller, Normal genes for the cholesterol side chain cleavage enzyme, P450scc, in congenital lipoid adrenal hyperplasia, J. Clin. Invest. 88 (1991) 1955–1962.
[36] Y. Sakai, T. Yanase, Y. Okabe, T. Hara, M. Waterman, R. Takayanagi, M. Haji, H. Nawata, No mutation in cytochrome P450 side chain cleavage in a patient with congenital lipoid adrenal hyperplasia, J. Clin. Endocrinol. Metab. 79 (1994) 1198–1201.
[37] M. Fukami, S. Sato, T. Ogata, N. Matsuo, Lack of mutations in P450scc gene (CYP11A) in six Japanese patients with congenital lipoid adrenal hyperplasia, Clin. Pediatr. Endocrinol. 4 (1995) 39–46.
[38] E. Okuyama, N. Nishi, S. Onishi, S. Itoh, Y. Ishii, H. Miyanaka, K. Fujita, Y. Ichikawa, A novel splicing junction mutation in the gene for the steroidogenic acute regulatory protein causes congenital lipoid adrenal hyperplasia, J. Clin. Endocrinol. Metab. 82 (1997) 2337–2342.
[39] P. Saenger, Z. Klonari, S.M. Black, N. Compagnone, S.H. Mellon, A. Fleischer, C.A.L. Abrams, C.H.L. Shackleton, W.L. Miller, Prenatal diagnosis of congenital lipoid adrenal hyperplasia, J. Clin. Endocrinol. Metab. 80 (1995) 200–205.
[40] W.L. Miller, Why nobody has P450scc (20,22 desmolase) deficiency (letter), J. Clin. Endocrinol. Metab. 83 (1998) 1399–1400.
[41] M.E. Toaff, H. Schleyer, J.F. Strauss III., Metabolism of 25-hydroxycholesterol by rat luteal mitochondria and dispersed cells, Endocrinology 111 (1982) 1785–1790.
[42] H.S. Bose, T. Sugawara, J.F. Strauss III., W.L. Miller, The pathophysiology and genetics of congenital lipoid adrenal hyperplasia, New Engl. J. Med. 335 (1996) 1870–1878.
[43] J.A. Harikrishna, S.M. Black, G.D. Szklarz, W.L. Miller, Construction and function of fusion enzymes of the human cytochrome P450scc system, DNA Cell Biol. 12 (1993) 371–379.
[44] T. Sugawara, J.A. Holt, D. Driscoll, J.F. Strauss III., D. Lin, W.L. Miller, D. Patterson, K.P. Clancy, I.M. Hart, B.J. Clark, D.M. Stocco, Human steroidogenic acute regulatory protein (StAR): functional activity in COS-1 cells, tissue-specific expression, and mapping of the structural gene to 8p11.2 and an expressed pseudogene to chromosome 13, Proc. Natl. Acad. Sci. U.S.A. 92 (1995) 4778–4782.
[45] M.K. Tee, D. Lin, T. Sugawara, J.A. Holt, Y. Guiguen, B. Buckingham, J.F. Strauss III., W.L. Miller, T → A transversion 11 bp from a splice acceptor site in the gene for steroidogenic acute regulatory protein causes congenital lipoid adrenal hyperplasia, Hum. Mol. Genet. 4 (1995) 2299–2305.
[46] H.S. Bose, O.H. Pescovitz, W.L. Miller, Spontaneous feminization in a 46,XX female patient with congenital lipoid adrenal hyperplasia caused by a homozygous frame-shift mutation in the steroidogenic acute regulatory protein, J. Clin. Endocrinol. Metab. 82 (1997) 1511–1515.
[47] J. Nakae, T. Tajima, T. Sugawara, F. Arakane, K. Hanaki, T. Hotsubo, N. Igarashi, Y. Igarashi, T. Ishii, N. Koda, T. Kondo, H. Kohno, Y. Nakagawa, K. Tachibana, Y. Takeshima, K. Tsubouchi, J.F. Strauss III., K. Fujieda, Analysis of the steroidogenic acute regulatory protein (StAR) gene in Japanese patients with congenital lipoid adrenal hyerplasia, Hum. Mol. Genet. 6 (1997) 571–576.
[48] S.H. Mellon, Neurosteroids: biochemistry, modes of action and clinical relevance, J. Clin. Endocrinol. Metab. 78 (1994) 1003–1008.
[49] S.E. Pollack, E.E. Furth, C.B. Kallen, F. Arakane, M. Kiriakidou, K.F. Kozarsky, J.F. Strauss III., Localization of steroidogenic acute regulatory protein in human tissues, J. Clin. Endocrinol. Metab. 82 (1997) 4243–4251.
[50] H. Watari, F. Arakane, C. Moog-Lutz, C.B. Callen, C. Tomasetto, G.L. Gerton, M.C. Rio, M.E. Baker, J.F. Strauss III., MLN64 contains a domain with homology to the steroidogenic acute regulatory protein (StAR) that stimulates steroidogenesis, Proc. Natl. Acad. Sci. U.S.A. 94 (1997) 8462–8467.
[51] X. Yang, K. Iwamoto, M. Wang, J. Artwohl, J.I. Mason, S. Pang, Inherited congenital adrenal hyperplasia in the rabbit is caused by

a deletion in the gene encoding cytochrome P450 cholesterol side-chain cleavage enzyme, Endocrinology 132 (1993) 1977–1982.
[52] W.L. Miller, Mitochondrial specificity of the early steps in steroidogenesis, J. Steroid. Biochem. Mol. Biol. 55 (1995) 607–616.
[53] A. Tanae, Y. Miki, I. Hibi, Pubertal presentation in patients with congenital lipoid adrenal hyperplasia (Prader's syndrome), Acta Pædiatr. Jpn. 30 (Suppl) (1988) 236–238.
[54] N. Matsuo, S. Tsuzaki, M. Anzo, T. Ogata, S. Sato, The phenotypic definition of congenital lipoid adrenal hyperplasia: analysis of the 67 Japanese patients (abstract), Horm. Res. 41 (suppl) (1994) 106 (Abstract).
[55] T.J. Cole, J.A. Blendy, A.P. Monaghan, K. Krieglstein, W. Schmid, A. Aguzzi, G. Fantuzzi, E. Hummler, K. Unsicker, G. Schutz, Targeted disruption of the glucocorticoid receptor gene blocks adrenergic chromaffin cell development and severly retards lung maturation, Genes. Dev. 9 (1995) 1608–1621.
[56] K. Fujieda, T. Tajima, J. Nakae, S. Sageshima, K. Tachibana, S. Suwa, T. Sugawara, J.F. Strauss III., Spontaneous puberty in 46,XX subjects with congenital lipoid adrenal hypreplasia, J. Clin. Invest. 99 (1997) 1265–1271.
[57] N. Katsumata, A. Tanae, T. Shinagawa, A. Nagashima-Miyokawa, M. Shimizu, T. Yasunaga, T. Tanaka, I. Hibi, Detection of the missense mutation A218V in the steroidogenic acute regulatory protein gene of a Japanese patient with congenital lipoid adrenal hyperplasia, Clin. Pediatr. Endocrinol. 6 (1997) 33–37.
[58] H.-W. Yoo, G.-H. Kim, Molecular and clinical characterization of Korean patients with congenital lipoid adrenal hyperplasia. J. Ped. Endocrinol. Metab. (in press).
[59] H.S. Bose, M.A. Baldwin, W.L. Miller, Incorrect folding of steroidogenic acute regulatory protein (StAR) in congenital lipoid adrenal hyperplasia, Biochemistry 37 (1998) 9768–9775.
[60] R. Voutilainen, W.L. Miller, Developmental expression of genes for the steroidogenic enzymes P450scc (20,22 desmolase), P450c17 (17α-hydroxylase/17,20 lyase) and P450c21 (21-hydroxylase) in the human fetus, J. Clin. Endocrinol. Metab. 63 (1986) 1145–1150.
[61] M. Kiriakidou, J.M. McAllister, T. Sugawara, J.F. Strauss III., Expression of steroidogenic acute regulatory protein (StAR) in the human ovary, J. Clin. Endocrinol. Metab. 81 (1996) 4122–4128.
[62] K. Caron, S.-C. Soo, W. Wetsel, D. Stocco, B. Clark, K. Parker, 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 (1997) 11540–11545.
[63] F. Arakane, T. Sugawara, H. Nishino, Z. Liu, J.A. Holt, D. Pain, D.M. Stocco, W.L. Miller, J.F. Strauss III., Steroidogenic acute regulatory protein (StAR) retains activity in the absence of its mitochondrial targeting sequence: implications for the mechanism of StAR action, Proc. Natl. Acad. Sci. U.S.A. 93 (1996) 13731–13736.
[64] F. Arakane, C.B. Kallen, H. Watari, J.A. Foster, N.B.V. Sepuri, D. Pain, S.E. Stayrook, M. Lewis, G.L. Gerton, J.F. Strauss III., The mechanism of action of steroidogenic acute regulatory protein (StAR): StAR acts on the outside of mitochondria to stimulate steroidogenesis, J. Biol. Chem. 273 (1998) 16339–16345.
[65] V. Papadopoulos, Peripheral-type benzodiazepine/diazepam binding inhibitor receptor: biological role in steroidogenic cell function, Endocr. Rev. 14 (1993) 222–240.
[66] H. Amri, S.O. Ogwuegbu, N. Boujrad, K. Drieu, V. Papadopoulos, *In vivo* regulation of peripheral-type benzodiazepine receptor and glucocorticoid synthesis by *Ginkgo biloba* extract EGb 761 and isolated ginkgolides, Endocrinology 137 (1996) 5707–5718.
[67] V. Papadopoulos, H. Amri, N. Boujrad, C. Cascio, M. Culty, M. Garnier, M. Hardwick, H. Li, B. Vidic, A.S. Brown, J.L. Reversa, J.M. Bernassau, K. Drieu, Peripheral benzodiazepine receptor in cholesterol transport and steroidogenesis, Steroids 62 (1997) 21–28.
[68] V. Papadopoulos, H. Amri, H. Li, N. Boujrad, B. Vidic, M. Garnier, Targeted disruption of the peripheral-type benzodiazepine receptor gene inhibits steroidogenesis in the R2C Leydig tumor cell line, J. Biol. Chem. 272 (1997) 32129–32135.
[69] F. Arakane, S.R. King, Y. Du, C.B. Kallen, L.P. Walsh, H. Watari, D.M. Stocco, J.F. Strauss III., Phosphorylation of steroidogenic acute regulatory protein (StAR) modules its steroidogenic activity, J. Biol. Chem. 272 (1997) 32656–32662.
[70] T. Sugawara, D. Lin, J.A. Holt, K.O. Martin, N.B. Javitt, W.L. Miller, J.F. Strauss III., The structure of the human steroidogenic acute regulatory (StAR) protein gene: StAR stimulates mitochondrial cholesterol 27-hydroxylase activity, Biochemistry 34 (1995) 12506–12512.