Chapter 2
Regulation of Adrenal Steroidogenesis

Marjut Pihlajoki, Markku Heikinheimo, and David B. Wilson

Introduction

The adrenal cortex is a major source of steroid hormones. Anatomically and functionally distinct adrenocortical zones synthesize specific classes of steroids in response to various stimuli. Adrenal steroids impact a myriad of physiological processes in the fetus and adult, including intrauterine homeostasis, organ maturation, salt/water balance, carbohydrate metabolism, and the response to stress. This chapter highlights the regulation of steroidogenesis in the adrenal cortex. Diseases associated with aberrant production of adrenal steroids are discussed.

Overview of Adrenal Steroidogenesis

The principal steroid hormones produced by the human adrenal cortex are the mineralocorticoid aldosterone, the glucocorticoid cortisol, and the 19-carbon (C_{19}) androgen precursor dehydroepiandrosterone (DHEA). Adrenal steroids are synthesized from cholesterol through the sequential actions of a series of cytochrome P450

M. Pihlajoki, Ph.D.
Children's Hospital, University of Helsinki,
Biomedicum Helsinki 2U, Tukholmankatu 8, 00290 Helsinki, Finland
e-mail: marjut.pihlajoki@helsinki.fi

M. Heikinheimo, M.D., Ph.D.
Children's Hospital, University of Helsinki, PO Box 22, 00014 Helsinki, Finland
e-mail: markku.heikinheimo@helsinki.fi

D.B. Wilson, M.D., Ph.D. (✉)
Departments of Pediatrics and Developmental Biology, Washington University School of Medicine, Box 8208, 660 S. Euclid Ave, St. Louis, MO 63110, USA
e-mail: wilson_d@wustl.edu

© Springer International Publishing AG 2018

A.C. Levine (ed.), *Adrenal Disorders*, Contemporary Endocrinology,
DOI 10.1007/978-3-319-62470-9_2

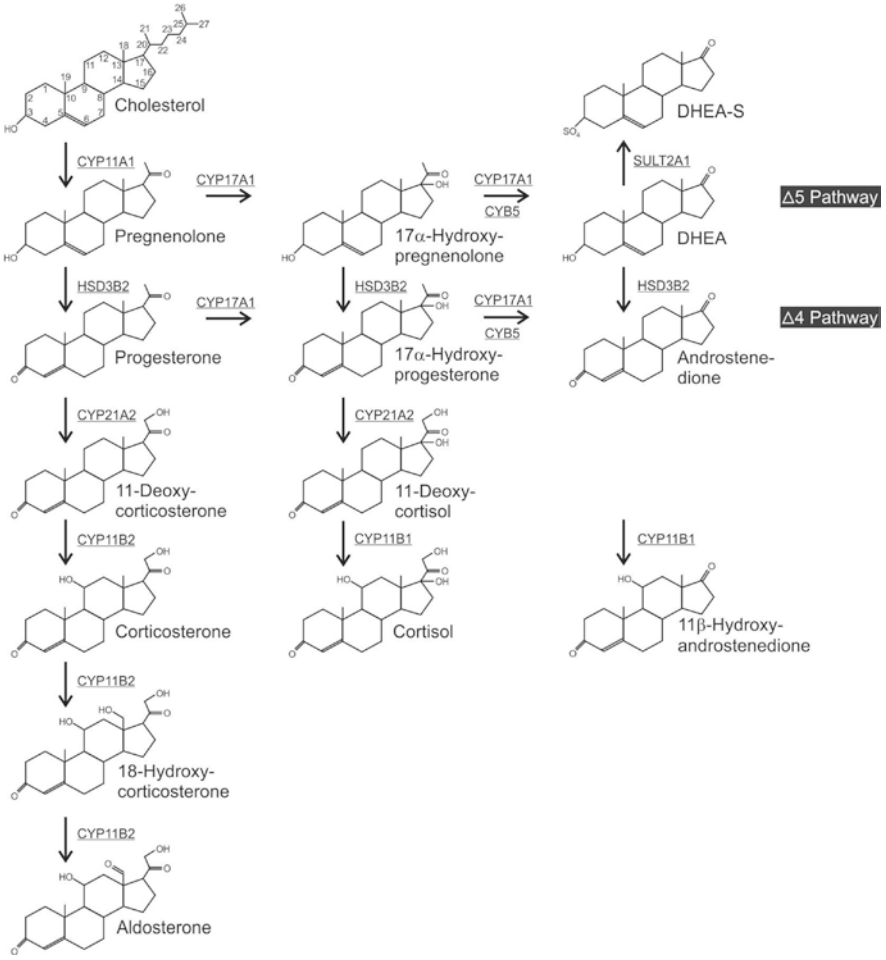

Fig. 2.1 Steroid biosynthetic pathways in the human adrenal cortex. Shown are enzymes (underlined) and intermediates in the biosynthesis of adrenal steroid hormones. 17α-Hydroxypregnenolone is the preferred substrate for the 17,20-lyase reaction of CYP17A1. Consequently, the Δ5 pathway to DHEA is favored over the Δ4 pathway to androstenedione. The adrenal gland produces small quantities of other steroids not shown here. An expanded view of adrenal androgen production is presented later

(CYP)-mixed function oxidases and hydroxysteroid dehydrogenases (HSDs) (Fig. 2.1) [1]. Steroid hormones are not stored in adrenocortical cells. Instead, adrenal steroid secretion relies on de novo synthesis, a process that requires a ready supply of cholesterol [2].

To initiate steroidogenesis, cholesterol undergoes facilitated transport from a replenishable pool in the outer mitochondrial membrane (OMM) to the inner mitochondrial membrane (IMM), where CYP11A1 (side-chain cleavage enzyme)

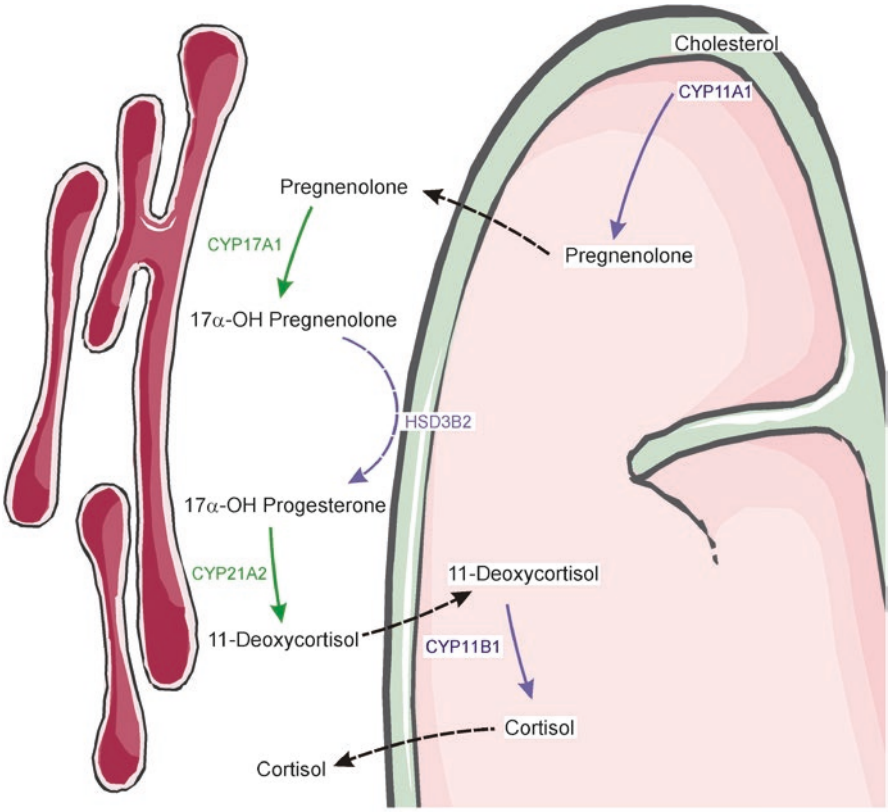

Fig. 2.2 Steroidogenic intermediates shuttle between mitochondria and the ER. The biosynthetic pathway for cortisol is shown; similar shuttling takes place during the synthesis of other adrenal steroid hormones. Enzymatic reactions that occur in mitochondria are shown in *purple*, whereas those that occur in the ER are in *green*. *Dashed lines* indicate passive diffusion across mitochondrial membranes. Prepared using image vectors from Servier Medical Art (www.servier.com), licensed under the Creative Commons Attribution 3.0 Unported License (http://creativecommons.org/license/by/3.0/)

catalyzes the conversion of cholesterol to pregnenolone [1]. Transcription of the *CYP11A1* gene is regulated in a hormonally-responsive manner and determines the net steroidogenic capacity of a cell [3]. Pregnenolone diffuses out of mitochondria and serves as the precursor for the ensuing steps of steroidogenesis, most of which take place in the endoplasmic reticulum (ER) (Fig. 2.2). The final steps of cortisol and aldosterone biosynthesis, catalyzed by the enzymes CYP11B1 and CYP11B2, respectively, occur in mitochondria. Thus, intermediates in the corticoid biosynthetic pathway shuttle between mitochondria and the ER. The electron donors for CYP enzymes in these two cellular compartments are summarized in Table 2.1.

Table 2.1 Cytochrome P450 enzymes involved in adrenal steroidogenesis

CYP classification	Location	Enzyme	Electron donor
Type I	Mitochondria	CYP11A1	NADPH via a flavoprotein (ferredoxin reductase) and an iron-sulfur protein (ferredoxin)
		CYP11B1	
		CYP11B2	
Type II	ER	CYP17A1	NADPH via a flavoprotein [P450-oxidoreductase (POR)]
		CYP21A2	

Each of these enzymes uses molecular oxygen and electrons from nicotinamide adenine dinucleotide phosphate (NADPH) to metabolize substrates

Zones of the Adrenal Cortex

In both the fetus and adult, the adrenal cortex is divided into concentric zones that produce different classes of steroid hormones [4, 5].

Human Fetal Adrenal Cortex

At the eighth week of human gestation, the fetal adrenal cortex comprises two morphologically distinct layers: an outer definitive zone (Dz) and an inner fetal zone (Fz) [6]. The Dz is thin and contains small basophilic cells, whereas the Fz is thick and contains large eosinophilic cells (Fig. 2.3). The Dz does not synthesize significant amounts of steroid hormones, but the Fz produces large quantities of DHEA and its sulfated counterpart DHEA-S. Cells of the Fz express *CYP17A1*, a dual function enzyme that catalyzes both a 17α-hydroxylation reaction and a 17,20-lyase reaction required for C_{19} steroid production [1]. The lyase reaction is selectively enhanced through allosteric interactions with cytochrome b_5 (CYB_5), a protein that is abundant in the Fz [1]. A third cortical zone, the transitional zone (Tz), develops shortly after the appearance of the Fz and Dz. The Tz secretes cortisol, a hormone that promotes maturation of the lungs and other organs [8].

C_{19} steroids secreted by the Fz are converted into estrogens through the actions of enzymes in the liver and/or placenta. The fetal pituitary, adrenal, liver, and placenta constitute a functional entity known the feto-placental unit [9] (Fig. 2.4). The concentration of estrogens in maternal plasma increases abruptly mid-gestation, reflecting production by this unit [10]. Estrogens support pregnancy by promoting maternal breast development, blood volume expansion, and uterine growth/contractility, although intact fetal adrenocortical function is not a prerequisite for term gestation or birth [11].

Adrenocorticotropic hormone (ACTH), a peptide secreted by the anterior pituitary gland, is a major regulator of fetal adrenal growth and function. ACTH promotes the production of both C_{19} steroids and cortisol in the fetal adrenal. Disruption of hypothalamic/pituitary function (e.g., in the anencephalic fetus) impairs Fz growth and decreases estrogen levels in the maternal circulation [8].

Another important regulator of steroidogenesis in the fetus is placenta-derived corticotropin-releasing hormone (CRH), a peptide that both directly and indirectly

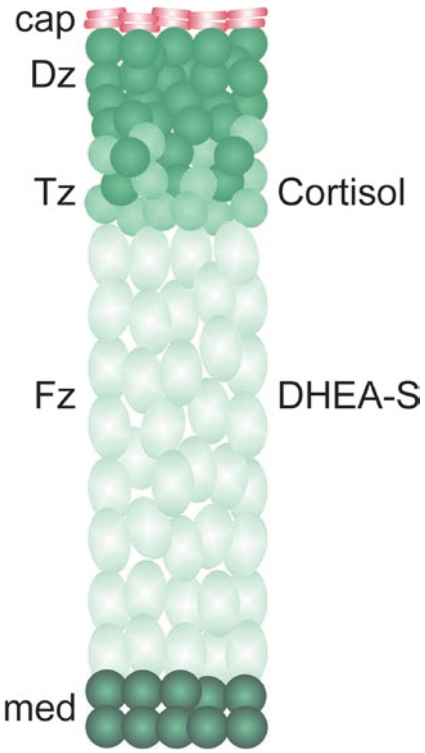

Fig. 2.3 Structure of the human fetal adrenal gland. The zones of the fetal cortex are the Dz, Tz, and Fz. The Tz and Fz produce cortisol and C_{19} androgen precursors, respectively. An early burst of cortisol production by the Tz during the 9th week of gestation, coinciding with a transient increase in expression of *HSD3B2*, is thought to safeguard female sexual development by limiting the production of androgen precursors by the Fz [7]. After birth the Dz differentiates into the functionally distinct zones of the adult cortex. *Cap* capsule, *DHEA-S* dehydroepiandrosterone sulfate, *Dz* definitive zone, *Fz* fetal zone, *med* medulla, *Tz* transitional zone

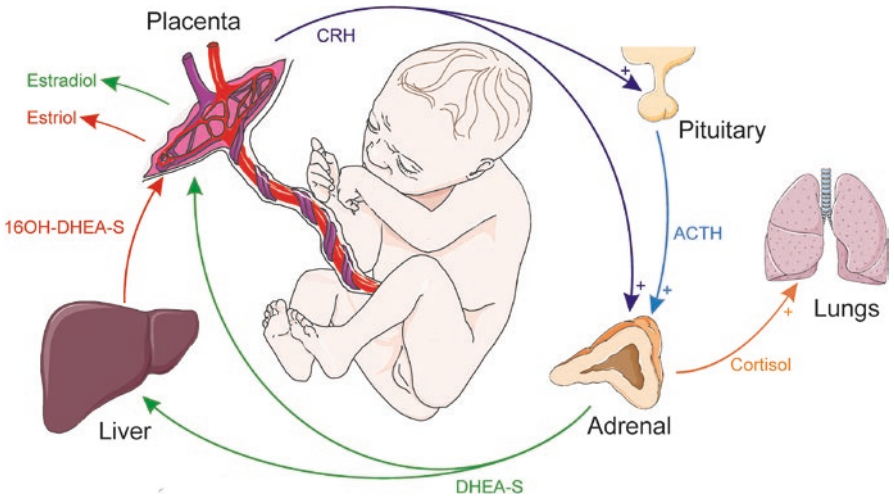

Fig. 2.4 Steroid production by the feto-placental unit. Placental CRH and pituitary-derived ACTH promote cortisol and DHEA-S secretion by the fetal adrenal gland. DHEA-S is converted into estrogens (estradiol and estriol) by enzymes in the liver and placenta. The resultant estrogens support pregnancy, while cortisol promotes the maturation of the lungs and other organs in the fetus. *ACTH* adrenocorticotropic hormone, *CRH* corticotropin-releasing hormone, *DHEA-S* dehydroepiandrosterone sulfate, *16OH-DHEA-S* 16-hydroxydehydroepiandrosterone sulfate. Prepared using image vectors from Servier Medical Art (www.servier.com), licensed under the Creative Commons Attribution 3.0 Unported License (http://creativecommons.org/license/by/3.0/)

stimulates fetal adrenal cells to produce cortisol and androgens [12, 13] (Fig. 2.4). Unlike hypothalamic CRH secretion, which is subject to feedback inhibition by glucocorticoids (see later), placental CRH release is enhanced by cortisol. At birth the placenta separates from the body, and this CRH feed-forward loop is interrupted. The newborn adrenal gland, which is almost as large as the kidney, shrinks dramatically over the first 2 weeks of life owing to apoptotic involution of the Fz. Regression of the Fz is accompanied by a reduction in C_{19} steroid production [1]. Postnatally, the Dz differentiates into the anatomically and functionally distinct zones of the adult cortex.

Human Adult Adrenal Cortex

The adult adrenal cortex of humans contains three layers: the zona glomerulosa (zG), zona fasciculata (zF), and zona reticularis (zR) (Fig. 2.5) [4, 14]. The cortex is enveloped by a capsule that provides structural support and houses stem/progenitor cells of the cortex. A complex network of blood vessels within the adrenal gland ensures

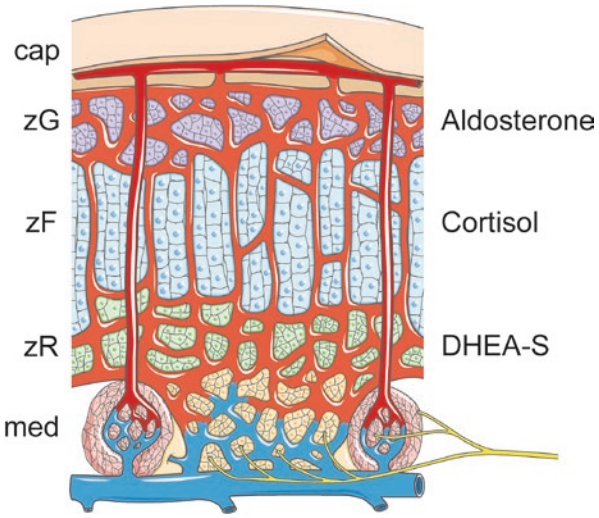

Fig. 2.5 Structure of the adult adrenal gland. The gland is covered by a capsule. The cortical zones are the zG, zF, and zR, which produce aldosterone, cortisol, and DHEA-S, respectively. Adrenal glands are highly vascularized, ensuring the efficient delivery of stimulants and export of steroid hormones into the systemic circulation. A subcapsular arteriolar plexus receives oxygenated blood and distributes it to the underlying tissue via two types of vessels: (1) sinusoids that supply the adrenal cortex and medulla and (2) medullary arteries that directly supply to the medulla. The adrenal medulla, which is innervated by preganglionic sympathetic fibers, functions as part of the sympathetic nervous system, releasing catecholamines into the circulation. *Cap* capsule, *DHEA-S* dehydroepiandrosterone sulfate, *med* medulla, *zF* zona fasciculata, *zG* zona glomerulosa, *zR* zona reticularis. Prepared using image vectors from Servier Medical Art (www.servier.com), licensed under the Creative Commons Attribution 3.0 Unported License (http://creativecommons.org/license/by/3.0/)

the efficient delivery of stimulants and the export of corticoids into the circulation [15, 16]. Blood flows centripetally in the gland, so inner cortical zones and the adrenal medulla are exposed to high concentrations of locally-produced steroids.

zG

The outermost zone, the zG, secretes aldosterone. The major physiological stimuli of aldosterone secretion are angiotensin II (Ang II) and extracellular K^+, but ACTH also enhances production of this steroid hormone [17]. Aldosterone binds to the mineralocorticoid receptor (MR) in cells of the distal nephron, leading to retention of Na^+ and excretion of K^+ and H^+ by the kidneys [18]. By modulating Na^+ balance, aldosterone impacts extracellular fluid volume and blood pressure. Aldosterone also plays important roles in certain cardiovascular, renal, and inflammatory diseases [19–21]. For example, aldosterone exerts direct actions on cardiomyocytes, contributing to cardiac fibrosis and congestive heart failure [22]. Distinctive molecular markers of the zG include CYP11B2 (the mitochondrial enzyme that catalyzes the final steps of aldosterone biosynthesis) and AT_1R (the Ang II receptor) [23]. The zG lacks expression of *CYP17A1* and therefore is not able to synthesize cortisol or androgen precursors.

zF

The largest cortical zone, the zF, produces the stress hormone, cortisol, as part of the hypothalamic-pituitary-adrenal (HPA) axis (Fig. 2.6). ACTH secreted by the pituitary is the principal stimulus for cortisol production [24]. Cells in the zF respond to

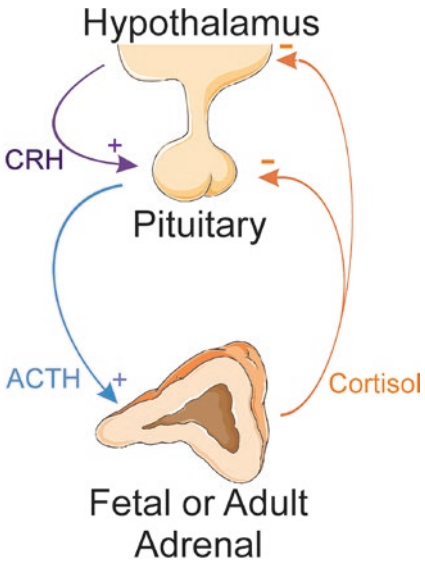

Fig. 2.6 HPA axis. In response to stress or other physiological cues, hypothalamic neurons secrete CRH, which stimulates the release of ACTH from the pituitary gland. ACTH promotes the secretion of cortisol by the adrenal cortex. Cortisol, in turn, inhibits the axis at the level of the pituitary and hypothalamus. *ACTH* adrenocorticotropic hormone, *CRH* corticotropin-releasing hormone, *HPA* hypothalamic-pituitary-adrenal. Prepared using image vectors from Servier Medical Art (www.servier.com), licensed under the Creative Commons Attribution 3.0 Unported License (http://creativecommons.org/license/by/3.0/)

ACTH via its G-protein-coupled receptor, the melanocortin 2 receptor (MC2R), working in conjunction with melanocortin 2 receptor accessory proteins (MRAPs). Adrenal-derived cortisol binds to the glucocorticoid receptor (GR), which is expressed in a wide array of cell types including hepatocytes, muscle cells, lymphocytes, neurons, and neuroendocrine cells. Cortisol functions to (1) increase blood glucose concentrations through gluconeogenesis; (2) facilitate the catabolism of proteins, lipids, and carbohydrates; (3) suppress the immune system; and (4) induce enzymes for catecholamine biosynthesis in the medulla [25, 26]. A distinctive molecular marker of the zF is CYP11B1, a mitochondrial enzyme required for the biosynthesis of cortisol [23].

zR

The innermost layer of the cortex, the zR, secretes the C_{19} steroids DHEA, DHEA-S, androstenedione, and 11β-hydroxyandrostenedione, which are often termed "adrenal androgens" [1]. DHEA-S is the most abundant adrenal steroid in the circulation of adults [27]. In contrast to more potent androgens, such as testosterone and dihydrotestosterone (DHT), adrenal androgens exhibit little or no binding/activation of the androgen receptor (AR) [28]. Consequently, adrenal C_{19} steroids function mainly as precursor molecules that can be converted into more potent androgens in peripheral tissues (e.g., hair follicles, sebaceous glands, prostate, and genital skin).

Cells of the zR, like those of the zG and zF, respond to ACTH. In contrast to other zones of the cortex, the zR does not become functionally active until 6–8 years of age (adrenarche), coinciding with increased expression of *CYB5*, the allosteric regulator that enhances the 17,20-lyase activity of CYP17A1 [1]. CYB5 serves as a distinctive molecular marker of the zR; another characteristic marker of this zone is SULT2A1, the enzyme responsible for sulfation of DHEA [29].

Comparative Adrenal Anatomy of Small Animal Models

Rodents are used as experimental models for the study of adrenal physiology, but there are noteworthy differences between the adrenocortical zonation patterns of rodents and humans [30]. The zR is absent from the adrenal gland of the mouse. The adrenal cortex of the young mouse contains an ephemeral juxtamedullary layer, the X-zone, that regresses during puberty in males and the first pregnancy in females [31]. Lineage tracing studies have shown that the X-zone is a derivative of the fetal cortex [31], but the physiological function of the X-zone remains unclear [32]. The rat adrenal cortex has an additional layer, the undifferentiated zone (zU), located between the zG and zF. The zU contains a transitional population of cells [33].

Species also differ in the complement of steroidogenic enzymes and cofactors expressed in the adrenal cortex, and these differences have biological ramifications. For example, cells in the zF and zR of humans express *CYP17A1*, so cortisol is the

principal glucocorticoid secreted by the adrenal gland of this species [34]. Cells in the zF of adult mice and rats lack CYP17A1, so corticosterone is the main glucocorticoid produced, and adrenal androgens are not made. A newly recognized rodent-like model is the spiny mouse (genus *Acomys*) [35]. In contrast to the laboratory mouse (genus *Mus*), the adrenal cortex of the spiny mouse has a zR. Cells in the zF and zR of the spiny mouse produce cortisol and DHEA, respectively. Thus, the adrenal physiology of the spiny mouse recapitulates that of humans [9].

Role of Zonal Remodeling in the Regulation of Steroidogenesis

The adrenal cortex is a dynamic tissue that undergoes continual renewal. Senescent cells are replenished through division and differentiation of stem/progenitor cells in the periphery of the gland (i.e., the adrenal capsule and subcapsule) [36–38]. The newly formed cells migrate centripetally to repopulate cortical zones. This cellular turnover facilitates adrenocortical remodeling in response to physiological demand for steroids. Cortical zones can reversibly expand, contract, or alter their biochemical profiles in response to physiological or pharmacological triggers (Table 2.2). Zonal remodeling is one of the major mechanisms by which steroid production is modulated.

Table 2.2 Impact of various stimuli on adrenocortical remodeling and function

Zone (species)	Physiological or pharmacological stimulus	Effect	References
zG (rat)	↓ Na^+ or ↑ K^+ in diet	Expands the zone, increasing aldosterone production	[17, 23, 39]
	↑ Na^+ or ↓ K^+ in diet	Contracts the zone, decreasing aldosterone production	
	Captopril or other ACE inhibitors	Contracts the zone, decreasing aldosterone production	
	Endothelin or serotonin	Stimulates aldosterone production	
	Dopamine or atrial natriuretic peptide	Inhibits aldosterone production	
	Arginine vasopressin	Expands the zone	
zF (rat)	ACTH	Expands the zone, increasing glucocorticoid production	[23]
	Dexamethasone	Contracts the zone, decreasing glucocorticoid production	
zR (primates)	Adrenarche	Increases *CYB5* expression, enhancing DHEA production	[1, 40–42]
	Social status in marmosets	Adult females develop a functional zR based on status in the group	
	Cortisol and androstenedione	Stimulates DHEA production through competitive inhibition of HSD3B2 activity	

Secreted Peptides Implicated in Adrenocortical Growth and Steroidogenesis

The archetypal peptide hormones impacting adrenocortical function are ACTH and Ang II, but many other hormones and growth factors, working alone or in combination, have been shown to regulate adrenocortical cell function [36, 38] (Table 2.3).

Table 2.3 Secreted proteins that regulate adrenocortical cell growth and function

	Factor	Function	References
Endocrine hormones	ACTH	Stimulates cortisol and androgen biosynthesis; some of its tropic actions are mediated indirectly via growth factors	[8]
	Ang II	Stimulates aldosterone production	[23]
	Placental CRH	Directly stimulates fetal adrenal cells to produce cortisol and androgens	[13]
	Human chorionic gonadotropin (hCG)	Drives the growth of the fetal adrenal gland during the first trimester of pregnancy	[8]
	Luteinizing hormone (LH)	The LH receptor has been shown to be functionally active in the adrenal of adults during pregnancy and other high gonadotropin states	[43, 44]
	Activins	Inhibit adrenocortical cell growth/survival and modulate steroidogenesis	[45, 46]
	Inhibins	Inhibit activin signaling in adrenocortical cells	[38, 46]
Growth factors	Insulin-related growth factors (IGF1/2)	Promote adrenocortical cell mitosis and survival; enhance the effect of ACTH on steroidogenesis in fetal and adult adrenocortical cells	[47, 48]
	Epidermal growth factor (EGF)	A potent mitogen for cultured fetal and definitive zone cells from mid-gestation human fetal adrenal glands	[49]
	Fibroblast growth factor-2 (FGF2)	Acts as an adrenocortical cell mitogen; binds to zG cells	[50]
Developmental signaling molecules	Sonic hedgehog (SHH)	Ligand secreted by subcapsular cells; promotes steroidogenic differentiation of stem/progenitor cells in the capsule	[51]
	Delta-like homologue-1 (DLK1)	Transmembrane protein that is cleaved and secreted by the rat zU; regulates the differentiation of steroidogenic cell progenitors in the capsule	[33]
	Wingless-related integration site-4 (WNT4)	Wnt/β-catenin signaling is critical for zG differentiation and maintenance; downregulation of this signaling is required for zG-to-zF conversion	[52, 53]
	R-spondin-3 (RSPO3)	Ligand that potentiates Wnt/β-catenin signaling and is required for zG differentiation	[54]

Hormones and paracrine factors traditionally associated with reproductive function, such as luteinizing hormone, activins, and inhibins, can affect adrenocortical steroidogenesis.

Uptake and Intracellular Trafficking of Cholesterol: Prelude to Steroidogenesis

Initiation of steroidogenesis entails the following steps: (1) mobilization of cholesterol, the precursor of all steroid hormones, from endogenous or exogenous sources, (2) transport of cholesterol to the OMM, and (3) transfer of cholesterol from the OMM to the IMM.

Mobilization of Free Cholesterol from Intracellular and Extracellular Sources

Cholesterol can be derived from a combination of sources: (1) de novo biosynthesis, (2) import of lipoprotein-associated cholesteryl esters (CEs) via endocytosis of the low-density lipoprotein receptor (LDLR), (3) uptake of esterified cholesterol through the high-density lipoprotein (HDL) scavenger receptor 1 (SR-B1), and (4) hydrolysis of CEs stored with lipid droplets. These redundant sources ensure that adequate cholesterol is available for steroidogenesis.

De Novo Cholesterol Biosynthesis

Cholesterol is synthesized from mevalonate and isoprenoid precursors [55]. The rate-limiting step of cholesterol biosynthesis is catalyzed by hydroxymethylglutaryl coenzyme A (HMG-CoA) reductase. Expression of HMG-CoA reductase is regulated by isoforms of sterol-regulatory element-binding protein (SREBP), a transcription factor that coordinate the synthesis, uptake, and metabolism of cholesterol and fatty acids [56]. Newly synthesized SREBP is membrane-bound and resides in the ER, where it interacts with SREBP cleavage-activating protein (SCAP), a key cellular cholesterol sensor [57] (Fig. 2.7). When cells are deficient in cholesterol, SCAP and other proteins escort SREBP from the ER to Golgi. Once in the Golgi, SREBP is proteolytically processed to generate a "mature" transcription factor that travels to the nucleus and activates genes required for de novo cholesterol synthesis (e.g., HMG-CoA reductase) and for internalization of LDL [58]. When cells are replete with cholesterol, SREBP is retained in the ER, thereby limiting its proteolytic activation.

De novo synthesis is not the principal source of cholesterol for steroidogenesis, as evidenced by the fact that patients treated with statins, inhibitors of HMG-CoA reductase, exhibit normal cortisol secretion [59, 60]. Nevertheless, de novo synthesis remains an important source of cholesterol under certain physiological and pathophysiological states [61].

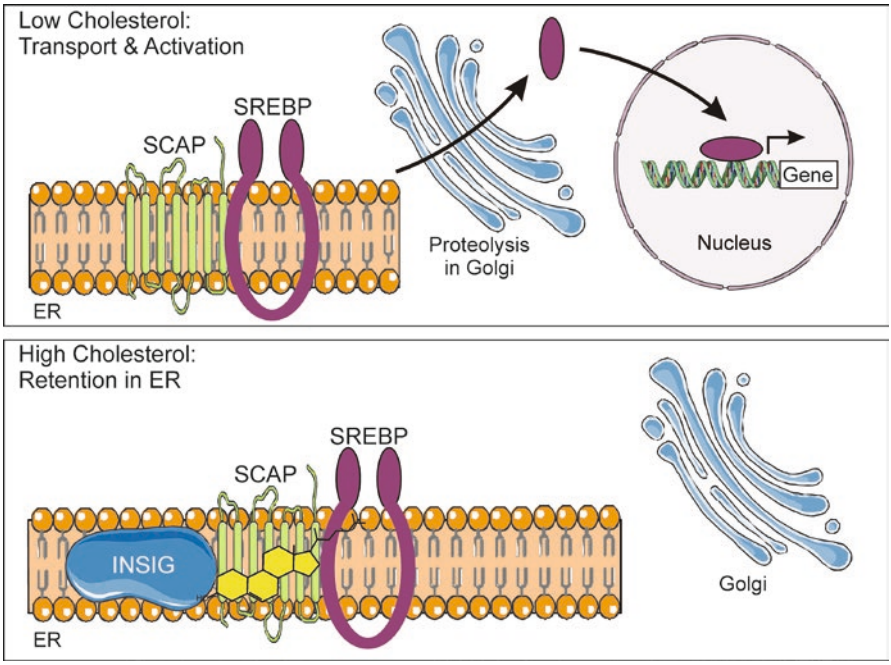

Fig. 2.7 Activation of SREBP, a key transcriptional regulator of steroidogenesis, is regulated by the cholesterol content of the ER membrane. Cholesterol homeostasis is maintained by SREBP, a membrane-associated transcription factor, working in conjunction with SCAP, a cholesterol sensor. When cholesterol levels are low, SCAP and other proteins escort SREBP from the ER to Golgi. Once in the Golgi, SREBP is proteolytically processed to generate a "mature" transcription factor that travels to the nucleus and increases the expression of genes required for cholesterol synthesis and uptake. High levels of cholesterol trigger a conformational change in SCAP, causing the SCAP-SREBP complex to associate with INSIG, an ER anchor protein. Consequently, SREBP is retained in the ER and not proteolytically activated. *ER* endoplasmic reticulum, *INSIG* insulin-induced gene, *SCAP* SREBP cleavage-activating protein, *SREBP* sterol-regulatory element-binding protein. Prepared using image vectors from Servier Medical Art (www.servier.com), licensed under the Creative Commons Attribution 3.0 Unported License (http://creativecommons.org/license/by/3.0/)

Import of Esterified Cholesterol via the LDL Receptor

Approximately 80% of the cholesterol used in the synthesis of adrenal steroids derives from uptake of plasma lipoproteins by LDLR (Fig. 2.8) [62]. Apolipoprotein E (apoE) and apolipoprotein B (apoB)-containing lipoprotein particles bind to LDLR and are internalized via clathrin-coated pits [63]. In late endosomes, lipoprotein-derived CEs are hydrolyzed by lysosomal acid lipase (LIPA) to produce free cholesterol. Loss-of-function mutations in *LIPA* cause Wolman disease and its milder variant, cholesteryl ester storage disease [64]. The liberation of LDL-derived cholesterol from late endosomes requires the protein products of two other genes *NPC1* and *NPC2* [65]. NPC2 binds and facilitates the hydrolysis of CEs by

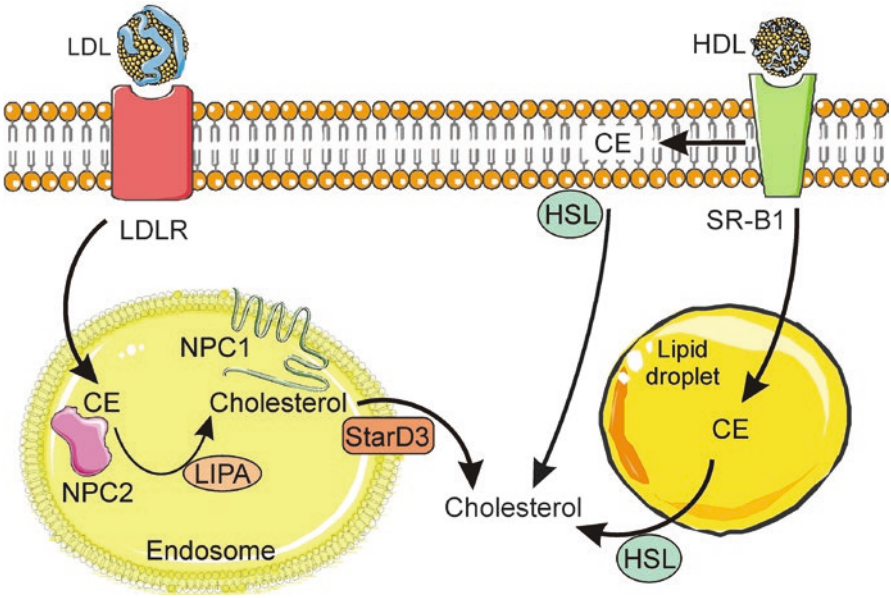

Fig. 2.8 Import of esterified cholesterol via lipoprotein receptors. Adrenocortical cells take up circulating LDL via receptor mediated endocytosis. In late endosomes, LDL-derived CE is bound by NPC2 and hydrolyzed by LIPA to yield free cholesterol, which is then transferred to NPC1, inserted into the endosomal membrane, and exported. A cholesterol-binding protein, StarD3, co-localizes with the NPC system and may participate in the egress of cholesterol from endosomes. SR-B1 mediates the uptake of CE from HDL. Some of the CE delivered via SR-B1 is incorporated into the plasma membrane, and some is directly incorporated into lipid droplets. HDL-derived CE is hydrolyzed to free cholesterol by HSL. *CE* cholesteryl ester, *HDL* high-density lipoprotein, *HSL* hormone-sensitive lipase, *LDL* low-density lipoprotein, *LDLR* LDL receptor, *LIPA* lysosomal acid lipase, *NPC1/NPC2* Niemann-Pick type C disease proteins 1 and 2, *StarD3* START domain protein 3, *SR-B1* scavenger receptor class B, type 1. Prepared using image vectors from Servier Medical Art (www.servier.com), licensed under the Creative Commons Attribution 3.0 Unported License (http://creativecommons.org/license/by/3.0/)

LIPA. The resultant-free cholesterol is transferred to NPC1, inserted into the endosomal membrane, and then exported. Mutations in *NPC1* or *NPC2* cause Niemann-Pick type C disease, another lysosomal storage disorder [66].

A cholesterol-binding protein, StarD3 (MLN64), co-localizes with NPC proteins and may participate in the egress of LDL-derived cholesterol from endocytic vesicles. StarD3 is a member of the START domain (StarD) family of proteins [67]. The START [StAR (*ST*eroidogenic *A*cute *R*egulatory protein)-related lipid *T*ransfer] domain is a conserved sequence that binds sterols and other lipids [68]. The human START domain family comprises 16 proteins, 5 of which (StarD1/D3/D4/D5/D6) bind cholesterol. StarD3 contains a leader sequence that targets the molecule to late endosomes. Enforced expression of a mutant form of StarD3 lacking the START domain causes accumulation of free cholesterol in lysosomes [69]. The roles of other START domain proteins, including the founding member of the family, StarD1 (better known as StAR), are discussed later.

Uptake of Esterified Cholesterol via SR-B1

Scavenger receptor class B, type 1 (SR-B1) mediates the uptake of HDL CEs through a mechanism that differs from the endocytic pathway used by the LDL receptor (Fig. 2.8) [70]. SR-B1 localizes to lipid rafts, membrane domains rich in cholesterol and sphingolipids. SR-B1 forms a nonaqueous channel [71]. Binding of HDL to SR-B1 results in the "selective uptake" of esterified cholesterol into the plasma membrane, followed by the release of the resultant lipid-depleted HDL particles back into the circulation. Some CEs delivered via SR-B1 appear to be incorporated directly into lipid droplets [72, 73]. CEs imported into the plasma membrane or lipid droplets by SR-B1 may be hydrolyzed to free cholesterol by hormone-sensitive lipase (HSL). Uptake through SR-B1 is the major source of cholesterol for steroidogenesis in rodents [74]. SR-B1 impacts steroidogenesis in human cell cells, too. Incubation of human HAC15 adrenocortical cells with cholesterol-free synthetic HDL leads to an efflux of cholesterol and a decrease in cortisol production [75].

Hydrolysis of Cholesteryl Esters Stored in Lipid Droplets

Hydrolysis of CEs stored within lipid droplets is the preferred source for cholesterol in the setting of acute hormonal stimulation. HSL, an enzyme activated in response to binding of ACTH or Ang II to their cognate receptors, catalyzes the hydrolysis of lipid droplet-associated CEs [73, 76]. ACTH activates HSL via PKA-mediated phosphorylation, whereas Ang II activates HSL via mitogen-activated protein kinases (MAPKs) (see later). In response to phosphorylation by these kinases, HSL is translocated from the cytoplasm to either the plasma membrane or the surface of lipid droplets, facilitating hydrolysis of CEs at these sites. In addition to catalyzing the hydrolysis of CEs, HSL interacts with various cholesterol-binding proteins to help direct the cholesterol to the OMM for steroidogenesis [77].

Esterification of Excess Free Cholesterol by SOAT1

Excess-free cholesterol, which is toxic to cells, may be esterified by the ER enzyme sterol O-acyltransferase 1 (SOAT1) [also called acyl-CoA:cholesterol O-acyltransferase 1 (ACAT1)]. The resultant CEs may be incorporated into lipid droplets for storage. Drugs that inhibit SOAT1 activity, such as mitotane and ATR-101, induce the accumulation of cholesterol and free fatty acids in the ER of adrenocortical cells (Fig. 2.9) [78–80]. This increase in cholesterol limits SREBP activation, resulting in downregulation of target genes that mediate cholesterol synthesis and uptake. The accumulation of cholesterol and free fatty acids induces ER stress, which triggers transcription of unfolded protein response (UPR) genes [81]. Persistent ER stress leads to upregulation of pro-apoptotic *BAX* and repression anti-apoptotic *BCL2*, thus inducing cell death [79]. Targeted deletion of *SOAT1* in an

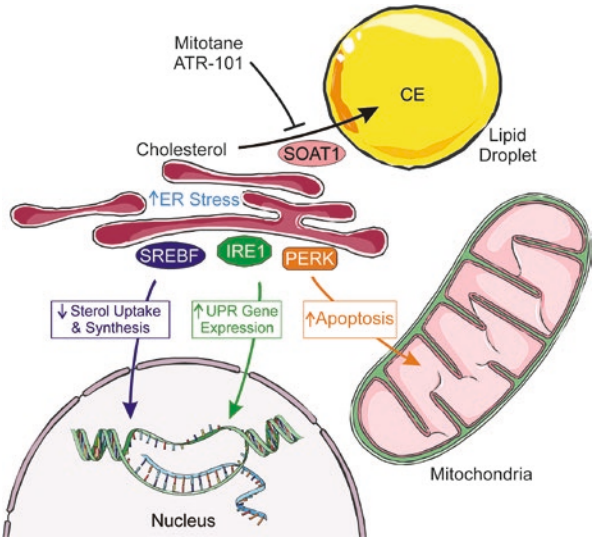

Fig. 2.9 Pharmacological inhibition of SOAT1, the enzyme that esterifies excess cholesterol, impairs adrenocortical steroidogenesis and cell survival. Inhibition of SOAT1 by either mitotane or ATR-101 leads to an increase in free cholesterol and fatty acids that (1) cause downregulation of SREBF-dependent genes that mediate cholesterol synthesis and uptake and (2) trigger ER stress, leading to IRE1-dependent splicing of XBP1 mRNA and subsequent transcription of UPR genes. As ER stress persists, increased expression of *PERK* induces increased *CHOP* expression, which triggers expression of pro-apoptotic *BAX* and repression of anti-apoptotic *BCL2*, thus inducing cell apoptosis. *CE* cholesteryl ester, *CHOP* CCAAT-enhancer-binding protein homologous protein, *ER* endoplasmic reticulum, *PERK* PKR-like ER kinase, *SREBF* sterol-regulatory element-binding protein, *SOAT1* sterol O-acyltransferase 1, *UPR* unfolded protein response, *XBP1* X-box-binding protein. Prepared using image vectors from Servier Medical Art (www.servier.com), licensed under the Creative Commons Attribution 3.0 Unported License (http://creativecommons.org/license/by/3.0/)

adrenocortical cell line recapitulates the effects of pharmacological inhibitors of SOAT1 [78]. Inhibition of cholesterol esterification is thought to be the mechanism of action of mitotane, the only drug approved for the treatment of patients with adrenocortical carcinoma (ACC) (Fig. 2.9). ATR-101 is undergoing preclinical and clinical testing as a new drug for the treatment of ACC [78].

Transport of Free Cholesterol to the OMM

Mitochondria contain a limited amount of cholesterol, the majority of which resides in the OMM [82, 83]. Therefore, cholesterol consumed during steroidogenesis must be continually replenished. Mitochondrial stores cannot be restocked with cholesterol via simple diffusion across aqueous cytoplasmic spaces, because this compound is relatively insoluble in water. Instead, “free” cholesterol is trafficked to

mitochondria via two general mechanisms: (1) vesicular transport, an energy-dependent process entailing budding and fusion of membrane vesicles, and (2) non-vesicular transport via soluble cholesterol-binding proteins [61, 71]. Close physical interactions between mitochondria and other subcellular compartments (e.g., ER, lipid droplets) promote cholesterol transfer.

Role of the Cytoskeleton in Cholesterol Delivery to the OMM

Intracellular vesicular trafficking of cholesterol requires rearrangement of an integrated network of cytoskeletal elements, composed of intermediate filaments, microtubules, and other structures [84–86]. Some cytoskeletal rearrangements appear to be driven by the protein myristoylated alanine-rich C-kinase substrate (MARCKS), which is phosphorylated in adrenocortical cells in response to hormonal stimulation [87] and can associate with actin filaments and the membrane surfaces [88]. Another key cytoskeletal protein is the intermediate filament vimentin, which binds to lipid droplets and to HSL and appears to regulate cholesterol delivery to mitochondria. Pharmacological or genetic disruption of cytoskeletal structure impacts cholesterol trafficking. For example, vimentin null mice exhibit decreased ACTH-stimulated corticosterone levels [89].

Role of SNARE Proteins in Cholesterol Delivery to Mitochondria

SNARE proteins [an acronym derived from "SNAP (*S*oluble *N*-ethylmaleimide-sensitive factor *A*ttachment Protein) *RE*ceptor"] are GTP-binding proteins that mediate vesicle fusion [90]. The most extensively studied SNARE proteins are those that mediate the fusion of synaptic vesicles with the presynaptic membrane in neurons, but recent studies suggest that these proteins also regulate cholesterol transfer [91]. Certain SNARE proteins, including α-SNAP and SNAP25, are associated with lipid droplets [92]. Gene-silencing experiments and in vitro reconstitution assays have shown that specific SNARE proteins [α-SNAP, SNAP25, syntaxin (STX)-5, and STX-17] are required for efficient cholesterol movement to mitochondria for steroidogenesis [91]. A subset of SNAREs, including STX-5, exhibit cholesterol-binding properties, and this may aid in the trafficking of cholesterol from lipid droplets to the OMM [93, 94].

MAM-Facilitated Transfer of Cholesterol to the OMM

Trafficking of cholesterol to mitochondria is also facilitated by the mitochondria-associated ER membrane (MAM), a distinct subdomain of the ER that is reversibly tethered to the OMM by lipids and protein filaments [71, 95, 96]. MAMs serve as hubs that coordinate metabolic interactions between these two organelles. MAMs have been implicated in a wide array of cellular processes, including lipid transport

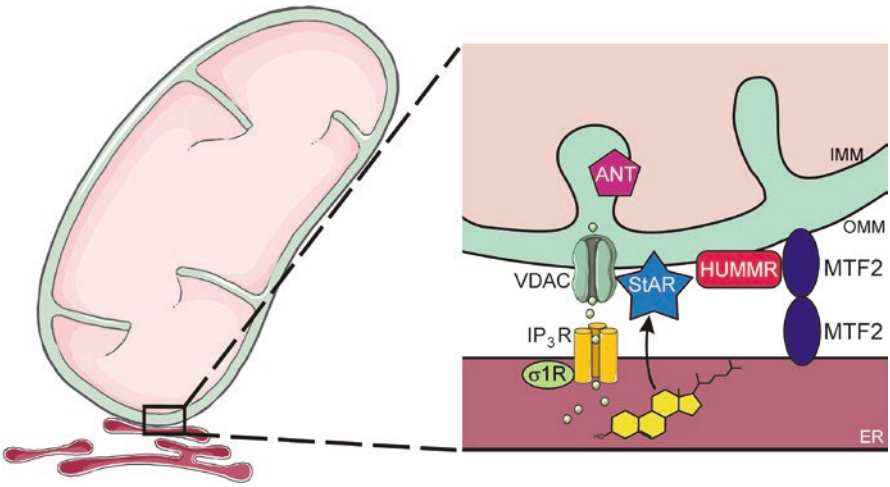

Fig. 2.10 Mitochondria-associated ER membrane (MAM). The ER communicates with mitochondria via the MAM, a specialized subdomain of the ER with the characteristics of a lipid raft. MAMs serve to coordinate metabolic interactions (e.g., lipid transport, Ca^{2+} signaling) between these two organelles. The following MAM components are illustrated: MTF2 (a tether), VDAC (a channel), σ-1 (a receptor), StAR (a transport protein), HUMMR (an OMM adaptor protein), ANT (an IMM nucleotide translocase), and IP_3R, (a receptor and Ca^{2+} channel). *ANT* adenine nucleotide translocase, *ER* endoplasmic reticulum, *IMM* inner mitochondrial membrane, *HUMMR* hypoxia-upregulated mitochondrial movement regulator, *IP_3R* inositol (1,4,5) trisphosphate receptor MTF2, mitofusin 2, *OMM* outer mitochondrial membrane, *σ1R* sigma-1 receptor, and *VDAC* voltage-dependent anion channel. Prepared using image vectors from Servier Medical Art (www.servier.com), licensed under the Creative Commons Attribution 3.0 Unported License (http://creativecommons.org/license/by/3.0/)

(e.g., mitochondrial import of phosphatidylserine), Ca^{2+} signaling, energy metabolism, mitochondrial dynamics, and apoptosis [97, 98].

MAMs are composed of cholesterol-rich lipid rafts and resident membrane proteins (Fig. 2.10). MAM proteins include: (1) mitofusin 2 (MTF2), which functions as a tether; (2) FATE1, which modulates ER-mitochondrial distance [99]; (3) the voltage-dependent anion channel (VDAC), an OMM-associated protein that allows for the passage of ions, metabolites, and signaling molecules (e.g., ATP) across the membrane (see later); and (4) sigma-1 receptor, which promotes cholesterol compartmentalization and trafficking [100, 101]. It has been proposed that cholesterol transfer from OMM to IMM occurs at specialized contact sites bridging the two membranes composed of VDAC and IMM adenine nucleotide translocase (ANT).

Genetic evidence supporting a role for the MAM in steroidogenesis has emerged from the isolation and characterization of mutant cell lines with altered intracellular cholesterol homeostasis. One such cell line harbors a mutation in the H/ACA small nucleolar RNA (snoRNA) U17, which regulates hypoxia-upregulated mitochondrial movement regulator (HUMMR), an OMM adaptor protein that promotes the formation of ER/mitochondrial contact sites [102].

Role of StarD4/D5/D6 in Cholesterol Trafficking

As mentioned earlier, there are five START proteins in the human genome that can bind cholesterol. Two of these have leader peptides that direct them to specific organelles: StarD1 (StAR itself), which is targeted to mitochondria, and StarD3, which is targeted to endosomes (see later). The remaining members of this subgroup—StarD4, StarD5, and StarD6—lack leader peptides and appear to be cytosolic proteins involved in non-vesicular cholesterol transport in various cell types, including steroidogenic cells. StarD4 is thought to be the principal agent delivering cholesterol to the OMM from elsewhere in the cell [67]. StarD5 binds cholesterol and bile acids, whereas StarD6 binds cholesterol and steroid hormones.

Transfer of Cholesterol from the OMM to the IMM

Once cholesterol reaches the OMM, a group of proteins led by steroidogenic acute regulatory protein (StAR) facilitate the transport of cholesterol to the IMM, the site of pregnenolone synthesis by CYP11A1. The delivery of cholesterol from the OMM to the IMM is the rate-limiting step in steroid production in adrenocortical cells. Hormones, such as ACTH and Ang II, rapidly stimulate this process. Transport occurs preferentially at sites of close contact between the OMM and IMM, and such sites are more plentiful in tubulovesicular than lamelliform mitochondria. The preponderance of tubulovesicular mitochondria in the zF compared to the zG reflects higher levels of steroidogenesis in the former [23].

StAR

The acute response to hormone stimulation is characterized by a rapid increase in the rate of steroid hormone biosynthesis, and classic studies indicated that the induction of steroidogenesis requires new protein synthesis (i.e., is cycloheximide-sensitive) [103, 104]. The acute steroidogenic response is controlled by StAR (StarD1), a rapidly synthesized and short-lived phosphoprotein [105, 106]. The principal function of StAR is to move cholesterol from the OMM to the IMM.

The expression and activation of StAR are tightly regulated by various signaling kinases, including PKA, protein kinase C (PKC), and extracellular signal-regulated kinases 1 and 2 (ERK1/2) [107, 108]. In response to hormonal stimulation, StAR is phosphorylated [109, 110], which enhances its activity [111]. Hormonal stimulation also modulates the activity of various transcription factors that regulate *StAR* expression [112, 113] (Fig. 2.11). For example, PKA-mediated activation of HSL leads to increased production of not only free cholesterol but also oxysterols, which activate liver X receptors (LXRs) that upregulate *StAR* transcription in a feed-forward manner for steroidogenesis [114, 115].

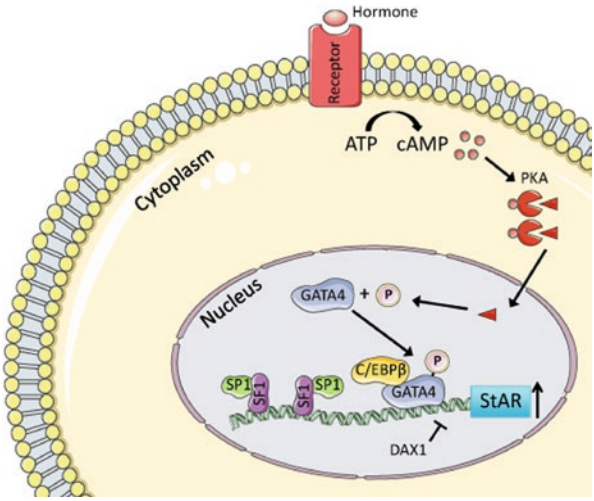

Fig. 2.11 Transcriptional regulation of *StAR* expression in steroidogenic cells. Shown is the regulation of *StAR* expression in testicular Leydig cells; similar mechanisms operate in fetal and adult adrenocortical cells. Hormonal stimulation leads to increases in the level of cAMP. Binding of cAMP to the regulatory subunit of PKA (PRKAR1A) allows dissociation of the catalytic subunit (triangle) and its translocation to the nucleus, where it phosphorylates target transcription factors, including GATA4. SF1 is essential for basal StAR expression and binds another widely expressed transcription factor, Sp1. CCAAT-enhancer-binding protein (C/EBPβ) acts synergistically with GATA4. DAX1 functions as a repressor. Prepared using image vectors from Servier Medical Art (www.servier.com), licensed under the Creative Commons Attribution 3.0 Unported License (http://creativecommons.org/license/by/3.0/)

The *StAR* gene encodes a 37 kDa "precursor" protein that harbors a mitochondrial targeting sequence [108, 116]. When StAR engages the OMM, portions of the molecule become membrane-associated, and StAR undergoes a conformational change that allows it to bind and release cholesterol [117]. As StAR is imported into the mitochondria, it undergoes proteolytic processing from a 37 kDa to a "mature" 30 kDa form [3]. This 30 kDa form localizes to the mitochondrial matrix and retains full StAR activity. Each molecule of StAR is thought to recirculate across the mitochondrial membranes many times, delivering up to 400 molecules of cholesterol to the IMM [118]. To prevent mitochondrial impairment from excess StAR accumulation, the 30 kDa form of the molecule is eventually degraded by proteases in the mitochondrial matrix [119]. Acute mitochondrial accumulation of StAR provokes a retrograde mitochondrial-nuclear signaling and transcriptional upregulation of the proteases involved in the degradation of StAR [119].

The importance of StAR for steroidogenesis is underscored by the disease lipoid congenital adrenal hyperplasia (lipoid CAH), an autosomal recessive disorder caused by loss-of-function mutations in *StAR* [120, 121]. Lipoid CAH is characterized by enlarged, lipid-laden adrenal glands that make minimal steroids. The condition is lethal in the neonatal period unless promptly diagnosed and treated with corticosteroids. Targeted ablation of the *Star* gene in mice recapitulates the phenotype of lipoid CAH in humans [122–124].

StAR's Entourage

StAR interacts with a variety of membrane-associated proteins. It has been proposed that these proteins are organized into a dynamic complex termed the transduceosome, which functions to translocate cholesterol efficiently to the IMM [125]. In addition to StAR, the transduceosome contains VDAC, the translocator protein (TSPO), a TSPO-associated protein PAP7, and other proteins [126]. In steroidogenic tissues that do not express StAR, such as the placenta, the remaining components of the transduceosome, working in conjunction with StarD3, may provide basal cholesterol transport to the IMM for production of pregnenolone.

Voltage-Dependent Anion Channel

VDAC is an OMM channel-forming protein that regulates the passage of ions and small molecules through the OMM. This function determines membrane potential, thereby regulating cell metabolism [127]. There are three isoforms of VDAC, termed VDAC-1, VDAC-2, and VDAC-3 [95]. In addition to controlling metabolism, VDAC isoforms influence programmed cell death by facilitating the release of apoptotic proteins located in the intermembranous space and serving as the proposed target of pro- and anti-apoptotic members of the BCL2 family [127].

VDAC, acting in conjunction with the σ-1 receptor in the MAM, helps facilitate cholesterol transfer for the initiation of pregnenolone synthesis [101]. VDAC interacts with the 37 kDa StAR precursor at the OMM and controls proteolytic processing, allowing the translocation of StAR into mitochondria as a mature 30 kDa protein [96, 128].

Translocator Protein: Necessity is the Mother of Contention

Translocator protein (TSPO), also known as the peripheral benzodiazepine receptor (PBR), is a highly conserved OMM protein [129]. *TSPO* is expressed in most mammalian tissues and found at highest levels in steroid-producing cells. Early evidence suggested that TSPO supports steroidogenesis by modulating cholesterol transport into mitochondria. The TSPO/PBR ligand PK11195 was shown to stimulate steroidogenesis in both adrenocortical and Leydig cell lines [130]. Mutagenesis and modeling studies indicated that TSPO could bind cholesterol with high affinity [131, 132]. TSPO and StAR were shown to be closely associated in fluorescence energy transfer experiments [133].

Recent experiments, however, have challenged the necessity of TSPO for steroidogenesis, inciting contentious debate [95, 129, 134–136]. Gene targeting studies in cell lines and mice have yielded conflicting results, ranging from no effect on

steroidogenesis to impairment of ACTH-stimulated glucocorticoid production [135, 137–140]. The precise function of TSPO remains an area of active investigation [141].

PAP7/ACBD3

PBR-associated protein 7 (PAP7) was identified in a yeast-two hybrid screen employing TSPO/PBR as bait [142]. In steroidogenic cells, PAP7 binds TSPO and the PKA regulatory subunit 1α (PKAR1A) [142, 143]. The PKA holoenzyme comprises two regulatory subunits and two catalytic subunits. Binding of cAMP to the regulatory subunits of PKA triggers the release of catalytic subunits, thereby activating the enzyme. PAP7 has been proposed to function as a kinase-anchoring protein that recruits and confines the PKA holoenzyme at key intracellular sites. A rise in cAMP leads to local release of the catalytic subunits, which phosphorylate StAR present at the OMM [144]. In addition to binding PKAR1A, PAP7 contains an acyl-coenzyme A (CoA)-binding domain, prompting its renaming to acyl-CoA-binding domain containing protein 3 (ACBD3).

Regulation of Cortisol Secretion

Glucocorticoids are essential for basal homeostasis and the response to stress, so production of cortisol is tightly regulated by HPA axis and locally acting factors.

ACTH Synthesis and Release

ACTH, a 39-amino acid peptide secreted by the anterior pituitary, is the principal stimulus for cortisol production [24]. When the HPA axis is activated by stress or other stimuli, neurons in the hypothalamic paraventricular nucleus secrete CRH and arginine vasopressin (AVP) into the hypophyseal portal circulation [145, 146]. CRH is the main stimulant of ACTH release. AVP acts synergistically with CRH to enhance ACTH secretion, although AVP is ineffective in the absence of CRH [147].

ACTH is derived from pro-opiomelanocortin (POMC), a 214-amino acid precursor synthesized in the anterior pituitary and limited other sites [148]. POMC can be proteolytically processed to generate ACTH and several other peptides, including α-melanocyte-stimulating hormone (α-MSH) and β-endorphin [149]. The N-terminal 18 amino acids of ACTH confer its biological activity, so shorter synthetic peptides [ACTH (1–24) and ACTH (1–18)] are used clinically in lieu of

full-length ACTH. The sequence of α-MSH, which is contained within the ACTH peptide, stimulates the production of melanin by melanocytes, resulting in skin hyperpigmentation when secreted in excess, as in the ACTH-dependent Cushing syndrome.

ACTH promotes steroidogenesis at three levels: (1) acute ACTH exposure rapidly (within minutes) stimulates the transport of cholesterol from the OMM to the IMM, (2) longer term exposure to ACTH (hours) promotes transcription of genes encoding steroidogenic enzymes, notably *CYP11A1*, and (3) prolonged exposure to ACTH (weeks to months) promotes zonal expansion and adrenal growth [126, 150].

Feedback Inhibition of the HPA Axis

Cortisol is the main negative regulator of the HPA axis (Fig. 2.6). Feedback inhibition by cortisol is mediated via binding to GRs in the pituitary and hypothalamus. Cortisol also acts on suprahypothalamic centers (e.g., hippocampus) to further limit the secretion of CRH [151]. Feedback inhibition in suprahypothalamic centers is mediated by cortisol binding to GRs and MRs [152].

Circadian Rhythm of Glucocorticoid Secretion

ACTH is secreted in regular pulses of varying amplitude. Peak ACTH secretion occurs in the early morning, coinciding with a rise in cortisol secretion [153]. The diurnal production of cortisol, which anticipates times of increased activity and energy demand, reflects an interplay between environmental cues (e.g., fluctuations in light intensity) and an internal circadian timekeeping system [154]. Disruption of this interplay contributes to the phenomenon of jetlag [155].

The circadian timekeeping system is composed of a central clock [the hypothalamic suprachiasmatic nucleus (SCN)] and subsidiary peripheral clocks in nearly every cell type, including adrenocortical cells [154]. The SCN is entrained to the light-dark cycle [156]. Highlighting the importance of the central clock, the diurnal glucocorticoid rhythm is altered by lesions of the SCN [157] or constant light exposure [158]. Signals from the SCN are relayed via the sympathetic nervous system to peripheral clocks in the adrenal cortex (Fig. 2.12). The rhythmic secretion of glucocorticoids from the adrenal gland is thought to synchronize peripheral circadian rhythms of tissues downstream of the SCN [155, 159].

The central and peripheral circadian clocks are composed of interlocking positive and negative transcriptional-translational feedback loops that oscillate with a periodicity of approximately 24 hours [154]. Genetically-engineered mice lacking of key components of the molecular clock exhibit either chronic elevation or

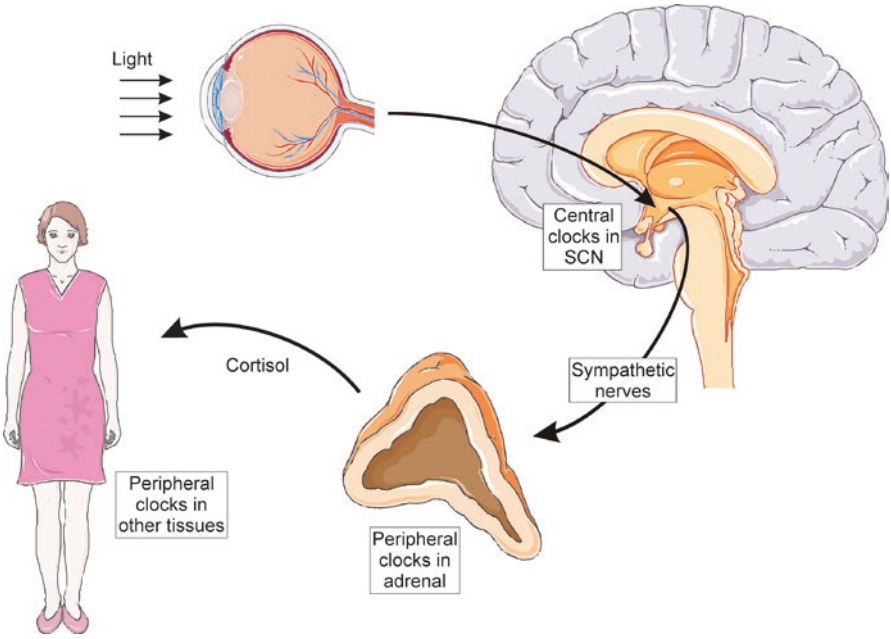

Fig. 2.12 Clock regulation of glucocorticoid secretion. Light received at the retina entrains central circadian clocks in the suprachiasmatic nucleus (SCN). Signals from the SCN are relayed via the sympathetic nervous system to peripheral clocks in the adrenal cortex. The rhythmic secretion of cortisol from the adrenal gland helps synchronize peripheral circadian rhythms of tissues downstream of the SCN. Prepared using image vectors from Servier Medical Art (www.servier.com), licensed under the Creative Commons Attribution 3.0 Unported License (http://creativecommons.org/license/by/3.0/)

suppression of glucocorticoid levels; rhythmic expression of certain steroidogenic genes, including *Star*, is altered in the adrenocortical cells of these animals [160–165]. Peripheral clocks in the adrenal cortex are thought to modulate sensitivity to ACTH, although the mechanistic basis for this effect is unclear.

MC2R and MRAPs

The trophic effects of ACTH are mediated through its plasma transmembrane G-protein-coupled receptor MC2R (Fig. 2.13). Melanocortin 2 receptor accessory proteins (MRAPs) are small membrane proteins that are essential for ACTH signaling. MRAP deficiency is one of the causes of hereditary unresponsiveness to ACTH [166]. Two types of MRAPs have been identified: MRAP1 and MRAP2. In humans, there are two distinct isoforms of MRAP1, termed MRAPα and MRAPβ, which share the same N-terminus and transmembrane domain but differ in their C-terminal

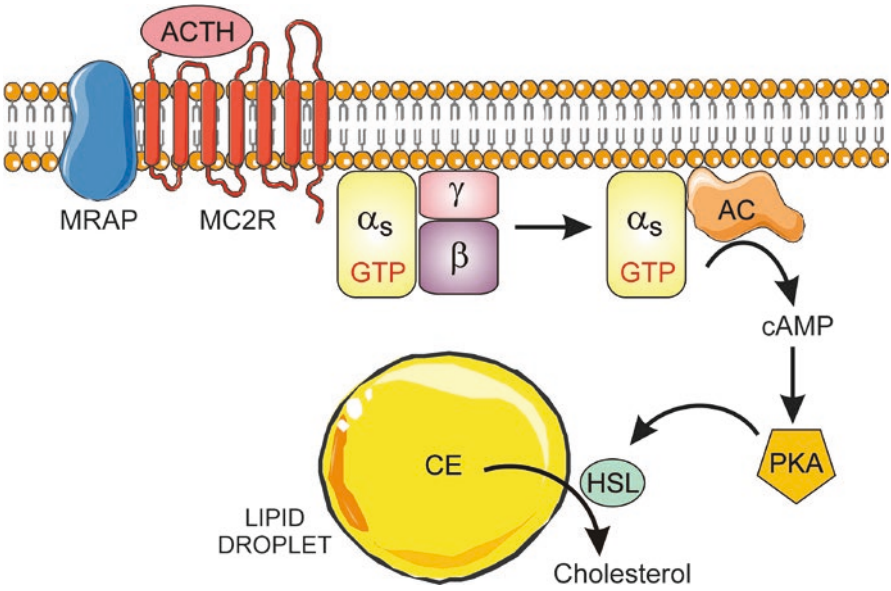

Fig. 2.13 ACTH-stimulated mobilization of cholesterol from lipid droplets. ACTH binds to its receptor MC2R. The ligand-bound receptor, in conjunction with the accessory protein MRAP, triggers the activation of AC via the heterotrimeric G-protein G_s. The resultant increase in cAMP activates PKA, leading to phosphorylation of HSL. The latter enzyme hydrolyzes CE in lipid droplets, producing free cholesterol for steroidogenesis. *AC* adenylate cyclase, *CE* cholesteryl ester, *HSL* hormone-sensitive lipase, *MC2R* melanocortin 2 receptor, *MRAP* melanocortin 2 receptor accessory protein, *PKA* protein kinase A. Prepared using image vectors from Servier Medical Art (www.servier.com), licensed under the Creative Commons Attribution 3.0 Unported License (http://creativecommons.org/license/by/3.0/)

domains. MRAP1 isoforms are involved in intracellular trafficking and signaling of MC2R. MRAPα has been implicated in targeting of MC2R to the plasma membrane, whereas MRAPβ appears to enhance cAMP production by ACTH-bound MC2R [153]. MRAP2 also is involved in trafficking of MC2R to the plasma membrane.

Activation of cAMP Signaling

Binding of ACTH to MC2R causes the α-subunit of the stimulatory heterotrimeric G-protein G_s to associate with adenylate cyclase (AC), resulting in cAMP production [167]. This rise in cAMP enhances lipid synthesis, protein synthesis, and protein phosphorylation, all of which promote cholesterol trafficking to mitochondria and the production of steroid hormones [167]. Most of these effects are mediated by PKA. cAMP binds to the regulatory subunits of PKA, allowing the catalytic subunits of PKA to phosphorylate downstream effectors. For example, PKA-induced

phosphorylation and activation of HSL enhances free cholesterol formation, while phosphorylation of StAR promotes transport of cholesterol from the OMM to the IMM [111]. PKA-dependent phosphorylation enhances CE uptake by enhancing the transcription and stability of SR-B1 [70]. PKA also phosphorylates transcription factors, leading to enhanced expression of steroidogenic genes such as CYP11A1 [167]. The transcription factors phosphorylated include steroidogenic factor 1 (SF1), cAMP response element-binding protein (CREB), and activator protein 1 (AP1) [150]. PKA-mediated phosphorylation of L-type Ca^{2+} channels activates a slow but sustained influx of Ca^{2+} that triggers additional signaling pathways.

Loss-of-function mutations in the protein kinase A regulatory subunit gene (*PRKAR1A*) cause excessive cAMP production. Such mutations underlie Carney complex, a syndrome associated with the pituitary-independent Cushing syndrome and adrenocortical neoplasia. Conditional deletion of *Prkar1a* in the adrenal cortex of mice leads to not only excess glucocorticoid production but also impaired apoptosis [168], mediated in part by crosstalk between the PKA and mammalian target of rapamycin (mTOR) pathways [169].

Somatic gain-of-function mutations in the *PRKACA* gene, which encodes the catalytic subunit of PKA, cause the ACTH-independent Cushing syndrome due to cortisol-producing adenomas [170, 171]. Germline duplications of this gene can cause bilateral adrenal hyperplasia [170].

Although the initial and most significant actions of ACTH are mediated through cAMP and the subsequent activation of PKA, there are also PKA-independent effects of cAMP, such as those mediated by Epac (the Exchange protein directly activated by cAMP) [172, 173]. One Epac isoform, Epac2, is highly expressed in the adrenal cortex and functions to activate Rap GTPases [174]. Epac proteins regulate various cellular processes via mechanisms that include modulation of gene expression and cytoskeletal rearrangements [153, 173, 174]. Treatment with an Epac-selective analogue of cAMP is sufficient to stimulate the expression of steroidogenic enzymes and cortisol secretion [175].

Phosphodiesterases

The level of intracellular cAMP is determined not only by its rate of synthesis by AC but also its rate of degradation by phosphodiesterases (PDEs) [176]. In mammals there are 11 families of PDEs, each with distinctive properties [177]. In mice, treatment with a PDE-selective inhibitor increases phosphorylation of HSL and basal corticosterone secretion [178]. Loss-of-function mutations in *PDE8B* or *PDE11A* have been linked to bilateral adrenal hyperplasia and Cushing syndrome in humans [176, 178].

Hydrolysis of cAMP by PDEs produces AMP, which in turn stimulates the AMP-activated protein kinase (AMPK) [179]. Activated AMPK represses the expression of transcription factors known to stimulate steroidogenesis (e.g., NUR77 and cJUN) and activates the expression of repressors of steroidogenesis (e.g., DAX1 and cFOS) [180] (Fig. 2.14).

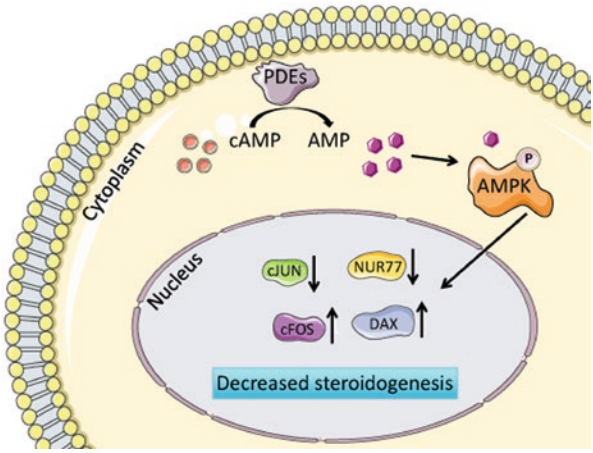

Fig. 2.14 Termination of cAMP signaling. Hydrolysis of cAMP by PDEs produces AMP, which in turn stimulates AMPK. Activated AMPK represses the expression of transcription factors known to stimulate steroidogenesis (e.g., NUR77 and cJUN) and activates the expression of repressors of steroidogenesis (e.g., DAX1 and cFOS). Prepared using image vectors from Servier Medical Art (www.servier.com), licensed under the Creative Commons Attribution 3.0 Unported License (http://creativecommons.org/license/by/3.0/)

Interplay of cAMP Signaling and Arachidonic Acid Metabolism

ACTH stimulation induces the release of arachidonic acid (AA) from phospholipid stores, and this fatty acid and its metabolites have been shown to enhance steroidogenesis [181, 182]. Intracellular levels of free AA are tightly regulated by not only phospholipases, such as phospholipase A_2 (PLA_2), but also by acyl-CoA synthetases and thioesterases. Most acyl-CoA synthetases exhibit a broad specificity with respect to fatty acid substrate, but acyl-CoA synthetase 4 (ACSL4) prefers AA as a substrate [183, 184]. Originally characterized as an activity in platelets [185], ACSL4 is highly expressed in steroidogenic tissues including the adrenal gland and gonads [183, 186]. The expression of *Acsl4* mRNA is upregulated through a cAMP-dependent pathway, and pharmacological inhibition of ACSL4 reduces steroidogenesis in adrenocortical cell lines [187]. The hormonal regulation of steroid synthesis requires the concerted action of ACS4 and a second enzyme, mitochondrial acyl-CoA thioesterase 2 (ACOT2), which cleaves arachidonoyl-CoA into its component parts. These two enzymes constitute an AA generation/export system, which releases AA in mitochondria in response to ACTH stimulation [188]. AA is then metabolized into lipoxygenated or epoxygenated products that induce the expression of *StAR* [182, 187, 188].

Other Signaling Pathways Activated by ACTH

ACTH triggers a transient increase in total protein tyrosine phosphatase (PTP) activity in zF cells and a concomitant decrease in the phosphotyrosine level of several proteins [189]. Treatment with PTP inhibitors reduces hormone-induced

stimulation of steroidogenesis [153], and PTPs have been implicated in the regulation of StAR induction and cholesterol transport to the IMM [189]. One particular PTP, PTPN11, has been shown to regulate the expression of *ACSL4* [190].

MAPKs have also been implicated in the action of ACTH [24]. This kinase family comprises the extracellular signal-related kinases ERK1/2 (p42/p44^{mapk}), the p38 MAPKs, and the p54 stress-activated JNK protein kinases [191]. MAPK targets in steroidogenic cells include HSL [76] and CYP17A1 (see later).

JAK/STAT (Janus kinase/signal transducer and activator of transcription) signaling is also activated by ACTH. Pharmacological or siRNA-mediated inhibition of JAK2 impairs ACTH-induced steroidogenesis [192]. Nuclear JAK2 regulates the amount of active CREB through tyrosine phosphorylation and prevention of proteasomal degradation, which in turn leads to transcriptional upregulation of *StAR* [192].

ACTH-Independent Mechanisms Regulating Cortisol Secretion

Although ACTH is the principal stimulus for glucocorticoid secretion, there are several ACTH-independent mechanisms that regulate cortisol release from the adrenal cortex. A variety of neuropeptides, neurotransmitters, and cytokines bind receptors on the surface of zF cells and modulate glucocorticoid secretion [193]. Additionally, factors secreted by endothelial cells and adipocytes influence the secretion of adrenal steroids [194, 195]. The close anatomical relationship among adrenocortical cells, medullary chromaffin cells, and nerve endings facilitates paracrine modulation of adrenal secretion of glucocorticoids [193].

Regulation of Aldosterone Secretion

The zG is optimized for the synthesis of aldosterone. Cells in this zone express *CYP11B2* (aldosterone synthase) but not *CYP17A1*, the enzyme that directs steroid substrates toward cortisol and androgen synthesis. The principal controllers of aldosterone production are Ang II and extracellular K^+, although ACTH also plays a role [17]. Ang II and extracellular K^+ act mainly by generating a cytosolic Ca^{2+} signal [196]. The signaling pathways and effectors employed include phospholipase C (PLC), inositol 1,4,5-trisphosphate (IP_3), Ca^{2+}/calmodulin-dependent protein kinases (CaMKs), diacylglycerol (DAG), PKC, MAPKs, tyrosine kinases, and PKA. Stimulation of these signaling pathways leads to the direct activation of several transcription factors. This, in turn, increases the expression of steroidogenic genes, including *CYP11B2*.

ACTH Signaling in the zG

In addition to regulating cortisol secretion in zF cells, ACTH can induce aldosterone production in the zG. As in the zF, ACTH acts on zG cells by binding to its receptor, MCR2, and activating AC via G_s [17]. This leads to increased cAMP/PKA

signaling, which facilitates the movement of cholesterol from the OMM to the IMM and activates transcription of key steroidogenic genes. Binding of ACTH to its receptor also impacts the electrical properties of zG cells [24].

Ang II Signaling

The zG controls extracellular fluid volume and salt balance as part of the renin-angiotensin-aldosterone system [17, 23]. Renin is a protease secreted by the juxtaglomerular apparatus of the kidney in response to extracellular fluid depletion, low sodium concentrations, or hypotension. Renin cleaves angiotensinogen, a glycoprotein constitutively secreted into the serum by the liver, yielding the decapeptide angiotensin I (Ang I). Angiotensin-converting enzyme (ACE), a protein expressed on the surface of pulmonary and renal endothelial cells, subsequently converts Ang I into to the vasoactive octapeptide Ang II.

The binding of Ang II to AT_1R induces coupling to $G_{\alpha q}$, resulting in activation of PLC [153, 196] (Fig. 2.15). The latter hydrolyzes phosphatidylinositol-4,5-bisphosphate

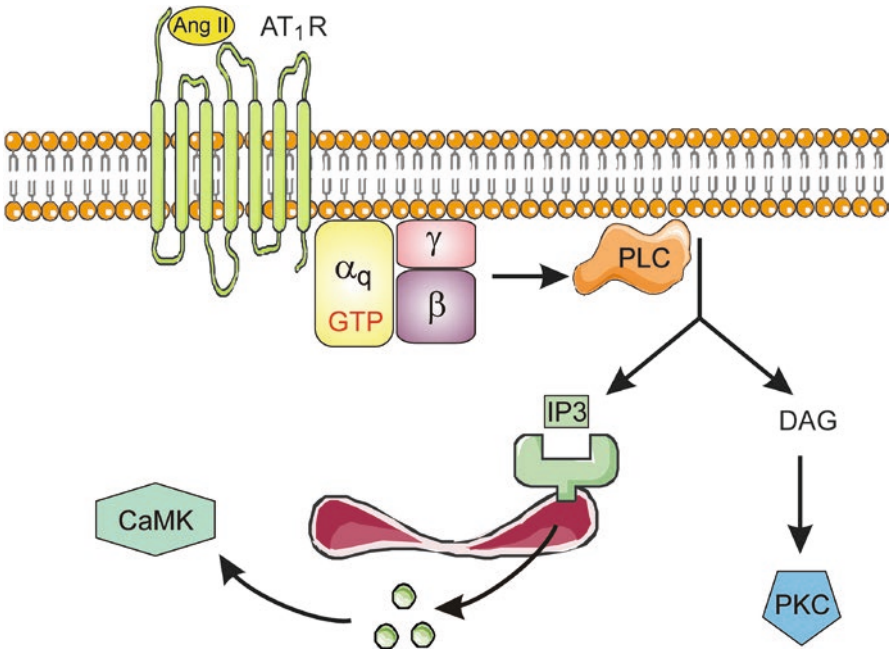

Fig. 2.15 Signaling pathways regulated by Ang II. Ang II binds to AT1R on zG cells and activates PLC via the heterotrimeric G-protein Gq, leading to the production of the second messengers IP_3 and DAG. IP_3 binds to its receptor and releases Ca^{2+} from intracellular stores into the cytoplasm. This triggers activation of CaMKs. DAG activates PKC. *Ang II* angiotensin II; *AT_1R* angiotensin II receptor, type 1; *CaMK* Ca^{2+}/calmodulin-dependent protein kinase; *DAG* diacylglycerol; IP_3, inositol (1,4,5) trisphosphate; *PKC* protein kinase C; *PLC* phospholipase C. Prepared using image vectors from Servier Medical Art (www.servier.com), licensed under the Creative Commons Attribution 3.0 Unported License (http://creativecommons.org/license/by/3.0/)

[PI(4,5)P_2] to produce the second messengers IP_3 and DAG. IP_3 binds to its receptor and mobilizes Ca^{2+} from intracellular depots into the cytoplasm. This leads to activation of CaMKs. DAG binds to PKC, resulting in the phosphorylation and activation of additional second messenger cascades that modulate the activity of transcription factors involved in aldosterone production [24, 197].

The binding of Ang II to its receptor also inhibits K^+ channels (Fig. 2.16). This inhibition causes cell depolarization, leading to the opening of voltage-gated Ca^{2+} channels. The ensuing increase in cytoplasmic Ca^{2+} activates CaMKs, which drive cell proliferation and the expression of *CYP11B2*, the enzyme that catalyzes the last steps of aldosterone synthesis [197]. In a fashion analogous to PKA in zF cells, the Ca^{2+} signal in zG cells leads to activation of HSL and StAR (via ERK1/2 or CaMK) [76, 198]. Additionally, the cytoplasmic Ca^{2+} signal is transferred to the mitochondrial matrix, where it enhances reduction of NAD^+ and $NADP^+$ [196].

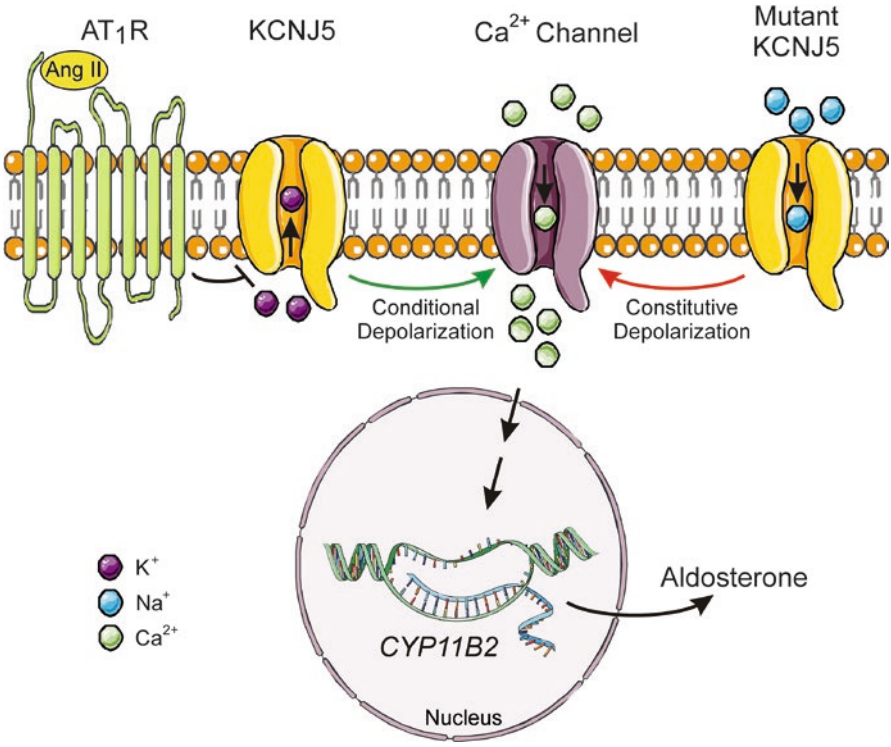

Fig. 2.16 Regulation of aldosterone production by voltage-gated channels in normal and pathological states. Ang II binds to its receptor (AT_1R) on zG cells and decreases efflux of K^+, thereby conditionally depolarizing the cell and increasing the entry of Ca^{2+} into the cytoplasm. This increase in cytoplasmic Ca^{2+} stimulates cell proliferation and transcription of CYP11B2 via CaMK and related signaling pathways, resulting in increased aldosterone production. *KCNJ5* gene mutations associated with familial hyperaldosteronism type 3 result in unselective potassium Kir 3.4 channels. This loss of selectivity causes constitutive cell depolarization, uncontrolled cell proliferation, and excessive aldosterone secretion. Prepared using image vectors from Servier Medical Art (www.servier.com), licensed under the Creative Commons Attribution 3.0 Unported License (http://creativecommons.org/license/by/3.0/)

The resultant increases in NADH and NADPH enhance ATP production and steroidogenesis, respectively.

The clinical consequences of excessive Ca^{2+} signaling are evident in familial hyperaldosteronism type 3 (FH3), a disease caused by mutations in *KCNJ5* [199]. These mutations perturb the selectivity filter of the inward rectifying potassium channel (Kir3.4) and allow Na^+ entry into the cell, resulting in constitutive membrane depolarization, Ca^{2+} influx, and enhanced aldosterone production (Fig. 2.16) [200].

Stimulation of Aldosterone Secretion by Extracellular K^+

Small changes in extracellular K^+ elicit changes in the plasma membrane potential of zG cells [201]. Elevated concentrations of extracellular K^+ depolarize the plasma membrane and activate voltage-dependent Ca^{2+} channels. The resulting increase in cytosolic Ca^{2+} triggers aldosterone production by the mechanisms detailed above. Aldosterone secretion increases linearly as the K^+ concentration exceeds 3.5 meq/L [202].

Other Signaling Pathways Activated by Ang II or K^+

Ang II also activates phospholipase D, which hydrolyzes phosphatidylcholine into phosphatidic acid, which can be metabolized to DAG by lipid phosphate phosphatases [153]. DAG activates PKC isoforms, which in turn activate ERK1/2. Ang II can activate PLA_2 to generate AA. Binding of Ang II to AT_1R can induce activation of the JAK/STAT pathway and receptor tyrosine kinases such as the EGF receptor [17, 153, 196].

Regulation of C_{19} Steroid Secretion

Spectrum of C_{19} Steroids Produced by the Adrenal

The zR secretes a variety of C_{19} steroids including DHEA, DHEA-S, androstenedione, and 11β-hydroxyandrostenedione [28, 203, 204]. The vast majority of circulating DHEA and DHEA-S is derived from the adrenal glands, and about half of circulating androstenedione is adrenal in origin, the balance arising from gonads [28]. To efficiently produce these C_{19} steroids, cells in the zR exhibit a specific biochemical profile: high CYP17A1 17,20 lyase activity (owing in part to

high levels of the allosteric regulator CYB_5), low HSD3B2 activity, and high SULT2A1 activity.

Most of the C_{19} steroids produced by the adrenal gland have little or no androgenic activity, but these compounds may be metabolized into more potent androgens and estrogens in peripheral tissues. For example DHEA and DHEA-S may be converted to androstenedione and then testosterone in tissues that express HSD3B1 and HSD17B1/5, such as adipose tissue or skin. The resultant testosterone can be metabolized further by 3-oxo-5α-steroid 4-dehydrogenase (SRD5A) to yield the highly potent androgen DHT or by aromatase to yield estradiol. The adrenal gland contributes ~1% to the total circulating testosterone in males and ~50% in females [205]. The peripheral conversion of adrenal C_{19} steroids to more potent androgens is of importance in the pathogenesis and treatment of castration-resistant prostate cancer [206].

Analysis of blood collected from adrenal veins has shown that the adrenal gland has the capacity to produce small quantities of more potent androgens such as testosterone and 11β-hydroxytestosterone [203]. The adrenal cortex lacks HSD17B3, the enzyme that converts androstenedione to testosterone in testicular Leydig cells. Instead, testosterone and 11β-hydroxytestosterone are synthesized in adrenal cells using another isoform of 17β-HSD termed AKR1C3 (HSD17B5). An expanded androgen biosynthetic pathway is diagrammed in Fig. 2.17. Metabolites in this expanded pathway may be useful for monitoring adrenal androgen production in certain disease states, such as 21-hydroxylase deficiency [207].

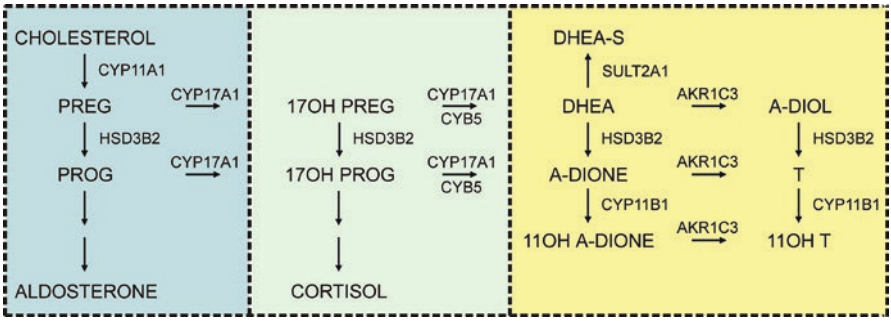

Fig. 2.17 Expanded view of the adrenal C_{19} steroid biosynthetic pathway. Adrenal vein sampling studies have shown that small quantities of potent androgens (T, 11OH T) are generated from C_{19} precursors. The enzymes responsible are highlighted in the yellow box. *11OH A-dione* 11β–hydroxyandrostenedione, *11OHT* 11β–hydroxytestosterone, *A-diol* androstenediol, *A-dione* androstenedione, *AKR1C3* 17β-hydroxysteroid dehydrogenase type 5, *CYB5* cytochrome b5, *CYP11A1* cytochrome P450 cholesterol side-chain cleavage, *CYP11B1* 11β-hydroxylase type 1, *DHEA* dehydroepiandrosterone, *DHEA-S* DHEA sulfate, *HSD3B2* 3β-hydroxysteroid dehydrogenase type 2, *SULT2A1* steroid sulfotransferase type 2A1, *T* testosterone

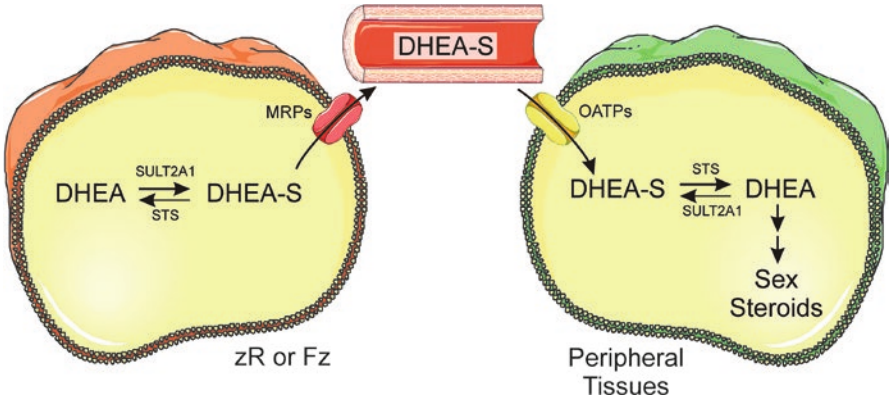

Fig. 2.18 Reversible sulfation allows DHEA, a hydrophobic steroid, to be transported to peripheral tissues where it can be metabolized into more potent androgenic and estrogenic steroids. After biosynthesis in the zR or Fz, DHEA is sulfated by SULT2A1 to facilitate transit in the circulation. Cellular efflux of DHEA-S occurs through MRPs, while OATPs allow import of DHEA-S into the target cell. Steroid sulfatase converts DHEA-S into DHEA. *MRPs* multidrug-resistant proteins, *OATPs* organic acid transport proteins, *STS* steroid sulfatase. Prepared using image vectors from Servier Medical Art (www.servier.com), licensed under the Creative Commons Attribution 3.0 Unported License (http://creativecommons.org/license/by/3.0/)

Reversible Sulfation of DHEA

Most of the DHEA produced by the adrenal is sulfated to increase water solubility and allow circulatory transport [208]. The sulfotransferase SULT2A1 converts DHEA to DHEA-S, an inactive steroid that serves as a reservoir for the peripheral formation of bioactive hormones. SULT2A1 requires the sulfate donor 3′-phosphoadenosine-5′-phosphosulfate (PAPS) for catalytic activity. In addition to facilitating circulatory transport of precursors, sulfation acts as a buffer to prevent adrenal androgen excess. Defects in DHEA-S sulfation caused by impaired synthesis of PAPS result in excessive amounts of DHEA, which is further converted to androstenedione and testosterone [209]. In affected females this manifests as premature pubarche and hyperandrogenic anovulation.

Cells can import DHEA-S via organic anion-transporting polypeptides for intracellular desulfation by steroid sulfatase (STS) and subsequent generation of androgenic and estrogenic compounds (Fig. 2.18) [208].

Role of ACTH in the Regulation of C_{19} Steroid Production

The regulation of adrenal C_{19} steroid production is not fully understood, but ACTH is thought to be the main stimulus for production of these compounds [203]. Dexamethasone suppression of ACTH levels decreases circulating adrenal C_{19} steroids. Like ACTH, adrenal C_{19} steroids exhibit a diurnal pattern of expression,

although the rhythmic fluctuation in DHEA-S is muted owing to its long half-life [210]. Further supporting a role for ACTH in the regulation of adrenal C_{19} steroid production, children with loss-of-function mutations in *MC2R* fail to experience adrenarche and the attendant increase in adrenal C_{19} steroids [211]. Clearly, however, signals other than ACTH influence adrenal C_{19} steroid production, as evidenced by the fact that in normal children DHEA-S levels increase at the time of adrenarche, whereas ACTH and cortisol do not. This age-related dissociation between circulating levels of cortisol and DHEA-S has spurred researchers to seek the still elusive adrenal androgen-stimulating hormone.

Modulation of CYP17A1 Activity

The 17,20-lyase activity of CYP17A1 is affected by phosphorylation of specific serine residues [204]. Dephosphorylation of CYP17A1 with protein phosphatase 2A decreases lyase activity and androgen synthesis [212]. Gene-silencing studies have shown that MAPK14 (p38a) is the kinase responsible for enhancing 17,20 lyase activity by phosphorylation [213]. Another kinase, ROCK1, appears to regulate MAPK signaling and target p38a for CYP17A1 phosphorylation [214].

Transcriptome profiling of adrenocortical zones has shown that bone morphogenetic protein 4 (BMP4) is differentially expressed between the zG and zR [215]. Cell culture studies suggest that BMP4 is an autocrine/paracrine negative regulator of C_{19} steroid synthesis and works by suppressing *CYP17A1* expression [215].

Other Factors Implicated in the Regulation of Androgen Production

When subjected to serum starvation, the human ACC cell line H295R adopts a hyperandrogenic phenotype, marked by increased production of DHEA, reduced HSD3B2 activity, increased CYP17A1 phosphorylation, and higher 17,20-lyase activity [216]. Transcriptional profiling of serum-starved H295R cells has implicated two additional factors, retinoic acid receptor-β (RARB) and angiopoietin-like protein 1 (ANGPTL1), in the regulation of androgen production [204]. The transcription factor RARB stimulates the *StAR*, *CYP17A1*, and *HSD3B2* promoters, while the secreted protein ANGPTL1 modulates CYP17A1 expression by inducing ERK1/2 phosphorylation.

Key Transcription Factors Involved in Steroidogenesis

Among the plethora of transcription factors implicated in the regulation of adrenal steroidogenesis, several play noteworthy roles.

SF1

SF1 (also called Ad4BP or NR5A1), the prototype of steroidogenic transcription factors, regulates a wide array of genes including enzymes involved in adrenal steroidogenesis [217, 218]. Enforced expression of SF1 in embryonic or mesenchymal stem cells is sufficient to activate steroidogenic gene expression [219, 220], and transgenic expression of *Sf1* in fetal adrenal progenitor cells leads to ectopic adrenal formation (Zubair et al. 2009).

Traditionally, SF1 has been classified as an orphan nuclear receptor, but studies have shown that certain lipids bind this transcription factor and regulate its activity [221]. For example, SF1 activity can be modulated by phosphorylation of the 3-position of the inositol head group of $PI(4,5)P_2$ while this phospholipid is bound to SF1 [222]. SF1 activity also is controlled by gene dosage, transcriptional regulation, posttranslational modification, and association with positive and negative cofactors [223–225].

Sf1$^{-/-}$ mice exhibit agenesis of both the adrenal glands and gonads [226]. Individuals with loss-of-function mutations in the DNA-binding domain of SF1 exhibit primary adrenal failure and gonadal dysgenesis. However, most loss-of-function mutations in human *SF1* are not associated with adrenal insufficiency but rather isolated XY gonadal dysgenesis. In addition to regulating steroidogenesis, SF1 has been implicated in the control of glycolysis, cell proliferation, cytoskeletal rearrangements, and apoptosis [217, 218].

DAX1

DAX1 (also called *NR0B1*) is an X-linked gene that encodes a repressor of steroidogenic gene expression [227]. DAX1 is an acronym for *D*osage-sensitive sex reversal, *A*drenal hypoplasia critical region on chromosome *X*. In response to ACTH, SF1-positive subcapsular progenitors downregulate *Dax1* and differentiate into corticoid-producing cells. DAX1 deficiency in humans and mice leads to excessive differentiation of subcapsular progenitors and eventual depletion of the stem/progenitor cell compartment [228, 229]. DAX1 deficiency typically affects boys and presents as primary adrenal insufficiency in early infancy or childhood, hypogonadotropic hypogonadism at puberty and impaired spermatogenesis [230]. Cytomegaly, a hallmark of adrenal dysfunction associated with *DAX1* deficiency [227, 228, 230, 231], is thought to be a compensatory response to a reduced number of cortical cells or to progenitor cell exhaustion [229].

CREB

CREB (cAMP response element-binding protein) is a transcription factor that binds to cAMP response elements (CREs) in the promoters or enhancers of genes [232]. CREB proteins are activated by phosphorylation on Ser142 by various kinases, including PKA and CaMKs. When activated, CREB recruits transcriptional coactivators,

including CREB-binding protein, thereby modulating gene expression [232]. CREB has been implicated in the regulation of multiple steroidogenic enzymes. CREB also plays a key role in the circadian clock; mutant mice lacking the Ser142 phosphorylation site in CREB have difficulty entraining to light-dark cycles [233].

GATA6

GATA6 is expressed in both the fetal and adult cortex [234–237]. GATA6 acts in synergy with SF1 and other transcription factors to enhance the expression of genes involved in adrenal steroidogenesis [237, 238]. In humans GATA6 is hypothesized to regulate the production of adrenal androgens and possibly glucocorticoids [234, 237, 239–241]. Heterozygous loss-of-function mutations in human *GATA6* have been linked to pancreatic agenesis, cardiac malformations, and biliary tract abnormalities, but not primary adrenocortical defects [242–244]. Targeted deletion of *Gata6* in $SF1^+$ cells of the mouse results in a thin adrenal cortex, cytomegaly, and blunted corticoid production [245]. *Gata4*/*Gata6* double-knockout mice generated with *Sf1*-cre exhibit severe adrenal hypoplasia. Female double-knockout mice die from adrenocortical insufficiency, whereas their male counterparts survive due to heterotopic corticoid production by cells in the testes [246–248].

Other Factors Involved in Steroidogenesis

Additional insights into the factors that regulate steroidogenesis have emerged from studies of patients with familial glucocorticoid deficiency (FGD) and other forms of congenital adrenal hypoplasia/insufficiency [166, 249, 250]. Such patients may be classified into two categories: those without extra-adrenal features (non-syndromic adrenal hypoplasia) and those with extra-adrenal features (syndromic adrenal hypoplasia). Causative genes for these two categories are shown in Table 2.4.

Table 2.4 Causes of primary adrenal hypoplasia/insufficiency that shed light on the regulation of steroidogenesis

Category	Disease	Gene	Reference
Non-syndromic (no extra-adrenal manifestations)	FGD	*MC2R*	[251]
	FGD	*MRAP*	[252]
	X-linked congenital adrenal hypoplasia	*DAX1 (NR0B1)*	[230]
	FGD	*NNT*	[253]
Syndromic (extra-adrenal manifestations)	FGD	*TXNRD2*	[254]
	AAA syndrome	*AAAS*	[255]
	IMAGe syndrome	*CDKN1C*	[256]
	Variant FGD	*MCM4*	[257]
	MIRAGE syndrome	*SAMD9*	[258]

Factors Affecting Redox Homeostasis

The adrenal cortex is particularly susceptible to oxidative stress, so inherited mutations that alter redox homeostasis often manifest clinically as adrenocortical insufficiency.

NNT

Reactive oxygen species (ROS), such as superoxide and hydrogen peroxide, are generated during steroidogenesis and oxidative phosphorylation. Detoxification of ROS in mitochondria depends on NADPH for regeneration of reduced glutathione (GSH) from oxidized glutathione (GSSG, glutathione disulfide). Nicotinamide nucleotide transhydrogenase (NNT) is a redox-driven H^+ pump of the IMM (Fig. 2.19). This enzyme uses energy from the mitochondrial H^+ gradient to reduce $NADP^+$ to NADPH [253]. Thus, NNT deficiency can negatively impact steroidogenesis in two ways: (1) by limiting production of NADPH, the electron donor for mitochondrial steroidogenic enzymes (CYP11A1, CYP11B1, CYP11B2), and (2) by allowing mitochondrial damage from excessive ROS. Loss-of-function mutations in *NNT* account for 5–10% of FGD patients [259]. Certain substrains of C57Bl/6 J mice, a widely used inbred line, harbor spontaneous *Nnt* mutations [260]. These mice exhibit higher levels of adrenocortical cell apoptosis and impaired glucocorticoid production. Consequently, experiments assessing adrenal steroidogenesis in C57Bl/6 J mice must be interpreted cautiously [261].

TXNRD2

The selenoprotein TXNRD2 is a mitochondrial thioredoxin reductase that contributes to redox homeostasis (Fig. 2.19). *TXNRD2* is highly expressed in the adrenal cortex, where it functions to limit oxidative stress by inactivating ROS. A homozygous loss-of-function mutation in the *TXNRD2* gene has been reported in consanguineous family [254]. TXNRD2 deficiency appears to be associated with extra-adrenal manifestations. *Txnrd2* ablation causes fatal cardiac and hematopoietic defects in mice [262, 263], and two novel heterozygous mutations in *TXNRD2* were identified in 3 of 227 patients with a diagnosis of dilated cardiomyopathy [264].

ALADIN

AAA syndrome, characterized by *A*drenal insufficiency, *A*lacrima, *A*chalasia, and a progressive neurological disorder, is caused by recessive mutations in *AAAS*. This gene encodes ALADIN, a nuclear pore complex gene [265]. Pathogenic mutations sequester ALADIN in the cytoplasm. Silencing of the *AAAS* gene in H295R ACC cells impairs redox homeostasis and inhibits steroidogenesis [255]. It is hypothesized that defective import of specific nuclear proteins allows oxidative damage in the adrenal glands and other tissues.

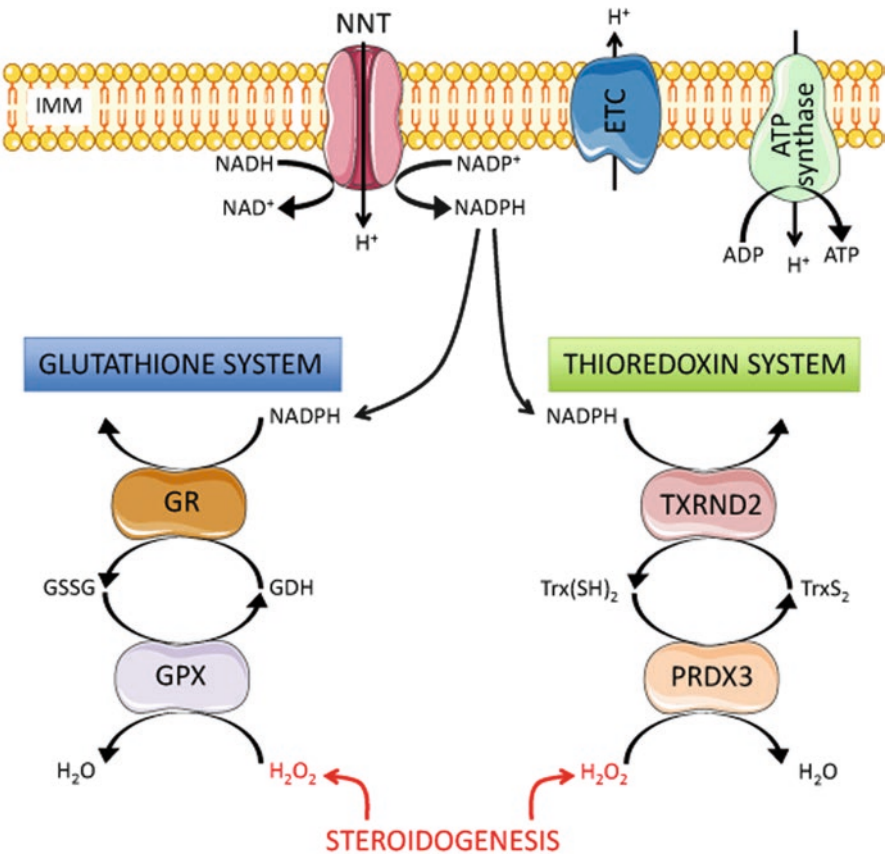

Fig. 2.19 Roles of NNT and TXRNR2 in steroidogenesis. NNT generates NADPH, the electron donor for the mitochondrial steroidogenic enzymes CYP11A1, CYP11B1, and CYP11B2. NNT and TXRND2 function to detoxify ROS that are the by-products of steroidogenesis and oxidative phosphorylation. *ETC* electron transport chain, *GSSG* glutathione disulfide, *GSH* glutathione, *GPX* glutathione peroxidase, *GR* glutathione reductase, *IMM* inner mitochondrial membrane, *TXNRD2* thioredoxin reductase 2, *PRDX3* peroxiredoxin 3. Prepared using image vectors from Servier Medical Art (www.servier.com), licensed under the Creative Commons Attribution 3.0 Unported License (http://creativecommons.org/license/by/3.0/)

Factors Affecting Adrenocortical Cell Growth

CDKN1C ($p57^{Kip2}$)

Heterozygous gain-of-function mutations in *CDKN1C*, which is paternally imprinted and encodes the cell cycle regulator $p57^{Kip2}$, cause IMAGe syndrome [256]. The hallmarks of IMAGe syndrome are *I*ntrauterine growth retardation, *M*etaphyseal dysplasia, *A*drenal hypoplasia congenita, and *G*enital abnormalities. *CDKN1C* is expressed in the adrenal cortex and is upregulated by ACTH treatment,

suggesting that its normal function in the adrenal cortex may be to limit cell proliferation. Supporting this premise, loss-of-function mutations in *CDKN1C* cause Beckwith-Wiedemann syndrome, an overgrowth syndrome associated with adrenal hyperplasia [266].

MCM4

Recessive mutations in the mini chromosome maintenance-deficient 4 (*MCM4*) homologue gene have been identified in a variant of FGD [257, 267]. Clinical manifestations of this FGD variant include short stature, chromosomal breakage, natural killer cell deficiency, and progressive adrenocortical insufficiency. The mechanistic basis for this loss in adrenal steroidogenic capacity is unclear. MCM4 is part of a DNA repair complex essential for DNA replication and genome stability in various cell types.

SAMD9

Sporadic heterozygous mutations in *SAMD9*, which encodes a facilitator of endosome fusion, cause MIRAGE syndrome. Clinical manifestations include *M*yelodysplasia, *I*nfection, *R*estriction of growth, *A*drenal hypoplasia, *G*enital phenotypes, and *E*nteropathy [258]. Expression of pathogenic SAMD9 variants in wild-type fibroblasts causes profound growth inhibition. Patient-derived fibroblasts exhibit restricted growth, increased size of early endosomes, and intracellular accumulation of giant vesicles carrying a late endosome marker. These abnormalities suggest that pathogenic *SAMD9* mutations enhance endosome fusion. Patient-derived fibroblasts have decreased plasma membrane expression of the EGF receptor, likely due to defective recycling of the receptor. The adrenal glands of affected individuals are small and disorganized, with foamy-appearing adrenocortical cells.

Acknowledgments We thank Karin Sanders, Sara Galac, and Audrey Odom John for the assistance with figure preparation. We thank Rebecca Cochran and Paul Hruz for reviewing the manuscript. This work was supported by the Sigrid Jusélius Foundation, the Academy of Finland, Department of Defense grants PC141008 and OC150105, Prostate Cancer Foundation, and the Paulo Foundation.

References

1. Miller WL, Auchus RJ. The molecular biology, biochemistry, and physiology of human steroidogenesis and its disorders. Endocr Rev. 2011;32:81–151.
2. Turcu AF, Auchus RJ. Adrenal steroidogenesis and congenital adrenal hyperplasia. Endocrinol Metab Clin N Am. 2015;44:275–96.
3. Miller WL. StAR search--what we know about how the steroidogenic acute regulatory protein mediates mitochondrial cholesterol import. Mol Endocrinol. 2007b;21:589–601.
4. Bornstein SR, Wilson DB. Anatomy of the adrenal cortex. In: Martini L, Huhtaniemi I, editors. Reference module in biomedical sciences. Oxford: Elsevier; 2015.

5. Vinson GP. Functional zonation of the adult mammalian adrenal cortex. Front Neurosci. 2016;10:238.
6. Monticone S, Auchus RJ, Rainey WE. Adrenal disorders in pregnancy. Nat Rev Endocrinol. 2012;8:668–78.
7. Goto M, Piper HK, Marcos J, Wood PJ, Wright S, Postle AD, Cameron IT, Mason JI, Wilson DI, Hanley NA. In humans, early cortisol biosynthesis provides a mechanism to safeguard female sexual development. J Clin Invest. 2006;116:953–60.
8. Mesiano S, Jaffe RB. Developmental and functional biology of the primate fetal adrenal cortex. Endocr Rev. 1997;18:378–403.
9. Quinn TA, Ratnayake U, Dickinson H, Castillo-Melendez M, Walker DW. The feto-placental unit, and potential roles of dehydroepiandrosterone (DHEA) in prenatal and postnatal brain development: a re-examination using the spiny mouse. J Steroid Biochem Mol Biol. 2016;160:204–13.
10. Peter M, Dorr HG, Sippell WG. Changes in the concentrations of dehydroepiandrosterone sulfate and estriol in maternal plasma during pregnancy: a longitudinal study in healthy women throughout gestation and at term. Horm Res. 1994;42:278–81.
11. Rainey WE, Rehman KS, Carr BR. The human fetal adrenal: making adrenal androgens for placental estrogens. Semin Reprod Med. 2004;22:327–36.
12. Sirianni R, Mayhew BA, Carr BR, Parker CR Jr, Rainey WE. Corticotropin-releasing hormone (CRH) and urocortin act through type 1 CRH receptors to stimulate dehydroepiandrosterone sulfate production in human fetal adrenal cells. J Clin Endocrinol Metab. 2005;90:5393–400.
13. Turcu A, Smith JM, Auchus R, Rainey WE. Adrenal androgens and androgen precursors-definition, synthesis, regulation and physiologic actions. Compr Physiol. 2014;4:1369–81.
14. Xing Y, Lerario AM, Rainey W, Hammer GD. Development of adrenal cortex zonation. Endocrinol Metab Clin N Am. 2015;44:243–74.
15. Ansurudeen I, Kopf PG, Gauthier KM, Bornstein SR, Cowley AW Jr, Campbell WB. Aldosterone secretagogues increase adrenal blood flow in male rats. Endocrinology. 2014;155:127–32.
16. Bassett JR, West SH. Vascularization of the adrenal cortex: its possible involvement in the regulation of steroid hormone release. Microsc Res Tech. 1997;36:546–57.
17. Bollag WB. Regulation of aldosterone synthesis and secretion. Compr Physiol. 2014;4:1017–55.
18. Cole TJ, Terella L, Morgan J, Alexiadis M, Yao YZ, Enriori P, Young MJ, Fuller PJ. Aldosterone-mediated renal sodium transport requires intact mineralocorticoid receptor DNA-binding in the mouse. Endocrinology. 2015;156:2958–68.
19. Brown NJ. Contribution of aldosterone to cardiovascular and renal inflammation and fibrosis. Nat Rev Nephrol. 2013;9:459–69.
20. Gomez-Sanchez CE. Non renal effects of aldosterone. Steroids. 2014a;91:1–2.
21. Pacurari M, Kafoury R, Tchounwou PB, Ndebele K. The renin-angiotensin-aldosterone system in vascular inflammation and remodeling. Int J Inflam. 2014;2014:689360.
22. Brown NJ. This is not Dr. Conn's aldosterone anymore. Trans Am Clin Climatol Assoc. 2011;122:229–43.
23. Yates R, Katugampola H, Cavlan D, Cogger K, Meimaridou E, Hughes C, Metherell L, Guasti L, King P. Adrenocortical development, maintenance, and disease. Curr Top Dev Biol. 2013;106:239–312.
24. Gallo-Payet N. 60 YEARS OF POMC: adrenal and extra-adrenal functions of ACTH. J Mol Endocrinol. 2016;56:T135–56.
25. Arlt W, Stewart PM. Adrenal corticosteroid biosynthesis, metabolism, and action. Endocrinol Metab Clin N Am. 2005;34:293–313.
26. Franchimont D. Overview of the actions of glucocorticoids on the immune response: a good model to characterize new pathways of immunosuppression for new treatment strategies. Ann N Y Acad Sci. 2004;1024:124–37.
27. Adams JB. Control of secretion and the function of C19-delta 5-steroids of the human adrenal gland. Mol Cell Endocrinol. 1985;41:1–17.

28. Davison SL, Bell R. Androgen physiology. Semin Reprod Med. 2006;24:71–7.
29. Rainey WE, Nakamura Y. Regulation of the adrenal androgen biosynthesis. J Steroid Biochem Mol Biol. 2007;108(3–5):281–6.
30. Beuschlein F, Galac S, Wilson DB. Animal models of adrenocortical tumorigenesis. Mol Cell Endocrinol. 2012;351:78–86.
31. Morohashi K, Zubair M. The fetal and adult adrenal cortex. Mol Cell Endocrinol. 2011;336:193–7.
32. Hershkovitz L, Beuschlein F, Klammer S, Krup M, Weinstein Y. Adrenal 20alpha-hydroxysteroid dehydrogenase in the mouse catabolizes progesterone and 11-deoxycorticosterone and is restricted to the X-zone. Endocrinology. 2007;148:976–88.
33. Guasti L, Cavlan D, Cogger K, Banu Z, Shakur A, Latif S, King PJ. Dlk1 up-regulates Gli1 expression in male rat adrenal capsule cells through the activation of beta1 integrin and ERK1/2. Endocrinology. 2013b;154:4675–84.
34. Galac S, Wilson DB. Animal models of adrenocortical tumorigenesis. Endocrinol Metab Clin N Am. 2015;44:297–310.
35. Quinn TA, Ratnayake U, Dickinson H, Nguyen TH, McIntosh M, Castillo-Melendez M, Conley AJ, Walker DW. Ontogeny of the adrenal gland in the spiny mouse, with particular reference to production of the steroids cortisol and dehydroepiandrosterone. Endocrinology. 2013;154:1190–201.
36. Pignatti E, Leng S, Carlone DL, Breault DT. Regulation of zonation and homeostasis in the adrenal cortex. Mol Cell Endocrinol. 2016;441:146–55.
37. Pihlajoki M, Dorner J, Cochran RS, Heikinheimo M, Wilson DB. Adrenocortical zonation, renewal, and remodeling. Front Endocrinol (Lausanne). 2015;6:27.
38. Walczak EM, Hammer GD. Regulation of the adrenocortical stem cell niche: implications for disease. Nat Rev Endocrinol. 2015;11:14–28.
39. Gallo-Payet N, Guillon G. Regulation of adrenocortical function by vasopressin. Horm Metab Res. 1998;30:360–7.
40. Pattison JC, Abbott DH, Saltzman W, Conley AJ, Bird IM. Plasticity of the zona reticularis in the adult marmoset adrenal cortex: voyages of discovery in the new world. J Endocrinol. 2009;203:313–26.
41. Topor LS, Asai M, Dunn J, Majzoub JA. Cortisol stimulates secretion of dehydroepiandrosterone in human adrenocortical cells through inhibition of 3betaHSD2. J Clin Endocrinol Metab. 2011;96:E31–9.
42. Thomas JL, Rajapaksha M, Mack VL, DeMars GA, Majzoub JA, Bose HS. Regulation of human 3beta-hydroxysteroid dehydrogenase type 2 by adrenal corticosteroids and product-feedback by androstenedione in human adrenarche. J Pharmacol Exp Ther. 2015;352:67–76.
43. Bernichtein S, Alevizaki M, Huhtaniemi I. Is the adrenal cortex a target for gonadotropins? Trends Endocrinol Metab. 2008;19:231–8.
44. Teo AE, Garg S, Shaikh LH, Zhou J, Karet Frankl FE, Gurnell M, Happerfield L, Marker A, Bienz M, Azizan EA, Brown MJ. Pregnancy, primary Aldosteronism, and adrenal CTNNB1 mutations. N Engl J Med. 2015;373:1429–36.
45. Beuschlein F, Looyenga BD, Bleasdale SE, Mutch C, Bavers DL, Parlow AF, Nilson JH, Hammer GD. Activin induces x-zone apoptosis that inhibits luteinizing hormone-dependent adrenocortical tumor formation in inhibin-deficient mice. Mol Cell Biol. 2003;23:3951–64.
46. Vanttinen T, Liu J, Kuulasmaa T, Kivinen P, Voutilainen R. Expression of activin/inhibin signaling components in the human adrenal gland and the effects of activins and inhibins on adrenocortical steroidogenesis and apoptosis. J Endocrinol. 2003;178:479–89.
47. Drelon C, Berthon A, Val P. Adrenocortical cancer and IGF2: is the game over or our experimental models limited? J Clin Endocrinol Metab. 2013;98:505–7.
48. Fottner C, Hoeflich A, Wolf E, Weber MM. Role of the insulin-like growth factor system in adrenocortical growth control and carcinogenesis. Horm Metab Res. 2004;36:397–405.
49. Crickard K, Ill CR, Jaffe RB. Control of proliferation of human fetal adrenal cells in vitro. J Clin Endocrinol Metab. 1981;53:790–6.

50. Guasti L, Candy Sze WC, McKay T, Grose R, King PJ. FGF signalling through Fgfr2 isoform IIIb regulates adrenal cortex development. Mol Cell Endocrinol. 2013a;371:182–8.
51. Finco I, LaPensee CR, Krill KT, Hammer GD. Hedgehog signaling and steroidogenesis. Annu Rev Physiol. 2015;77:105–29.
52. Drelon C, Berthon A, Mathieu M, Martinez A, Val P. Adrenal cortex tissue homeostasis and zonation: a WNT perspective. Mol Cell Endocrinol. 2015;408:156–64.
53. Heikkila M, Peltoketo H, Leppaluoto J, Ilves M, Vuolteenaho O, Vainio S. Wnt-4 deficiency alters mouse adrenal cortex function, reducing aldosterone production. Endocrinology. 2002;143:4358–65.
54. Vidal V, Sacco S, Rocha AS, da Silva F, Panzolini C, Dumontet T, Doan TM, Shan J, Rak-Raszewska A, Bird T, Vainio S, Martinez A, Schedl A. The adrenal capsule is a signaling center controlling cell renewal and zonation through Rspo3. Genes Dev. 2016;30:1389–94.
55. Burns MP, Rebeck GW. Intracellular cholesterol homeostasis and amyloid precursor protein processing. Biochim Biophys Acta. 2010;1801:853–9.
56. Horton JD, Goldstein JL, Brown MS. SREBPs: transcriptional mediators of lipid homeostasis. Cold Spring Harb Symp Quant Biol. 2002;67:491–8.
57. Goldstein JL, DeBose-Boyd RA, Brown MS. Protein sensors for membrane sterols. Cell. 2006;124:35–46.
58. Brown MS, Goldstein JL. A proteolytic pathway that controls the cholesterol content of membranes, cells, and blood. Proc Natl Acad Sci U S A. 1999;96:11041–8.
59. Braamskamp MJ, Kusters DM, Wiegman A, Avis HJ, Wijburg FA, Kastelein JJ, van Trotsenburg AS, Hutten BA. Gonadal steroids, gonadotropins and DHEAS in young adults with familial hypercholesterolemia who had initiated statin therapy in childhood. Atherosclerosis. 2015;241:427–32.
60. Laue L, Hoeg JM, Barnes K, Loriaux DL, Chrousos GP. The effect of mevinolin on steroidogenesis in patients with defects in the low density lipoprotein receptor pathway. J Clin Endocrinol Metab. 1987;64:531–5.
61. Miller WL. Disorders in the initial steps of steroid hormone synthesis. J Steroid Biochem Mol Biol. 2016;165(Pt A):18–37.
62. Capponi AM. Regulation of cholesterol supply for mineralocorticoid biosynthesis. Trends Endocrinol Metab. 2002;13:118–21.
63. Miller WL, Bose HS. Early steps in steroidogenesis: intracellular cholesterol trafficking. J Lipid Res. 2011;52:2111–35.
64. Burton BK, Balwani M, Feillet F, Baric I, Burrow TA, Camarena Grande C, Coker M, Consuelo-Sanchez A, Deegan P, Di Rocco M, Enns GM, Erbe R, Ezgu F, Ficicioglu C, Furuya KN, Kane J, Laukaitis C, Mengel E, Neilan EG, Nightingale S, Peters H, Scarpa M, Schwab KO, Smolka V, Valayannopoulos V, Wood M, Goodman Z, Yang Y, Eckert S, Rojas-Caro S, Quinn AG. A phase 3 trial of Sebelipase Alfa in Lysosomal acid lipase deficiency. N Engl J Med. 2015;373:1010–20.
65. Peake KB, Vance JE. Defective cholesterol trafficking in Niemann-Pick C-deficient cells. FEBS Lett. 2010;584:2731–9.
66. Vanier MT. Complex lipid trafficking in Niemann-Pick disease type C. J Inherit Metab Dis. 2015;38:187–99.
67. Strauss JF 3rd, Kishida T, Christenson LK, Fujimoto T, Hiroi H. START domain proteins and the intracellular trafficking of cholesterol in steroidogenic cells. Mol Cell Endocrinol. 2003;202:59–65.
68. Iyer LM, Koonin EV, Aravind L. Adaptations of the helix-grip fold for ligand binding and catalysis in the START domain superfamily. Proteins. 2001;43:134–44.
69. Alpy F, Stoeckel ME, Dierich A, Escola JM, Wendling C, Chenard MP, Vanier MT, Gruenberg J, Tomasetto C, Rio MC. The steroidogenic acute regulatory protein homolog MLN64, a late endosomal cholesterol-binding protein. J Biol Chem. 2001;276:4261–9.
70. Shen WJ, Azhar S, Kraemer FB. ACTH regulation of adrenal SR-B1. Front Endocrinol (Lausanne). 2016;7:42.

71. Rone MB, Fan J, Papadopoulos V. Cholesterol transport in steroid biosynthesis: role of protein-protein interactions and implications in disease states. Biochim Biophys Acta. 2009;1791:646–58.
72. Connelly MA, Kellner-Weibel G, Rothblat GH, Williams DL. SR-BI-directed HDL-cholesteryl ester hydrolysis. J Lipid Res. 2003;44:331–41.
73. Kraemer FB, Shen WJ, Harada K, Patel S, Osuga J, Ishibashi S, Azhar S. Hormone-sensitive lipase is required for high-density lipoprotein cholesteryl ester-supported adrenal steroidogenesis. Mol Endocrinol. 2004;18:549–57.
74. Plump AS, Erickson SK, Weng W, Partin JS, Breslow JL, Williams DL. Apolipoprotein A-I is required for cholesteryl ester accumulation in steroidogenic cells and for normal adrenal steroid production. J Clin Invest. 1996;97:2660–71.
75. Taylor MJ, Sanjanwala AR, Morin EE, Rowland-Fisher E, Anderson K, Schwendeman A, Rainey WE. Synthetic high-density lipoprotein (sHDL) inhibits steroid production in HAC15 adrenal cells. Endocrinology. 2016;157:3122–9.
76. Cherradi N, Pardo B, Greenberg AS, Kraemer FB, Capponi AM. Angiotensin II activates cholesterol ester hydrolase in bovine adrenal glomerulosa cells through phosphorylation mediated by p42/p44 mitogen-activated protein kinase. Endocrinology. 2003;144:4905–15.
77. Shen WJ, Patel S, Natu V, Hong R, Wang J, Azhar S, Kraemer FB. Interaction of hormone-sensitive lipase with steroidogenic acute regulatory protein: facilitation of cholesterol transfer in adrenal. J Biol Chem. 2003;278:43870–6.
78. LaPensee CR, Mann JE, Rainey WE, Crudo V, Hunt SW 3rd, Hammer GD. ATR-101, a selective and potent inhibitor of acyl-CoA Acyltransferase 1, induces apoptosis in H295R adrenocortical cells and in the adrenal cortex of dogs. Endocrinology. 2016;157:1775–88.
79. Sbiera S, Leich E, Liebisch G, Sbiera I, Schirbel A, Wiemer L, Matysik S, Eckhardt C, Gardill F, Gehl A, Kendl S, Weigand I, Bala M, Ronchi CL, Deutschbein T, Schmitz G, Rosenwald A, Allolio B, Fassnacht M, Kroiss M. Mitotane inhibits sterol-O-acyl Transferase 1 triggering lipid-mediated endoplasmic reticulum stress and apoptosis in adrenocortical carcinoma cells. Endocrinology. 2015;156:3895–908.
80. Scheidt HA, Haralampiev I, Theisgen S, Schirbel A, Sbiera S, Huster D, Kroiss M, Muller P. The adrenal specific toxicant mitotane directly interacts with lipid membranes and alters membrane properties depending on lipid composition. Mol Cell Endocrinol. 2016;428:68–81.
81. Kaufman RJ. Stress signaling from the lumen of the endoplasmic reticulum: coordination of gene transcriptional and translational controls. Genes Dev. 1999;13:1211–33.
82. Crivello JF, Jefcoate CR. Intracellular movement of cholesterol in rat adrenal cells. Kinetics and effects of inhibitors. J Biol Chem. 1980;255:8144–51.
83. Privalle CT, Crivello JF, Jefcoate CR. Regulation of intramitochondrial cholesterol transfer to side-chain cleavage cytochrome P-450 in rat adrenal gland. Proc Natl Acad Sci U S A. 1983;80:702–6.
84. Hall PF, Almahbobi G. Roles of microfilaments and intermediate filaments in adrenal steroidogenesis. Microsc Res Tech. 1997;36:463–79.
85. Li D, Sewer MB. RhoA and DIAPH1 mediate adrenocorticotropin-stimulated cortisol biosynthesis by regulating mitochondrial trafficking. Endocrinology. 2010;151:4313–23.
86. Sewer MB, Li D. Regulation of steroid hormone biosynthesis by the cytoskeleton. Lipids. 2008;43:1109–15.
87. Arbuzova A, Schmitz AA, Vergeres G. Cross-talk unfolded: MARCKS proteins. Biochem J. 2002;362:1–12.
88. Betancourt-Calle S, Bollag WB, Jung EM, Calle RA, Rasmussen H. Effects of angiotensin II and adrenocorticotropic hormone on myristoylated alanine-rich C-kinase substrate phosphorylation in glomerulosa cells. Mol Cell Endocrinol. 1999;154:1–9.
89. Kraemer FB, Khor VK, Shen WJ, Azhar S. Cholesterol ester droplets and steroidogenesis. Mol Cell Endocrinol. 2013;371:15–9.
90. Barbosa AD, Savage DB, Siniossoglou S. Lipid droplet-organelle interactions: emerging roles in lipid metabolism. Curr Opin Cell Biol. 2015;35:91–7.

91. Lin Y, Hou X, Shen WJ, Hanssen R, Khor VK, Cortez Y, Roseman AN, Azhar S, Kraemer FB. SNARE-mediated cholesterol movement to mitochondria supports Steroidogenesis in rodent cells. Mol Endocrinol. 2016;30:234–47.
92. Jagerstrom S, Polesie S, Wickstrom Y, Johansson BR, Schroder HD, Hojlund K, Bostrom P. Lipid droplets interact with mitochondria using SNAP23. Cell Biol Int. 2009;33: 934–40.
93. Enrich C, Rentero C, Hierro A, Grewal T. Role of cholesterol in SNARE-mediated trafficking on intracellular membranes. J Cell Sci. 2015;128:1071–81.
94. Kraemer FB, Shen WJ, Azhar S. SNAREs and cholesterol movement for steroidogenesis. Mol Cell: Endocrinol; 2016.
95. Midzak A, Papadopoulos V. Adrenal mitochondria and Steroidogenesis: from individual proteins to functional protein assemblies. Front Endocrinol (Lausanne). 2016;7:106.
96. Prasad M, Kaur J, Pawlak KJ, Bose M, Whittal RM, Bose HS. Mitochondria-associated endoplasmic reticulum membrane (MAM) regulates steroidogenic activity via steroidogenic acute regulatory protein (StAR)-voltage-dependent anion channel 2 (VDAC2) interaction. J Biol Chem. 2015;290:2604–16.
97. Doghman-Bouguerra M, Lalli E. The ER-mitochondria couple: in life and death from steroidogenesis to tumorigenesis. Mol Cell Endocrinol. 2016;441:176–84.
98. Vance JE. MAM (mitochondria-associated membranes) in mammalian cells: lipids and beyond. Biochim Biophys Acta. 2014;1841:595–609.
99. Doghman-Bouguerra M, Granatiero V, Sbiera S, Sbiera I, Lacas-Gervais S, Brau F, Fassnacht M, Rizzuto R, Lalli E. FATE1 antagonizes calcium- and drug-induced apoptosis by uncoupling ER and mitochondria. EMBO Rep. 2016;17(9):1264–80.
100. Hayashi T, Su TP. Sigma-1 receptors (sigma(1) binding sites) form raft-like microdomains and target lipid droplets on the endoplasmic reticulum: roles in endoplasmic reticulum lipid compartmentalization and export. J Pharmacol Exp Ther. 2003;306:718–25.
101. Marriott KS, Prasad M, Thapliyal V, Bose HS. Sigma-1 receptor at the mitochondrial-associated endoplasmic reticulum membrane is responsible for mitochondrial metabolic regulation. J Pharmacol Exp Ther. 2012;343:578–86.
102. Jinn S, Brandis KA, Ren A, Chacko A, Dudley-Rucker N, Gale SE, Sidhu R, Fujiwara H, Jiang H, Olsen BN, Schaffer JE, Ory DS. snoRNA U17 regulates cellular cholesterol trafficking. Cell Metab. 2015;21:855–67.
103. Ferguson JJ Jr. Protein synthesis and Adrenocorticotropin responsiveness. J Biol Chem. 1963;238:2754–9.
104. Garren LD, Ney RL, Davis WW. 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. 1965;53:1443–50.
105. Clark BJ, Wells J, King SR, Stocco DM. The purification, cloning, and expression of a novel luteinizing hormone-induced mitochondrial protein in MA-10 mouse Leydig tumor cells. Characterization of the steroidogenic acute regulatory protein (StAR). J Biol Chem. 1994;269:28314–22.
106. Miller WL. Mechanism of StAR's regulation of mitochondrial cholesterol import. Mol Cell Endocrinol. 2007a;265-266:46–50.
107. Duarte A, Castillo AF, Podesta EJ, Poderoso C. Mitochondrial fusion and ERK activity regulate steroidogenic acute regulatory protein localization in mitochondria. PLoS One. 2014;9:e100387.
108. Manna PR, Dyson MT, Stocco DM. Regulation of the steroidogenic acute regulatory protein gene expression: present and future perspectives. Mol Hum Reprod. 2009;15:321–33.
109. Pon LA, Hartigan JA, Orme-Johnson NR. Acute ACTH regulation of adrenal corticosteroid biosynthesis. Rapid accumulation of a phosphoprotein. J Biol Chem. 1986;261:13309–16.
110. Pon LA, Orme-Johnson NR. Acute stimulation of corpus luteum cells by gonadotrophin or adenosine 3′,5′-monophosphate causes accumulation of a phosphoprotein concurrent with acceleration of steroid synthesis. Endocrinology. 1988;123:1942–8.

111. Arakane F, King SR, Du Y, Kallen CB, Walsh LP, Watari H, Stocco DM, Strauss JF 3rd. Phosphorylation of steroidogenic acute regulatory protein (StAR) modulates its steroidogenic activity. J Biol Chem. 1997;272:32656–62.
112. Manna PR, Wang XJ, Stocco DM. Involvement of multiple transcription factors in the regulation of steroidogenic acute regulatory protein gene expression. Steroids. 2003;68:1125–34.
113. Tremblay JJ, Viger RS. Novel roles for GATA transcription factors in the regulation of steroidogenesis. J Steroid Biochem Mol Biol. 2003;85:291–8.
114. Cummins CL, Volle DH, Zhang Y, McDonald JG, Sion B, Lefrancois-Martinez AM, Caira F, Veyssiere G, Mangelsdorf DJ, Lobaccaro JM. Liver X receptors regulate adrenal cholesterol balance. J Clin Invest. 2006;116:1902–12.
115. Manna PR, Cohen-Tannoudji J, Counis R, Garner CW, Huhtaniemi I, Kraemer FB, Stocco DM. Mechanisms of action of hormone-sensitive lipase in mouse Leydig cells: its role in the regulation of the steroidogenic acute regulatory protein. J Biol Chem. 2013;288:8505–18.
116. Arakane F, Sugawara T, Nishino H, Liu Z, Holt JA, Pain D, Stocco DM, Miller WL, Strauss JF 3rd. Steroidogenic acute regulatory protein (StAR) retains activity in the absence of its mitochondrial import sequence: implications for the mechanism of StAR action. Proc Natl Acad Sci U S A. 1996;93:13731–6.
117. Bose HS, Whittal RM, Baldwin MA, Miller WL. The active form of the steroidogenic acute regulatory protein, StAR, appears to be a molten globule. Proc Natl Acad Sci U S A. 1999;96:7250–5.
118. Artemenko IP, Zhao D, Hales DB, Hales KH, Jefcoate CR. Mitochondrial processing of newly synthesized steroidogenic acute regulatory protein (StAR), but not total StAR, mediates cholesterol transfer to cytochrome P450 side chain cleavage enzyme in adrenal cells. J Biol Chem. 2001;276:46583–96.
119. Bahat A, Perlberg S, Melamed-Book N, Lauria I, Langer T, Orly J. StAR enhances transcription of genes encoding the mitochondrial proteases involved in its own degradation. Mol Endocrinol. 2014;28:208–24.
120. Bose HS, Sugawara T, Strauss JF 3rd, Miller WL. The pathophysiology and genetics of congenital lipoid adrenal hyperplasia. N Engl J Med. 1996;335:1870–8.
121. Lin D, Sugawara T, Strauss JF 3rd, Clark BJ, Stocco DM, Saenger P, Rogol A, Miller WL. Role of steroidogenic acute regulatory protein in adrenal and gonadal steroidogenesis. Science. 1995;267:1828–31.
122. Caron KM, Soo SC, Wetsel WC, Stocco DM, Clark BJ, Parker KL. 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. 1997;94:11540–5.
123. Hasegawa T, Zhao L, Caron KM, Majdic G, Suzuki T, Shizawa S, Sasano H, Parker KL. Developmental roles of the steroidogenic acute regulatory protein (StAR) as revealed by StAR knockout mice. Mol Endocrinol. 2000;14:1462–71.
124. Sasaki G, Ishii T, Jeyasuria P, Jo Y, Bahat A, Orly J, Hasegawa T, Parker KL. Complex role of the mitochondrial targeting signal in the function of steroidogenic acute regulatory protein revealed by bacterial artificial chromosome transgenesis in vivo. Mol Endocrinol. 2008;22:951–64.
125. Rone MB, Midzak AS, Issop L, Rammouz G, Jagannathan S, Fan J, Ye X, Blonder J, Veenstra T, Papadopoulos V. Identification of a dynamic mitochondrial protein complex driving cholesterol import, trafficking, and metabolism to steroid hormones. Mol Endocrinol. 2012;26:1868–82.
126. Papadopoulos V, Miller WL. Role of mitochondria in steroidogenesis. Best Pract Res Clin Endocrinol Metab. 2012;26:771–90.
127. Shoshan-Barmatz V, Keinan N, Zaid H. Uncovering the role of VDAC in the regulation of cell life and death. J Bioenerg Biomembr. 2008;40:183–91.
128. Bose M, Whittal RM, Miller WL, Bose HS. Steroidogenic activity of StAR requires contact with mitochondrial VDAC1 and phosphate carrier protein. J Biol Chem. 2008;283:8837–45.

129. Selvaraj V, Stocco DM. The changing landscape in translocator protein (TSPO) function. Trends Endocrinol Metab. 2015;26:341–8.
130. Krueger KE, Papadopoulos V. Peripheral-type benzodiazepine receptors mediate translocation of cholesterol from outer to inner mitochondrial membranes in adrenocortical cells. J Biol Chem. 1990;265:15015–22.
131. Lacapere JJ, Delavoie F, Li H, Peranzi G, Maccario J, Papadopoulos V, Vidic B. Structural and functional study of reconstituted peripheral benzodiazepine receptor. Biochem Biophys Res Commun. 2001;284:536–41.
132. Li H, Yao Z, Degenhardt B, Teper G, Papadopoulos V. Cholesterol binding at the cholesterol recognition/interaction amino acid consensus (CRAC) of the peripheral-type benzodiazepine receptor and inhibition of steroidogenesis by an HIV TAT-CRAC peptide. Proc Natl Acad Sci U S A. 2001b;98:1267–72.
133. West LA, Horvat RD, Roess DA, Barisas BG, Juengel JL, Niswender GD. Steroidogenic acute regulatory protein and peripheral-type benzodiazepine receptor associate at the mitochondrial membrane. Endocrinology. 2001;142:502–5.
134. Papadopoulos V, Aghazadeh Y, Fan J, Campioli E, Zirkin B, Midzak A. Translocator protein-mediated pharmacology of cholesterol transport and steroidogenesis. Mol Cell Endocrinol. 2015;408:90–8.
135. Selvaraj V, Stocco DM, Tu LN. Minireview: translocator protein (TSPO) and steroidogenesis: a reappraisal. Mol Endocrinol. 2015;29:490–501.
136. Selvaraj V, Tu LN, Stocco DM. Crucial role reported for TSPO in viability and Steroidogenesis is a misconception. Commentary: Conditional Steroidogenic cell-targeted deletion of TSPO unveils a crucial role in viability and hormone-dependent steroid formation. Front Endocrinol (Lausanne). 2016;7:91.
137. Banati RB, Middleton RJ, Chan R, Hatty CR, Kam WW, Quin C, Graeber MB, Parmar A, Zahra D, Callaghan P, Fok S, Howell NR, Gregoire M, Szabo A, Pham T, Davis E, Liu GJ. Positron emission tomography and functional characterization of a complete PBR/TSPO knockout. Nat Commun. 2014;5:5452.
138. Fan J, Campioli E, Midzak A, Culty M, Papadopoulos V. Conditional steroidogenic cell-targeted deletion of TSPO unveils a crucial role in viability and hormone-dependent steroid formation. Proc Natl Acad Sci U S A. 2015;112:7261–6.
139. Tu LN, Morohaku K, Manna PR, Pelton SH, Butler WR, Stocco DM, Selvaraj V. Peripheral benzodiazepine receptor/translocator protein global knock-out mice are viable with no effects on steroid hormone biosynthesis. J Biol Chem. 2014;289:27444–54.
140. Tu LN, Zhao AH, Stocco DM, Selvaraj V. PK11195 effect on steroidogenesis is not mediated through the translocator protein (TSPO). Endocrinology. 2015;156:1033–9.
141. Tu LN, Zhao AH, Hussein M, Stocco DM, Selvaraj V. Translocator protein (TSPO) affects Mitochondrial fatty acid oxidation in Steroidogenic cells. Endocrinology. 2016;157:1110–21.
142. Li H, Degenhardt B, Tobin D, Yao ZX, Tasken K, Papadopoulos V. Identification, localization, and function in steroidogenesis of PAP7: a peripheral-type benzodiazepine receptor- and PKA (RIalpha)-associated protein. Mol Endocrinol. 2001a;15:2211–28.
143. Liu J, Rone MB, Papadopoulos V. Protein-protein interactions mediate mitochondrial cholesterol transport and steroid biosynthesis. J Biol Chem. 2006;281:38879–93.
144. Poderoso C, Maloberti P, Duarte A, Neuman I, Paz C, Cornejo Maciel F, Podesta EJ. Hormonal activation of a kinase cascade localized at the mitochondria is required for StAR protein activity. Mol Cell Endocrinol. 2009;300:37–42.
145. Carrasco GA, Van de Kar LD. Neuroendocrine pharmacology of stress. Eur J Pharmacol. 2003;463:235–72.
146. Habib KE, Gold PW, Chrousos GP. Neuroendocrinology of stress. Endocrinol Metab Clin N Am. 2001;30:695–728.
147. Itoi K, Seasholtz AF, Watson SJ. Cellular and extracellular regulatory mechanisms of hypothalamic corticotropin-releasing hormone neurons. Endocr J. 1998;45:13–33.

148. Clark AJ. 60 YEARS OF POMC: the proopiomelanocortin gene: discovery, deletion and disease. J Mol Endocrinol. 2015;56(4):T27–37.
149. Raffin-Sanson ML, de Keyzer Y, Bertagna X. Proopiomelanocortin, a polypeptide precursor with multiple functions: from physiology to pathological conditions. Eur J Endocrinol. 2003;149:79–90.
150. Ruggiero C, Lalli E. Impact of ACTH Signaling on transcriptional regulation of Steroidogenic genes. Front Endocrinol (Lausanne). 2016;7:24.
151. Richards EM, Hua Y, Keller-Wood M. Pharmacology and physiology of ovine corticosteroid receptors. Neuroendocrinology. 2003;77:2–14.
152. Gomez-Sanchez EP. Brain mineralocorticoid receptors in cognition and cardiovascular homeostasis. Steroids. 2014b;91:20–31.
153. Gallo-Payet N, Battista MC. Steroidogenesis-adrenal cell signal transduction. Compr Physiol. 2014;4:889–964.
154. Dibner C, Schibler U, Albrecht U. The mammalian circadian timing system: organization and coordination of central and peripheral clocks. Annu Rev Physiol. 2010;72:517–49.
155. Kiessling S, Eichele G, Oster H. Adrenal glucocorticoids have a key role in circadian resynchronization in a mouse model of jet lag. J Clin Invest. 2010;120:2600–9.
156. Ota T, Fustin JM, Yamada H, Doi M, Okamura H. Circadian clock signals in the adrenal cortex. Mol Cell Endocrinol. 2012;349:30–7.
157. Moore RY, Eichler VB. Loss of a circadian adrenal corticosterone rhythm following suprachiasmatic lesions in the rat. Brain Res. 1972;42:201–6.
158. Park SY, Walker JJ, Johnson NW, Zhao Z, Lightman SL, Spiga F. Constant light disrupts the circadian rhythm of steroidogenic proteins in the rat adrenal gland. Mol Cell Endocrinol. 2013;371:114–23.
159. Balsalobre A, Brown SA, Marcacci L, Tronche F, Kellendonk C, Reichardt HM, Schutz G, Schibler U. Resetting of circadian time in peripheral tissues by glucocorticoid signaling. Science. 2000;289:2344–7.
160. Barclay JL, Shostak A, Leliavski A, Tsang AH, Johren O, Muller-Fielitz H, Landgraf D, Naujokat N, van der Horst GT, Oster H. High-fat diet-induced hyperinsulinemia and tissue-specific insulin resistance in cry-deficient mice. Am J Physiol Endocrinol Metab. 2013;304:E1053–63.
161. Lamia KA, Papp SJ, Yu RT, Barish GD, Uhlenhaut NH, Jonker JW, Downes M, Evans RM. Cryptochromes mediate rhythmic repression of the glucocorticoid receptor. Nature. 2011;480:552–6.
162. Leliavski A, Shostak A, Husse J, Oster H. Impaired glucocorticoid production and response to stress in Arntl-deficient male mice. Endocrinology. 2014;155:133–42.
163. Oster H, Damerow S, Hut RA, Eichele G. Transcriptional profiling in the adrenal gland reveals circadian regulation of hormone biosynthesis genes and nucleosome assembly genes. J Biol Rhythm. 2006;21:350–61.
164. Son GH, Chung S, Choe HK, Kim HD, Baik SM, Lee H, Lee HW, Choi S, Sun W, Kim H, Cho S, Lee KH, Kim K. Adrenal peripheral clock controls the autonomous circadian rhythm of glucocorticoid by causing rhythmic steroid production. Proc Natl Acad Sci U S A. 2008;105:20970–5.
165. Turek FW, Joshu C, Kohsaka A, Lin E, Ivanova G, McDearmon E, Laposky A, Losee-Olson S, Easton A, Jensen DR, Eckel RH, Takahashi JS, Bass J. Obesity and metabolic syndrome in circadian clock mutant mice. Science. 2005;308:1043–5.
166. Guran T, Buonocore F, Saka N, Ozbek MN, Aycan Z, Bereket A, Bas F, Darcan S, Bideci A, Guven A, Demir K, Akinci A, Buyukinan M, Aydin BK, Turan S, Agladioglu SY, Atay Z, Abali ZY, Tarim O, Catli G, Yuksel B, Akcay T, Yildiz M, Ozen S, Doger E, Demirbilek H, Ucar A, Isik E, Ozhan B, Bolu S, Ozgen IT, Suntharalingham JP, Achermann JC. Rare causes of primary adrenal insufficiency: genetic and clinical characterization of a large Nationwide cohort. J Clin Endocrinol Metab. 2016;101:284–92.

167. de Joussineau C, Sahut-Barnola I, Levy I, Saloustros E, Val P, Stratakis CA, Martinez A. The cAMP pathway and the control of adrenocortical development and growth. Mol Cell Endocrinol. 2012;351:28–36.
168. Sahut-Barnola I, de Joussineau C, Val P, Lambert-Langlais S, Damon C, Lefrancois-Martinez AM, Pointud JC, Marceau G, Sapin V, Tissier F, Ragazzon B, Bertherat J, Kirschner LS, Stratakis CA, Martinez A. Cushing's syndrome and fetal features resurgence in adrenal cortex-specific Prkar1a knockout mice. PLoS Genet. 2010;6:e1000980.
169. de Joussineau C, Sahut-Barnola I, Tissier F, Dumontet T, Drelon C, Batisse-Lignier M, Tauveron I, Pointud JC, Lefrancois-Martinez AM, Stratakis CA, Bertherat J, Val P, Martinez A. mTOR pathway is activated by PKA in adrenocortical cells and participates in vivo to apoptosis resistance in primary pigmented nodular adrenocortical disease (PPNAD). Hum Mol: Genet; 2014.
170. Beuschlein F, Fassnacht M, Assie G, Calebiro D, Stratakis CA, Osswald A, Ronchi CL, Wieland T, Sbiera S, Faucz FR, Schaak K, Schmittfull A, Schwarzmayr T, Barreau O, Vezzosi D, Rizk-Rabin M, Zabel U, Szarek E, Salpea P, Forlino A, Vetro A, Zuffardi O, Kisker C, Diener S, Meitinger T, Lohse MJ, Reincke M, Bertherat J, Strom TM, Allolio B. Constitutive activation of PKA catalytic subunit in adrenal Cushing's syndrome. N Engl J Med. 2014;370:1019–28.
171. Ronchi CL, Di Dalmazi G, Faillot S, Sbiera S, Assie G, Weigand I, Calebiro D, Schwarzmayr T, Appenzeller S, Rubin B, Waldmann J, Scaroni C, Bartsch DK, Mantero F, Mannelli M, Kastelan D, Chiodini I, Bertherat J, Reincke M, Strom TM, Fassnacht M, Beuschlein F. Genetic landscape of sporadic unilateral adrenocortical adenomas without PRKACA p.Leu206Arg mutation. J Clin Endocrinol Metab. 2016;101(9):3526–38.
172. Aumo L, Rusten M, Mellgren G, Bakke M, Lewis AE. Functional roles of protein kinase a (PKA) and exchange protein directly activated by 3′,5′-cyclic adenosine 5′-monophosphate (cAMP) 2 (EPAC2) in cAMP-mediated actions in adrenocortical cells. Endocrinology. 2010;151:2151–61.
173. Lewis AE, Aesoy R, Bakke M. Role of EPAC in cAMP-mediated actions in adrenocortical cells. Front Endocrinol (Lausanne). 2016;7:63.
174. Bos JL. Epac proteins: multi-purpose cAMP targets. Trends Biochem Sci. 2006;31:680–6.
175. Enyeart JA, Enyeart JJ. Metabolites of an Epac-selective cAMP analog induce cortisol synthesis by adrenocortical cells through a cAMP-independent pathway. PLoS One. 2009;4:e6088.
176. Horvath A, Stratakis CA. Unraveling the molecular basis of micronodular adrenal hyperplasia. Curr Opin Endocrinol Diabetes Obes. 2008;15:227–33.
177. Azevedo MF, Faucz FR, Bimpaki E, Horvath A, Levy I, de Alexandre RB, Ahmad F, Manganiello V, Stratakis CA. Clinical and molecular genetics of the phosphodiesterases (PDEs). Endocr Rev. 2014;35:195–233.
178. Horvath A, Giatzakis C, Tsang K, Greene E, Osorio P, Boikos S, Libe R, Patronas Y, Robinson-White A, Remmers E, Bertherat J, Nesterova M, Stratakis CA. A cAMP-specific phosphodiesterase (PDE8B) that is mutated in adrenal hyperplasia is expressed widely in human and mouse tissues: a novel PDE8B isoform in human adrenal cortex. Eur J Hum Genet. 2008;16(10):1245–53.
179. Abdou HS, Bergeron F, Tremblay JJ. A cell-autonomous molecular cascade initiated by AMP-activated protein kinase represses steroidogenesis. Mol Cell Biol. 2014;34:4257–71.
180. Tremblay JJ. Molecular regulation of steroidogenesis in endocrine Leydig cells. Steroids. 2015;103:3–10.
181. Dada L, Cornejo Maciel F, Neuman I, Mele PG, Maloberti P, Paz C, Cymeryng C, Finkielstein C, Mendez CF, Podesta EJ. Cytosolic and mitochondrial proteins as possible targets of cycloheximide effect on adrenal steroidogenesis. Endocr Res. 1996;22:533–9.
182. Wang X, Walsh LP, Reinhart AJ, Stocco DM. The role of arachidonic acid in steroidogenesis and steroidogenic acute regulatory (StAR) gene and protein expression. J Biol Chem. 2000;275:20204–9.

183. Kang MJ, Fujino T, Sasano H, Minekura H, Yabuki N, Nagura H, Iijima H, Yamamoto TT. A novel arachidonate-preferring acyl-CoA synthetase is present in steroidogenic cells of the rat adrenal, ovary, and testis. Proc Natl Acad Sci U S A. 1997;94:2880–4.
184. Lewin TM, Van Horn CG, Krisans SK, Coleman RA. Rat liver acyl-CoA synthetase 4 is a peripheral-membrane protein located in two distinct subcellular organelles, peroxisomes, and mitochondrial-associated membrane. Arch Biochem Biophys. 2002;404:263–70.
185. Wilson DB, Prescott SM, Majerus PW. Discovery of an arachidonoyl coenzyme a synthetase in human platelets. J Biol Chem. 1982;257:3510–5.
186. Soupene E, Kuypers FA. Mammalian long-chain acyl-CoA synthetases. Exp Biol Med (Maywood). 2008;233:507–21.
187. Cornejo Maciel F, Maloberti P, Neuman I, Cano F, Castilla R, Castillo F, Paz C, Podesta EJ. An arachidonic acid-preferring acyl-CoA synthetase is a hormone-dependent and obligatory protein in the signal transduction pathway of steroidogenic hormones. J Mol Endocrinol. 2005;34:655–66.
188. Maloberti P, Castilla R, Castillo F, Cornejo Maciel F, Mendez CF, Paz C, Podesta EJ. Silencing the expression of mitochondrial acyl-CoA thioesterase I and acyl-CoA synthetase 4 inhibits hormone-induced steroidogenesis. FEBS J. 2005;272:1804–14.
189. Cooke M, Mele P, Maloberti P, Duarte A, Poderoso C, Orlando U, Paz C, Cornejo Maciel F, Podesta EJ. Tyrosine phosphatases as key regulators of StAR induction and cholesterol transport: SHP2 as a potential tyrosine phosphatase involved in steroid synthesis. Mol Cell Endocrinol. 2011;336:63–9.
190. Paz C, Cornejo Maciel F, Gorostizaga A, Castillo AF, Mori Sequeiros Garcia MM, Maloberti PM, Orlando UD, Mele PG, Poderoso C, Podesta EJ. Role of protein phosphorylation and tyrosine phosphatases in the adrenal regulation of steroid synthesis and Mitochondrial function. Front Endocrinol (Lausanne). 2016;7:60.
191. Houslay MD, Kolch W. Cell-type specific integration of cross-talk between extracellular signal-regulated kinase and cAMP signaling. Mol Pharmacol. 2000;58:659–68.
192. Lefrancois-Martinez AM, Blondet-Trichard A, Binart N, Val P, Chambon C, Sahut-Barnola I, Pointud JC, Martinez A. Transcriptional control of adrenal steroidogenesis: novel connection between Janus kinase (JAK) 2 protein and protein kinase a (PKA) through stabilization of cAMP response element-binding protein (CREB) transcription factor. J Biol Chem. 2011;286:32976–85.
193. Bornstein SR, Engeland WC, Ehrhart-Bornstein M, Herman JP. Dissociation of ACTH and glucocorticoids. Trends Endocrinol Metab. 2008;19:175–80.
194. Ansurudeen I, Willenberg HS, Kopprasch S, Krug AW, Ehrhart-Bornstein M, Bornstein SR. Endothelial factors mediate aldosterone release via PKA-independent pathways. Mol Cell Endocrinol. 2009;300:66–70.
195. Ehrhart-Bornstein M, Lamounier-Zepter V, Schraven A, Langenbach J, Willenberg HS, Barthel A, Hauner H, McCann SM, Scherbaum WA, Bornstein SR. Human adipocytes secrete mineralocorticoid-releasing factors. Proc Natl Acad Sci U S A. 2003;100:14211–6.
196. Spät A, Hunyady L, Szanda G. Signaling interactions in the adrenal cortex. Front Endocrinol (Lausanne). 2016;7:17.
197. Nogueira EF, Bollag WB, Rainey WE. Angiotensin II regulation of adrenocortical gene transcription. Mol Cell Endocrinol. 2009;302:230–6.
198. Clark BJ, Combs R. Angiotensin II and cyclic adenosine 3′,5′-monophosphate induce human steroidogenic acute regulatory protein transcription through a common steroidogenic factor-1 element. Endocrinology. 1999;140:4390–8.
199. Zennaro MC, Boulkroun S, Fernandes-Rosa F. An update on novel mechanisms of primary aldosteronism. J Endocrinol. 2015;224:R63–77.
200. Vaidya A, Hamrahian A, Auchus RJ. Genetics of primary Aldosteronism. Endocr Pract. 2015;21(5):1–15.
201. Spät A. Glomerulosa cell--a unique sensor of extracellular K+ concentration. Mol Cell Endocrinol. 2004;217:23–6.

202. Himathongkam T, Dluhy RG, Williams GH. Potassim-aldosterone-renin interrelationships. J Clin Endocrinol Metab. 1975;41:153–9.
203. Rege J, Nakamura Y, Satoh F, Morimoto R, Kennedy MR, Layman LC, Honma S, Sasano H, Rainey WE. Liquid chromatography-tandem mass spectrometry analysis of human adrenal vein 19-carbon steroids before and after ACTH stimulation. J Clin Endocrinol Metab. 2013;98:1182–8.
204. Udhane SS, Flück CE. Regulation of human (adrenal) androgen biosynthesis-new insights from novel throughput technology studies. Biochem Pharmacol. 2016;102:20–33.
205. Kirschner MA, Bardin CW. Androgen production and metabolism in normal and virilized women. Metabolism. 1972;21:667–88.
206. Ferraldeschi R, Sharifi N, Auchus RJ, Attard G. Molecular pathways: inhibiting steroid biosynthesis in prostate cancer. Clin Cancer Res. 2013;19:3353–9.
207. Turcu AF, Nanba AT, Chomic R, Upadhyay SK, Giordano T, Shields JJ, Merke DP, Rainey W, Auchus R. Adrenal-derived 11-oxygenated 19-carbon steroids are the dominant androgens in classic 21-hydroxylase deficiency. Eur J Endocrinol. 2016;174(5):601–9.
208. Mueller JW, Gilligan LC, Idkowiak J, Arlt W, Foster PA. The regulation of steroid action by Sulfation and Desulfation. Endocr Rev. 2015;36:526–63.
209. Noordam C, Dhir V, McNelis JC, Schlereth F, Hanley NA, Krone N, Smeitink JA, Smeets R, Sweep FC, Claahsen-van der Grinten HL, Arlt W. Inactivating *PAPSS2* mutations in a patient with premature pubarche. N Engl J Med. 2009;360:2310–8.
210. Migeon CJ, Keller AR, Lawrence B, Shepard TH 2nd. Dehydroepiandrosterone and androsterone levels in human plasma: effect of age and sex; day-to-day and diurnal variations. J Clin Endocrinol Metab. 1957;17:1051–62.
211. Brett EM, Auchus RJ. Genetic forms of adrenal insufficiency. Endocr Pract. 2015;1-17
212. Pandey AV, Mellon SH, Miller WL. Protein phosphatase 2A and phosphoprotein SET regulate androgen production by P450c17. J Biol Chem. 2003;278:2837–44.
213. Tee MK, Miller WL. Phosphorylation of human cytochrome P450c17 by p38alpha selectively increases 17,20 lyase activity and androgen biosynthesis. J Biol Chem. 2013;288:23903–13.
214. Miller WL, Tee MK. The post-translational regulation of 17,20 lyase activity. Mol Cell Endocrinol. 2015;408:99–106.
215. Rege J, Nishimoto HK, Nishimoto K, Rodgers RJ, Auchus RJ, Rainey WE. Bone morphogenetic protein-4 (BMP4): a paracrine regulator of human adrenal C19 steroid synthesis. Endocrinology. 2015;156:2530–40.
216. Kempna P, Marti N, Udhane S, Flück CE. Regulation of androgen biosynthesis - a short review and preliminary results from the hyperandrogenic starvation NCI-H295R cell model. Mol Cell Endocrinol. 2015;408:124–32.
217. Baba T, Otake H, Sato T, Miyabayashi K, Shishido Y, Wang CY, Shima Y, Kimura H, Yagi M, Ishihara Y, Hino S, Ogawa H, Nakao M, Yamazaki T, Kang D, Ohkawa Y, Suyama M, Chung BC, Morohashi K. Glycolytic genes are targets of the nuclear receptor Ad4BP/SF-1. Nat Commun. 2014;5:3634.
218. Ruggiero C, Doghman M, Lalli E. How genomic studies have improved our understanding of the mechanisms of transcriptional regulation by NR5A nuclear receptors. Mol Cell Endocrinol. 2014;408:138–44.
219. Crawford PA, Sadovsky Y, Milbrandt J. Nuclear receptor steroidogenic factor 1 directs embryonic stem cells toward the steroidogenic lineage. Mol Cell Biol. 1997; 17:3997–4006.
220. Mizutani T, Kawabe S, Ishikane S, Imamichi Y, Umezawa A, Miyamoto K. Identification of novel steroidogenic factor 1 (SF-1)-target genes and components of the SF-1 nuclear complex. Mol Cell Endocrinol. 2015;408:133–7.
221. Urs AN, Dammer E, Kelly S, Wang E, Merrill AH Jr, Sewer MB. Steroidogenic factor-1 is a sphingolipid binding protein. Mol Cell Endocrinol. 2007;265-266:174–8.
222. Blind RD, Suzawa M, Ingraham HA. Direct modification and activation of a nuclear receptor-PIP2 complex by the inositol lipid kinase IPMK. Sci Signal. 2012;5:ra44.

223. Doghman M, Karpova T, Rodrigues GA, Arhatte M, De MJ, Cavalli LR, Virolle V, Barbry P, Zambetti GP, Figueiredo BC, Heckert LL, Lalli E. Increased steroidogenic factor-1 dosage triggers adrenocortical cell proliferation and cancer. Mol Endocrinol. 2007;21:2968–87.
224. Figueiredo BC, Cavalli LR, Pianovski MA, Lalli E, Sandrini R, Ribeiro RC, Zambetti G, DeLacerda L, Rodrigues GA, Haddad BR. Amplification of the steroidogenic factor 1 gene in childhood adrenocortical tumors. J Clin Endocrinol Metab. 2005;90:615–9.
225. Lee FY, Faivre EJ, Suzawa M, Lontok E, Ebert D, Cai F, Belsham DD, Ingraham HA. Eliminating SF-1 (NR5A1) sumoylation in vivo results in ectopic hedgehog signaling and disruption of endocrine development. Dev Cell. 2011;21:315–27.
226. Parker KL. The roles of steroidogenic factor 1 in endocrine development and function. Mol Cell Endocrinol. 1998;145:15–20.
227. Lalli E, Melner MH, Stocco DM, Sassone-Corsi P. DAX-1 blocks steroid production at multiple levels. Endocrinology. 1998;139:4237–43.
228. Achermann JC, Meeks JJ, Jameson JL. Phenotypic spectrum of mutations in DAX-1 and SF-1. Mol Cell Endocrinol. 2001;185:17–25.
229. Scheys JO, Heaton JH, Hammer GD. Evidence of adrenal failure in aging Dax1-deficient mice. Endocrinology. 2011;152:3430–9.
230. Suntharalingham JP, Buonocore F, Duncan AJ, Achermann JC. DAX-1 (NR0B1) and steroidogenic factor-1 (SF-1, NR5A1) in human disease. Best Pract Res Clin Endocrinol Metab. 2015;29:607–19.
231. Zazopoulos E, Lalli E, Stocco DM, Sassone-Corsi P. DNA binding and transcriptional repression by DAX-1 blocks steroidogenesis. Nature. 1997;390:311–5.
232. Sands WA, Palmer TM. Regulating gene transcription in response to cyclic AMP elevation. Cell Signal. 2008;20:460–6.
233. Gau D, Lemberger T, von Gall C, Kretz O, Le Minh N, Gass P, Schmid W, Schibler U, Korf HW, Schutz G. Phosphorylation of CREB Ser142 regulates light-induced phase shifts of the circadian clock. Neuron. 2002;34:245–53.
234. Jimenez P, Saner K, Mayhew B, Rainey WE. GATA-6 is expressed in the human adrenal and regulates transcription of genes required for adrenal androgen biosynthesis. Endocrinology. 2003;144:4285–8.
235. Kiiveri S, Liu J, Westerholm-Ormio M, Narita N, Wilson DB, Voutilainen R, Heikinheimo M. Differential expression of GATA-4 and GATA-6 in fetal and adult mouse and human adrenal tissue. Endocrinology. 2002;143:3136–43.
236. Nakamura Y, Suzuki T, Sasano H. Transcription factor GATA-6 in the human adrenocortex: association with adrenal development and aging. Endocr J. 2007;54:783–9.
237. Nakamura Y, Xing Y, Sasano H, Rainey WE. The mediator complex subunit 1 enhances transcription of genes needed for adrenal androgen production. Endocrinology. 2009;150:4145–53.
238. Viger RS, Guittot SM, Anttonen M, Wilson DB, Heikinheimo M. Role of the GATA family of transcription factors in endocrine development, function, and disease. Mol Endocrinol. 2008;22:781–98.
239. Flück CE, Miller WL. GATA-4 and GATA-6 modulate tissue-specific transcription of the human gene for P450c17 by direct interaction with Sp1. Mol Endocrinol. 2004;18:1144–57.
240. Huang YH, Lee CY, Tai PJ, Yen CC, Liao CY, Chen WJ, Liao CJ, Cheng WL, Chen RN, Wu SM, Wang CS, Lin KH. Indirect regulation of human dehydroepiandrosterone sulfotransferase family 1A member 2 by thyroid hormones. Endocrinology. 2006;147:2481–9.
241. Martin LJ, Taniguchi H, Robert NM, Simard J, Tremblay JJ, Viger RS. GATA factors and the nuclear receptors SF-1/LRH-1 are key mutual partners in the regulation of the human HSD3B2 promoter. Mol Endocrinol. 2005;19:2358–70.
242. Allen HL, Flanagan SE, Shaw-Smith C, De Franco E, Akerman I, Caswell R, Ferrer J, Hattersley AT, Ellard S. GATA6 haploinsufficiency causes pancreatic agenesis in humans. Nat Genet. 2012;44:20–2.
243. Bonnefond A, Sand O, Guerin B, Durand E, De Graeve F, Huyvaert M, Rachdi L, Kerr-Conte J, Pattou F, Vaxillaire M, Polak M, Scharfmann R, Czernichow P, Froguel P. GATA6 inactivating mutations are associated with heart defects and, inconsistently, with pancreatic agenesis and diabetes. Diabetologia. 2012;55(10):2845–7.

244. Maitra M, Koenig SN, Srivastava D, Garg V. Identification of GATA6 sequence variants in patients with congenital heart defects. Pediatr Res. 2010;68:281–5.
245. Pihlajoki M, Gretzinger E, Cochran R, Kyrönlahti A, Schrade A, Hiller T, Sullivan L, Shoykhet M, Schoeller EL, Brooks MD, Heikinheimo M, Wilson DB. Conditional mutagenesis of *Gata6* in SF1-positive cells causes gonadal-like differentiation in the adrenal cortex of mice. Endocrinology. 2013;154:1754–67.
246. Heikinheimo M, Pihlajoki M, Schrade A, Kyronlahti A, Wilson DB. Testicular steroidogenic cells to the rescue. Endocrinology. 2015;156:1616–9.
247. Padua MB, Jiang T, Morse DA, Fox SC, Hatch HM, Tevosian SG. Combined loss of the GATA4 and GATA6 transcription factors in male mice disrupts testicular development and confers adrenal-like function in the testes. Endocrinology. 2015;156(5):1873–86.
248. Tevosian SG, Jimenez E, Hatch HM, Jiang T, Morse DA, Fox SC, Padua MB. Adrenal development in mice requires GATA4 and GATA6 transcription factors. Endocrinology. 2015;156:2503–17.
249. Kyritsi, E. M., A. Sertedaki, G. Chrousos, and E. Charmandari, 2000. Familial or sporadic adrenal hypoplasia syndrome.
250. Malikova J, Flück CE. Novel insight into etiology, diagnosis and management of primary adrenal insufficiency. Horm Res Paediatr. 2014;82:145–57.
251. Weber A, Clark AJ. Mutations of the ACTH receptor gene are only one cause of familial glucocorticoid deficiency. Hum Mol Genet. 1994;3:585–8.
252. Metherell LA, Chapple JP, Cooray S, David A, Becker C, Ruschendorf F, Naville D, Begeot M, Khoo B, Nurnberg P, Huebner A, Cheetham ME, Clark AJ. Mutations in MRAP, encoding a new interacting partner of the ACTH receptor, cause familial glucocorticoid deficiency type 2. Nat Genet. 2005;37:166–70.
253. Meimaridou E, Kowalczyk J, Guasti L, Hughes CR, Wagner F, Frommolt P, Nurnberg P, Mann NP, Banerjee R, Saka HN, Chapple JP, King PJ, Clark AJ, Metherell LA. Mutations in NNT encoding nicotinamide nucleotide transhydrogenase cause familial glucocorticoid deficiency. Nat Genet. 2012;44:740–2.
254. Prasad R, Chan LF, Hughes CR, Kaski JP, Kowalczyk JC, Savage MO, Peters CJ, Nathwani N, Clark AJ, Storr HL, Metherell LA. Thioredoxin Reductase 2 (TXNRD2) mutation associated with familial glucocorticoid deficiency (FGD). J Clin Endocrinol Metab. 2014;99:E1556–63.
255. Prasad R, Metherell LA, Clark AJ, Storr HL. Deficiency of ALADIN impairs redox homeostasis in human adrenal cells and inhibits steroidogenesis. Endocrinology. 2013;154:3209–18.
256. Arboleda VA, Lee H, Parnaik R, Fleming A, Banerjee A, Ferraz-de-Souza B, Delot EC, Rodriguez-Fernandez IA, Braslavsky D, Bergada I, Dell'angelica EC, Nelson SF, Martinez-Agosto JA, Achermann JC, Vilain E. Mutations in the PCNA-binding domain of CDKN1C cause IMAGe syndrome. Nat Genet. 2012;44(7):788–92.
257. Hughes CR, Guasti L, Meimaridou E, Chuang CH, Schimenti JC, King PJ, Costigan C, Clark AJ, Metherell LA. MCM4 mutation causes adrenal failure, short stature, and natural killer cell deficiency in humans. J Clin Invest. 2012;122:814–20.
258. Narumi S, Amano N, Ishii T, Katsumata N, Muroya K, Adachi M, Toyoshima K, Tanaka Y, Fukuzawa R, Miyako K, Kinjo S, Ohga S, Ihara K, Inoue H, Kinjo T, Hara T, Kohno M, Yamada S, Urano H, Kitagawa Y, Tsugawa K, Higa A, Miyawaki M, Okutani T, Kizaki Z, Hamada H, Kihara M, Shiga K, Yamaguchi T, Kenmochi M, Kitajima H, Fukami M, Shimizu A, Kudoh J, Shibata S, Okano H, Miyake N, Matsumoto N, Hasegawa T. *SAMD9* mutations cause a novel multisystem disorder, MIRAGE syndrome, and are associated with loss of chromosome 7. Nat Genet. 2016;48(7):792–7.
259. Roucher-Boulez F, Mallet-Motak D, Samara-Boustani D, Jilani H, Asmahane L, Souchon PF, Simon D, Nivot S, Heinrichs C, Ronze M, Bertagna X, Groisne L, Leheup B, Catherine NS, Blondin G, Lefevre C, Lemarchand L, Morel Y. NNT mutations: a cause of primary adrenal insufficiency, oxidative stress and extra-adrenal defects. Eur J Endocrinol. 2016;175(1):73–84.
260. Toye AA, Lippiat JD, Proks P, Shimomura K, Bentley L, Hugill A, Mijat V, Goldsworthy M, Moir L, Haynes A, Quarterman J, Freeman HC, Ashcroft FM, Cox RD. A genetic and physiological study of impaired glucose homeostasis control in C57BL/6J mice. Diabetologia. 2005;48:675–86.

261. Figueira TR. A word of caution concerning the use of Nnt-mutated C57BL/6 mice substrains as experimental models to study metabolism and mitochondrial pathophysiology. Exp Physiol. 2013;98:1643.
262. Conrad M, Jakupoglu C, Moreno SG, Lippl S, Banjac A, Schneider M, Beck H, Hatzopoulos AK, Just U, Sinowatz F, Schmahl W, Chien KR, Wurst W, Bornkamm GW, Brielmeier M. Essential role for mitochondrial thioredoxin reductase in hematopoiesis, heart development, and heart function. Mol Cell Biol. 2004;24:9414–23.
263. Kiermayer C, Northrup E, Schrewe A, Walch A, de Angelis MH, Schoensiegel F, Zischka H, Prehn C, Adamski J, Bekeredjian R, Ivandic B, Kupatt C, Brielmeier M. Heart-specific knockout of the Mitochondrial Thioredoxin Reductase (Txnrd2) induces metabolic and contractile dysfunction in the aging myocardium. J Am Heart Assoc. 2015;4
264. Sibbing D, Pfeufer A, Perisic T, Mannes AM, Fritz-Wolf K, Unwin S, Sinner MF, Gieger C, Gloeckner CJ, Wichmann HE, Kremmer E, Schafer Z, Walch A, Hinterseer M, Nabauer M, Kaab S, Kastrati A, Schomig A, Meitinger T, Bornkamm GW, Conrad M, von Beckerath N. Mutations in the mitochondrial thioredoxin reductase gene TXNRD2 cause dilated cardiomyopathy. Eur Heart J. 2011;32:1121–33.
265. Handschug K, Sperling S, Yoon SJ, Hennig S, Clark AJ, Huebner A. Triple a syndrome is caused by mutations in AAAS, a new WD-repeat protein gene. Hum Mol Genet. 2001;10:283–90.
266. Brioude F, Netchine I, Praz F, Le Jule M, Calmel C, Lacombe D, Edery P, Catala M, Odent S, Isidor B, Lyonnet S, Sigaudy S, Leheup B, Audebert-Bellanger S, Burglen L, Giuliano F, Alessandri JL, Cormier-Daire V, Laffargue F, Blesson S, Coupier I, Lespinasse J, Blanchet P, Boute O, Baumann C, Polak M, Doray B, Verloes A, Viot G, Le Bouc Y, Rossignol S. Mutations of the imprinted CDKN1C Gene as a cause of the overgrowth Beckwith-Wiedemann syndrome: clinical Spectrum and functional characterization. Hum Mutat. 2015;36:894–902.
267. Casey JP, Nobbs M, McGettigan P, Lynch S, Ennis S. Recessive mutations in MCM4/PRKDC cause a novel syndrome involving a primary immunodeficiency and a disorder of DNA repair. J Med Genet. 2012;49:242–5.