[Frontiers in Bioscience 17, 824-846, January 1, 2012]

Gonadal transactivation of STARD1, CYP11A1 and HSD3B

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1. ABSTRACT

The steroidogenic acute regulatory protein, cytochrome P450 cholesterol side-chain cleavage enzyme and specific 3beta-hydroxysteroid dehydrogenase/delta5-delta4 isomerases initiate the essential process of steroidogenesis in the gonads. Testicular and ovarian expression of their respective genes, STARD1, CYP11A1 and gonadal HSD3B, is primarily controlled by gonadotropins with contributions by various growth factors. Gonadotropins through their receptors switch on cyclic AMP signaling pathways that recruit NR5A, GATA and often CREB, NR4A, or Sp1 transcription factors to regulatory regions of each gene's promoter to elicit transcription. The specific combination of transcription factors involved depends on the cellular context. In this review, we summarize current understanding of the factors that control transactivation of the STARD1, CYP11A1 and gonadal HSD3B genes in Leydig cells in the testis and the theca, differentiating granulosa and luteal cells in the ovary.

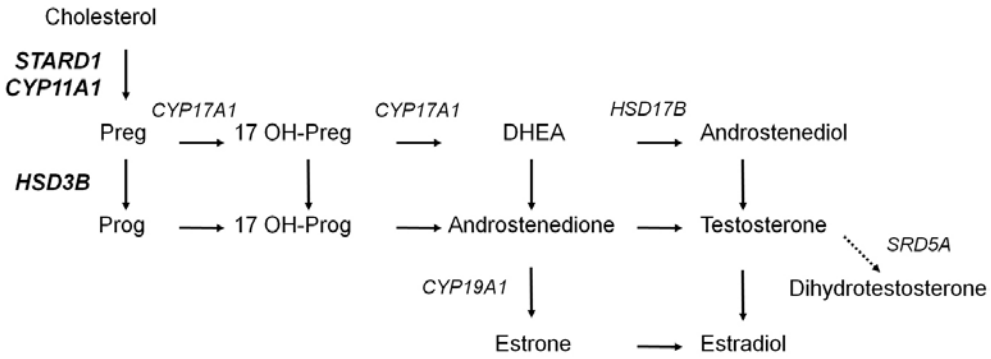

Figure 1. Overview of genes coding for mediators of gonadal steroidogenesis. Cholesterol is the substrate for *de novo* steroid synthesis. The STARD1 gene encodes the steroidogenic acute regulatory protein which controls cholesterol delivery to the inner mitochondrial membrane. CYP11A1 encodes the P450 cholesterol side-chain cleavage enzyme which forms part of the P450scc complex. Genes encoding the enzymes mediating each step are indicated in *italics*. The genes HSD3B, CYP17A1, CYP19A1, HSD17B and SRD5A encode the enzymes 3beta-hydroxysteroid dehydrogenase/delta5-delta4 isomerase, P450 17alpha-hydroxylase/17,20-lyase, P450 aromatase, 17beta-hydroxysteroid dehydrogenase and 5alpha-reductase, respectively. Other abbreviations are Preg (pregnenolone) and Prog (progesterone). Dashed arrow indicates most of this enzyme activity is extragonadal. The pathway taken by pregnenolone is species and tissue dependent.

2. INTRODUCTION

Sex steroid hormones such as progesterone, testosterone and estradiol are generated from cholesterol by the gonads and are essential for reproduction (1). The proteins that initiate their synthesis include the steroidogenic acute regulatory protein (StAR; encoded by the STARD1 gene), cytochrome P450 cholesterol side-chain cleavage enzyme (P450scc; encoded by the CYP11A1 gene) and 3beta-hydroxysteroid dehydrogenase/delta5-delta4 isomerases (3beta-HSDs; encoded by HSD3B genes).

Cytochrome P450scc catalyzes the initial conversion of cholesterol to pregnenolone, the precursor to all other steroids. This reaction occurs at the inner mitochondrial membrane and is solely limited by the availability of substrate. Hence, the transport of hydrophobic cholesterol across the aqueous intermembrane space to the inner mitochondrial membrane where the P450scc complex resides is rate limiting. The rate of this transport is controlled by StAR (2,3). Trophic hormones rapidly induce expression of StAR and in turn stimulate cholesterol transfer to P450scc and thus, steroidogenesis.

Pregnenolone is next metabolized to progesterone by select 3beta-hydroxysteroid dehydrogenase/delta5-delta4 isomerases (4). Specific steroidogenic cell types alternatively convert pregnenolone to 17alpha-hydroxyprogesterone by cytochrome P450 17alpha-hydroxylase/17,20-lyase (P450c17alpha) prior to the actions of 3beta-HSD. Progesterone and products further downstream of the P450c17alpha reaction, testosterone and estrogens, comprise the major sex steroid hormones in mammals. A summary of the gonadal steroidogenic pathways is shown in Figure 1.

Adult Leydig cells in the interstitium of the testis rapidly generate testosterone in response to stimulation by the pituitary gonadotropin, luteinizing hormone (LH), and its consequent generation of StAR (5,6). Ovarian theca cells in the outer layer of the pre-surge developing follicle express STARD1, CYP11A1 and HSD3B genes and generate androgens in response to LH. These steroids filter across the basement membrane to granulosa cells where the follicle-stimulating hormone (FSH) regulated enzyme, cytochrome P450 aromatase, converts them to estrogens. With ovulation, both cell types luteinize in forming the corpus luteum, with increased transcription of STARD1, CYP11A1 and HSD3B and a new focus on progesterone production to support pregnancy (7).

Since steroid hormones are secreted upon synthesis rather than stored, the expression of these genes is a key regulatory point in steroidogenesis. This article briefly reviews the transcriptional regulation of STARD1, CYP11A1 and HSD3B in the primary steroidogenic cell types in the gonads.

3. THE STARD1 GENE

3.1. Hormonal control

Hormonal control of StAR mRNA levels largely determines the level of gonadal steroid synthesis. The major regulator of STARD1 in Leydig cells is LH through stimulation of the cAMP pathway. This effect is mimicked by human chorionic gonadotropin (hCG), which also binds and activates the LH receptor. Growth factors such as insulin-like growth factor 1 (IGF-1), epidermal growth factor (EGF), fibroblast growth factor (FGF) and transforming growth factor alpha (TGFalpha), also increase STARD1 mRNA in Leydig cells in part through protein kinase C (PKC) signaling (8), an effect mimicked by phorbol esters (9). While gonadotropins and cAMP analogs positively regulate STARD1 by themselves, many factors often only serve to augment or inhibit gonadotropin stimulation of the gene (8,10-12). Additionally, steroids may exert effects on STARD1. Glucocorticoids for

Table 1. Hormones and growth factors regulating the STARD1, CYP11A1, and HSD3B genes in Leydig, granulosa and luteal cells

	Leydig Cells			Granulosa/Luteal Cells			
Factor	**STARD1**	**CYP11A1**	**HSD3B**	**STARD1**	**CYP11A1**	**HSD3B**	**References**
Gonadotropins							
LH/hCG	+	+	+	+	+	+	(22,120-122,129,155,177,178)
FSH				+	+	+	(34,35,125,179-182)
Growth Factors							
IGF-1	+	+		+	+	+	(8,10,11,22,146,179,183-185)
IGF-2				+			(184)
Insulin		+		+	+		(177,183,184,186)
EGF	+			-	+		(8,187,188)
FGF	+						(8)
Amphiregulin				+	+	+	(189)
GDF-9				+/-	-		(190-192)
Steroids							
Glucocorticoid	-	-	-				(119,193)
Progesterone	+				+		(194,195)
Testosterone			-				(119,196)
Cytokines							
TNFalpha	-	-	-	-	-		(197-203)
LIF	-						(204)
Interferon gamma	-	-		-			(205-207)
TGFbeta				+/-	+/-	+/-	(208-210)
TGFalpha	+	+					(8,118)
IL-1	+	-	-				(8,202,211)
BMP-2				-		-	(212,213)
BMP-4 and -6				-	-	-	(212-215)
BMP-5				-			(216)
BMP-7				-			(213,217)
BMP-15[1]				-	-	-	(218)
Others							
Activin A	+			-	+/-	-	(192,219,220)
Leptin[2]	-	-		+/-			(92,221)
GnRH I/II	+	+	+				(222)
PGF2alpha				-	-	-	(23,28,105,223,224)
PGE2				+	+	+	(225)
Thyroid hormone	+/-	+					(226,227)
Prolactin[2,3]	+/-				+	+/-	(131,228)

LH/hCG: Luteinizing hormone/human chorionic gonadotropin, FSH: follicle-stimulating hormone, IGF: insulin-like growth factor, EGF: epidermal growth factor, FGF: fibroblast growth factor, GDF-9: growth and differentiation factor-9, TNF: tumor necrosis factor, LIF: leukemia inhibitory factor, IL-1: interleukin 1, BMP: bone morphogenic protein, PGF2: prostaglandin F2, PGE2: prostaglandin E2, [1]BMP-15 repression most likely due to its suppression of FSH receptor, [2]Responses in ovarian cells are dose-dependent, [3]Responses in Leydig cells may be due to alteration of LH receptor

instance utilize the glucocorticoid receptor to suppress steroid production and STARD1 expression by rat progenitor Leydig cells (13). Table 1 presents a summary of regulators of Leydig cell STARD1.

Rat and human Sertoli cells in the testis also express low levels of STARD1 mRNA (14,15), which can be increased by cAMP and FSH (14,16) and decreased by interleukin 1beta (IL-1beta) (17). Since there is no detectable P450scc basally, the role of StAR in this cell type is unclear.

As with Leydig cells, LH and mimetics like hCG and cAMP analogs induce STARD1 gene expression in steroidogenic cell types in the ovary (18-24). Known regulators of STARD1 in theca cells include LH (20,21), stem cell factor (SCF) in combination with IGF-1 (25), and insulin alone or in combination with LH (21).

In general, the granulosa cell layers do not express StAR until the LH surge and the protein is maintained in corpus lutea (18,19,26,27). Unlike Leydig cells, activation of PKC by factors like prostaglandin F2alpha (PGF2alpha) or phorbol esters in luteal cells decreases STARD1 mRNA levels (23,28). In some granulosa cell culture models, phorbol ester can either stimulate or inhibit STARD1 expression (29,30). However, phorbol ester in combination with the adenylyl cyclase agonist forskolin mimics LH stimulation of granulosa cells from rat preovulatory follicles to increase STARD1 mRNA (31). Vasoactive intestinal peptide (VIP) which activates both PKA and PKC pathways also robustly stimulates STARD1 mRNA levels in immortalized KK1 and primary mouse granulosa cells (32). As in Leydig cells, some hormones only serve to augment the action of other hormones. For example, in granulosa neuregulin increases amphiregulin induction of STARD1 but has no activity by itself (33).

Known regulators of STARD1 in granulosa and luteal cells are summarized in Table 1. Note that most of the studies have been performed in culture, where granulosa cells in particular have a wide variety of phenotypes, ranging from less differentiated to advanced luteinized cells. This fact is especially relevant to studies of FSH in cultured granulosa. While it is widely accepted

that endogenous FSH does not stimulate STARD1 *in vivo*, it does so in primary cultures by mimicking LH activation of the cAMP-signaling pathway (18,19,34,35).

3.2. Transactivators

The proximal 5′-flanking region of the STARD1 gene has a high degree of homology between species with the first 100 bp upstream of the transcriptional start site of human exhibiting 87%, 86%, 84%, and 80-82% with pig, cow, sheep, and rodents, respectively (36). Research on the STARD1 gene reveals that key regulatory elements of the promoter reside between -119 to -58 bp relative to the transcription start site (36). Numerous transcription factors including CCAAT/enhancer binding protein beta (C/EBPbeta), NR5A, NR4A and cAMP-response binding protein (CREB) family members affect gene transactivation within this proximal region. Other important elements lie considerably more distant.

3.2.1. Steroidogenic Factor 1 (SF-1/NR5A1) and Liver Receptor Homologue 1 (LRH-1/NR5A2)

Members of the NR5A family represented by SF-1 and LRH-1 are essential regulators of steroidogenic tissue development and function, including through their effects on STARD1 expression. The human STARD1 promoter contains NR5A-binding sites at -105 to -95, -42 to -35 and -926 to -918 (37). One or more sites are required for maximal basal and/or cAMP-stimulated promoter activity in Leydig and luteinized granulosa cells in culture (37-41). Mutation of the homologous -105 to -95 site or a unique site at -137 to -132 in the mouse slashes basal and EGF-stimulated responses in Leydig cells (12). The proximal NR5A sites do not participate in STARD1 promoter activity in less differentiated granulosa cells (35,40,42), but gain this function with luteinization. Correspondingly, mutagenesis of the -105 to -95 element reduces promoter activity in granulosa-luteal cells (40). Recently, three additional functional NR5A recognition elements were identified between -3400 to -3000 bp in the human promoter which may be relevant at least in granulosa cells (43).

Gonadal regulation of STARD1 from the NR5A elements often involves SF-1 (44,45). Global deletion of SF-1 ablates StAR expression in the murine testes (46). Studies in mouse Leydig tumor cell lines collectively indicate that overexpression of SF-1 increases STARD1 promoter basal transcriptional activity as well as responses to cAMP analog alone or in the presence of IGF-1 (10,47).

The contribution of fellow NR5A member LRH-1 in STARD1 regulation is yet to be fully discerned. Separate studies indicate that an ovulatory stimulus of hCG in wild-type mice recruits SF-1 (47) and/or LRH-1 (48) to the endogenous STARD1 promoter in ovarian tissue. In general, the specific NR5A family member that binds to the STARD1 promoter may change according to tissue type and differentiation state of the cell. Like SF-1, LRH-1 transactivates the STARD1 promoter in clonal and human granulosa tumor cells, likely through the -105 to -95 site (49,50). Targeted loss of LRH-1 in granulosa cells disrupts STARD1 mRNA levels and progesterone production in hCG-treated transgenic mice, causing a failure to ovulate (48). In the corpus luteum, LRH-1 expression exceeds that of SF-1 (51-53), suggesting luteal STARD1 transcription is likely regulated by LRH-1. Indeed, LH increases the association of LRH-1 with the bovine STAR promoter in luteal cells (54).

3.2.2. Nur77

The STARD1 promoter contains an NR4A or NGFI-B response element (NBRE), that coincides with the -105 to -95 NR5A site (55). This element is bound by the most abundant NR4A protein in steroidogenic cells, Nur77 (NGFI-B/NR4A1) and cAMP stimulation of Leydig cells recruits Nur77 to the portion of the STARD1 promoter containing this element (55).

Expression of Nur77 activates the murine STARD1 promoter to various degrees in Leydig tumor cells (55-57). Stimulation of second messenger pathways utilized by LH upregulates Nur77 in rodent primary Leydig cells and Leydig tumor cells (55,58) but not SF-1. These data raise the question: are the effects of cAMP and EGF on this promoter element actually due to Nur77 and not SF-1 (12,55)? One clue is provided by Martin and Tremblay who found that mutation of the NBRE/NR5A site specifically decreases stimulation by Nur77 in MA-10 cells, while mutation of the other NR5A sites solely affects SF-1-mediated transactivation (59). They further found that suppression of cAMP stimulation by dexamethasone may involve loss of binding of the -95 site by Nur77, not SF-1 as indicated by promoter and chromatin immunoprecipitation (ChIP) assays (60).

While Nur77 may displace NR5A protein binding to the NRBE, SF-1 can act at the other NR4A-binding sites to additively increase STARD1 gene expression in Leydig cells (59). At the same time however, Nur77 can potentially interfere with SF-1 transactivation by promoting the expression of the SF-1 repressor protein, gonadotropin-inducible ovarian transcription factor-1 (GIOT-1) (61). Transactivation of the murine gene by Nur77 is itself sensitive to inhibition by androgen receptor corepressor-19 kDa (ARR19) (62).

Nur77 and other NR4A proteins are also reported in cAMP and phorbol ester-stimulated human granulosa tumor cells, human corpora lutea and rat granulosa cells in post-surge preovulatory follicles (63-65). Like StAR, Nur77 is responsive to cAMP-stimulation in theca cells (66). Androgen and STARD1 mRNA production in human theca cells increases with Nur77 overexpression and declines with reduced endogenous Nur77 levels (66). However, existing data in ovarian cells support SF-1 activation of the STARD1 promoter through the proximal site rather than Nur77 (45,63).

3.2.3. GATA family

Recombinant GATA4 and GATA6 bind a highly conserved GATA recognition motif in the STARD1 promoter, located at -63 to -58 in the human and -66 to -61 in the mouse (36). Both proteins are expressed in somatic steroidogenic cells of the ovary and testis where they can

influence StAR expression (67). The GATA site contributes to maximal responsiveness of the murine promoter to cAMP in MA-10 Leydig tumor cells (39), FSH in cultured granulosa cells (35,42) and EGF in the mouse Leydig tumor cell line mLTC-1 as well as basal activity (12). Further studies in a heterologous cell line indicate that GATA6-driven STARD1 promoter activity synergizes with SF-1 and is vulnerable to DAX-1 repression (68).

Treatment of MA-10 cells with cAMP analog recruits GATA4 to the endogenous STARD1 proximal promoter (47). There, GATA4 can increase murine STARD1 promoter activity by itself and augment stimulation by cAMP analog or cAMP analog and IGF-1 as shown in mLTC-1 cells (10,69). Co-expression with the homeobox transcription factor distal-less homeobox 5 (Dlx5) further enhances GATA4-mediated transactivation (69).

Similarly, both exogenous GATA4 and GATA6 can increase porcine STARD1 promoter activity in granulosa cells through a -70 to -64 GATA site (42,70). Granulosa cells collected from mouse periovulatory follicles following an ovulatory dose of hCG display increased association of GATA4 with the proximal promoter simultaneous to the induction of STARD1 expression (47). Luteinization sees GATA4 levels decline *in vivo* (70-73). The persistence of GATA6 expression suggests that GATA6 takes over for GATA4 in promoting STARD1 gene transcription in luteal cells. Interestingly, reduction of GATA4 levels in luteinizing porcine granulosa cells increases the level of cAMP-stimulated STARD1 mRNA, but not when GATA6 is also lowered (74). These data together indicate that the GATA family member that drives the STARD1 promoter changes with differentiation and that GATA6 may be a more potent inducer of STARD1 expression.

3.2.4. C/EBPbeta

The proximal STARD1 promoter harbors a highly conserved C/EBP recognition sequence located at -119 to -110 in the human, denoted C1 in the mouse (36). Other more proximal sites exist that vary in location, number and sequence by species. For instance, the human gene possesses a C/EBP-binding site at -50 to -41 (75), whereas a -58 to -49 site in the porcine promoter which is conserved in ruminants is recognized by C/EBPbeta in porcine granulosa cell nuclear extracts (42).

Mutational analysis of C1 and a second downstream C/EBP site, C2 in cAMP analog-stimulated MA-10 cells and C1 in EGF-stimulated mLTC-1 cells indicate that C/EBP proteins do not drive the cAMP-responsiveness of the promoter, but rather increase the size of basal and stimulated transactivation by other factors (12,76). In fact, exogenous expression of C/EBPbeta in mLTC-1 cells solely alters the magnitude, not the relative response of promoter activity to cAMP analog with or without IGF-1 (10) and the level of C/EBPbeta found at the endogenous STARD1 promoter in MA-10 cells is unchanged by stimulation with a cAMP analog (47).

For periovulatory granulosa cells in the mouse, C/EBPbeta is recruited to the endogenous proximal promoter upon initiation of differentiation to a luteal cell phenotype via stimulation of the cAMP pathway, such as by hCG administration *in vivo* (47). Targeted deletion of both C/EBPalpha and beta in granulosa cells of mice drastically reduces STARD1 induction by combined forskolin and phorbol ester in culture (77). Mutagenesis studies indicate that basal promoter activity with luteinization using rat granulosa-luteal cells partly relies on the C1 site (40). Both C/EBP elements in human luteinized granulosa cells are essential for maximal basal and cAMP-stimulated STARD1 promoter activity but dispensable for the fold-response to cAMP (75).

Current models suggest that C/EBPbeta may complement or recruit SF-1 at the STARD1 promoter, possibly through physical interactions as indicated by *in vitro* studies (76). Promoter assays in a heterologous epithelial-like cell line indicate that SF-1-mediated transactivation of the murine promoter requires both C1 and C2 (76).

Studies using porcine granulosa cells indicate that C/EBPbeta, may also coordinately work with GATA proteins. The magnitude of porcine promoter activity in response to FSH with or without IGF-1 in this cell type relies on a proximal C/EBP site in conjunction with the neighboring GATA element (42). Overexpression studies in porcine granulosa and clonal cell types reveal cooperation between C/EBPbeta and GATA4 to elicit STARD1 promoter activity (42,73). A physical interaction between the proteins is described in MA-10 cells (78). Altogether, the data draw the picture of a transcription factor that supports basal and stimulated STARD1 gene expression in coordination with NR5A and GATA factors.

3.2.5. CREB family

The sensitivity of the STARD1 promoter to cAMP is partly due to the presence of cAMP-response element (CRE) half-sites. Three half-sites, referred to as CRE1, CRE2 and CRE3, populate the -110 to -67 region of the murine promoter (79) and are recognized by CRE-binding proteins (CREB) (80). Of these sites, the -81 to -78 CRE2 contributes the most to cAMP-responsiveness of the promoter in MA-10 cells (41,80).

This region is important for the STARD1 promoter response to FSH in rat granulosa cells (35). While CRE2 encompasses a rodent-specific -81 to -72 C/EBP element (C3) (35), selective nucleotide mutagenesis points to CREB not a C/EBP protein as the crucial factor involved in this region (40). While CREB family member CRE modulator CREM-tau can mimic the action of CREB, current evidence indicates that CREB is the family member that binds CRE2, perhaps as a heterodimer with activating transcription factor ATF-1 (80,81).

The binding of CREB to this region is apparently cell-type specific, owing to a one base-pair overlap of CRE2 with C2 characterized in ChIP assays with MA-10 and preovulatory granulosa cells purified from hCG-treated mice (47). Upon cAMP stimulus of MA-10 cells, the level of CREB/CREM but not C/EBPbeta associated with the

proximal promoter increases within 30 minutes. A separate study suggests that only phosphorylated not total CREB increases (81). For granulosa cells, the concentration of C/EBPbeta associated with this region increases within one hour of hCG (47). Association of CREB with the proximal promoter rises after a delay, suggesting C/EBPbeta binding to C2 is more relevant in this cell type.

As suggested by these studies, exogenous expression of CREB in MA-10 cells elevates basal activity as well as the size and fold of the response to cAMP analog of the murine promoter; inhibitors of CREB as well as a nonphosphorylatable CREB blunt promoter activation (41,80). Results in steroidogenic adrenocortical cells indicate that longer term stimulation of STARD1 could involve unphosphorylated CREB complexed with transducer of regulated CREB activity (TORC) (82). On the other hand, the high basal promoter activity observed in more differentiated rodent granulosa-luteal cells is immune to CREB inhibition (40).

Like C/EBPbeta, CREB cooperates with other transcription factors to promote STARD1 expression. CREB and SF-1 additively increase cAMP-stimulated promoter activity in MA-10 cells (41). Combinations of mutations of SF-1 and CRE sites or of the shared base between CRE1 and the overlapping SF-1 site reduce basal and cAMP analog-induced activity (80). GATA4 and CREB also cooperate through CRE2 as demonstrated by mutational analysis (35,40).

3.2.6. Activator Protein 1 (AP-1)

The effects on STARD1 by AP-1 Jun and Fos related proteins are apparently limited to rodents through a conserved order-specific AP-1-binding or TPA response element (TRE) at -81 to -75 (83,84). Steroidogenic stimuli such as phorbol ester (*e.g.*, phorbol-12-myristate-13-acetate (PMA)), cAMP analog, hCG and to some extent, EGF elevate levels of c-Fos and Jun proteins in Leydig and MA-10 cells (85,86). Fos and Jun proteins bind the TRE in nuclear extracts from cAMP analog-stimulated MA-10 cells (83).

Basal promoter activity in various Leydig tumor cell lines is enhanced by Jun proteins and FosB and generally inhibited by Fos-related antigen (Fra) 1 and Fra-2 (83,84). c-Fos may slightly elevate or inhibit activity in a dose-dependent manner (12,59,83,84). The most potent AP-1 factor is c-Jun, whose loss ablates basal promoter activity (10,83).

Stimulation by EGF is largely independent of c-Jun and JunD but is attenuated by c-Fos and Fra-1 (12). However, c-Jun increases PMA-stimulated promoter activity (86). FosB and c-Jun proteins elevate cAMP-induced promoter activity in transfected Leydig tumor cells while c-Fos, Fra1 and Fra2 are inhibitory (10,83,84). A dominant negative c-Jun dampens stimulation by cAMP analog or PMA (10,86). The level of phosphorylated c-Jun affiliated with the promoter increases with either treatment (81,84,86). Similarly, the levels of c-Fos and phosphorylated c-Fos protein as well as the amount of phosphorylated c-Fos at the proximal promoter rise with cAMP stimulation (84).

Characterization of the roles of AP-1 proteins and CREB in the mouse is complicated by the complete overlap of the TRE and CRE2 sites. Unlike CREB, AP-1 factors help determine the size of the cAMP response but not the response itself. Indeed, both basal and stimulated promoter activity generally decline with mutation of CRE2/TRE (12,39,76,81,83). However, binding to the endogenous promoter by AP-1 and CREB with cAMP stimulation changes over time as observed in MA-10 cells (84). At first, levels of phosphorylated c-Jun, c-Fos and CREB associated with the region encompassing the CRE2/TRE site increase. Later, these levels of phosphorylated c-Jun and c-Fos decline, while phosphorylated CREB remains high (84).

c-Jun also interacts with other transcription factors, enhancing for example promoter transactivation by NR5A proteins through the proximal -45 SF-1 element in MA-10 cells (59). c-Jun increases promoter stimulation by Nur77 even when the CRE2/TRE site is mutated, indicating that it recognizes a second site in the promoter or directly interacts with the NR4A factor (59).

Interestingly, co-expression of CREB and c-Jun or c-Fos blunts increases in basal and cAMP-stimulated activity elicited by either factor alone (84). One explanation is that the formation of transactivating complexes is disrupted by the competition of AP-1 proteins and CREB for the restricted amounts of the cofactor CREB-binding protein (CBP) (81,84).

Unlike Leydig cells, the murine CRE2/TRE element may solely be bound by CREB in rat granulosa cells. Differentiation to a luteal cell phenotype is accompanied by a change in the transcription factor binding to this site, where Fra-2 now displaces CREB to foster cAMP-independent transcription (40). Furthermore, promoter activity is suppressed by a dominant negative form of Fra2/Fos in luteal cells but not in less differentiated granulosa cells.

3.2.7. Intermediaries of oxysterol regulation

Oxysterols play an integral role in StAR regulation as observed in several cell types (87-89). It may partly involve sterol regulatory element binding protein (SREBP) family transcription factors which are activated under conditions of sterol depletion and promote the synthesis of sterol biosynthetic and lipogenic enzymes. Surprisingly, overproduction of SREBP-1a, SREBP-1c and variably SREBP-2 drives production of sterol-depleting STARD1 mRNA in human granulosa-derived or heterologous cells (87,90-93). *In vitro* studies indicate this upregulation at least for SREBP1a is realized through an SRE corresponding to -81 to -70 in the human that overlaps the C3 site in the rodent (90,91). Exogenous expression of SREBP1 augments cAMP analog stimulation (10). Mutation of a putative more proximal SRE immediately downstream of the GATA site in the murine promoter decreases basal and EGF-stimulated activity in mLTC-1

cells (12). Nuclear transcription factor Y (NF-Y) and SF-1 enhance recognition of a distal -740 to -731 SRE in the rat by SREBP-1 *in vitro* and promoter activity evoked by SREBP-1a in nonsteroidogenic cells (93).

Importantly though, endogenous SREBP has not been shown to increase STARD1 gene expression under the low sterol conditions that promote SREBP cleavage and translocation to the nucleus. Conversely, the presence of high levels of oxysterols, which inhibit SREBP activation, increases STARD1 mRNA levels in MA-10 cells (88). Thus, the involvement of SREBP proteins in transactivation of the STARD1 gene remains unclear.

The role of oxysterols may be more complicated. Not only does StAR transfer cholesterol for pregnenolone production into the mitochondria, but it similarly elicits the production of the liver X receptor (LXR) ligand 27-hydroxycholesterol (87,94) by a resident mitochondrial hydroxylase. StAR-dependent 27-hydroxycholesterol generation offers a means of feedback regulation through the activation of LXR (95).

The mouse promoter harbors an LXR response element (LXRE) at -200 to -185 (96,97). Disruption of LXRalpha/beta expression in null mice or by RNAi in an adrenocortical cell line increases STARD1 mRNA levels (96,97). On the other hand, the promoter responds positively to LXR expression in co-transfection experiments in nonsteroidogenic cells (97) and STARD1 and SREBP1c rise with LXR agonist in the murine ovary and testis but not in LXRalpha/beta null mice (98,99). The accompanying rise of SREBP1c points to a potential explanation for the results of the SREBP overexpression studies. Notably, 27-hydroxycholesterol, which inhibits SREBP-regulated LDL receptor promoter activity, has no effect on STARD1 promoter activity in human granulosa-derived and heterologous cells cultured in delipidated media (90). Thus, LXR control of STARD1 expression appears to be mulitfactorial, perhaps involving heterodimer formation with retinoid X receptor (RXR) alpha (97). Indeed, retinoids upregulate STARD1 mRNA and promoter activity in K28 Leydig cells (100) and human theca cells (101). Whether LXR binds the promoter in the context of gonadal cells has not been determined, although LXRalpha has been shown to associate with the promoter in the adrenal (97).

3.3. Transrepressors

3.3.1. Dosage-sensitive sex reversal, adrenal hypoplasia congenita, critical region on the X chromosome, gene 1 (DAX-1/NR0B1)

The actions of SF-1 in steroidogenic tissues is opposed by DAX-1 (102). Rat R2C Leydig tumor cells maintain low levels of DAX-1 and consequently express robust levels of StAR and steroid (103). Exogenous expression of DAX-1 suppresses StAR and steroidogenesis in R2C cells as well as basal and forskolin-activated promoter activity and endogenous STARD1 gene expression in other clonal cell lines (102-105). Furthermore, DAX-1 suppresses the activation of the STARD1 promoter by Nur77 and NR5A proteins in various gonadal cell lines (50,56,106). Silencing of its expression via RNAi increases basal and cAMP analog and phorbol ester-stimulated StAR and steroid production in MA-10 cells (56).

Although a nuclear receptor, DAX-1 does not recognize a specific DNA sequence; it may instead act by binding DNA hairpin structures. Hairpins in the STARD1 promoter may form within -61 to -27 for the human (102) and -44 to -20 in mouse (56). Murine STARD1 expression is inhibited by binding of the hairpin by DAX-1 in MA-10 cells as indicated by ChIP and *in vitro* DNA binding assays (56). Other studies insist that DAX-1 represses Nur77, SF-1 and LRH-1 via uncharacterized direct protein-protein interactions (107,108).

Inhibitors of steroidogenesis may act through DAX-1. In the rat ovary, a luteolytic factor and inhibitor of luteal steroid production, PGF2alpha, simultaneously increases DAX-1 while decreasing STARD1 mRNA (105). Conversely, steroidogenic activators antagonize DAX-1 expression. Stimulation of the promoter by cAMP analog or phorbol 12-myristate 13-acetate is linked to reduced DAX-1 association with the STARD1 promoter and increased promoter activity in MA-10 cells (56).

This said, controversial evidence exists for a coactivator function for DAX-1 on SF-1 activity in MA-10 cells. Here, basal STARD1 transcription may rely on DAX-1 at least in part through recruitment of steroid receptor RNA activator (SRA) (109).

3.3.2. Kruppel family

The pleiotropic GLI-Kruppel family member Yin Yang-1 (YY1) associates with a site -1382 to -1193 bp upstream of the transcription start site and inhibits rat promoter activity in rat luteal cells (110). In the rat ovary, PGF2alpha increases YY1 levels, promotes YY1 binding to the upstream site and likely YY1-associated recruitment of histone deacetylase 1 (HDAC1), coinciding with a sharp decline in STARD1 mRNA (91,105,110). Silencing of YY1 expression blocks the effect of PGF2alpha on STARD1 expression (110).

This transcription factor also inhibits SREBP-1a binding and activation in clonal cells (91), possibly independent of a direct interaction between the two proteins (110). In the human promoter, the proximal SREs overlap a nonconserved YY1 binding site that lies within -90 to -65 (90). Accordingly, SREBP-1a-sponsored promoter activity in clonal cells is enhanced by selective YY1 site mutations (90).

Promoter activity is further suppressed by other Kruppel-like factors (KLFs), specifically KLF4, 9 and 13, in luteinized porcine granulosa cells (111). At the same time, endogenous STARD1 mRNA levels are elevated by lentiviral expression of KLF13 in these cells, making the role of KLF13 a target for future investigation.

3.4. Other mediators of STARD1 transcription

The regulation of the STARD1 gene includes many other components. The ubiquitous transcription

factor Sp1 stimulates human promoter activity in coordination with SF-1 in a steroidogenic cell line (112) and murine promoter activity in the absence or presence of a cAMP analog in mLTC-1 cells (10). Additionally, mutation of the murine -146 to -137 Sp1 site cuts the size of basal activity and the response to EGF in Leydig cells (12). Other positive regulators include NF-Y in clonal cells (91), beta-catenin in coordination with LRH-1 in bovine luteal cells (54) and PPARgamma agonist in MA-10 Leydig cells and KK1 immortalized granulosa cells (113).

A second Sp family member, Sp3, alternatively utilizes the -180 and -150 bp region to recruit HDACs and repress basal murine promoter activity in MA-10 cells (114). Ovarian factor forkhead transcription factor L2 (FOXL2) also represses STARD1 transactivation through an element within the first -95 bp of the human promoter (115) following sumoylation (116). FOXO1 is also implicated in tonic inhibition of StAR and progesterone production by rodent granulosa cells prior to the LH surge (117).

4. THE CYP11A1 GENE

4.1. Hormonal control

As with STARD1, CYP11A1 gene expression is primarily regulated by gonadotropins in gonadal steroidogenic cells. A summary of the positive and negative regulators of CYP11A1 mRNA levels in Leydig, granulosa and luteal cells appears in Table 1. Some differences in CYP11A1 expression are observed between immature and mature Leydig cells. Immature Leydig cells generate more CYP11A1 mRNA in response to TGFalpha in the rat (118), whereas mature cells possess high basal levels of CYP11A1 mRNA that rise at a slower pace in response to cAMP than STARD1 (119-122). In contrast to STARD1, CYP11A1 is basally absent in Sertoli cells, but does appear in immature cells with chronic FSH or cAMP analog treatment (16).

Growth hormone augments IGF-1 stimulation of CYP11A1 in porcine granulosa cells but has no effect alone (123). Theca cell expression of CYP11A1 mRNA is regulated by LH, appearing with antrum formation and increasing during follicular development (18,19,26,124-126). CYP11A1 promoter activity is further upregulated by IGF-1 in rat theca cells and mRNA levels are opposed by BMP-4, -6 and -7 in bovine theca cells (25,126,127). CYP11A1 expression is also stymied by GDF-9 in bovine theca cells (128).

Levels of CYP11A1 rise with the ovulatory LH surge in granulosa and theca cells. In primates the corpus luteum of the menstrual cycle also depends on LH (129). On the other hand during pregnancy, continued expression of CYP11A1 mRNA levels in luteal cells depends on rescue of the corpus luteum by species-specific luteotrophic factors including hCG in humans and prolactin in rodents (130,131). Once induced in the pregnant rat, luteal CYP11A1 is constitutively expressed (125).

4.2. Transactivators

The upstream 4.4 kb of the human gene is sufficient to replicate the pattern of endogenous expression in transgenic mice, albeit incompletely in granulosa cells (132). Responsiveness to steroidogenic stimuli is primarily determined by elements within the first 120 bp (36,133). This first 95 bp upstream of the transcriptional start site of human shares approximately 72%, 72%, 68%, 68%, and 64% with the corresponding sheep, pig, cow, mouse, and rat regions, respectively, with conserved NR5A and Sp1 sites (36).

4.2.1. NR5A family

A NR5A binding element appears within -50 to -43 (SCC1) in the rat and -46 to -38 in the human that recognizes recombinant LRH-1 and SF-1 in granulosa and luteal cell extracts (134,135). Studies using transgenic mice that express a lacZ fusion gene under the control of the human promoter indicate that CYP11A1 expression relies on this proximal NR5A site (136). Further such studies suggest that testicular expression of human CYP11A1 also requires another element that is unconserved and lies within an upstream cAMP-responsive sequence at -1617 to -1609 (136). Basal and cAMP-inspired bovine promoter activity in luteal cells involves an NR5A site lying within -57 to -32 (137,138).

Murine generation of CYP11A1 is less dependent on the proximal NR5A-binding element. While *in vitro* studies with granulosa cells suggest that forskolin-stimulated rat promoter activity partially relies on SCC1 (134), loss of the site in the endogenous gene in the mouse principally results in reduced steroidogenesis in the testes and adrenal without discernable effects on the ovary or reproduction (139). A second site at -79 to -71 (SCC2) appears to be uninvolved. Mutation of both sites reduces basal activity with little effect on the cAMP-stimulated response in MA-10 cells (140). However, CYP11A1 expression is reliant on NR5A proteins through some mechanism, as cytochrome P450scc content is reduced in testes from SF-1-knockout mice and targeted deletion of LRH-1 in granulosa cells sharply lowers CYP11A1 mRNA levels in hCG-treated mice (46,48). Correspondingly, mRNA levels are responsive to SF-1 overexpression in bovine theca cells as is rat promoter activity in K28 cells (57,141). Human promoter activity is similarly affected by SF-1 and LRH-1 in granulosa and heterologous cells (49,63,135).

4.2.2. GATA family

Studies on the role of GATA proteins on CYP11A1 gene expression are still in their infancy. Several putative GATA sites are identifiable *in silico* in the first 1000 bp of the CYP11A1 promoter in the human, cow, pig and rodents with the number and location of the sites being unconserved (data not shown). A site between -475 to -470 in the murine promoter recognizes GATA4 using nuclear extracts of rat granulosa cells and mediates FSH stimulation of the promoter (142). Silencing of GATA4 and GATA6 by RNAi modestly lifts basal CYP11A1 mRNA levels in luteinizing porcine granulosa cells with no effect on its

response to cAMP, suggesting GATA is not important for the cAMP-responsiveness of the CYP11A1 gene in this context (74). Overexpression of either GATA protein can drive human promoter activity in non-gonadal cell lines (68,143,144).

4.2.3. Sp family

A G-rich stretch in the proximal promoter harbors at least one Sp1 site. In the bovine promoter, Sp1 and/or Sp3 bind the -118 to -100 region in luteal nuclear extracts (138,145). Basal and cAMP-stimulated promoter activity in luteal cells relies on cooperation between this region and the proximal NR5A site as indicated by mutagenesis studies (138).

An IGF-1 response element (IGFRE) that contains a functional Sp1-binding site lies within -130 to -100 of the porcine promoter. Forskolin and IGF-1 drive promoter activity in porcine granulosa cells through this IGFRE (146). The 3'-end of the element recognizes Sp1 while the 5' end binds polypyrimidine tract-binding protein-associated splicing factor (PSF) (147). Basal promoter activity in granulosa cells is elevated by Sp1 and decreased by PSF, which also opposes Sp1 induction (147,148). The ability of IGF-1 to drive porcine promoter activity in steroidogenic Y1 adrenocortical cells is reliant on exogenous expression of Sp1 or mutation of the PSF-binding site (147).

4.3. Transrepressors

Endogenous gene expression and forskolin-stimulated human promoter activity in Y1 cells are opposed by DAX-1 (104). This factor further stymies human promoter activation by LRH-1 in granulosa cells as well as by GATA6 and SF-1 in nonsteroidogenic cells (68,135). However, evidence surprisingly exists for a basal coactivator role for DAX-1 in MA-10 cells (109). Basal promoter activity and endogenous mRNA levels are reduced as well by KLF13 overexpression in luteinized porcine granulosa cells (111). KLF4 and 9 have similar effects on the CYP11A1 promoter. As with STARD1, FOXO1 may also repress CYP11A1 expression in rodent granulosa cells prior to the LH surge (117).

4.4. Other mediators of CYP11A1 transcription

Reduction of Nur77 levels reduces CYP11A1 mRNA levels in human theca cells while increased expression elevates them (66). Rat CYP11A1 gene expression is increased by Nur77 in K28 cells, but the protein has little effect on promoter activity in human granulosa tumor cells (57,63). A multi-functional CRE half-site exists at -450 to -447, which like the murine STARD1 CRE2/AP-1 site, binds CREB-1 in less differentiated rat granulosa cells and Fra-2 with more differentiation (142).

Conflicting data from cell culture and knockout mouse models muddy current understanding of LXR involvement in CYP11A1 gene transcription (96,97). Ovarian but not testicular CYP11A1 mRNA levels rise in response to LXR agonist for the mouse (98,99), but ovarian hyperstimulation in LXRalpha/beta null mice causes abnormally high mRNA levels and cyst formation in ovaries, suggesting LXR restrains CYP11A1 gene expression (98).

Select retinoids stimulate gene expression and promoter activity in human theca cells through a putative retinoic acid receptor binding element (RARE) (101), or heterodimerization with known existing regulators such as LXR. Further elements that specify expression in Leydig cells may lie within -2.5 to -5 kb in the mouse promoter (5).

5. THE HSD3B GENES

5.1. Hormonal control

While many species possess several genes encoding for 3beta-HSD, the protein generated in gonadal steroidogenic cells generally arises from the HSD3B2 gene in humans and HSD3B1 in other species -- collectively referred to herein as HSD3B (4,149). The mouse and human HSD3B promoters bear little similarity, while those of many species remain uncharacterized, making progress on the study of its regulation more difficult. The gene is expressed in Leydig cells, the theca in developing follicles, luteal cells and late follicular phase granulosa cells, except in the bovine where expression is observed in granulosa developmentally earlier (5,150-152). LH is a major regulator of HSD3B and is mimicked by cAMP-signaling agonists (119,129,153-159). Many other factors affect 3beta-HSD protein levels or enzymatic activity in gonadal cells, however, in most cases their effects on HSD3B mRNA have not yet been examined (4). Glucocorticoid receptor-mediated suppression of HSD3B is observed in rat progenitor Leydig cells (13). A summary of the known regulators of HSD3B mRNA in Leydig, granulosa and luteal cells is shown in Table 1.

While IGF-1 elevates HSD3B mRNA levels in rat theca cells, insulin, IGF-1 and IGF-2 only affect expression in human thecal tumor cells through enhancement of the response to forskolin (158,160). Basal mRNA levels in theca cells decline with BMP-4, -6 and -7 (127).

5.2. Transactivators and transrepressors

5.2.1. NR5A family and DAX-1

A phorbol ester-sensitive region in the human promoter includes an NR5A-binding element at -64 to -56 as shown in non-gonadal steroidogenic cells (161). SF-1 putatively drives basal promoter activity through this site in human granulosa tumor and non-gonadal cell lines (53,63,162) while LRH-1 by itself or additively with cAMP analog increases human promoter activity in human granulosa tumor cells (53). Other sites at -315 to -309 and -906 to -900 recognize LRH-1 in human granulosa tumor and luteinized granulosa cell extracts. Mutation of either site detrimentally affects the response to LRH-1 in the former cell type. Stimulation of the promoter by LRH-1 is also opposed by DAX-1 (53).

Still, NR5A family members may only play a minor role in HSD3B gene regulation *in vivo*. Granulosa

cell expression of HSD3B is unaffected by granulosa-cell targeted loss of LRH-1 in hCG-treated transgenic mice (48). While promoter activity in MA-10 cells is insensitive to SF-1 and LRH-1 (162), mutational analysis does point to a requirement for the NR5A site for full basal promoter activity (163).

5.2.2. GATA family

The promoter responds to both GATA4 and GATA6 overexpression in adrenal cell lines and GATA4 alone in MA-10 cells (162). As well, co-expression of either GATA4 or GATA6 synergistically increases NR5A protein-stimulated promoter activity in both kinds of cells. A -196 site in the human can account for promoter responsiveness to GATA factors (162). At least three other potential sites exist at -966, -570 and -364. RNAi studies reveal that GATA4 mediates basal mRNA production in luteinizing porcine granulosa cells while neither GATA4 nor GATA6 affect the response to cAMP (74).

5.3. Other mediators of HSD3B transcription

Overexpression of Nur77 in human granulosa tumor cells and Nur77, Nurr1 and Nor1 in MA-10 cells drive the human promoter through an NBRE at -133 to -125 (63,163). HSD3B levels in human theca cells rise with Nur77 overexpression and decline with reduced endogenous Nur77 (66). A protein constitutively expressed in the testis, hypoxia inducible factor (HIF) 1alpha recognizes a putative HIF response element at -1012 in the murine gene (164). Exogenous expression of HIF-1alpha transactivates murine HSD3B in a model immature Leydig cell line, TM3 cell.

In prolactin receptor-expressing nonsteroidogenic cells, prolactin instigates binding of Stat5 to a -118 to -110 recognition site in the human gene, which in turn activates the promoter (165). This study may model how prolactin regulates HSD3B expression in the rodent corpus luteum (166).

5.4. The hsd3b6 gene

Less is known about a second isoform of 3beta-HSD also found in adult rodent Leydig cells, hsd3b6 (167). Due to its homology to gonadal HSD3B (168), the results of work carried out prior to its identification in the testis could actually reflect changes in hsd3b6 gene expression. Because of this, the differential regulation of the two isoforms in the testis requires further study. Interestingly, transgenic mice lacking the LH receptor exhibit a selective loss of hsd3b6 mRNA in the testes, but not HSD3B (169). Accordingly, LH does induce murine hsd3b6 promoter activity in K28 cells (57). This induction is opposed by TNFalpha.

Unlike HSD3B, both SF-1 and Nur77 activate the murine hsd3b6 promoter in Leydig cells and are opposed by TNFalpha (57). TNFalpha further reduces hsd3b6 mRNA levels and steroidogenesis in R2C cells. Like STARD1, Nur77 transactivation of hsd3b6 is sensitive to inhibition by ARR19 (62).

6. GENERAL REGULATION OF TRANSACTIVATORS AND TRANSREPRESSORS

Acute activation of the transcription factors that impact STARD1, CYP11A1, and HSD3B transcription is regulated by the same second messenger pathways that regulate the genes themselves. The majority of work on the regulation of these transcription factors has been performed in the context of the STARD1 promoter, but findings from this research may equally apply to the CYP11A1 or HSD3B genes when transactivating factors and signaling pathways are in common. Most evidence reflects that their response to LH/hCG stimulation is governed by cAMP pathways. PKC, MAP kinases (ERK1/ERK2) and CAM kinase pathways play supplementary or modulatory roles in LH/hCG or growth factor signaling. These pathways can alter the abundance of transcription factors or their activity. In MA-10 Leydig cells cAMP agonist increases Nur77 and c-Fos protein levels (55,56,84) and decreases the level of DAX-1 (56), but has no effect on CREB, Sp1, C/EBPbeta, SF-1, c-Jun, GATA4 or SREBP-1 (29,84,170). Phorbol ester activation of PKC elevates protein levels of C/EBPbeta, c-Jun, c-Fos and Nur77 (29,55,56) and decreases DAX-1, but does not affect Sp1, SF-1, GATA4 or SREBP-1 (29). The combination of PMA and cAMP analog further enhances expression of C/EBPbeta, c-Jun and c-Fos over PMA alone. In murine granulosa cells, hCG, presumably acting via cAMP pathways, leaves CREB, SF-1 and GATA4 unchanged, while increasing C/EBPbeta (170). In MA-10 cells, Nur77 expression falls with DAX-1 overexpression (56). Inhibition of CAMK1 decreases forskolin-stimulated (PKA pathway) Nur77 protein levels (55).

These second messenger pathways also promote activating post-translational modifications of relevant transcription factors, such as GATA4 phosphorylation by PKA signaling (171). Phosphorylation of CREB, c-Jun and c-Fos by PKA and PKC correlates with their increased association with the STARD1 promoter (see above) (84). Cooperative phosphorylation of CREB also occurs when both kinases are active (29). Silencing of PKC alpha, delta, epsilon or mu (protein kinase D; PKD) in MA-10 cells reduces STAR protein and progesterone production (172). PKCzeta similarly regulates granulosa cell expression of Nurr77 in response to LH (65). The cause, at least in the case of PKD silencing, is traceable to reductions in the level of phospho-CREB, phospho-c-Jun and phospho-c-Fos associated with the STARD1 promoter following PMA stimulation (172). Phosphorylation of C/EBPbeta by ERK1/2 downstream of PKA signaling contributes to STARD1 expression in murine granulosa cells (173). On the other hand, activation of PKA inhibits phosphorylation by salt-inducible kinase (SIK) and nuclear export of TORC, permitting the cofactor to work with CREB to enhance STARD1 gene transcription (174).

In addition to post-translational regulation, activation of trophic signaling pathways also enhances interactions of relevant transcription factors with coactivators and corepressors. Ligand binding also plays a role for certain nuclear receptors. While a detailed

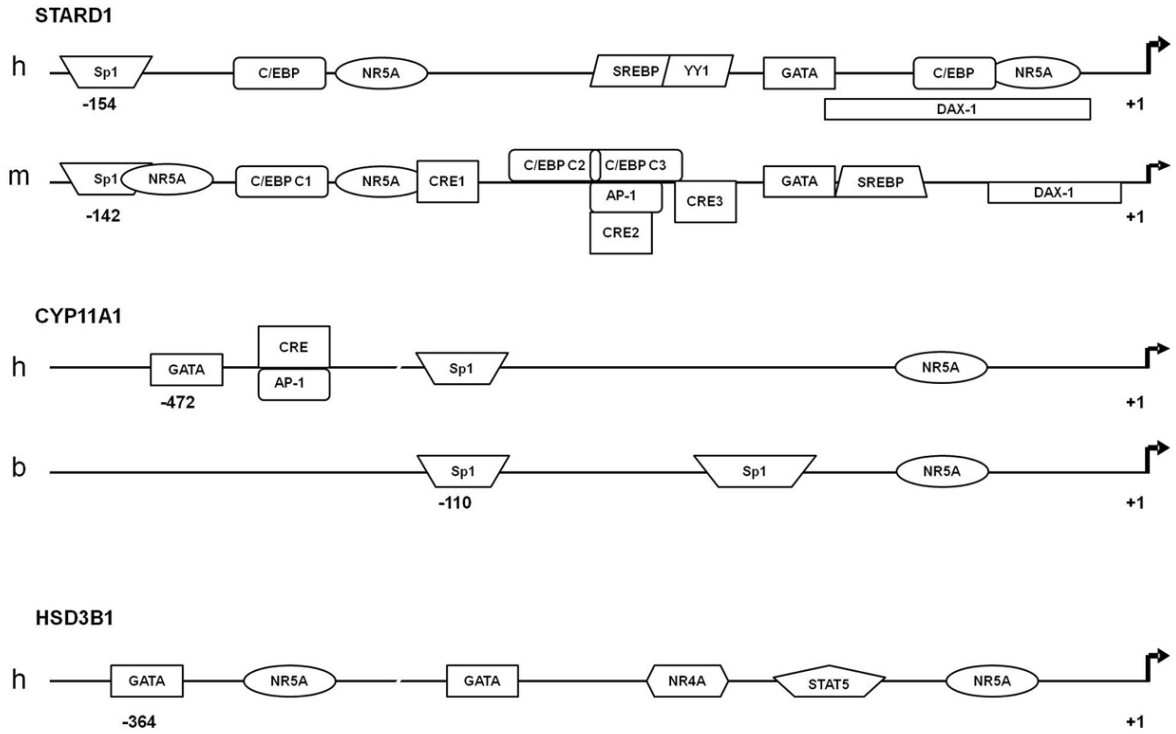

Figure 2. Schematic representation showing the most studied proximal 5'-flanking regions for the STARD1, CYP11A1 and HSD3B genes. Elements relevant to gonadal cell transactivation of these genes is shown for the human (h), selected murine (m) and bovine (b) genes. CRE indicates CRE half-site. Numbers indicate position relative to the transcriptional start site (+1). GATA sites and NR5A sites which can bind SF-1 or LRH-1 are common to all gene promoters. See main text for references.

discussion of these aspects of regulation is beyond the scope of this article, perhaps the most illuminating example of how all of these factors come together is SF-1. The activity of this key transactivator is impacted by cofactors like glucocorticoid receptor-interacting protein 1 (GRIP1) and nuclear receptor corepressor (N-CoR), post-translational modifications such as phosphorylation and sumoylation, and possibly binding of certain phospholipids (175). The SF-1 regulated gene product DAX-1 further alters SF-1 activity. Multiple signaling pathways also control GATA4 generation and activity in gonadal cells (reviewed in (176)). Regulation of Nur77 expression by LH/hCG within the ovary appears to mirror that of STARD1 and CYP11A1 in the ovary (65).

7. SUMMARY AND PERSPECTIVE

De novo steroid synthesis in the gonads requires the coordinated activities of StAR, P450scc and select 3beta-HSD subtypes. The basal level of each protein's mRNA varies by tissue type and developmental status. Increases in gene expression elicited by trophic hormones primarily rely mostly on cAMP signaling and concomitant recruitment and activation of key transcription factors. A diagrammatic summary of the most studied promoters including conserved elements is shown in Figure 2. Promoter studies in several gonadal cellular settings reveal that NR5A and GATA family proteins are common transactivators and DAX-1 is a common suppressor. At least two of these genes positively respond to CREB, NR4A or Sp1 family members. The roles of SREBPs, LXRs, Kruppel factors and other candidate transcription factors identified as regulating the genes in other tissues bear further investigation.

Although overexpression studies implicate numerous transcription factors in the transactivation of these genes, the challenge is to verify their proposed activities in the animal. Refinements of the ChIP technique using validated antibodies in combination with fresh tissues or cells isolated during different physiological states can verify the direct interaction of transcription factors with the promoter as well as with each other. Certainly such work has been performed for several transcription factors in the case of the STARD1 gene, but it needs to be expanded to draw a more accurate picture of how steroidogenesis is regulated in the gonads.

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Abbreviations: 3beta-HSD: 3beta-hydroxysteroid dehydrogenase/delta4-delta5 isomerase, AP-1: activator protein 1, ARR19: androgen receptor corepressor-19 kDa, BMP: bone morphogenic protein, C/EBP: CCAAT/enhancer binding protein, cAMP: 3'-5'-cyclic adenosine monophosphate, CBP: CREB-binding protein, ChIP: chromatin immunoprecipitation, CRE: consensus cAMP response element, CREB: cyclic AMP response element binding protein, CREM: CRE modulator, CRS: cAMP response sequences, CYP11A1: P450scc gene, DAX-1: dosage-sensitive sex reversal, adrenal hypoplasia critical region, on chromosome X, gene 1, Dlx5: distal-less homeobox 5, EGF: epidermal growth factor, ERK1/2: extracellular signal-regulated kinases 1/2, FGF: fibroblast growth factor, FSH: follicle stimulating hormone, FOXL2: forkhead box protein L2, FOXO1: forkhead box protein O1, Fra: Fos-related antigen, GDF-9: growth differentiation factor-9, GIOT-1: gonadotropin-inducible ovarian transcription factor-1, GnRH: gonadotropin releasing hormone, GRIP1: glucocorticoid receptor-interacting protein 1, hCG: human chorionic gonadotropin, HSD3B: gene(s) coding for 3beta-HSD, IGF: insulin-like growth factor, IGFRE: insulin-like growth factor response element, IL-1: interleukin 1, KLF: Kruppel-like factor, LDL: low density lipoprotein, LIF: leukemia inhibitory factor, LRH-1/NR5A2: liver receptor homologue-1, LH: luteinizing hormone, LXR: liver X receptor, LXRE: liver X receptor response element, mLTC-1: murine Leydig tumor cells, N-CoR: nuclear receptor corepressor, NF-Y: nuclear transcription factor Y, NR4A: nuclear receptor 4A subgroup, NR5A: nuclear receptor 5A subgroup, P450c17alpha: cytochrome P450 17alpha-hydroxylase/17,20-lyase, P450scc: cytochrome P450 cholesterol side-chain cleavage enzyme, PGE2: prostaglandin E2, PGF2: prostaglandin F2, PKA: protein kinase A, PKC: protein kinase C, PMA: phorbol-12-myristate-13-acetate, PSF: polypyrimidine tract-binding protein-associated splicing factor, Preg: Pregnenolone, Prog: progesterone, SCF: stem cell factor, SF-1/NR5A1: steroidogenic factor 1, Sp1: stimulatory protein 1, SREBP:

sterol regulatory element binding protein, SRE: sterol response element, StAR: steroidogenic acute regulatory protein, STARD1: steroidogenic acute regulatory protein gene, STAT: signal transducers and activators of transcription, TGF: transforming growth factor, TNF: tumor necrosis factor, TRE: phorbol ester response element, VIP: vasoactive intestinal peptide, YY1: Yin Yang-1

Key Words: STARD1, CYP11A1, HSD3B1, HSD3B2, Hsd3b6, Steroidogenesis, Promoter, GATA, SF-1, LRH-1, Sp1, CREB, CREM, AP-1, DAX-1, C/EBP, Nur77, SREBP, YY1, NR4A, Stat5, HIF-1alpha, LXR, RXR/RAR, Testis, Ovary, Granulosa, Leydig, Luteal, Review

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