



Transcription Factors in the Regulation of Leydig Cell Gene Expression and Function

Karine de Mattos¹, Robert S. Viger^{1,2} and Jacques J. Tremblay^{1,2*}

¹ Reproduction, Mother and Child Health, Centre de recherche du centre hospitalier universitaire de Québec, Université Laval, Québec City, QC, Canada, ² Centre de recherche en Reproduction, Développement et Santé Intergénérationnelle, Department of Obstetrics, Gynecology, and Reproduction, Faculty of Medicine, Université Laval, Québec City, QC, Canada

Cell differentiation and acquisition of specialized functions are inherent steps in events that lead to normal tissue development and function. These processes require accurate temporal, tissue, and cell-specific activation or repression of gene transcription. This is achieved by complex interactions between transcription factors that form a unique combinatorial code in each specialized cell type and in response to different physiological signals. Transcription factors typically act by binding to short, nucleotide-specific DNA sequences located in the promoter region of target genes. In males, Leydig cells play a crucial role in sex differentiation, health, and reproductive function from embryonic life to adulthood. To better understand the molecular mechanisms regulating Leydig cell differentiation and function, several transcription factors important to Leydig cells have been identified, including some previously unknown to this specialized cell type. This mini review summarizes the current knowledge on transcription factors in fetal and adult Leydig cells, describing their roles and mechanisms of action.

Keywords: transcription factors, gene expression, regulatory element, DNA binding motif, steroidogenesis, Leydig cells

OPEN ACCESS

Edited by:

Vassilios Papadopoulos,
University of Southern California,
United States

Reviewed by:

Diane Rebourcet,
The University of Newcastle, Australia
Peter O'Shaughnessy,
University of Glasgow,
United Kingdom

*Correspondence:

Jacques J. Tremblay
Jacques-J.Tremblay@
crchudequebec.ulaval.ca

Specialty section:

This article was submitted to
Reproduction,
a section of the journal
Frontiers in Endocrinology

Received: 22 February 2022

Accepted: 15 March 2022

Published: 07 April 2022

Citation:

de Mattos K, Viger RS and
Tremblay JJ (2022) Transcription
Factors in the Regulation of Leydig Cell
Gene Expression and Function.
Front. Endocrinol. 13:881309.
doi: 10.3389/fendo.2022.881309

1 INTRODUCTION

Localized in the testicular interstitium, Leydig cells are the principal source of testosterone and insulin-like 3 (INSL3), two hormones that regulate male reproductive development and function. In mammals, there are at least two distinct populations of Leydig cells, fetal Leydig cells (FLC) and adult Leydig cells (ALC), which are responsible for the synthesis of steroid hormones in the prenatal and postnatal testes, respectively [reviewed in (1, 2)]. Steroidogenesis is a multi-step process requiring various transporters and enzymes to convert cholesterol into a steroid hormone [reviewed in (3)]. The expression of the genes coding for these steroidogenic proteins is finely regulated to avoid steroid hormone insufficiency or excess across the lifespan.

Transcription factors (TFs) are fundamental to the regulation of gene expression. They are specialized proteins that recognize and bind to regulatory DNA sequences, modulating the rate of gene transcription [reviewed in (4)]. TFs typically recruit or interact with other TFs forming a unique molecular code that is key for specifying temporal- and tissue-specific gene expression as well as hormone responsiveness in hormone-sensitive target tissues. Moreover, TFs exhibit a dynamic behaviour that is characterized by their ability to interact with various partner proteins and

to regulate different target genes according to many determinants such as cell type, development stage, and signal stimulus, among others.

In recent years, the development of novel and powerful methodological approaches in molecular genetics has led to the emergence of new information regarding the role of TFs in the regulation of Leydig cell differentiation and function, and by extension, in male fertility and reproductive health. In this mini review, we provide a brief overview of the roles and mechanisms of action of some of the most characterized TFs in Leydig cells. We have adopted the most recent classification of TFs, which is based both on amino acid sequence homology and the tertiary structure of their DNA-binding domains (5). Using this classification, TFs that have been identified in Leydig cells are presented in **Table 1**; **Table 2** lists the target genes for these TFs in Leydig cells.

2 SUPERCLASS OF BASIC DOMAINS

2.1 Class of Basic Leucine Zipper Factors (BZIP)

2.1.1 AP-1 Factors

The activator protein 1 (AP-1) is a dimeric complex that includes members of the JUN, FOS, activating transcription factor (ATF), and musculoaponeurotic fibrosarcoma (MAF) families of TFs (54). Among the AP-1 members, JUN and FOS are the best characterized. The JUN subfamily comprises three members (cJUN, JUNB, and JUND) while four members compose the FOS subfamily [cFOS, FOSB, Fos-related antigens 1 (FRA-1, FOSL1), and Fos-related antigens 2 (FRA-2, FOSL2)]. Members of the JUN family can homodimerize or heterodimerize, whereas FOS family members only form heterodimers. The DNA sequence recognized by AP-1 members differs according to the dimer involved. JUN : JUN and FOS : JUN dimers recognize the TPA-response element (TRE; TGA(C/G)TCA) and the cAMP-responsive element (CRE; TGACGTCA), whereas ATF dimers preferentially recognize the CRE motif, and MAF dimers bind to MAF recognition elements (MAREs), a long palindromic sequence that contains TRE or CRE motifs (55) [reviewed in (56)].

AP-1 members were first described in Leydig cells in the late 1990s (57). AP-1 factors regulate several genes in Leydig cells such as the steroidogenic acute regulatory protein (*Star*) gene, which is activated by cJUN (6, 7, 12). In addition, cJUN cooperates with other TFs, including GATA4, STAT5B, and NUR77 leading to a stronger activation of the *Star* promoter (7–9). Both cJUN and cFOS regulate *Star* promoter activity by recruiting CREB and CBP (10). Transcription of the gap junction protein alpha1 [*Gja1*, also known as connexin43 (*Cx43*)] gene, involved in the initiation and maintenance of sperm production, is also controlled by cJUN, JUNB, and FOSL2, and by a cJUN/cFOS cooperation (11, 58). Furthermore, the ferredoxin 1 (*Fdx1*) promoter is activated by a cJUN/SF1 cooperation (12). *Fdx1* is a partner of *Cyp11a1*, participating in the conversion of cholesterol into pregnenolone, the first and rate-limiting step in steroidogenesis. It is important to note that the nature of the cJUN dimerization partner

influences its role in gene regulation. For example, the combination of either FOSL2 or cFOS with cJUN inhibits the stimulatory effect of cJUN on the *Star* promoter (6, 10, 59). AP-1 factors in Leydig cells have been reviewed elsewhere (56).

2.1.2 CREB-Related Factors

CREB-related factors include three members: CRE-binding protein (CREB), cAMP response element modulator (CREM), and CRE-activating transcription factor (ATF-1). CREB factors homodimerize and heterodimerize with other CREB members and with other bZIP TFs, such as AP-1 members (60). CREB factors regulate transcription by binding to a CRE motif (TGACGTCA) similar to that recognized by AP-1 members, leading to overlap and redundancy in their activities (61). Although CREM is the most abundant member in MA-10 Leydig cells, all CREB members activate *Star* transcription through CRE elements located in the proximal promoter region (13, 14). Moreover, CREB factors cooperate with SF1 (NR5A1, Ad4BP) to enhance *Star* transcription (15). CREB also stimulates *CKLFSF2B* promoter activity in response to LH/cAMP (16). *Cklfsf2b* codes for a protein that inhibits steroidogenesis in Leydig cells (16). Therefore, CREB is involved in both activation and repression of steroidogenesis in Leydig cells depending on its target genes.

2.1.3 C/EBP-Related Factors

Members of the CCAAT/enhancer binding protein (C/EBP) subfamily contain a bZIP DNA-binding domain and regulate gene expression by binding to the sequence (A/G)TTGCG(C/T)AA(C/T) as homo- or heterodimers (62). C/EBP β is the predominant member in Leydig cells (17, 63) where it activates *Star* transcription alone and in cooperation with SF1 and GATA4 (17–19). C/EBP β also cooperates with NF- κ B p50 to stimulate *Nur77* promoter activity in Leydig cells (20). The *Nur77* gene encodes the orphan nuclear receptor NUR77, which regulates several genes involved in steroidogenesis in Leydig cells (see Section 3.1.2, *NGFI-B/NR4A Receptors*, below).

3 SUPERCLASS OF ZINC-COORDINATING DNA-BINDING DOMAINS

3.1 Class of Nuclear Receptors With C4 Zinc Fingers

TFs belonging to the nuclear receptor class respond to extracellular and intracellular signals to regulate gene expression. They also regulate cellular functions within the cytoplasm (64). In this section we present the nuclear receptors for which the roles and mechanisms of action are, or have begun to be, characterized in Leydig cells. Detailed information can be found in a review article dedicated to nuclear receptors in Leydig cells (65).

3.1.1 COUP-Like/NR2F Receptors

The nuclear receptor subclass 2, group F (NR2F) subfamily consists of three members: chicken ovalbumin upstream promoter transcription factor I (COUP-TFI, NR2F1, EAR3),

TABLE 1 | Classification of transcription factors identified in Leydig cells.

Superclass	Class	Family	Subfamily	Transcription factor
Basic Domains	Basic leucine zipper factors (bZIP)	Jun-related	Jun	cJUN JUNB
			NF-E2-like factors	NFE2L2 (NRF2)
		Fos-related	Fos	cFOS FRA-2 (FOSL2)
		CREB-related	CREB-like	CREB CREM
		C/EBP-related	C/EBP	C/EBP β
	Basic helix-loop-helix factors (bHLH)	PAS domain	Arnt-like factors	ARNTL (BMAL1)
			SREBP factors	SREBP
			USF	USF1 USF2
		AP-2	n.a.	SPZ1
			n.a.	AP-2
Zinc-Coordinating DNA-Binding Domains	Nuclear receptors with C4 zinc fingers	Steroid Hormone Receptors (NR3)	GR-like receptors (NR3C)	NR3C1 (GR) NR3C2 (MR) NR3C3 (PR) NR3C4 (AR)
			ER-like (NR3A)	Er α ; Er β
		Thyroid hormone receptor-related (NR1)	Retinoic acid receptors (RAR - NR1B)	RAR α , RAR β , RAR γ
			Thyroid hormone receptors (THR - NR1A)	TR α , TR β
			PPAR (NR1C)	PPAR α , PPAR β / δ , PPAR γ
		RXR-related receptors (NR2)	LXR (NR1H)	LXR α , FXR
			Retinoid X receptors (NR2B)	RXR α , RXR β , RXR γ
		NGFI-B-related receptors (NR4A)	Testicular receptors (NR2C)	TR2 (NR2C1)
			COUP-like receptors (NR2F)	COUP-TFII (NR2F2)
		FTZ-F1-related receptors (NR5A)	n.a.	NR4A1 (NUR77, NGFI-B) NR4A2 (NURR1)
			n.a.	NR5A1 (SF-1, FTZ-F1)
			n.a.	NR5A2 (LRH1)
		DAX-related receptors (NR0B)	n.a.	NROB1 (DAX1) NROB2 (SHP)
GATA-type zinc fingers	Two zinc-finger GATA factors		GATA4	
Helix-Turn-Helix domains	Homeodomain factors	Paired-related HD	Sp1-like	SP1 SP3
			Kr-like	KLF6
		EGR	EGR1 (NGFI-A)	
		ZNF44-2-like (unclassified)	ZNF44 (GIOT2) ZNF461 (GIOT1)	
		ARX	ARX	
Alpha-helices exposed by beta-structures	Fork head/winged helix factors	HD-LIM	LHX2-like	RHOX4 PBX1 LHX9
			FOXA	FOXA3 (HNF-3 γ)
		MEF2	MEF2A MEF2C MEF2D	
Immunoglobulin fold	Rel homology region (RHR) factors	NF-kappa β -related	NF-kappa β p50 subunit-like NF-kappa β p65 subunit-like	NF- κ B p50 NF- κ B p65 (RelA)
		STAT	n.a.	STAT5B

n.a., not applicable.

COUP-TFII (NR2F2, ARP1) and COUP-TFIII (NR2F6, EAR2). NR2Fs have been implicated in various physiological and developmental processes by regulating the expression of numerous genes [reviewed in (66, 67)]. *Via* their double zinc

finger DNA-binding domain, NR2F factors bind as monomers to the nuclear receptor element AGGTCA and its variants. They also bind as dimers to direct (DR), inverted (IR), and everted (ER) repeats separated by 1-12 nucleotides (68).

TABLE 2 | Transcription factors and their target genes in Leydig cells.

Transcription Factor	Target Gene*	Select References
AP-1 (cJUN/cFOS)	<i>h, mStar</i> <i>mGja1</i> <i>mFdx1</i>	(6–10) (11) (12)
CREB/CREM	<i>mStar</i> <i>hCKLFSF2B</i>	(10, 13–15) (16)
C/EBP β	<i>mStar</i> <i>rNr4a1 (Nur77)</i>	(17–19) (20)
BMAL1	<i>mStar</i>	(21)
AP-2	<i>m, rLhr</i>	(22, 23)
NR2F2 (COUP-TFII)	<i>mStar</i> <i>mInsl3</i> <i>mAmhr2</i> <i>mAkr1c14</i> <i>mGsta3</i> <i>mInhha</i>	(24) (25) (26) (27) (28) (28)
NR4A1 (NUR77, NGFI-B)	<i>mStar</i> <i>m, hHsd3b</i> <i>h, mInsl3</i> <i>rCyp17a1</i>	(9, 29, 30) (31, 32) (33, 34) (35, 36)
NR5A1 (SF1, FTZ-F1)	<i>m, hStar</i> <i>rCyp19a1</i> <i>hHSD3B2</i> <i>hCyp11a1</i> <i>rCyp17a1</i> <i>rPrlr</i> <i>rAmhr2</i> <i>mVanin-1</i> <i>m, hInsl3</i> <i>mFdx1</i>	(9, 15, 17–19, 37) (38) (32) (37) (39, 40) (41) (42) (43) (33, 34) (12)
NR5A2 (LRH1)	<i>mStar</i> <i>rCyp19a1</i> <i>m, hInsl3</i>	(9) (44) (33)
NR0B1 (DAX1)	<i>mStar</i>	(45)
GATA4	<i>h, mStar</i> <i>hHSD3B2</i> <i>mAmhr2</i> <i>rSrd5a1</i> <i>rSrb1</i> <i>mLhr</i>	(7, 18, 19, 46, 47) (32) (46) (46) (48) (22)
SP1	<i>mVegf</i> <i>mPbr</i>	(49) (50)
SP1/SP3	<i>hINSL3</i> <i>rPdgfra</i>	(34) (51)
KLF6	<i>mStar</i>	(47)
FOXA3 (HNF-3 γ)	<i>rNr4a1 (Nur77)</i> <i>mGsta1-4</i> <i>mAkr1c14</i>	(52) (53) (27)
MEF2	<i>rNr4a1 (Nur77)</i> <i>rCyp17a1</i>	(20) (31)
NF- κ B p50	<i>mStar</i>	(8)
NF- κ B p65 (RelA)	<i>rNr4a1 (Nur77)</i>	(8)
STAT5B		

*The letter preceding the name of the gene refers to the species: m, mouse; r, rat; h, human.

Of the NR2F subfamily members, COUP-TFII is by far the most abundant in Leydig cells. Although COUP-TFII is present in mice interstitial cells from early fetal life throughout adulthood, it is only associated with steroidogenically active ALC in postnatal life (24). COUP-TFII is a marker of stem cells giving rise to the ALC population (24, 69). *In vivo* studies using mouse models have shown that COUP-TFII is crucial for Leydig cell development and male reproductive function (70, 71). In Leydig cells, COUP-TFII regulates the expression of several

genes involved in lipid metabolism, male gonad development, and steroidogenesis (28). COUP-TFII activates *Star*, *Insl3*, and *Amhr2* expression by binding to their respective promoter sequences (24–26). It cooperates with SF1 on the *Star* and *Insl3* promoters (24, 25) and with SP1 on the *Amhr2* promoter (26). The *Akr1c14* gene, which codes for the 3 α -HSD enzyme that catalyzes the interconversion of dihydrotestosterone (DHT) into 5 α -androstane-3 α ,17 β -diol (3 α -diol), is activated by COUP-TFII in cooperation with MEF2 (27). COUP-TFII also

activates the expression of *Gsta3* and *Inha*, genes involved in the inactivation of reactive oxygen species and in the homeostasis of the hypothalamic-pituitary-gonadal axis, respectively (28). Expression of several other Leydig cell genes including *Cyp17a1*, *Hsd3b1* and *Cyp11a1* is reduced in *Coup-tfii* null mice (71) and in COUP-TFII-depleted MA-10 Leydig cells (28), implying a role for COUP-TFII in their expression.

3.1.2 NGFI-B/NR4A Receptors

The NR4A family consists of three orphan nuclear receptors: neuron-derived clone 77 (NR4A1, NUR77, NGFI-B, TR3), nuclear receptor related 1 (NR4A2, NURR1) and neuron-derived orphan receptor 1 (NR4A3, NOR1). NR4A members can bind to DNA either as monomers, homodimers, or heterodimers. NUR77 and NURR1 also heterodimerize with RXR. As monomers, they bind to a NGFI-B-response element (NBRE; AAAGGTCA), as homodimers and heterodimers to a Nur-response element (NurRE; TGATATTTN₆AAATGCCA), and as heterodimers with RXR to a DR5 sequence [reviewed in (72, 73)]. NR4A factors are immediate early response genes involved in the regulation of several physiological and pathological processes, including steroidogenesis (74) [reviewed in (75)].

Leydig cells contain mainly NUR77, followed by NURR1 where both are important regulators of basal and hormone-induced gene transcription (76). *Nur77* expression is strongly increased by LH (76) *via* the CAMKI pathway (29, 77) consistent with its role as a key regulator of several genes in Leydig cells including *Cyp17a1* (31, 35), *Hsd3b1* (31), *HSD3B2* (32), *Insl3* (33, 34), and *Star* (29, 30). NUR77 regulates the expression of these genes by cooperating with CAMKI (29), cJUN (9), KLF6 (34), and SF1 (34). In Leydig cells, *Nur77* expression is controlled by distinct regulatory elements for both basal and hormone-induced expression (77), through mechanisms involving MEF2 (52), STAT5B (8), CREB (77), cJUN (9), C/EBP β (20), and NF- κ B p50 (20).

3.1.3 FTZ-F1-Related/NR5A Receptors

The nuclear receptor 5A (NR5A) family comprises two members: steroidogenic factor 1 (NR5A1, Ad4BP, SF1) and liver receptor homolog 1 (NR5A2, LRH1, FTF). Both factors share high sequence similarity, bind to the same DNA motif, regulate common target steroidogenic genes, and exhibit overlapping expression in several tissues [reviewed in (78, 79)]. Despite this, they have nonredundant roles and cannot fully compensate for each other [reviewed in (78, 79)]. NR5A members regulate gene expression by binding as monomers to the sequence (T/C)CAAGGTCA located in the promoter region of target genes.

SF1 was initially identified as a tissue-specific activator of several cytochrome P450 steroid hydroxylase genes (38, 80). SF1 is essential for steroidogenesis, reproduction, and male sex differentiation, as revealed by mutations in the *SF1* gene in humans and in mouse models where adrenal and gonadal development and function are impaired (37, 81–84) [reviewed in (85, 86)]. Interestingly, *Sf1* knockdown in MLTC-1 Leydig cells leads to downregulation of *Star* and *Cyp11a1* and accumulation of neutral lipids and cholesterol (37). Moreover, SF1 is one of only a handful of TFs that can convert fibroblasts

into functional Leydig-like cells, revealing the pivotal role of this nuclear receptor in Leydig cells (87, 88).

In vitro analysis of regulatory elements has shown that the expression of several Leydig cell genes is regulated by SF1. These include *Star* (9, 17, 37), *Cyp19a1* (38), *HSD3B2* (32), *Cyp17a1* (39, 40), *Cyp11a1* (37), *Prlr* (41), *Amhr2* (42), *Vanin-1* (43), *Insl3* (33), and *Fdx1* (12). SF1 activity relies on interactions with a long list of protein partners, such as C/EBP β (17), cJUN (9, 12), DAX1 (45), GATA4 (89), and KLF6 (34).

Like SF1, LRH1 influences steroidogenesis and fertility. To date, only a few genes are known to be regulated by LRH1 in Leydig cells, including *Star* (in cooperation with cJUN) (9), *Cyp19a1* (44), and *Insl3* (33).

3.1.4 DAX-Related/NROB Receptors

The DAX-related receptor (NROB) family comprises two members: critical region on the X chromosome gene 1 (NROB1, DAX1) and small heterodimer partner (NROB2, SHP). They lack the typical zinc finger DNA-binding domain and therefore act mainly as transcriptional repressors by inhibiting the activity of other TFs (90, 91). Both members are present in Leydig cells and act as homodimers or heterodimers (92).

In *Dax1*-deficient mice, testis cord organization is compromised and FLC development is arrested (93). *In vitro* studies in Leydig cell lines revealed that DAX1 represses steroidogenesis by inhibiting *Star* expression, while silencing *Dax1* expression increases *Star* transcription leading to enhanced steroidogenesis (45). DAX1 interacts with and represses the activity of NUR77 and SF1, inhibiting *Star* expression (36, 45). Interestingly, *Dax1* knockdown in MA-10 Leydig cells decreases *Cyp11a1* and *Star* expression suggesting that DAX1 could also act as a coactivator in addition to its repressor role (94).

SHP is a repressor of steroidogenesis. In mouse Leydig cells, *Shp* expression is reduced by hCG treatment (95). In *Shp*-deficient mice, testosterone levels as well as *Star*, *Cyp11a1*, and *Hsd3b1* mRNA levels are increased leading to premature sexual maturation (96). SHP inhibits steroidogenesis by interacting and repressing the activity of LRH1 (96). *Shp* mRNA levels are significantly reduced in COUP-TFII- and MEF2-depleted Leydig cells, indicating that *Shp* expression requires these two TFs (28, 97).

3.2 Class of Other C4 Zinc Finger-Type Factors

3.2.1 Two Zinc-Finger GATA Factors

The six GATA members (GATA1 to 6) are crucial for the development and function of several tissues, including the male gonad [reviewed in (98, 99)]. GATA factors regulate gene expression by binding *via* their two zinc fingers to the DNA sequence (A/T)GATA(A/G) in the promoter region of target genes. Of the six GATA factors, GATA4 is the most abundant in Leydig cells *in vivo* (100–102). Its expression is also the broadest being present from the onset of testis morphogenesis and into adult life (103). Considered one of the first gonadal markers in both sexes, GATA4 is required for urogenital ridge development in mice and later for mammalian gonadal differentiation (103, 104).

A *Sfl*-Cre mouse line, which expresses the Cre recombinase in several tissues including Leydig, Sertoli and adrenal cells, was used to conditionally inactivate *Gata4*. The resulting males were undervirilized and had small testes lacking mature sperm (105), thereby supporting a role for this factor in male reproductive function. Transcriptomic analysis of GATA4-depleted MA-10 Leydig cells revealed several deregulated pathways, including cholesterol metabolism and steroidogenesis (46). Consistent with this, GATA4 stimulates the transcription of several genes expressed in Leydig cells such as *HSD3B2* (32), *Cyp19a1* (106), *Star* (46, 106), *Inha* (106), *Sfl* (106), *Amhr2* (46), and *Srd5a1* (46). GATA4 also cooperates with cJUN, C/EBP β , and MEF2 to upregulate *Star* expression (7, 18, 47). These results emphasize the indispensable role of GATA4 in the differentiation and function of FLC and ALC (46, 107). The critical nature of GATA4 in the Leydig cell differentiation is further supported by the demonstration that GATA4, along with SF1 and DMRT1 or NUR77, are sufficient to reprogram fibroblasts toward the Leydig-like cell fate (87, 88).

4 SUPERCLASS OF HELIX-TURN-HELIX DOMAINS

4.1 Class of Forkhead/Winged Helix Factors

4.1.1 Forkhead Box (FOX) Factors

The forkhead box A3 (FOXA3) is the only member of the FOXA subfamily present in the testes, mainly in ALC (51, 108, 109). So far, the only direct target identified for FOXA3 in Leydig cells is the gene coding for the platelet-derived growth factor receptor alpha (*Pdgfra*) (51), that in response to PDGF signaling, acts in Leydig cell differentiation and testis organogenesis (110). In cAMP-induced steroidogenesis, FOXA3 is proposed to repress *Nur77* expression, which in turn reduces steroidogenic gene expression and testosterone production (111). These findings indicate that FOXA3 participates actively in the control of Leydig cell function and male fertility.

5 SUPERCLASS OF α -HELICES EXPOSED BY β -STRUCTURES

5.1 Class of MADS Box Factors

5.1.1 MEF2 Subfamily

The Myocyte Enhancer Factor 2 (MEF2) factor subfamily comprises four members (MEF2A-2D) that share two highly conserved domains, a MADS box and a MEF2 domain, involved in dimerization and DNA binding [reviewed in (112)]. MEF2 factors form homo- and heterodimers that bind the sequence YTAWWWWTAR (Y=C/T, W=A/T, R=G/A) in the promoter region of their target genes. Because of their conserved DNA-binding domain, MEF2 members share common targets and can compensate for each other. MEF2 members also display unique spatiotemporal patterns in different tissues. Due to their divergent transactivation domain, MEF2 factors respond to

different signals and interact with different partners, leading to specific gene expression [reviewed in (112)].

Although widely studied in other organs, the presence of MEF2 in the testes, more specifically in Sertoli and Leydig cells, was only reported in 2014 (52). In Leydig cells, MEF2A and MEF2D and to a lesser extent MEF2C, are expressed from early gonadal development into adulthood (52). MEF2A/2D-depleted MA-10 Leydig cells produce less steroid hormone demonstrating that MEF2 factors have a role in male reproductive function (47). Consistent with this, microarray analysis of MEF2A/2D-depleted MA-10 Leydig cells identified several differently regulated genes known to be involved in fertility, gonad morphology, and steroidogenesis (97). To date, direct gene targets for MEF2 factors in Leydig cells include *Nur77* (52), *Gsta1-4* (53), *Star* (involving a MEF2/GATA4 cooperation) (47), and *Akr1c14* (through a cooperation with COUP-TFII) (27). The complete network of genes regulated by MEF2 factors in Leydig cells as well as MEF2 interacting partners remain to be fully elucidated.

6 SUPERCLASS OF IMMUNOGLOBULIN FOLD

6.1 Class of STAT Domain Factors

6.1.1 STAT Factors

The signal transducer and activator of transcription (STAT) family consists of seven proteins [reviewed in (113)]. Cytokines and growth factors activate STAT members through the Janus kinase (JAK) signaling pathway. In the nucleus, STAT factors regulate gene transcription by binding as homo- or heterodimers to the γ -interferon-activated sequence (GAS; TTCN₃GAA) in the promoter region of target genes. So far, STAT5B is the only STAT factor identified in Leydig cells (114). In these cells, STAT5B is activated by growth hormone, an important regulator of steroidogenesis (8). STAT5B activates *Star* expression directly by binding to a GAS element and in cooperation with cJUN (8). STAT5B also activates the *Nur77* promoter (8).

7 OTHER TRANSCRIPTION FACTORS PRESENT IN LEYDIG CELLS

Other TFs have been described in Leydig cells, but their mechanisms of action remain poorly characterized. This includes the nuclear factor E2-related factor-2 (NRF2, NFE2L2), which is an important modulator of reactive oxygen species levels, especially in aging Leydig cells (115–117). Furthermore, the brain and muscle arnt-like protein-1 (BMAL1), a component of the circadian clock system, is also directly involved in the control of Leydig cell function in different species, by regulating the expression of *Star*, *Hsb3b*, and *Cyp11a1* (21, 118, 119). Finally, members of the nuclear factor kappa-beta (NF- κ B) family, involved in immune and inflammatory responses, also contribute to the regulation of steroidogenesis in Leydig cells (20, 31, 120).

8 CONCLUDING REMARKS

As described in this mini review, several TFs belonging to different classes and families are pivotal to ensure proper Leydig cell differentiation and function. This underscores the complex regulatory mechanisms involved. Most of the knowledge acquired so far has relied on *in vitro* analyses of regulatory elements of genes expressed in Leydig cells. Although we are far from fully understanding all the signals, pathways, and TFs involved, technological advances and novel mouse models will certainly lead to significant discoveries in the coming years.

REFERENCES

- Shima Y. Development of Fetal and Adult Leydig Cells. *Reprod Med Biol* (2019) 18:323–30. doi: 10.1002/rmb2.12287
- Teerds KJ, Huhtaniemi IT. Morphological and Functional Maturation of Leydig Cells: From Rodent Models to Primates. *Hum Reprod Update* (2015) 21:310–28. doi: 10.1093/humupd/dmv008
- Selvaraj V, Stocco DM, Clark BJ. Current Knowledge on the Acute Regulation of Steroidogenesis. *Biol Reprod* (2018) 99:13–26. doi: 10.1093/biolre/i0y102
- Lambert SA, Jolma A, Campitelli LF, Das PK, Yin Y, Albu M, et al. The Human Transcription Factors. *Cell* (2018) 172:650–65. doi: 10.1016/j.cell.2018.01.029
- Wingender E, Schoeps T, Haubrock M, Krull M, Dönitz J. TFClass: Expanding the Classification of Human Transcription Factors to Their Mammalian Orthologs. *Nucleic Acids Res* (2018) 46:D343–7. doi: 10.1093/nar/gkx987
- Manna PR, Eubank DW, Stocco DM. Assessment of the Role of Activator Protein-1 on Transcription of the Mouse Steroidogenic Acute Regulatory Protein Gene. *Mol Endocrinol* (2004) 18:558–73. doi: 10.1210/me.2003-0223
- Martin LJ, Bergeron F, Viger RS, Tremblay JJ. Functional Cooperation Between GATA Factors and cJun on the Star Promoter in MA-10 Leydig Cells. *J Androl* (2012) 33:81–7. doi: 10.2164/jandrol.110.012039
- Hébert-Mercier P-O, Bergeron F, Robert NM, Mehanovic S, Pierre KJ, Mendoza-Villarreal RE, et al. Growth Hormone-Induced STAT5B Regulates Star Gene Expression Through a Cooperation With cJun in Mouse MA-10 Leydig Cells. *Endocrinology* (2022) 163:1–13. doi: 10.1210/endo/bqab267
- Martin LJ, Tremblay JJ. The Nuclear Receptors NUR77 and SF1 Play Additive Roles With C-JUN Through Distinct Elements on the Mouse Star Promoter. *J Mol Endocrinol* (2009) 42:119–29. doi: 10.1677/JME-08-0095
- Manna PR, Stocco DM. Crosstalk of CREB and Fos/Jun on a Single Cis-Element: Transcriptional Repression of the Steroidogenic Acute Regulatory Protein Gene. *J Mol Endocrinol* (2007) 39:261–77. doi: 10.1677/JME-07-0065
- Ghouili F, Martin LJ. Cooperative Regulation of Gjal Expression by Members of the AP-1 Family cJun and cFos in TM3 Leydig and TM4 Sertoli Cells. *Gene* (2017) 635:24–32. doi: 10.1016/j.gene.2017.09.017
- Roumaud P, Rwigemera A, Martin LJ. Transcription Factors SF1 and cJun Cooperate to Activate the Fdx1 Promoter in MA-10 Leydig Cells. *J Steroid Biochem Mol Biol* (2017) 171:121–32. doi: 10.1016/j.jsbmb.2017.03.003
- Manna PR, Dyson MT, Eubank DW, Clark BJ, Lalli E, Sassone-Corsi P, et al. Regulation of Steroidogenesis and the Steroidogenic Acute Regulatory Protein by a Member of the cAMP Response-Element Binding Protein Family. *Mol Endocrinol* (2002) 16:184–99. doi: 10.1210/mend.16.1.0759
- Clem BF, Hudson EA, Clark BJ. Cyclic Adenosine 3',5'-Monophosphate (cAMP) Enhances cAMP-Responsive Element Binding (CREB) Protein Phosphorylation and Phospho-CREB Interaction With the Mouse Steroidogenic Acute Regulatory Protein Gene Promoter. *Endocrinology* (2005) 146:1348–56. doi: 10.1210/en.2004-0761
- Manna PR, Eubank DW, Lalli E, Sassone-Corsi P, Stocco DM. Transcriptional Regulation of the Mouse Steroidogenic Acute Regulatory Protein Gene by the cAMP Response-Element Binding Protein and Steroidogenic Factor 1. *J Mol Endocrinol* (2003) 30:381–97. doi: 10.1677/jme.0.0300381
- Kumar S, Kang H, Park E, Park HS, Lee K. The Expression of CKLFSP2B Is Regulated by GATA1 and CREB in the Leydig Cells, Which Modulates Testicular Steroidogenesis. *Biochim Biophys Acta - Gene Regul Mech* (2018) 1861:1063–75. doi: 10.1016/j.bbagr.2018.10.002
- Reinhart AJ, Williams SC, Clark BJ, Stocco DM. SF-1 (Steroidogenic Factor-1) and C/EBP Beta (CCAAT/Enhancer Binding Protein-Beta) Cooperate to Regulate the Murine Star (Steroidogenic Acute Regulatory) Promoter. *Mol Endocrinol* (1999) 13:729–41. doi: 10.1210/mend.13.5.0279
- Tremblay JJ, Hamel F, Viger RS. Protein Kinase A-Dependent Cooperation Between GATA and CCAAT/Enhancer-Binding Protein Transcription Factors Regulates Steroidogenic Acute Regulatory Protein Promoter Activity. *Endocrinology* (2002) 143:3935–45. doi: 10.1210/en.2002-220413
- Hu Y, Dong C, Chen M, Lu J, Han X, Qiu L, et al. Low-Dose Monobutyl Phthalate Stimulates Steroidogenesis Through Steroidogenic Acute Regulatory Protein Regulated by SF-1, GATA-4 and C/EBP-Beta in Mouse Leydig Tumor Cells. *Reprod Biol Endocrinol* (2013) 11:1–1. doi: 10.1186/1477-7827-11-72
- El-Asmar B, Giner XC, Tremblay JJ. Transcriptional Cooperation Between NF-Kb P50 and CCAAT/Enhancer Binding Protein Beta Regulates Nur77 Transcription in Leydig Cells. *J Mol Endocrinol* (2009) 42:131–8. doi: 10.1677/JME-08-0016
- Alvarez JD, Hansen A, Ord T, Bebas P, Chappell PE, Giebultowicz JM, et al. The Circadian Clock Protein BMAL1 Is Necessary for Fertility and Proper Testosterone Production in Mice. *J Biol Rhythm* (2008) 23:26–36. doi: 10.1177/0748730407311254
- Nikula H, Koskimies P, El-Hefnawy T, Huhtaniemi I. Functional Characterization of the Basal Promoter of the Murine LH Receptor Gene in Immortalized Mouse Leydig Tumor Cells. *J Mol Endocrinol* (2001) 26:21–9. doi: 10.1677/jme.0.0260021
- Tsai-Morris CH, Geng Y, Xie XZ, Buczko E, Dufau ML. Transcriptional Protein Binding Domains Governing Basal Expression of the Rat Luteinizing Hormone Receptor Gene. *J Biol Chem* (1994) 269:15868–75. doi: 10.1016/S0021-9258(17)40761-7
- Mendoza-Villarreal RE, Robert NM, Martin LJ, Brousseau C, Tremblay JJ. The Nuclear Receptor NR2F2 Activates Star Expression and Steroidogenesis in Mouse MA-10 and MLTC-1 Leydig Cells. *Biol Reprod* (2014) 91:26. doi: 10.1095/biolreprod.113.115790
- Mendoza-Villarreal RE, Di-Luoffo M, Camiré E, Giner XC, Brousseau C, Tremblay JJ. The INSL3 Gene Is a Direct Target for the Orphan Nuclear Receptor, COUP-TFII, in Leydig Cells. *J Mol Endocrinol* (2014) 53:43–55. doi: 10.1530/JME-13-0290
- Mehanovic S, Mendoza-Villarreal RE, Viger RS, Tremblay JJ. The Nuclear Receptor COUP-TFII Regulates Amhr2 Gene Transcription via a GC-rich Promoter Element in Mouse Leydig Cells. *J Endocr Soc* (2019) 3:2236–57. doi: 10.1210/js.2019-00266
- Di-Luoffo M, Brousseau C, Tremblay JJ. MEF2 and NR2F2 Cooperate to Regulate Akr1c14 Gene Expression in Mouse MA-10 Leydig Cells. *Andrology* (2016) 4:335–44. doi: 10.1111/andr.12150

AUTHOR CONTRIBUTIONS

KM wrote the first draft of the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

Supported by a grant from the Canadian Institutes of Health Research (funding reference number MOP-81387) to JT. KM is the recipient of a studentship from the Fonds de recherche du Québec-Santé.

28. Mehanovic S, Mendoza-Villarreal RE, de Mattos K, Talbot P, Viger RS, Tremblay JJ. Identification of Novel Genes and Pathways Regulated by the Orphan Nuclear Receptor COUP-TFII in Mouse MA-10 Leydig Cells. *Biol Reprod* (2021) 105:1283–306. doi: 10.1093/biolre/iaob131
29. Martin LJ, Boucher N, Brousseau C, Tremblay JJ. The Orphan Nuclear Receptor NUR77 Regulates Hormone-Induced Star Transcription in Leydig Cells Through Cooperation With Ca²⁺/Calmodulin-Dependent Protein Kinase I. *Mol Endocrinol* (2008) 22:2021–37. doi: 10.1210/me.2007-0370
30. Manna PR, Huhtaniemi IT, Stocco DM. Mechanisms of Protein Kinase C Signaling in the Modulation of 3',5'-Cyclic Adenosine Monophosphate-Mediated Steroidogenesis in Mouse Gonadal Cells. *Endocrinology* (2009) 150:3308–17. doi: 10.1210/en.2008-1668
31. Hong CY, Park JH, Ahn RS, Im SY, Choi H-S, Soh J, et al. Molecular Mechanism of Suppression of Testicular Steroidogenesis by Proinflammatory Cytokine Tumor Necrosis Factor Alpha. *Mol Cell Biol* (2004) 24:2593–604. doi: 10.1128/mcb.24.7.2593-2604.2004
32. Martin LJ, Taniguchi H, Robert NM, Simard J, Tremblay JJ, Viger RS. GATA Factors and the Nuclear Receptors, Steroidogenic Factor 1/Liver Receptor Homolog 1, Are Key Mutual Partners in the Regulation of the Human 3 β -Hydroxysteroid Dehydrogenase Type 2 Promoter. *Mol Endocrinol* (2005) 19:2358–70. doi: 10.1210/me.2004-0257
33. Robert NM, Martin LJ, Tremblay JJ. The Orphan Nuclear Receptor NR4A1 Regulates Insulin-Like 3 Gene Transcription in Leydig Cells. *Biol Reprod* (2006) 74:322–30. doi: 10.1095/biolreprod.105.044560
34. Tremblay MA, Mendoza-Villarreal RE, Robert NM, Bergeron F, Tremblay JJ. KLF6 Cooperates With NUR77 and SF1 to Activate the Human INSL3 Promoter in Mouse MA-10 Leydig Cells. *J Mol Endocrinol* (2016) 56:163–73. doi: 10.1530/JME-15-0139
35. Zhang P, Mellon SH. Multiple Orphan Nuclear Receptors Converge to Regulate Rat P450c17 Gene Transcription: Novel Mechanisms for Orphan Nuclear Receptor Action. *Mol Endocrinol* (1997) 11:891–904. doi: 10.1210/mend.11.7.9940
36. Song KH, Park YY, Ki CP, Cheol YH, Jin HP, Shong M, et al. The Atypical Orphan Nuclear Receptor DAX-1 Interacts With Orphan Nuclear Receptor Nur77 and Represses Its Transactivation. *Mol Endocrinol* (2004) 18:1929–40. doi: 10.1210/me.2004-0043
37. Hatano M, Migita T, Ohishi T, Shima Y, Ogawa Y, Morohashi KI, et al. SF-1 Deficiency Causes Lipid Accumulation in Leydig Cells via Suppression of STAR and CYP11A1. *Endocrine* (2016) 54:484–96. doi: 10.1007/s12020-016-1043-1
38. Lynch JP, Lala DS, Peluso JJ, Luo W, Parker KL, White BA. Steroidogenic Factor 1, an Orphan Nuclear Receptor, Regulates the Expression of the Rat Aromatase Gene in Gonadal Tissues. *Mol Endocrinol* (1993) 7:776–86. doi: 10.1210/mend.7.6.8395654
39. Givens CR, Zhang P, Bair SR, Mellon SH. Transcriptional Regulation of Rat Cytochrome P450c17 Expression in Mouse Leydig MA-10 and Adrenal Y-1 Cells: Identification of a Single Protein That Mediates Both Basal and cAMP-Induced Activities. *DNA Cell Biol* (1994) 13:1087–98. doi: 10.1089/dna.1994.13.1087
40. Zhang P, Mellon SH. The Orphan Nuclear Receptor Steroidogenic Factor-1 Regulates the Cyclic Adenosine 3',5'-Monophosphate-Mediated Transcriptional Activation of Rat Cytochrome P450c17 (17 Alpha-Hydroxylase/C17-20 Lyase). *Mol Endocrinol* (1996) 10:147–58. doi: 10.1210/mend.10.2.8825555
41. Hu Z, Zhuang L, Guan X, Meng J, Dufau ML. Steroidogenic Factor-1 Is an Essential Transcriptional Activator for Gonad-Specific Expression of Promoter I of the Rat Prolactin Receptor Gene. *J Biol Chem* (1997) 272:14263–71. doi: 10.1074/jbc.272.22.14263
42. Teixeira J, Kehas DJ, Antun R, Donahoe PK. Transcriptional Regulation of the Rat Müllerian Inhibiting Substance Type II Receptor in Rodent Leydig Cells. *Proc Natl Acad Sci USA* (1999) 96:13831–8. doi: 10.1073/pnas.96.24.13831
43. Wilson MJ, Jeyasuria P, Parker KL, Koopman P. The Transcription Factors Steroidogenic Factor-1 and SOX9 Regulate Expression of Vanin-1 During Mouse Testis Development. *J Biol Chem* (2005) 280:5917–23. doi: 10.1074/jbc.M412806200
44. Pezzi V, Sirianni R, Chimento A, Maggolini M, Bourguiba S, Delalande C, et al. Differential Expression of Steroidogenic Factor-1/Adrenal 4 Binding Protein and Liver Receptor Homolog-1 (LRH-1)/Fetoprotein Transcription Factor in the Rat Testis: LRH-1 as a Potential Regulator of Testicular Aromatase Expression. *Endocrinology* (2004) 145:2186–96. doi: 10.1210/en.2003-1366
45. Manna PR, Dyson MT, Jo Y, Stocco DM. Role of Dosage-Sensitive Sex Reversal, Adrenal Hypoplasia Congenita, Critical Region on the X Chromosome, Gene 1 in Protein Kinase A and Protein Kinase C-Mediated Regulation of the Steroidogenic Acute Regulatory Protein Expression in Mouse Leydig Tumor. *Endocrinology* (2009) 150:187–99. doi: 10.1210/en.2008-0368
46. Bergeron F, Nadeau G, Viger RS. GATA4 Knockdown in MA-10 Leydig Cells Identifies Multiple Target Genes in the Steroidogenic Pathway. *Reproduction* (2015) 149:245–57. doi: 10.1530/REP-14-0369
47. Daems C, Di-Luoffo M, Paradis É, Tremblay JJ. MEF2 Cooperates With Forskolin/cAMP and GATA4 to Regulate Star Gene Expression in Mouse MA-10 Leydig Cells. *Endocrinology* (2015) 156:2693–703. doi: 10.1210/en.2014-1964
48. Mizutani T, Yamada K, Minegishi T, Miyamoto K. Transcriptional Regulation of Rat Scavenger Receptor Class B Type I Gene. *J Biol Chem* (2000) 275:22512–9. doi: 10.1074/jbc.M001631200
49. Schwarzenbach H, Chakrabarti G, Paust HJ, Mukhopadhyay AK. Gonadotropin-Mediated Regulation of the Murine VEGF Expression in MA-10 Leydig Cells. *J Androl* (2004) 25:128–39. doi: 10.1002/j.1939-4640.2004.tb02768.x
50. Giatzakis C, Papadopoulos V. Differential Utilization of the Promoter of Peripheral-Type Benzodiazepine Receptor by Steroidogenic Versus Nonsteroidogenic Cell Lines and the Role of Sp1 and Sp3 in the Regulation of Basal Activity. *Endocrinology* (2004) 145:1113–23. doi: 10.1210/en.2003-1330
51. Garon G, Bergeron F, Brousseau C, Robert NM, Tremblay JJ. FOXA3 Is Expressed in Multiple Cell Lineages in the Mouse Testis and Regulates Pdgfra Expression in Leydig Cells. *Endocrinology* (2017) 158:1886–97. doi: 10.1210/en.2016-1736
52. Daems C, Martin LJ, Brousseau C, Tremblay JJ. MEF2 Is Restricted to the Male Gonad and Regulates Expression of the Orphan Nuclear Receptor Nr4a1. *Mol Endocrinol* (2014) 28:886–98. doi: 10.1210/me.2013-1407
53. Di-Luoffo M, Brousseau C, Bergeron F, Tremblay JJ. The Transcription Factor MEF2 Is a Novel Regulator of Gsta Gene Class in Mouse MA-10 Leydig Cells. *Endocrinology* (2015) 156:4695–706. doi: 10.1210/en.2015-1500
54. Eferl R, Wagner EF. AP-1: A Double-Edged Sword in Tumorigenesis. *Nat Rev Cancer* (2003) 3:859–68. doi: 10.1038/nrc1209
55. Kataoka K, Noda M, Nishizawa M. Maf Nuclear Oncoprotein Recognizes Sequences Related to an AP-1 Site and Forms Heterodimers With Both Fos and Jun. *Mol Cell Biol* (1994) 14:700–12. doi: 10.1128/mcb.14.1.700-712.1994
56. Nguyen HT, Najih M, Martin LJ. The AP-1 Family of Transcription Factors Are Important Regulators of Gene Expression Within Leydig Cells. *Endocrine* (2021) 74:498–507. doi: 10.1007/s12020-021-02888-7
57. Li X, Hales KH, Watanabe G, Lee RJ, Pestell RG, Hales DB. The Effect of Tumor Necrosis Factor- α and cAMP on Induction of AP-1 Activity in MA-10 Tumor Leydig Cells. *Endocrine* (1997) 6:317–24. doi: 10.1007/BF02820509
58. Noelke J, Wistuba J, Damm OS, Fietz D, Gerber J, Gaehle M, Brehm R. A Sertoli Cell-Specific Connexin43 Knockout Leads to Altered Interstitial Connexin Expression and Increased Leydig Cell Numbers. *Cell Tissue Res* (2015) 361:633–44. doi: 10.1007/s00441-015-2126-7
59. Shea-Eaton W, Sandhoff TW, Lopez D, Hales DB, McLean MP. Transcriptional Repression of the Rat Steroidogenic Acute Regulatory (Star) Protein Gene by the AP-1 Family Member C-Fos. *Mol Cell Endocrinol* (2002) 188:161–70. doi: 10.1016/s0303-7207(01)00715-8
60. Cai DH, Wang D, Keefer J, Yeamans C, Hensley K, Friedman AD. C/EBP Alpha: AP-1 Leucine Zipper Heterodimers Bind Novel DNA Elements, Activate the PU.1 Promoter and Direct Monocyte Lineage Commitment More Potently Than C/EBP Alpha Homodimers or AP-1. *Oncogene* (2008) 27:2772–9. doi: 10.1038/sj.onc.1210940
61. Rutberg SE, Adams TL, Olive M, Alexander N, Vinson C, Yuspa SH. CRE DNA Binding Proteins Bind to the AP-1 Target Sequence and Suppress

- AP-1 Transcriptional Activity in Mouse Keratinocytes. *Oncogene* (1999) 18:1569–79. doi: 10.1038/sj.onc.1202463
62. Osada S, Yamamoto H, Nishihara T, Imagawa M. DNA Binding Specificity of the CCAAT/Enhancer-Binding Protein Transcription Factor Family. *J Biol Chem* (1996) 271:3891–6. doi: 10.1074/jbc.271.7.3891
 63. Nalbant D, Williams SC, Stocco DM, Khan SA. Luteinizing Hormone-Dependent Gene Regulation in Leydig Cells May Be Mediated by CCAAT/Enhancer-Binding Protein- β . *Endocrinology* (1998) 139:272–9. doi: 10.1210/endo.139.1.5663
 64. Zhang Z, Burch PE, Cooney AJ, Lanz RB, Pereira FA, Wu J, et al. Genomic Analysis of the Nuclear Receptor Family: New Insights Into Structure, Regulation, and Evolution From the Rat Genome. *Genome Res* (2004) 14:580–90. doi: 10.1101/gr.2160004
 65. Martin LJ, Tremblay JJ. Nuclear Receptors in Leydig Cell Gene Expression and Function. *Biol Reprod* (2010) 83:3–14. doi: 10.1095/biolreprod.110.083824
 66. Lin FJ, Qin J, Tang K, Tsai SY, Tsai MJ. Coup D'etat: An Orphan Takes Control. *Endocr Rev* (2011) 32:404–21. doi: 10.1210/er.2010-0021
 67. Ashraf UM, Sanchez ER, Kumarasamy S. COUP-TFII Revisited: Its Role in Metabolic Gene Regulation. *Steroids* (2019) 141:63–9. doi: 10.1016/j.steroids.2018.11.013
 68. Cooney AJ, Tsai SY, O'Malley BW, Tsai MJ. Chicken Ovalbumin Upstream Promoter Transcription Factor (COUP-TF) Dimers Bind to Different GGTC A Response Elements, Allowing COUP-TF to Repress Hormonal Induction of the Vitamin D₃, Thyroid Hormone, and Retinoic Acid Receptors. *Mol Cell Biol* (1992) 12:4153–63. doi: 10.1128/mcb.12.9.4153
 69. Kilcoyne KR, Smith LB, Atanassova N, Macpherson S, McKinnell C, van den Driesche S, et al. Fetal Programming of Adult Leydig Cell Function by Androgenic Effects on Stem/Progenitor Cells. *Proc Natl Acad Sci USA* (2014) 111:E1924–32. doi: 10.1073/pnas.1320735111
 70. Pereira FA, Qiu Y, Zhou G, Tsai MJ, Tsai SY. The Orphan Nuclear Receptor COUP-TFII Is Required for Angiogenesis and Heart Development. *Genes Dev* (1999) 13:1037–49. doi: 10.1101/gad.13.8.1037
 71. Qin J, Tsai MJ, Tsai SY. Essential Roles of COUP-TFII in Leydig Cell Differentiation and Male Fertility. *PLoS One* (2008) 3:1–11. doi: 10.1371/journal.pone.0003285
 72. Maxwell MA, Muscat GEO. The NR4A Subgroup: Immediate Early Response Genes With Pleiotropic Physiological Roles. *Nucl Recept Signal* (2006) 4:e002. doi: 10.1621/nrs.04002
 73. Kurakula K, Koenis DS, van Tiel CM, de Vries CJM. NR4A Nuclear Receptors Are Orphans But Not Lonesome. *Biochim Biophys Acta - Mol Cell Res* (2014) 1843:2543–55. doi: 10.1016/j.bbamcr.2014.06.010
 74. Wilson TE, Mouw AR, Weaver CA, Milbrandt J, Parker KL. The Orphan Nuclear Receptor NGFI-B Regulates Expression of the Gene Encoding Steroid 21-Hydroxylase. *Mol Cell Biol* (1993) 13:861–8. doi: 10.1128/mcb.13.2.861-868.1993
 75. Eells J, Witta J, Otridge J, Zuffova E, Nikodem V. Structure and Function of the Nur77 Receptor Subfamily, a Unique Class of Hormone Nuclear Receptors. *Curr Genomics* (2005) 1:135–52. doi: 10.2174/1389202003351580
 76. Song KH, Park JI, Lee MO, Soh J, Lee K, Choi HS. LH Induces Orphan Nuclear Receptor Nur77 Gene Expression in Testicular Leydig Cells. *Endocrinology* (2001) 142:5116–23. doi: 10.1210/endo.142.12.8525
 77. Martin LJ, Boucher N, El-Asmar B, Tremblay JJ. cAMP-Induced Expression of the Orphan Nuclear Receptor Nur77 in MA-10 Leydig Cells Involves a CaMKI Pathway. *J Androl* (2009) 30:134–45. doi: 10.2164/jandrol.108.006387
 78. Fayard E, Auwerx J, Schoonjans K. LRF-1: An Orphan Nuclear Receptor Involved in Development, Metabolism and Steroidogenesis. *Trends Cell Biol* (2004) 14:250–60. doi: 10.1016/j.tcb.2004.03.008
 79. Meinsohn M-C, Smith OE, Bertolin K, Murphy BD. The Orphan Nuclear Receptors Steroidogenic Factor-1 and Liver Receptor Homolog-1: Structure, Regulation, and Essential Roles in Mammalian Reproduction. *Physiol Rev* (2019) 99:1249–79. doi: 10.1152/physrev.00019.2018
 80. Morohashi K, Zanger UM, Honda S, Hara M, Waterman MR, Omura T. Activation of CYP11A and CYP11B Gene Promoters by the Steroidogenic Cell-Specific Transcription Factor, Ad4BP. *Mol Endocrinol* (1993) 7:1196–204. doi: 10.1210/mend.7.9.8247022
 81. Luo X, Ikeda Y, Parker KL. A Cell-Specific Nuclear Receptor Is Essential for Adrenal and Gonadal Development and Sexual Differentiation. *Cell* (1994) 77:481–90. doi: 10.1016/0092-8674(94)90211-9
 82. Sadovsky Y, Crawford PA, Woodson KG, Polish JA, Clements MA, Tourtellotte LM, et al. Mice Deficient in the Orphan Receptor Steroidogenic Factor 1 Lack Adrenal Glands and Gonads But Express P450 Side-Chain-Cleavage Enzyme in the Placenta and Have Normal Embryonic Serum Levels of Corticosteroids. *Proc Natl Acad Sci USA* (1995) 92:10939–43. doi: 10.1073/pnas.92.24.10939
 83. Jeyasuria P, Ikeda Y, Jamin SP, Zhao L, De Rooij DG, Themmen APN, et al. Cell-Specific Knockout of Steroidogenic Factor 1 Reveals Its Essential Roles in Gonadal Function. *Mol Endocrinol* (2004) 18:1610–9. doi: 10.1210/me.2003-0404
 84. Karpova T, Ravichandiran K, Insisienmay L, Rice D, Agbor V, Heckert LL. Steroidogenic Factor 1 Differentially Regulates Fetal and Adult Leydig Cell Development in Male Mice. *Biol Reprod* (2015) 93:1–15. doi: 10.1095/biolreprod.115.131193
 85. Parker KL, Rice DA, Lala DS, Ikeda Y, Luo X, Wong M, et al. Steroidogenic Factor 1: An Essential Mediator of Endocrine Development. *Recent Prog Horm Res* (2002) 57:19–36. doi: 10.1210/rp.57.1.19
 86. Schimmer BP, White PC. Minireview: Steroidogenic Factor 1: Its Roles in Differentiation, Development, and Disease. *Mol Endocrinol* (2010) 24:1322–37. doi: 10.1210/me.2009-0519
 87. Yang Y, Li Z, Wu X, Chen H, Xu W, Xiang Q, et al. Direct Reprogramming of Mouse Fibroblasts Toward Leydig-Like Cells by Defined Factors. *Stem Cell Rep* (2017) 8:39–53. doi: 10.1016/j.stemcr.2016.11.010
 88. Hou YP, Zhang ZY, Xing XY, Zhou J, Sun J. Direct Conversion of Human Fibroblasts Into Functional Leydig-Like Cells by SF-1, GATA4 and NGFI-B. *Am J Transl Res* (2018) 10:175–83.
 89. Tremblay JJ, Viger RS. Transcription Factor GATA-4 Enhances Mullerian Inhibiting Substance Gene Transcription Through a Direct Interaction With the Nuclear Receptor SF-1. *Mol Endocrinol* (1999) 13:1388–401. doi: 10.1210/mend.13.8.0330
 90. Zanaria E, Muscatelli F, Bardoni B, Strom TM, Guioli S, Guo W, et al. An Unusual Member of the Nuclear Hormone Receptor Superfamily Responsible for X-Linked Adrenal Hypoplasia Congenita. *Nature* (1994) 372:635–41. doi: 10.1038/372635a0
 91. Iyer AK, McCabe ERB. Molecular Mechanisms of DAX1 Action. *Mol Genet Metab* (2004) 83:60–73. doi: 10.1016/j.ymgme.2004.07.018
 92. Iyer AK, Zhang Y-H, McCabe ERB. Dosage-Sensitive Sex Reversal Adrenal Hypoplasia Congenita Critical Region on the X Chromosome, Gene 1 (DAX1) (NR0B1) and Small Heterodimer Partner (SHP) (NR0B2) Form Homodimers Individually, as Well as DAX1-SHP Heterodimers. *Mol Endocrinol* (2006) 20:2326–42. doi: 10.1210/me.2005-0383
 93. Meeks JJ, Russell TA, Jeffs B, Huhtaniemi I, Weiss J, Jameson JL. Leydig Cell-Specific Expression of DAX1 Improves Fertility of the Dax1-Deficient Mouse. *Biol Reprod* (2003) 69:154–60. doi: 10.1095/biolreprod.102.011429
 94. Xu B, Yang W-H, Gerin I, Hu C-D, Hammer GD, Koenig RJ. Dax-1 and Steroid Receptor RNA Activator (SRA) Function as Transcriptional Coactivators for Steroidogenic Factor 1 in Steroidogenesis. *Mol Cell Biol* (2009) 29:1719–34. doi: 10.1128/mcb.01010-08
 95. Vega A, Martinot E, Baptissart M, De Haze A, Saru JP, Baron S, et al. Identification of the Link Between the Hypothalamo-Pituitary Axis and the Testicular Orphan Nuclear Receptor NR0B2 in Adult Male Mice. *Endocrinology* (2015) 156:660–9. doi: 10.1210/en.2014-1418
 96. Volle DH, Duggavathi R, Magnier BC, Houten SM, Cummins CL, Lobaccaro JMA, et al. The Small Heterodimer Partner Is a Gonadal Gatekeeper of Sexual Maturation in Male Mice. *Genes Dev* (2007) 21:303–15. doi: 10.1101/gad.409307
 97. Di-Luoffo M, Daems C, Bergeron F, Tremblay JJ. Novel Targets for the Transcription Factors MEF2 in MA-10 Leydig Cells. *Biol Reprod* (2015) 93:1–12. doi: 10.1095/biolreprod.114.127761
 98. Viger RS, Taniguchi H, Robert NM, Tremblay JJ. Role of the GATA Family of Transcription Factors in Andrology. *J Androl* (2004) 25:441–52. doi: 10.1002/j.1939-4640.2004.tb02813.x
 99. Tremblay M, Sanchez-Ferraz O, Bouchard M. GATA Transcription Factors in Development and Disease. *Development* (2018) 145:1–20. doi: 10.1242/dev.164384
 100. Yomogida K, Ohtani H, Harigae H, Ito E, Nishimune Y, Engel JD, et al. Developmental Stage- and Spermatogenic Cycle-Specific Expression of Transcription Factor GATA-1 in Mouse Sertoli Cells. *Development* (1994) 120:1759–66. doi: 10.1242/dev.120.7.1759

101. Ketola I, Rahman N, Toppari J, Bielinska M, Porter-Tinge SB, Tapanainen JS, et al. Expression and Regulation of Transcription Factors GATA-4 and GATA-6 in Developing Mouse Testis. *Endocrinology* (1999) 140:1470–80. doi: 10.1210/endo.140.3.6587
102. Robert NM, Tremblay JJ, Viger RS. Friend of GATA (FOG)-1 and FOG-2 Differentially Repress the GATA-Dependent Activity of Multiple Gonadal Promoters. *Endocrinology* (2002) 143:3963–73. doi: 10.1210/en.2002-220280
103. Viger RS, Mertineit C, Trasler JM, Nemer M. Transcription Factor GATA-4 Is Expressed in a Sexually Dimorphic Pattern During Mouse Gonadal Development and Is a Potent Activator of the Mullerian Inhibiting Substance Promoter. *Development* (1998) 125:2665–75. doi: 10.1242/dev.125.14.2665
104. Hu YC, Okumura LM, Page DC. Gata4 Is Required for Formation of the Genital Ridge in Mice. *PLoS Genet* (2013) 9:1–12. doi: 10.1371/journal.pgen.1003629
105. Manuylov NL, Zhou B, Ma Q, Fox SC, Pu WT, Tevosian SG. Conditional Ablation of Gata4 and Fog2 Genes in Mice Reveals Their Distinct Roles in Mammalian Sexual Differentiation. *Dev Biol* (2011) 353:229–41. doi: 10.1016/j.ydbio.2011.02.032
106. Tremblay JJ, Viger RS. GATA Factors Differentially Activate Multiple Gonadal Promoters Through Conserved GATA Regulatory Elements. *Endocrinology* (2001) 142:977–86. doi: 10.1210/endo.142.3.7995
107. Bielinska M, Seehra A, Toppari J, Heikinheimo M, Wilson DB. GATA-4 Is Required for Sex Steroidogenic Cell Development in the Fetal Mouse. *Dev Dyn* (2007) 236:203–13. doi: 10.1002/dvdy.21004
108. Kaestner KH, Hiemisch H, Luckow B, Schütz G. The HNF-3 Gene Family of Transcription Factors in Mice: Gene Structure, cDNA Sequence, and mRNA Distribution. *Genomics* (1994) 20:377–85. doi: 10.1006/geno.1994.1191
109. Behr R, Sackett SD, Bochkis IM, Le PP, Kaestner KH. Impaired Male Fertility and Atrophy of Seminiferous Tubules Caused by Haploinsufficiency for Foxa3. *Dev Biol* (2007) 306:636–45. doi: 10.1016/j.ydbio.2007.03.525
110. Brennan J, Tilmann C, Capel B. Pdgfr-Alpha Mediates Testis Cord Organization and Fetal Leydig Cell Development in the XY Gonad. *Genes Dev* (2003) 17:800–10. doi: 10.1101/gad.1052503
111. Kim H, Kumar S, Lee K. FOXA3, a Negative Regulator of Nur77 Expression and Activity in Testicular Steroidogenesis. *Int J Endocrinol* (2021) 2021:1–8. doi: 10.1155/2021/6619447
112. Potthoff MJ, Olson EN. MEF2: A Central Regulator of Diverse Developmental Programs. *Development* (2007) 134:4131–40. doi: 10.1242/dev.008367
113. Dodington DW, Desai HR, Woo M. JAK/STAT – Emerging Players in Metabolism. *Trends Endocrinol Metab* (2018) 29:55–65. doi: 10.1016/j.tem.2017.11.001
114. Kanzaki M, Morris PL. Lactogenic Hormone-Inducible Phosphorylation and Gamma-Activated Site-Binding Activities of Stat5b in Primary Rat Leydig Cells and MA-10 Mouse Leydig Tumor Cells. *Endocrinology* (1998) 139:1872–82. doi: 10.1210/endo.139.4.5956
115. Nakamura BN, Lawson G, Chan JY, Banuelos J, Cortés MM, Hoang YD, et al. Knockout of the Transcription Factor Nrf2 Disrupts Spermatogenesis in an Age-Dependent Manner. *Free Radic Biol Med* (2010) 49:1368–79. doi: 10.1016/j.freeradbiomed.2010.07.019
116. Chen H, Jin S, Guo J, Kombairaju P, Biswal S, Zirkin BR. Knockout of the Transcription Factor Nrf2: Effects on Testosterone Production by Aging Mouse Leydig Cells. *Mol Cell Endocrinol* (2015) 409:113–20. doi: 10.1016/j.mce.2015.03.013
117. Chung J-Y, Chen H, Zirkin B. Sirt1 and Nrf2: Regulation of Leydig Cell Oxidant/Antioxidant Intracellular Environment and Steroid Formation. *Biol Reprod* (2021) 105:1307–16. doi: 10.1093/biolre/iaob150
118. Xiao Y, Zhao L, Li W, Wang X, Ma T, Yang L, et al. Circadian Clock Gene BMAL1 Controls Testosterone Production by Regulating Steroidogenesis-Related Gene Transcription in Goat Leydig Cells. *J Cell Physiol* (2021) 236:6706–25. doi: 10.1002/jcp.30334
119. Ding H, Zhao J, Liu H, Wang J, Lu W. BMAL1 Knockdown Promoted Apoptosis and Reduced Testosterone Secretion in TM3 Leydig Cell Line. *Gene* (2020) 747:144672. doi: 10.1016/j.gene.2020.144672
120. Mealy K, Robinson B, Millette CF, Majzoub J, Wilmore DW. The Testicular Effects of Tumor Necrosis Factor. *Ann Surg* (1990) 211:470–5. doi: 10.1097/0000658-199004000-00014

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Copyright © 2022 de Mattos, Viger and Tremblay. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.