CHAPTER

15

Transcription Factors in Sertoli Cells

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I. INTRODUCTION

Sertoli cells throughout development differentiate and undergo a number of functional and structural modifications [1]. These modifications start with Sertoli cell fate determination at the time of male sex determination in embryonic day 11–13 mice [2]. Following this initial event, Sertoli cells undergo a rapid phase of proliferation and differentiation [1]. The functional maturation at puberty is achieved by exit from the cell cycle that corresponds with the formation of the blood–testis barrier [3]. In the adult, the primary functions of Sertoli cells are to support spermatogenesis by (1) providing the microenvironment for the growth and development of the germ cells, (2) transmitting hormonal/environmental cues to the developing germ cells through paracrine signaling mechanisms, and (3) providing the structural support for the developing germ cells [4, 5]. Therefore, a number of distinct differentiated and functional states of the Sertoli cell exist from the earliest period in the embryo with their role in sex determination [6–8] to the adult stage of maintaining spermatogenesis. Each of these is associated with, and regulated by, a series of coordinated gene expression events at various periods of development [9–12]. For example, the molecular events leading to the initiation of Sertoli cell differentiation at the time of sex determination involves a coordinated and sequential expression of a number of DNA binding proteins such as WT-1, SF-1, Sry, Sox9, GATA-6, Dmrt1, and DAX-1 [13–17]. The expression of these proteins is achieved through an intricate and complex transcriptional regulatory network.

The transcriptional regulation of Sertoli cell gene expression in large part determines the unique differentiated state and functional status of the cell throughout development. Therefore, the unique set of transcription factors present and how they interact to coordinate gene expression determines the state of Sertoli cell differentiation required to maintain testis function and spermatogenesis. This chapter reviews what is currently known regarding the transcription factors present in Sertoli cells and how they may coordinate specific cellular functions.

II. TRANSCRIPTION FACTORS

Transcription factors are DNA binding proteins (*trans*-acting) that bind to DNA regulatory elements located *cis*- to the target genes. Transcription factors are generally considered final targets of signal-transduction pathways [18–20]. The level of expression of transcription factors and their activities determines whether their target genes are transcribed and to what extent.

These regulators of gene expression in turn are tightly regulated by a multitude of signaling pathways influenced by cell–cell interaction, growth factors, hormones, extracellular matrix, and the stage of development. This is exemplified by the observations that the gene expression profile of Sertoli cells associated with germ cells at different stages of spermatogenesis are different [9, 21–29]. In response to follicle stimulating hormone (FSH), the prepubertal cells express genes involved in proliferation and, following puberty, the cells express genes involved in maintaining differentiation and spermatogenesis [30, 31]. Therefore, alterations in the transcriptional regulation of the Sertoli cell determine changes in cellular differentiation and function.

In general, the transcriptional control unit, or the promoter, present immediately upstream of the transcriptional start site of the gene is a cluster of binding sites for the RNA polymerase complex and transcription factors [32, 33] (Fig. 15.1). Most of the Sertoli cell gene promoters have a conserved TATA box, which binds the basal transcription factor initiation complex (TFIID, IIB, and IIF). These TATA box-containing promoters are highly regulated in that these promoters are switched on or off in response to a specific stimulus. Alternatively, some promoters of the housekeeping genes such as the androgen binding protein (ABP) [34] and FSH receptor [35, 36] are devoid of TATA boxes but instead contain a G/C-rich sequence and an initiator region (Inr) [37, 38]. These TATA-less promoters can allow initiation of transcription at multiple sites over a broad region, often generating transcripts with multiple 5′ ends [39]. The organization of the typical promoter is shown in Figure 15.1. In addition to the promoter, an enhancer element can also stimulate transcription in an orientation-independent manner [40, 41] (Fig. 15.1). These enhancer elements can be located distal to the promoter or in the introns and 3′ UTR regions. The enhancer elements can also bind multiple gene-specific transcription factors. As an added level of control, the bound transcription factors can interact to form cell specific transcription factor complexes. These interactions can be mediated by regulatory proteins (coactivators/repressors), which may not directly bind to the DNA, but facilitate interactions between DNA bound transcription factors [42, 43] (Fig. 15.1). Equally important are the transcription factors and regulatory proteins that repress the transcription and are often identified as silencer regions on the promoter or enhancers [44]. These reversible modifications are important steps in the control of cellular gene expression.

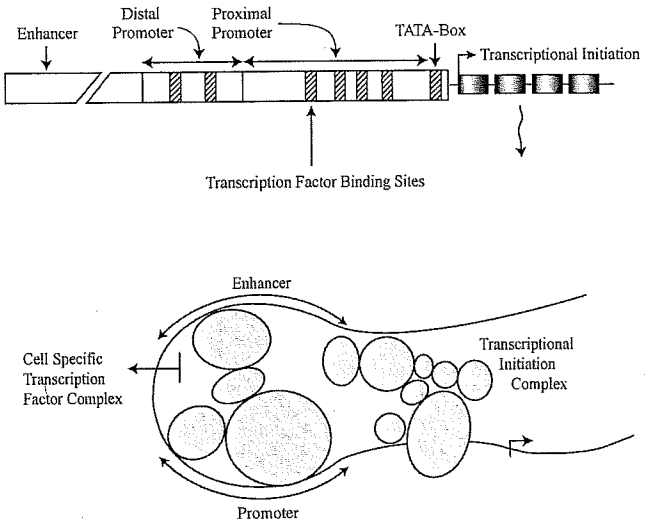

FIGURE 15.1 The organization of a typical eukaryotic promoter. In general, the eukaryotic promoters contain a TATA box within the proximal –30 bp of the transcriptional initiation site, which binds the basal transcriptional machinery. The promoter upstream of the TATA box can be organized into proximal (about –500 bp), distal (–500 to –1000 bp), and enhancer (more than –1000 bp). These regions can bind sequence-specific transcription factors. The bound transcription factor can also physically interact either directly or through accessory proteins in order to form a cell-specific transcriptional complex.

III. TRANSCRIPTIONAL CONTROL OF SERTOLI CELL FUNCTION

The functional and developmental changes associated with Sertoli cell differentiation and functions are a result of stage-specific activation/repression of specific transcription factors. Some of these transcription factors such as Sry [45] and DAX-1 [46, 47] are expressed only during a particular stage of development. Factors, such as C/EBPβ [48] and androgen receptor, are dependent on the presence/absence of specific stimuli such as FSH and specific stages of spermatogenesis [49], respectively. Thus, to achieve the complexity of functions required by Sertoli cells, such as sex determination and spermatogenesis, it is apparent that these cells achieve a degree of transcriptional control that can be achieved by modulation of the activities of specific transcription factors by one or more of the following mechanisms:

1. Phosphorylation/dephosphorylation, for example, CREB phosphorylation in response to FSH [50]
2. Protein–protein interactions, for example, interactions between bHLH, CREB, and CBP in transferrin promoter [51]
3. Ligand-dependent activation of the nuclear hormone receptors, for example, activation of

androgen receptor and retinoic acid receptors by binding of their cognate ligands, androgen and retinoic acid, respectively
4. Expression during a particular stage of development, for example, spermatogenesis stage-dependent expression of androgen receptor
5. Availability of DNA binding sites (methylation/demethylation), for example, Sertoli cell–specific demethylation of the E-box response element in FSH receptor promoter [52]

A large number of transcription factors have been identified in Sertoli cells, mostly through comparing genetic disorders in *Drosophila*, *Caenorhabditis elegans*, humans, and mice, leading to a male reproductive phenotype such a sex reversal (ambiguous gonads, vanishing testes, persistent Müllerian duct syndrome, loss or decrease in fertility and testis size). These elegant studies have resulted in the identification of key Sertoli cell–specific transcription factors and their postulated mechanism of action. These include Sry, DAX-1, Dmrt1, CREB, AR, and GATA among many others listed in Table 15.1. The complex interplay and hierarchy of these factors at the Sertoli cell "systems level" remain to be investigated.

TABLE 15.1 Categorization of Transcription Factors in Sertoli Cells

1. Transcription factors with basic domains
   a. Basic leucine zipper (bZIP); jun, fos, CREB, ATF, C/EBP
   b. Basic helix–loop–helix (bHLH); E2A, E2–2, Id1, Id2, Id3, Id4, REB
   c. bZIP-bHLH: USF, USF2, c-myc
2. Transcription factors with zinc-finger domains
   a. Nuclear receptors: AR, ER, RAR, RXR, PPAR, TR, SF-1, DAX-1
   b. Cys4 zinc fingers: GATA, FOG
   c. Cys2His2 zinc-finger domains: TFIIIA, Sp1, SP3, Sp4, WT1
3. Transcription factors with helix–turn–helix motifs
   a. Paired box: Pem
   b. LIM homeodomain: Lhx9
   c. Winged helix/Forkhead: Trident
4. β scaffold factors with minor groove contacts
   a. Rel/Ankyrin: NFκB family
   b. HMG: Sox, Sry
5. Others
   a. STAT: STAT-1 and -2
   b. CBP
   c. Dmrt1

IV. TRANSCRIPTION FACTORS EXPRESSED IN SERTOLI CELLS

Recent progress in high-throughput gene expression profiling technology has allowed us to develop a comprehensive understanding of the Sertoli cell transcription factor "Transcriptome." The Sertoli cells cultured from postnatal 20-day-old rats express a large and diverse group of transcription factors as listed in Table 15.2. The expression of some of these transcription factors is based on previous studies such as the retinoic acid receptor α, upstream transcription factor USF-1, or GATA-4 and GATA-6. The high levels of expression of p53, orphan nuclear receptors, *Drosophila* hairy, and enhancer of split (HES) are novel observations and suggest a functional significance of these factors in Sertoli cells. In response to FSH, the expression of some of the transcription factors remains unchanged, suggesting that they may be involved in maintaining basal Sertoli cell functions or may undergo phosphorylation or other post-transcriptional modifications in response to specific stimuli in order to be active. As expected, the expression of the majority of transcription factors is increased in response to FSH (Table 15.2). Most notable among these are C/EBPβ, which may be involved in mediating the FSH-cAMP-PKA response. The highest increase in the expression levels is observed for the orphan nuclear receptors Nr4a2 and Nr4a3 (40-fold average) within 2 hr of FSH stimulation (Table 15.2B). A large number of transcription factors also decrease in response to FSH, which include C/EBPδ, retinoic acid receptors, GATA-4, and thyroid hormone receptor α. The dynamics of activation of certain transcription factors in response to FSH over a period of time (24 hr) also correlates well with the previously published reports. These include cAMP-responsive element modulator (CREM), which is induced after 4 hr of FSH stimulation, a delayed response when compared to C/EBPβ. The data provided in Table 15.2 should serve as a comprehensive overview of the basal and FSH-induced Sertoli cell transcription factor profile. The physiological function and significance of each of these transcription factors should be considered in relation to mechanisms that can modify their activities as listed earlier. In the following sections, the Sertoli cell transcription factors are discussed that have a previously identified functional significance in terms of stage-specific expression (outlined in Table 15.3), expression in response to hormones and growth factors, and/or that have a

TABLE 15.2A Sertoli Cell Transcription Factor: FSH Responsiveness

Transcription Factor Class/Sub class/name/Access. no.			0 hr Signal	2 hr xChange	4 hr xChange
1. Transcription Factors with Basic Domains					
1.a Basic Leucine Zipper					
Jun	AI175959	c-jun	429	0.5	1.0
Atf3	M63282	Activating transcription factor 3 (ATF3)	234	1.4	0.5
Junb	AA891041	jun B proto-oncogene	171	5.7	**2.7**
Crem	S66024	cAMP responsive element modulator (CREM)	113	5.8	**11.2**
Creb1	X14788	cAMP response element binding protein 1 (CREB)	22	1.6	1.5
Cebpd	M65149	CCAAT/enhancerbinding, protein (C/EBP) delta	106	0.4	0.4
Cebpb	X60769	CCAAT/enhancer binding protein (C/EBP), beta	67	7.5	2.4
Dbp	J03179	D site albumin promoter binding protein	247	0.3	0.0
1.b. Basic Helix–Loop–Helix					
Hes1	D13417	Hairy and enhancer of split 1 (*Drosophila*)	212	**2.1**	1.2
rE12	S77532	Helix–loop–helix transcription factor E12	64	0.9	1.0
Hif1a	Y09507	Hypoxia inducible factor 1, alpha subunit	43	1.7	**2.1**
1.c. bHLH-ZIP					
Usf1	AA891717	Upstream transcription factor 1	702	0.7	0.5
Usf2	X90823	Transcription factor USF2	46	1.0	1.4
1.d. bHLH-PAS					
Arnt	U61184	Aryl hydrocarbon receptor nuclear translocator	65	0.9	1.0

Note: The data are the mean of two different experiments and are within a 15% coefficient of variation (CV). The ***Xchange*** represents increases (bold) or decreases (underline) of the transcription in cultured rat (20-day-old) Sertoli cells treated with FSH for various time intervals. The data were generated by using the rat Affymetrix chip 434. The analysis was obtained from a data set previously described by Dr. Derek McLean and Dr. Michael Griswold [324].

TABLE 15.2B Sertoli Cell Transcription Factor FSH Responsiveness

Transcription Factor Class/Sub class/name/Access. no.			0 hr Signal	2 hr xChange	4 hr xChange	8 hr xChange	24 hr xChange
2. Transcription Factors with Zinc-Finger Domains							
2.a. Nuclear Receptors							
Rara	U15211	Retinoic acid receptor, α	749	0.5	0.5	0.6	0.6
Nr1h2	U14533	Ubiquitous Receptor/Liver X receptor β	662	0.6	0.7	0.8	0.5
Thra	M31174	Thyroid hormone receptor α	608	0.7	0.7	0.6	0.9
Nfyc	AA875121	Nuclear transcription factor-Y γ	302	1.0	1.1	1.2	1.1
Nr0b1	X99470	DAX-1	265	0.9	0.7	0.5	1.1
Nr5a1	D42156	SF-1	133	0.3	0.2	0.2	0.2
Nr0b2	D86580	SHP (Small Heterodimer Partner)	109	0.2	0.2	0.2	0.4
Rxra	L06482	Retinoid X receptor alpha	83	0.3	0.6	0.3	0.2
Ppard	U40064	Peroxisome proliferator activated receptor δ	68	0.7	0.8	0.6	0.7
Nr4a1	U17254	IEG transcription factor NGFI-B	65	**24.0**	**13.9**	1.8	**2.1**
Nfya	M34238	Nuclear transcription factor-Y α	56	0.6	1.0	1.2	0.7
Nfix	AB012234	Nuclear factor I/X	52	0.5	0.7	0.6	1.1
Nr1d2	U20796	Rev-Erb Alpha	51	**3.7**	**3.1**	1.9	**2.0**
Nr4a3	AI176710	Nuclear Orphan Receptor 1	8	**39.5**	**30.5**	**8.0**	**2.5**

Continued

TABLE 15.2B Sertoli Cell Transcription Factor FSH Responsiveness—cont'd

Transcription Factor Class/Sub class/name/Access. no.			0 hr Signal	2 hr xChange	4 hr xChange	8 hr xChange	24 hr xChange
Nr4a2	U01146	NOT, RNR-1, H2F-3, NURR1	6	**40.6**	**26.8**	**4.9**	**3.6**
Nr1d1	M25804	NR subfamily 1, group D, member 1	205	0.4	0.4	0.3	0.7
Nr2f6	AF003926	NR subfamily 2, group F, member 6	181	1.3	2.5	1.7	1.0
2.b. Cys4 Zinc Fingers							
Gata4	L22761	GATA-binding protein 4	306	0.5	0.9	0.7	0.4
Gata6	L22760	GATA-binding protein 6	161	1.7	1.9	1.2	0.9
2.c. Cys2His2 Zinc-Finger Domains							
Tieg	AI172476	TGFB inducible early growth response	112	1.8	1.6	1.6	1.3
Wt1	X69716	Wilms tumor 1	70	1.6	1.8	1.4	1.4
Egr1	M18416	Early growth response 1	86	1.2	0.6	0.5	1.1
Egr4	AI145177	Early growth response 4	74	1.8	2.0	1.3	1.2
Klf9	D12769	Kruppel-like factor 9	47	**4.1**	**3.8**	**2.1**	**1.1**

Note: The data are the mean of two different experiments and are within a 15% coefficient of variation (CV). The ***Xchange*** represents increases (bold) or decreases (underline) of the transcription in cultured rat (20-day-old) Sertoli cells treated with FSH for various time intervals. The data were generated by using the rat Affymetrix chip 434. The analysis was obtained from a data set previously described by Dr. Derek McLean and Dr. Michael Griswold [324].

TABLE 15.2C Sertoli Cell Transcription Factor FSH Responsiveness

Transcription Factor Class/Sub class/name/Access. no.			0 hr Signal	2 hr xChange	4 hr xChange	8 hr xChange	24 hr xChange
3. Transcription Factors with Helix–turn–Helix motifs							
3.a. Homeo Domain							
Tcf1; HNF1	X54423	Transcription factor 1	56	0.7	0.6	0.4	0.6
3.b. Paired Box							
Pax4	AF053100	Paired box gene 4	51	0.5	0.5	0.2	0.8
3.c. Tryptophan Clusters							
Ets1	AI175900	v-ets E26 oncogene homologue 1 (avian)	81	0.6	0.9	0.9	0.8
Irf1	M34253	Interferon regulatory factor 1	222	1.6	0.8	1.0	**1.6**
4. Beta Scaffold Factors with Minor Groove Contacts							
4.a. Rel/ Ankyrin							
Nfkb1	L26267	Nuclear factor κ B p105 subunit	138	1.5	**4.6**	**2.9**	1.0
4.b. STAT							
Stat1	AA892553	Signal transducer and activator of transcription 1	104	0.5	0.4	0.1	0.6
Stat3	X91810	Signal transducer and activator of transcription 3	82	0.6	0.5	0.7	0.4
Stat5b	X91988	Signal transducer and activator of transcription 5b	41	1.7	1.3	1.2	**1.5**
4.c. Class p53							
Tp53	X13058	Tumor protein p53	306	0.6	0.7	0.7	0.6

Note: The data are the mean of two different experiments and are within a 15% coefficient of variation (CV). The ***Xchange*** represents increases (bold) or decreases (underline) of the transcription in cultured rat (20-day-old) Sertoli cells treated with FSH for various time intervals. The data were generated by using the rat Affymetrix chip 434. The analysis was obtained from a data set previously described by Dr. Derek McLean and Dr. Michael Griswold [324].

TABLE 15.3 Expression of Transcription Factors in Relation to Developmental Stage of Sertoli Cells[a]

Transcription factor	Undifferentiated gonad	Sex determination	Embryonic differentiation	Prenatal/prepubertal	Puberty/adult
FSH receptor			++	++	++
Androgen receptor	?	?	++	++	++
Aromatase			++	++	–
Transferrin	–	–	+	+	++
Sry	–	+++	–	–	–
SF–1	++	+++	++	+	+
FOG2	+++	+++	+/–	+	++
Sox9	++	+++	+	+	++
FOG1	–	–	+	++	++
GATA–2	++	–	–	–	–
GATA–1	–	–	–	+	++
GATA–4	+	++	+	+	+
NFκB					
CREB			+(?)	+(?)	++
CREM			+(?)	+ (?)	++
WT–1	++	++	+	+	+
Jun					
Fos	?	?	?	++	++
Lhx9	?	++	++	?	?
DAX–1	?	+	++	++	++

[a] The expression of FSH receptor, androgen receptor, aromatase, and transferrin are also indicated as markers of functional differentiation of Sertoli cell. Legend: +, present; –, absent; ?, expression not known.

potential mechanism of action in regulating Sertoli cell genes. This is discussed in regards to the transcription factor categorization outlined in Table 15.1.

A. Transcription Factors with Basic Domains

1. *Basic Leucine Zipper*

The characteristic feature of basic leucine zipper (bZIP) transcription factors is the conserved 60- to 80-amino-acid bipartite alpha helix. The alpha-helix at the C terminal contains leucine every seven amino acids. The sequence-specific DNA binding is achieved by the highly charged basic region toward the N-terminal of bZIP transcription factors. The bZIP transcription factors bind to a double-stranded consensus DNA sequence as homodimers or heterodimers (Fig. 15.2). Unlike other transcription factors, the presence of bZIP proteins has been shown only in eukaryotes [53].

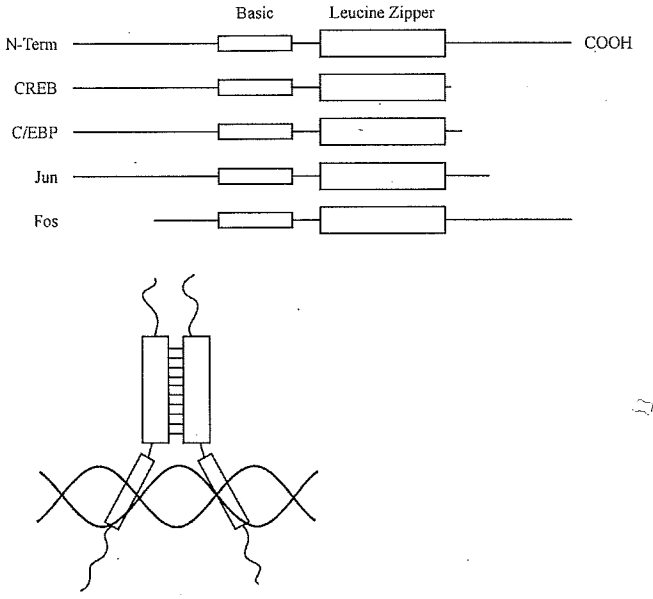

FIGURE 15.2 Schematic of basic leucine zipper transcription factors. The leucine zipper mediates dimerization and the charged basic domain is involved in DNA binding. Significant variation between the length of the N and C terminals is observed within the family members and may affect the dimerization.

a. C/EBP

CCAAT/enhancer binding protein (C/EBP) transcription factors are involved in diverse cellular

processes such as proliferation, differentiation, metabolism, inflammation, and numerous other responses and are highly expressed in hepatocytes, adipocytes, and hematopoietic cells. C/EBP dimers bind to the palindromic DNA consensus sequence 5′: –A/GTTGCG/CTCAA/T–3′ [54, 55]. Structure–function analyses of these proteins have identified several *trans*-activation domains, some of which can physically interact with general transcription factors present in the initiation complex. At least six members of the family have been isolated and characterized (C/EBPα–C/EBPζ). The functional diversity of the C/EBP family is further expanded by the generation of polypeptides through the use of alternative translation initiation sites and through interactions within the family and with other transcription factors [56]. Heterodimeric interaction partners of C/EBP include Fos, Jun [57], ATF4 [58, 59], ATF2 [60], and CREB [61].

Largely considered constitutively acting factors, recently C/EBPs have also been shown to mediate cAMP responses. A number of cAMP-responsive gene promoters also contain binding sites for C/EBP. Generally, these binding sites are located within the region of the promoter that is responsible for mediating the acute responsiveness to cAMP. Collective evidence now suggests that C/EBPs may have both constitutive and cAMP-inducible activities, and are now considered a cAMP-responsive nuclear regulator. The role of C/EBP in mediating cAMP responses can also be due to the ability of C/EBP dimers to bind related CRE sites [62].

The functions of the C/EBP family of transcription factors in energy metabolism and as a downstream effecter of cAMP responses are also functions in Sertoli cells. C/EBPα, β, δ, and ζ messenger RNA (mRNA) and proteins are present in Sertoli cell primary cultures but only the β and δ isoforms are induced in the presence of FSH or cAMP [48]. The β isoform appears to be predominant since this is the primary isoform observed in oligonucleotide gel shift experiments. The kinetics of C/EBP expression suggests that the early induction of C/EBP isoforms by cAMP may play a role in FSH-dependent regulation of late response genes in Sertoli cells. These observations are also supported by data presented in Table 15.2. C/EBPβ may mediate the cAMP responses elicited by FSH because its expression is increased within 2 hr by more than 25-fold in response to FSH. In contrast, the expression of C/EBPδ expression is decreased more than 2-fold in response to FSH. The significance of C/EBPδ downregulation following FSH treatment is intriguing and suggests that isoform-specific homo- and/or heterodimerization may be involved in limiting FSH action.

b. CREB

CREB (cAMP response element binding protein) is a large superfamily and consists of the closely related factors CREM [63], ICER (an alternate spliced form of CREM) [64], and ATF-1 (activating transcription factor 1) (Fig. 15.3). A high degree of homology within the bZIP domain of CREB, CREM, and ATF-1 allows them to form homo- and heterodimers and to bind to the same palindromic *cis*-regulatory element TGACGTCA [65]. While many CREB binding sites are comprised of variations of this consensus motif, almost all harbor the core sequence CGTCA.

The activation of the cAMP pathway by the binding of FSH to its cognate receptor and subsequent activation of CREB and associated downstream events influencing spermatogenesis have been extensively studied and reviewed elsewhere [66, 67]. The immediate effect of cAMP or FSH on Sertoli cells is the phosphorylation of CREB, which usually occurs within 1 to 5 min [50]. The almost instant phosphorylation and activation of CREB at serine 133 ensures the expression of downstream regulatory genes such as c-fos [66]. The presence of at least two CRE sites in the proximal 300 bp of the CREB promoter is required for cAMP-inducible expression of the gene and supports the model of FSH- and cAMP-mediated CREB autoregulation of its own promoter [50]. This autoregulatory mechanism may explain the dramatic stage-specific oscillations in Sertoli cells of CREB messenger RNA levels during the 12-day cycles of spermatogenesis in rat seminiferous tubules [21].

c. CREM

CREM and its spliced variants α, β, and γ bind to a consensus CRE with high affinity and specificity similar to CREB. Unlike CREB, CREM lacks the transcriptional

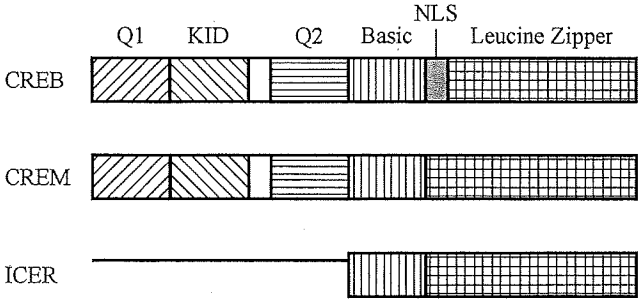

FIGURE 15.3 Typical organization of the CREB family of transcription factors. **Q1, Q2:** represent the transactivation domain and are present in all CREB isoforms and CREMt, but absent in ICER and CREMα; **KID:** kinase inducible domain is present in all CREB and CREM isoforms but not in ICER; **Basic:** mediates binding to CRE sequence; **leucine zipper**: mediates dimerization, **NLS:** nuclear localization signal.

activation domain. Therefore, CREM competes with the binding of CREB to the CRE (Fig. 15.3).

In addition to CREB, the Sertoli cells also express the repressor ICER, an isoform of CREM [68] (Fig. 15.3). ICER is expressed in response to FSH and its induction accompanies downregulation of CREB and FSH receptor transcript by binding to a CRE-like sequence in the regulatory region of the gene leading to long-term desensitization [26]. The gene expression profile of the transcriptional repressor CREM reported in Table 15.2 validates the previous results, in that it is induced at least 4 hr after stimulation of Sertoli cells by FSH [69]. This delayed expression of ICER may be involved in regulating the expression of early event genes such as C/EBPβ, which are induced within 2 hr of FSH stimulation.

d. The AP-1 Complex

The AP-1 transcription factor complex consists of a dimer between members of the fos, jun, and ATF families of proteins [70, 71]. In addition to fos, jun, and ATF, CREB proteins can also form part of the AP-1 complex. Whereas fos proteins can only heterodimerize, the jun proteins can both homo- and heterodimerize with fos [72], ATF2 [73], and ATF3 members to form transcriptionally active complexes. The jun-fos dimers preferentially bind to the TRE (TGACTCA, TPA response element) [74]. The preference for the binding changes to the consensus CRE response element, TGACGTCA, when one of the members of the dimer is ATF or CREB [75, 76]. The jun homodimers bind DNA at low affinity and are generally considered transcriptionally inactive [77]. Preliminary results from our laboratory have also shown that members of the AP-1 family, specifically, ATF-3 can interact with scleraxis, a member of the basic helix–loop–helix (bHLH) transcriptional family (unpublished observations).

cfos Given its proto-oncogene status, cfos is the most actively studied transcription factor in any cell system. In Sertoli cells cfos has been implicated as an early event gene that is rapidly and transiently induced in response to FSH [78–80]. Upon stimulation with FSH and cAMP, the expression of cfos is observed within 30 min. The kinetics of induction of cfos in relation to other Sertoli cell genes such as transferrin, plasminogen activator [81], ABP, and inhibin-α subunit [81] support the notion that cfos is an immediate early gene. This is also supported by the observation that treatment of Sertoli cells with cfos blocks FSH-mediated activation of transferrin [82] and their attachment and spreading on contact with extracellular component [83]. The later observation can be extended to suggest the role of cfos in the formation and maintenance of the blood–testis barrier and basal lamina. In addition to FSH, other growth factors, possibly from germ cells, such as basic fibroblast growth factor (FGF) [84], leukemia inhibitory factor (LIF) [85], growth hormone releasing hormone (GHRH), nerve growth factor (NGF), interleukin 6 (IL-6), and interferon γ [86] also have a rapid stimulatory effect on cfos expression. In cultured immature porcine Sertoli cells, FGF has been shown to stimulate cell proliferation and plasminogen activator activity by early induction of the cfos gene independent of cAMP and the calcium/phospholipid pathways [84, 87]. Collectively, these studies suggest that cfos expression in Sertoli cells is regulated by diverse signaling pathways. The kinetics of induction of cfos by hormones and growth factors ranges from 15 min to 2 hr with return to basal levels between 6 and 18 hr [81, 85, 86]. Some of these differences observed in the time of induction of cfos following treatments may be due to culture conditions, concentrations of hormone used, and time of treatment after Sertoli cell isolation. These results suggest that cfos activation may be an important early event and lack of its function/expression may lead to gross Sertoli cell abnormalities.

Mice lacking cfos are viable and fertile but lack osteoclasts [88]. But the homozygous chimeric mice generated by embryonic stem cells targeted at the c-fos locus show reduced placental and fetal weights and significant loss of viability at birth. Among other abnormalities these mice also show delayed or absent gametogenesis, lymphopenia, and altered behavior [89]. The effect on gametogenesis supports a potential critical role for cfos in Sertoli cells.

Jun (c-jun, junB, and junD) Consistent with its expression elsewhere, junD is also highly expressed in the embryonic testis and in purified postnatal and prepubertal Sertoli cells (unpublished observation). High levels of expression of JunD suggest the importance of the AP-1 transcription factor complex in testicular development and Sertoli cell differentiation. Consistent with this observation, the male junD knockouts have reproductive phenotypes that include reduced fertility with age, increased inhibin, and decreased FSH serum levels [90]. In spite of being highly expressed, the regulation of junD has not been extensively studied in Sertoli cells. The expression of other members of the jun family such as junB is increased in response to FGF and FSH [81]. JunB can alternate or sometimes antagonize the activity of c-jun. Consistent with these observations, FSH negatively regulates the expression of c-jun [81]. Interestingly, the c-jun mRNA levels are increased, following retinoid (vitamin A) stimulation of the Sertoli cells [91] and in response to tumor necrosis factor alpha (TNFα) it is phosphorylated [92]. The differential dynamics of c-jun and junB expression in

response to FSH and vitamin A is interesting and will need further evaluation regarding the role of the jun proto-oncogene family in Sertoli cell function. [92].

2. *Basic Helix–Loop–Helix Transcription Factors*

The bHLH transcription factors are critical cell-type determinants and play important roles in cell growth, differentiation, and sex determination [93]. A bHLH domain that is conserved from yeast to mammals characterizes members of this family [94]. The bHLH domain consists of two amphipathic helixes separated by a loop that mediates homo- and heterodimerization adjacent to a DNA-binding region rich in basic amino acids [95, 96] (Fig. 15.4). The bHLH dimers bind to an E-box (CANNTG) DNA consensus sequence present in a wide variety of promoters [96, 97]. The E-box response element has been shown to influence the promoters of a number of Sertoli cell genes [98–101]. The bHLH proteins have been classified into two distinct classes. The ubiquitously expressed class A bHLH proteins consist of E2–2 [102], HEB [103], and E12 and E47 (the differentially spliced products of the E2A gene) [96]. These dimerize with tissue-restricted and developmentally regulated class B proteins such as MyoD and neuroD [104, 105]. The members of the Id (*I*nhibitor of *d*ifferentiation/DNA binding) family modulate the transcriptional activity of class A and B bHLH heterodimers. The four known Id proteins, Id1 [106], Id2 [106], Id3 [107], and Id4 [108], share a homologous HLH domain, but lack the basic DNA binding region. Thus, the Id proteins act to sequester bHLH proteins (preferentially binding to class A) by forming inactive dimers to prevent binding of bHLH proteins to the E-box sites [109] (Fig. 15.4). Therefore, Id proteins function as dominant negative bHLH transcription factors [110]. Alternatively, Id proteins can also interact with several other non-bHLH proteins and influence cellular function. For example, Id proteins can interact with proteins such as retinoblastoma (Rb) tumor suppressor protein to promote cell division [111] and ETS-TCF proteins to modulate the intracellular response to growth factors [112].

The potential role of bHLH proteins in regulating Sertoli cell function was hypothesized based on the observations that in *Drosophila, daughterless* and *ASC* (acheate-scute-complex) have a role in sex determination [113]. Subsequent promoter analysis of Sertoli cell genes also showed the presence of conserved E-box response elements. Systematic studies involving mutations in the E-box used reverse transcriptase–polymerase chain reaction (RT-PCR) to determine known and ubiquitously expressed bHLH proteins; these studies strongly suggested their significance in regulating Sertoli cell function [98, 99]. Sertoli cell expressed genes

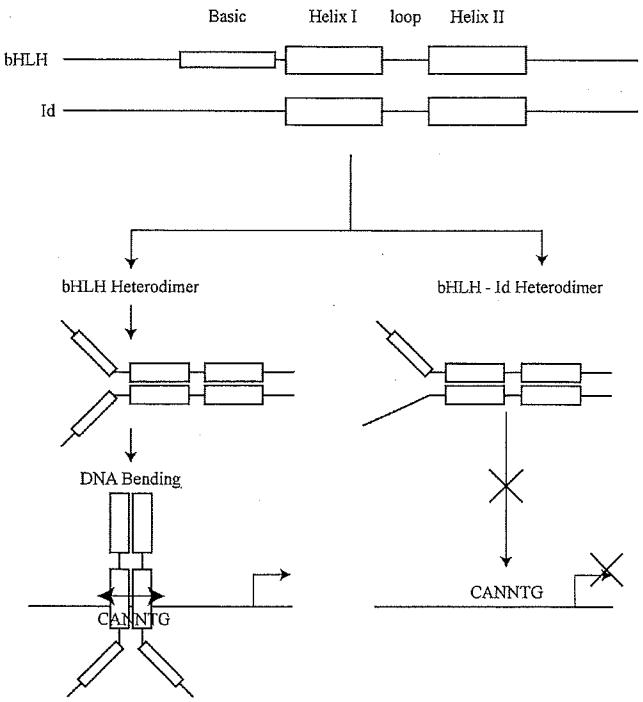

FIGURE 15.4 Schematic of basic helix–loop–helix family of transcription factors. In general, the bHLH transcription factor heterodimerizes. The dimerization is mediated by the HLH domain. The changed basic domains of the dimer bind the conserved E-box response element (CANNTG). The inhibitor of differentiation (Id) family of HLH transcription factors lacks the basic domain. The heterodimers between bHLH and Id proteins therefore fail to bind DNA and initiate transcription.

indicate that transferrin, ABP, FSHR [114, 115], SF-1 [116], c-fos [99], and the RIIβ subunit of cAMP-dependent protein kinase promoter [117] are regulated by an E-box response element. The ubiquitously expressed proteins E47 and REBα are expressed in Sertoli cells [118]. Surprisingly, the negatively acting bHLH proteins Id1, Id2, Id3, and Id4 are also expressed in postpubertal terminally differentiated Sertoli cells [28, 51]. The suppression of proliferation of prepubertal proliferating Sertoli cells treated with thyroid hormone, retinoic acid, and testosterone is accompanied by an increased expression of Id2 and Id3 [119]. Long-term exposure (48 hr) of postpubertal Sertoli cells to FSH induces Id4, but downregulates Id1 expression. In contrast, serum induces the expression of Id1, Id2, and Id3 but suppresses Id4 [51]. Short-term treatment of postpubertal Sertoli cells (4 hr) with FSH significantly upregulates Id3 (unpublished observations).

In a similar experiment, a biphasic expression of Id2 is observed. The first peak of expression is observed at around 4 hr and a second peak appearing at about 8 hr. Such a biphasic expression pattern of Id2 is also observed in human diploid fibroblasts after serum

stimulation, which corresponds to the G_1 phase and G_1–S transition, supporting their role in proliferation [120]. The postpubertal Sertoli cells fail to proliferate after FSH induction, but together these results suggest that these cells may be competent to reenter the cell cycle. The mechanisms by which Id proteins promote the cell cycle are diverse but may involve suppression of p21, p27, cyclin A, or E/CdK2 and interactions with pRb [121]. Given the role of Id proteins in cell proliferation, the functional significance of Id protein expression in terminally differentiated and postmitotic Sertoli cells is unclear. Contrary to the hypothesis that Id proteins have redundant functions, the results in these studies suggest that Id1, Id2, Id3, and Id4 are differentially regulated and may have distinct functions [51]. Id1 may act to maintain Sertoli cell growth potential, whereas Id2, Id3, and Id4 may be involved in the differentiation and hormone regulation of Sertoli cells. Recent observations from our laboratory have suggested that the additional bHLH proteins such as scleraxis, ITF-4, and HEY (hairy and enhancer of split, *Drosophila* homologue) are also expressed by Sertoli cells (unpublished observation). The significance of these transcription factors in Sertoli cells is currently being investigated.

3. *bZIP-bHLH: USF, USF2, and cMyc*

The bZIP bHLH family can be considered a family of composite transcription factors with two domains, the bZIP and the bHLH. The bHLH domain is considered the primary DNA binding domain, whereas the bZIP domain may be involved in protein–protein interactions. The presence of the bZIP domain also raises the possibility that these transcription factors may interact with other bZIP proteins such as cfos, jun, and CREB to form cell-specific transcription factor complexes.

a. USF

The two isoforms of USF, USF1 [122] and USF2 [123], are ubiquitously expressed in many cell types [124]. The highly conserved C-terminal bZIP-bHLH domain allows identical dimerization and DNA-binding specificities. Both of the isoforms bind to E-boxes as heterodimers [125]. USF1 and USF2 have been shown to regulate the expression of genes involved in the metabolism of glucose response. Consequently, the USF1 and USF2 knockout mice have a diminished glucose response [126, 127]. USF1 is expressed at high levels in Sertoli cells and in response to FSH its expression levels decrease by more than twofold.

USF2 is expressed at a significantly lower level than USF1, but may be important in negatively regulating certain components of the FSH signaling pathway, such as RIIβ, the regulatory subunit of cAMP-dependent protein [128]. The role of USF1 and USF2 in regulating Sertoli cell-specific expression of the FSHR gene has been extensively studied [36, 100, 115] and reviewed [129]. Both USF1/USF2, possibly as a heterodimer pair, bind to the E-box in the proximal FSHR promoter [100, 114]. The downregulation of USF1, 4 hr after FSH stimulation, a profile similar to ICER, may also be involved in negatively regulating FSHR expression (Table 15.2). In cells expressing FSHR genes such as Sertoli cells, the USF1/USF2 binding E-boxes sites are unmethylated, whereas these sites are methylated in non-FSHR-expressing cells. The methylated sites may interfere with the binding of general transcription factors such as USF leading to repressive chromatin structure in the nonexpressing cells [52].

b. c-myc

In general, c-myc promotes cell growth and proliferation, inhibits terminal differentiation and sensitizes cells to apoptosis [130]. Consistent with these functions, c-myc expression in response to FSH [131] and testosterone [132] is observed in proliferating prepubertal and early pubertal Sertoli cells, but not in nonproliferating, postpubertal Sertoli cells [131, 132]. The presence of the E-box (CANNTG), which can bind the c-myc-max heterodimer in the promoter of Sertoli cell genes, may suggest its importance in regulating their expression. C-myc expression is also induced by IL-6 and interferon gamma (IFNγ) in primary Sertoli cells established from immature testis, suggesting its role in Sertoli cell proliferation in response to these mitogens [133].

B. Transcription Factors with Zinc-Finger Domains

The transcription factors within this family are characterized by a short stretch of ~30 amino acids containing at least two conserved cysteines and histidines. The conserved C_2H_2 residues coordinate a zinc ion, allowing the finger to assume a compact β-turn with cysteines and an α-helix with histidine. DNA recognition by zinc-finger transcription factors is typically determined by amino acid sequence within the 30 amino acids and number of zinc fingers (three or more) [134].

1. *Nuclear Receptors*

The nuclear receptors are composed of three domains: (1) the NH_2-terminal transcriptional activation domain (A/B domain), (2) the DNA-binding domain (DBD), and (3) the C-terminal ligand-binding domain [135] (Fig. 15.5). The nuclear receptors containing zinc

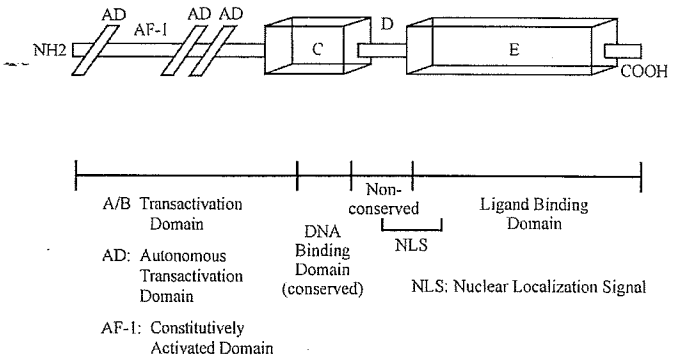

FIGURE 15.5 Structure of a typical nuclear hormone receptor. The N-terminal region (A/B transactivation domain) contains constitutively active transactivation region(s) (AF-1) and several autonomous transactivation domains (ADs). The highly conserved C domain functions as a DNA-binding domain and may also serve as a dimerization domain with other nuclear receptors. The ligand-dependent transactivation function of nuclear receptors is achieved by the binding of respective ligands to the E domain or ligand-binding domain.

fingers bind DNA as a dimer with each monomer recognizing one of the two hexanucleotides as an inverse or a direct repeat. The nuclear receptor superfamily has more than 48 known members [136, 137]. The nuclear hormone receptors binds specific steroids, terpene-derived molecules, and peptides. The majority of superfamily members have no recognized ligand and are therefore termed orphan receptors [138]. In Sertoli cells the following major groups of this superfamily are known:

Group 1: Steroid receptors (bind DNA as homodimers); androgen receptors (ARs), GGTACAnnnTGTTCT; and estrogen receptors (ERs): AGGTCAnnnTGACCT

Group 2: Receptors that dimerize with RXR (*cis* retinoic acid receptor) such as thyroid hormone (TR), AGGTCATGACCT; all-*trans* retinoic acid (RAR), AGGTCAnnnnAGACCA; and peroxisome proliferator-activated receptors (PPAR)

Group 3: Orphan nuclear receptors (bind DNA as a monomers and/or dimers): SF-1, DAX-1

a. Androgen Receptor

The androgen receptor is one of the earliest [139, 140] and most studied transcription factors in Sertoli cells [141, 142]. Over the years a number of studies have shown that both FSH and androgens are involved in regulating its expression. In cultured immature Sertoli cells, short-term (5-hr) exposure to FSH downregulates androgen receptor expression [143]. Long-term exposure of cultured immature Sertoli cells to FSH increases both AR protein and mRNA. The effects of FSH on AR expression can be mimicked by cAMP suggesting that the effect of FSH on AR are mediated by the PKA pathway [143]. Activation of the PKC pathway leads to the downregulation of AR. *In vivo*, a complex interplay between androgens and FSH influencing transcriptional, translational, and post-translation regulation of androgen receptor expression has been proposed.

The earliest time point when androgen receptor expression appears in Sertoli cells is postnatal day 5, and it persists through adulthood. By day 21, at the initiation of spermatogenesis, the AR expression in Sertoli cells is predominantly observed in stages II–VII of the spermatogenic cycle [49, 144]. This stage-specific expression of AR may be an important mechanism for regulating Sertoli cell responsiveness to testosterone and its subsequent control of spermatogenesis [145].

The stage-specific expression of AR in adult Sertoli cells suggests that germ cell regulatory factors may also influence its expression. TNFα, a secretory product of round spermatids, has been shown to stimulate the expression of AR in Sertoli cells [146]. The highly regulated AR gene expression at the promoter lever is due to three κB enhancer elements that interact with Sertoli cell p50 and RelA nuclear factor kappa B (NFκB) proteins. The overexpression of these NFκB subunits in Sertoli cells stimulates AR promoter activity. *In vivo*, TNFα may stimulate NFκB binding to the AR promoter, leading to the increased promoter activity and AR expression in primary cultures of Sertoli cells [146, 147].

The androgen receptor gene has a repetitive DNA sequence in exon 1 that encodes a polyglutamine stretch. Within the normal polymorphic range this (CAG)(n) length is inversely related to the transcriptional activity of the androgen receptor and is directly reflected in the efficiency of spermatogenesis, but not overall fertility. For example, men with short CAG repeats have the highest sperm output within the normal fertile population. AR gene mutations are also common and cause androgen insensitivity syndrome with altered sexual differentiation in XY individuals [148, 149].

In addition to binding to its cognate response element, the AR also directs the assembly and stabilization of the basal transcription apparatus. Androgen-dependent cofactors and CREB-binding protein (CBP) are influenced by AR target gene promoters to enhance transcription. Regulation of AR activity in response to diverse signaling pathways have also been reported. For example, interactions between AR and members of the STAT family, PIASx and PIAS1 (protein inhibitor of activated signal transducer), have been demonstrated. The expression of PIAS1 is observed in Sertoli cells after puberty. In addition to Sertoli cells, PIAS1 expression is also present in Leydig cells and germ cells

suggesting its role in androgen-dependent initiation and maintenance of spermatogenesis [150].

A small nuclear RING finger protein, SNURF (or RNFN), also regulates androgen-receptor dependent transcription. The 3.0-kb SNURF transcript is predominantly expressed in Sertoli cells of both immature and mature testis [151]. The molecular actions of AR on Sertoli cells remain to be fully elucidated.

b. Estrogen Receptors

The male mice homozygous for a mutation in the estrogen receptor gene (ER knockout; ERKO) are infertile [152]. These earlier studies showing lack of fertility in ERKO knockout mice were confirmed to be that of the ERα because male mice lacking the estrogen receptor beta (ERβ) exhibit no compromised fertility [153]. Because ERα is essentially absent in the Sertoli cells [154, 155], it was postulated that the infertility was a result of a defect in germ cell function. Recently, sperm transplantation studies have shown that the altered sperm function that is characteristic of the ERα knockout male [152] is the result of the loss of ERα actions in the reproductive tract rather than in the germ cell. In the double ERKO males, the α-βERKO indicates an overall phenotype of that of αERKO [156].

ERα is expressed only in Leydig cells, whereas ERβ is widely expressed in various testicular cell types such as Leydig, some peritubular cells, Sertoli cells, and germ cells, except spermatids and meiotic spermatocytes [157]. In the human testis, a spliced variant of the ERβ is also observed. The wild-type ERβ1 and its spliced variant, the ERβ2, are both detected in human testis. The expression of ERβ1 is most intense in pachytene spermatocytes and round spermatids, but low levels of expression are also detected in Sertoli cells. Highest levels of expression of ERβ2 protein are detected in Sertoli cells and spermatogonia. In the fetal human testis (12–19 wk of gestation) ERβ1 and ERβ2 are also expressed in some, but not all Sertoli cells [158]. Given the expression profile of both the estrogen receptors and the reproductive phenotypes in the knockouts, the functions of these two estrogen receptors are not redundant. Specific Sertoli cell actions of ER are unclear at the present time.

c. Retinoic Acid Receptors

The retinoic acid receptors (RAR) can be largely divided into two broad families with each family having at least three subtypes (α, β, and γ) encoded by a different gene. RARα, RARβ, and RARγ bind all-*trans* and 9-*cis* retinoic acid. RXRα, RXRβ, and RXRγ preferentially bind 9-*cis* retinoic acids. The heterodimerization between RAR and RXR, homodimerization between RXR, and heterodimerization of RXR and RAR with other transcription factors can generate a large repertoire of transcriptional complexes that bind specific retinoic acid response element(s) and/or other DNA response elements [159–161].

The various retinoic acid receptors are widely expressed in fetal, neonatal, and adult Sertoli cells, suggesting the significance of vitamin A–dependent signaling in Sertoli cell function [162–164]. RARβ and RARγ, and RXRγ expression is first observed in the Sertoli cells of fetal testis and continues to be expressed in the adult [165, 166]. In the juvenile testis, the α isoforms of RAR and RXR are also observed. In the adult, both RAR and RXR and all of their subtypes are expressed [167]. Retinoic acid increases Sertoli cell proliferation in testicular explants from 14.5-day-old rats, an effect mediated by RARβ [165].

Some redundancy may exist between the expression of RAR subtypes in the Sertoli cells. The expression of RARα and RXRβ seems to be required for proper Sertoli cell function because the mice homozygous for deletion of either of these receptors are sterile due to altered Sertoli cell function [168–170]. The Sertoli cells in the RXRβ homozygous mutants show progressive accumulation of unsaturated triglycerides and those with RARα mutants show frequent vacuolation. The overall phenotype of the two-retinoid receptor knockout males is similar to the Sertoli cells of vitamin A–deficient animals, which are infertile [171].

The pathways regulated by RAR subtypes in the Sertoli cells are complex and are regulated at multiple levels. The dimerization between various subtypes and other transcription factors such as PPAR allows the dimer to bind different response elements, allowing activation/repression of selective genes. The nuclear/cytoplasmic localization and expression of individual receptor subtypes in response to hormones/growth factors can also determine the timing and magnitude of expression of target genes [163]. For example, using cultured Sertoli cells from 20-day-old rats, retinoic acid in a ligand-dependent manner induces the translocation of RARα to the nucleus, which can be blocked by FSH [172]. The translocation of RARα to the nucleus can also be achieved in a ligand-independent manner by PKC and MAPK pathway [173]. Retinoic acid itself increases the expression of RARβ, whereas in vitamin A-deficient rats retinol increases the expression of RARα [174].

d. Thyroid Hormone Receptors

Overall thyroid hormones (THs) can increase testis size and sperm function [175, 176]. The increased testicular size may be due to the effect of TH on increasing Sertoli cell proliferation [177–179] by regulating p27 (Kip1) activity. In the Sertoli cells, the thyroid

hormone receptor α isoform is observed [180]. The two α isoforms 1 and 2 are expressed differentially during prepubertal and postpubertal stages of Sertoli cell development. The thyroid hormone binding α 1 isoform is expressed at higher levels during the prepubertal stages, whereas the nonthyroid hormone binding α 2 isoform is the predominant isoform in postpubertal Sertoli cells [181]. Thyroid hormone may also be involved in regulating the expression of AR mRNA [177, 182].

e. Orphan Nuclear Receptors

SF-1 SF-1 belongs to the steroid hormone receptor superfamily with an N-terminal zinc-finger DNA binding domain. SF-1 (steroidogenic factor 1, also known as adrenal 4-binding protein, Ad4-BP), initially identified as a tissue-specific transcriptional regulator of cytochrome P450 steroid hydroxylases, is vital for endocrine development and function [183, 184]. The mouse Ftz-F1 gene locus coding for SF-1 closely resembles the *Drosophila* fushi tarazu factor 1 (Ftz-F1), which regulates the developmental homeobox gene fushi tarazu [185]. Unlike most other nuclear receptors, SF-1 binds to the extended CCA/GAGGTC sites as a monomer [186]. Additional DNA binding specificity is achieved through amino acid residues C terminal to the DNA binding domain, which recognizes specific nucleotides 5′ to the AGGTCA site [187]. The C-terminal ligand binding domain has no known ligand; hence, it is referred to as an orphan nuclear receptor.

SF-1 expression is observed in the indifferent gonad at embryonic day 9 in mice, the earliest stage of gonadal development when the intermediate mesoderm condenses into the urogenital ridge. At embryonic day 12.5, the time of sex determination, SF-1 expression becomes dimorphic, increasing in the testis and decreasing in the ovaries. In the developing testis, SF-1 transcripts are detected in both the Leydig cells and the Sertoli cells [188, 189].

The SF-1 knockout mice die shortly after birth from adrenocortical insufficiency and exhibit male-to-female sex reversal of the external genitalia. Because their gonads regress before male sexual differentiation normally occurs, the internal and external urogenital tracts of SF-1 knockout mice are female irrespective of genetic sex [190, 191]. In humans, a mutation in SF-1 leading to sex reversal has also been reported [192].

DAX-1 DAX-1 (dosage-sensitive sex reversal-adrenal hypoplasia congenital critical region on the X chromosome, gene 1) belongs to the nuclear receptor superfamily of ligand activated transcription factors based on a sequence/structure similarity with other nuclear receptors, especially at the C-terminal domain [193, 194]. DAX-1 lacks the zinc-finger DNA binding domain [195]. The absence of any known ligand puts DAX-1 in the orphan receptor category. DAX-1 contains a unique amino terminal domain with 3–5 repeats of a 65- to 67-amino-acid motif that forms two putative zinc-finger domains. Unlike other nuclear receptors, this zinc-finger domain may not function as a central DNA binding domain but may mediate protein–protein interactions with SF1 [196, 197], ER [198], and AR [199]. The role of DAX-1 at a post-transcriptional level has also been proposed based on the observation that DAX-1 can be found associated with polyribosomes, complexed with polyadenylated RNA [200].

Functionally, DAX-1 seems to act at multiple levels to repress the expression of genes involved in steroid hormone metabolism through a potent transcriptional repression domain present in its C terminus, which is similar to the nuclear receptors' ligand binding domain [196]. Most of the known clinical manifestations involving DAX-1 mutations such as hypoplasia congenita (AHC), which is consistently associated with hypogonadotropic hypogonadism (HHG), are present in the C-terminal domain of DAX-1 protein. These mutations may lead to cytoplasmic retention of DAX-1 and transcriptional silencing due to the lack of interactions with transcriptional cofactors [201].

DAX-1 plays a significant role in the sex determination pathway. The DAX-1 deficiency in male mice leads to hypogonadism and sterility [202, 203]. The progressive degeneration of the germinal epithelium, independent of abnormal gonadotropin and testosterone production suggests that lack of DAX-1 impairs the function of somatic cells early during development [203]. The earliest expression of DAX-1 is seen in the Sertoli cells at the time of cord formation in the testis at embryonic day 12.5 (E12.5) in mice [204]. Between days E13.5 and E17.5, the expression of DAX-1 markedly decreases in Sertoli cells and increases in the interstitial cells [204]. In the female, DAX-1 is expressed in the entire ovarian primordium from E12.5 until E14.5. After E14.5, the expression of DAX-1 decreases and between E17.5 and postnatal day 0 (P0) is limited to cells near the ovarian surface epithelium. In contrast, DAX-1 expression is persistent in postnatal Sertoli cells but rare in Leydig cells [204]. The expression of DAX-1 in the embryonic Sertoli cells may also be regulated by Wnt-4 [205]. In humans, duplication of 1p31-p35 harboring the Wnt-4 gene is associated with upregulation of DAX-1. Interestingly, this upregulation of DAX-1 leads to XY sex reversal [205]. These results suggest that sex determination is sensitive to the dosage of DAX-1 and excess DAX-1 may in fact be antagonistic to Sry.

The DAX-1 expression in postpubertal Sertoli cells peaks with the first spermatogenic wave and is

subsequently regulated and increases during androgen-sensitive phases of the spermatogenic cycle [46]. In cultured Sertoli cells, DAX-1 expression is downregulated by the pituitary hormone FSH [46] (Table 15.2B).

f. Cys4 Zinc Fingers

GATA The GATA transcription factor family (GATA-1 through -6) contains a highly conserved DNA binding domain, consisting of two zinc-finger domains (CX2CX17CX2C), and it binds to a nucleotide sequence (A/T)GATA(A/G). Subtle sequence variations within the core response element (i.e., GATA, GATT, GATC) and flanking region may allow differential binding among coexpressed GATA family members within a specific tissue. The GATA family of transcription factors is widely expressed during embryonic stages and in the adult [206]. The targeted deletions of individual GATA proteins have revealed important functions in the development of various organs and systems, such as hematopoiesis (GATA-1, -2, and -3) and cardiogenesis (GATA-4) [206–208].

The coordinated expression of GATA-4 has been implicated in testicular development and function. GATA-4 protein is detected as early as E11.5 in the developing somatic cell lineages in the gonads of both XX and XY mouse embryos [209]. Following sex determination by E12.5–E13.5, GATA-4 expression is confined to the Sertoli cells and is markedly downregulated in the XX somatic cells, suggesting its role in sexual differentiation and determination [209, 210]. In humans, GATA-4 is expressed in the early fetal testicular development to adulthood and peaks at 19–22 weeks of gestation in Sertoli cells [211]. A similar role for GATA-4 in swine testicular development has also been proposed [212]. In support of the essential role of GATA-4 in testis differentiation, a number of studies have been performed to identify the genes activated by GATA-4, which include steroidogenic enzymes [213], steroidogenic acute regulatory protein [214], inhibin-α [215], and Müllerian inhibiting substance [209, 216]. In addition to GATA-4, the expression of GATA-6 has also been observed in late fetal, neonatal, juvenile, and adult Sertoli cells [210]. In the postpubertal Sertoli cells, GATA-6 and -4 levels are unaltered, but those of GATA-1 decrease in response to FSH [217]. GATA-1 expression is also observed in prepubertal Sertoli cells. After puberty, the GATA-1 expression is observed in Sertoli cells associated with stage VII, VIII, and IX seminiferous tubules [29]. The uniform expression of GATA-1 in the testis of germ-cell–depleted models such as W/Wv, jsd/jsd, or cryptorchid mice has led to the hypothesis that GATA-1 expression may be negatively regulated by germ cells [22]. In the absence of GATA-1, the testes develop normally with no obvious effect on spermatogenesis and no change in the expression of putative GATA-1 target genes and other GATA factors [218].

GATA-4 knockout mice die by 9.5 days postcoitus and exhibit profound defects in ventral morphogenesis, including abnormal foregut formation and a failure of fusion of the bilateral myocardial primordial [219, 220]. This early embryonic lethality of GATA-4 knockout mice has been a limitation for studying the role of GATA-4 during various stages of gonadal development. This limitation has been partially addressed by generating knockout mice for FOG-2 (friends of GATA-2) or mice homozygous for a targeted mutation in GATA-4 that abrogates the interaction of GATA-4 with FOG [221] as discussed next.

FOG FOG family proteins contain between eight and nine zinc-finger domains. These highly conserved C-X2-5CX12HX2-5H domains (CCHH ZnFs) have been shown to interact with the N-terminal domain of GATA proteins [222]. The interactions between the two members of the FOG family, FOG-1 and FOG-2, with GATA-1 and GATA-4 have been extensively studied and appear to repress the GATA-dependent transcription in cardiac morphogenesis [223, 224].

The interaction between GATA-4 and FOG-2 has also been proposed as an important step in gonadal development [29, 225]. FOG-2 expression correlates with that of GATA-4 and can be observed in the male urogenital ridge [29]. FOG-2 is transiently expressed in the mouse Sertoli cells at E12.5 [29]. The downregulation of FOG-2 expression after E12.5 may have important implications because FOG-2 can repress the GATA-4 dependent *trans*-activation of the MIS promoter [29, 216, 225].

The expression of FOG-2 and GATA-4 in the urogenital ridge may also be required for Sry expression. The mouse embryos homozygous for a null allele of FOG-2 exhibit significantly decreased levels of the Sry transcript at E11.5, whereas WT1 and SF1 expression is normal. In contrast, the expression of Sox9, MIS, and Dhh genes downstream of Sry were decreased. Similar results obtained in mice homozygous for a targeted mutation in GATA4 (GATA4(ki)) abrogates the interaction of GATA-4 with FOG cofactors. Collectively, these results suggest that FOG-2 and GATA-4 interactions play significant roles in various stages of sex determination and Sertoli cell differentiation [221].

g. Cys2 His2 Zinc-Finger Domains

SP1 Family of GC-Box Binding Transcription Factors The presence of GC boxes has been shown to play a critical role in directing promoter specificity to the Sertoli cell. A multitude of transcription factors

expressed by Sertoli cells such as Sp1, Sp3, Egr-1, Rnf6, Ap-2, and Sp3 bind the GC box. The significance of the GC boxes is not whether they bind any cell-specific transcription factors, but more in terms of formation of a cell-specific complex that aids in stabilization of the transcriptional initiation complex. The GC-box binding transcription factors have been shown to influence Sertoli cell expressed genes such as the specificity of KL (Kit ligand) expression by Sertoli cells in response to FSH [226], ABP expression [227], stage-specific expression of L-cathepsin promoter [228], SF-1 expression [229], Dmrt1 expression [230], clusterin promoter activity [231], and activation of the TATA-less promoter Mer, a receptor tyrosine kinase [232]. Inhibin-α subunit and CREB [233] have also been shown to be directly dependent on the presence of a GC-rich region in the respective proximal promoters.

Wilms' Tumor 1 The Wilms' tumor 1 (WT1) transcription factor has four contiguous C_2H_2 zinc-finger motif at the C terminal and an N-terminal transcriptional regulatory domain. Loss of function of WT1 was initially identified in Wilms' tumor, a pediatric kidney cancer [234, 235]. Subsequently, several other complex phenotypes such as gonadoblastoma, congenital malformations, and 46XY gonadal dysgenesis were associated with the loss of WT1. The role of WT1 as a sex-determining gene was proposed based on mutations in WT1 in individuals with Danys-Drash syndrome or with Frasier syndrome, both of which resulted in gonadal dysgenesis [236].

Mice with targeted deletion of the WT1 gene may survive to birth but have no gonads regardless of their genetic sex. This observation supports the notion that, like SF-1, WT1 may act upstream of Sry. These homozygous null mice also have no kidneys and exhibit a wide range of anomalies in heart, lung, and mesothelium [237]. Consistent with these phenotypes, WT1 gene expression has been localized to Sertoli cell [238].

WT1 is a complex gene. At least 24 isoforms are now known as a result of alternative start sites, splicing, and RNA editing (U => C editing). These isoforms can lead to diverse activities of WT1 such as RNA processing and interactions with DNA as a homodimer. The transcriptional activity of WT1 is dependent on the presence of the KTS domain, a string of lysine, threonine, and serine between zinc fingers 3 and 4. The presence or absence of the KTS tripeptide can determine the strength of WT1 transcriptional activity. In general WT1 (–)KTS is considered to be a transcriptional activator, whereas the (+)KTS isoform is generally localized to the spliceosomal site [236, 239, 240]. The (–)KTS isoform can regulate the expression of MIS, Sry, and androgen receptor in Sertoli cells [241–243].

C. Transcription Factors with a Helix–turn–Helix Motif

1. *Homeobox Genes*

The homeobox genes contain a conserved 183-bp sequence coding for a 61-amino-acid homeodomain, which is responsible for sequence-specific DNA binding [244]. Functionally, the homeobox genes are involved in biological processes such as control of cell identity [245], cell growth and differentiation [246], cell–cell interactions, and cell–extracellular matrix interactions [247]. The diversity of regulatory pathways influenced by homeobox genes suggests that these transcription factors may have a significant role in Sertoli cell biology, from initiation of Sertoli cell phenotype to regulation of Sertoli cell function in the adult testis. Members of the homeobox family, such as Oct4 [248, 249] and PAX, have been shown to be involved in germ cell development. The only homeobox gene reported to have a role in Sertoli cell function is the Pem gene.

a. Pem

The orphan homeobox gene Pem encodes a homeodomain transcription factor related to the Prd/Pax gene family [250]. Pem is induced in Sertoli cells at day 9 postnatal in mice, at the initiation of meiosis in germ cells [251]. Following puberty, Pem expression is restricted to androgen-dependent stages IV–VIII of the seminiferous epithelium cycle [251]. Consistent with this observation, Pem gene expression is regulated by androgens and other cell types [252, 253]. The proximal 0.6-kb 5′-flanking sequence is sufficient for age- and stage-specific expression of the mouse Pem gene in Sertoli cells. Transgenic mice overexpressing Pem specifically in Sertoli cells have an increased number of preleptotene spermatocytes and elongated spermatocytes with double- and single-stranded breaks, respectively, with no obvious anomalies in spermatogenesis, fertility, or fecundity. Pem gene expression in Sertoli cells may be involved in the regulation of the expression of secreted or cell-surface proteins that may serve to control premeiotic DNA replication, DNA repair, and/or chromatin remodeling in the adjacent germ cells [254].

2. *LIM Homeodomain: Lhx9*

Lhx9 (LIM/homeobox gene 9) encodes a transcription factor implicated in various developmental processes, including gonadogenesis [13]. In the rat, Lhx9 expression is observed in the undifferentiated gonads but disappears as epithelial cells differentiate into Sertoli cells and begin to express MIS. The mutually exclusive expression of Lhx9 and MIS suggests that Hx9 may negatively regulate MIS gene expression [255].

3. *Winged Helix: WIN-1/Trident*

Winged helix (WH) proteins are a large family of putative transcription factors that may regulate mesenchymal-to-epithelial transitions and maintain cellular differentiation [256, 257]. Family members share a highly conserved 100-amino-acid DNA-binding domain, which was first identified in HNF-3 proteins [257]. Targeted disruptions of a number of WH genes have revealed essential functions of WH proteins in development for cell fate determination, cellular proliferation, and cellular differentiation [257–260]. The role of WH proteins in mesenchymal-to-epithelial transitions during kidney and brain development was established by targeted disruption of BF-2 and BF-1, respectively [261, 262]. An increasing number of WH proteins are also involved in the transcriptional regulation of cell-specific genes, which suggests a role in integrating transcriptional gene networks [263–265].

A novel member of the WH family, rWIN, is expressed in Sertoli cells [266]. During embryogenesis, the expression of Trident, the mouse homologue of rWIN/HFH-11, is observed in all proliferative cells, but not in resting cells [267]. The human homologue HFH-11 is highly expressed in spermatocytes and spermatids [268]. In contrast, spermatogonia undergoing active proliferation reportedly do not express HFH-11 [268]. Long-term stimulation (72 hr) of cultured Sertoli cells with FSH decreases rWIN expression. The dynamics of the regulation of rWIN expression suggest that rWIN is transiently upregulated within 30 min of FSH stimulation. Such a transient increase in rWIN expression is analogous to the induction of the immediate early gene c-*fos*. The transient upregulation of rWIN expression in response to FSH may be required for the expression of Sertoli cell functional genes such as transferrin [266].

D. Beta Scaffold Factors with Minor Groove Contact

1. *Rel/Ankyrin*

NFκB

NFκB is a family of closely related transcription factors that bind to the kB DNA motif as a dimer [269]. NFκB was originally discovered in the nucleus of B cells, where it binds to the κ chain of the immunoglobulins. The various members of the NFκB family and the dimerization/processing scheme are outlined in the Figure 15.6. Within the nucleus, the activity of NFκB is also modulated by phosphorylation. The possibility of preferential dimerization and phosphorylation in response to appropriate stimuli makes NFκB an attractive transcription factor family to study mechanisms of cell-specific gene regulation in both normal and diseased states. The roles of NFκB in tumorigenesis have been extensively studied and include regulating the expression of immunoregulatory and inflammatory genes, antiapoptotic cell survival genes, cell proliferation genes, and differentiation genes [270, 271].

The RelA and p50 NFκB DNA binding activity is also present in Sertoli cells [27, 272]. Importantly, NFκB proteins can specifically bind to the κB enhancer motifs within the promoter of the CREB gene [273]. The role of NFκB in germ cell–Sertoli cell interactions has also been proposed [272]. TNFα, an NFκB-activating cytokine produced by round spermatids located adjacent to Sertoli cells, stimulates the elimination of IκB, translocates additional NFκB to the nucleus, and increases NFκB binding to CREB promoter κB enhancer elements [146, 273]. TNFα also stimulates transcriptional activity of the CREB promoter. These data demonstrate that NFκB contributes to the upregulation

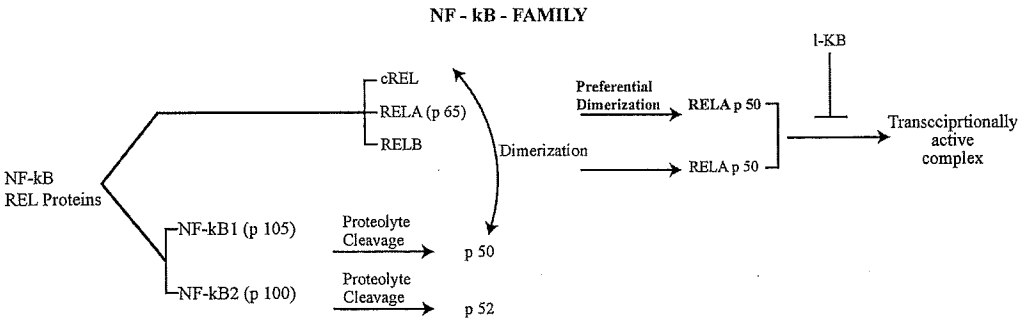

FIGURE 15.6 Mechanism of action of REL proteins. The reticuloendotheliosis family (REL) of proteins is divided into two broad categories. The first consists of CREL, RELA, and RELB. The proteins are synthesized in mature forms as opposed to the second group of REL proteins the NFκB1 (p105) and NFκB2 (p100). The p105 and p100 undergo proteolytic cleavage to generate the p50 and p52 forms, respectively. This proteolytic cleavage removes the C-terminal ANKYRIN repeats. Together, the REL proteins can form the transcriptionally active homo- or heterodimers. The transcriptional activity of these dimers can be inhibited by inhibitor of κB (IκB). IκB proteins consist of an N-terminal regulatory domain followed by a series of ANKYRIN repeats.

of CREB expression in Sertoli cells and raises the possibility that NFκB may induce other Sertoli genes required for spermatogenesis [273]. In addition, the activators of the PKA signaling pathway such as forskolin or FSH also increase NFκB DNA binding activity [27]. The limited studies and the potential role of NFκB in the regulation of cellular function make this family an attractive candidate for further research.

2. *High Mobility Group*

High mobility group (HMG) proteins are nonhistone chromosomal proteins that contain two highly conserved DNA binding HMG-box domains. The superfamily is largely divided into three subfamilies: HMG1, HMG2, and HMGB4. Acting primarily as architectural proteins, they facilitate the assembly of nucleoprotein complexes by bending and binding preferentially to distorted DNA, thus effecting recombination and initiation of transcription [274, 275]. In contrast to other transcription factors, the HMG proteins have no transactivation domain. HMG-box proteins might be targeted to particular DNA sites in chromatin by either protein–protein interactions or recognition of specific DNA structures [276]. The significance of these proteins can be appreciated from the observations that HMG1 is one of the most abundant nuclear proteins in all mammalian cells.

a. Sry

Sry, a member of the Sox family of HMG box proteins, is considered a testis-determining gene and is located on the Y chromosome [277–279]. Sry is expressed for a short period of time (10.5–12 and 11.5–13 days postcoitus in the mouse and rat, respectively) in selected epithelial cells of the genital ridge and is sufficient for initiating their differentiation into Sertoli cells [2, 45, 280] (Table 15.3). The differentiation of Sertoli cells initiates testis differentiation from the indifferent gonad. Transgenic mice overexpressing Sry develop as males even with an XX karyotype and, when deleted, the chromosomally male mice adopt a female phenotype [278, 281, 282].

The molecular mechanism of Sry function is an area of intense research and is complicated by the fact that Sry has been evolving very fast, as indicated by significant sequence differences among various mouse strains [283]. Outside the consensus HMG box, the Sry sequences are highly divergent between species. Based on the function and mechanism of action of other HMG-box proteins, it can be predicted that Sry may have other partners with which it interacts via its HMG box [190, 284, 285]. In humans a single 34-kDa protein called SIP-1 (Sry interacting protein) has been identified through yeast two-hybrid interaction studies [286]. SIP-1 is ubiquitous, nuclear, and interacts via its two PDZ protein binding domains with the most C-terminal seven amino acids in human Sry. The murine homologues of SIP-1 have not been found, possibly because the non-HMG-box segments of the Sry protein are significantly different from the human Sry. Another possibility that needs careful evaluation is that more than two proteins may be involved in this interaction, and formation of the complex is dependent on the availability of all of the proteins of the complex. Alternatively, the presence of a C-terminal *trans*-activation domain in the mouse Sry and its absence in the human gene may suggest that a "functional Sry unit" in humans may require interactions with SIP-1 and not in mouse. Potential phosphorylation of a protein kinase capable of phosphorylating an N-terminal domain serine residue may modulate the DNA-binding activity of Sry. A similar phosphorylation residue is absent in the mouse Sry [287]. The rapid evolution of the Sry sequence, divergence between species of regions outside the HMG box, selective protein interactions, and functional modification such as phosphorylation suggest the HMG-box alone is sufficient for the activity of Sry.

b. Sox Family of Transcription Factors

The expression of at least two Sox proteins, Sox3 and Sox9, is also observed in the Sertoli cells of the gonad. Sox3 is most closely related to Sry and is largely considered to be the evolutionary precursor of Sry [288]. Sox3 is expressed in both XX and XY gonads during the critical period of sex determination [289]. On the other hand, Sox9 expression is more widespread and is present in the chondrocytes, pancreas, and heart among other tissues [290, 291]. In the developing gonad, Sox9 is initially expressed in both males and females [292]. The Sox9 expression becomes restricted to the male gonad following the initiation of Sry expression, suggesting that after sex determination Sry may be involved in regulating the expression of Sox9 [13]. The localization of the Sox9-positive cells around the testicular cords suggests that it is expressed primarily in Sertoli cells [292, 293]. Unlike Sry, the expression of Sox9 is biphasic and decreases gradually until birth but is increased immediately before puberty at 15 days postnatal and persists in the adult mice seminiferous tubules [293]. The expression profile of Sox9 suggests that it is required for the important phases of aggregation and reorganization of the Sertoli cells and germ cell differentiation. The consensus motif for Sox proteins has been defined as the heptameric sequence 5′-(A/T)(A/T)CAA(A/T)G-3′ [294].

The close association between Sox9 and Sry expression suggests that it may be important for Sertoli cell differentiation and sex determination [295–296]. Such a function is also supported by the fact that ~75% of karyotypically male patients with heterozygous Sox9 mutations develop as intersexes or XY females. Thus, Sox9 is expressed in Sertoli cells that also express Sry. However, whereas Sry expression is transient, Sox9 expression is maintained throughout further testis development. Sox8, another member of the Sox family, is also expressed in the developing mouse testis around the time of sex determination [297].

E. Others

1. *STATs*

The STAT (signal transducer and activator of transcription) family is a group of transcription factors that translocates signals from the cytokine, hormone, or growth factor activated receptors to the nucleus. The receptor-recruited STATs are phosphorylated on a single C-terminal tyrosine. The homo- and heterodimers of STATs between phosphotyrosine of one STAT and the SH2 domain of the interacting STAT bind to sequence-specific DNA sites. Out of seven known STAT family members (STAT1–7), only STAT-3 and STAT-1 activities have been observed in response to cytokines such as LIF [85], IL-6, and IFNγ [86] in Sertoli cells. FSH appears to suppress STAT-1 and STAT-3 expression, but stimulates STAT-5 (Table 15.2C).

2. *CBP*

Both CBP and p300 are highly homologous nuclear proteins originally cloned for their ability to interact with phosphorylated CREB [298] and with the adenovirus E1A protein [299], respectively. Both CBP and p300 are thought to function as crucial links between diverse signal transduction and transcriptional pathways and to play an essential role in the cellular processes of growth and differentiation [300–302]. Several protein motifs in CBP and p300, such as a bromodomain, a KIX domain, and three regions rich in Cys/His residues (C/H domains), are conserved in CBP/p300 species ranging from *Drosophila* to mammals. The CBP/p300 consists of flexible modules that can accommodate interactions with multiple activators of transcription and integrate signals derived from multiple pathways. These modules serve as binding sites for sequence-specific transcription factors and other components that regulate gene expression. The CBP/p300 interactions with transcription factors include the bHLH myogenic proteins; CREB; the oncogene product Myb; the retinoic acid, estrogen, glucocorticoid, and thyroid hormone receptors; AP1 complex; components of the basal transcription machinery such as transcription factor IIB (TFIIB); and TATA-binding protein [300–302]. These observations suggest that CBP/p300 might constitute a physical nexus between enhancer binding proteins and components of the basal transcription machinery. In addition, CBP/p300 may disrupt repressive chromatin structures through its intrinsic or associated histone acetyl transferase activity [303, 304].

In Sertoli cells, the coactivators, CBP/p300, integrate the synergistic actions of the bHLH proteins and CREB on the transferrin promoter and are required for optimal FSH stimulation of the transferrin promoter (Fig. 15.7). Based on the effect of CBP/p300 antisense oligonucleotides on wild-type and E-box and PRII mutants of the transferrin promoter, it is speculated that CBP/p300 may form a ternary complex involving bHLH and CREB transcription factors. The formation of a ternary complex by CBP/p300 may facilitate integration of transcriptional regulators such as bHLH proteins and CREB in Sertoli cells. This is postulated to result in positive cross-talk between the transcription factors and the signal transduction pathways in Sertoli cells. The possibility that CBP/p300 may also integrate the basal transcription machinery to the bHLH and CREB transcription factors is also likely [305].

3. *Dmrt1*

The presence of Dmrt1 as one of the key sex-determining genes in mammals was proposed from the studies on *C. elegans* and *Drosophila* genes MAB-3

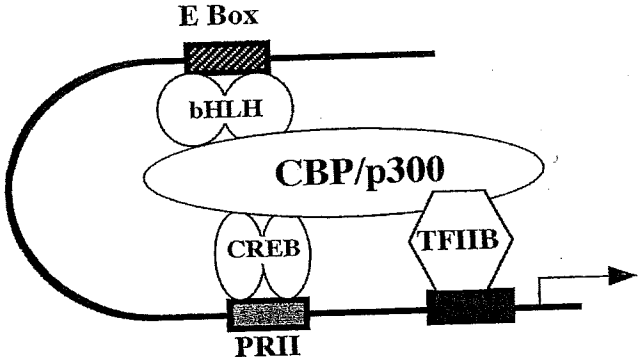

FIGURE 15.7 Proposed model for the regulation of transferrin gene expression in Sertoli cells. bHLH and CREB proteins are required for the transcriptional activity of the transferrin promoter. An interaction between the bHLH and CREB proteins is facilitated by CBP/p300. This cooperative interaction may be required for Sertoli cell-specific transferrin gene expression.

and doublesex, respectively [306]. MAB-3 and doublesex genes have a highly conserved DM domain with cysteines and histidines [307, 308]. The DM chelates zinc, but this zinc-binding domain is distinct from other zinc-binding motifs [309]. The DM domain is probably the most conserved sex-determining domain across phyla. Consistent with its role in sex determination, overexpression of Dmrt1 has been shown to induce sex reversal in many species.

In humans, the role of Dmrt1 as a potential sex-determining gene was obtained through karyotyping sex-reversed males that showed monosomy in chromosome 9p with a loss of the Dmrt locus that harbors the Dmrt1 gene [308, 310, 311]. In mice, the Dmrt1 gene was cloned by using a degenerate PCR approach. Dmrt1 in mouse is expressed exclusively in the XX and XY genital ridge prior to sexual differentiation. The expression becomes restricted to seminiferous cords during progression of testis development and is observed in both Sertoli cells and germ cells [312]. In the postnatal testis, Dmrt1 expression increases by postnatal day 10. As germ cells proliferate, the expression of Dmrt1 drops around the third week of postnatal development [313]. In cultured Sertoli cells, FSH and cAMP (PKA pathway) are known to upregulate expression, whereas phorbol esters (PKC pathway) downregulate Dmrt1 expression [314]. The expression of Dmrt1 in Sertoli cells is regulated by a combination of ubiquitously expressed transcription factors Sp1, Sp3, and Egr1 [230].

V. REGULATION OF SERTOLI CELL GENES BY COMBINATORIAL INTERACTIONS OF TRANSCRIPTION FACTORS

The cumulative observations from a number of studies suggest that only a small number of transcription factors and their combinatorial interactions may be required to regulate the expression of large numbers of genes expressed by Sertoli cells. Examples of some of the genes known to be regulated by the combinatorial interactions are discussed next. In most of these studies, these mechanisms have been studied using *in vitro* systems of cultured Sertoli cells transfected with a specific promoter region of genes of interest. The strength/activity of the promoter is usually measured by its ability to induce the expression of a reporter gene such as β-galactosidase, luciferase, chloromphericol, or acetyl transferase. Alternatively, the timing of expression of a particular transcription factor (e.g., DAX-1) in relation to a potential gene of interest (e.g., MIS) can also provide some clues as to the regulatory mechanisms in the cell. Although these *in vitro* studies have yielded a great deal of useful information, these data should also be evaluated in terms of the complexity of *in vivo* chromatin dynamics and interactions.

A. Regulation of the Transferrin Gene Expression

Cell-specific expression of the iron-binding protein transferrin is in part mediated through the regulation of its promoter [315]. Serum transferrin is produced by hepatocytes, but cells that create a blood barrier such as Sertoli cells in the testis and choroid plexus epithelium in the brain also express the transferrin to provide iron to cells sequestered within the serum-free environment. Both mouse and human transferrin promoters have been used to identify the potential conserved response elements and/or regions involved in Sertoli cell-specific expression of the transferrin gene. A remarkable (80%) homology between the proximal human and mouse transferrin promoters suggests that the transcriptional control mechanisms between the species are conserved. Comparisons of the regulatory regions and cell-specific expression (hepatocytes versus Sertoli cells) have revealed that the enhancer element in the –3600/–3300 region are functional in hepatoma cells, but are unable to increase transcription in Sertoli cells. The conserved proximal region I and II (PRI and PRII) in the proximal 150 bp of the promoter are sufficient to achieve optimal transferrin gene expression in Sertoli cell cultures. In response to FSH, CREB, or CREB-like cAMP responsive proteins, possibly C/EBPβ, can bind to the PRII site [316, 317]. The presence of E-box response elements in the proximal promoter is also known to regulate the transferrin promoter activity in Sertoli cells [98]. As an added level of control to achieve specific gene expression, the bHLH proteins bound to the E-box can physically interact with transcription factors binding to the PRII site. This interaction can be mediated through CBP/p300 [305]. The CBP and p300 are the only known histone acetyltransferases (HATs) that are capable of acetylating all four core histones. The E-box and PRII are approximately 220 bp apart, which corresponds to one turn of the DNA around the nucleosome complex. Such proximity and the intrinsic HAT activity of CBP/p300 further support the hypothesis that CBP/p300 may form a ternary complex involving bHLH and CREB proteins [305] (Fig. 15.7). The presence of negatively acting bHLH Id proteins and their expression profile in response to FSH may help to fine tune the transferrin gene expression by inhibiting the bHLH proteins [51].

B. Regulation of the MIS Promoter

In males MIS is secreted normally by fetal and adult Sertoli cells and in the females by postnatal granulosa cells. MIS regulates testicular development by initiating the regression of Müllerian ducts. The dynamics of its expression in Sertoli cells led to the hypothesis that MIS may be one of the earliest sex-specific targets of the Sry transcription factor. The role of other transcription factors involved in the sex determination pathway in the regulation of the MIS promoter also has been reported. For example, upon ligand binding, SF-1 may regulate Sertoli cell–specific expression of the MIS promoter [318]. It has been speculated that this ligand may be specific to Sertoli cells. The cell specificity of MIS expression has been localized to the proximal 180 bp of the promoter, which harbors the consensus SF-1 binding site [318]. SF-1 may help to maintain quantitative levels of MIS expression, but loss of HMG-box sequences that bind Sox9 may lead to abnormal male sex differentiation.

Interactions between GATA-4 and SF-1 [319], Sox9 and SF-1 [320], and WT-1 and SF-1 [242] have also been proposed in the regulation of the MIS promoter. These interactions are mediated by direct protein–protein interactions. The transcriptional synergism between GATA-4 and SF-1 can be disrupted by DAX-1. The repression of the MIS promoter by DAX-1 may involve direct interactions with SF-1 bound to DNA and may not necessarily involve GATA-4 [321]. The mutually exclusive expression of GATA-1 and MIS during prepubertal development of Sertoli cells has led to the hypothesis that GATA-1 may inhibit MIS gene expression [322]. The presence of FOG2 in Sertoli also suggests that it may modulate GATA-4–dependent MIS expression [216]. The presence of SF-1, FOG2, GATA-4, DAX-1, and Sox9 in multiple cell lineages, but restricted MIS expression to Sertoli cells, supports a mechanism involving a Sertoli cell–specific cooperative interaction between these transcription factors.

C. Regulation of SF-1 Promoter

The sexually dimorphic pattern of SF-1 expression observed during male gonadal differentiation suggests the role of similarly expressed transcription factors in its regulation. For example, the proximal SF-1 promoter harbors a conserved Sox binding site AACAAAG (Sox-BS1) [323]. This site binds Sox9 with high affinity, but other Sox proteins, which are also coexpressed with SF-1 such as Sox8 and Sox3, can also bind this site with lower affinity. In addition to the Sox-BS1 site, other sites such as the USF1 and USF2 binding E-box site, the CAAT site, sequences with overlapping E-box and CCAAT sites, and multiple GC-rich Sp1/Sp3 are also required for optimal SF-1 promoter activity [229].

VI. CONCLUSION AND FUTURE DIRECTIONS

Over the years and with an ever-increasing knowledge of the genome, the studies addressing the mechanisms involved in Sertoli cell gene regulation have matured from a linear approach that is based on characterization of specific *cis*-acting regulatory sequences and their cognate transcription factors to a more global approach that considers regulation in terms of the complete functional unit that simulates a specific gene expression pattern *in vivo*. This global approach is required to acknowledge the role of transcription factors, coactivators, corepressors, chromatin remodeling enzymes, histone acetylases, deacetylases, kinases, and methyl-transferases in regulating Sertoli cell-specific gene expression. For example, E-box response elements are required for the expression of many Sertoli cell genes, as discussed in this chapter. The consensus site for an E-box (CANNTG) occurs approximately once every 300 bp in the human genome. All of these sites are obviously not bound by bHLH transcription factors. Therefore, it is reasonable that some E-box sites have a preference in terms of sequences surrounding them or their state of methylation. In addition, accessory mechanisms may be involved that can include the presence or absence of other transcription factor coactivators such as CREB, C/EBP, and CBP/p300, as in the case of the transferrin promoter. Together these mechanisms may serve as a "homing mechanisms" that steer a particular transcription factor toward a very small subset of potential target sites.

In recent studies the concept of cell-specific transcription factor complex involving many of the concepts discussed earlier is also being evaluated. For example, the transferrin gene expression may involve combinatorial interactions between bHLH, CREB, and CBP/p300 [305]. The formation of a complex on the serum response element of the cfos promoter may involve interactions between bHLH proteins and serum response factors, which may be specific to Sertoli cells [99]. A complex interplay of stage-specific expression of transcription factors and the corresponding expression of functional genes such as MIS supports this hypothesis.

Promoter analysis for conserved binding sites in coregulated genes can also be useful to develop a consensus of the Sertoli cell transcriptional regulatory networks. Microarray experiments in combination with

other techniques such as genome-wide analysis of *in vivo* transcription factor–binding using "ChIP on a chip" (chromatin immunoprecipitation on a chip using transcription factor–specific antibodies) and specific computer algorithms will help in assembling complete Sertoli cell–specific transcriptional regulatory maps. For example, in a large-scale gene expression profiling of Sertoli cell genes, the genes can be grouped in a pattern definition process such as selecting coregulated genes with similar transcription factor core motifs. The complexity of this initial data can be further reduced by selecting a second common transcription factor motif. Following the process of verification, such as biologically relevant overexpression, reverse genetics and knockout experiments are needed. A consensus can be developed on the potential regulatory networks involving similar transcription factors and their interactions. For example, the role of the E-box elements in regulating the transferrin gene promoter was considered as a potential conserved process due to its presence and validation in a number of other Sertoli cell gene promoters.

The current review helps categorize the various transcription factors in Sertoli cells. The FSH regulation of these transcription factors (Table 15.2) and developmental regulation (Table 15.3) have been also reviewed. Currently very few cell-specific transcription factors have been shown to promote or induce Sertoli cell differentiation. In contrast, observations support the proposal that unique combinations of more widely expressed transcription factors are likely involved in Sertoli cell–specific gene expression. How this wide variety of transcription factors interacts to form unique regulatory networks in Sertoli cells will be the primary future research to elucidate the transcription regulation of Sertoli cell differentiation and development.

Acknowledgments

The authors wish to thank Dr. Derek McLean and Dr. Michael Griswold for sharing the rat Sertoli cell microarray data. The authors also wish to thank Mr. Jim Shima and Ms. Jacqueline Ague for analysis of the microarray data.

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