Chapter 7

Transcription Regulation in Spermatogenesis

Wing-Yee Lui* and C. Yan Cheng

Introduction

Spermatogenesis is a highly coordinated process in which diploid spermatogonia (2n) differentiate into mature haploid (1n) spermatozoa in the seminiferous epithelium. In this process, spermatogonia undergo several mitotic divisions and either enter a stem cell renewal pathway, or commit themsevles for further development. Diploid spermatocytes subsequently undergo two meiotic divisions and result in the production of haploid round spermatids. They then enter the process of spermiogenesis in which profound morphological and biochemical restructuring, such as the formation of acrosome and flagellum occur, and give rise to mature spermatozoa.

The cyclic and synchronous nature of spermatogenesis leads to specific pattern of cellular associations at a given segment in the tubules in which germ cells at particular stages of differentiation will associate with one another. Such cellular associations have been classified into the stages of the seminiferous epithelium. There are twelve (stages I-XII) and fourteen stages (stages I-XIV) of the seminiferous epithelium in mouse and rat, respectively[1,2] according to their cellular associations. Such differentiation pattern apparently requires precise regulation of specific genes at a given stage. In order to have a better understanding how transcription factors exerts their regulatory function to modulate cellular and stage-specific gene expression during spermatogenesis, we summarize herein some of the recent findings in the study of transcription regulation during spermatogenesis into five categories: (i) general transcription factors, (ii) nuclear receptor superfamily of transcription factors, (iii) other transcription factors involved in testicular functions, (iv) testis-specific gene transcription, and (v) transcriptional regulation of cell junction dynamics. The chapter is not intended to be exhaustive, rather, it serves as a guide for future studies based on latest findings in the field.

Transcription Regulation in Spermatogenesis

General Transcription Factors

Regulation of stringent stage-specific gene expression in testicular cells and the massive wave of transcriptional activity in germ cells following meiosis are governed by a highly specialized transcriptional mechanism.[3] Such temporal and restricted pattern of gene transcription is achieved by the presence of germ cell-specific transcription factors (Table 1). In addition, various general transcription factors, in term of their expression levels and their testis-specific isoforms, are differentially regulated in germ cells and in testes. It is believed that the differential expression of general transcription factors also play a crucial role to ensure proper and efficient transcription in germ cells throughout spermatogenesis.[4] For instance, TFIIB (a transcription factor that serves as a positioning factor for polymerase), TATA-binding protein (TBP)

*Corresponding Author: Wing-Yee Lui—School of Biological Sciences, The University of Hong Kong, Pokfulam Road, Hong Kong, China. Email: wylui@hku.hk

Molecular Mechanisms in Spermatogenesis, edited by C. Yan Cheng.

Table 1. List of the genes encoding the transcription factors whose deletion in the mice generate defects in males

Gene Disrupted	Male Phenotype	Female Phenotype	References
AR	Complete arrest at pachytene spermatocyte stage Female-like appearance Small testis with a decrease in serum testosterone concentration	Fertile	Yeh, 2002[23]
RARα	Complete arrest and severe degeneration of the seminiferous epithelium	Fertile	Lufkin, 1993[34]
RXRβ	All survivers are sterile Partial arrest at primary spermatocyte stage Structural abnormalities in spermatozoa	Fertile	Kastner, 1996[35]
GCNF	Embryonic lethality	Embryonic lethality	Chung, 2001[156]
TR2	Functional testis having normal sperm number and motility	Fertile	Shyr, 2002[73]
TR4	Delay in the first wave of spermatogenesis Prolonged stages XI to XII of spermatogenesis Reduced fertility	Fertile	Mu, 2004[72]
CREM	Complete arrest at pachytene spermatocyte stage	Fertile	Nantel, 1996;[81] Blendy, 1996[157]
CREB (α and δ isoforms)	Fertile	Fertile	Hummler, 1994[158]
CREB (α, β and δ)	Die shortly after birth	Die shortly after birth	Rudolph, 1998[159]
Rhox5	Subfertile Increased frequency of apoptotic meiotic spermatocytes	Fertile	Pitman, 1998;[95] MacLean, 2005[26]
Sperm-1	Subfertile	Fertile	Pearse, 1997[97]
Plzf	Exhibit progressive loss of spermatogonia and increase in apoptosis with age	Fertile	Costoya, 2004[102] Buaas, 2004[103]
WT1	Conditional knockout mice show impaired spermatogenesis and predicted to be fertile	—	Gao, 2006[106]
GATA-1	Embryonic lethality	Embryonic lethality	Pevny, 1991[160]
GATA-4	Embryonic lethality	Embryonic lethality	Narita, 1997[161]
GATA-6	Embryonic lethality	Embryonic lethality	Koutsourakis, 1999[162]
MSY2	Infertile	Infertile	Yang, 2005[139]
CAF1	Infertile	Fertile	Nakamura, 2004[143]; Berthet, 2004[144]

and RNA polymerase II were found to be accumulated in early haploid germ cells. Their levels in haploid germ cells are much higher than in somatic cells.[5] Adult rodent testes contain 80-200 molecules of TBP mRNA per haploid genome-equivalent, whereas adult spleen and liver contains 0.7 and 2.3 molecules of TBP mRNA per haploid genome-equivalent, respectively.[5] Such organization of transcription factors enable early spermatids accumulate enough mRNA for their development until the final stages of spermiogenesis.

In addition to the unique expression pattern of various general transcription factors in germ cells, the presence of their testis-specific isoforms may play a specialized function in spermatogenesis. ALF or TFIIA-τ is a testis-specific isoform of TFIIA which may have specificity for a subset of transcriptional activators.[6,7]

Nuclear Receptor Superfamily

Lipophilic Hormone Nuclear Receptors

Androgen Receptor

Androgens are crucial steroid hormones in male reproduction and their actions ranging from regulating sexual differentiation, sexual maturation, spermatogenesis to production of gonadotropins.[8-12] Androgens exert their effects through the androgen receptor (AR). AR is a ligand-inducible transcription factor (110 kDa) that regulates the expression of target genes in response to its cognate ligand (androgen) through binding to an androgen response element (ARE).[12,13]

Similar to other members of the nuclear receptor superfamily, AR can be divided into four functional domains. They are: NH_2-terminal transactivation domain, DNA-binding domain (DBD), hinge region, and ligand-binding domain (LBD). AR has two separate NH_2-terminal transactivation domains which possibly interact with different coregulators or transcription factors in a promoter content-dependent manner.[14] The DBD contains two zinc fingers that recognize specific DNA consensus sequences. AR homodimer binds to the inverted repeat ARE, GGTACAnnnTGTTCT.[15-18] Apart from the formation of homodimer, it was reported that AR is capable of forming the heterodimers with TR4 (human testicular receptor 4, TR4, is an orphan member of the nuclear receptor superfamily) or ERα (estrogen receptor α), which results in a decrease in AR transcriptional activity.[19,20] The LBD is responsible for the formation of the ligand-binding pocket, facilitating the interaction between AR and heat shock protein, and also interacting with AR NH_2 terminus to stabilize the bound androgen.[21,22]

AR is of particular interest because of the observation that knockout of AR produced male mice displaying female-like appearance with arrested spermatogenesis.[23] Although AR plays an indispensable role in spermatogenesis, only a few number of genes have been identified so far that are directly regulated by AR in the testis. The expression of X-linked Rhox5/PEM homeobox gene is a typical example of AR-mediated gene regulation in the testis.[24-26] Barbulescu et al. have identified two functional AREs within 300-bp upstream of the Rhox5 transcription start site.[27,28] The promoter region containing the regulatory sequences that directs AR-dependent expression specifically in Sertoli cells and confers AR stage-specific expression in adult testis.[29,30] Recent studies from MacLean et al. have shown that another four Rhox genes (namely Rhox2, 3, 10 and 11) are dramatically upregulated in response to incubation with testosterone and cotransfection with an AR expression plasmid. Although the promoter sequences of the four Rhox genes have not yet been characterized, it is apparent that they all are androgen-dependent.[26]

Apart from AR knockout mice, a tissue-specific knockout mouse with the AR gene deleted in Sertoli cells [SC AR knockout mice] was generated to investigate how androgen/AR in Sertoli cells influence spermatogenesis.[31,32] It was found that the SC AR knockout male exhibit similar phenotypes as that of $AR^{-/-}$ mice with more severe testis atrophy. SC AR knockout mice showed alterations in the expression of anti-Mullerian hormone (AMH), cyclin A1, Pem and sperm-1.[31,32] The increase in the expression of AMH in mice leads to the reduction of testosterone production in Leydig cells. Significant reduction in germ cell number in SC AR knockout is associated with increased germ cell apoptosis and reduced expression of cyclin A1, Pem and sperm-1 genes that are important for late stage of germ cell development.[31,32] Sertoli cell-specific AR knockout mice clearly demonstrated the functional significance of AR in Sertoli cells in maintaining spermatogenesis and steroidogenesis.

Using another SC AR knock-out model,[32a] it was shown that the loss of androgen receptors in Sertoli cells led to a disruption of the blood-testis barrier (BTB) integrity since biotin could

diffuse through the BTB.[32b] Using techniques of gene profiling, it was shown that the gene responsible for the "leaky" BTB in SC AR knock-out mice is likely to be claudin 3, which displays transient expression in newly formed tight junctions.[32b] However, it is noted that the SC AR knock-out mice used in this study were made with a floxed exon 1, yet the floxed animals had already displayed marked hypomorphic phenotype and the ultimate AR knockout was neither complete nor Sertoli cell selective,[32a] which may explain why did the SC AR knock-out mice had a serum testosterone level almost 40-fold of that of the wild type.[32a] Furthermore, the testicular claudin 3 level in adult rat testes was extremely low,[32b] and it is virtually undetectable beyond 45 days of age (Yan and Cheng, unpublished observations), making claudin 3 hardly an important structural component of the BTB in adult rats. Nonetheless, it is likely that testosterone and its receptor are important components that regulate BTB dynamics, much work is needed in the field to define the precise molecular target(s) of testosterone and AR at the BTB.

Retinoid Receptors

Retinoic acid receptors (RARs) and retinoid X receptors (RXRs) are two members of this family found in the testis. Ligand-dependent activation of RAR and RXR are essential to spermatogenesis based on the fact that infertility was observed in vitamin A-deficient rats and in RARα and RXRβ transgenic mice.[33-36] In vitro binding studies have demonstrated that the natural metabolites all-trans-RA and 9-cis-RA are high-affinity ligands for RARs, whereas only 9-cis-RA has been shown to bind RXRs. Each family consists of three genes, namely α, β, and γ, and each of them exists as multiple isoforms. RXR is capable of forming homodimers (RXR/RXR), heterodimers with RAR (RXR/RAR) and with other types of nuclear receptors such as thyroid hormone receptor (RXR/TR),[37] such characteristic enables this receptor family exerts combinatorial regulatory properties.

The homodimer and heterodimer function as RA-inducible transcriptional regulatory proteins through binding to DNA sequences called retinoic acid response element (RARE) or retinoid X response element (RXRE) located within the promoter of target genes. The consensus sequence of RARE is AGGTCAnnnnnAGGTCA, whilst RXRE is direct repeats of AGGTCA with one nucleotide spacing (AGGTCAnAGGTCA). The RAR/RXR heterodimer binds to the RARE, with RXR occupying the 5' upstream half-site and RAR occupying the 3' downstream half-site.[38]

Extensive studies using RARα and RXRβ transgenic mice have clearly demonstrated that retinoic acid-mediated gene regulation via RAR, and RXR play a crucial role in spermatogenesis. For instance, detailed morphological analysis in RARα knockout mouse testes showed that the typical characteristic of stage VIII tubule, where mature step 16 spermatids aligning along the tubular lumen, was not observed.[39] Instead, a mixed population of germ cells was found in stage VIII tubule in RARα knockout male.[39] For RXRβ knockout mice, failure of spermatid release occurred within the germinal epithelium and the epididymis contained very few spermatozoa. Although knockout of RARα and RXRβ resulted in male infertility, they displayed different seminiferous tubule morphology. These observations suggest that the downstream targets of RARα and RXRβ are not the same. Genes expressed in different testicular cells, namely Stra8 and bone morphogenetic protein 4 (BMP4) in germ cells, prostaglandin D2 synthetase in Sertoli cells, and fibronectin and laminin in myoid cells, were shown to be regulated by retinoic acid or retinol.[40-44] However, the precise mechanisms of vitamin A-mediated gene regulations have yet to be elucidated. Whether the regulation of those genes are direct effects mediated through the interaction of retinoid receptors and their corresponding promoters, or whether other retinoid-regulated proteins mediate indirect regulatory effects remain to be determined. Identification of the regulatory mechanism on RA-RAR-mediated BMP4 expression in other cell lines has provided a blueprint to study the transcription regulation of BMP4 gene in germ cells.[45]

Orphan Receptors

Germ Cell Nuclear Factor

Germ cell nuclear factor (GCNF), which is also known as retinoid receptor-related testis-associated receptor (RTR), is a novel member of the nuclear receptor superfamily of ligand-activated transcription factors.[46,47] Since the natural ligand for GCNF has not been identified, GCNF is classified as an orphan receptor. GCNF binds as a homodimer either to direct repeat response elements (AGGTCA) without additional nucleotide or to extended half-site such as TCAAGGTCA (XRE).[46,48-51] It does not form heterodimer with other nuclear receptors such as RXR.[48] In vitro studies have revealed that GCNF is a sequence-specific repressor of transcription and it folds into a β-sheet that contributes to dimerization and the recruitment of corepressors.[51-54] It can interact with other nuclear corepressors and with the repressor, RAP80, that is highly expressed in the testis.[54-56]

GCNF expression is restricted to the developing nervous system during embryogenesis, whereas the receptor is expressed during specific stages in maturing germ cells. Two transcripts of GCNF gene with sizes of 7.4 kb and 2.3 kb have been identified in spermatogenic cells. The 7.4 kb transcript is expressed during testicular development and is the predominant form in pachytene spermatocytes, whereas the 2.3 kb transcript is expressed predominantly in round spermatids.[47,57,58] In situ hybridization studies have shown that the GCNF transcript levels remain low during the meiotic prophase in rats and mice, and increase substantially and reach maximal level in round spermatids at stages VI-VIII.[58]

Up to now, several genes expressed in the testis were found to be regulated by GCNF. The temporal expression of protamine genes, prm-1 and prm-2, at stage I round spermatid is regulated reciprocally by GCNF and cAMP-response element modulator, CREMτ.[59-61] Binding of GCNF to GCNF response elements of prm-1 and prm-2 promoters represses both basal and CREMτ-activated transcription, thus GCNF may play a role to shut down protamine gene expression in elongating spermatids.[61] Apart from prm-1 and prm-2 genes, mitochondrial glycerol-3-phosphate dehydrogenase (mGPDH) and endozepine-like peptide (ELP) are two other testis-specific genes that are regulated by GCNF.[62,63] Both promoters of mGPDH and ELP genes contain CRE/GCNF elements that can effectively bind to GCNF. The binding of GCNF to these motifs can interfere with CREMτ-transactivation. Apparently, GCNF is a crucial transcription regulator that regulates the temporal and spatial expression of several testicular genes during meiosis and the early haploid phase of spermatogenesis.

Testicular Orphan Receptors 2 and 4

Testicular orphan receptor 2 (TR2) and testicular orphan receptor 4 (TR4) constitute a subfamily of nuclear receptors.[64,65] The TR2 and TR4 can modulate its target gene expression by forming homodimers and binding to the AGGTCA direct repeat (DR) sequences in its target genes.[66,67] TR4 can modulate transactivation mediated by other steroid nuclear receptors through interaction with these steroid receptors. For instance, TR4 could interact with the androgen receptor (AR) and the estrogen receptor (ER) that suppress AR- and ER-mediated transactivation.[19,68]

TR2 and TR4 have been shown to be expressed in mouse testes. TR2 is confined to meiotic and postmeiotic germ cells,[69,70] whereas TR4 is predominantly expressed in primary spermatocytes, especially in late-stage pachytene spermatocytes.[71,72] The expression of TR4 in round spermatid is stage-dependent and is confined to stage VII.[72] Although the male knockout mice of TR2 and TR4 are fertile, the disruption of TR4 gene does affect spermatogenesis at the end of late meiotic prophase and subsequent meiotic divisions, thus delays the first wave of spermatogenesis in the $TR4^{-/-}$ mice.[72,73] Gene disruption analyses indicated that TR4, but not TR2, is essential for normal spermatogenesis in mice.

Recent studies has demonstrated that TR4 can suppress the expression of 25-hydroxyvitamin D3 24-hydroxylase, Cyp24a1, through direct binding of TR4 to the vitamin D3 receptor response element (VDRE) in Chinese hamster ovary (CHO) cells. The VDRE shares similarity

with the hormone response element for the TR4, which contains two repeated half sites of AGGTCA; however, it is separated by a 3-nucleotide space. Using the TR4$^{-/-}$ knockout mice model, Mu et al. showed that the expression level of Cyp24a1 increased in adult mice testes when TR4 gene was knocked out. Such observation indicates that testicular Cyp24a1 expression is also under the precise control of TR4.[72] Cyp24a1 is the only gene identified so far that is regulated by TR in the testis, identification of the molecular targets, such as putative ligands of TR2 and TR4, and the mechanisms that affect meiosis may help in a better understanding of the role of TR in spermatogenesis.

Transcription Factors Involved in Testicular Functions

Basic-Domain-Leucine-Zipper (b-zip) Family

Members of the b-zip family that are known to be expressed in the testis include cAMP response element modulator (CREM), cAMP response element binding protein (CREB), and activating transcription factor 1 (ATF1).[74,75] These proteins contain a basic DNA-binding domain with an adjacent leucine zipper that is required for dimerization and binding to a specific cis-acting element.[76,77] CREM, CREB and ATF-1 are capable of forming homodimers and heterodimers in response to cAMP signaling pathway and bind to a regulatory DNA sequence, known as cAMP responsive element (CRE). A CRE is constituted by the palindromic consensus sequence, TGACGTCA.[76-78]

CREM

Many isoforms of CREM are generated by alternative splicing. Among them, CREMτ is the isoform which has been extensively studied as its expression is restricted to the testis and is highly regulated during spermatogenesis.[79] CREMτ mRNA transcript is found at high levels in pachytene spermatocytes and more advanced germ cells, while its protein is present only in post-meiotic spermatids, suggesting that CREMτ plays a role in late stages of spermatogenesis.[80] The importance of CREMτ in spermatogenesis could be reflected in the gene knockout studies since spermatogenic arrest was observed at pachytene spermatocyte stage.[81] A list of postmeiotic genes encoding structural proteins required for spermatid differentiation, including the transition proteins (TP1 and TP2), protamines (prm1 and prm2), RT7, testis angiotensin-converting enzyme (ACE), proacrosin and calspermin, were found to be the direct targets of CREMτ.[82-85] All of these genes contain the putative CREs for the binding of CREMτ. It is apparent that CREMτ is a key transcription factor that controls postmeiotic germ cell differentiation.

Different from other CREM isoforms, the activation of CREMτ requires the association of a coactivator known as activator of CREM in testes (ACT).[86] ACT is exclusively expressed in testes.[86] ACT shows similar developmental expression pattern as CREMτ in testes and they are colocalized in spermatids.[86-88] ACT displays intrinsic transactivation potential capable of converting CREMτ into a potent transcriptional activator, leading to the activation of CREMτ in a phosphorylation-independent manner.[87,88] The presence of ACT in post-meiotic germ cells enables stage-specific activation of CREMτ-mediated gene transcription.

To elucidate the significance of ACT in CREMτ-mediated gene transcription in testes, gene targeting disruption in mice has been performed. It is surprising that male mice lacking ACT are fertile, which is different from the CREM knockout counterpart. Mice lacking ACT show some male reproductive defects including abnormalities in sperm heads and tails and reduced sperm motility.[89] However, the expressions of CREMτ-dependent genes, such as TP1 and prm1, were not affected in ACT knockout mice. These results seemingly suggest that other yet-to-be identified coactivators exist in testes could compensate for the loss of ACT to modulate CREMτ-dependent gene transcription.

CREB

Similar to CREM, many CREB isoforms are generated by alternative splicing in the testis. Although the gene knockout analyses of CREB isoforms have been performed, the role of CREB

in spermatogenesis has not been fully elucidated. Since mice carrying mutations in all CREB isoforms exhibited severe developmental disorders and died shortly after birth. In situ hybridization analysis has shown that CREB mRNA is present in Sertoli cells in stages I-VIII tubules and the amount decreases to an undetectable level at stages IX-XIV.[90] The cellular localization of CREB in the testis is quite different from CREM, whose protein is present only in post-meiotic spermatids. Several genes involved in spermatogenesis such as murine spermatogenesis-associated protein, claudin-II and nectin-2 have been found to be regulated directly by CREB via the CRE motif in the corresponding promoters.[91,92,153] Interaction of CREB with other transcription factors, such as c-Jun, was found to be involved in regulating the nectin-2 gene transcription in Sertoli cells.[92] In addition, overexpression of dominant-negative CREB in primary Sertoli cells could completely inhibit the FSH-induced c-Fos expression. Taken collectively, these data illustrate that CREB seems to play an intriguing role in regulating gene transcription in Sertoli cells.

Homeobox Family

Transcription factors belonging to this family contain the homeobox motif that is a highly conserved DNA-binding domain constituted by 61 amino acids. Transcription factors belonging to this family are grouped in subfamilies based on the homeodomain sequence as well as the gene structure.[93] Our chapter does not attempt to cover all members of this family but highlights two subfamilies that show intimate relationships with spermatogenesis. They are the reproductive homeobox X-linked (Rhox) gene cluster and the POU-domain gene family.

Reproductive Homeobox X-Linked (Rhox) Gene Cluster

Rhox gene cluster presents a newly homeobox subfamily that contains 12 related homeobox genes.[24,26] All 12 Rhox genes are organized into three subclusters, namely α (Rhox 1-4), β (Rhox 5-9) and γ (Rhox 10-12) on the X chromosome and are expressed in male and female reproductive tissues. All of them exhibit cell type-specific expression.[26] In testes, all Rhox genes are restricted to Sertoli cells except Rhox4 which is predominantly expressed in Leydig cells.[26] Apart from cell-type specificity, these 12 Rhox genes exhibit a colinear expression pattern in which an expression gradient is achieved spatially, temporally, or quantitatively, pertinent to their relative position within subclusters. For instance, the genes in subcluster α display both temporal and quantitative colinearity.[26] Rhox1, the gene located at the distal 5' end of subcluster α express first (days 7-12 postpartum) followed by Rhox2 (day 12 postpartum), Rhox3 and Rhox4 (days 20-22 postpartum).[26] Among them, Rhox1 is expressed at the highest level during testis development than other gene members in same subcluster and each subsequent gene in the same subcluster exhibits a stepwise decline in its expression level.[26] It is believed that such colinear expression pattern observed in the Rhox cluster might provide Sertoli cells with a precise regulatory system to transduce temporally variable signals to germ cells at all stages of development.[94] Clearly, future studies such as targeted disruption or knockdown approaches will be required to reveal the individual and overlapping function of these Rhox genes in spermatogenesis.

The importance of the Rhox gene cluster in spermatogenesis could be demonstrated at least by target disruption of Rhox5 gene in male mice.[26,95] Ablation of Rhox5 gene by homologous recombination was subsequently found that mutant male are subfertile.[26] Reduced sperm count and sperm motility along with increased germ cell apoptosis were observed in Rhox5$^{-/-}$ mice. Since the expression of Rhox5 is restricted to Sertoli cells,[24,26,95] it is likely that Rhox5 plays a role in regulating the expression of Sertoli-cell genes that can modulate germ cell survival. Efforts should be made to elucidate the functional significance of each Rhox member in spermatogenesis and identify target genes that are regulated by the Rhox gene cluster.

POU Homedomain Proteins

*S*perm-1

Sperm-1, belonging to the family of the POU (Pit, Oct, Unc) homeodomain proteins, is selectively expressed in male germ cells immediately preceding the first meiotic division and in

the haploid spermatids.[96,97] Sperm-1 preferentially binds to an octamer DNA-response element with sequence of 5'-GCATATGTTATT-3' in which the optimal sequence differs from that preferred by other POU protein members.[96]

Knockout studies of Sperm-1 in mice have been performed, null mice develop normal testis, apparently with normal spermatogenesis and produce normal number of motile sperms as those of normal mice, except that the Sperm-1 null male mice are subfertile.[97] However, the molecular basis for this subfertile phenotype has not yet been elucidated. Thus, identification of the molecular targets and mechanism of action of sperm-1 may help in a better understanding its role in spermatogenesis.

Oct-4

Oct-4 is expressed in the postproliferative prospermatogonia until after birth in male embryos. Oct-4 expression continues in undifferentiated type A spermatogonia as spermatogenesis starts, and is downregulated when germ cells enter their differentiation pathway. There is no reexpression of Oct-4 in germ cells at any developemental stages of spermatogenesis.[98,99] The downregulation of Oct-4 seems to be one of the molecular triggers in the commitment of meiosis in male germ cells, although the target gene(s) involved in such event has not been identified.

C_2H_2 Zinc Finger Family

Transcription factors belong to this family must contain C_2H_2 zinc finger motif (also known as Krüppel zinc finger motif), which is generally present in tandem arrays with the sequence of Y/F-X-C-X_{2-4}-C-X_3-F-X_5-L-X_2-H-X_{3-5}-H, where X can be variable amino acids.[100] These conserved cysteine and histidine residues are able to bond tetrahedrally to a zinc ion. Plzf and WT1 are two transcription factors that are known to possess a C_2H_2 zinc finger and have been reported to have significant impact on spermatogenesis.[101]

Plzf

Plzf is also known as zinc-finger protein 145 (zfp145) that is expressed in the developing male gonad.[102] In postnatal and adult testes, Plzf is restricted to spermatogonia that exhibit stem-cell like properties and is coexpressed with Oct-4, a transcription factor implicated in maintaining stem-cell population.[102,103] The functional importance of Plzf has been revealed by two in vivo studies. Studies of naturally occurring Plzf-mutant (luxoid) mice and Plzf knockout mice have shown that both mutant mice exhibit a progressive loss of spermatogonia with age, associated with an increase in apoptosis, but without apparent defects in Sertoli cells.[102,103] Spermatogonial transplantation experiments demonstrated that Plzf is a spermatogonia-specific transcription factor that is required to regulate self-renewal and maintenance of the stem cell pool as transplantation of spermatogonia isolated from Plzf-null mice failed to repopulate gonads that had been chemically depleted of germ cells.[102] Up to now, no direct target gene of Plzf regulation has been identified. Apparently, it is an area that needs further investigation.

Wilms' Tumor Protein (WT1)

WT1 protein contains four COOH-terminal C_2H_2 zinc fingers for DNA binding and one of each transcriptional repression and activation domains at its NH_2 terminus.[104] WT1 plays a crucial role in the development of the genitourinary system.[105] Conditional knockout of WT1 protein in Sertoli cells by embryonic day 14.5 could result in disruption of developing seminiferous tubules and progressive loss of Sertoli cells and germ cells.[106] Using tissue-specific RNA interference (RNAi) approach that disrupts the expression of WT1 in mouse testis, studies have shown that increased germ cell apoptosis, loss of adherens junctions and impaired spermatogenesis were observed in siRNA-WT1 mice.[107] Microarray analysis on siRNA-WT1 testes has found that a spectrum of genes encoding signaling molecules and structural proteins whose expressions were altered.[107] For instance, integrin cytoplasmic domain associated protein 1α (Icap1-α) and epidermal growth factor receptor pathway substrate 8 (Eps8), which are signaling molecules that regulate actin-mediated cytoskeletal events, are altered in siRNA-WT1 testes.[107] These results

suggest that Icap1-α and Eps8 are the target proteins of WT1 and WT1 is a crucial transcription factor in regulating spermatogenesis.

GATA Family

All GATA proteins contain a DNA-binding domain composed of two conserved multifunctional zinc fingers, C-X_2-C-X_{17}-C-X_2-C, where X represents variable amino acids.[108,109] GATA proteins recognize and bind to the DNA consensus motif, WGATAR.[109] The N-terminal zinc finger is required for the specificity and stability of the DNA binding, whilst the C-terminal zinc finger is for the recognition and binding to the core GATA motif.[110-113] GATA interacts with cofactors such as Friend of GATA-1 and -2 (FOG-1 and FOG-2) and p300/CBP via the N-terminal or C-terminal zinc fingers, resulting in either activation or repression of gene transcription.[114-119]

GATAs are essential transcription factors in mammalian reproductive development and function. Among six members of this family, GATA-1, -4 and -6 are found in testes. GATA-1 is expressed in mouse Sertoli cells from stages VII to IX of the seminiferous epithelial cycle.[120] GATA-4 is present in mouse testis throughout all developmental stages and localized to Sertoli cells and Leydig cells.[121-123] GATA-6 is expressed in neonatal, prepubertal, and adult testes and localized in Sertoli cells.[116,122] The GATA family members play equally important role in gonadal development, testosterone production and regulation of gene expression in testicular somatic cells such as Sertoli and Leydig cells.[118,119,124] For instance, GATA-4 is capable of activating the promoters of testicular genes including Mullerian-inhibiting substance (MIS), PII aromatase (Cyp19), SF-1, StAR and inhibin α.[124] The examples mentioned herein are not intended to be exhaustive, readers are strongly encouraged to read earlier review to gain a more comprehensive view of this protein family.[118,119]

Nuclear Factor Kappa B (NF-κB) Family

The NF-κB family of transcription factors regulates a wide variety of genes involved in spermatogenesis. The NF-κB family is composed of p50, p52, p65 (RelA), RelB and c-Rel,[125,126] which regulates transcription by binding as homo- or heterodimers to κB enhancer elements in the regulatory region of genes. Among five protein subunits, p50 and p65 have been shown to express in rat testes. Nuclear expression of p50 and p65 are cell-type and stage-specific. Nuclear p50 and p60 are highest at stages XIV-VII in Sertoli cells and stages VII-XI in spermatocytes.[127]

Like another transcription factors, the NF-κB family of transcription factors can activate and repress testicular gene transcription. For example, TNF-α induces NF-κB binding to the cAMP-response element-binding protein (CREB) in AR promoters and elevates their promoter activities in Sertoli cells.[128,129] TNF-α has been reported to downregulate SF-1 transactivation of Mullerian inhibiting substance (MIS) gene in the testis by NF-κB. The SF-1-bound NF-κB could recruit histone deacetylases to inhibit the SF-1-mediated MIS gene activation.[130] Since TNF-α is a major cytokine secreted by germ cells, it is believed that the effect of TNF-α and its downstream regulators, NF-κB, may not be limited to those identified genes. Clearly, there is much remains to be investigated with regard to the function of NF-κB in spermatogenesis.

Y-Box

The family of Y-box proteins contains a conserved cold-shock domain (CSD) for DNA binding, a variable N-terminal domain thought for transactivation and a C-terminal tail for protein-protein interaction.[131,132] YB-1 was the first identified transcription factor that bound to the Y-box and the consensus DNA sequence was determined as CTGATTGGYYUU, a reverse sequence motif of the CCAAT box.[133]

Mammalian germ cell homologues of *Xenopus* FRG Y1 and FRG Y2 have been identified in mouse testis, namely MSY1 and MSY2 respectively.[134,135] Similar to *Xenopus* homologues, MSY1 is ubiquitously expressed in somatic tissues; whereas MSY2 is expressed in meiotic and postmeiotic germ cells.[134,136] Several studies have revealed that Y-box proteins are needed to activate gene transcription in male germ cells, such as protamine 2 and cytochrome c genes.[137,138] Recent knockout studies further confirmed the functional significance of MSY2 in spermatogenesis.

Spermatogenesis is disrupted in postmeiotic null germ cells with many misshapen and multinucleated spermatids.[139]

Apart from MSY2, at least two other Y-box proteins, MSY1 and MSY4, are expressed in meiotic and postmeiotic germ cells.[134,140] However, their roles on gene transcription pertinent to spermatogenesis remain entirely unknown.

CAF1

Chromatin assembly factor-1 (CAF1), also called as Cnot7, is the mammalian homolog of yeast CAF1.[141] It is a component of the CCR4-NOT complex that has multiple roles in regulating transcription.[142] CAF1-deficient male mice are sterile owning to oligo-astheno-teratozoospermia shown in two independent knockout studies.[143,144] Maturation of spermatids is unsynchronized and impaired. Further studies have shown that the proper function of retinoid X receptor β (RXRβ)-mediated transcription in the testis requires the interaction of CAF1 through the AF-1 domain of RXRβ, suggesting CAF1 functions as a coregulator of RXRβ in regulating transcription in testicular somatic cells as RXRβ is expressed in somatic Sertoli cells and Leydig cell.[143]

Testis-Specific Gene Expression

Testis-specific gene expression could be in part achieved through the expression of testis-specific transcription factors, such as CREMτ, and cell type-specific components of the general or core transcription machinery as an increasing number of tissue or cell type-specific components of general transcription factors has been identified, such as TFIIA-τ, a testis-specific isoform of TFIIA.[145-147]

An alternative approach to achieve tissue-specific gene expression is by permanent transcriptional repression of that particular promoter in nonexpressing cells via DNA methylation.[148] A testis-specific expression of histone H1t is one of the examples belonging to this category. The repression of the histone H1t gene in nonexpressing cells is achieved by partial and full methylation of all seven CpG dinucleotides within the H1t proximal promoter, while these CpG dinucleotides are completely unmethylated in primary spermatocytes.[149,150]

Transcriptional Regulation of Cell Junction Dynamics

The translocation of germ cells across the seminiferous epithelium during spermatogenesis requires extensive restructuring of cell junctions at the Sertoli-germ and Sertoli-Sertoli interface.[151] It is believed that the transcriptional, post-transcriptional and post-translational regulations of cell junction proteins play crucial roles in controlling the assembly and disassembly of cell junctions, resulting in the progressive movement of germ cells to the adluminal from the basal compartment for the completion of spermatogenesis.[152] Therefore, studies of the transcriptional regulation of junction proteins found at the ectoplasmic specialization (ES) and the blood-testis barrier (BTB) are crucial for the thorough understanding of spermatogenesis. In our laboratory, the transcriptional regulations of nectin-2 and claudin-11 in Sertoli cells have been studied.[92,153] Nectin-2 is a junction protein localized at Sertoli cells and interacts at nectin-3 that is expressed in germ cells to form the heterotypic interlock between Sertoli and germ cells at the apical ES.[154] Our recent studies have demonstrated that CREB and c-Jun are bound to the cAMP responsive element (CRE) motif of the nectin-2 promoter located between nucleotides -316 and -211 (relative to the translation start site), resulting in the upregulation of nectin-2 gene transcription. Apart from CREB and c-Jun, two members of Sp1 family, Sp1 and Sp3, are also positive regulators of the nectin-2 transcription.[92] Analysis of the staged tubules has confirmed that the cyclic expressions of CREB and nectin-2 coincide with the event of apical ES restructuring between Sertoli cells and germ cells. It is believed that the tight regulation of the basal nectin-2 transcription by CREB, c-Jun and Sp1 are crucial to regulate the disassembly of adherens junctions between Sertoli cells and germ cells during spermiation (Fig. 1).

Apart from adherens junction proteins, we have also studied the transcriptional regulation of tight junction (TJ) proteins in Sertoli cells. Claudin-11 is a TJ integral protein found in testis and

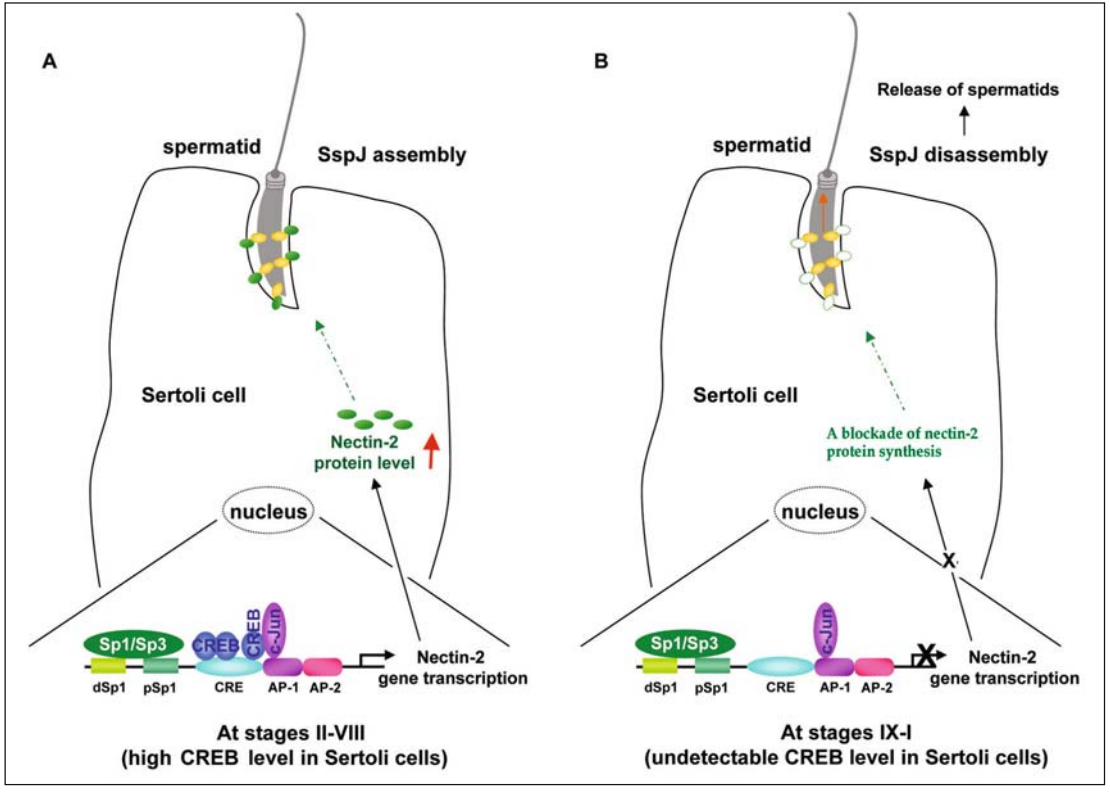

Figure 1. A-B) A proposed model for the regulation of nectin-2 expression in testis. This model accounts for the functional cooperation of multiple transcription factors (Sp1 protein family, CREB and c-Jun) in regulating the basal nectin-2 gene transcription. It also illustrates how the cyclic expression of CREB in a spermatogenic cycle influences the nectin-2 gene transcription, which in turn regulates the assembly of SspJ (A) at stages II-VIII and disassembly at stages IX-I (B), resulting in spermiation.

CNS (central nervous system) myelin.[155] In our study, we demonstrated that the overlapping GATA/NF-Y motif within the core promoter of claudin-11 gene is modulated by differential binding of various transcription factors, resulting in dual transcriptional control.[153] We confirmed that GATA, nuclear factor YA (NF-YA), and cAMP response element-binding protein (CREB) form a complex in vivo and bind to the GATA/NF-Y region to promote claudin-11 gene transcription. GATA and CREB transactivation could be further modulated by the presence of Smad3 and Smad4 proteins. Binding of Smad proteins at the GATA/NF-Y motif could repress the GATA and CREB transactivation of claudin-11 gene. Such repression required the recruitment and physical interactions of histone deacetylase 1 and its corepressor, mSin3A, with Smad proteins. It is believed that cyclic changes in the ratio of positive regulators (GATA, NF-YA and CREB) to negative regulators (Smads) in the seminiferous epithelium during the spermatogenic cycle might provide the precise control in claudin-11 gene transcription.

Concluding Remarks and Future Perspectives

As we briefly reviewed and discussed herein, much work on the transcriptional regulation of spermatogenesis conducted in the past two decades was focused on individual transcription factor, and most of these studies relied solely on changes in phenotypes of the knock-out mice to assess the function of different transcription factors. However, the physiological linkage between different transcription factors during spermatogenesis remains unknown. Also, the molecular target genes of these transcription factors at different stages of the seminiferous epithelial cycle are largely unknown. Furthermore, how these genes and their proteins regulate different facets of spermatogenesis, such as germ cell cycle, meiosis, spermatogonial proliferation and renewal, germ cell apoptosis, cell adhesion and junction restructuring, germ cell migration, biochemical and morphological events pertinent to spermiogenesis, and others, remain unexplored. Nonetheless, with the recent advances in genomics and proteomics research, such as the use of gene profiling techniques coupled with mass spectrometry to identify target genes (proteins) important to transcriptional regulation in knock-out mice versus wild types, this shall provide an unprecedented opportunity for investigators in the field.

Acknowledgement

This work was supported in part by grants from CRCG Seed Funding Programme for Basic Research and Hong Kong Research Grant Council (HKU7609/06M and and HKU771507M to WYL). CYC was supported by grants from NIH (NICHD, U01 HD045908; U54 HD029990, Project 3), and the CONRAD Program (CICCR, CIG 01-72).

References

1. Oakberg EF. Duration of spermatogenesis in the mouse and timing of stages of the cycle of the seminiferous epithelium. Am J Anat 1956; 99:507-516.
2. Russell L. Movement of spermatocytes from the basal to the adluminal compartment of the rat testis. Am J Anat 1977; 148:313-328.
3. Eddy EM. Male germ cell gene expression. Recent Prog Horm Res 2002; 57:103-128.
4. Sassone-Corsi P. Transcriptional checkpoints determining the fate of male germ cells. Cell 1997; 88:163-166.
5. Schmidt EE, Schibler U. High accumulation of components of the RNA polymerase II transcription machinery in rodent spermatids. Development 1995; 121:2373-2383.
6. Upadhyaya AB, Lee SH, DeJong J. Identification of a general transcription factor TFIIAα/β homolog selectively expressed in testis. J Biol Chem 1999; 274:18040-18048.
7. Ozer J, Moore PA, Lieberman PM. A testis-specific transcription factor IIA (TFIIAτ) stimulates TATA-binding protein-DNA binding and transcription activation. J Biol Chem 2000; 275:122-128.
8. McLachlan RI, Wreford NG, O'Donnell L et al. The endocrine regulation of spermatogenesis: Independent roles for testosterone and FSH. J Endocrinol 1996; 148:1-9.
9. Sheckter CB, Matsumoto AM, Bremner WJ. Testosterone administration inhibits gonadotropin secretion by an effect directly on the human pituitary. J Clin Endocrinol Metab 1989; 68:397-401.
10. Keller ET, Ershler WB, Chang C. The androgen receptor: A mediator of diverse responses. Front Biosci 1996; 1:d59-71.

11. Roy AK, Lavrovsky Y, Song CS et al. Regulation of androgen action. Vitam Horm 1999; 55:309-352.
12. Heinlein CA, Chang C. Androgen receptor (AR) coregulators: An overview. Endocr Rev 2002; 23:175-200.
13. Bagchi MK, Tsai MJ, O'Malley BW et al. Analysis of the mechanism of steroid hormone receptor-dependent gene activation in cell-free systems. Endocr Rev 1992; 13:525-535.
14. Jenster G, van der Korput HA, Trapman J et al. Identification of two transcription activation units in the N-terminal domain of the human androgen receptor. J Biol Chem 1995; 270:7341-7346.
15. Kasper S, Rennie PS, Bruchovsky N et al. Cooperative binding of androgen receptors to two DNA sequences is required for androgen induction of the probasin gene. J Biol Chem 1994; 269:31763-31769.
16. Zhou Z, Corden JL, Brown TR. Identification and characterization of a novel androgen response element composed of a direct repeat. J Biol Chem 1997; 272:8227-8235.
17. Verrijdt G, Schoenmakers E, Alen P et al. Androgen specificity of a response unit upstream of the human secretory component gene is mediated by differential receptor binding to an essential androgen response element. Mol Endocrinol 1999; 13:1558-1570.
18. Claessens F, Verrijdt G, Schoenmakers E et al. Selective DNA binding by the androgen receptor as a mechanism for hormone-specific gene regulation. J Steroid Biochem Mol Biol 2001; 76:23-30.
19. Lee YF, Shyr CR, Thin TH et al. Convergence of two repressors through heterodimer formation of androgen receptor and testicular orphan receptor-4: A unique signaling pathway in the steroid receptor superfamily. Proc Natl Acad Sci USA 1999; 96:14724-14729.
20. Panet-Raymond V, Gottlieb B, Beitel LK et al. Interactions between androgen and estrogen receptors and the effects on their transactivational properties. Mol Cell Endocrinol 2000; 167:139-150.
21. He B, Kemppainen JA, Voegel JJ et al. Activation function 2 in the human androgen receptor ligand binding domain mediates interdomain communication with the NH(2)-terminal domain. J Biol Chem 1999; 274:37219-37225.
22. Fang Y, Fliss AE, Robins DM et al. Hsp90 regulates androgen receptor hormone binding affinity in vivo. J Biol Chem 1996; 271:28697-28702.
23. Yeh S, Tsai MY, Xu Q et al. Generation and characterization of androgen receptor knockout (ARKO) mice: An in vivo model for the study of androgen functions in selective tissues. Proc Natl Acad Sci USA 2002; 99:13498-13503.
24. Lindsey JS, Wilkinson MF. Pem: A testosterone- and LH-regulated homeobox gene expressed in mouse Sertoli cells and epididymis. Dev Biol 1996; 179:471-484.
25. Maiti S, Doskow J, Li S et al. The Pem homeobox gene: Androgen-dependent and -independent promoters and tissue-specific alternative RNA splicing. J Biol Chem 1996; 271:17536-17546.
26. MacLean IInd JA, Chen MA, Wayne CM et al. Rhox: A new homeobox gene cluster. Cell 2005; 120:369-382.
27. Barbulescu K, Geserick C, Schuttke I et al. New androgen response elements in the murine pem promoter mediate selective transactivation. Mol Endocrinol 2001; 15:1803-1816.
28. Geserick C, Meyer HA, Barbulescu K et al. Differential modulation of androgen receptor action by deoxyribonucleic acid response elements. Mol Endocrinol 2003; 17:1738-1750.
29. Rao MK, Wayne CM, Wilkinson MF. Pem homeobox gene regulatory sequences that direct androgen-dependent developmentally regulated gene expression in different subregions of the epididymis. J Biol Chem 2002; 277:48771-48778.
30. Rao MK, Wayne CM, Meistrich ML et al. Pem homeobox gene promoter sequences that direct transcription in a Sertoli cell-specific, stage-specific, and androgen-dependent manner in the testis in vivo. Mol Endocrinol 2003; 17:223-233.
31. Chang C, Chen YT, Yeh SD et al. Infertility with defective spermatogenesis and hypotestosteronemia in male mice lacking the androgen receptor in Sertoli cells. Proc Natl Acad Sci USA 2004; 101:6876-6881.
32. De Gendt K, Swinnen JV, Saunders PT et al. A Sertoli cell-selective knockout of the androgen receptor causes spermatogenic arrest in meiosis. Proc Natl Acad Sci USA 2004; 101:1327-1332.
32a. Holdcraft RW, Braun RE. Androgen receptor function is required in Sertoli cells for the terminal differentiation of haploid spermatids. Development 2004; 131:459-467
32b. Meng J, Holdcraft RW, Shima JE et al. Androgens regulate the permeability of the blood-testis barrier. Proc Natl Acad Sci USA 2005; 102:16696-16700.
33. van Pelt AM, van den Brink CE, de Rooij DG et al. Changes in retinoic acid receptor messenger ribonucleic acid levels in the vitamin A-deficient rat testis after administration of retinoids. Endocrinology 1992; 131:344-350.
34. Lufkin T, Lohnes D, Mark M et al. High postnatal lethality and testis degeneration in retinoic acid receptor α mutant mice. Proc Natl Acad Sci USA 1993; 90:7225-7229.

35. Kastner P, Mark M, Leid M et al. Abnormal spermatogenesis in RXRβ mutant mice. Genes Dev 1996; 10:80-92.
36. Akmal KM, Dufour JM, Vo M et al. Ligand-dependent regulation of retinoic acid receptor α in rat testis: In vivo response to depletion and repletion of vitamin A. Endocrinology 1998; 139:1239-1248.
37. Lee S, Privalsky ML. Heterodimers of retinoic acid receptors and thyroid hormone receptors display unique combinatorial regulatory properties. Mol Endocrinol 2005; 19:863-878.
38. Soprano DR, Qin P, Soprano KJ. Retinoic acid receptors and cancers. Annu Rev Nutr 2004; 24:201-221.
39. Chung SS, Sung W, Wang X et al. Retinoic acid receptor α is required for synchronization of spermatogenic cycles and its absence results in progressive breakdown of the spermatogenic process. Dev Dyn 2004; 230:754-766.
40. Ricci G, Catizone A, Scarcella MF et al. Vitamin A modulation of basement membrane production by purified testicular myoid cells. Exp Cell Res 1999; 249:102-108.
41. Samy ET, Li JC, Grima J et al. Sertoli cell prostaglandin D2 synthetase is a multifunctional molecule: Its expression and regulation. Endocrinology 2000; 141:710-721.
42. Oulad-Abdelghani M, Bouillet P, Decimo D et al. Characterization of a premeiotic germ cell-specific cytoplasmic protein encoded by Stra8, a novel retinoic acid-responsive gene. J Cell Biol 1996; 135:469-477.
43. Rogers MB, Hosler BA, Gudas LJ. Specific expression of a retinoic acid-regulated, zinc-finger gene, Rex-1, in preimplantation embryos, trophoblast and spermatocytes. Development 1991; 113:815-824.
44. Baleato RM, Aitken RJ, Roman SD. Vitamin A regulation of BMP4 expression in the male germ line. Dev Biol 2005; 286:78-90.
45. Thompson DL, Gerlach-Bank LM, Barald KF et al. Retinoic acid repression of bone morphogenetic protein 4 in inner ear development. Mol Cell Biol 2003; 23:2277-2286.
46. Chen F, Cooney AJ, Wang Y et al. Cloning of a novel orphan receptor (GCNF) expressed during germ cell development. Mol Endocrinol 1994; 8:1434-1444.
47. Hirose T, O'Brien DA, Jetten AM. RTR: A new member of the nuclear receptor superfamily that is highly expressed in murine testis. Gene 1995; 152:247-251.
48. Borgmeyer U. Dimeric binding of the mouse germ cell nuclear factor. Eur J Biochem 1997; 244:120-127.
49. Yan ZH, Medvedev A, Hirose T et al. Characterization of the response element and DNA binding properties of the nuclear orphan receptor germ cell nuclear factor/retinoid receptor-related testis-associated receptor. J Biol Chem 1997; 272:10565-10572.
50. Greschik H, Schule R. Germ cell nuclear factor: An orphan receptor with unexpected properties. J Mol Med 1998; 76:800-810.
51. Greschik H, Wurtz JM, Hublitz P et al. Characterization of the DNA-binding and dimerization properties of the nuclear orphan receptor germ cell nuclear factor. Mol Cell Biol 1999; 19:690-703.
52. Bauer UM, Schneider-Hirsch S, Reinhardt S et al. Neuronal cell nuclear factor—A nuclear receptor possibly involved in the control of neurogenesis and neuronal differentiation. Eur J Biochem 1997; 249:826-837.
53. Cooney AJ, Hummelke GC, Herman T et al. Germ cell nuclear factor is a response element-specific repressor of transcription. Biochem Biophys Res Commun 1998; 245:94-100.
54. Yan Z, Jetten AM. Characterization of the repressor function of the nuclear orphan receptor retinoid receptor-related testis-associated receptor/germ cell nuclear factor. J Biol Chem 2000; 275:35077-35085.
55. Fuhrmann G, Chung AC, Jackson KJ et al. Mouse germline restriction of Oct4 expression by germ cell nuclear factor. Dev Cell 2001; 1:377-387.
56. Yan Z, Kim YS, Jetten AM. RAP80, a novel nuclear protein that interacts with the retinoid-related testis-associated receptor. J Biol Chem 2002; 277:32379-32388.
57. Katz D, Niederberger C, Slaughter GR et al. Characterization of germ cell-specific expression of the orphan nuclear receptor, germ cell nuclear factor. Endocrinology 1997; 138:4364-4372.
58. Zhang YL, Akmal KM, Tsuruta JK et al. Expression of germ cell nuclear factor (GCNF/RTR) during spermatogenesis. Mol Reprod Dev 1998; 50:93-102.
59. Mali P, Kaipia A, Kangasniemi M et al. Stage-specific expression of nucleoprotein mRNAs during rat and mouse spermiogenesis. Reprod Fertil Dev 1989; 1:369-382.
60. Hummelke GC, Meistrich ML, Cooney AJ. Mouse protamine genes are candidate targets for the novel orphan nuclear receptor, germ cell nuclear factor. Mol Reprod Dev 1998; 50:396-405.
61. Hummelke GC, Cooney AJ. Reciprocal regulation of the mouse protamine genes by the orphan nuclear receptor germ cell nuclear factor and CREMτ. Mol Reprod Dev 2004; 68:394-407.

62. Rajkovic M, Middendorff R, Wetzel MG et al. Germ cell nuclear factor relieves cAMP-response element modulator τ-mediated activation of the testis-specific promoter of human mitochondrial glycerol-3-phosphate dehydrogenase. J Biol Chem 2004; 279:52493-52499.
63. Valentin M, Balvers M, Pusch W et al. Structure and expression of the mouse gene encoding the endozepine-like peptide from haploid male germ cells. Eur J Biochem 2000; 267:5438-5449.
64. Chang C, Kokontis J. Identification of a new member of the steroid receptor super-family by cloning and sequence analysis. Biochem Biophys Res Commun 1988; 155:971-977.
65. Chang C, Da Silva SL, Ideta R et al. Human and rat TR4 orphan receptors specify a subclass of the steroid receptor superfamily. Proc Natl Acad Sci USA 1994; 91:6040-6044.
66. Lee YF, Young WJ, Lin WJ et al. Differential regulation of direct repeat 3 vitamin D3 and direct repeat 4 thyroid hormone signaling pathways by the human TR4 orphan receptor. J Biol Chem 1999; 274:16198-16205.
67. Young WJ, Lee YF, Smith SM et al. A bidirectional regulation between the TR2/TR4 orphan receptors (TR2/TR4) and the ciliary neurotrophic factor (CNTF) signaling pathway. J Biol Chem 1998; 273:20877-20885.
68. Shyr CR, Hu YC, Kim E et al. Modulation of estrogen receptor-mediated transactivation by orphan receptor TR4 in MCF-7 cells. J Biol Chem 2002; 277:14622-14628.
69. Lee CH, Chang L, Wei LN. Molecular cloning and characterization of a mouse nuclear orphan receptor expressed in embryos and testes. Mol Reprod Dev 1996; 44:305-314.
70. Lee CH, Chang L, Wei LN. Distinct expression patterns and biological activities of two isoforms of the mouse orphan receptor TR2. J Endocrinol 1997; 152:245-255.
71. Hirose T, Fujimoto W, Tamaai T et al. TAK1: Molecular cloning and characterization of a new member of the nuclear receptor superfamily. Mol Endocrinol 1994; 8:1667-1680.
72. Mu X, Lee YF, Liu NC et al. Targeted inactivation of testicular nuclear orphan receptor 4 delays and disrupts late meiotic prophase and subsequent meiotic divisions of spermatogenesis. Mol Cell Biol 2004; 24:5887-5899.
73. Shyr CR, Collins LL, Mu XM et al. Spermatogenesis and testis development are normal in mice lacking testicular orphan nuclear receptor 2. Mol Cell Biol 2002; 22:4661-4666.
74. Lalli E, Lee JS, Masquilier D et al. Nuclear response to cyclic AMP: Central role of transcription factor CREM (cyclic-AMP-responsive-element modulator). Biochem Soc Trans 1993; 21:912-917.
75. Lalli E, Sassone-Corsi P. Signal transduction and gene regulation: The nuclear response to cAMP. J Biol Chem 1994; 269:17359-17362.
76. Sassone-Corsi P. Transcription factors responsive to cAMP. Annu Rev Cell Dev Biol 1995; 11:355-377.
77. Montminy M. Transcriptional regulation by cyclic AMP. Annu Rev Biochem 1997; 66:807-822.
78. Montminy MR, Bilezikjian LM. Binding of a nuclear protein to the cyclic-AMP response element of the somatostatin gene. Nature 1987; 328:175-178.
79. Foulkes NS, Mellstrom B, Benusiglio E et al. Developmental switch of CREM function during spermatogenesis: From antagonist to activator. Nature 1992; 355:80-84.
80. Nantel F, Sassone-Corsi P. CREM: A transcriptional master switch during the spermatogenesis differentiation program. Front Biosci 1996; 1:d266-269.
81. Nantel F, Monaco L, Foulkes NS et al. Spermiogenesis deficiency and germ-cell apoptosis in CREM-mutant mice. Nature 1996; 380:159-162.
82. Delmas V, van der Hoorn F, Mellstrom B et al. Induction of CREM activator proteins in spermatids: Down-stream targets and implications for haploid germ cell differentiation. Mol Endocrinol 1993; 7:1502-1514.
83. Zhou Y, Sun Z, Means AR et al. cAMP-response element modulator τ is a positive regulator of testis angiotensin converting enzyme transcription. Proc Natl Acad Sci USA 1996; 93:12262-12266.
84. Sassone-Corsi P. CREM: A master-switch governing male germ cells differentiation and apoptosis. Semin Cell Dev Biol 1998; 9:475-482.
85. Steger K, Klonisch T, Gavenis K et al. Round spermatids show normal testis-specific H1t but reduced cAMP-responsive element modulator and transition protein 1 expression in men with round-spermatid maturation arrest. J Androl 1999; 20:747-754.
86. Fimia GM, De Cesare D, Sassone-Corsi P. A family of LIM-only transcriptional coactivators: Tissue-specific expression and selective activation of CREB and CREM. Mol Cell Biol 2000; 20:8613-8622.
87. De Cesare D, Fimia GM, Sassone-Corsi P. Signaling routes to CREM and CREB: Plasticity in transcriptional activation. Trends Biochem Sci 1999; 24:281-285.
88. Fimia GM, De Cesare D, Sassone-Corsi P. CBP-independent activation of CREM and CREB by the LIM-only protein ACT. Nature 1999; 398:165-169.

89. Kotaja N, De Cesare D, Macho B et al. Abnormal sperm in mice with targeted deletion of the act (activator of cAMP-responsive element modulator in testis) gene. Proc Natl Acad Sci USA 2004; 101:10620-10625.
90. Waeber G, Meyer TE, LeSieur M et al. Developmental stage-specific expression of cyclic adenosine 3',5'-monophosphate response element-binding protein CREB during spermatogenesis involves alternative exon splicing. Mol Endocrinol 1991; 5:1418-14130.
91. Slongo M, Zotti L, Onisto M. Cloning and characterization of the promoter region of human spata2 (spermatogenesis-associated protein 2) gene. Biochim Biophys Acta 2003; 1625:192-196.
92. Lui WY, Sze KL, Lee WM. Nectin-2 expression in testicular cells is controlled via the functional cooperation between transcription factors of the Sp1, CREB, and AP-1 families. J Cell Physiol 2006; 207:144-157.
93. Samuel S, Naora H. Homeobox gene expression in cancer: Insights from developmental regulation and deregulation. Eur J Cancer 2005; 41:2428-2437.
94. Hogeveen KN, Sassone-Corsi P. Homeobox galore: When reproduction goes RHOX and roll. Cell 2005; 120:287-288.
95. Pitman JL, Lin TP, Kleeman JE et al. Normal reproductive and macrophage function in Pem homeobox gene-deficient mice. Dev Biol 1998; 202:196-214.
96. Andersen B, Pearse IInd RV, Schlegel PN et al. Sperm 1: A POU-domain gene transiently expressed immediately before meiosis I in the male germ cell. Proc Natl Acad Sci USA 1993; 90:11084-11088.
97. Pearse II RV, Drolet DW, Kalla KA et al. Reduced fertility in mice deficient for the POU protein sperm-1. Proc Natl Acad Sci USA 1997; 94:7555-7560.
98. Pesce M, Gross MK, Scholer HR. In line with our ancestors: Oct-4 and the mammalian germ. Bioessays 1998; 20:722-732.
99. Pesce M, Wang X, Wolgemuth DJ et al. Differential expression of the Oct-4 transcription factor during mouse germ cell differentiation. Mech Dev 1998; 71:89-98.
100. Pavletich NP, Pabo CO. Crystal structure of a five-finger GLI-DNA complex: New perspectives on zinc fingers. Science 1993; 261:1701-1707.
101. Chen Z, Brand NJ, Chen A et al. Fusion between a novel Kruppel-like zinc finger gene and the retinoic acid receptor-α locus due to a variant t(11;17) translocation associated with acute promyelocytic leukaemia. EMBO J 1993; 12:1161-1167.
102. Costoya JA, Hobbs RM, Barna M et al. Essential role of Plzf in maintenance of spermatogonial stem cells. Nat Genet 2004; 36:653-659.
103. Buaas FW, Kirsh AL, Sharma M et al. Plzf is required in adult male germ cells for stem cell self-renewal. Nat Genet 2004; 36:647-652.
104. Rauscher FJ, Morris JF, Tournay OE et al. Binding of the Wilms' tumor locus zinc finger protein to the EGR-1 consensus sequence. Science 1990; 250:1259-1262.
105. Scholz H, Kirschner KM. A role for the Wilms' tumor protein WT1 in organ development. Physiology (Bethesda) 2005; 20:54-59.
106. Gao F, Maiti S, Alam N et al. The Wilms tumor gene, Wt1, is required for Sox9 expression and maintenance of tubular architecture in the developing testis. Proc Natl Acad Sci USA 2006; 103:11987-11992.
107. Rao MK, Pham J, Imam JS et al. Tissue-specific RNAi reveals that WT1 expression in nurse cells controls germ cell survival and spermatogenesis. Genes Dev 2006; 20:147-152.
108. Tsai SF, Martin DI, Zon LI et al. Cloning of cDNA for the major DNA-binding protein of the erythroid lineage through expression in mammalian cells. Nature 1989; 339:446-451.
109. Lowry JA, Atchley WR. Molecular evolution of the GATA family of transcription factors: Conservation within the DNA-binding domain. J Mol Evol 2000; 50:103-115.
110. Martin DI, Orkin SH. Transcriptional activation and DNA binding by the erythroid factor GF-1/NF-E1/Eryf 1. Genes Dev 1990; 4:1886-1898.
111. Yang HY, Evans T. Distinct roles for the two cGATA-1 finger domains. Mol Cell Biol 1992; 12:4562-4570.
112. Omichinski JG, Trainor C, Evans T et al. A small single-"finger" peptide from the erythroid transcription factor GATA-1 binds specifically to DNA as a zinc or iron complex. Proc Natl Acad Sci USA 1993; 90:1676-1680.
113. Omichinski JG, Clore GM, Schaad O et al. NMR structure of a specific DNA complex of Zn-containing DNA binding domain of GATA-1. Science 1993; 261:438-446.
114. Fox AH, Kowalski K, King GF et al. Key residues characteristic of GATA N-fingers are recognized by FOG. J Biol Chem 1998; 273:33595-33603.

115. Tevosian SG, Deconinck AE, Cantor AB et al. FOG-2: A novel GATA-family cofactor related to multitype zinc-finger proteins Friend of GATA-1 and U-shaped. Proc Natl Acad Sci USA 1999; 96:950-955.
116. Robert NM, Tremblay JJ, Viger RS. Friend of GATA (FOG)-1 and FOG-2 differentially repress the GATA-dependent activity of multiple gonadal promoters. Endocrinology 2002; 143:3963-3973.
117. Dai YS, Cserjesi P, Markham BE et al. The transcription factors GATA4 and dHAND physically interact to synergistically activate cardiac gene expression through a p300-dependent mechanism. J Biol Chem 2002; 277:24390-24398.
118. LaVoie HA. The role of GATA in mammalian reproduction. Exp Biol Med (Maywood) 2003; 228:1282-1290.
119. Viger RS, Taniguchi H, Robert NM et al. Role of the GATA family of transcription factors in andrology. J Androl 2004; 25:441-452.
120. Yomogida K, Ohtani H, Harigae H et al. Developmental stage- and spermatogenic cycle-specific expression of transcription factor GATA-1 in mouse Sertoli cells. Development 1994; 120:1759-1766.
121. Viger RS, Mertineit C, Trasler JM et al. Transcription factor GATA-4 is expressed in a sexually dimorphic pattern during mouse gonadal development and is a potent activator of the Mullerian inhibiting substance promoter. Development 1998; 125:2665-2675.
122. Ketola I, Rahman N, Toppari J et al. Expression and regulation of transcription factors GATA-4 and GATA-6 in developing mouse testis. Endocrinology 1999; 140:1470-1480.
123. Ketola I, Anttonen M, Vaskivuo T et al. Developmental expression and spermatogenic stage specificity of transcription factors GATA-1 and GATA-4 and their cofactors FOG-1 and FOG-2 in the mouse testis. Eur J Endocrinol 2002; 147:397-406.
124. Tremblay JJ, Viger RS. GATA factors differentially activate multiple gonadal promoters through conserved GATA regulatory elements. Endocrinology 2001; 142:977-986.
125. Baldwin Jr AS. The NF-κB and IκB proteins: New discoveries and insights. Annu Rev Immunol 1996; 14:649-683.
126. Ghosh S, May MJ, Kopp EB. NF-κB and Rel proteins: Evolutionarily conserved mediators of immune responses. Annu Rev Immunol 1998; 16:225-260.
127. Delfino F, Walker WH. Stage-specific nuclear expression of NF-κB in mammalian testis. Mol Endocrinol 1998; 12:1696-1707.
128. Delfino FJ, Walker WH. NF-κB induces cAMP-response element-binding protein gene transcription in Sertoli cells. J Biol Chem 1999; 274:35607-35613.
129. Delfino FJ, Boustead JN, Fix C et al. NF-κB and TNF-α stimulate androgen receptor expression in Sertoli cells. Mol Cell Endocrinol 2003; 201:1-12.
130. Hong CY, Park JH, Seo KH et al. Expression of MIS in the testis is downregulated by tumor necrosis factor α through the negative regulation of SF-1 transactivation by NF-κB. Mol Cell Biol 2003; 23:6000-6012.
131. Kohno K, Izumi H, Uchiumi T et al. The pleiotropic functions of the Y-box-binding protein, YB-1. Bioessays 2003; 25:691-698.
132. Ladomery M, Sommerville J. A role for Y-box proteins in cell proliferation. Bioessays 1995; 17:9-11.
133. Didier DK, Schiffenbauer J, Woulfe SL et al. Characterization of the cDNA encoding a protein binding to the major histocompatibility complex class II Y box. Proc Natl Acad Sci USA 1988; 85:7322-7326.
134. Tafuri SR, Familari M, Wolffe AP. A mouse Y box protein, MSY1, is associated with paternal mRNA in spermatocytes. J Biol Chem 1993; 268:12213-12220.
135. Kwon YK, Murray MT, Hecht NB. Proteins homologous to the Xenopus germ cell-specific RNA-binding proteins p54/p56 are temporally expressed in mouse male germ cells. Dev Biol 1993; 158:99-100.
136. Oko R, Korley R, Murray MT et al. Germ cell-specific DNA and RNA binding proteins p48/52 are expressed at specific stages of male germ cell development and are present in the chromatoid body. Mol Reprod Dev 1996; 44:1-13.
137. Yiu GK, Hecht NB. Novel testis-specific protein-DNA interactions activate transcription of the mouse protamine 2 gene during spermatogenesis. J Biol Chem 1997; 272:26926-26933.
138. Yiu GK, Murray MT, Hecht NB. Deoxyribonucleic acid-protein interactions associated with transcriptional initiation of the mouse testis-specific cytochrome c gene. Biol Reprod 1997; 56:1439-1449.
139. Yang J, Medvedev S, Yu J et al. Absence of the DNA-/RNA-binding protein MSY2 results in male and female infertility. Proc Natl Acad Sci USA 2005; 102:5755-5760.
140. Giorgini F, Davies HG, Braun RE. Translational repression by MSY4 inhibits spermatid differentiation in mice. Development 2002; 129:3669-3679.

141. Rouault JP, Prevot D, Berthet C et al. Interaction of BTG1 and p53-regulated BTG2 gene products with mCaf1, the murine homolog of a component of the yeast CCR4 transcriptional regulatory complex. J Biol Chem 1998; 273:22563-22569.
142. Draper MP, Salvadore C, Denis CL. Identification of a mouse protein whose homolog in Saccharomyces cerevisiae is a component of the CCR4 transcriptional regulatory complex. Mol Cell Biol 1995; 15:3487-3495.
143. Nakamura T, Yao R, Ogawa T et al. Oligo-astheno-teratozoospermia in mice lacking Cnot7, a regulator of retinoid X receptor β. Nat Genet 2004; 36:528-533.
144. Berthet C, Morera AM, Asensio MJ et al. CCR4-associated factor CAF1 is an essential factor for spermatogenesis. Mol Cell Biol 2004; 24:5808-5820.
145. Hochheimer A, Tjian R. Diversified transcription initiation complexes expand promoter selectivity and tissue-specific gene expression. Genes Dev 2003; 17:1309-1320.
146. Kimmins S, Kotaja N, Davidson I et al. Testis-specific transcription mechanisms promoting male germ-cell differentiation. Reproduction 2004; 128:5-12.
147. Monaco L, Kotaja N, Fienga G et al. Specialized rules of gene transcription in male germ cells: The CREM paradigm. Int J Androl 2004; 27:322-327.
148. Jaenisch R, Bird A. Epigenetic regulation of gene expression: How the genome integrates intrinsic and environmental signals. Nat Genet 2003; 33:245-254.
149. Singal R, van Wert J, Bashambu M et al. Testis-specific histone H1t gene is hypermethylated in nongerminal cells in the mouse. Biol Reprod 2000; 63:1237-1244.
150. Grimes SR, Wilkerson DC, Noss KR et al. Transcriptional control of the testis-specific histone H1t gene. Gene 2003; 304:13-21.
151. Mruk DD, Cheng CY. Sertoli-Sertoli and Sertoli-germ cell interactions and their significance in germ cell movement in the seminiferous epithelium during spermatogenesis. Endocr Rev 2004; 25:747-806.
152. Lui WY, Lee WM. Regulation of junction dynamics in the testis—Transcriptional and post-translational regulations of cell junction proteins. Mol Cell Endocrinol 2006; 250:25-35.
153. Lui WY, Wong EWP, Guan Y et al. Dual transcriptional control of claudin-11 via an overlapping GATA/NF-Y motif: Positive regulation through the interaction of GATA, NF-YA and CREB and negative regulation through the interaction of Smad, HDAC1 and mSin3A. J Cell Physiol 2007, 211:638-648.
154. Ozaki-Kuroda K, Nakanishi H, Ohta H et al. Nectin couples cell-cell adhesion and the actin scaffold at heterotypic testicular junctions. Curr Biol 2002; 12:1145-1150.
155. Morita K, Sasaki H, Fujimoto K et al. Claudin-11/OSP-based tight junctions of myelin sheaths in brain and Sertoli cells in testis. J Cell Biol 1999; 145:579-588.
156. Chung AC, Katz D, Pereira FA et al. Loss of orphan receptor germ cell nuclear factor function results in ectopic development of the tail bud and novel posterior truncation. Mol Cell Biol 2001; 21:663-677.
157. Blendy JA, Kaestner KH, Weinbauer GF et al. Severe impairment of spermatogenesis in mice lacking the CREM gene. Nature 1996; 380:162-165.
158. Hummler E, Cole TJ, Blendy FA et al. Targeted mutation of the CREB gene: compensation within the CREB/ATF family of transcription factors. Proc Natl Acad Sci USA 1998; 95:4481-4486.
159. Rudolph D, Tafuri A, Gass P et al. Impaired fetal T cell development and perinatal lethality in mice lacking the cAMP response element binding protein. Proc Natl Acad Sci USA 1998; 95:4481-4486.
160. Pevny L, Simon MC, Robertson E et al. Erythoid differentiation in chimaeric mice blocked by a targeted mutation in the gene for transcription factor GATA-1. Nature 1991 349:257-260.
161. Narita N, Bielinska M, Wilson DB. Wild-type endoderm abrogates the ventral developmental defects associated with GATA-4 deficiency in the mouse. Dev Biol 1997; 189:270-274.
162. Koutsourakis M, Langeveld A, Patient R et al. The transcription factor GATA6 is essential for early extraembryonic development. Development 1999; 126:723-732.