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REVIEW

Unique Chromatin Remodeling and Transcriptional Regulation in Spermatogenesis

Paolo Sassone-Corsi

Most of our knowledge of transcriptional regulation comes from studies in somatic cells. However, increasing evidence reveals that gene regulation mechanisms are different in haploid germ cells. A number of highly specialized strategies operate during spermatogenesis. These include a unique chromatin reorganization program and the use of distinct promoter elements and specific transcription factors. Deciphering the rules governing transcriptional control during spermatogenesis will provide valuable insights of biomedical importance.

The developmental process of spermatogenesis relies on a number of distinct regulatory programs involving sophisticated hormonal control from the hypothalamic-pituitary axis (*1*). This review concentrates on recent advances about the unique rules governing postmeiotic transcription in male germ cells. One very special feature concerns the process of chromatin remodeling, which involves various steps that are unlike those in somatic cells (*2*). Many generally expressed genes use alternative promoters in male germ cells, and several genes have a homolog whose expression is specific for the male germ line. Transgenesis experiments have revealed that various cis-acting regulatory elements direct expression exclusively to the testis, demonstrating the presence of germ cell–specific factors (*2*, *3*–*5*).

Chromatin Dynamics

In somatic cells, specific chromatin remodeling events have been directly coupled to transcriptional activation and silencing (*6*–*8*). Are the same events operating in male germ cells? During spermatogenesis, the haploid genome undergoes extensive reorganization through meiosis and DNA compaction. Meiosis involves homologous chromosome pairing at synapsis and meiotic recombination (Fig. 1). After desynapsis and completion of meiosis, gene transcription increases, but then the haploid genome is compacted within the sperm head to a volume of about 5% of that of a somatic cell nucleus. This remarkable repackaging event is achieved by replacing histones with protamines (*9, 10*), arginine- and cysteine-rich proteins that organize the haploid male genome into a highly specialized, doughnut-shaped chromatinic structure that is fundamentally different from the classical nucleosomal architecture (*9, 10*). The reason for the histone-protamine transition is probably related to the high compaction potential of nucleoprotamines and the requirement for a unique chromatin architecture that would enable a specific transcription schedule after fertilization.

In mammals, histones are not replaced directly by protamines (Fig. 1). Transition proteins (TP1 and TP2) are small, basic nuclear proteins that appear when histones are displaced and chromatin condensation initiates. Targeted mutation of each transition protein suggests a redundant role for the transition proteins (*11, 12*). Both TP1- and TP2-mutant mice are fertile and display only minor spermiogenesis abnormalities, indicating that histone replacement and chromatin compaction are transition protein–independent processes. Indeed, precocious chromatin con-

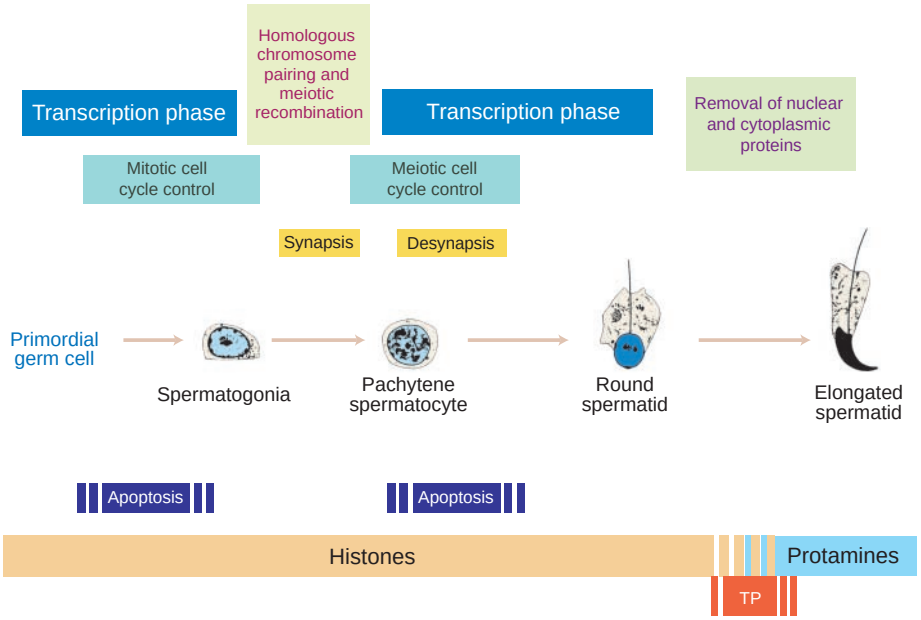

Fig. 1. Spermatogenesis is a cyclic developmental process by which spermatogonia cells generate the mature spermatozoon. These events are characterized by important modifications in chromatin organization, basically during two periods, meiosis—which includes the synapsis and desynapsis of the chromosomes—and the histone-protamine transition. Postmeiotically, a powerful wave of transcription occurs in haploid cells, which is governed by highly specialized molecular mechanisms. Specific genes operate at distinct steps of the spermatogenic process.

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densation occurs in transgenic mice with premature protamine-1 translation (*13*).

The dynamic displacement of transition proteins is controlled by phosphorylation-dephosphorylation events, including direct phosphorylation of TP2 by protein kinase A, which greatly reduces TP2's DNA condensation property (*14*). Thus, activation of specific signaling pathways plays a critical role in the timing and accuracy of the histone-to-protamine transition. The ubiquitin system, which also is signaling-regulated, appears implicated in the histone-protamine conversion by controlling the half-life, stabilization, refolding, and translocation of these proteins. Indeed, ablation of the HR6B gene, which encodes a ubiquitin-conjugating DNA repair enzyme, results in impaired spermatogenesis and in structural alteration of postmeiotic chromatin (*15*). The ubiquitin system is highly active postmeiotically, regulating apoptosis and the massive breakdown of cellular proteins at that time.

Protamines are also modified posttranslationally, but the physiological significance of these modifications is mostly unknown. At least, phosphorylation has been shown to be essential. Mutation of the calmodulin-dependent protein kinase *Camk4*, which phosphorylates protamine-2, results in defective spermiogenesis and male sterility (*16*). Although *Camk4* is expressed in various tissues, no other phenotypic effect is observed.

The classical view is that replacement of histones by protamines results in silencing of transcription. Silencing has been shown to be coupled to heterochromatin condensation and recognition of methylated residues on histone tails by the protein HP1 (heterochromatin protein-1) (*6*–*8*). During chromatin reorganization in spermiogenesis, HP1 is localized in the heterochromatic chromocenter of spermatids, a structure believed to function as an organizer of higher order chromatin fibers. Because spermatid nuclei are nearly devoid of histones, the target of HP1 may be methylated protamines or residual histones that could contribute to chromocenter architecture. One histone-like protein that remains associated with the chromatin during the transition to protamines is the centromeric-specific H3-like protein CENP-A (*17*), which thereby behaves as a heritable centromeric molecule. CENP-A also becomes phosphorylated, although both functional significance and modality of this modification are yet undetermined.

Transcription Mechanisms

A significant number of gene promoters display an activity highly restricted to male germ cells (*2*). In these cells, there is a balance of common regulators and germ cell–specific factors (*3*–*5*). After meiosis, the beginning of spermiogenesis is characterized by a massive wave of transcriptional activity, which results in the activation of a number of essential postmeiotic genes in early haploid cells. To insure this efficient and timely transcription, various general transcription factors are differentially regulated in germ cells. TBP (TATA-binding protein), TFIIB, and RNA polymerase II accumulate in early haploid germ cells in much higher amounts than in any somatic cell. For example, although adult spleen and liver cells contain 0.7 and 2.3 molecules of TBP mRNA per haploid genome-equivalent, respectively, adult testes contain 80 to 200 molecules of TBP transcript per haploid genome equivalent (*18*).

Germ cells differ from somatic cells not only for the unusual expression levels of these general factors, but also because of the presence of testis-specific isoforms. TFIIA is a good example. TFIIA is an RNA polymerase II–associated factor that stimulates transcription by stabilizing TBP association with promoter DNA and by facilitating activator-dependent conformational changes in the preinitiation complex. A TFIIA testis-specific isoform, ALF or TFIIAτ has been identified (*19, 20*). The expression pattern of ALF indicates a specialized function in spermiogenesis. Although TFIIAτ or ALF is able to functionally substitute for TFIIA in a transcription assay, it seems to have unique association properties with some activators, probably underscoring its testis-specific function (*19, 20*).

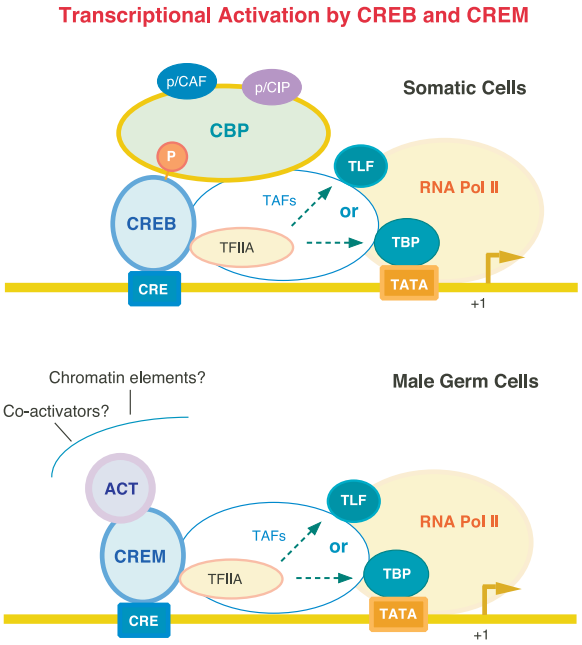

Fig. 2. CREB- and CREM-mediated transcription can take place after interaction with different coactivators. (Top) Schematic representation of the classical view by which CREB elicits its function in somatic cells. A key event is CREB phosphorylation, which promotes the recruiting of CBP, a large coactivator with HAT (histone acetyltransferase) activity. In male germ cells (bottom), CREM is not phosphorylated, and activation is elicited by association with ACT, a testis-specific coactivator that has no HAT activity. In both models, activation involves contacts with factors of the general transcription machinery, such as TBP (TATA-binding protein). TLF (TBP-like factor) may operate on TATA-less promoters, and as TBP does, TLF associates with TFIIA. Ablation of CREM and TLF genes in the mouse germ line causes severe spermiogenesis deficiency and increased germ cell apoptosis.

The RNA polymerase II–associated complex TFIID is composed of TBP and various TBP-associated proteins (TAF_{II}s). Some of the TAFs function in transcriptional regulation, chromatin modification, and DNA repair. Whereas TBP and TAF_{II}s are ubiquitous, testis-specific TBP and TAF_{II} paralogs have been identified. For example, a testis-specific paralog of $TAF_{II}80$ exists in *Drosophila* that has been shown to be essential for spermatogenesis (*21*). In mammals, a testis-specific isoform of $TAF_{II}55$ has been found, named $TAF_{II}Q$, whose function is still unclear (*22*). It is interesting that $TAF_{II}Q$ is encoded by the X chromosome, as well as $TAF_{II}250$, an essential factor for RNA polymerase II transcription. Differently from the autosomes, the X and Y chromosomes are transcriptionally silenced in mammalian spermatocytes, because they are condensed in heterochromatin (*23, 24*). The sexual chromosomes are segregated into a special nuclear compartment, the "sex vesicle," where RNA polymerase II is absent (*25*). Thus, in order to ensure efficient transcription, alternative isoforms of these critical transcription factors are likely synthesized from the autosomes in haploid cells.

Transcription of TATA-less promoters in somatic cells is thought to utilize TBP-like factors that have no direct TATA binding function. As several testis-specific promoters do not contain a canonical TATA element, the role of TBP-like factors during spermatogenesis is relevant. Notable is the case of the TBP-related protein TRF2 or TLF, which displays a highly specific developmental pattern of expression, in contrast to TBP, which is ubiquitously present in all stages of spermatogenesis. TLF plays a specialized role in mammalian spermatogenesis (*26, 27*), as revealed by the mutation of the gene that causes a complete arrest of late spermiogenesis and increased germ cell apoptosis (*26, 27*). An unexpected feature of the TLF-deficient mice is that early spermatids display a fragmentation of the chromocenter and aberrant HP1 distribution (*28*), indicating that this transcription factor participates also in the organization of the centromeric heterochromatin.

The role of TLF in mammalian spermatogenesis contrasts to its function in *Caenorhabditis elegans*, *Xenopus*, and zebrafish (*29*–*31*), where TLF appears to be critical in early embryogenesis—possibly acting as a surrogate of TBP at specific promoters whose expression is required at the onset of zygotic transcription in the developing embryo.

Specificity of Activation

The molecular and functional connections between the general transcription machinery and cell-specific activators have been extensively studied in somatic cells. Many genes activated postmeiotically contain CREs (cAMP-responsive elements), which recruit members of the CREB family of transcription factors (*32*).

In somatic cells, CREB binds to CREs and activates transcription when it becomes phosphorylated. The event of phosphorylation triggers the recruiting of a large coactivator, CBP (CREB-binding protein). CBP has a dual function. It contacts other elements of the transcription machinery and acetylates histones, and thus possibly contributes to chromatin decondensation events that precede transcription (*33*).

CREB is poorly expressed in testis. Instead, another member of the CREB family, CREM, is present at very high levels. In germ cells, CREM interacts with TFIIA and selected TAFs of the TFIID complex. CREM was thought to play a critical role during the postmeiotic transcriptional phase. Indeed, CREM-null mice display a complete block of the differentiation program at the first step of spermiogenesis (*32*). Thus, CREM appears to directly influence the fate of male germ cells. As TLF interacts with TFIIA, which interacts with CREM, a germ cell–specific complex appears to operate (Fig. 2). It is noteworthy that mice lacking either CREM or TLF display phenotypic similarities, including an important increase in germ cells apoptosis.

The complex including CREM has additional unique features. CREM does not seem to be phosphorylated in germ cells, and thus the classical signaling-dependent mechanism that uses CBP is not operating. Instead, CREM is activated by a tissue-specific coactivator, the LIM-only protein ACT, whose expression is exquisitely restricted to male germ cells (*34*). The study of the mechanism by which ACT operates is likely to reveal some important features of gene regulation in male germ cells.

Conclusion

The developmental process of spermatogenesis is governed by a unique genetic and molecular program. A fine-tuning of the regulatory mechanisms devoted to the differentiation of male germ cells is essential, because errors at any level could have dramatic consequences for the maintenance of the species. It is essential that we pursue a deeper understanding of the molecular processes regulating gene expression during spermatogenesis, as it will be highly valuable for biomedical and therapeutic work.

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REVIEW

Intercellular Communication in the Mammalian Ovary: Oocytes Carry the Conversation

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The production of functional female gametes is essential for the propagation of all vertebrate species. The growth of oocytes within ovarian follicles and their development to mature eggs have fascinated biologists for centuries, and scientists have long realized the importance of the ovarian follicle's somatic cells in nurturing oogenesis and delivering the oocyte to the oviduct by ovulation. Recent studies have revealed key roles of the oocyte in folliculogenesis and established that bidirectional communication between the oocyte and companion somatic cells is essential for development of an egg competent to undergo fertilization and embryogenesis. The challenge for the future is to identify the factors that participate in this communication and their mechanisms of action.

Germ cells are uniquely specialized to transmit the genome to succeeding generations. In animal species, sexual reproduction requires meiotic division to produce haploid gametes (i.e., eggs and spermatozoa), which upon fertilization give rise to the totipotent embryo. In both sexes, interactions between the developing gametes and neighboring somatic cells are crucial for fertility (*1*). The importance of this communication in spermatogenesis is underscored by clinical cases of male infertility, transgenic mouse models, and xenogeneic germ cell transplantation experiments (*1, 2*). Similarly, in females, complex intercellular dialogs have evolved to regulate oogenesis in species as wide-ranging as fruit flies (*3*) and mice.

In the mammalian perinatal ovary, oocytes arrested in the diplotene stage of meiosis I become surrounded by a single, squamous layer of somatic cells to form a finite population of nongrowing primordial follicles (*4*) (Fig. 1). Primary follicles are recruited from the primordial pool as oocytes grow and the surrounding somatic cells (called granulosa cells) become cuboidal and proliferative. This transition is associated with a