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Regulation of gene expression during spermatogenesis

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Spermatogenesis occurs in successive mitotic, meiotic and post-meiotic phases and genes expressed during this process encode proteins necessary for processes specific to the different phases of germ cell development. Some genes encode proteins with essential roles in structures or functions specific to spermatogenic cells, are expressed in developmentally regulated patterns and are transcribed only in, or produce mRNAs unique to, spermatogenic cells. They are referred to as chauvinist genes, because male germ cells favor their expression with such strong prejudice. The expression of these genes is influenced by extrinsic cues, but is determined primarily by the intrinsic genetic program of spermatogenic cells. These processes are subject to transcriptional, translational and post-translational regulation. However, many aspects of the mechanisms regulating gene expression in spermatogenic cells remain to be determined.

Key words: gene expression / spermatogenesis / testis / transcription / translation

The process of spermatogenesis

SPERMATOGENESIS INVOLVES a number of unique processes, including meiosis, haploid gene expression, formation of the acrosome and the flagellum, removal of histones from the chromatin and their replacement with protamines, and nuclear condensation. However, it also has features of a typical differentiating tissue, including a self-renewing stem cell population, a series of cell divisions closely associated with stepwise developmental processes and progression from morphologically undifferentiated to highly differentiated cells. Genes are expressed during spermatogenesis for proteins that serve these different purposes, as well as for maintaining the general house-keeping functions of all cells. Although all of these genes are important for germ cell development, the focus of this review is on the regulation of genes required specifically for the unique processes of spermatogenesis.

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Phases of spermatogenesis

Spermatogenesis occurs in successive mitotic, meiotic and post-meiotic phases (Figure 1). In the mouse, the mitotic phase lasts ~ 10 days, the meiotic phase ~ 11 days and the post-meiotic phase ~ 14 days. During the mitotic phase, the stem cells divide six times to form successively type A, intermediate and type B spermatogonia. The final division produces preleptotene spermatocytes, which begin the meiotic phase and undergo the last cell cycle S phase of spermatogenesis. During the meiotic phase (leptotene, zygotene, pachytene, diplotene and diakinesis stages), chromosomes condense, synaptonemal complexes form, and homologous chromosomes synapse and recombine to exchange genetic materials. This is followed by two meiotic divisions that occur in rapid succession without DNA replication to produce spermatids, the post-meiotic phase cells. Spermatids are then remodeled into spermatozoa by the processes of acrosome formation, nuclear condensation, flagellar development, and loss of the majority of the cytoplasm.

The testis contains spermatogenic cells in many different stages of development, complicating the study of germ cell gene expression. Without the development and refinement of procedures to isolate highly enriched populations of individual spermatogenic cell types,[1] many of the studies on which this review is based might not have occurred.

Chauvinist genes

Genes expressed during spermatogenesis encode

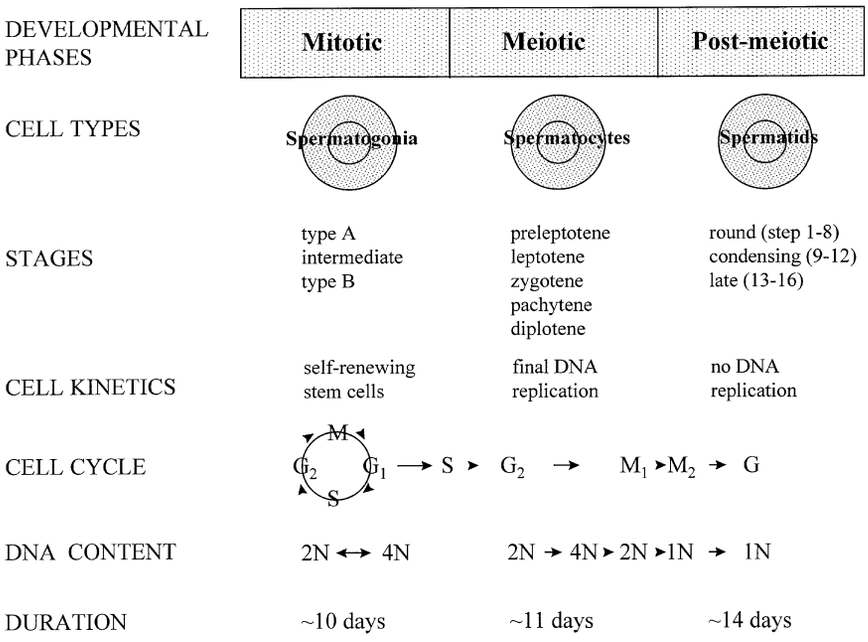

Figure 1. The developmental process of spermatogenesis includes mitotic, meiotic and post-meiotic phases. The stages for the cell types in each of the developmental phases are shown. The last chromosome replication during spermatogenesis occurs in preleptotene spermatocytes. Two cell divisions occur between the meiotic and the post-meiotic phases without chromosomal replication, resulting in spermatid nuclei that contain a haploid amount of DNA. The duration of each phase during spermatogenesis is shown for the mouse.

proteins necessary both for general activities and for processes specific to germ cells. From 15,000 to 20,000 different transcripts are present in a cell population[2] and only a few percent of the genes expressed in germ cells have been identified.[3] Some of these genes: (1) encode proteins with essential roles for structures or functions specific to spermatogenic cells; (2) are expressed in developmentally regulated patterns; and (3) are transcribed only in spermatogenic cells or produce mRNAs unique to spermatogenic cells. Genes with these characteristics have been named chauvinist genes, because male germ cells favor their expression with such strong prejudice.[4] Nearly 100 chauvinist genes have been reported that are expressed during the meiotic phase alone.[3]

Chauvinist genes can be grouped into three general categories: homologous genes, unique genes and genes expressing unique transcripts. Homologous genes are those expressed only in spermatogenic cells, but they are related closely to genes expressed in somatic cells and are often members of gene families. Some examples are the genes for histone H1t, heat shock protein HSP70-2 and lactate dehydrogenase C. Unique genes encode spermatogenic cell proteins with no significant overall similarity to those expressed in any other cells. Some of the proteins encoded by these genes are synaptonemal complex protein 1, transition proteins 1 and 2 (TSP1 and TSP2) and protamines 1 and 2. Although they may have motifs similar to those found in other proteins, they are not significantly homologous overall to any known proteins in other cell types. Genes can express spermatogenic cell-specific transcripts that differ from those produced in somatic cells because alternative transcription start sites, polyadenylation signals, or spermatogenic cell-specific exons are used in germ cells (Figure 2). Examples are spermatogenic cell-specific transcripts from genes for angiotensin converting enzyme, hexokinase 1 and cystic fibrosis transmembrane regulator.[3,5]

Regulation of gene expression

The precise pattern of germ cell development, the unique gene products required for spermatogenesis and the conserved nature of the process imply that the primary regulator of spermatogenesis is an intrin-

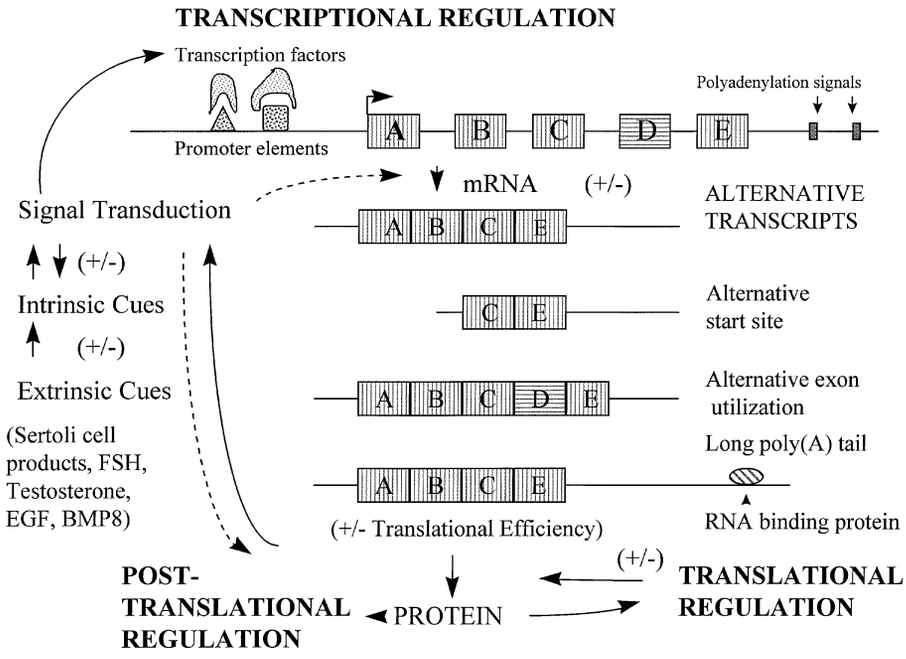

Figure 2. The regulation of gene expression during spermatogenesis occurs at the transcription, translation and post-translation levels. Transcription most likely is regulated by the intrinsic genetic program acting through signal transduction processes. The intrinsic program probably is influenced by extrinsic cues from the endocrine system and neighboring cells. Alternative transcripts are relatively common in spermatogenic cells and some of the ways that these transcripts are produced are indicated. Translational regulation results in mRNA storage and subsequent utilization for proteins needed after transcription ends in condensing spermatids. Post-translational regulation modulated through signal transduction mechanisms probably has a significant role in influencing these processes.

sic genetic program. This was shown dramatically when rat spermatogonial stem cells transplanted to the mouse testis were able to give rise to normal-appearing rat sperm[6] (see review by Brinster and Nagano, this volume). However, spermatogenesis is not an autonomous process. It is subject to secondary regulation by endocrine cues transmitted indirectly through surrounding somatic cells, by growth factors, are by short-loop paracrine and autocrine signals.[7] Precisely how these extrinsic cues influence germ cell development is not understood, but they may do so through their effects on converging signal transduction pathways that modulate the primary effects of the intrinsic genetic program (Figure 2). These pathways probably effect gene expression by causing post-translational modifications of transcription factors and other proteins that regulate alternative transcript processing mechanisms that occur in spermatogenic cells (Figure 2). Components of signal transduction pathways have been identified in spermatogenic cells,[3] but how the different pathways are activated and interact in these cells remains to be determined.

Extrinsic regulators of spermatogenesis

Testosterone and follicle stimulating hormone (FSH) usually are considered to be the primary hormonal regulators of spermatogenesis (see review by Zirkin, this volume). Testosterone is a steroid hormone produced by Leydig cells located between the seminiferous tubules of the testis. Testosterone acts through the androgen receptor, a ligand-activated transcription factor and a member of the nuclear receptor superfamily. Treatment of male rats with ethane dimethane sulfonate (EDS) destroys Leydig cells and disrupts spermatogenesis, but spermatogenesis can be maintained in these animals at nearly normal levels with exogenous testosterone. Testosterone affects spermatogenesis indirectly through androgen receptors in Sertoli and peritubular cells, but the nature of

the signals from these cells to germ cells are unknown. Testosterone is necessary for spermatogenesis, but it is unlikely to regulate directly specific events of gene expression in germ cells.

FSH is a peptide hormone produced in the anterior lobe of the pituitary. It binds to receptors on the cell surface to activate signal transduction processes. Such receptors are present on Sertoli cells but not germ cells. Suppression of hormone action with antibodies to FSH or receptor antagonists causes modest decreases in testis weight, germ cell numbers and sperm output in rats.[8,9] FSH influences Sertoli cell replication during fetal and neonatal life, but male mice with a targeted deletion of the FSH β-subunit gene are fertile.[10] These studies suggest that FSH supports spermatogenesis, but does not have a significant role in regulating gene expression in germ cells.

Other extrinsic factors have been identified that act through receptors to influence spermatogenesis. The testis of adult, vitamin A-deficient (VAD) rats contains few cells more advanced than type A spermatogonia, but treatment with retinol restores spermatogenesis.[11] The RAR α and RXR β retinoid receptors are members of the nuclear receptor superfamily and gene knock-out studies indicate that they are critical for spermatogenesis.[12,13] RAR α receptors are present in spermatogenic cells[14] while RXR β receptors are located in Sertoli cells.[13] A receptor related to those for retinoids, called GCNF or RTR, is present in round spermatids and developing oocytes. It is a member of the nuclear receptor superfamily, but the ligand and the role of this receptor in germ cells are unknown.[15,16]

The proteins or mRNAs for several growth factors and growth factor receptors have been detected in the testis,[17,18] but so far only two of these have been shown to have a role in regulating spermatogenesis. Epidermal growth factor (EGF) receptors are present on Sertoli, Leydig and peritubular cells. Removal of the submandibular gland, a major source of EGF, results in decreased sperm counts and lower fertility.[19,20] The EGF precursor is present also in pachytene spermatocytes and round spermatids, suggesting that EGF may influence spermatogenesis through both juxtacrine and endocrine routes.[21] Targeted inactivation of genes for several TGFβ superfamily members expressed in the testis (inhibin α, activin βB, Müllerian inhibiting substance, TGFβ1) does not cause primary defects in spermatogenesis, suggesting that they do not have a regulatory role in this process. An exception is the bone morphogenetic protein 8B (BPM8B), a TGFβ superfamily member that is expressed in spermatogenic cells. Inactivation of the *Bmp8b* gene leads to variable degrees of germ-cell deficiency and infertility.[22] Although these studies strongly suggest that EGF and BMP8B are local regulators of spermatogenesis, their effects on germ cells may be mediated through Sertoli cells. Since this appears to be true for many and perhaps all of the extrinsic factors that influence spermatogenesis, it is surprising that so little is known about intercellular communication between these cell types. However, ligands bearing the mannose 6-phosphate moiety are produced by Sertoli cells and receptors for these ligands are present on spermatogenic cells,[23] making these ligands and receptors attractive candidates for mediating Sertoli germ-cell communication.

Intrinsic regulators of spermatogenesis

The intrinsic regulation of gene expression in spermatogenesis occurs at three levels: transcription, translation and post-translation. Transcriptional regulation is the primary determinant of gene expression, as in other cell types. However, translational regulation probably has a greater role in germ cells than in other cell types, particularly for proteins synthesized during the post-meiotic phase. Post-translational regulation occurs through modifications of proteins, perhaps in response to extrinsic and intrinsic cues, to effect transcription and translation during spermatogenesis (Figure 2).

Regulation of transcription

Gene transcription is regulated by the binding of combinations of transcription factors to characteristic promoter motifs in the DNA sequence upstream of the protein coding region, thereby inducing changes in chromatin structure and modulating activity of the transcriptional machinery (Figure 2). Some transcription factors are ubiquitous and are involved in regulating many genes expressed in diverse tissues, whereas others are restricted in distribution and regulate tissue-specific gene expression. Unique transcription factors have been identified in spermatogenic cells (e.g. SPRM1, TAK-1, ZFY-2, OCT-2), but it remains to be determined which genes they regulate.[3,5,24] The best characterized are the cAMP-responsive element binding protein (CREB) and the closely related cAMP-responsive element modulator

(CREM) transcription factors that are activated by cyclic AMP/protein kinase A signaling pathways. Different CREB and CREM protein isoforms encoded by alternative transcripts suppress or activate transcription by binding to cAMP response elements (CRE) in gene promoters during specific phases of spermatogenesis (see review by Sassone-Corsi, this volume).

In vitro studies, by using band shift and foot-print assays, strongly suggest that unique promoter-binding proteins are present, that regulate the transcription of genes in spermatogenic cells. They bind to DNA promoter sequence motifs unlike those for known transcription factors and are present in nuclei of spermatogenic cells, but not of somatic cells.[3] Examples of those believed to activate gene expression during spermatogenesis include the histone H1t/TE element-binding proteins[25] and the phosphoglycerate kinase 2 promoter-binding proteins.[26] However, other promoter-binding proteins are present in nuclei of somatic cells, but not in spermatogenic cells. One such protein apparently binds to a negative regulatory element (NRE) of the *c-mos* promoter to repress expression in somatic tissues, but not in germ cells.[27] Sequences nearly identical to the NRE of *c-mos* are present in the promoter regions of other genes expressed only in spermatogenic cells (e.g. protamine 2, phophoglycerate kinase 2, cytochrome c_T and heat shock protein Hst70) suggesting this may be a common mechanism for suppressing their expression in somatic cells. These promoter-binding proteins have not been identified, but their restricted tissue distribution and association with unique promoter motifs, suggests that they are transcription factors involved in regulating spermatogenic cell-specific gene expression.

Regulation of translation

The nucleus condenses in step 9–12 spermatids of the mouse as the histones are shed from the chromatin and are replaced by TSP1 and TSP2, and then by the protamines. This results in cessation of transcription several days before many of the proteins required for assembling the spermatozoon are synthesized. Germ cells accommodate for this loss of transcriptional ability by synthesizing and storing mRNAs in an inactive form and then activating them when the proteins are needed. This delay appears to be accomplished by mRNA-binding proteins (Figure 2) that associate with transcripts and prevent their utilization, and/or by changes in mRNA translational efficiency.[28]

It has been shown that RNA-binding proteins can interact with specific sequence elements in the mRNA to suppress translation in spermatids (see review by Braun, this volume). Studies in transgenic mice determined that sequences in the last 62 nucleotides of the 3′ untranslated region (UTR) regulates translation of protamine 1 mRNA in spermatids.[29] It was found subsequently that a 40-kDa protamine 1 RNA-binding protein (PRBP) that binds to this sequence is present in spermatids when the mRNA is repressed translationally.[30] Other studies demonstrated that an 18-kDa phosphoprotein present in testis and brain extracts binds to YH sequence elements in the 3′ UTR of protamine 2 mRNA and represses its translation *in vitro*.[31] In addition, 48- and 50-kDa proteins bind to a conserved 20- to 22-bp element in the 3′ UTR of protamine 1 and 2 mRNAs.[32] These results suggest that either multiple mRNA binding proteins interact to repress mRNA transcription, or that some of these proteins have functions other than translational control, such as effecting mRNA stability and localization.[28]

Translational activity is influenced by the rate of initiation, which is reduced when mRNAs are not associated with ribosomes or if ribosomes are spaced far apart.[28] Although a high proportion of mRNAs in spermatogonia or early spermatocytes are translated efficiently, some of those in later spermatocytes and spermatids show a low rate of initiation or a temporally regulated change from low to high efficiency. The stored mRNAs in spermatids usually have poly[A^+] tails greater than 150 nucleotides in length and are not associated with ribosomes. They appear to undergo shortening of the poly[A^+] tract to approx. 30 nucleotides coincident with becoming associated with polysomes and translationally active.[33] However, the mechanisms and the specific time of poly[A^+] shortening and translational activation remain unclear, and there appear to be substantial differences between these characteristics for various spermatogenic cell transcripts.[28] It also is unclear whether transcript shortening is a cause or an effect of translational activation of stored mRNAs.

Alternative transcripts

Another way that gene expression is regulated during spermatogenesis is by the generation of alternative transcripts that encode different isoforms of proteins (Figure 2). Although alternative transcripts are not

unique to spermatogenic cells, they are relatively common in this cell type and often are produced in a specific phase of spermatogenesis.[3,5,34–36] Alternative transcripts can be generated in spermatogenic cells by utilization of different promoters that activate unique transcription start sites, either up-stream of the usual start site, as occurs for cytosolic aspartate aminotransferase,[37] or down-stream of the usual start site, as occurs for angiotensin-converting enzyme.[38] They may also be produced by utilization of alternative exons, as occurs for hexokinase 1,[39] or of alternative poly-adenlyation signals, as for β1,4-galactosyltransferase.[40] However, the mechanisms responsible for producing most alternative transcripts in spermatogenic cells are not known. Because several genes are expressed only in spermatogenic cells that are members of transcriptional machinery or RNA processing gene families,[3] there may be significant differences in the ways that transcripts are processed in spermatogenic cells, compared to other cells.

Patterns of gene expression during spermatogenesis

The patterns of gene expression in spermatogenic cells are likely to be the result of multiple processes (Figures 1 and 2). Because of the complexity and uniqueness of the process, it is not surprising that a large number of chauvinist genes are expressed during spermatogenesis. However, there are two particularly notable features of chauvinist gene expression that may be important for gametogenesis and for ensuring the integrity and effectiveness of genetic processes affecting evolution. First, many of the genes are regulated developmentally. These genes often encode proteins for unique structural components of spermatogenic cells, such as the synaptonemal complex, acrosome and flagellum, and for unique functional processes, such as meiotic recombination and transcriptional regulation. They also may encode unique components of signal transduction pathways, unique proteins that chaperone other proteins and assist them during their folding and assembly into functional complexes, and unique meiotic cell cycle components[3] (see review by Cobb and Handel, this volume). Second, genes expressed in somatic tissues are often down-regulated in germ cells, whereas chauvinist genes that encode highly similar proteins with apparently comparable functions are up-regulated.[4] These features probably are important for gametogenesis, and for the integrity of genetic processes that effect survival and evolution of the species.

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