Review

Sexual selection, genetic conflict, selfish genes, and the atypical patterns of gene expression in spermatogenic cells

Kenneth C. Kleene*

Department of Biology, University of Massachusetts Boston, Boston, MA 02125-3393, United States

Received for publication 12 April 2004, revised 23 June 2004, accepted 3 September 2004

Abstract

This review proposes that the peculiar patterns of gene expression in spermatogenic cells are the consequence of powerful evolutionary forces known as sexual selection. Sexual selection is generally characterized by intense competition of males for females, an enormous variety of the strategies to maximize male reproductive success, exaggerated male traits at all levels of biological organization, co-evolution of sexual traits in males and females, and conflict between the sexual advantage of the male trait and the reproductive fitness of females and the individual fitness of both sexes. In addition, spermatogenesis is afflicted by selfish genes that promote their transmission to progeny while causing deleterious effects. Sexual selection, selfish genes, and genetic conflict provide compelling explanations for many atypical features of gene expression in spermatogenic cells including the gross overexpression of certain mRNAs, transcripts encoding truncated proteins that cannot carry out basic functions of the proteins encoded by the same genes in somatic cells, the large number of gene families containing paralogous genes encoding spermatogenic cell-specific isoforms, the large number of testis-cancer-associated genes that are expressed only in spermatogenic cells and malignant cells, and the overbearing role of Sertoli cells in regulating the number and quality of spermatozoa.

© 2004 Elsevier Inc. All rights reserved.

Keywords: Spermatogenesis; Gene expression; Sexual selection; Selfish genes; Genetic conflict; Transcriptional promiscuity; Cancer-testis associated genes; Translational regulation

Introduction

Spermatozoa are produced by spermatogenesis, a complex process involving cell proliferation, cell differentiation, meiosis, and powerful selective pressures on male reproductive success. Spermatogenesis is divided into three phases, each of which contains a distinct cell type (Russell et al., 1990). Spermatogonia, mitotically dividing diploid cells, generate large numbers of cells that eventually become spermatozoa. Some spermatogonia withdraw from the cell cycle and enter the 15-day meiotic phase, cells known as spermatocytes. The meiotic divisions produce haploid cells, spermatids, which undergo a 2-week period of differentiation, spermiogenesis. Early haploid cells, round spermatids, are transcriptionally active, while late haploid cells, elongated spermatids, are transcriptionally inert due to changes in chromatin structure (Kierszenbaum and Tres, 1975). The differentiation of spermatogenic cells involves profound changes in the proteome, and the ultrastructure of the nucleus, flagellum, mitochondria, and Golgi (reviewed in Eddy and O’Brien, 1998). All stages of spermatogenic cells are intimately associated with Sertoli cells, a somatic cell type that regulates the development, number, and quality of spermatogenic cells (Eddy, 2002).

The patterns of gene expression in meiotic and haploid spermatogenic cells in mammals differ profoundly from those in somatic cells. Analyses of expressed sequence tags and microarrays indicate that an unusually diverse set of mRNAs is expressed in mouse, human, and Drosophila testes and that a large number of these mRNAs are expressed only in spermatogenic cells (Andrews et al., 2000; Kerr et al., 1994; Hoog, 1991; Pawlak et al., 1999;
Yuan et al., 1995). It is not surprising that spermatogenic cells express a unique set of genes, because specialized cells are defined by their cell-type-specific proteins. The unusual feature of spermatogenesis is that many genes that are expressed in both spermatogenic and somatic cells produce transcripts that differ in structure due to alternative promoters, alternative splicing, and upstream polyadenylation sites (reviewed in Kleene, 2001; Venables, 2002; Walker et al., 1999). Spermatogenesis is also unusual because many gene families include paralogs that are expressed in somatic cells, and paralogs that are expressed solely in spermatogenic cells (Eddy and O’Brien, 1998).

Gene expression in spermatogenic cells exhibits several other peculiar features. Many mRNAs are expressed at grossly higher levels in meiotic and haploid spermatogenic cells than in somatic cells, and some spermatogenic cell-specific transcripts encode truncated proteins that lack domains essential for the functions of the proteins encoded by the same genes in somatic cells (reviewed in Kleene, 2001). Furthermore, all mRNA species are at least partially translationally repressed, but the repression often does not serve the developmental function of directing the synthesis of proteins to a specific stage, because many mRNA species undergo no developmental changes in translational activity (Kleene, 1996, 2001). In addition, some relatively abundant mRNAs in spermatogenic cells exhibit little or no translationally active polysomal mRNA (Kleene, 2001).

The reasons why the patterns of gene expression in spermatogenic cells differ so greatly from those in somatic cells are poorly understood. The atypical patterns of expression of individual genes are usually attributed to important functions in spermatogenesis, without specifying what these functions might be or why atypical expression is so pervasive. The explanations that have been proposed include the complex differentiation of spermatocytes and haploid spermatids, and the widespread alterations in the patterns of gene expression at the transcriptional and post-transcriptional levels. It seems clear that powerful evolutionary forces are at work, because complex adaptations are necessary to create and compensate for the profound alterations in gene expression.

This review proposes that the unusual patterns of gene expression in spermatogenic cells are related to an evolutionary phenomenon known as sexual selection. The basic principles of sexual selection were understood by Darwin (1871), and sexual selection is discussed in a vast literature that is mentioned in introductory biology textbooks. Although the relevance of sexual selection to gene expression in spermatogenic cells is clear to evolutionary biologists (Meiklejohn et al., 2003), this topic has received negligible discussion by molecular biologists who study spermatogenesis.

Precopulatory sexual selection at the organismal and behavioral levels

When Darwin published The Descent of Man, and Selection in Relation to Sex in 1871, he recognized that sexual selection is a distinct form of natural selection. Natural selection acts on genetic variation to select individuals who survive to reproduce, while sexual selection acts on genetic variation in each sex to maximize the number of offspring bearing each parent’s genes. The traits that are influenced by sexual selection produce advantages in competition with members of the same sex and interactions with members of the opposite sex, but these traits frequently increase natural selection.

The basis of many aspects of sexual selection is that the males and females of most species have conflicting strategies for maximizing the number of offspring bearing their genes (Anderson, 1994; Birkhead, 2000). In many species, the contribution of the male to the survival of the offspring is negligible, restricted to a small quantity of semen. Males often maximize their reproductive success by inseminating the eggs of as many females as possible, and preventing other males from inseminating the eggs of females that they have inseminated. In contrast, the female is usually more selective to ensure that her offspring are of high quality and that her resources are sufficient to secure their survival. Since sexually mature females devote a large amount of resources to generating eggs (and sometimes to nurturing offspring), females are normally sexually receptive for a short period. Thus, sexually active males typically outnumber sexually receptive females, so the competition for mates is more intense in males than in females. These general principles have several consequences:

First, the evolution of exaggerated sexual traits is normally restricted to males, the more competitive sex. The traits that advance male reproductive success are extremely diverse, species-specific, and often extravagant: stags have antlers, male elephant seals gain a fighting advantage with bulk, various bird species have different kinds of feather displays, male bowerbirds build large and highly decorated nests to attract females, male dung flies wrestle with other males for the opportunity to copulate with females (reviewed in Birkhead, 2000).

The generalization that sexual traits are more developed in the more competitive sex is illustrated by phalaropes, a genus of shore birds in which the roles of males and females are reversed (Delehanty et al., 1998). Male phalaropes brood the eggs and rear the offspring without female assistance, so they are rarely sexually receptive. Thus, female phalaropes, the more competitive sex, are larger, more decorated, and sexually aggressive than the males.
Second, Darwin (1871) recognized that the characteristics that advance male reproductive success often increase the risk of natural selection. The primary function of the antlers of stag deer is fighting other males for the opportunity to mate with does, and the long, ornate feathers of the peacock are a display to make males more attractive to peahens. However, both characteristics decrease the fitness of individual males: producing feathers and antlers uses biosynthetic resources, spectacular feathers attract predators, and fighting weakens stags. The conflict between sexual selection and natural selection is illustrated graphically by the observation that male garden spiders die spontaneously, immediately after locking their genitals into the female’s genitals, a maneuver that blocks mating by other males (Foellmer and Fairbairn, 2003).

Third, the evolution of male characteristics that attract females would not have reproductive advantages if the females did not find the male traits appealing (Birkhead, 2000; Wiens, 2001). Thus, the evolution of male reproductive traits is accompanied by the co-evolution of female preference for those traits. Co-evolution can lead to exaggeration of male traits, because the expression of the male trait and the female preference for that trait evolve cooperatively. Greater expression is accompanied by greater preference, and the exaggeration of the traits spirals until the costs become too great and natural selection imposes limits.

Phylogenetic studies reveal that male sexually selected traits are lost frequently (Wiens, 2001). The factors that lead to a loss of male sexual traits include diminished female preference for a male trait, or strong selection against the trait because of conspicuousness to predators, limited resource availability, and aggressive competition with other males (Wiens, 2001). In some cases, diminished female preference can initiate an evolutionary arms race, in which the male sexual traits become increasingly exaggerated as the female preference for the male traits declines.

In summary, sexual selection at the organismal and behavioral levels is characterized by intense competition of males for females, enormous variety in the strategies to maximize reproductive success, co-evolution of sexual traits in males and females, conflict between the sexual advantage of the male trait and the individual fitness of males, and conflicts between the reproductive fitness of males and females. The following sections document that similar phenomena operate after copulation and at the cellular and molecular levels.

Post-copulatory sexual selection

The females of most species mate with more than one male, so sexual competition between males continues in the female reproductive tract after copulation, a phenomenon known as sperm competition (Birkhead, 2000). The following examples illustrate that post-copulatory sexual selection has fostered diverse strategies to enhance reproductive success and conflicts between the reproductive advantages and costs to the fitness of individuals.

Most terrestrial species use internal fertilization, thus the sperm of one male compete with the sperm of other males in the female reproductive tract (Birkhead, 2000). The males of many species generate seminal plugs that interfere with the subsequent mating of other males, and the penises of the males of some species are specialized for removing the seminal plugs or sperm derived from previous matings (Simmons and Siva-Jothy, 1998). In other species, the males first copulate with a female, then guard her to prevent her from mating with another male. The seminal fluid of Drosophila melanogaster males contains a complex set of proteins that enhances their reproductive success by increasing the female’s egg-laying rate, reducing her receptivity to subsequent mating with other males, promoting sperm storage, and killing the sperm of other males (Wolfner, 2002). In addition, the toxins that kill the sperm of other males are toxic to the female and reduce her life span.

The selective pressures on sperm are intense because the millions of sperm in the ejaculate of a single male and the sperm ejaculated by multiple males compete to be the first sperm to fertilize the egg (Parker, 1993, 1998). The highly polar head–tail organization of sperm in most vertebrates and invertebrates is ideally suited for motility and gamete interactions during fertilization (reviewed in Travis and Kopf, 2002). The head contains the haploid nuclear DNA, enzymes used in penetrating the egg membranes, and proteins involved in the attachment of sperm to the egg, while the tail contains the flagellum, which propels the sperm and the enzymes that provide energy for the flagellum. To enhance motility, the hydrodynamic resistance of the sperm of most animals is reduced by eliminating the cytoplasm and reducing the nuclear volume by packaging the chromosomal DNA with sperm-specific basic chromosomal structural proteins, protamines, or special histones (Allen et al., 1996; Poccia, 1986). Two glycolytic enzymes involved in energy production in mice have sperm-specific intracellular localization signals resulting in the deposition immediately adjacent to the flagellar axoneme. The localization signal of glyceraldehyde-3-phosphate dehydrogenase is encoded by a paralogous gene, while the localization signal of hexokinase I is contained in an amino terminal extension generated by a spermatogenic cell-specific transcription start site of the same gene that is expressed in somatic cells (Bunch et al., 1998; Travis et al., 1998).

Mammalian sperm have accessory structures surrounding the flagellar axoneme, outer dense fibers, and the fibrous sheath, which are thought to enhance the propulsive force of the flagellum in the viscous fluids of the female reproductive tract (Eddy et al., 2003). The fibrous sheath also functions as a scaffold for the localization of energy-producing enzymes and signal transduction factors (Eddy et al., 2003; Travis and Kopf, 2002).

Sperm specializations that enhance reproductive success are not limited to motility, sperm–egg adhesion, and sperm
number. Human sperm use chemotaxis, mediated by odorant receptors, to locate eggs (Spehr et al., 2003). Wood mice have evolved a complex specialization, sperm cooperation, in which hundreds of sperm are linked together in aggregates that have greater motility than individual sperm. These aggregates dissociate into individual sperm during the final approach to the egg (Moore et al., 2002). The water snail, *Viviparus ater*, produces nonfertilizing sperm containing a small fraction of the haploid genome, known as oligopyrene sperm, in addition to normal sperm (Oppliger et al., 2003). Oligopyrene sperm, particularly large oligopyrene sperm, confer an advantage to normal sperm in competitive matings. Sperm are also subject to a variety of selective pressures in the female reproductive tract such as resistance to toxicity, promotion of storage, and longevity of sperm motility (Birkhead, 2000). It is likely that there are intense selective pressures on signal transduction pathways involved in the acrosome reaction, hypermotility, chemotaxis, and sperm cooperation.

In a striking example of an extreme male trait, the flagella of *Drosophila bifurca* sperm are 5.8-cm long, ~40 times the length of the female’s body. The disadvantages of these long flagella are obvious; the flagella are knotted and useless in propulsion, and sperm number is drastically reduced (Karr and Pitnick, 1996; Pitnick et al., 1995). *D. melanogaster* also have giant sperm of a more modest size, ~2.2 mm (Miller and Pitnick, 2002). Sperm length is a determinant of mating success in *D. melanogaster*, and co-evolves with the size of a female organ that stores sperm, the seminal receptacle (Miller and Pitnick, 2002). Increases in sperm and seminal receptacle length can be selected experimentally in less than 10 generations. Thus, the advantage of sperm length is dictated by the size of the seminal receptacle. Similarly, by intensifying sperm competition in *Caenorhabditis elegans* with mutations that block self-fertilization of hermaphrodites, increases in the size of amoeboid sperm can be observed in only 15 generations (LaMunyon and Ward, 2002). It is important to note that both sets of experiments utilized outbred populations, so the rapid evolution of sperm size results from intense selective pressures on pre-existing polymorphisms rather than new mutations.

### Sexual selection at the genetic and molecular levels

The diversity of adaptations to sexual selection described above at the organismal and cellular levels reflects the expression and evolution of the genes underlying these traits. Therefore, genes that are involved in sexual reproduction are evolving rapidly, sometimes co-evolving in males and females, and sometimes in conflict between males and females, and between natural selection and reproductive advantages of the individual. The rates of evolution of some of the genes involved in reproduction are unusually rapid (reviewed in Swanson and Vacquier, 2002). Evolutionary biologists say that these genes are fixed in a selective sweep, which means that a single allele has spread rapidly throughout the population. Some of these rapidly evolving genes encode proteins that function in the attachment of sperm to eggs and penetrating the egg membranes during fertilization (Swanson and Vacquier, 2002). In some cases, complementary proteins that promote the binding of sperm and eggs are co-evolving rapidly under the influence of positive Darwinian selection for advantageous alleles that promote fertilization and advantages in competition with the sperm of other males. However, the reproductive isolation resulting from this co-evolution may create the ultimate risk, extinction.

As noted previously, the seminal fluid of *D. melanogaster* contains toxins that kill the sperm of other males and reduce the life span of females. Rice (1996) has shown that when female *D. melanogaster* are prevented from co-evolving with males, male adaptation leads to a reduction in female survivorship in only 30 generations. Conversely, when male and female *D. melanogaster* are forced to be monogamous, the conflict between male and female interests in reproduction is reduced, and male adaptation leads to an increased female survivorship in 47 generations (Rice and Holland, 1999).

A series of mutations and genetic rearrangements have created a multigene family encoding a sperm-specific dynein intermediate chain (*Sdic*) that was fixed in a selective sweep during the last 3 million years in *D. melanogaster* (Nurminsky et al., 1998). Spiess et al. (2003) have identified a similar gene family in mice: the SPEER family of 14 genes that is expressed in spermatocytes and spermatids with no detectable homologues in other species of mammals.

Meiklejohn et al. (2003) have documented using microarrays that mRNA levels in the male germ lines of various species of *Drosophila* are more variable than those in somatic tissues and the female germ line, implying that regulatory mechanisms controlling mRNA transcription and stability are evolving rapidly. It should be remembered here that increases in sperm length and size can be selected experimentally in tens of generations in outbred populations of *D. melanogaster* and *C. elegans* (LaMunyon and Ward, 2002; Miller and Pitnick, 2002).

### Selfish genes and genetic conflict

A class of genes, referred to commonly as selfish genes (SGs), or perhaps more appropriately as self-promoting genes, can effect an advantage in transmission to progeny relative to other genes in the genome even though SGs impair reproductive or individual fitness. Despite their costs, SGs can spread rapidly to fixation in the population (Hatcher, 2000; Hurst and Werren, 2001). SGs also create advantages for new mutations that counteract the deleterious effects of the SGs, or enhance the transmission advantage of...
other genes. The terms, genetic and intragenomic conflict, describes situations in which the selective advantage of one gene opposes the selective advantages of other genes. The effects of SGs differ from sexual selection because the selection targets competition between the sperm in the ejaculate of a single male instead of competition between sperm from multiple males. However, SGs resemble sexual selection by creating conflicts between the transmission advantage of the SG and the costs of diminished individual fitness and reproductive success (Hatcher, 2000; Hurst and Werren, 2001).

Many genetic systems in males and females exist in which SGs gain an advantage in the frequency of transmission to progeny by distorting the normal Mendelian 50:50 ratio of transmission of heterozygous alleles (Pennisi, 2003; Zollner et al., 2004). One of the best studied of these systems is a group of tightly linked genes on mouse chromosome 17, known as t-haplotypes. In heterozygous males, the t-haplotype is transmitted to progeny with frequencies that deviate markedly from normal Mendelian ratios, >90% t-haplotype and <10% wild type. The low transmission of wild-type haplotypes to progeny is caused by deleterious effects of the t-haplotypes on the motility of sperm from multiple males. However, SGs resemble sexual selection by creating conflicts between the transmission advantage of the SG and the costs of diminished individual fitness and reproductive success (Ardlie, 1998).

Mutations that distort transmission ratios are thought to be relatively common, but are rarely observed, because the distorting genes are fixed rapidly and their activities are masked by the rapid fixation of mutations that suppress the distortion of transmission ratios. In support of this idea, Tao et al. (2001) demonstrated that Drosophila mauritiana, which exhibits a normal 50:50 sex ratio, contains a cryptic distorter and suppressor system. The sex ratio of D. simulans is distorted markedly by the introduction of a small chromosomal segment from D. mauritiana in a homozygous state. This observation implies that D. mauritiana contains a sex ratio distorter that is inactivated by a suppressor in the D. mauritiana background, but is active in the D. simulans background that lacks this suppressor. However, D. simulans has its own suppressors of sex-ratio distorters, at least one on every autosome and the Y-chromosome (Cazemajor et al., 1997).

The conflicting evolutionary strategies of male and female mammals to maximize the number of offspring bearing the genes of each parent underlies genomic imprinting, a phenomenon in which the parental alleles in mammals are differentially expressed in offspring. The majority of imprinted genes are pairs of genes that have antagonistic effects on fetal growth (Tilghman, 1999). For example, the paternal allele of insulin-like growth factor 2 gene (IGF2), which stimulates fetal growth, is hypomethylated in the male germ line and active in offspring, while the maternal allele is hypermethylated in the female germ line and inactive in offspring. The opposite pattern of imprinting is observed for the insulin-like growth factor 2 receptor gene (IGF2R), which encodes a protein that degrades the insulin growth factor 2: the maternal allele is hypomethylated and active in offspring, while the paternal allele is hypermethylated and inactive in offspring. The patterns of imprinting of the IGF2 and IGF2R genes parallel a conflict between the reproductive strategies of the father and mother in maximizing the number of offspring bearing each parent’s genes (Moore and Haig, 1991). The IGF2 gene, which is active when it is derived from the father, promotes fetal growth, draining as many maternal resources as possible for the father’s offspring, even though this may jeopardize the ability of the mother to care for the fetuses of her present pregnancy, and her capacity for future pregnancies. The IGF2R gene, which is active when it is derived from the mother, limits the growth of the fetuses, which maximizes the number of her offspring by ensuring that she has sufficient resources to sustain all of her current fetuses and by preserving her health for future pregnancies. The antagonism between imprinted genes in males and females can produce a co-evolutionary arms race in which increased levels of IGF2 are counterbalanced by increased levels of IGF2R. Genomic imprinting also has costs because the imprinted loci are functionally hemizygous, thus eliminating the benefits of diploidy in masking recessive mutations.

Gene expression runs amok: sexual selection and the atypical patterns of gene expression in spermatogenic cells

Several examples of atypical patterns of gene expression in spermatogenic cells are described below, which exhibit the hallmarks of sexual selection.

Overexpression of the TATA-binding protein (TBP) mRNA

The gross overexpression of mRNAs is one of the distinctive features of the atypical patterns of gene expression in mammalian spermatogenic cells (Kleene, 2001). An extreme example is the mRNA encoding the transcription factor, TBP, which has been reported to be expressed at 50–1000-fold higher levels in round spermatids in rats and mice than in somatic tissues (Persengiev et al., 1996; Schmidt and Schibler, 1995). The mRNAs encoding poly(A) binding protein, polyadenylation cleavage stimulation factors CstF–64 and CPSF-160, and translation initiation factor eIF4E are also expressed at 50–100-fold higher levels in spermatogenic cells than in somatic cells (Dass et al., 2001; Kleene et al., 1994; Miyagi et al., 1995).

Of course, mRNAs might be grossly overexpressed because the corresponding protein is needed at much higher levels in spermatogenic cells than in somatic cells. A clear
example is phospholipid hydroperoxide glutathione peroxidase (PHGPx), which functions as a cytosolic enzyme that reduces oxidized phospholipids in somatic cells, and as an enzymatically inactive structural protein in the outer membranes of the sperm mitochondria (Ursini et al., 1999). The PHGPx mRNA is the most efficiently translated mRNA in meiotic and early haploid spermatogenic cells yet characterized, 75% loaded on polysomes (Cataldò et al., 1999; Kleene, unpublished). Evidently, large amounts of PHGPx protein are produced by relatively efficient translation of high levels of mRNA.

However, the levels of TBP in testis are elevated much less than the levels of Tbp mRNA compared with somatic tissues, only 50- to several-fold (Perletti et al., 1999; Persengiev et al., 1996; Schmidt and Schibler, 1995). This is at least partly due to strong translational repression of the Tbp mRNA (Persengiev et al., 1996; Schmidt and Schibler, 1997). Although one might expect that the high levels of TBP are correlated with a plethora of genes that use TATA-dependent promoters in spermatogenic cells, in reality relatively few promoters in meiotic and haploid spermatogenic cells use a TATA-box, notably the genes encoding the testis-specific member of the histone H1 family (H1t), the 27-kDa outer dense fiber protein, and all of the members of the protamine/transition protein gene family (Clare et al., 1997; Kleene et al., 1992; van der Hoorn and Tarnasky, 1992 and references therein). A large number of promoters in spermatogenic cells lack TATA boxes (Fitzgerald et al., 1992; McCarrey and Thomas, 1987; Means et al., 1991; Schmidt et al., 1997), and several genes use a TATA-dependent promoter in somatic cells and a TATA-independent promoter in spermatogenic cells (Garrity and Wold, 1990; Gu et al., 1994; Kilpatrick et al., 1990).

In theory, the extremely high levels of expression of the Tbp mRNA can be explained by two features of sexual selection that cause grossly exaggerated male traits at the cellular and organismal levels, female preference or a genetic arms race. Presumably, the high levels of Tbp mRNA evolved to enhance the expression of unidentified downstream genes that augment male reproductive success. For example, it is likely that the factors that promote transcription of the mRNAs encoding the proteins in the gigantic sperm tail of D. bifurca are grossly overexpressed (Pitnick et al., 1995). As pointed out by Schmidt (1996), the overexpression of TBP is also likely to have side effects by activating transcription of promoters containing elements that deviate from the TATA-consensus sequence resulting in the expression of inappropriate transcripts. The strong translational repression of the Tbp mRNA and the inactivation of a subset of TATA-promoters in spermatogenic cells may have selective advantages in neutralizing the deleterious effects of TBP overexpression. Since TATA-related factor 2 inhibits transcription of certain TATA-containing promoters, one advantage of this Tbp paralog may be in reducing the negative effects of overexpressing TBP (Moore et al., 1999).

The transcripts encoding a truncated protein, calsperrin

The Ca$^{2+}$/calmodulin-dependent kinase IV (CCDKIV) gene is expressed in the early stages of meiosis, and haploid spermatogenic as well as several somatic tissues (Means et al., 1997). Knockout of the CCDKIV gene results in male infertility and a block to spermatogenesis in the elongated spermatid stage in mice (Wu et al., 2000). CCDKIV phosphorylates protamine 2, which is important for chromatin remodeling in elongated spermatids. In addition, rats and mice express a second transcript of the CCDKIV gene using a promoter and transcription start site in intron 4. The resulting truncated protein lacks the kinase domain and is known as calsperrin (Means et al., 1991). The calsperrin mRNA is expressed at high levels in spermatogenic cells in rats and at low levels in mice (Wu et al., 2001), but BLAST searches using human CCDKIV intron 4 as a query detect no equivalent EST sequences in human testis (Kleene, unpublished). The knockout of the calsperrin transcript in mice has no phenotype (Wu et al., 2001).

A variety of explanations could account for the existence of the calsperrin mRNA. First, calsperrin may never have had a positive function in mammals, because its mRNA is the adventitious by-product of a transcription factor that is grossly overexpressed in spermatogenic cells. Second, calsperrin may have had an important function in the distant past, when very high levels of CCDKIV had important advantages in male reproductive success. For example, calsperrin might be a competitive inhibitor that suppresses the deleterious effects of high levels of CCDKIV by binding to a protein that is phosphorylated by CCDKIV. However, other suppressors of CCDKIV may have evolved subsequently, and the advantage of calsperrin is drastically reduced in extant mice. Third, calsperrin may have a function, but the phenotype of the knockout cannot be detected by cursory examination of laboratory animals. Perhaps, calsperrin confers a subtle reproductive advantage that would be evident only if the knockout mice were studied for hundreds of generations, or in natural populations of mice, because environmental stress and sperm competition are reduced in laboratory conditions. Fourth, the function of calsperrin may be dependent on interactions with other genes. For example, the knockouts of the transition protein 1, transition protein 2, and sperm–mitochondrial associated cysteine-rich protein genes decrease fertility on homozygous backgrounds, but do not affect fertility on mixed backgrounds (Adham et al., 2001; Nayernia et al., 2002; Yu et al., 2000).

The absence of the kinase domain in calsperrin exemplifies a number of mRNAs encoding severely truncated proteins in spermatogenic cells that cannot carry out the basic functions of the proteins encoded by the same genes in somatic cells such as c-kit, c-fer, vasopressin, and α-tubulin (Albanesi et al., 1996; Dobner et al., 1987; Fischman et al., 1990; Foo et al., 1991). In other cases, truncation of proteins may produce advantageous changes in...
functions. For example, the mRNA encoding the transcription factor sterol response element binding protein (SREBP) is alternatively spliced in meiotic and haploid spermatogenic cells generating a factor, SREBPg, which lacks the carboxy terminus domain containing a cytoplasmic localization signal that is necessary for regulation of transcriptional activity by sterols in somatic cells (Wang et al., 2002). This converts SREBP, which is inducible by sterols in somatic cells, into SREBPg, which is constitutively active in spermatogenic cells.

It has been suggested that transcription in spermatogenic cells is promiscuous or leaky, based on the predicted effects of overexpression of TBP and the possibility that the severely truncated proteins encoded by certain transcripts are nonfunctional (Ivell, 1992; Schmidt, 1996). Others have criticized this term because of difficulties in determining whether the truncated spermatogenic cell-specific transcripts are truly nonfunctional (Eddy and O’Brien, 1998; Hecht, 1998). However, it seems inevitable that alterations in the transcriptional apparatus that produce high levels of transcripts with strong advantages in male reproductive success will generate adventitious transcripts with negative or neutral effects on reproductive or individual fitness. Such adventitious transcripts clearly qualify as transcriptional promiscuity. In practice, rigorous experimental identification of promiscuous transcripts may be very difficult because it requires measurements of reproductive and individual fitness in natural populations.

Expression of genes that promote cell growth

The number of sperm in an ejaculate is an important factor in the competition for fertilization in species in which females mate with more than one male (Birkhead, 2000). In general, males in these species have larger testes relative to body size and produce many more sperm, while closely related species that remain monogamous for life have smaller testes and produce fewer sperm.

This line of reasoning leads to the prediction that genes that promote cell growth will exhibit atypical patterns of expression in spermatogenic cells. A number of mRNAs that promote cell growth are overexpressed in spermatogenic cells in mice such as the ribosomal protein L32 (Rpl32) (Kleene et al., 2003), ribosomal protein S16 (Kleene, unpublished), translation initiation factor Eif4e (Miyagi et al., 1995), ornithine decarboxylase (Alcivar et al., 1989), and protooncogene c-mos and c-abl mRNAs (Meijer et al., 1987; Propst et al., 1987; Zakeri et al., 1988). The Rpl32 mRNA is also transcribed from a transcription start site that eliminates a cis-element, a 5′ terminal oligo(cytosine) tract (TOP), that inactivates translation of ~100 mRNAs with functions in the activity of the transcriptional apparatus in nongrowing cells (Kleene et al., 2003; Meyuhas and Horstein, 2000). The absence of the 5′TOP of the Rpl32 mRNA would result in efficient translation in nongrowing cells. High mobility group proteins, HMGA1 and HMGA2, are expressed at high levels in rapidly dividing embryonic cells, malignant cells, and meiotic and haploid spermatogenic cells (Chieffi et al., 2002), and expression of histone H1.1 is restricted to rapidly proliferating somatic cells and spermatogenic cells (Franke et al., 1998).

LeGrand (2001) and Summers et al. (2002) have proposed that the selective pressures on sperm production could enhance the occurrence of selfish genes that transmit a propensity for cancer to progeny. It is therefore intriguing that there are striking similarities in the patterns of gene expression in spermatogenic and malignant cells. The mRNAs encoding many constituents of the translational apparatus, including ribosomal proteins, eIF4E, eIF4G, and PABPC1, are overexpressed in malignant cells (Dua et al., 2001). The growth-promoting activity of eIF4E is demonstrated by findings that overexpression induces malignant transformation of NIH-3T3 cells (Lazaris-Karatzas et al., 1990). Therefore, it is notable that the levels of Eif4e mRNA in spermatogenic cells are much higher than the levels in malignant cells (Miyagi et al., 1995). Perhaps, the most striking example of similarities in gene expression in malignant and spermatogenic cells is the 88 cancer-testis-associated genes that are expressed only in malignant cells and spermatagonia, spermatocytes, and/or spermatids (reviewed in Zendman et al., 2003). Cancer-testis-associated genes are likely SGs that evolved originally to enhance sperm-number, and are activated abnormally in malignant cells. Although there is no reason to believe that all genes that promote cell growth are expressed atypically, the 97 genes mentioned above suggest that this is a common phenomenon.

The regulation of growth of spermatogenic cells also exhibits signs of genetic conflict between advantages in large numbers of sperm, and potential costs such as draining metabolic resources and deleterious effects on progeny. The overexpression of the ornithine decarboxylase, c-mos, c-abl, Eif4e, Pabpc1, and Rpl32 mRNAs is accompanied by strong translational repression and/or by relatively low levels of the corresponding protein, presumably reflecting the actions of antagonistic genes that repress translation (Alcivar et al., 1989; Kleene et al., 1994, 2003; Meijer et al., 1987; Miyagi et al., 1995; Propst et al., 1987; Zakeri et al., 1988).

LeGrand (2001) has suggested an intriguing explanation for the importance of Sertoli cells in regulating spermatogenesis. The development of spermatogenic cells occurs in intimate association with Sertoli cells, which are absolutely necessary for spermatogenesis and eliminate excess and abnormal spermatogenic cells (Print and Loveland, 2000). Entrusting this function to a somatic cell type minimizes the possibility that SGs that inactivate apoptosis or deregulate cell proliferation will be transmitted to progeny where they may cause cancer. Presumably, the interactions of Sertoli cells and spermatogenic cells are in constant conflict between genetic alterations that create new SGs that enable
spermatogenic cells to escape surveillance by Sertoli cells, and the evolution of new surveillance mechanisms that enable Sertoli cells to detect and eliminate spermatogenic cells expressing deleterious SGs.

Selective pressures on the expression of genes on the X- and Y-chromosomes in spermatogenic cells

The X- and Y-chromosomes in mammalian males, the heterogametic sex, also favor novel patterns of gene expression in spermatogenic cells. The X-chromosome becomes condensed and transcriptionally inactive during meiosis in male mammals as a mechanism of preventing recombination between the X- and Y-chromosomes (McKee and Handel, 1993). The inactivation of the X-chromosome during male meiosis creates a strong selective advantage for mechanisms that replace the functions of genes on the X-chromosome. Many intron-containing genes on the X-chromosome have been copied into autosomal genes that are expressed only in meiotic and haploid spermatogenic cells (Emerson et al., 2004). These duplicate genes are often retrogenes, intronless-genes that are created by reverse transcribing mRNAs and inserting the DNA copy into nuclear DNA (Boer et al., 1987; Emerson et al., 2004; McCarrey and Thomas, 1987). The observation that ~90% of retrogenes that are expressed in spermatogenic cells are autosomal and have intron-containing progenitor genes on the X-chromosome implicates the selective advantage of replacing the functions of genes on the X-chromosome (Emerson et al., 2004). Of course, these retrogenes may evolve spermatogenic cell-specific functions subsequently under the influence of sexual selection.

The opposite situation is also observed in an over-abundance of X-linked genes that are expressed in spermatogonia, a stage when the X-chromosome is transcriptionally active (Wang et al., 2001). This phenomenon can be explained by sex-dependent antagonistic effects of genes on the X-chromosome (Gibson et al., 2002; Wang et al., 2001). Recessive mutations in X-linked genes that have a large advantage in males and a small disadvantage in females will rapidly spread to fixation because males are hemizygous for these genes. This will be followed by fixation of mutations that suppress expression in females. Interestingly, many cancer-testis-associated genes are X-linked and expressed in spermatogonia (Zendman et al., 2003).

Conclusions

This review argues that the patterns of gene expression in spermatogenic cells are shaped by selection for mutations that enhance male reproductive success and selfish genes and a variety of genetic conflicts. mRNAs that are expressed specifically in spermatogenic cells have been divided into three classes (Willison and Ashworth, 1987): (1) transcripts of genes that are expressed only in spermatogenic cells; (2) transcripts of members of gene families encoding spermatogenic cell-specific isoforms; and (3) transcripts produced by genes that are expressed in somatic and spermatogenic cells with altered structures due to alternative promoters and processing. This review proposes that the genes encoding three classes of mRNAs have four different functions: (1) Genes encoding proteins that are necessary for meiosis and development of a functional spermatozoon. At least some of these genes are conserved in distantly related organisms (Eddy, 2002). (2) SGs and sexually selected genes with advantages in transmission to offspring, frequently associated with negative effects on individual fitness or reproductive success in either sex. (3) Opposing genes with selective advantages that reduce the deleterious side effects of SGs and sexually selected genes, or augment the transmission of genes that are disadvantaged by SGs. (4) Transcripts with neutral or deleterious effects produced as the adventitious by-products a genetic arms race or genes with a strong sexual advantage. The phenotypic advantages and disadvantages of genes in the last three categories are exceedingly complex and difficult to predict, since they will depend on the history of random mutations in both sexes in each species, and interactions between proteins that are expressed at abnormal levels, in atypical intracellular localizations, and/or with altered amino acid sequences. Clearly, the patterns of gene expression in spermatogenic cells are not simply the optimum end product of a conserved developmental pathway that has been fine-tuned by millions of years of strong selective pressures.

Sexual selection provides a simple explanation for gene families with different paralogs that are expressed in somatic cells and spermatogenic cells. Presumably, the function of the somatic isoforms is shaped by natural selection, while the function of the spermatogenic cell-specific isoforms is shaped by sexual selection. The identification of many spermatogenic cell-specific isoforms is consistent with overwhelming evidence that strong selective pressure exist in males, the more competitive sex.

It also makes excellent biological sense that the atypical patterns of gene expression in spermatogenic cells begin in meiotic cells, the stage when many genes that encode proteins in the spermatozoon are first expressed. Genetic arms races and male–female co-evolution provide plausible explanations for the gross overexpression of certain mRNAs during spermatogenesis. The observation that certain highly expressed mRNAs in spermatogenic cells are strongly translationally repressed throughout their lifetime can be understood as a mechanism of minimizing the deleterious effects of mRNA overexpression (Kleene, 2001). This idea is consistent with evolutionary theory and the role of translational regulation in fine-tuning protein accumulation (Mathews et al., 2000).

Students of spermatogenesis have always been impressed by its complexity. This review contains bad
news: spermatogenesis is even more complex than most molecular biologists suspected, and the functions of atypical gene expression in spermatogenic cells will be difficult to predict because they represent evolutionary accidents. This review also carries good news: studies of gene expression in spermatogenic cells will produce insights into some of the fundamental problems in biology and medicine such as specification, the advantages of sexual reproduction, cancer, male infertility and the multitude of factors that determine enhance reproductive success.

Acknowledgments

This research was supported by NSF grants MCB-9874491 and BCE-0348497. The author is grateful to Rick Kesseli, Paige Dennis, and Melinda Stern for thoughtful comments on the manuscript.

References


Spieß, A.N., Walther, N., Muller, N., Balvers, M., Hansis, C., Iveli, R.,...