Published in final edited form as:

Mol Cell Endocrinol. 2009 July 10; 306(1-2): 24–32. doi:10.1016/j.mce.2009.03.003.

Male infertility caused by spermiogenic defects: Lessons from gene knockouts

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Abstract

Spermiogenesis refers to the process by which postmeiotic spermatids differentiate into elongated spermatids and eventually spermatozoa. During spermiogenesis, round spermatids undergo dynamic morphologic changes, which include nuclear condensation and elongation, formation of flagella and acrosome, reorganization of organelles and elimination of cytoplasm upon spermiation. This cellular differentiation process is unique to male haploid germ cells, which may explain why ~half of the testis-specific genes are exclusively expressed in spermiogenesis. The spermiogenesis-specific expression implies that these genes contribute to either structural or functional aspects of future sperm. Many such genes have been inactivated in mice and some of these gene knockout mice display male infertility due to nonfunctional sperm which display no or various degrees of structural abnormalities. Since the majority of these spermiogenesis-specific genes are highly conserved between mice and humans, findings from knockout mouse studies may be applicable to human infertility. Here, I briefly review some of these spermatid-specific gene knockouts. The mouse studies strongly suggest that sperm quality rather than quantity is a better indicator of male fertility and novel assays should be developed to determine sperm functionality.

Keywords

Sperm count; Sperm motility; Fertility; Haploid; Contraceptive; Drug targets

1. Spermiogenesis is unique to male germ cell development and many of the testis-specific genes are expressed in spermiogenesis

Spermatogenesis is a complex differentiation process through which highly specialized haploid spermatozoa are differentiated from diploid germline stem cells (Clermont, 1972). The developmental process begins in the basal compartment of the seminiferous epithelium with spermatogonia proliferating by mitosis and differentiating into spermatocytes. Since mitotic multiplication of spermatogonia is the main event in this phase, it has been termed the mitotic phase. Primary spermatocytes then undergo two meiotic divisions and become haploid round spermatids in the meiotic phase of spermatogenesis. The last phase, which is called spermiogenesis, is characterized by complex morphogenesis through which round

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spermatids differentiate into elongated spermatids and eventually spermatozoa (Fig. 1) (Fawcett, 1975; Oakberg, 1956). During spermiogenesis, apparatuses necessary for sperm function are formed, which include not only grossly or microscopically visible structures like the flagella and acrosome, but also molecular structures like surface receptors and ion channels (Dadoune, 1994). To facilitate swimming, round spermatids undergo an elongation process, during which both the nucleus and cytoplasm elongate in a coordinated way. Nuclear elongation is always accompanied by nuclear condensation, tail formation/growth and cytoplasm elongation. Meanwhile, mitochondria are rearranged along the mid-piece of the tail and the cytoplasm gradually retreats towards the tail. Upon spermiation (the process of releasing fully developed spermatids, called spermatozoa, into the lumen of seminiferous tubules), the cytoplasm is shed off and spermatozoa are released into the lumen of the seminiferous tubules and are carried by the Sertoli cell secretion to the epididymis for further maturation. Spermiogenesis is unique to male haploid germ cells. Unique processes often require unique genes/gene products to execute specific functions. Therefore, among ~600 testis-specific protein-coding genes identified by *in silico* database mining and microarray-based expression profiling (Lin and Matzuk, 2005; Schultz et al., 2003; Shima et al., 2004), ~350 have been found to be exclusively expressed in haploid male germ cells (our unpublished data). Gene knockout studies have identified essential roles of numerous such genes in spermiogenesis (Table 1). Here, I briefly describe the genes involved in each of the major steps of spermiogenesis (Fig. 1 and Table 1). Lessons learned from these knockout studies are also discussed.

2. Mouse models of male infertility caused by spermiogenic defects

2.1. Genes required for acrosome formation

The acrosome is a structure attached to the nucleus of a mature spermatozoon (Fig. 2). The acrosome contains proteolytic enzymes which are released to dissolve zona pellucida proteins during fertilization. Acrosome formation involves three consecutive phases: the Golgi phase, also called the granule phase, starts when numerous pro-acrosomic granules are formed in trans-Golgi stacks followed by a fusion into a single, large acrosomic granule that associates with the nuclear envelope (Abou-Haila and Tulsiani, 2000). In the subsequent cap and acrosomic phases, the acrosome increases in size and spreads over the anterior nuclear pole. Disruption in the acrosome formation can cause malformation of the acrosome. In humans, this disruption results in a rare human infertility condition (~0.1% of incidence) termed globozoospermia (also called round-headed spermatozoa) (Aitken et al., 1990; Holstein et al., 1973; Kullander and Rausing, 1975; Lalonde et al., 1988; Singh, 1992).

Gopc and *Agfg1* (previously called *Hrb*) have been identified to be essential for the Golgi phase of acrosome biogenesis. The *Gopc* gene encodes the mouse Golgi-associated PDZ- and coiled-coil motif-containing protein (GOPC), and deletion of *Gopc* in mice causes poorly formed pro-acrosomic granules (Ito et al., 2004). These male mice display infertility due to globozoospermia, which is characterized by round sperm heads lacking the acrosome (Yao et al., 2002). In addition, *Gopc*-null sperm also display marked defects in forming stable mitochondrial sheath (Yao et al., 2002). AGFG1 is an HIV-1 Rev-binding/interacting protein (Cochrane, 2004) and is required for docking and/or fusion of pro-acrosomic

vesicles during acrosome biogenesis. Male mice with a null mutation in *Agfg1* are infertile and display round-headed spermatozoa lacking the acrosome (Kang-Decker et al., 2001). *Csnk2a2* encodes the alpha catalytic subunit of protein casein kinase II and is preferentially expressed in spermatids (Escalier et al., 2003; Xu et al., 1999). Although a direct link between CSNK2A2 and acrosome biogenesis has not been established, male mice lacking *Csnk2a2* are infertile, displaying oligospermia and globozoospermia. GBA2 encodes the endoplasmic reticulum-resident enzyme beta-glucosidase 2 and is expressed in Sertoli cells in the testis. GBA2 deficiency in mice does not affect bile acid and cholesterol metabolism, but causes lipid accumulation in the endoplasmic reticulum of testicular Sertoli cells, round-headed sperm (globozoospermia), and impaired male fertility (Roy et al., 2006; Yildiz et al., 2006). It appears that the lack of acrosome formation in *Gba2*-null mice results from Sertoli cell defects which in turn disrupt acrosome formation of spermatids. This suggests that spermiogenesis is not an autonomous process, but is highly dependent on interactions between developing spermatids and their supporting Sertoli cells. A recent study has shown that *SPATA16*, a testis-specific gene, is involved in human male infertility due to globozoospermia (Dam et al., 2007). The involvement of human orthologs of the above-mentioned mouse genes in human globozoospermia needs further investigation.

2.2. Genes essential for sperm tail formation

Motility is essential for sperm to reach and penetrate the zone pellucida of the egg. Motility is generated by sperm flagella (Mortimer, 1997). The functional unit of flagella is the axoneme which is composed of structural elements and motor proteins that work in a coordinated and regulated fashion to generate waveforms and to produce progressive movement (Goodenough and Heuser, 1985; Haimo and Rosenbaum, 1981; King, 2000; Mortimer, 1997; Porter and Sale, 2000; Smith and Lefebvre, 1997). The axoneme extends continuously throughout the length of the flagellum and is composed of a “9+2” array of microtubules and associated proteins (Fig. 2) (Fawcett, 1975; Oko et al., 1990). In the mid-piece of the flagellum, nine outer dense fibers surround the axoneme and are paired with nine peripheral doublets of microtubules, forming a “9+9+2” pattern (Fig. 2C–G). The mitochondrial sheath wraps around the outer dense fibers in the mid-piece (Fig. 2C) and these fibers continue to extend through the principal piece of the tail (Fig. 2D–F). The outer dense fibers and the fibrous sheath taper off at the beginning of the end piece of the tail (Fig. 2G).

Tektins are a family of cytoskeletal proteins that are associated with microtubules and are preferentially expressed in tail-forming spermatids in the testis. Five Tektin proteins (TEKTs 1–5) have been identified so far (Iguchi et al., 1999, 2002; Iida et al., 2006; Larsson et al., 2000; Matsuyama et al., 2005; Roy et al., 2004; Wolkowicz et al., 2002; Xu et al., 2001) and *Tekt2* and *Tekt4* have been found to be required for normal flagellar structure and function. TEKT2 is localized to the axoneme and *Tekt2*-null male mice are infertile. *Tekt2*-null sperm display flagellar bending and reduced motility (Iguchi et al., 1999; Tanaka et al., 2004) due to a disruption in the dynein inner arm. TEKT4 is located on outer dense fibers, not associated with the axonemal tubulins of flagella in mice (Roy et al., 2007, 2008). Male mice lacking TEKT4 are subfertile, and *Tekt4*-null sperm exhibit drastically reduced

forward progressive velocity and uncoordinated waveform propagation along the flagellum (Roy et al., 2007).

VDACs are small channel proteins involved in the translocation of metabolites across the mitochondrial outer membrane and three voltage-dependent anion channels (VDACs1–3) have been identified in mice and humans (Sorgato and Moran, 1993). Mice lacking *Vdac3* are infertile due to structural defects in the axoneme characterized by a loss of a single microtubule doublet at a conserved position within the axoneme (Hinsch et al., 2004; Sampson et al., 2001). Since the defects are observed mainly in epididymal, not testicular, sperm, it appears that the axoneme becomes unstable during sperm epididymal maturation. Despite normal sperm counts, sperm in the *Vdac3*^{-/-} mice exhibit markedly reduced motility. Since VDACs are mitochondrial channels, these findings suggest that normal mitochondrial function is required for the stability of the axoneme. Selenoprotein plasma 1 (SEPP1) is an extracellular glycoprotein localized to the flagella of developing spermatids (Burk and Hill, 2005; Burk et al., 2003; Motsenbocker and Tappel, 1984). *Sepp1*^{-/-} male mice are infertile (Olson et al., 2005) because SEPP1-null spermatozoa display structural defects of the flagella including a truncated mitochondrial sheath and the extrusion of axonemal microtubules and outer dense fibers from the principal piece. Consequently, a hairpin-like bend is formed at the junction of the mid-piece and the principal piece. AKAPs are scaffold proteins for kinases and phosphatases which can form macromolecular complexes with other proteins involved in signal transduction (Edwards and Scott, 2000). AKAP4 is a member of the AKAP protein family and is abundantly expressed in the fibrous sheath of developing spermatids (Brown et al., 2003; Eddy et al., 2003; Miki et al., 2002). *Akap4*^{-/-} male mice are infertile despite their normal sperm count. AKAP4-null sperm (Brown et al., 2003; Miki et al., 2002) lack a fibrous sheath, which results in shortened flagella and loss of progressive motility. The defects have been suggested to result from failure in signal transduction in the absence of AKAP4. *PF16* is a gene first identified in the green alga *Chlamydomonas* and inactivation of PF16 results in flagellar paralysis (Dutcher et al., 1984; Smith and Lefebvre, 1997). Human and murine orthologs of PF16, named sperm-associated antigen 6 (SPAG6), display the eight armadillo repeats which are required for its association with microtubules and thus for flagellar function (Neilson et al., 1999; Sapiro et al., 2002, 2000; Zhang et al., 2005, 2002). Male mice lacking SPAG6 are infertile because their sperm display disorganized flagellar structures including the lack of a central pair and alterations in the fibrous sheath and/or outer dense fibers (Sapiro et al., 2002).

2.3. Genes essential for energy, metabolism, and signaling aspects of sperm motility

Structural defects in the sperm tail can result in abnormal or abolished motility. Since the coordinated movement of the sperm tail requires energy (e.g., ATP) and signaling (e.g., cAMP and Ca²⁺) from their surroundings, disruptions in energy supply and signaling transduction can also cause motility defects. Mammalian sperm display minimal or no motility in the epididymis, whereas they gain vigorous forward movement, termed progressive motility, upon ejaculation or collection into a physiological medium. The motility waveform changes once the sperm are in the female reproductive tract, characterized by increases in both the amplitude and the asymmetry of flagellar bending. These changes lead to a whiplash-like motion, termed hyperactivated motility, which is

believed to facilitate sperm transport to the oviduct and penetration of the zona pellucida during fertilization (Ho and Suarez, 2001; Suarez and Ho, 2003). ATP provides energy essential for coordinated movement of the central axoneme and accessory flagellar structures (Mann and Lutwak-Mann, 1981). Sources of ATP to support sperm motility are compartmentalized along the length of the flagellum. Oxidative phosphorylation is confined to the proximal segment of the flagellum where the mitochondria are localized (mid-piece), whereas glycolysis is restricted to the principal piece (Beyler et al., 1985; Bunch et al., 1998; Mori et al., 1998; Welch et al., 2000; Westhoff and Kamp, 1997). Both metabolic pathways are believed to provide sufficient ATP along the entire length of the flagellum to support activated and hyperactivated motility.

Several unique isozymes of glycolysis, including glyceraldehyde 3-phosphate dehydrogenase-S (GAPDHS), phosphoglycerate kinase-2 (PGK2), and lactate dehydrogenase-C₄ (LDHC4), are found in mammalian sperm (Boer et al., 1987; McCarrey and Thomas, 1987; Millan et al., 1987; Sakai et al., 1987; Welch et al., 2000, 1992). GAPDHS is the sole GAPDH isozyme in sperm (Bunch et al., 1998) and it is tightly bound to the fibrous sheath. GAPDHS-null males are infertile and display sluggish movement without forward progression (Miki et al., 2004). Despite unchanged mitochondrial oxygen consumption, levels of ATP are significantly reduced in GAPDHS-null sperm compared to those in wild-type sperm, suggesting that a significant amount of the energy required for sperm motility is generated by glycolysis rather than oxidative phosphorylation (Miki et al., 2004; Tanii et al., 2007; Welch et al., 2006).

Capacitation is a bicarbonate-induced, cAMP-dependent process through which sperm acquire the ability to fertilize eggs (Fraser, 1981; Lefievre et al., 2002; Osheroff et al., 1999; Visconti et al., 1995). Bicarbonate- and calcium-responsive soluble adenylyl cyclase (sAC) is encoded by *Adcy10* and is the major source of cAMP in sperm. Male mice deficient in sAC are infertile because their sperm show no motility despite normal sperm morphology and counts (Esposito et al., 2004). Although membrane-permeable cAMP analogs can rescue the motility defect, the “rescued” null sperm fail to develop hyperactivated motility and remain unable to fertilize eggs *in vitro*. sAC thus may serve as a signaling molecule required for sperm motility.

Ca²⁺ is another important messenger during capacitation (Yanagimachi, 1994). Changes in swimming patterns during sperm transit in the female reproductive tract are mediated by elevation of Ca²⁺ (Suarez et al., 1983). However, the identity of the channel responsible for the influx of Ca²⁺ remains unclear. Four cation channel-like proteins, CATSPER1–4, possess pore-lining residues and their amino acid sequences resemble those of a single repeat of the conventional four-repeat voltage-gated Ca²⁺ channel (Jin et al., 2005; Lobley et al., 2003; Quill et al., 2001; Ren et al., 2001; Serrano et al., 1999; Wennemuth et al., 2000; Westenbroek and Babcock, 1999). All 4 proteins are localized to the mid-piece and principal piece of the sperm tail, suggesting their roles in flagellar function. Indeed, lack of any of the 4 proteins results in male infertility and sperm that fail to develop hyperactivated motility (Carlson et al., 2005; Jin et al., 2007; Qi et al., 2007; Quill et al., 2003). These 4 CATSPER proteins form a tetramer and each subunit of the tetramer is essential for the full function of

this cation channel (Qi et al., 2007). Recent evidence suggests that defects in the *CATSPER* genes may be involved in human male infertility (Nikpoor et al., 2004).

2.4. Genes essential for spermatid nuclear condensation

Both the cytoplasm and the nucleus of a round spermatid elongate during spermiogenesis. Nuclear somatic histones are gradually replaced by transition nuclear proteins (TNPs) and eventually by protamine proteins. Transition nuclear proteins 1 and 2 (TNPs1 and 2) account for 90% of the chromatin basic proteins in spermatids at steps between histone removal and protamine deposition (Meistrich et al., 2003). Inactivation of either *Tnp1* or *Tnp2* in mice (Yu et al., 2000; Zhao et al., 2004) generates a subfertile phenotype, showing less condensed sperm nuclei (Yu et al., 2000). In addition, an elevated level of breaks in the DNA strand and other shape defects, such as heads bent back on mid-pieces, mid-pieces in hairpin configurations, coils, and clumps, is observed with high incidence. Double *Tnp* knockout mice (*Tnp1*^{-/-}–*Tnp2*^{-/-}) are completely infertile due to defects similar but more severe than *Tnp1* or *Tnp2* single knockout mice (Zhao et al., 2004), suggesting that TNP1 and TNP2 may have identical functions, but two genes are required to obtain a sufficiently high concentration of the proteins to support normal nuclear condensation. Protamines are the major DNA-binding proteins in the sperm nucleus and can pack the DNA into less than 5% of the volume of a somatic cell nucleus in most vertebrates (Lewis et al., 2003; Meistrich et al., 2003; O'Brien and Zini, 2005). High percentage male chimeric mice derived from either *Prm1*^{+/-} or *Prm2*^{+/-} embryonic stem cells are completely infertile (Cho et al., 2003, 2001) due to disrupted nuclear condensation, protamine 2 processing, and sperm function, suggesting an essential role for sperm nuclear condensation and head formation. TNPs and protamine proteins are major players in sperm nuclear condensation, but it appears that other nuclear factors also participate in this process. This notion is based upon the identification of at least two somatic histone isoforms, H1FNT (formerly H1T2) and HILS1, which are expressed in spermatids undergoing nuclear condensation (Iguchi et al., 2004; Martianov et al., 2005; Yan et al., 2003). Their expression patterns in spermiogenesis partially overlap with those of transition proteins and protamines. Since histone-like proteins are usually involved in chromatin modification, it is plausible to assume that these histone variants are also players participating in the sperm nuclear condensation process. Indeed, inactivation of H1FNT causes male infertility due to delayed nuclear condensation and aberrant elongation (Martianov et al., 2005; Tanaka et al., 2005). The role of HILS1 remains unknown.

2.5. Genes essential for the expression of late spermatid genes

Genes required for late spermiogenesis (steps 9–16 spermatids in mice) have to be transcribed in round spermatids (steps 1–8) because transcription ceases when nuclear elongation and condensation start at step 9 in mice. The transcripts are stabilized and stored until they are translated in later steps where their encoded proteins are needed (Eddy, 2002; Sassone-Corsi, 1997, 2002). This uncoupling of transcription and translation is a feature of gene regulation in spermiogenesis, which is achieved by means of unique transcriptional control, chromatin remodeling, and post-transcriptional modulations (Sassone-Corsi, 2002).

The master switch for the expression of late spermiogenic genes has been identified, which includes several spermatid-specific transcriptional regulators. The *Crem* (cAMP-responsive

element modulator) gene encodes the transcriptional activator CREM_T, which is highly expressed in round spermatids (Foulkes et al., 1992) and is essential for the expression of many important postmeiotic genes, such as *Prm1*, *Prm2*, *Tnp1* and *Tnp2* (Blendy et al., 1996; Nantel et al., 1996; Sassone-Corsi, 1998). Transcription initiation by RNA polymerase II in eukaryotes requires the coordinated action of a set of general transcription factors (Hampsey, 1998; Hampsey and Reinberg, 1999; Orphanides et al., 1996). One of the major factors in transcription initiation is the TATA binding protein (TBP). TATA box binding protein-like 1 (TBPL1) is a protein with high homology to the TBP core domain that displays a stage-specific expression pattern during mouse spermatogenesis (Sassone-Corsi, 1997). *Tbpl1*^{-/-} male mice are sterile due to a late and complete arrest of spermiogenesis at step 7 (Martianov et al., 2001). Several spermiogenesis genes transcribed in late round spermatids appear to be under TBPL1 control (Martianov et al., 2001), including *MTEST640*, *MTEST641*, and *MTEST643*. PAPOLB (also called TPAP) is a testis-specific, cytoplasmic polyadenylate (polyA) polymerase (Kashiwabara et al., 2002, 2000). Knockout of *Papolb* results in an arrest of spermiogenesis (Kashiwabara et al., 2002). *Papolb*-deficient mice display significant down-regulation of expression of haploid-specific genes that are required for spermiogenesis, such as *Mcsp* (encoding mitochondrial capsule selenoprotein), *Hspa11* (heat shock protein 1-like), *Akap4* (A-kinase anchoring proteins), *Odfl* (sperm outer dense fiber protein RT7), *Tnp1* (transition protein 1), and *Prm1* and *-2* (protamine 1 and 2). The TPAP deficiency also causes incomplete elongation of the polyA tails of particular transcription factor mRNAs that are important for spermiogenesis, including TBPL1, TFIIA, TAF10, TAF12, and TAF13.

PIWIL1 (also called MIWI) is the mouse ortholog of *Drosophila* Piwi (for p-element-induced wimpy testis) protein (Cox et al., 1998, 2000). In mice and humans three MIWI homologs exist: PIWIL1, PIWIL2 (also called MIWI2) and PIWIL4 (also called MILI for MIWI-like protein) (Carmell et al., 2007; Kuramochi-Miyagawa et al., 2004, 2001). All three MIWI proteins have been found to bind a class of non-coding small RNA species called piRNAs (for Piwi-interacting RNAs) (Aravin et al., 2006; Girard et al., 2006; Grivna et al., 2006a; Lau et al., 2006). Gene knockout studies have shown that lack of either *Piwil2* or *Piwil4* causes meiotic arrest in spermatogenesis (Carmell et al., 2007; Kuramochi-Miyagawa et al., 2004), suggesting their indispensability in the meiotic phase of male germ cell development. Interestingly, *Piwil1*-null mice display spermiogenic arrest at step 7, implying an essential role of PIWIL1 in late spermiogenesis (Deng and Lin, 2002). Coincidentally, this phenotype is similar to the phenotype of the *Crem* knockouts described above, which lacks transcriptional regulators of late spermiogenic gene expression. PIWIL1 has been shown to be associated with translational machinery (Grivna et al., 2006b), suggesting that PIWIL1 alone or together with its-bound piRNAs participates in post-transcriptional regulation of late spermatid gene expression. Failure in either transcription of late spermiogenic genes or in translation of these late spermiogenic mRNAs can result in an identical phenotype: the lack of late spermiogenesis.

2.6. Genes essential for proper cytoplasm removal

Upon the release of spermatozoa into the lumen of the seminiferous tubule (termed spermiation), the cytoplasm and its contents are shed off and the resultant residual bodies are

then phagocytosized by Sertoli cells (Clermont, 1972). Cytoplasm removal appears to be a strictly regulated process. In mice the spermatid cytoplasm starts to move posteriorly toward the tail when the nucleus starts to elongate and condense. The Sertoli cell cytoplasmic membrane invades the cytoplasm of spermatid during nuclear elongation and some spermatid cytoplasmic contents are engulfed by Sertoli cells (Sakai et al., 1988; Sakai and Yamashina, 1989). With further elongation of the nucleus, the spermatid cytoplasm also elongates and becomes more rectangular in shape and moves further to the posterior. Once spermatids are fully differentiated into spermatozoa, the cytoplasm peels off, presumably from multiple points along mid- to principal pieces of the tail (Sakai et al., 1988; Sakai and Yamashina, 1989). In mice, cytoplasmic droplets are small vesicles located at the junction between the mid-piece and principal piece of the tail (Fig. 2A). This structure is believed to be remnant of the shed cytoplasm and the majority of mouse epididymal sperm possess it (Cooper, 2005; Kuster et al., 2004). *Spem1* encodes a spermatid-specific protein expressed in the cytoplasm of steps 9–16 spermatids (Zheng et al., 2007). *Spem1*-null mice produce normal numbers of sperm, but all sperm heads are bent back on mid-pieces, and cytoplasmic remnants resembling cytoplasmic droplets are trans-located to the region surrounding the bent heads. A high incidence of sperm with bent back heads has been observed in several lines of mice deficient in proteins involved in nuclear condensation, such as *Prm1* or *Prm2* high percentage chimeras (Cho et al., 2003, 2001), *Tnp1* and *Tnp2* knockout mice (Yu et al., 2000; Zhao et al., 2004). These suggest that improper nuclear condensation is related to the head-bent-back phenotype. The misallocation of cytoplasmic droplets implies that the shedding of cytoplasm during spermiation occurs in a wrong place and the completely bent back heads suggest that the elongation of the nucleus and the growth of the flagella are not well coordinated. Further molecular characterization of the *Spem1*-null mice is required to fully explain the phenotype and reveal the function of SPEM1. Nevertheless, *Spem1* is involved in synchronized development of the sperm head and the tail and in proper removal of spermatid cytoplasm.

3. What do these knockout mice tell us?

Since virtually all spermiogenic genes are dedicated for transforming round spermatids into spermatozoa, it is not surprising that structural and functional defects arise in the absence of these genes. The knockout mouse lines discussed above show many commonalities, which are of importance for us to understand the mechanisms underlying the regulation of haploid male germ cell development. Some of the findings suggest that more sensitive assays should be developed to efficiently detect subtle functional defects of sperm in fertility clinics.

3.1. Lack of “checkpoint” during late spermiogenesis

Severe germ cell depletion is common when essential early spermatid (steps 1–8) genes are inactivated, as observed in *Crem*, *Tbpl1*, *Papalb* and *Piwil1* knockout mice (Blendy et al., 1996; Deng and Lin, 2002; Kashiwabara et al., 2002; Martianov et al., 2001; Nantel et al., 1996). The depletion of round spermatids is usually mediated by either enhanced apoptosis, formation of multinucleated “giant cells” or detachment from Sertoli cells followed by sloughing into the lumen of the seminiferous tubules. Consequently, many round spermatids can be observed in the epididymis and these KO mice display decreased testis weights. If

spermiogenesis is arrested in early spermatids, no sperm or significantly decreased sperm counts are expected. In contrast, mice lacking proteins required for late spermiogenesis (steps 9–16) show no obvious germ cell depletion and seemingly normal testis histology at light microscopic levels. Consistently, both testis weights and sperm counts are normal. These include mice lacking *Spem1*, *Akap4*, *Tekt2*, *Tekt4*, *Vdac3*, *Sepp1*, *Akap4*, *Spag6*, *Gapdhs*, *Adcy10*, *Catsper1–4*, *Tnp1*, *Tnp2*, *H1fnt* or *Spem1* (see Table 1). These observations suggest that defective round spermatids can be depleted, whereas malformed or deformed spermatozoa can be produced in full quantity within the seminiferous epithelium without triggering depletive effects. This phenomenon implies that a checkpoint may be present in early spermiogenesis up to step 8 round spermatids, but a similar quality control mechanism does not exist in later spermiogenesis because the development of defective late spermatids can proceed all the way through to spermiation. It was previously thought that a ubiquitination-mediated quality control mechanism could eliminate structurally and/or functionally defective spermatozoa during their transition through the epididymis (Sutovsky, 2003). These knockout mice displaying malformed and deformed spermatozoa due to loss of function of late spermiogenic genes show normal sperm counts (using caudal epididymal spermatozoa) and even normal initial motility (e.g., *Catsper1–4* knockout mice), suggesting that this epididymal checkpoint may not exist at all.

3.2. Sperm count is not a good indicator of fertility and novel assays are needed to detect dysfunctional sperm

It appears that the loss of function of late spermiogenic genes does not affect the final number of sperm despite the fact that mutant sperm are misshapen and/or dysfunctional. Therefore, sperm quantity is not a good indicator of fertility. This notion has been suggested by clinical studies as well, showing that it is the shape of sperm, not sperm counts, that correlates better with the male fertility (Guzick et al., 2001). Some obvious structural defects can be determined at the light microscopic levels (e.g., lack of acrosome, heads bent back, shortened tail, or swollen heads), whereas some defects are at molecular levels and cannot be detected microscopically (e.g., sperm with defects in energy production, signaling transduction and metabolism). Sperm lacking certain components necessary for full competence of motility and fertility usually display slightly decreased motility when analyzed within 30 min after collection (e.g., *Catsper3* or *4*-null sperm) and an accelerated loss of motility is common in this type of sperm (Jin et al., 2007). *Catsper1–4*-null mice display both normal counts and initial motility, but fail to develop hyperactivated motility (Carlson et al., 2005; Jin et al., 2007; Qi et al., 2007; Quill et al., 2003). This type of functional defects can be detected by observing swimming patterns of the sperm after activation in capacitating medium, or by computer-assisted semen analysis (CASA) using modified settings. Since the current CASA tracks the movement of sperm heads (Krause, 1995; Lenzi, 1997), while defects of these sperm lie in waveforms of the tail, novel analyses based upon tail movement may be a more effective way to detect tail waveform abnormalities.

3.3. Spermiogenesis-specific gene and proteins as male contraceptive targets

Most of the spermiogenic genes are highly conserved between mice and humans. If inactivation of a gene leads to an infertility phenotype in mice, specific modulation of the

same gene product in humans theoretically could result in a contraceptive effect. Therefore, these gene knockout studies not only reveal essential roles of these genes in multiple developmental steps of haploid male germ cells, but also point to the interesting notion that these genes and their products are good targets for male contraceptives for the following reasons: (1) haploid germ cells express numerous unique genes, therefore targeting these proteins would have minimal or no side effects; (2) many of the haploid germ cell-specific genes encode membrane proteins, enzymes, ion channels, carrier proteins, and signaling molecules (Schultz et al., 2003), which are excellent pharmaceutical targets for potential male contraceptives (Nass and Strauss, 2004a,b); (3) unlike spermatocytes or spermatogonia, anomalies of which often affect the micro-environment of the seminiferous epithelium resulting in depletion of other germ cell populations including spermatogonial stem cells, blocking differentiation of haploid germ cells rarely affects early spermatogenic cell types. Therefore, reversibility can be expected if these spermatid-specific genes are targeted; and (4) all major chromosomal remodeling events (e.g., crossover by homologous recombination and reestablishment of paternal gene imprinting patterns) are accomplished before the haploid stage. Targeting haploid germ cells is therefore less risky in terms of transmitting paternal genome with compromised integrity and stability to offspring in case of accidental pregnancy.

Evaluation of molecular targets of all known drugs based upon safety and effectiveness has resulted in a list of ideal drug targets at the order of cell surface receptors (45%) > enzymes (28%) > hormones and growth factors (11%) > ion channels (5%) > nuclear receptor (2%) (Drews, 2000). *Csnk2a2*, *Gapdhs*, *Adcy10* and *Pap0lb* encode enzymes and *Vdac3* and *Catsper1–4* encode ion channels. These genes or gene products should serve good contraceptive targets. So far only ~10% of the genes that are specifically/preferentially expressed in haploid germ cells have been analyzed using gene knockout technology. The knockout mouse project (KOMP) aims at inactivating all mouse protein-coding genes, and mice bearing null mutations in each of ~30,000 genes will be available by 2011 (Austin et al., 2004). By then, characterization of knockout mice with male infertility phenotype will allow us to compile a complete list of genes that play essential roles in each step of spermiogenesis. Furthermore, small compounds targeting some of the most “druggable” gene products will be identified. With the rapid development of the high throughput, small compound screening technology, it is anticipated that new male contraceptive drugs that target haploid germ cell-specific gene products will be available in the near future.

Acknowledgments

David Young is acknowledged for editing the text and comments. I apologize to colleagues whose work is not cited here due to publisher's space limit. The research in the Yan Laboratory is supported by NIH grants HD048855 and HD050281, as well as a startup grant from the University of Nevada, Reno.

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Fig. 1.

Genes essential for each of the multiple steps of spermiogenesis, as revealed by gene knockout studies in mice. Mouse spermiogenesis consists of 16 steps (labeled with 1–16) and specific associations between stages of the seminiferous epithelial cycle (marked with Roman numerals I–VIII) and developing steps of spermatids are shown.

Fig. 2.

Ultrastructure of the mouse epididymal sperm. (A) Scanning electron microscopic image of a mouse epididymal sperm. (B) Transmission electron microscopic (TEM) image of the longitudinal section of the head and neck regions of a sperm. (C) TEM image of a cross-section from the mid-piece of the sperm tail. (D) TEM image of a cross-section from the junction between the mid-piece and principal piece of the sperm tail where the cytoplasmic droplet is located. (E and F) TEM images of cross-sections from middle and lower portions of the principal piece of the sperm tail, respectively. (G) TEM image of a cross-section from the end piece of the sperm tail.

Table 1

Knockout mouse models of male infertility due to spermiogenic defects.

Gene symbol (old symbol)	Protein name	Spermiogenic process	Testis histology	Testis weight	Sperm count	Percentage of motile sperm
<i>Gopc</i>	Golgi-associated PDZ and coiled-coil motif containing	Acrosome biogenesis	Normal	Normal	Normal	Reduced
<i>Aggl1 (Hrb)</i>	ArfGAP with FG repeats 1	Acrosome biogenesis	Normal	Normal	1/15 of WT	Reduced
<i>Csnk2a2</i>	Casein kinase 2, alpha prime polypeptide	Acrosome biogenesis	Normal	Normal	1/3 of WT	N/A
<i>Gba2</i>	Beta glucosidase 2	Acrosome biogenesis	Normal	Slightly reduced	1/2 of WT	Reduced
<i>Tekt2</i>	Tektin 2	Tail formation	Normal	Normal	Normal	Reduced
<i>Tekt4</i>	Tektin 4	Tail function	Normal	Normal	Normal	Reduced
<i>Vdac3</i>	Voltage-dependent anion channel 3	Tail formation	Normal	Normal	Normal	1/4 of WT
<i>Sepp1</i>	Selenoprotein P, plasma, 1	Tail formation	Normal	Normal	Normal	Reduced
<i>Akap4</i>	A kinase (PRKA) anchor protein 4	Tail formation	Normal	Normal	Normal	Reduced
<i>Spag6</i>	Sperm associated antigen 6	Tail formation	Normal	Normal	1/3 of WT	1/7 of WT
<i>Gapdh</i>	Glyceraldehyde-3-phosphate dehydrogenase, spermatogenic	Energy supply to tail movement	Normal	Normal	Normal	1/23 of WT
<i>Adcy10 (sAC)</i>	Adenylate cyclase 10, or soluble adenylyl cyclase	Signaling molecule required for sperm motility	Normal	Normal	Normal	N/A
<i>Caspr1, 2, 3, 4</i>	Cation channel, sperm associated 1, 2, 3, 4	Ion channel-mediated signaling required for hyperactivated motility	Normal	Normal	Normal	Slightly reduced initial motility, quick loss of motility over time, and lack of hyperactivated motility
<i>Top1, 2</i>	Transition protein 1, 2	Nuclear condensation	Normal	Normal	Normal	Slightly Reduced
<i>H1mt (H1t2)</i>	H1 histone family, member N, testis-specific	Nuclear condensation	Normal	Normal	Normal	Reduced
<i>Crem</i>	cAMP responsive element modulator	Late spermiogenesis gene expression	Lack of spermatids	Reduced	None	None
<i>Tbpl1</i>	TATA box binding protein-like 1	Late spermiogenesis gene expression	Spermiogenic arrest at step 7	Reduced	None	None
<i>Papalb (Tpap)</i>	Poly (A) polymerase beta (testis-specific)	Late spermiogenesis gene expression	Lack of spermatids	Reduced	None	None
<i>Piwi1 (Miwi)</i>	Piwi-like homolog 1 (Drosophila)	Late spermiogenesis gene expression	Spermiogenic arrest at step 7	Reduced	None	None
<i>Spem1</i>	Sperm maturation 1	Coordinated nuclear elongation, tail growth and cytoplasm removal	Normal	Normal	Normal	Reduced