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Invited Review

Mammalian spermatogenesis investigated by genetic engineering

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Summary. Genes involved in mammal spermatogenesis can now be identified through mutants created by genetic engineering. Information has been obtained on male meiosis, but also on the factors regulating the proliferation, maintenance and differentiation of male germ cells. Its has also increased our knowledge of the germ cell phenotype emerging from an altered germ cell genotype. This review is focused on data from genes expressed in male germ cells and on the question of how germ cells and Sertoli cells cope with the molecular lesions induced. The conservation of a wild-type phenotype of male germ cells in mutant mice is discussed, and how the mouse genetic background can lead to different germ cell phenotypes for a given gene mutation.

Key words: Meiosis, Sperm, Spermatogenesis, Transgenic, Testis

What happens to germ cells altering germ cell gene expression?

Considerable information on factors involved in the differentiation of male germ cells has now been obtained from mutant mice created by genetic engineering. It includes genes involved in the development of the male reproductive tract, in the production of hormones and their receptors and in Sertoli cell function (reviewed in Lamb and Niederberger, 1994; Simoni, 1994; Sassone-Corsi, 1997; Okabe et al., 1998). This review is focused only on genes expressed in male germ cells and how germ cells cope with the induced molecular lesions. Tables 1-3 present the testicular characteristics of these mutant mice generated by genetic engineering.

Genes expressed in primordial germ cells and/or spermatogonia

TIAR is an RNA recognition motif/ribonucleoprotein-type RNA-binding protein that is highly expressed in the nucleus of primordial germ cells (PGCs) (Beck et al., 1998). In *tiar*$^{-/-}$ embryos at day 11.5, a reduced number of PGCs was present at the genital ridges but was absent from day-13.5 *tiar*$^{-/-}$ embryos, suggesting an inability of *tiar*$^{-/-}$ PGCs to respond to a survival signal. At 3 months of age, testes of *tiar*$^{-/-}$ mice were approximately one-third the normal size and lacked spermatogonia, spermatids and spermatozoa. The germ cell defect of *tiar*$^{-/-}$ mice could be compared to the PGC defects characterizing mutations in the *c-kit* tyrosine kinase receptor (white spotting) and its ligand, *Steel* factor. A role for TIAR in growth factor signaling was suggested (Beck et al., 1998).

The telomerase holoenzyme consists of an essential RNA template and protein components. Telomerase mediates the synthesis and maintenance of telomeric repeats in proliferative cells. Telomerase activity is strong in type A spermatogonia, down-regulated during spermatogenesis, and absent in the differentiated spermatozoa (Ravindranath et al., 1997). From the third generation of mice lacking telomerase RNA (*mTR*$^{-/-}$ mice), the spermatogonia proliferation rate decreased and germ cell apoptosis increased. By the fifth generation, germ cells became depleted and, by the sixth generation, most seminiferous tubules showed a striking absence of spermatogenesis. The spermatogonial stem cell loss could be due to a decline in telomere length with each successive stem cell generation and to fusion and loss of chromosomes. The long latency in the appearance of defects in the mouse may simply relate to the long telomere lengths of the laboratory mouse strain *Mus Musculus* (Lee et al., 1998).

ZFX, a zinc-finger protein, which may function as sequence-specific transcription activator, seems to be expressed in male germ cells, as in other tissues. Although its expression pattern remains to be determined (this is why *Zfx*$^{-/-}$ mice are not included in the tables), the phenotype obtained in *Zfx* null mutants provided evidence for a role of ZFX in the growth and maintenance of germ cell populations. In male embryos from *Zfx* null mutant mice, the number of PGCs was less than half of that in the wild-type embryos, resulting later in less than half the number of sperm in the

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Table 1. Early spermatogenesis failure following germline genetic engineering in the mouse.

GENOTYPE	GERM CELL PHENOTYPE	REFERENCES
Tiar$^{-/-}$	primordial germ cell deficiency	Beck et al., 1998
mTR$^{-/-}$	spermatogonia proliferation failure	Lee et al., 1998
Dazla$^{-/-}$	spermatogonia arrest	Ruggiu et al., 1997
c-myc (exogenous)	spermatogonia/early prophase I arrest	Suzuki et al., 1996
Bcl-2 (exogenous and misexpression)	spermatogonia accumulation	Furuchi et al., 1996
Bax$^{-/-}$	spermatogonia/preleptotene accumulation	Knudson et al., 1995
Dmc1$^{-/-}$	early zygotene arrest	Pittman et al., 1998; Yoshida et al., 1998
Mlh1 $^{-/-}$	postsynapsis arrest (pachytene or metaphase I), microsatellite instability	Baker et al., 1996; Edelmann et al., 1996
Pms2 $^{-/-}$	pachytene synapsis abnormalities, microsatellite instability	Baker et al., 1995
Atm$^{-/-}$	zygotene/pachytene arrest	Barlow et al., Xu et al., 1996; Elson et al., 1996
Abl$^{-/-}$	pachytene arrest	Kharbanda et al., 1998
Hsp70-2$^{-/-}$	mid-late pachytene arrest, desynapsis failure	Dix et al., 1997; Zhu et al., 1997
A-myb$^{-/-}$	early pachytene arrest	Toscani et al., 1997
p53$^{-/-}$ or reduced	giant-cell degenerative syndrome	Rotter et al., 1993
Bmp8b$^{-/-}$	pachytene loss, proliferation defects	Zhao et al., 1996
Bmp8a$^{-/-}$	more or less meiotic germ cell degeneration	Zhao et al., 1998
c-mos (overexpression)	increased number of spermatocytes and spermatids	Higgy et al., 1995
v-*Mos* (overexpression)	metaphase I arrest, tubulin anomalies	Rosenberg et al., 1995
Bsg$^{-/-}$	metaphase I arrest, some round spermatids	Igakura et al., 1998

epididymides in males and one eighth the number of oocytes seen in females. Considering the reduced body mass of mutants, the low PGC counts in embryos could be explained by a reduction in the number of epiblast cells allocated to the germ cell lineage (Luoh et al., 1997).

The autosomal *Daz* gene (*Dazla*) encodes a protein containing RNA-binding motifs. In the mouse testis, Dazla is found in type-B spermatogonia, preleptotene and zygotene spermatocytes with a non-uniform cytoplasmic localization (Ruggiu et al., 1997). *Dazla* is also expressed before sex-differentiation in male and female embryonic gonads containing PGCs (Seligman and Page, 1998). Disruption of the *Dazla* gene (Ruggiu et al., 1997) has led to loss of germ cells in both males and females. At 15 days post-coitum, the testes from homozygous and heterozygous mice appeared normal but a sparcity of the seminiferous tubules was seen at 19 days post-coitum due to one-half the amount of germ cells. Testes from homozygous mice at 9 weeks showed an almost complete absence of germ cells. The phenotype of heterozygous mice was surprising: they produced one-half the wild-type sperm number and 60% abnormal sperm, 57% of spermatozoa lacking heads and 25% having tail abnormalities. Data show that the mouse *Dazla* gene is essential for the development and survival of germ cells. The cytoplasmic localization of Dazla suggests that Dazla may be related to a translational control by packaging or localizing of mRNAs.

The *c-myc* protooncogene is expressed in PGCs and in type-B spermatogonia. In other cell lineages, *c-myc* is thought to function in cellular proliferation and/or differentiation. Expression of rat *c-myc* in mice (Suzuki et al., 1996) has led to germ cell death as early as postnatal day 4, and at day 14, few cells survived beyond the pachytene stage. Data indicate that high *c-myc* level is probably deleterious to spermatocyte differentiation, and to a lesser extent, to spermatogonial differentiation. Since *c-myc* reaches a peak in B spermatogonia, it is possible that the decrease in *c-myc* level thereafter is an obligatory step for the completion of spermatogenesis.

The product of the *Bcl-2* gene, which encodes a membrane-associated protein, prevents the normal course of cell death without altering proliferation in cultured cells. Transgenic mice misexpressing the human *Bcl-2* gene in spermatogonia (Furuki et al., 1996) had inhibition of germ cell death at 2 weeks of age. At 4 weeks, the testis weight was about twice that of normal mice due to accumulation of spermatogonia while only some spermatocytes were present. A significant proportion of accumulated germ cells was degenerating. This suggested that atypical spermatogonia, forced to survive, may be at a disadvantage for further differentiation or that *Bcl-2* could affect the progression of meiosis. By 7 weeks of age, most germ cells were deleted in the tubules and the weight of testes had decreased to about one-third that of normal littermates. By 6 months of age, in the seminiferous tubules for which the exogenous *Bcl-2* expression had ceased, spermatogenesis was partially recovered and about one-third of the tubules contained elongated spermatids and even spermatozoa. Because *Bcl-2* is not expressed in spermatogonia in wild-type mice, it is suggested that the death-survival balance may be controled by unidentified molecules of the *Bcl-2* family.

BAX is known to promote cell death. Testes from *Bax*$^{-/-}$ mice (Knudson et al., 1995) exhibited an accumulation of spermatogonia and preleptotene spermatocytes. These cells had an atypical distribution of decondensed chromatin and irregular size and shape. Pachytene spermatocytes were reduced in number, round spermatids were rare and elongated spermatids were absent. Clustered apoptotic germ cells were present and the most severely affected tubules were partially or

Table 2. Other germ cell phenotypes following genetic engineering in the mouse.

GENOTYPE	GERM CELL PHENOTYPE	REFERENCES
calmegin$^{-/-}$	normal	Ikawa et al., 1997
Crem$^{-/-}$	round spermatid arrest/$^{+/-}$sperm tail anomalies	Blendy et al., 1996; Nantel et al., 1996
mHR6B$^{-/-}$	shedding of spermatids, sperm shape anomalies	Roest et al., 1996
Bclw$^{-/-}$	elongating spermatid arrest	Ross et al., 1998
Acr (exogenous)	spermatid arrest, spermatid head abnormalitites	O'Brien et al., 1996
Acr$^{-/-}$	normal	Baba et al., 1994; Adham et al., 1997;
protamine (exogenous)	chromatin and head-tail function instablity	Maleszewski et al., 1998
Ace $^{-/-}$	normal	Hagaman et al., 1998
Pcsk4$^{-/-}$	normal	Mbikay et al., 1997
Sprm1$^{-/-}$	normal	Pearse et al., 1997
gt$^{-/-}$	normal	Lu and Shur, 1997
Xist $^{-/-}$	normal	Marahens et al., 1997
Cenpb$^{-/-}$	normal	Hudson et al., 1998

completely devoid of germ cells. The massive apoptosis and disorganized spermatogenesis may indicate a role for *Bax* in regulating the meiotic cycle. The accumulation of atypical premeiotic germ cells may reflect their prolonged survival or a differentiation block that ultimately results in cell death.

Genes expressed during the meiotic prophase I stages

The *Dmc1* (disrupted meiotic cDNA) gene encodes a protein with homology to RecA, a bacterial protein that promotes recognition of homologous DNA and catalyzes strand exchange. The mouse DMC1 protein is detected in leptotene-to-zygotene spermatocytes and may be a component of early recombination nodules. Homozygous male mice with null mutation in the *Dmc1* gene (Pittman et al., 1998; Yoshida et al., 1998) showed an arrest of meiosis at the early zygotene stage. At 8 weeks of age, three types of tubules were observed in *Dmc1*-deficient mouse testes. The first type was enriched in leptotene and zygotene spermatocytes. The second type contained spermatocytes undergoing apoptotic cell death. In the third type, only a single layer of type-B spermatogonia was seen. *Dmc1*-deficient spermatocytes revealed extensive asynapsis of homologues, and chromosome degeneration. Sites of unusual synapsis (homologous or nonhomologous) were encountered in 16.6% (one site) and 11.8% (two sites) of spermatocytes (Yoshida et al., 1998). Synapsis anomalies and the meiotic arrest stage were examined with antibodies known to stain the synaptonemal complexes (SCs). Anti-SYCP1 stains only when synapsis and formation of tripartite SCs have occurred. Arrested spermatocytes from *Dmc1*$^{-/-}$ mice were unstained with anti-SYCP1 but stained positively with anti-SYCP3 and anti-RAD51 antibodies that are markers of the zygotene stage (Pittman et al., 1998). Taken together, these results demonstrate that in *Dmc1*$^{-/-}$ mice, the homologue synapsis, as well as SC formation, were completely blocked and suggest that *Dmc1* is essential for the chromosomes to find their corresponding homologues.

Null mutation in the *Mlh1* gene (encoding a protein required for DNA mismatch repair) has led to complete absence of spermatozoa due to blockage of pachytene spermatocytes I. The chromosomes in pachytene showed normal pairing but post-synapsis meiotic arrest. Then spermatocytes became apoptotic and fragmented (Edelmann et al., 1996). In another report, diplotene and metaphasic spermatocytes I were found (Baker et al., 1996). In mid-pachytene spermatocytes, the X and Y chromosomes were completely separated. A steady increase in the frequency of autosomal univalents was observed and chiasma formation was reduced 10- to 100-fold, suggesting failure of homologues to cross over or to maintain chiasmata. The male germline also exhibited increased mutation in microsatellite DNA sequences. Data suggested that failure to repair heteroduplexes is likely to trigger arrest (Edelmann et al., 1996). Therefore, *Mlh1* appears to be required for normal levels of chiasma formation or stabilization and to be involved in meiotic cross-over (Baker et al., 1996). Because mutants deficient in the *Mlh1* cannot form or maintain normal chiasmata and spermatocytes arrest at metaphase I (Baker et al., 1996), the existence in the mouse of a conserved tension-sensing checkpoint has been suggested (Page and Orr-Weaver, 1997).

The *Pms2* gene also encodes a protein required for mismatch repair and PMS2 and *MLH1* form a functional heterodimer. However, findings suggest that PMS2 and MLH1 have distinct functions in male meiosis. In *Pms2*$^{-/-}$ males (Baker et al., 1995), primary spermatocytes were vacuolized suggesting disruption at the primary spermatocyte stage. A prominent feature was frequent asynapsis or extensive single axial element formation with very little synapsis. Also, different types of nonhomologous synapsis, interlocked bivalents and association of an autosomal bivalent with the XY body were found. All these features indicated that *Pms2* deficiency results in abnormalities in chromosome synapsis, more particularly at the stage of homologue searching. The number of round, elongating and elongated spermatids was also reduced, and many of these cells had abnormally-shaped heads. Consequently, the number of spermatozoa was less then 25% of a

normal male and spermatozoa were grossly abnormal, with misshaped heads and truncated, irregular flagella. The genomic instability at microsatellite loci in the male germline showed that *Pms2* is involved in DNA mismatch repair. The overall observations in *Pms2*-deficient mice suggests links among mismatch repair, genetic recombination and initiation of chromosome synapsis during meiosis.

Atm is a gene mutated consistently in Ataxia Telangiectasia patients. It codes for a phosphoprotein with a carboxyl-terminal region similar to the catalytic domain of phosphatidylinositol 3-kinases. The ATM protein is involved in sensing double-stranded breaks in DNA and signaling cell-cycle arrest after this type of DNA damage. Its similarity to the DNA-PK catalytic subunit suggests that ATM could also play a direct role in processing or repair of DNA damage. In male germ cells, ATM localizes along synapsed chromosomal axes (Keegan et al., 1996) and is likely to be a component of the recombination nodules (reviewed in Hawley and Friend, 1996). $Atm^{-/-}$ mice did not produce mature sperm (Barlow et al., 1996; Elson et al., 1996; Xu et al., 1996). Spermatocytes in $Atm^{-/-}$ mice (Xu et al., 1996) appeared to halt development between the zygotene and pachytene stages of meiosis prophase I and in 6-week-old males, spermatids were completely absent. In zygotene nuclei, there were frequently univalents and partially synapsed bivalents suggesting a general delay in synapsis. Nevertheless, most nuclei progressed into pachynema and most bivalents were synapsed. However, they were fragmented, and progressive chromosome damage resulted in fragmentation and erosion of the entire genome. Nuclei appeared apoptotic and late pachytene were never observed. Lack of functional ATM would disrupt chromosome pairing and compromise chromosome integrity, allowing conversion of nascent double-strand breaks into chromosome breaks (Xu et al., 1996). Germ cell anomalies of $Atm^{-/-}$ mice suggest that damage is caused by a missing checkpoint at the zygotene stage or by a missing repair function (Page and Orr-Weaver, 1997).

The c-Abl is known to associate with the DNA-dependent protein kinase (DNA-PK) and with ATM (both activating c-Abl) and to bind *p53 in vitro*. Pachytene spermatocytes express high levels of c-Abl tyrosine kinase. Lower levels of c-Abl are found in spermatogonia, preleptotene spermatocytes and spermatids. c-Abl localizes near the ends of pachytene chromosomes and seems to interact directly with meiotic chromosomes. $Abl^{-/-}$ mice (Kharbanda et al., 1998) were generally infertile. Round and elongated spermatids were markedly diminished or absent in certain seminiferous tubules. The majority of germinal cells showed mild to striking degenerative changes. The $Abl^{-/-}$ phenotype was variable but observations indicated defects in meiotic progression beyond the pachytene stage. Data suggested that c-Abl is involved either in telomere movement or in meiotic recombination.

The Hsp70-2 heat shock protein is expressed during prophase of meiosis I in male germ cells. $Hsp70\text{-}2^{-/-}$ mice (Dix et al., 1997; Zhu et al., 1997) have demonstrated that HSP70-2 is required for SC desynapsis. Desynapsis impairment leads to mid-late pachytene spermatocyte apoptosis. Electron microscopy showed degenerated cells with decondensing sex vesicle and degradated SCs. $Hsp70\text{-}2^{-/-}$ mice failed to assemble CDC2/cyclin B1 complex, the CDC2 kinase activity was not present in the testis and the phosphorylation status of CDC2 was altered (Zhu et al., 1997). Cyclin B-dependent CDC2 protein kinase activity is required for G2/M phase transition and HSP70-2 is known to be a chaperone for CDC2 in pachytene spermatocytes. Impairment of these processes may therefore explain failure to complete meiosis I. Data suggest that CDC2 requires direct or indirect interaction with HSP70-2 and that this interaction establishes and/or maintains the CDC2 protein in a conformation that is competent for cyclin B1 binding and to acquire kinase activity in mouse spermatocytes (Zhu et al., 1997). HSP70-2 is normally associated with SCs in pachytene spermatocytes. Loss of the ability of CDC2 to phosphorylate SCP1/SYN1 (that are SC proteins containing a carboxyl-terminus basic domain which is a potential target site for CDC2 protein kinase) may lead to failure of desynapsis (Dix et al., 1997).

In mice homozygous for a germline mutation in *A-myb* gene (a regulator of transcription predominantly expressed in testis) (Toscani et al., 1997), spermatogenesis was arrested at the spermatocyte I stage, probably at early pachytene. Spermatogonia and preleptotene spermatocytes were normal whereas most pachytene spermatocytes showed degeneration and a large number of apoptotic cells were observed in testis. $A\text{-}myb^{-/-}$ mice exhibited an increased expression of genes in spermatogonia and preleptotene. This may reflect either a compensatory feedback regulatory effect due to the lack of fully differentiated end-product cell types or may be due to a relative increase in the proportion of the populations of early germ cells.

The *p53* tumor-suppressor gene is regarded as the "genome guardian" which arrests cells to permit the repair of genetic damage occurring spontaneously during normal DNA replication. Testes of *p53* promoter-CAT transgenic mice (Rotter et al., 1993) showed a reduction in the expression of p53 protein which could be attributable to competition of the molecular excess concentration of the transgene *p53* promoter sequences with the endogenous *p53* promoter sequences. Testes contained multinucleated giant cells spreading from the spermatogonia layer into the lumen of the seminiferous tubules, suggesting that they represent abnormal progenies of the 4N primary spermatocytes. Depending on the level of the reduction in the p53 mRNA, they ranged from a sporadic appearance of a single giant cell, to large numbers of giant cells per tubule, to total depletion of the seminiferous tubules. Homozygous *p53* null mice of the genetic 129/Sv background from two separate studies also exhibited multinucleated giant cells

and a high incidence of seminomas (Donehower et al., 1992; Rotter et al., 1993). To account for the giant-cell degenerative syndrome, it has been suggested that either cells undergo additional rounds of DNA replication, possibly accompanied by uncontrolled gene amplifications or that spermatocytes are unable to complete the process of the cytoplasmic bridges (Rotter et al., 1993). The use of *p53* null mutation in mice sharing synapsis anomalies (X^Y*0 and T16/Y) has demonstrated that apoptotic elimination of spermatocytes with synaptic errors is p53-independent (Odorisio et al., 1998).

Genes expressed at meiotic and post-meiotic stages

Bone morphogenetic proteins (BMPs) constitute a large group of secreted molecules of the transforming growth factor-ß (TGFß) superfamily. *Bmp8b* is expressed at low level in spermatogonia and early meiotic germ cells (early postnatal period), at low level in spermatogonia and spermatocytes (before 3.5 weeks of age) and at high level in round spermatids (after 3.5 weeks of age). Male mice with an inactivated *Bmp8b* (Zhao et al., 1996) exhibited two separable phenotypes during spermatogenesis. During early puberty, there was a block of germ-cell proliferation in 20-30% of the testis, suggesting a major role of BMP8B in the initiation of meiosis. During mid- and late-puberty, there was a recovery of spermatogenesis, possibly due to the presence of BMP8A. During early adulthood, the increased apoptosis of pachytene spermatocytes resulted in a decreased number of spermatids, showing that BPM8B is required for the maintenance of spermatogenesis. Therefore *Bmp8b* is required for both the initiation and maintenance of spermatogenesis in the mouse. By contrast, without a functional *Bmp8a* (Zhao et al., 1998), no obvious germ cell deficiency was observed in the majority of mice during the initiation of spermatogenesis. However, as the mice aged, 47% of the *Bmp8a* homozygous mutants showed different degrees of germ cell degeneration suggesting that *Bmp8a* plays a role in the maintenance of spermatogenesis. Epididymis degeneration after inactivation of *Bmp8a* suggested that BMP8A acts as a paracrine molecule on the epithelium of the epididymis to maintain its survival.

The *c-mos* proto-oncogene encodes a serine-threonine protein kinase and is known to have a transforming potential. In male germ cells, it is expressed predominantly in round spermatids and at a low level in pachytene spermatocytes. A level of *c-mos* below the detection in spermatogonia has not been excluded. Overexpression of *c-mos* as early as the pachytene step (Higgy et al., 1995) induced a marked increase in germ cell number, contrary to overexpression of *c-mos* in only spermatids. The *c-mos* RNAs accumulated in spermatocytes and increased in spermatids, the *c-mos* protein $p43^{c\text{-}mos}$ being increased in both cell types. There was a 78% increase in numbers of total male germ cells (46.5% in increase spermatocytes and 45% in increase spermatids) and 11% increase in testis weight. Nevertheless, testes did not reveal abnormalities in the germ cell epithelium or the interstitial cells and *c-mos* transgenic mice were normally fertile. One explanation of the observed phenotype is a change in the timing of meiosis: *c-mos* in transgenic mice could lead to reduction or inactivation of an intercellular signal from pachytene spermatocytes that normally inhibits proliferation of less mature germ cells.

The v-*Mos* can associate with microtubules, has a kinase activity, phosphorylates tubulin and *Mos* RNA is present in post-meiotic spermatids. The overexpression of v-*Mos* in primary spermatocytes has led to arrest of spermatogenesis at the metaphase plate of the first meiotic division (Rosenberg et al., 1995). The number of mitotically active cells, of leptotene-zygotene spermatocytes and of metaphasic spermatocytes I were increased, indicating an alteration in the timing of meiosis. Metaphase spindles failed to stain with anti-α and anti-ß tubulin antibodies and a difference in γ-tubulin was found in arrested cells. An increased level of phosphoproteins with metaphase-specific epitopes and induction of MAP kinase and MAP kinase-related proteins was also observed.

Basigin (Bsg) is a highly glycosylated transmembrane protein and a member of the immunoglobulin (Ig) superfamily with two Ig-like domains. Basigin mRNAs are strongly expressed in spermatocytes and spermatids. $Bsg^{-/-}$ embryos (Igakura et al., 1998) mainly died in the peri-implantation stage. The surviving mice had reduced body weights and $Bsg^{-/-}$ males were sterile. In the adult, germ cells were mostly arrested at the metaphase of the first meiosis and degenerated. Only a small number of these cells differentiated to step 1 spermatids; they degenerated at this point and no germ cells beyond this stage were found. Before the metaphase arrest, germ cells looked morphologically normal. The Ig superfamily could arise as a means to regulate complex reproductive processes in mammals.

Calmegin is a testis-specific, Ca^{2+}-binding protein, homologous to calnexin, an ubiquitous ER chaperone. Calmegin binds to nascent polypeptides during spermatogenesis and is expressed from pachytene spermatocyte to spermatid stage. Spermatogenesis was normal in $calmegin^{-/-}$ mutant mice (Ikawa et al., 1997) although they were sterile. All sperm parameters were normal and no differences were found in sperm proteins tested between -/- and +/+ mice. Sperm from -/- males failed to adhere to the oocyte *in vitro* despite frequent collisions with the zona pellucida. Calmegin could ensure the proper maturation of certain sperm surface protein(s) required for binding to the oocyte extracellular matrix.

Genes expressed in haploid germ cells

The transcriptional activator CREM (cyclic AMP-responsive element modulator) is highly expressed in postmeiotic cells. Heterozygous *Crem*-deficient mice

(Blendy et al., 1996; Nantel et al., 1996) showed a reduction in the number of spermatozoa and in the ratio of motile spermatozoa, most spermatozoa having a kink and bubble-like structure midway along the tail. In homozygous *Crem*-deficient mice, spermatogenesis was arrested at the first step of spermiogenesis, before elongating spermatids. Multinucleated giant cells and highly refractile smaller bodies contained high concentrations of DNA-free ends. Expression of premeiotic germ cells genes appeared unchanged while RNAs for several genes normally expressed in post-meiotic germ cells were not found in *Crem*-deficient mice. Crem may be responsible for the activation of haploid germ-cell specific genes such as protamines 1 and 2, and transition proteins 1 and 2.

The ubiquitin-conjugating yeast enzyme RAD6 is known to ubiquinate histones 2A and 2B *in vitro* and HR6A and HR6B are mammalian homologues of RAD6. Murine HR6B is designated *mHR6B*. Testis of adult *mHR6B*$^{-/-}$ mice (Roest et al., 1996) showed shedding of spermatids to total absence of all germ cell types depending on the males. In most knockout males with spermatogenesis, only few spermatozoa were found. At least 70% of these spermatozoa had an aberrant head morphology, in most cases combined with middle piece deformation. The residual spermatozoa appeared almost immotile and few spermatozoa displayed a sluggish progression or nonprogressive motility. In testis, round and elongated spermatids displayed an irregular orientation and distribution and were prematurely released. The small number of elongating/condensing spermatids present had an abnormal morphology. Transition proteins were synthesized but were not uniformly located in the nucleus. Data suggest that in mammalian spermatids, the functional homologues of RAD6 polyubiquinate histones for their degradation and replacement by transition proteins and, subsequently, by protamines.

Bclw is a death-protecting member of the *Bcl2* family which is expressed in elongating spermatids and Sertoli cells. Homozygous *Bclw*-deficient mice (Ross et al., 1998) were sterile and the spermatogenesis was arrested at the elongating spermatid stage. There was apoptosis of spermatocytes and degeneration of elongating spermatids, and at 5-6 months of age, most seminiferous tubules lacked germ cells. At 8 months, most Sertoli cells were sloughed or lost from the tubules. *Bclw* may be required for haploid germ cell differentiation in a cell-intrinsic manner but it was not possible to demonstrate whether germ cells were also affected at all stages by an indirect effect of aberrant Sertoli cell function.

In mice expressing the boar proacrosin at the post-meiotic steps, the exogenous proacrosin failed to reach the acrosome (O'Brien et al., 1996). Boar proacrosin appeared primarily in vesicles of condensing spermatids, then was dispersed throughout the cytoplasm of spermatids or confined to vesicular structures near the tubule lumen. Delays in transcription and/or translation of the transgene may account for the acrosomal targeting defect. Few spermatozoa were produced that were frequently rounded and blunt at the apical tip. Irregularly-shaped giant cells with several flagella and multinucleated cells were common and the seminiferous tubules were disorganized. Infertile males produced fewer spermatozoa because many spermatids did not reach the condensing stage. An explanation may be that boar proacrosin was activated and that its proteolytic activity disrupted spermatogenesis during spermatid formation. The conversion to an active protease could result from either species differences in praocrosin structure and cleavage of the molecule or lack of specific proteins that bind to the inactive precursor.

Mice carrying a mutation at the acrosin locus ($Acr^{-/-}$) produced sperm with an acrosin-like activity, either very low (the residual activity could be due to other serine proteases) (Adham et al., 1997) or null (Baba et al., 1994). Capacitated homozygous mouse sperm penetrated the zona pellucida and fertilized the oocytes but had a selective disadvantage (Adham et al., 1997) due to a delayed penetration and fertilization (almost 30 min, Baba et al., 1994). However, $Acr^{-/-}$ mice were fertile and yielded litters of comparable number and size to those of $Acr^{+/+}$ mice.

Mice expressing the galline protamine (Maleszewski et al., 1998) had an apparently normal germ cell phenotype, but the spermatozoa were vulnerable to mechanical handling, as about 40% of heads and tails were separated after gentle pipetting in suspension. The chromatin was heterogenous and unstable and the motility was markedly reduced. The spermatozoa completely failed to fertilize and bound to zona pellucida, and none of them inserted their heads into the zona. Their ability to fertilize zona-free oocytes *in vitro* indicated ability to undergo capacitation and acrosome reaction *in vitro*.

Angiotensin-converting enzyme (ACE) catalizes the cleavage of C-terminal dipeptides from several substrates. The gene for ACE codes for both a somatic and a smaller testis-specific isozyme. Testis ACE protein in the mouse is first detected in haploid spermatids. It has been generated mice carrying a disruption of the *Ace* gene which prevents the synthesis of both testis and somatic ACE (Hagaman et al., 1998). Sperm from homozygous males exhibits normal parameters. However, they achieved *in vitro* fertilization at a reduced frequency and showed defects in binding to zona pellucida. Moreover, few spermatozoa from homozygous males were found in oviduct regions above the intramural uterotubal junctions 1 hr after mating. This raises the possibility that the release of testis ACE is important for the detachment of sperm from the oviduct epithelium at capacitation. Perhaps the membrane domain that binds to the oviduct epithelium may share ACE and would leave behind a specific remnant on the sperm surface by ACE proteolysis (Hagaman et al., 1998).

Transcripts of the PC4 serine protease have been

Table 3. Germ cell behaviour, testis injuries and fertility. In all cases knock-out null mutant mice were infertile (heterozygous were fertile)

GENOTYPE	GERM CELL FATE	TESTIS	FEMALE
Tiar$^{-/-}$	absent		oogonia deficiency
mTR$^{-/-}$	apoptosis	germ cell loss Leydig hyperplasia	reduced ovary size, morula stage arrest
Dazla$^{-/-}$	depleted		atretic ovary
c-myc (exogenous)	apoptosis	Sertoli injury	
Bcl-2 (exogenous and misexpression) •	multinucleated giant cells, apoptosis, depleted	Sertoli injury	reduced fertility
Bax $^{-/-}$	apoptosis, multinucleated cells	disorganized, Sertoli injury	ovary atrasia or not
Dmc1$^{-/-}$	apoptosis (5-10% of tubules)	germ cell loss	oocyte loss
Mlh1$^{-/-}$	apoptosis, multinucleated cells		meiosis I arrest
Pms2 $^{-/-}$	few abnormal spermatozoa	Sertoli injury	
Atm$^{-/-}$	apoptosis, chromosome fragmentation	Sertoli injury	no oocytes I
Abl$^{-/-}$	degenerative	disorganized	
Hsp 70-2$^{-/-}$	apoptosis	Sertoli injury	
A-myb$^{-/-}$	apoptosis	Sertoli injury	(defective breast tissue)
p53$^{-/-}$ or reduced	depleted		
Bmp8b $^{-/-}$*	depleted, apoptosis	Sertoli injury	
Bmp8a $^{-/-}$	apoptosis		
v-*Mos* (overexpression)	mitotic/meiotic cell accumulation	Leydig hyperplasia	
Bsg$^{-/-}$	spermatocyte I abundant		defective implantation
Crem$^{-/-}$	apoptosis, multinucleated cells		
mHR6B$^{-/-}$	apoptosis, depleted	disorganized	
Bclw$^{-/-}$	apoptosis, degeneration	testis depletion	
Acr (exogenous)	multinucleated cells	disorganized	

•: bcl2 $^{-/-}$ and Bclx$^{-/-}$ were fertiles; *: some fertile males

detected in spermatocytes and spermatids. Male fertility was found to be reduced in *Pcsk4*$^{-/-}$ mice in the absence of any evident spermatogenic abnormality, except that the percentage of hyperactivated spermatozoa was lower (Mbikay et al., 1997). Sprm-1 is the Oct-3/4-like POU factor (POU-homeodomain gene family) expressed predominantly in spermatids. *Sprm*-1$^{-/-}$ mice were subfertile on the 129/Sv genetic background, although the sperm parameters appeared normal and the fertility potential was comparable to the wild-type mice following *in vitro* fertilization. It was suggested that *Sprm-1* may regulate a haploid gene pathway that is required for optimum fertility (Pearse et al., 1997). Beta 1,4-galactosyltransferase is a candidate receptor for ZP3. Beta-1,4-galactosyltransferase null (*gt*$^{-/-}$) males were fertile but spermatozoa from *gt* $^{-/-}$ mice were defective for sperm-egg interaction *in vitro*, in particular for the acrosome reaction in response to ZP3 and for the penetration of the egg coat. This suggests that ZP3 binding and subsequent induction of the acrosome reaction are dispensable for fertilization but impart advantage to the fertilizing sperm (Lu et al., 1997). In contrast, alpha-1,3-galactosyltransferase null males had normal sperm-zona pellucida interaction and fertilization *in vitro* (Liu et al., 1997).

Germ cell phenotypes in cases of insertional mutations

Data from germline technology which is relevant for the male reproductive function can also be obtained by chance as a result of insertional mutation (Simoni, 1994) which may lead to identification of new genes, such as for the ligand of the *c-Kit* receptor in the *Steel* locus (Keller et al., 1990).

Symplastic spermatids is a recessive insertional mutation in mice affecting an unidentified gene on chromosome 14 (MacGregor et al., 1990). Spermatids from homozygous (*sys/sys*) males form multinucleated syncytia (symplasts) and did not complete maturation, leading to azoospermia. Symplasts result from premature opening of the intercellular bridges that connect round spermatids. Young round spermatids and spermatocytes were occasionally seen to degenerate, and some seminiferous tubules appeared depleted of germ cells, except in the basal compartment. Sertoli cells exhibited prominent defects, such as cytoplasmic vacuolation and degeneration, suggesting that Sertoli cell abnormalities were responsible for germ cell degeneration (Russell et al., 1991). Spermatids in symplasts (Fig. 6B in Russell et al., 1991) initiated nuclear elongation and formed an acrosome, while chromatin condensation and flagellar growth did not occur.

Expression of the human epidermal growth factor receptor (EGF receptor) (Merlino et al., 1991) was found at the cell surface of late spermatids, then concentrated within residual bodies and was not detected on mature sperm. A transgenic line of this study showed spermatozoa with a rigid immobile middle piece, and rapid motion restricted to the end piece and part of the principal piece. An average of 71% of sperm were missing axonemal outer doublets, resulting in a 5+2 pattern in the middle piece. These flagellar anomalies were observed in the vas deferens and the epididymis but not in the testis, suggesting extensive microtubule

disruption and/or disassembly after spermiation. It was proposed that the EGF receptor transgene was integrated into an important testis-specific gene. The endogenous gene could encode a highly expressed, haploid-specific axonemal protein or, alternatively, a protein that indirectly dictates axonemal stability.

Mutation *Lvs* (lacking vigourous sperm) has been found in mice carrying the *HCK* protooncogene as a transgene. The spermatid nuclei were misshapen, short and wide. The acrosome and the tail appeared normal, as was also the morphology of Sertoli cells. The abnormal phenotype was probably caused by the insertion itself disrupting a gene (or genes) important in spermatogenesis (Magram and Bishop, 1991).

Mice homozygous for a tyrosinase minigene (Russell et al., 1994) exhibited two different germ cell phenotypes depending on the insertional mutation caused by genetic engineering. In some males the flagellum fails to develop normally. It appeared as a round process containing disorganized microtubules and crystalline tubulin. In other males, there were anomalies of the acrosome spreading over the nucleus, of the nuclear envelope and of nuclear elongation due to manchette anomalies. Modification of the nuclear envelope may be the primary event that leads to the other morphogenesis anomalies of the spermatid head structures (Russell et al., 1994). Impairment of spermatozoa release and of sperm cytoplasm elimination had been induced following an inserted additional renin gene in rat (Sharpe et al., 1995).

Germ cell molecular lesions frequently lead to germ cell apoptosis

Table 3 shows that in many cases, arrested germ cells undergo apoptosis, the initiating signals of apoptosis being dependent on the molecular lesions induced. Explanations for this germ cell behaviour have been proposed in some studies. The *c-myc* is thought to function in the induction of apoptosis of growth-arrested cells *in vitro*. An analogous mechanism could occur in germ cells since type-B spermatogonia and spermatocytes (which undergo apoptosis in mice overexpressing *c-myc*) may be considered to be in a physiological condition similar to the growth-arrested condition *in vitro* (Suzuki et al., 1996). *Hsp70-2*$^{-/-}$ mice exhibited apoptotic late pachytene spermatocytes as early as the first wave of spermatogenesis, suggesting that a process activating apoptosis is potentiated, or a process inhibiting apoptosis is compromised in the absence of HSP70-2. Proteins involved in DNA repair or recombination that require HSP70-2 chaperone activity may be incorrectly folded, transported, or assembled, thereby disrupting the balance between inhibitors and inducers of apoptosis and thus leading to germ cell death (Dix et al., 1996). In *Crem*-deficient mice, apoptosis was 10-fold higher than in normal mice and appeared coincidentally with the maturation of haploid spermatids during prepubertal development, suggesting that Crem may be linked to cell cycle gene regulation and the induction of apoptosis in certain cell cycle types (Nantel et al., 1996). *mHR6B*$^{-/-}$ mice presented a 4-fold increase in the number of apoptotic germ cells that were clustered and localized in the layers that contain primary spermatocytes. An explanation is that the ubiquitin system plays a role in numerous cellular processes, among them metabolic homeostasis, cell cycle regulation, apoptosis and gene expression (Roest et al., 1996).

In *A-myb*$^{-/-}$ mice, both degeneration (cellular breakdown) and apoptosis have been observed (Toscani et al., 1997). However, in most cases of mutant mice created by genetic engineering, arrested germ cells undergo active cell death (apoptosis) rather than failure of homeostasis (necrosis). It has been suggested that lack of an essential gene can cause germ cells to cease differentiation and that apoptosis could be a general pathway for eliminating germ cells whose differentiation is compromised (Mori et al., 1997). The defects in spermatogenesis in both *Bax* and p53 models signify a role for the apoptotic pathway in monitoring male gametogenesis, and *Bax*-deficient mice provide evidence for an interrelationship of proliferation, differentiation, and cell death (Knudson et al., 1995). Misexpression of *Bcl-2* in spermatogonia indicates that renewal, differentiation and apoptosis of spermatogonia are closely related and that a pathway affected by *Bcl-2* plays a major role in switching the developmental fates of spermatogonia (Furuki et al., 1996).

Germ cell apoptosis is more frequently encountered in cases of disruption of a gene expressed in primary spermatocytes. Apoptosis of arrested pachytene spermatocytes in mice deficient in *Mlh1* suggest a meiotic checkpoint arrest and that cells that are arrested become slated for apoptosis (Edelmann et al., 1996). Primary spermatocytes are particularly sensitive to dysregulation of the apoptotic pathway, as seen by the induction of apoptosis both in mice with exogenous *Bcl-2* (inhibiting apoptosis factor) (Furuki et al., 1996) and inactivated *Bax* (promoting apoptosis factor) (Knudson et al., 1995). Apoptosis of pachytene spermatocytes was observed in double-homozygous mutant males (*Hsp70-2*$^{-/-}$, *p53*$^{-/-}$) showing that apoptosis is activated by a p53-independent mechanism (Dix et al., 1997).

What happens to Sertoli cells when germ cells are genetically modified?

Table 3 shows the intriguing fate of many of the genetically modified testes which exhibit Sertoli cell injury and other testis alterations which can be attributed to Sertoli deficiencies, such as germ cell sloughing and giant cell formation. Sertoli cell injury was observed in mice overexpressing *c-myc*, with the Sertoli cells either exhibing giant vacuoles or being detached from the basement membrane (Suzuki et al., 1996). Similar Sertoli cell behaviour was seen in *mHR6B*$^{-/-}$ mice (Roest et al., 1996) and in mice with a *Dazla* gene disruption

(Ruggiu et al., 1997). In *Hsp70-2*$^{-/-}$ mice (Dix et al., 1996) and in testes of *A-myb*$^{-/-}$ mice (Toscani et al., 1997) seminiferous tubules contained highly vacuolized Sertoli cells. In *Atm*$^{-/-}$ mice at 6 weeks old (Xu et al., 1996), Sertoli cells were also highly vacuolized and at 2 months, many tubules were barren of all germ cell types (Barlow et al., 1996). Vacuolization of Sertoli cell was not observed in *Crem*$^{-/-}$ mice, but multinucleated giant cells were numerous (Blendy et al., 1996; Nantel et al., 1996). Vacuolized Sertoli cells and multinucleated spermatids were found in mice expressing boar proacrosin (O'Brien et al., 1996) and in *Bax*$^{-/-}$ mice (Knudson et al., 1995).

Death of Sertoli cells by the apoptotic program was not common, contrary to spermatocytes (Mori et al., 1997). In the most representative cases, the Sertoli cells first underwent vacuolization, then fragmentation of the cytoplasmic processes and finally exfoliated. This could lead to testis atrophy with complete depletion of the seminiferous epithelium at 2-3 months of age. Since this involution is progressive, testis examination of 5-8-week-old mice allows the observation of the germ cell fate following genetic engineering. Therefore, data suggest that germ cell factors related to germ cell differentiation and survival may also affect Sertoli cells. This is particularly obvious for genes expressed in the first meiotic prophase.

Testis depletion and involution, as frequently induced in mice by genetic engineering, have been well documented in toxicity studies on rodents (Lee et al., 1993). Soon after puberty, most transgenic mice exhibit testicular changes that can be compared to those induced by expression of tetanus toxin in Sertoli cells (Eisel et al., 1993). Nevertheless, genetic engineering procedure(s) must not be involved. The germ cells overexpressing *c-mos* did not undergo cell death and the increased germ cell ratio obtained did not lead to Sertoli cell injury (Higgy et al., 1995). From constructs using the same promoter, mice expressing the human transcription factor E2F-1 exhibited germ cell apoptosis and depletion (the Sertoli cells did not seem to be affected) but not those expressing the human DP-1 (a partner of E2F-1) (Holmberg et al., 1998). The insertional mutation *Lvs* affecting spermatids did not affect Sertoli cell morphology (Magram and Bishop, 1991).

The presence of several growth factors in spermatocytes suggests that these cells synthesize and secrete paracrine factors which interact with neighboring Sertoli cells (reviewed in Eddy and O'Brien, 1998) and it is known that in particular circumstances, germinal cells affect Sertoli cell function (Jegou, 1993). This fact is illustrated after gene targeting of the *Bpm8b* gene (Zhao et al., 1996) and the *Dhh* gene (*Desert hedgehog*, encoding a protein thought to be involved in cell signaling) (Bitgood et al., 1996). In both cases, a change of Sertoli cell gene expression was detected in tubules after germ cell depletion. It has also been suggested that Sertoli cell injuries can be due to their contact with detrimental germ cell products. In *mHR6B*$^{-/-}$ mice, the formation of vacuoles in Sertoli cells can be explained by the release of protamines known to exert toxic effects on epithelial cells (Roest et al., 1996). In mice expressing boar proacrosin, it has been suggested that an active protease could modify adhesion molecules or junctional specializations between spermatids and Sertoli cells resulting in germ cell sloughing (O'Brien et al., 1996).

However, Sertoli cells are thought to be notoriously resistant to many treatments that affect germ cells (Russell et al., 1990). This is also found following genetic engineering such as in *bclw*-deficient mice (*bclw* is expressed in spermatids and in Sertoli cells) showing extensive apoptosis of spermatocytes but no evidence of simultaneous apoptosis-mediated death of Sertoli cells. Moreover, Sertoli cells persist in the absence of *Bclw* for up to six months (Ross et al., 1998). It is considered that in rodents, deficits in Sertoli cells can result in major germ cell abnormalities but not *vice versa* (Russell et al., 1991). This is illustrated by disruption of the Sertoli cell factor RXRß resulting in male sterility owing to oligo-astheno-teratozoospermia, a consequence of failure of spermatid release and progressive degeneration of the germinal epithelium in addition to acrosome and tail abnormalities (Kastner et al., 1996).

Sertoli cell survival does not necessitate the presence of germ cells, as shown by the well known Sertoli Cell Only syndrome in man. The mice mutation *weaver* (*wv*) (related to an inwardly rectifying potassium channel) (Verina et al., 1995) has been a model to explore this question (Harrison and Roffler-Tarlov, 1998). In homozygous *wv/wv* mice, some seminiferous tubules become depleted of germ cells, but not spermatogonia. The blood-testis barrier is normal in these mice indicating that *wv/wv* Sertoli cells are able to receive and respond appropriately to hormone signals. Moreover, the aged *wv/wv* mice did not show differences in overall testis morphology of their testes from younger *wv/wv* adults, indicating that spermatogenesis is initiated continually in homozygous *weavers* by spermatogonial stem cells and functional Sertoli cells.

In case of germ cell maturation arrest in men, germ cell apoptosis is significantly increased (Lin et al., 1997). However, in the human, developmental stops at meiotic steps are often identified far from puberty due to germ cell renewal for several decades, and the Sertoli cells are maintained (Escalier, 1999). This situation can be compared to mice with a null mutation of the *Dmc1* gene. In 10-week-old mice with a complete arrest at the zygotene stage, only 5-10% of the seminiferous tubules contained apoptotic cells. This suggested that apoptosis occurs in only a restricted region of the tubules, in a rather short time and that the incomplete differentiation cycle occurs repeatedly (Yoshida et al, 1998). Nevertheless, data on knockout mice question the destiny of the human germ cells carrying mutations of genes which could lead to extensive testis depletion (as in the mouse) and whether these mutations can be related to some

Table 4. Male fertility in cases of normal germ cell phenotype and normal testis histology.

GENOTYPE	MALE FERTILITY*
c-mos (overexpression)	normal
Calmegin$^{-/-}$	no, ZP binding failure
protamine (exogenous)	no
Acr$^{-/-}$	delayed *in vitro*
Ace$^{-/-}$	defective
Pcsk4$^{-/-}$	reduced
Sprm1$^{-/-}$	subnormal
gt$^{-/-}$	defective *in vitro*
Xist$^{-/-}$	normal
Cenpb$^{-/-}$	normal

*: knock-out null mutant mice; heterozygous were fertile; no case of affected female fertility

Sertoli Cell Only syndromes in adults.

How can the wild germ cell phenotype be conserved when the germ cell genotype is modified?

Table 2 and 4 present cases of absence of obvious anomalies in the phenotype and functions of male germ cells in null mutants for a germ-cell gene. This fact may be related to functional redundancy of gene products regulating complex developmental processes as strongly suggested in the case of mice carrying a null mutation in the centromere protein B (Cenpb) gene which have normal meiosis (Hudson et al., 1998). Genes that belong to the same family or parallel pathways are often functionally redundant (Lobe and Nagy, 1998). A normal phenotype and function of germ cells could result from the expression of back-up genes induced by loss of function of an inactivated gene.

The numerous reports on mice with inactivation of gene expressed in male germ cells reveal that, in the heterozygous state, testicular changes were infrequent and in no cases was fertility altered. Considering genes expressed at the haploid stage, this suggests that genetically distinct spermatids can be phenotypically equivalent. That haploid spermatids are functionally diploid was earlier demonstrated for transgene expressed post-meiotically: the product of the transgene was found in all spermatids from hemizygous mice (Braun et al., 1989). Male germ cells are characterized by incomplete cytokinesis at each of the mitotic and meiotic cell divisions, as seen by intercellular bridges connecting the cells. Consequently, all the cells derived from a single spermatogonial cell develop within a common syncytium. The testis-brain RNA-binding protein (TR-RBP) moves through intercellular bridges suggesting that TB-RBP has a role in the distribution of equal amounts of mRNAs in haploid male germ cells (Morales et al., 1998). Therefore in heterozygous mutant mice, cytoplasmic communications could provide factors and mRNAs that rescue cells carrying the mutation. Limitations can be the availability of these factors (dosage, stability, functional localization, binding properties). Additional differences may result from recombination placing either an inactivated gene or a transgene in a different environment depending on the cells. The position effect on gene expression can lead to either enhancement or repression of other genes or of the transgene.

Animal models of male reproduction?

It is well known that there are some inherent limitations of transgenic insertions and embryonic stem cell "knockouts" as animal models of human genetic disease. Many "knock-out" mouse models show great variation in phenotype on different genetic backgrounds (reviewed in Erickson, 1996). This may be due to modifiers that are allelic variants at loci other than the one being modified. Genetic modifiers can suppress or enhance the expression of genes involved in the pathophysiological pathway. They may alter transcription rates or mRNA stability. This also concerns the male reproductive function, as illustrated by the following examples. Homozygous *p53* null mice of genetic 129/Sv background exhibited multinucleated giant germ cells and a high incidence of seminomas, but this phenotype was not found on the C57BL/6 background (Rotter et al., 1993). On a mixture of the inbred strains CBA and C57BL/6, mice overexpressing the transcription factor E2F-1 did not get testis tumors. On a mixture comprising, in addition, the 129 background, they developed testicular teratoma (Holmberg et al., 1998). *Sprm-1*$^{-/-}$ mice on the C57BL/6 background were fertile. On the 129/Sv genetic background the effective fertility of *Sprm-1* $^{-/-}$ males was so low that it was not possible to sustain a 129/Sv colony using only mutants (Pearse et al., 1997). It is noteworthy that the 129 strain is known to have a low fertility potential, and that teratoma of the testis is the most common tumor in strain 129 (Stevens, 1973). Also, the embryonic stem cells usually used for gene inactivation are obtained from the 129 strain. The determination of the best genetic background may enhance the usefulness of a model.

Other influences such as position-effect variegation may have indirect modifying effects, particularly in the case of transgenic insertion. Also, confounding effects due to the promoter used may be particularly important regarding the highly regulated proliferation and differentiation process of the spermatogenesis. It has already been suggested that (over)expressing transgenes in the testis may result in complex perturbations of testicular function that are of yet indefinite relevance to clinical andrology (Simoni, 1994). Moreover, transgenic experiments often involve the insertion of tandem multiple-copy arrays at single sites and transgene arrays often show unpredictable levels of expression. It has been demonstrated in *Drosophila* that repeat arrays condense into heterochromatin inducing their silencing. In transgenic mouse lines, the reduction of copy number

of silenced transgenes leads to a striking increase in their expression demonstrating that the silencing is intrinsic to the array. Repeat-induced gene silencing (RIGS) seems to be a genome defense mechanism (reviewed in Henikoff, 1998).

An insight into other data of particular interest

Some results from mutant mice were unexpected. Targeted mutation of the *FSHβ* gene, a factor considered essential for gonadal function, did not affect male fertility (Kumar et al., 1997). Inactivation of the α-nuclear oestrogen receptor (oestrogen considered as the "female" hormone) results in male infertility (Eddy et al., 1996; Hess et al., 1997). *Dazla*, first suspected as a male fertility factor, appears implicated in oocyte maturation (Ruggiu et al., 1997). Disruption of the *A-myb* gene predominantly expressed in male germ cells leads to male infertility, but also to breast tissue underdevelopment (Toscani et al., 1997). Of particular interest, deletion of the *Xist* gene revealed no effect on male fertility, suggesting no role of *Xist* in meiotic X chromosome inactivation (Marahens et al., 1997). Genetic engineering reveals that defects can appear only after several generations, as found in mice lacking the telomerase RNA (Lee et al., 1998).

Results from knock-out mice have shown the genetic factors common to germ and somatic cell lines. This could be related to gene products involved in highly reproductive organs, such as the telomerase RNA (Lee et al., 1998). Immune system development failure, neurological anomalies and growth retardation can be associated with male sterility such as for the gene mutated in Ataxia Telangiectasia (*Atm*) (Barlow et al., 1996). Moreover, targeted mutagenesis has revealed that genes involved in meiotic recombination may have a role in tumorigenesis such as for the *Atm* gene (Hawley and Friend, 1996) and the DNA mismatch repair gene *Pms2* (Baker et al., 1995). Also those mutants have shown that genes involved in the recombination machinery may be sex-specific (Table 3) (Baker et al., 1996; Edelmann et al., 1996). When genes are known to be involved in human diseases, knock-out mice provide the basis for medical advances, as in Ataxia Telangiectasia (Keegan et al., 1996) and Fanconi anemia (Whitney et al., 1996).

Conclusion

Data obtained by genetic engineering have shown the contribution of many genes in spermatogenesis. Taken together, these data are the source of much information on the initiation and maintenance of spermatogenesis, germ cell survival and proliferation, meiosis developmental program and meiotic checkpoints, somatic and germ cell interrelations, spermiogenesis and fertilizing ability.

Knock-outing or misexpression of genes that are expressed early in spermatogenesis appears to have the effect of aborting spermatogenesis rather than changing the cellular fate of germ cells. This could be of interest for studies on the relationships between germ cell checkpoint controls and signals for apoptosis. When investigated, such as in *Hsp70-2*$^{-/-}$ mice (Dix et al., 1997) and in *A-myb*$^{-/-}$ mice (Toscani et al., 1997), there is neither premature down-regulation of genes expressed during prophase of meiosis nor premature initiation of expression of genes transcribed in postmeiotic spermatids. Targeting a gene expressed in post-meiotic germ cells frequently leads to azoospermia or teratozoospermia. In other cases, modifying gene expression does not change the germ cell phenotype and can be compatible with fertility (with reduced performances or not). A variety of germ cell phenotypes has been obtained by insertional mutations affecting expression of a gene(s) involved in spermatogenesis. Although these gene(s) remain to be identified, these mutants represent models for azoospermia, oligozoospermia and asthenozoospermia (Lamb and Niederberger, 1994; Simoni, 1994).

So far, in most cases, gene inactivation concerned proteins involved in meiosis and/or in metabolism. This may explain why this results in germ cell death in many cases and sometimes in seminiferous epithelium extensive injuries. Targeting germ cell-specific genes related to structural proteins should lead to very different germ cell phenotypes. Moreover, promising alternatives to initial gene-targeting approaches are to create conditional genome alterations by controlling their onset, frequency, spatial location, and tissue/cell type specificity (reviewed in Lobe and Nagy, 1998) and which will address specific aspects of mutant phenotypes.

Notes added in proof

Genetic engineering has allowed to identify additional genes involved in the mouse spermatogenesis. Most often, disruption of genes expressed in germ cells have led to germ cell apoptosis, testis depletion and Sertoli cells injuries: *movo1* (Dai et al., Gene Dev., 1998, 12: 3452), *bcl-w* (Print et al., Proc. Natl. Acad. Sci. USA, 1998, 95: 12424), *PPlcγ* (Varmuza et al., Dev. Biol., 1999, 205: 98). A diplotene I arrest has been obtained disrupting the *cyclin A1* gene (Liu et al., Nature Genet., 1998, 20: 377) and an insertional mutation in the *morc* locus has resulted in a leptotene/zygotene I arrest (Watson et al., Proc. Natl. Acad. Sci. USA, 1998, 95: 14361).

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