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Identification of *morc* (*microrchidia*), a mutation that results in arrest of spermatogenesis at an early meiotic stage in the mouse

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ABSTRACT **The *microrchidia*, or *morc*, autosomal recessive mutation results in the arrest of spermatogenesis early in prophase I of meiosis. The *morc* mutation arose spontaneously during the development of a mouse strain transgenic for a tyrosinase cDNA construct. *Morc* −/− males are infertile and have grossly reduced testicular mass, whereas −/− females are normal, indicating that the *Morc* gene acts specifically during male gametogenesis. Immunofluorescence to synaptonemal complex antigens demonstrated that −/− male germ cells enter meiosis but fail to progress beyond zygotene or leptotene stage. An apoptosis assay revealed massive numbers of cells undergoing apoptosis in testes of −/− mice. No other abnormal phenotype was observed in mutant animals, with the exception of eye pigmentation caused by transgene expression in the retina. Spermatogenesis is normal in +/− males, despite significant transgene expression in germ cells. Genomic analysis of −/− animals indicates the presence of a deletion adjacent to the transgene. Identification of the gene inactivated by the transgene insertion may define a novel biochemical pathway involved in mammalian germ cell development and meiosis.**

The genetic control of spermatogenesis is complex (1). Mutations at multiple loci and in structurally and functionally disparate genes in the mouse genome affect gametogenesis (2). Most mutations are pleiotropic, causing multisystem pathologies rather than isolated spermatogenic abnormalities. For example, the autosomal recessive mutation *weaver,* which results in degeneration of germ cells, also causes loss of the cerebellar granular cell layer and ataxia in affected mice (3). The histologic phenotypes of mutations that affect germ cells are varied and include both reduced cell numbers and abnormal cell morphologies. Further complicating our understanding of germ cell biology is the fact that genes known to be essential for spermatogenesis participate in multiple cellular processes, including transcriptional control (4, 5), cell proliferation (6), protein folding (7), and DNA repair (8, 9).

In this study, we report a mouse mutant, *morc* (for *microrchidia*), that arose by insertional mutation. The mutation causes complete arrest of spermatogenesis at an early meiotic stage. Our immunofluorescence results show that *morc* −/− germ cells enter meiotic prophase, but arrest before the pachytene stage, suggesting that *Morc* may regulate early meiotic prophase events involving chromosome synapsis or recombination. Initial molecular characterization of the *morc* locus demonstrates a deletion of genomic sequences flanking the transgene. These data indicate that *morc* sterility results from a deletion of all or part of a gene required for progression of male germ cells through meiosis.

MATERIALS AND METHODS

Transgenic Mice. FVB/n inbred mice were obtained from Harlan Laboratories (Houston, TX). The transgene construct included the mouse RNA polymerase II 5′ regulatory region (GenBank no. M14101, nucleotides 1–712), a loxP recognition site (5′-GGAACCCTTAATATAACTTCGTATAATG-TATGCTATACGAAGTTATTAGGTCCCTCGAC-3′) and the mouse tyrosinase coding region (GenBank no. D00440 nucleotides 1–1976) cloned into the *Bam*HI site of pBluescript SK^-. The insert was excised with *Not*I, purified, and injected into male pronuclei by using standard transgenesis protocols (10).

Morphometrics and Statistical Analysis. Mice were euthanized with CO_2, and weights were obtained for total body, testes, and epididymides. One-way ANOVA was used to test whether the testes weights differed significantly for mice of different genotypes.

Histologic Assays. Tissues were immediately fixed in 10% buffered formalin or Bouin's fixative for 16 h at 4°C, followed by equilibration in 70% ethanol with standard processing and paraffin embedding. Five-micrometer-thick sections were stained with hematoxylin/eosin or periodic-acid Schiff-base for routine histology. For immunohistochemistry, sections were deparaffinized, preblocked with rabbit serum, and treated with either antityrosinase antibody (rabbit antipeptide serum PEP7, ref. 11) at 1:1,000 dilution, rabbit control serum (1:1,000), or PBS alone. Bound antibodies were detected with biotinylated goat anti-rabbit antibody (Vector Laboratories), then streptavidin horseradish peroxidase (Vector) followed by chromagen development (diamino benzidine, SigmaFast D-4293) for 5 min with final hematoxylin counterstain. Immunofluorescent detection of synaptonemal complex antigens was as described (12), using polyclonal mouse antibodies anti-COR1, recognizing mouse SYCP3, and rabbit anti-SYN1, recognizing mouse SYCP1 (13). Apoptosis in testes sections was detected by using a modified fluorescent terminal deoxynucleotidyltransferase-mediated UTP end labeling (TUNEL) assay (14). Briefly, biotinylated dCTP (GIBCO/

Abbreviations: *morc*, *microrchidia*; TUNEL, terminal deoxynucleotidyltransferase-mediated UTP end labeling; STS, sequence-tagged site; SC, synaptonemal complex; dpp, postpartum day.

Data deposition: The sequence reported in this paper has been deposited in the GenBank database (accession no. AF089712).

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BRL) was incorporated by using terminal deoxynucleotidyl transferase (TdT, GIBCO/BRL), and detected by binding fluorescein isothiocyanate-labeled streptavidin (Vector). Proteinase K digestion was 30 min at 37°C, and TdT incubation was 90 min at 37°C. Control tissues included small intestine and thymus.

Tyrosinase Assays. Cells and tissues were lysed in buffer containing 2% 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate, 50 mM Hepes, 200 mM NaCl, pH 7.5, and protease inhibitors. Tyrosinase assays were performed as described (15). One unit of tyrosinase was defined as the amount of enzyme that catalyzed the oxidation of 1 mmol of tyrosine in 1 min.

Molecular Biology. A phage library was constructed in vector EMBL3 from a complete *Bam*HI restriction digest of −/− mouse DNA, size selected for 15- to 20-kb fragments. Screening the library with a polII-loxP chimeric probe yielded five clones, four of which had identical restriction patterns. A subclone of one of these four phages was sequenced to generate a sequence-tagged site (STS). This STS was used to screen a commercial mouse P1 genomic library (Genome Systems, St. Louis), and two clones were obtained. Subcloning of one of the P1 clones yielded a 3.9-kb *Eco*RI fragment containing the transgene insertion site. Standard protocols were used for preparation of genomic DNA from tails or livers, extraction of total RNA from testes, and Southern and northern blotting (16). Genotyping was performed by using STSs specific for either the wild-type locus or the rearranged transgenic locus. The transgenic STS produced a 200-bp PCR product using a polymerase II primer (AGTTAGCCGTTATTAGTGGAGAGG) and a primer from *morc* −/− flanking genomic sequences (AACTTGTAACTCAGGCTACAT). The wild-type STS (primers GGTTGGCTTCAAATTCATGGT and CATGGAGGTGTGAGCTAGGTG) was derived from sequences deleted in *morc* −/− mice and amplified a 93-bp product. PCR was performed by using standard conditions and products analyzed on 3% agarose gels. The *morc* (*microrchidia*) locus name and symbol has been approved and reserved by the International Committee on Standardized Nomenclature for Mice (17). The transgene we describe is designated TgN(Tyr)1Az, and the allele of the *morc* locus identified by the insertion is designated *morc*$^{TgN(Tyr)1Az}$ in accordance with the committee's rules.

RESULTS

Origin of the *morc* Mutation. To generate a strain of mice in which coat color could be used to assay for subsequent cre-mediated recombination events, FVB/n fertilized eggs were injected with a mouse polII-loxP-tyrosinase construct that drives tyrosinase expression from an RNA polymerase II promoter (see *Materials and Methods*). In the single line produced, the transgene rescued eye pigmentation in a dosage-dependent fashion [wild type (+/+) = pink; heterozygous transgenic (+/−) = brown; homozygous transgenic (−/−) = black], but coat color remained white regardless of genotype (Fig. 1, *Upper*). Thus despite having a "ubiquitous" promoter, the transgene was expressed in a tissue-restricted fashion. The eye color phenotype was used advantageously as an indicator of genotype for crosses.

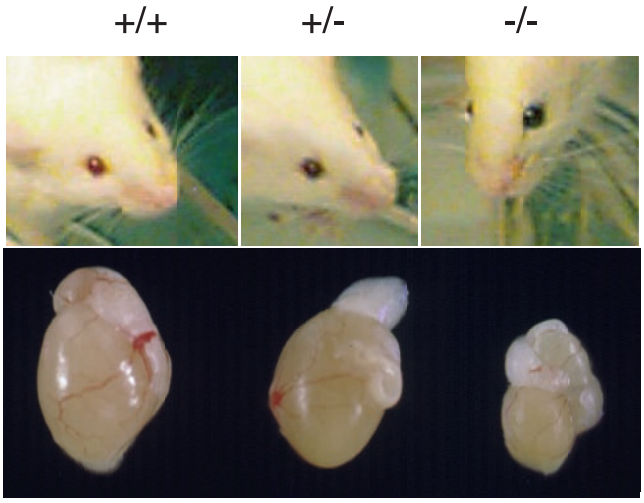

FIG. 1. Gross appearance of *morc* mice. (*Upper*) The wild-type FVB (+/+) pink (albino) eye color contrasts with the brown (+/− mice) or black (−/− mice) eye pigmentation caused by one or two copies of the *morc* transgene, respectively. (*Lower*) A marked size reduction is noted in the −/− testis compared with +/+ or +/− testis. Epididymides were of similar size in all three genotypes (the normal epididymis was inadvertently removed from the +/− specimen). The +/− testis is slightly pigmented because of transgene expression (see text).

When F_3 transgene-positive animals were mated, it became apparent that −/− males never sired any offspring, despite normal copulatory behavior. Test matings were set up between wild-type and +/− or −/− animals of both sexes. Homozygous or heterozygous transgenic females mated with wild-type FVB males gave birth to normal-sized litters with the expected mendelian ratio of genotypes. Heterozygous males were also fertile, indicating that the infertility phenotype was recessive.

Description of the *morc* −/− Phenotype. At autopsy, testes of −/− males were grossly smaller than testes of +/+ or +/− animals (Fig. 1, *Lower*). On average, −/− testes weighed less than one-third as much as those of +/+ FVB mice (Table 1). In contrast, other urogenital structures such as the epididymides were normal (Table 1). Careful necropsy revealed no other gross abnormalities. We thus named the mutation *microrchidia*, a medical term for abnormally small testes. Testes from older +/− (but not +/+ or −/−) animals displayed a faint brown pigmentation, probably caused by tyrosinase expression (Fig. 1, *Lower*).

Histologic abnormalities in adult −/− males were striking. Sections from −/− epididymides revealed a complete absence of spermatozoa (not shown). In contrast, +/+ and +/− males had epididymides that were filled with spermatozoa (not shown). When seminiferous tubules from all three genotypes were examined, −/− males had marked abnormalities (Fig. 2 *B* and *D*), whereas +/+ (not shown) and +/− (Fig. 2 *A* and *C*) males had normal testis histophenotype. Numerous cells in the adluminal compartment of mutants had hyperchromatic, condensed nuclei. These appeared to be pyknotic spermatocytes that had undergone degeneration during early prophase of meiosis I (Fig. 2*D*). No secondary spermatocytes or sper-

Table 1. Adult testes and epididymides weights (mean ± SD) with ratio to body mass for 6-week-old wild-type (+/+), heterozygous (+/−), and homozygous (−/−) *morc* mice

Mice	Testes wt, mg	Epididymides wt, mg	Body mass, g	Testes/Bm, 10^{-3}	Epi/Bm, 10^{-3}
+/+	163.17 ± 19.35 (15)	63.53 ± 18.38 (14)	23.40 ± 2.85 (15)	6.99 ± 0.61 (15)	2.72 ± 0.79 (14)
+/−	165.15 ± 30.65 (35)	69.46 ± 18.45 (31)	26.08 ± 3.81 (31)	6.36 ± 1.23 (31)	2.81 ± 0.55 (27)
−/−	44.30 ± 4.73 (29)	50.59 ± 7.65 (19)	23.88 ± 2.53 (21)	1.91 ± 0.23 (21)	2.16 ± 0.48 (19)

Each column shows mean ± SD, followed by the number of animals analyzed (*n*). Epi, epididymides. Bm, body mass.

matids were ever seen in −/− animals. In 6-week-old mice, spermatogonia were present in normal numbers and appeared to be actively undergoing mitoses. By age 6 months, the germinal epithelium of mutant mice contained only Sertoli cells, with no mature germ cells (not shown). This phenotype is reminiscent of the "Sertoli only" histophenotype seen in some sterile men (18–20). The Leydig cells appeared to be increased in number in −/− mice; however, the hyperproliferation was accentuated by loss of germ cells (not shown). Comprehensive tissue surveys demonstrated that the sole histologic defect in −/− males was the germ cell maturation arrest. Females of all genotypes had normal ovarian histology (not shown).

To ascertain more precisely the stage of spermatogenic breakdown in *morc* −/− mice, we investigated the assembly of components of the synaptonemal complex (SC), a structure unique to meiotic cells. Numerous cells from testes of −/− mice contained SC antigens, indicating definitively that mutant germ cells enter meiotic prophase. We found mutant cells with characteristics of both the leptotene and zygotene stages of meiotic prophase, but none with the characteristics of the pachytene stage (Fig. 3). In mutant leptotene spermatocytes we detected focal staining with the COR1 antibody, which recognizes mouse SYCP3, a component of the lateral axes that eventually synapse to form the SC (13). We also found mutant zygotene spermatocytes (Fig. 3 *C* and *D*) had well-developed axes detected by the COR1 antibody, as well as regions of synapsis, detected by the SYN1 antibody that recognizes mouse SYCP1, a protein of the central element of the SC (13). There were occasional late zygotene spermatocytes, but no pachytene spermatocytes with complete synapsis in −/− mice. Pachytene spermatocytes, however, were prevalent in the +/− mice. Thus spermatogenesis does not progress beyond early meiotic prophase, the leptotene and zygotene stages, in −/− mice.

Longitudinal studies comparing mutant versus wild-type testes (not shown) indicated that −/− mice had histologically normal testes with normal numbers of prospermatogonia at postpartum day (dpp) 5. By dpp 10, −/− mice had produced significant numbers of spermatocytes, but even at this early time point, degeneration of spermatocytes was apparent. By dpp 18, cell death in spermatocytes of −/− mice was marked, and by dpp 24 no morphologically intact spermatocytes were seen. In contrast, +/+ animals had produced numerous secondary spermatocytes and immature spermatids at dpp 24. Thus, the first wave of spermatogenesis in *morc* −/− mice is abortive, and −/− germ cells never progress through meiosis to form haploid spermatids.

Apoptosis in *morc* −/− Testis. Because apoptosis is an important mechanism by which the testis regulates the number and quality of germ cells, we investigated the manner of germ cell death in our mutant. A modified TUNEL assay was performed on testis sections from 6-week-old mice of all three genotypes. Normal levels of apoptosis were found in +/+ (Fig. 4*A*) and +/− testes (not shown). However, there was extensive germ cell apoptosis in testes of −/− animals (Fig. 4*B*).

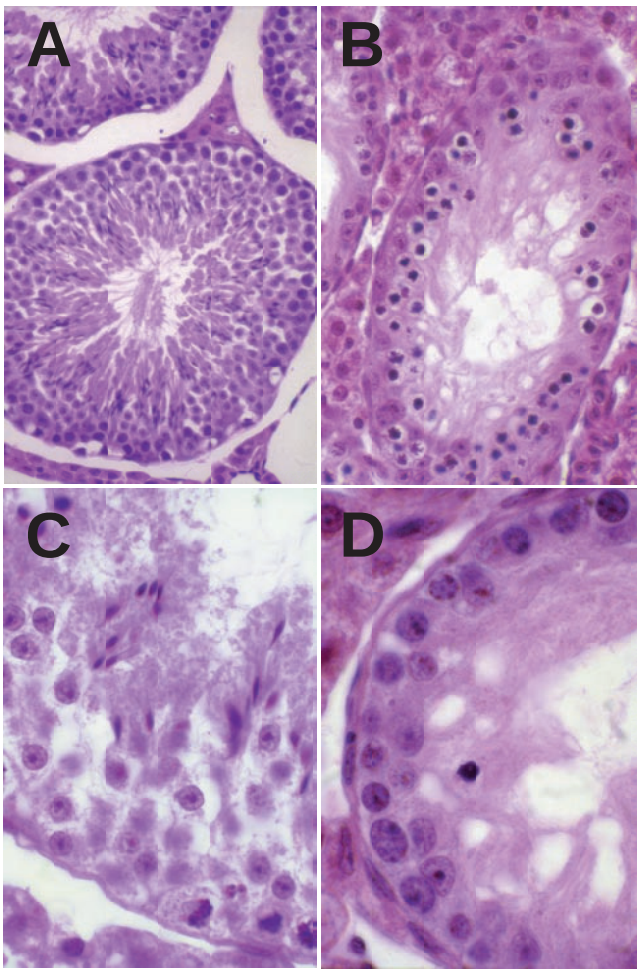

FIG. 2. Testes histology of *morc* mice. Representative hematoxylin/eosin sections from testes of adult +/− *(A* and *C)* or −/− *(B* and *D)* mice demonstrate absence of spermatocytes in the −/− animals. Numerous pyknotic cells were present in −/− mice at the location where primary spermatocytes are found. (Magnifications: *A* and *B,* ×400; *C* and *D,* ×1,000).

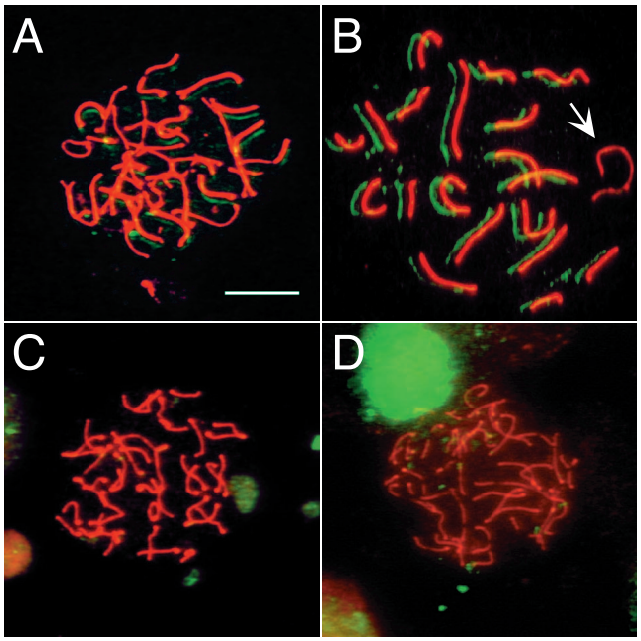

FIG. 3. Molecular analysis of *morc* spermatogenic arrest. Immunofluorescence analysis of spermatocytes from 24-day-old *morc* +/− *(A* and *B)* or −/− *(C* and *D)* mice, with anti-COR1 (red), recognizing mouse SYCP3, and anti-SYN1 (green), recognizing mouse SYCP1. The green image is deliberately offset from the red image to facilitate visualization of coincidence of staining. *(A)* Normal zygotene spermatocyte from a +/− mouse, with well-developed axes staining with anti-COR1; these axes are not completely paired, and initiation of synapsis is recognized by staining with anti-SYN1. *(B)* Normal pachytene spermatocyte from a +/− mouse; note coincident immunoreactivity for SYN1 with all axes indicating full and complete synapsis, except for the sex chromosomes (arrow), which normally synapse only in the most terminal region. *(C)* Abnormal zygotene-like spermatocyte from a −/− animal. Even though full axial elements are formed, no pairing is seen. *(D)* A *morc* −/− zygotene spermatocyte with focal regions of initiation of synapsis. (Bar = 10 μm.)

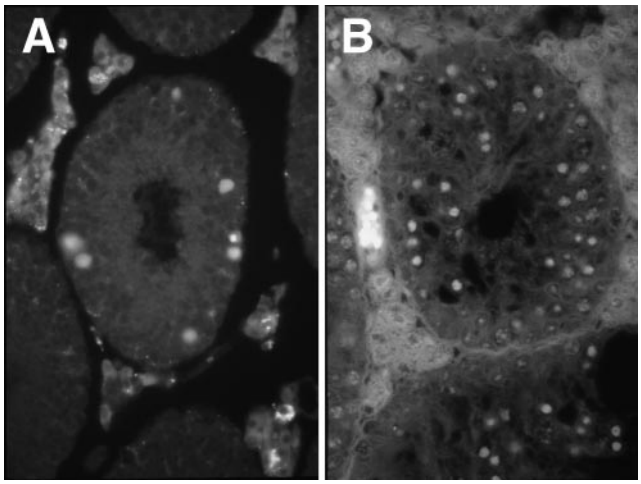

FIG. 4. Apoptosis in testes of adult *morc* mice. *(A)* Most (>90%) wild-type tubules revealed no TUNEL positivity; the section shown here demonstrates the occasional apoptotic cells present in wild-type animals. *(B)* In contrast, the majority of germ cells from −/− mice are undergoing apoptosis. (Magnification: ×200.)

Apoptotic cells coincided with the pyknotic cells seen by light microscopy in the adluminal compartment of the germinal epithelium in −/− mice, and the fluorescent TUNEL assay appeared to enhance the nuclear karyorrhexis typical of apoptosis (Fig. 4*B*). The increased apoptosis in −/− animals was seen as early as dpp18 (not shown). Thus, germ cells that fail to progress through meiosis I in −/− mice appear to be lost through apoptosis.

Tyrosinase Expression. We investigated transgene expression to identify which cell types might be affected by the *morc* mutation. Tyrosinase activity in testes extracts from 6-week-old +/− mice was approximately 15% that of cultured mouse melanocytes, whereas no activity was detected in testis extracts of +/+ or −/− mice (Fig. 5*A*). Western blot analysis (not shown) confirmed the activity results.

We hypothesized that the lack of tyrosinase activity in −/− mice could reflect expression of the transgene specifically in late primary/secondary spermatocytes, which are absent in these animals. To test this hypothesis, we performed tyrosinase immunohistochemistry on fixed testis sections. Specific staining with PEP7 antiserum (11) was demonstrated in germinal epithelial cells of both +/− and −/− but not +/+ animals (Fig. 5*B*). Staining of +/− testes was most prominent in postmitotic germ cells. The fainter staining of −/− testes could represent tyrosinase levels below the sensitivity of the activity assay or Western blotting. A survey of other tissues showed staining only in testis and retina. The abundant expression of tyrosinase in germ cells of histologically normal, fertile +/− males makes it unlikely that the *morc* phenotype results from cytotoxicity caused by ectopic tyrosinase activity.

Linkage of the Transgene to *morc* Phenotype. A P1 genomic clone containing the *morc* transgene integration site was isolated, and a 3.9-kb *Eco*RI fragment containing the insertion site was subcloned and sequenced (*Materials and Methods*). BLAST searches (21, 22) of GenBank with this sequence did not identify any known genes or expressed sequence tags. Using the 3.9-kb sequence, we designed a PCR assay to genotype mice irrespective of eye color. We then compared the testicular masses of animals genotyped by this assay. The testis weights clearly fell into two groups: normal and *morc* phenotypes. None of 15 +/+ and two of 35 +/− mice had small testes, whereas 30 of 30 −/− animals had the *morc* phenotype of small testes. ANOVA comparing the weights from the three groups demonstrated that −/− mice had significantly smaller testes ($P < 8.4 \times 10^{-36}$). There were no significant differences

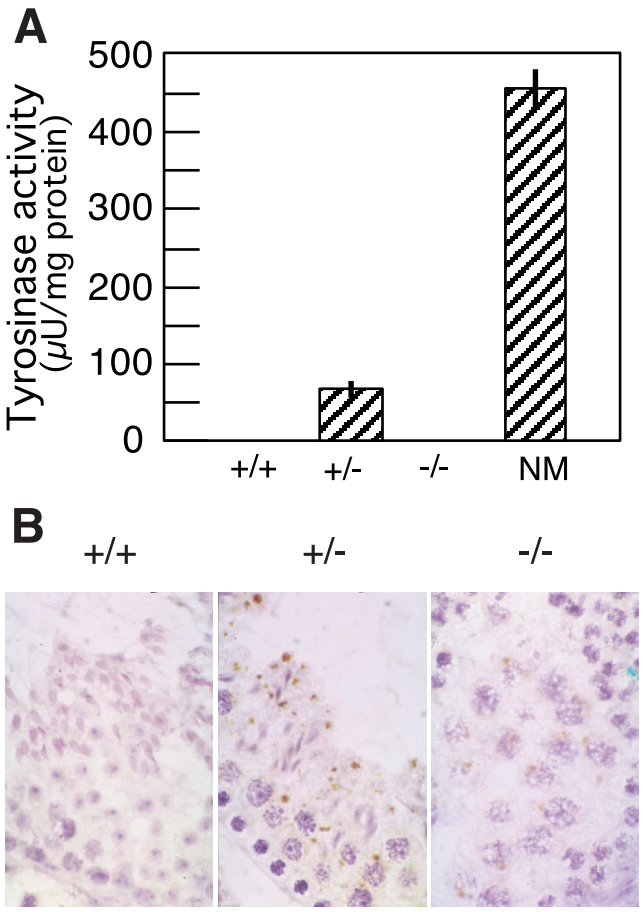

FIG. 5. Expression of tyrosinase in *morc* mice. *(A)* Tyrosinase activity in testes extracts from *morc* +/+, +/−, or −/−, as compared with cultured mouse melanocytes (NM). Histograms represent average of triplicate measurements, and bars indicate SD of the mean. *(B)* Immunohistochemical staining of tyrosinase in testes of *morc* mice. Wild-type controls (+/+) show no antibody staining. Intense staining is seen in the germ cell cytoplasm of +/− testes. Faint staining is also present in −/− germ cells. (Magnification: ×400.)

in mean body mass between the groups. The two +/− mice with small testes were littermates. These could be phenocopies caused by an environmental insult, or alternatively *morc* may be semidominant with very low penetrance in heterozygotes.

The *morc* Locus Contains a Deletion. A Southern blot probed with a fragment from one end of the 3.9-kb *Eco*RI subclone containing the transgene insertion site showed the expected wild-type 3.9-kb band in +/+ animals and a rearranged 2.8-kb band in +/− and −/− animals (Fig. 6*A*). A probe from the other end of the subclone detected the same 3.9-kb *Eco*RI fragment in +/+ and +/− animals, but failed to detect any fragment in −/− DNA (Fig. 6*B*). We concluded that there was a deletion of genomic sequences to one side of the transgene. Failure to PCR-amplify flanking sequences from DNA of −/− mice confirmed the deletion (not shown).

Additional bands were detected in Southern blots probed with a tyrosinase cDNA, suggesting that the transgene integrated into more than one chromosomal site (not shown). Further genotyping indicated that the additional transgenes became genetically fixed during the propagation of the *morc* line. However, these bands segregated independently from either the eye color or testis phenotype (not shown), demonstrating that the additional transgene(s) are silent with respect to tyrosinase expression and are unrelated to the *morc* −/− phenotype.

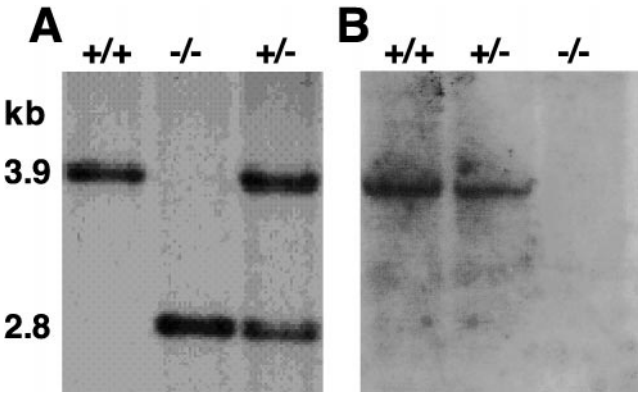

FIG. 6. Southern blotting using probes flanking the transgene. DNA from +/+, +/−, or −/− animals was digested with *Eco*RI and hybridized with each end of a 3.9-kb *Eco*RI fragment containing the transgene insertion site (see text). *(A)* One end detects an altered fragment (2.8 kb) for the *morc* chromosome. *(B)* The opposite end detects the 3.9-kb wild-type fragment with half intensity in +/− animals and no fragment in −/− mice, demonstrating a deletion to one side of the transgene.

DISCUSSION

We have described an autosomal recessive mouse mutation that arrests germ cell development in early meiotic prophase, before the pachytene stage, a critical phase of spermatogenesis when events are initiated in preparation for the later segregation of meiotic chromosomes. The *morc* mutant phenotype is especially interesting because our results show that *morc*'s biologic effects are restricted to male gametogenesis, in contrast to many other infertility mutations.

Infertility is a common clinical problem, affecting about 10% of all couples. Many cases are likely to have genetic etiologies, and some could be caused by mutations in the biochemical pathway(s) in which *morc* acts. The histophenotype of older *morc* −/− mice is reminiscent of the "Sertoli cell only" class of human male infertility. In addition, genes such as *morc* that are required specifically for spermatogenesis also may provide attractive new targets for male contraception.

The search for genes that are specifically responsible for human infertility has yielded several candidates. An example is the identification of *DAZ* (deleted in azoospermia), a gene that maps to a Y chromosomal region deleted in 10% of sterile men (23, 24). The murine homologue of *DAZ* (*dazla*) has been knocked out (25), and like *morc*, *dazla* −/− male mice show severe disruption of spermatogenesis from an early age. However, germ cell proliferation and tubule formation is markedly decreased during early testis development in *dazla* −/− mice, suggesting that *Dazla* acts premeiotically. In contrast, *morc* −/− males have normal premeiotic development and show germ cell arrest only on entry into meiosis. Further, *dazla* −/− females are infertile and show a severe lack of germ cells entering meiosis from 15 days postcoitum onward (25), in contrast to the histologically normal ovaries in fertile *morc* −/− females.

Other "spermatogenesis genes" have been identified by the phenotypes of mouse knockouts. An interesting example of a rapidly growing class of such genes are those functioning in recombination, especially the mismatch repair (MMR) family members, which include the mouse homologues of the bacterial *mutS* and *mutL* genes. The initial focus in MMR was in relation to this system's ability to remove unpaired stretches of DNA formed by polymerase errors or misincorporation (26). However, it soon became apparent from studies of knockout mice that MMR is required for completion of meiosis. Mice deficient for *mutL* homologues *Mlh1* or *Pms2* have meiotic arrest phenotypes (8, 27). Of note, the *mlh1* −/− phenotype is different from that of *morc* (and *pms2* −/− mice) in that both sexes are infertile. The *mlh1* −/− testes histophenotype is similar to *morc* in that arrest occurs early in meiosis I and produces condensed pyknotic primary spermatocytes (27). The *pms2* −/− phenotype is male specific, as is *morc*. However, in contrast to *morc* −/− males, *pms2* −/− males produce round and elongating spermatids, although with many morphological abnormalities. During meiotic prophase, meiotic chromosomes from *pms2* −/− spermatocytes exhibit aberrant synapsis formation, resulting in large numbers of asynaptic bivalents (8).

Other interesting DNA repair and genome stability genes that resulted in germ cell abnormalities when knocked out are the Ataxia telangiectasia mutated (*ATM*) and *BRCA2* genes (28–31). In fact, a major role for *ATM* may be to monitor DNA damage during meiosis (32, 33). These and many other mutant mice have unequivocally linked DNA repair with gametogenesis, and similarities between the phenotypes of *morc* and DNA repair or recombination deficient mice raise the possibility that *morc* functions in recombination.

Our TUNEL data show that germ cells in testes lacking *Morc* die by programmed cell death. Apoptosis also has been shown to be a mechanism of germ cell loss in mutants such as the *mlh1* −/− mice (27) or *bblw* knockout mice (34). Multicellular organisms have exploited apoptosis for many purposes, including regulation of germ cell quantity and quality, but also tissue renovation, organogenesis, immune cell selection, and cancer protection. Apoptosis occurs via a complex and highly regulated sequence of events. In the testis, apoptosis is a normal feature of germinal epithelium, producing a final wave of sperm that reflects the constant removal of both defective and probably also normal cells (35–37). Teleologically it makes sense that there exists a low threshold for apoptosis of male gametes with genomic errors, because their progeny could waste precious female reproductive resources if they were able to fertilize an oocyte (38). Two germ cell apoptotic pathways have been distinguished. The first depends on the presence of normal p53, for example, the germ cell apoptosis induced by ionizing radiation (39, 40). The second is a p53-independent pathway that is triggered by chromosomal abnormalities such as asynapsis during prophase I of meiosis (40). Whether apoptosis in *morc* −/− testis is p53 dependent remains to be seen. Another question that may be answered by cloning the *Morc* gene and characterizing its function is whether *Morc* acts directly in apoptosis as a negative regulator like *Bcl2* or *Bclw* genes, or whether *Morc*'s absence triggers the normal apoptosis process at an upstream point.

Random insertional mutagenesis has found wide application in *Drosophila* genetics, where it is possible to systematically screen for desired phenotypes, including male sterility (41). Such screens are impractical in mammals, but a number of mutations have been identified serendipitously during planned creation of transgenic mice. These mutations have provided major insights into mammalian development. For example, phenotypes involving germ cell proliferation (42), limb formation (43), and aging (44) have been reported, and in the latter two cases novel genes were identified (44, 45). Similarly, the insertional mutation we report should facilitate cloning of the *Morc* gene and will likely yield new insight into male germ cell development and human infertility.

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