Absence of the DNA-/RNA-binding protein MSY2 results in male and female infertility

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MSY2, a germ-cell-specific member of the Y-box family of DNA-/RNA-binding proteins, is proposed to function as a coactivator of transcription in the nucleus and to stabilize and store maternal and paternal mRNAs in the cytoplasm. In mice lacking *Msy2*, a normal Mendelian ratio is observed after matings between heterozygotes with equal numbers of phenotypically normal but sterile male and female homozygotes (*Msy2*$^{-/-}$). Spermatogenesis is disrupted in postmeiotic null germ cells with many misshapen and multinucleated spermatids, and no spermatozoa are detected in the epididymis. Apoptosis is increased in the testes of homozygotes, and real-time RT-PCR assays reveal large reductions in the mRNA levels of postmeiotic male germ cell mRNAs and smaller reductions of meiotic germ cell transcripts. In females, there is no apparent decrease in either the number of follicles or their morphology in ovaries obtained from 2- and 8-day-old *Msy2*$^{-/-}$ mice. In contrast, follicle number and progression are reduced in 21-day-old *Msy2*$^{-/-}$ ovaries. In adult *Msy2*$^{-/-}$ females, oocyte loss increases, anovulation is observed, and multiple oocyte and follicle defects are seen. Thus, *Msy2* represents one of a small number of germ-cell-specific genes whose deletion leads to the disruption of both spermatogenesis and oogenesis.

mice | contraception | transcription | translation | Y-box protein

The highly conserved family of Y-box proteins, expressed in organisms ranging from bacteria to humans, contains a cold-shock domain essential for nucleic-acid binding and variable N and C termini that confer binding specificity (1). As DNA-binding proteins, Y-box proteins serve as transcription coactivators, recognizing DNA motifs such as CTGATTGGC/TC/TAA (2). As RNA-binding proteins, Y-box proteins help stabilize mRNAs and, depending on their concentration, can inhibit or stimulate mRNA translation (3, 4).

Among DNA-/RNA-binding proteins, the mouse Y-box protein MSY2 is one of the most abundant, constituting 0.7% (Fig. 1) and 2% of total protein in male germ cells and fully grown oocytes, respectively (5, 6). MSY2 is the mouse ortholog of the *Xenopus laevis* FRGY2 (2) and human Contrin proteins (7), Y-box proteins proposed to be solely expressed in germ cells (8). In the testis, *Msy2* is expressed in meiotic and postmeiotic germ cells, where it is believed to function in long-term mRNA storage and stabilization because cessation of transcription in postmeiotic germ cells necessitates posttranscriptional regulation for many mRNAs encoding late-stage germ cell and spermatozoan proteins (8). In addition, MSY2 marks specific mRNAs in the nucleus for storage in the cytoplasm, providing a linkage between transcription and mRNA storage for a subset of male germ cell mRNAs (9).

In the female, MSY2 protein accumulates during oocyte growth, but after fertilization, it is totally degraded by the late two-cell stage (6). As in postmeiotic male germ cells, MSY2 is located in the cytoplasm in oocytes, but, in contrast to male germ cells, where MSY2 is soluble, ≈70% of MSY2 is retained after permeabilization procedures that release >70% of total oocyte protein (6, 10). This retention depends on the ability of MSY2 to bind RNA and the integrity of the maternal mRNAs (10). These properties suggest that MSY2 plays a global role in regulating oocyte mRNA stability and translation. For example, the pronounced stability of oocyte mRNAs during the growth phase [with a half-life of ≈8–12 days (11)] may be due, in large part, to their sequestering of oocyte mRNAs, through association with MSY2, from the translation machinery, thereby minimizing degradation.

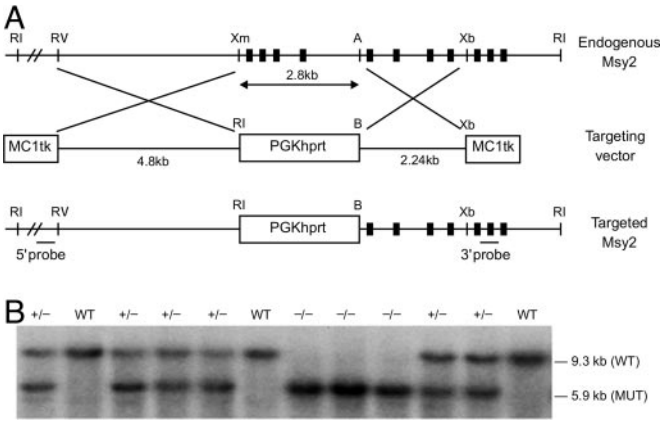

Fig. 1. Strategy for targeted disruption of the *Msy2* gene. (*A*) Exons are represented by vertical bars, and introns are represented by intervening horizontal lines. Exons 1, 2, 2′, and 3 and flanking region were replaced by homologous recombination with a hypoxanthine phosphoribosyl transferase (*Hprt*) gene driven by the *Pgk* promoter. Restriction sites: A, *Apa*I; B, *Bam*HI; RI, *Eco*RI; RV, *Eco*RV; Xb, *Xba*I; and Xm, *Xma*I. (*B*) Southern blot genotyping of DNA from wild-type, heterozygous, and homozygous mice. The mice were genotyped by Southern blot analysis of purified tail DNA digested with *Eco*RI and *Bam*HI and hybridized with a probe 5′ to the disrupted gene. A 5.9-kb DNA band was seen in targeted mice replacing the 9.3-kb DNA fragment of wild-type mice.

More than 200 different mutations can cause reproductive defects (12). Many reproductive mutations are male- or female-specific because of the different patterns of gene expression needed to produce the gametes. Other mutations in genes expressed during meiosis lead to meiotic arrest in both sexes. Here, we show that gene-targeting of *Msy2* in mice leads to infertile males and females that are otherwise healthy and phenotypically normal. Spermatogenesis terminates in postmeiotic germ cells with no sperm seen in the epididymis and a marked increase of apoptosis during meiosis. In adult females,

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few growing follicles and no corpora lutea are observed. This infertility phenotype is one of a small number in which the deletion of a germ-cell-specific gene leads to the disruption of both spermatogenesis and oogenesis.

Materials and Methods

Targeting Construct and Generation of Mice. The *Msy2*$^{-/-}$ targeting construct (Fig. 1*A*) was produced by using a 129S6/SvEv mouse genomic library that is isogenic with the AB2.2 ES cells used for electroporation. ES cell clones were electroporated, selected, and screened by Southern blotting as described in ref. 13. Eight of 87 (9.2%) ES cell clones analyzed were correctly targeted on both the 5′ and 3′ sides by using the probes as shown in Fig. 1*A*. Germ-line transmission was achieved from ES cell clone MSY2-188-A6. Mice were genotyped by Southern blotting as described in ref. 13, and the mutation was maintained on a 129S5/SvEvBrd hybrid genetic background.

Northern Blot and Immunoblot Analyses. Total RNA was analyzed by Northern blotting as described in ref. 14. Tissue extracts were prepared and analyzed by immunoblot as described in refs. 6 and 15.

Histologic and TUNEL Analyses. Freshly dissected testes from wild-type and mutant mice were fixed in Bouin's solution and embedded in paraffin. Wild-type and mutant ovaries were fixed in Bouin's solution or in 10% formalin. Tissue sections were stained with periodic acid/Schiff hematoxylin. Apoptosis was analyzed by TUNEL of paraffin sections of testes with an ApopTag peroxidase staining kit (Chemicon). The sections were counterstained with hematoxylin.

Real-Time RT-PCR Quantitation of Testicular mRNAs. Primers were checked by PCR to ensure that they generated single bands of the predicted size. PCR was performed by using the SYBR Green PCR Master Mix and the ABI 7700 thermal cycler (Applied Biosystems) at typical amplification parameters (50°C for 2 min and 95°C for 10 min, followed by 40 cycles at 95°C for 15 s and 60°C for 1 min), and differences were displayed as the cycle of threshold (C_t) value for each gene. The expression level of each mRNA was normalized to 18S rRNA, and the Delta-Delta C_t method was used for the relative quantification analysis (9).

Results

MSY2 Is Essential for Male and Female Fertility. To define the roles of *Msy2* in mammalian development, a targeted deletion of *Msy2* exons 1–3 (*Msy2*^{tm1Nbh}, herein called *Msy2*$^{-}$) was produced in ES cells (Fig. 1*A*). Exons 1–3 encode key amino acids essential for the nucleic-acid-binding cold-shock domain. Heterozygous (*Msy2*$^{+/-}$) mice were viable and fertile. χ^2 analysis of 285 F_2 offspring from these intercrosses demonstrated a normal Mendelian ratio [70 wild type (24.6%), 140 heterozygotes (49.1%), and 75 homozygotes (26.3%)] with similar numbers of male (n = 39) and female (n = 36) *Msy2*$^{-/-}$ mice. Intercrosses of eight *Msy2*$^{+/-}$ males and females for 6 months resulted in litter sizes that averaged 7.16 ± 0.37 pups (n = 49 litters) with 1.02 litters ± 0.02 per month. These numbers are similar to the eight pups per litter and one litter per month seen for wild-type mice. In contrast to *Msy2*$^{+/-}$ males and females, phenotypically normal *Msy2*$^{-/-}$ males (n = 10) and females (n = 10) were infertile when bred to control wild-type partners over a 6-month period. Thus, MSY2 is required for fertility in both sexes.

To confirm that the targeted deletion functionally disrupted the *Msy2* gene, Northern blot and immunoblots were used to analyze *Msy2* mRNA and MSY2 protein levels, respectively. In total RNA from the testes of 7-week-old mice, *Msy2* mRNA was reduced to ≈50% in *Msy2*$^{+/-}$ mice and was absent in the testes of *Msy2*$^{-/-}$ mice (Fig. 2*A*). Hybridization of the same blots with probes for the Sertoli cell marker, clusterin, and for actin revealed no significant differences in testis mRNA levels in wild-type, *Msy2*$^{+/-}$, and *Msy2*$^{-/-}$ mice.

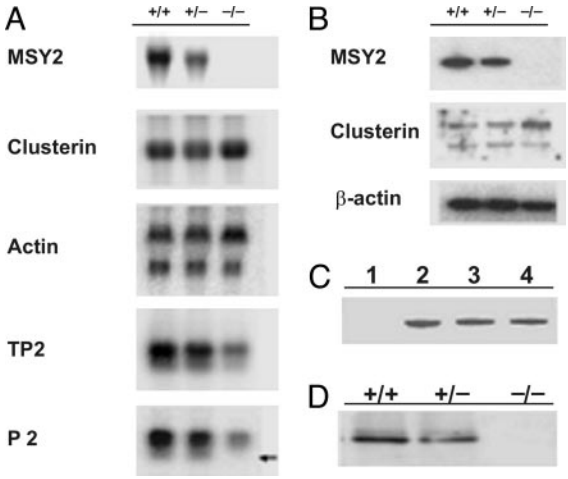

Fig. 2. *Msy2* mRNA is absent in the testes of *Msy2*$^{-/-}$ mice. (*A*) Northern blot of total testis RNA (10 μg) from wild-type, heterozygous, and homozygous mice hybridized with probes for *Msy2*, clusterin, actin, *Tnp2*, and *Prm2*. (*B*) Immunoblot of testes extracts (5 μg for MSY2 and 20 μg for clusterin and actin) were probed with anti-MSY2, anti-clusterin, and anti-actin. (*C*) Immunoblot of extracts (5 μg) of brain (lane 1), pachytene spermatocytes (lane 2), round spermatids (lane 3), and a mixed population of dissociated male germ cells (lane 4). (*D*) Immunoblot obtained with 25 (lane 1) wild-type oocytes, 25 heterozygote oocytes (lane 2), and 25 *Msy2*-null oocytes (lane 3). The oocytes were transferred to sample buffer that was then directly applied to the gel, thus ensuring no loss of protein in the samples.

Immunoblots of testis extracts from wild-type, *Msy2*$^{+/-}$, and *Msy2*$^{-/-}$ 7-week-old mice revealed that MSY2 protein was reduced to ≈50% of wild-type littermates in heterozygotes and absent in homozygotes. No significant differences were seen for clusterin or actin. Thus, MSY2 protein levels depend on gene copy number, and the gene targeting successfully deleted *Msy2* to produce a null allele (Fig. 2*B*).

Spermatogenesis Arrests in Late Spermiogenesis in *Msy2*$^{-/-}$ Males. To determine the cause of the infertility in the *Msy2*$^{-/-}$ males, testes were initially analyzed at 6 weeks of age. Testes of the *Msy2*$^{-/-}$ males (63.95 ± 3.52 mg; n = 6) were statistically smaller ($P < 0.001$) than testes of the wild-type males (88.68 ± 3.77 mg; n = 6). At low-power magnification, no mature spermatozoa were detected in the tubules of adult *Msy2*$^{-/-}$ males (Fig. 3*A*) in contrast to wild-type and *Msy2*$^{+/-}$ males (data not shown). At higher magnification, a block in spermatogenesis as spermatids elongate was seen in a number of the tubules (Fig. 3 *B* and *D*) compared with a wild-type control (Fig. 3*C*). Large, perhaps diploid, vacuolated spermatids and multinucleated condensed spermatids were frequently seen. Although small numbers of normal-looking condensed spermatids were found at step 12, most spermatids were misshapen, showed variable condensation, and were often in multinucleate bodies. Consistent with the block in germ cell development, epididymides from adult *Msy2*$^{-/-}$ males lacked mature spermatozoa (Fig. 3*F*), whereas wild-type epididymides were packed with spermatozoa (Fig. 3*E*). Also consistent with these findings, testes from 21-day-old *Msy2*$^{-/-}$ males were morphologically and histologically indistinguishable from controls (data not shown). Thus, the absence of MSY2 results in a block late in spermatogenesis, likely secondary to the altered regulation of key spermatogenic genes that are expressed after the meiotic division.

Messenger RNAs encoding the transition proteins (TNPs) and

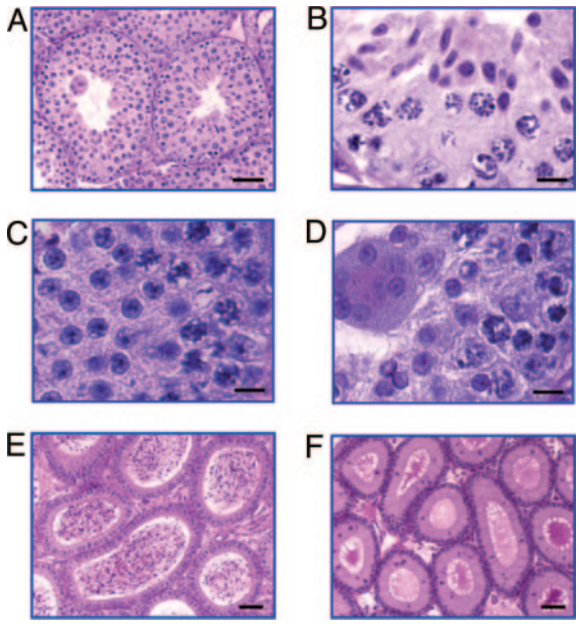

Fig. 3. Histology of testes and epididymis from 6- and 7-week-old wild-type and *Msy2*-null mice. (*A* and *B*) *Msy2*$^{-/-}$ testis. (*C*) *Msy2*$^{+/+}$ testis. (*D*) *Msy2*$^{-/-}$ testis. (*E*) *Msy2*$^{+/+}$ epididymis. (*F*) *Msy2*$^{-/-}$ epididymis. (Scale bars: *A*, *E*, and *F*, 20 μm ; *B–D*, 80 μm.)

the protamines (PRMs) are transcribed and stored in the cytoplasm with poly(A) tails that undergo partial deadenylation at the time of translation in later-stage cells, thereby providing good markers for staging the differentiating spermatids (16). *Tnp2* mRNAs are first detected in step-7 round spermatids, whereas TNP2 proteins appear at step 12 (17). Northern blot analysis of *Tnp2* revealed that a reduced but detectable amount of both the ribonucleoprotein and polysomal forms of *Tnp2* mRNA is present in total RNA from *Msy2*$^{-/-}$ mice, consistent with the histological presence of early-stage spermatids (Figs. 2*A* and 3 *C* and *D*). In contrast, although the longer, fully adenylated protamine 2 (*Prm2*) mRNA that is transcribed in step-7 round spermatids was detected, the shorter *Prm2* mRNA, which becomes partially deadenylated at the time of translation in step-12 to -15 spermatids (16), was not detectable in the testes of *Msy2*$^{-/-}$ mice (see arrow in Fig. 2*A*). The shorter form of *Prm2* mRNA was not detected even in overexposed gels (data not shown). The morphological absence of most normal step-12 spermatids (Fig. 3) and the absence of the shorter PR transcripts (Fig. 2) establishes that cessation of spermatogenesis occurs in mid- to late spermiogenesis.

Postmeiotic Germ Cell mRNA Levels Are Greatly Reduced in *Msy2*$^{-/-}$ Testes. In the testis, *Msy2* is expressed in meiotic spermatocytes and postmeiotic spermatids (8). Real-time RT-PCR analysis of purified RNA from populations of pachytene spermatocytes and round spermatids indicate that they contained similar amounts of *Msy2* mRNA (Table 1), and immunoblot analysis with an affinity-purified antibody to recombinant MSY2 protein detected similar amounts of MSY2 protein (Fig. 2*C*). Male germ cell mRNAs, such as *Msy1*, *Msy2*, *Msy4*, *Ldh3* (LdhC4), *Tsn* (TB-RBP), and *Tsnax* (TRAX), are present in both meiotic and postmeiotic male germ cells, whereas other mRNAs, such as *Prm2* and *Act*, are first expressed in postmeiotic male germ cells (Table 1).

By using real-time RT-PCR assays normalized to amounts of 18S rRNA, the impact of the *Msy2* deletion on a number of the temporally expressed male germ cell mRNAs was measured.

Table 1. Quantification of relative amounts of mRNA in pachytene spermatocytes and round spermatids by real-time RT-PCR

Gene	C_t-Pach	C_t-RS	Pach:RS
Prm2	27.58 ± 0.04	22.99 ± 0.05	1:24.08
Act	26.87 ± 0.02	23.24 ± 0.05	1:12.38
Pgk2	29.05 ± 0.07	26.36 ± 0.03	1:6.45
Acr	23.56 ± 0.01	22.51 ± 0.01	1:2.07
Msy1	23.92 ± 0.01	23.82 ± 0.04	1:1.07
Msy2	21.42 ± 0.08	21.45 ± 0.15	1:1.02
Msy4	19.45 ± 0.07	19.39 ± 0.08	1:1.04
Ldh3	20.48 ± 0.00	20.55 ± 0.12	1:1.05
Tsn (TB-RBP)	29.05 ± 0.06	30.58 ± 0.03	1:0.34
Tsnax (TRAX)	23.31 ± 0.04	24.90 ± 0.01	1:0.33

Pach, pachytene spermatocytes; RS, round spermatids.

Germ-cell-specific mRNAs that are solely expressed or highly enriched in postmeiotic cells (e.g., *Prm1*, *Prm2*, *Tnp1*, *Tnp2*, *Akap4*, and *Act*) were greatly reduced in *Msy2*$^{-/-}$ mice compared with their wild-type littermates, consistent with the loss of late-stage spermatids (Table 2). Reductions were also seen for male germ cell mRNAs, such as *Acr*, *Ldh3*, *Msy4*, *Tsn*, *Tsnax*, and *Ace*, that are expressed in both meiotic and postmeiotic cells, likely a reflection of both meiotic (Fig. 4) and postmeiotic cell loss. No significant changes were detected for clusterin, a marker for Sertoli cells. Thus, in agreement with the loss of late-stage postmeiotic cells, a marked reduction of mRNAs expressed in postmeiotic cells is seen in the absence of MSY2.

The Testes of *Msy2*$^{-/-}$ Mice Have Markedly Increased Levels of TUNEL-Positive Cells. The infertility and the reductions in the weight of the testes from *Msy2*$^{-/-}$ mice suggest germ cell loss. To determine the cause of the germ cell loss in the testes of the same *Msy2*$^{-/-}$ mice analyzed above, TUNEL assays were performed. Compared with wild-type littermate controls, a marked increase in the number of TUNEL-positive cells was detected in the testes of the 7-week-old *Msy2*$^{-/-}$ mice (Fig. 4). At higher magnification, most of the TUNEL-positive cells were seen to be in meiotic prophase, with a smaller number of later-stage germ cell types.

An Early Loss of Oocytes Occurs in *Msy2*$^{-/-}$ Females. We have used transgenic RNA interference to study *Msy2* function in mouse oocytes and noted that reducing MSY2 protein by 60–70% resulted in reduced fertility, whereas a 95% reduction resulted in infertility (18). The amount of MSY2 in oocyte extracts derived from *Msy2* heterozygotes was reduced ≈50% when compared with wild type, and none could be detected in oocyte extracts prepared from *Msy2*$^{-/-}$ mice (Fig. 2*D*). This finding further confirms that in males and females, the *Msy2* mutant allele is null.

To characterize the infertility of the *Msy2*$^{-/-}$ females, ovaries were analyzed at various time points. At 2 and 8 days of age, ovaries of wild-type and *Msy2*$^{-/-}$ females were indistinguishable (data not shown). By 21 days of age, there were fewer follicles in the *Msy2*$^{-/-}$ female ovaries (Fig. 5*C*), and in many of those present, the granulosa cells appeared to be disorganized. This finding is in contrast to the age-matched littermate control ovaries that had multiple secondary and earlier follicles (Fig. 5 *A* and *B*). By 8 weeks of age, whereas wild-type ovaries had a full range of follicles and corpora lutea (Fig. 5*D*), *Msy2*$^{-/-}$ ovaries had fewer follicles and occasional hemorrhagic cysts (Fig. 5*E*). By 20 weeks, there was a range of phenotypes in the *Msy2*$^{-/-}$ ovaries that likely depended on the number of oocytes lost earlier in life. Some ovaries had cysts, oocytes lacking cumulus cells (Fig. 5*F*), and significant oocyte death that included an inter-

Table 2. Quantification of relative amounts of mRNA in +/+, +/−, and −/− *Msy2* mice by real-time RT-PCR

mRNA	C_t (+/+)	C_t (+/−)	C_t (−/−)	−/−:+/+
Msy2	22.37 ± 0.01	23.32 ± 0.01	29.66 ± 0.04	0.00
Postmeiotic				
Prm2	29.22 ± 0.11	29.54 ± 0.12	32.40 ± 0.08	0.08
Prm1	26.36 ± 0.05	26.88 ± 0.06	29.48 ± 0.01	0.09
Tnp1	25.54 ± 0.05	25.91 ± 0.01	29.17 ± 0.01	0.06
Tnp2	21.15 ± 0.01	21.58 ± 0.01	24.30 ± 0.12	0.09
Akap4	27.87 ± 0.00	27.84 ± 0.01	29.55 ± 0.02	0.24
Act	25.32 ± 0.05	25.46 ± 0.01	28.17 ± 0.12	0.11
Meiotic/postmeiotic				
Acr	25.38 ± 0.03	25.38 ± 0.02	26.01 ± 0.00	0.49
Ldh3	23.57 ± 0.01	23.89 ± 0.11	24.61 ± 0.02	0.37
Msy1	31.94 ± 0.02	32.03 ± 0.02	33.16 ± 0.04	0.33
Msy4	21.93 ± 0.01	21.86 ± 0.05	23.15 ± 0.04	0.33
Tsn (TB-RBP)	31.21 ± 0.06	31.15 ± 0.04	31.68 ± 0.02	0.55
Tsnax (TRAX)	27.88 ± 0.07	27.92 ± 0.06	28.46 ± 0.00	0.51
Ace	32.93 ± 0.07	33.03 ± 0.04	34.29 ± 0.02	0.30
Clusterin	23.89 ± 0.02	23.53 ± 0.03	23.72 ± 0.07	0.85
18S rRNA	15.79 ± 0.02	15.56 ± 0.02	15.40 ± 0.01	

The expression level of mRNA was normalized to the 18S rRNA (as a loading control).

stitium full of zona pellucida remnants and zonae pellucidae being "invaded" by granulosa cells (Fig. 5*G*). These latter findings are reminiscent of $Gdf9^{-/-}$ ovaries (19) and $Gdf9^{+/-}Bmp15^{-/-}$ ovaries (20). Other ovaries had mostly preantral follicles, with an occasional follicle containing two oocytes and oocytes that appeared larger than normal (data not shown). By 8 months of age, $Msy2^{-/-}$ ovaries continued to vary in size and follicle number. Three of nine $Msy2^{-/-}$ mice had ovaries that were small and, typically, devoid of oocytes and follicles (Fig. 5 *H* and *I*). The ovaries of all nine $Msy2^{-/-}$ mice had an abundance of interstitial tissue, likely remnants of degenerating follicles, and a range of zona pellucida remnants. Interestingly, the ovaries of five of nine $Msy2^{-/-}$ mice had corpora lutea that contained centrally "trapped" oocytes, similar to knockout models with ovulation defects, e.g., progesterone-receptor-knockout mice (21) and pentraxin-3-knockout mice (22). Consistent with these latter findings, four of four $Msy2^{-/-}$ juvenile females failed to ovulate in response to gonadotropin treatment. Thus, the absence of *MSY2* in oocytes leads to early loss of oocytes, defects in ovulation, and infertility.

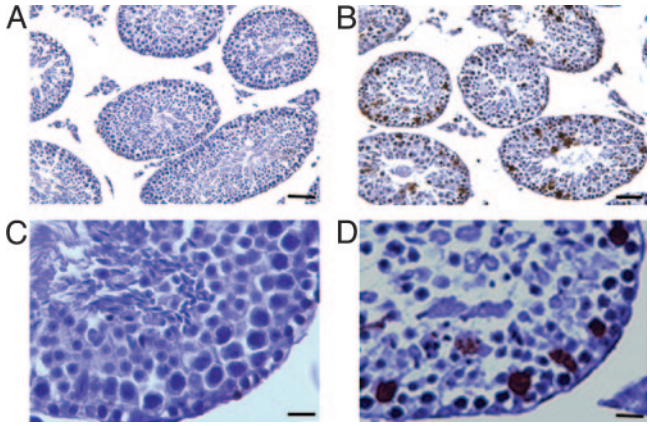

Fig. 4. TUNEL assay of testes from wild-type and *Msy2*-null mice. Apoptotic cells (brown) were detected by *in situ* TUNEL peroxidase staining in testis sections from wild-type mice. (Scale bars: *A* and *B*, 20 μm; *C* and *D*, 100 μm.)

Discussion

The complexities of normal gamete formation require the synthesis of many different proteins whose expression is temporally regulated before, during, and after meiosis in the testis and ovary. Numerous infertilities arise from deficiencies in primor-

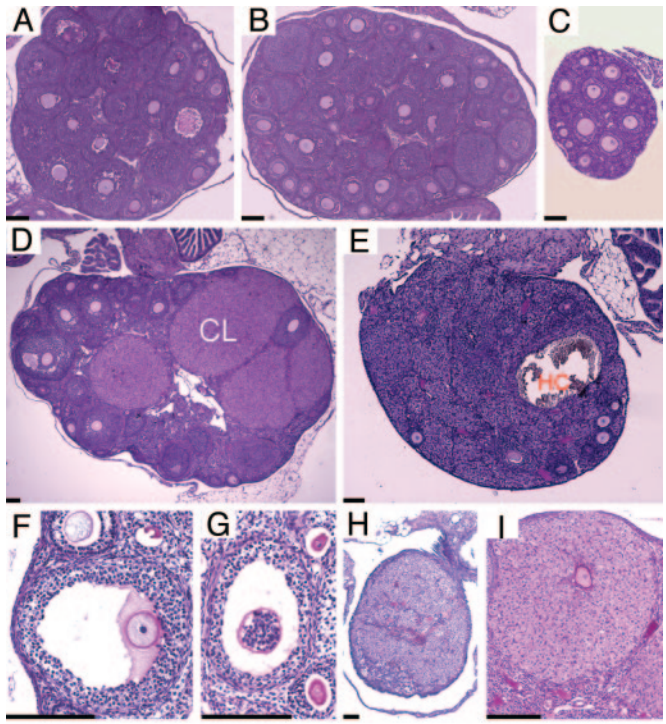

Fig. 5. Histology of ovaries of *Msy2*-null and control mice. (*A–C*) Three-week-old wild-type (*A*), $Msy2^{+/-}$ (*B*), and $Msy2^{-/-}$ (*C*) ovaries captured at the same magnification. (*D*) Wild-type 8-week-old ovary. (*E–I*) $Msy2^{-/-}$ ovaries at 8 weeks (*E*), 20 weeks (*F* and *G*), and 8 months (*H* and *I*). Note the numerous follicles in *A* and *B* compared with *C*. Note the numerous corpora lutea (CL) and follicles at various stages of development in *D* and the absence of corpora lutea, reduced number of follicles, hemorrhagic cyst (HC), and increased interstitial tissue in *E*. (Scale bars: 100 μm.)

dial germ cells and disruption of many essential male or female germ-cell-specific proteins causes sex-specific infertility (12). Gene targeting of certain meiotic proteins leads to meiotic arrest and infertility in both sexes. *Msy2* is one of a small number of genes that is selectively expressed in both male and female germ cells and whose deletion leads to infertility in both sexes without altering phenotypes or sexual behavior.

The multifunctionality of Y-box proteins provides numerous sites for reproductive deficiencies leading to infertility in mice lacking *Msy2* (23). MSY2 functions in both the nucleus and the cytoplasm and binds both DNA and RNA. In the nucleus, the *X. laevis* ortholog of MSY2, FRGY2, serves as a coactivator of *in vitro* transcription from the mouse protamine promoter (24). Deletion of *Msy2* affects the expression of regulatory genes such as *ACT*, the activator of the cAMP-responsive element up-modulator, CREM-tau, a transcription factor in the testis. CREM-tau is proposed to serve as a master-switch gene directing the onset of spermiogenesis (25, 26), and its inactivation in mice leads to the down-regulation of many postmeiotic transcripts and the arrest of spermatogenesis in early round spermatids. Thus, not only does the deletion of *Msy2* affect its own target mRNAs, but the accompanying decrease of ACT mRNA levels to ≈10% of wild-type levels (Table 2) could affect other mRNAs by indirectly controlling their transcription.

In the cytoplasm, it is proposed that MSY2 serves as a major structural protein stabilizing and regulating the translation of stored mRNAs (6). In addition to the global role of mRNA stabilization, bacterially expressed recombinant MSY2 can suppress the translation of mRNAs in a sequence-independent manner in *in vitro* mRNA-binding assays (27). Moreover, the somatic cell Y-box protein YB-1 enhances mRNA translation at low concentrations and suppresses protein synthesis at higher concentrations (4, 28). Immunoprecipitation of mRNAs with an affinity-purified MSY2 antibody combined with suppressive subtractive hybridization studies has identified a large number of male germ cell mRNAs that are bound or not bound by MSY2 (9). The MSY2-bound mRNAs contain the consensus Y-box DNA-binding sequence in their promoters, suggesting that a linkage exists between the nucleus and cytoplasm that coregulates the transcription and subsequent stabilization of specific germ cell mRNAs. A similar association has been reported for a p50-like Y-box protein in the dipteran *Chironomus tentans*, which is added to nascent transcripts and remains in association during nucleocytoplasmic transport and in the cytoplasm (29). A similar linkage occurs in male germ cells for mRNAs transported by another DNA/RNA-binding protein, TB-RBP (30). ACT, the regulator of CREM-tau, is transported from the cytoplasm to the nucleus by the kinesin KIF17b (31). KIF17b, in association with TB-RBP and other interacting proteins, transports mRNAs both inter- and intracellularly, thereby also linking events in the nucleus and cytoplasm (32). Assuming that MSY2 affects the stability or time of translation of the mRNAs it binds, in the absence of MSY2, the posttranscriptional regulation of numerous genes required for spermatid or oocyte development would be altered. Thus, a deficiency of MSY2 could affect transcription, mRNA stability, and/or time of translation of numerous germ cell mRNAs required for spermatid and oocyte maturation.

At least two other Y-box proteins, *Msy1* and *Msy4*, are expressed in meiotic and postmeiotic male germ cells (33, 34). MSY2 interacts with MSY4, forming a complex that binds to the 3′ UTR of the *Prm1* mRNA (35). About 75% of polyadenylated RNA in the testis is estimated to be complexed with MSY2 and MSY4 (34). Overexpression of *Msy4* leads to the disruption of spermatogenesis and sterility with severe defects in sperm morphology (36). Whether MSY2 levels are altered in the presence of higher levels of MSY4 was not reported. The decrease of *Msy4* mRNA in the testes of *Msy2*-null males (Table 2) may indicate that MSY2 and MSY4 must be maintained in a specific stoichiometry for proper function, as seen for the RNA-binding protein TB-RBP and its interacting protein, TRAX (37).

Because the meiotic pachytene spermatocytes and postmeiotic round spermatids contain about equal amounts of *Msy2* mRNA (Table 1) and MSY2 protein (Fig. 2), it is noteworthy that the differentiation of many germ cells continues past meiosis and arrests postmeiotically in the absence of MSY2. Although spermatogenesis ceases during spermiogenesis, the increase in apoptosis in spermatocytes (Fig. 4) and the reductions of mRNAs in meiotic/postmeiotic cells suggests that the deficiency in MSY2 affects earlier stages of germ cells. By using a *Zp3*-promoter-based transgenic RNAi approach with oocytes, reduced fertility is observed when *Msy2* mRNA is targeted in oocytes arrested in the first meiotic prophase. Females harboring transgenic oocytes that contain reduced amounts of MSY2 protein are subfertile or infertile (depending on the degree of MSY2 reduction) (18). These observations are consistent with the proposed function of MSY2 in oocytes to stabilize mRNAs and thereby facilitate mRNA accumulation during the growth phase. These interactions may require specific stoichiometries, because no significant fertility deficiencies are seen in heterozygote matings, despite their 50% reduction in MSY2 protein.

It is now widely recognized that the oocyte plays a pivotal role in coordinating follicle development (38). For example, the appropriate expression of several genes in follicle cells depends on the presence of the developing oocyte. The disruption of normal follicle development when MSY2 is not present in sufficient amounts in the oocyte is likely a manifestation of the impact of compromised oocyte function on the development of the companion follicle cells.

Although MSY2 is highly expressed and is an essential protein for both male and female germ cells, several interesting differences exist based on different functional needs of the male and female gametes. In the male, even though MSY2 binding to mRNAs appears to be primarily independent of mRNA sequence, MSY2 associates with specific mRNAs and regulates their translation. As previously mentioned, this specificity of binding appears to be linked to the transcripts derived from a Y-box-containing promoter (9). We do not know whether specific transcripts are associated with MSY2 in oocytes, but a reduction of MSY2 leads to a general reduction in protein synthesis (18). A second major difference is that MSY2 in male germ cells is soluble after Triton X-100 permeabilization, whereas >70% of MSY2 in oocytes remains insoluble and likely accounts for the majority of oocyte mRNAs (>80%) being retained in this insoluble fraction (6, 10). Finally, MSY2 is lost during spermatogenesis as a consequence of being eliminated in the residual body, i.e., MSY2 is discarded along with a host of other cellular components. In oocytes, MSY2 is inactivated by degradation initiated during oocyte maturation and, essentially, complete by the end of the two-cell stage (6). The destruction of MSY2 may provide the trigger for the general degradation of maternal mRNAs that parallels MSY2 loss. Thus, although MSY2 serves similar general posttranscriptional functions in both male and female germ cells, the way in which it executes this function is sex-specific.

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