TEX14 is essential for intercellular bridges and fertility in male mice

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Cytokinesis in somatic cells concludes with the formation of a midbody, which is abscised to form individual daughter cells. In contrast, germ cell cytokinesis results in a permanent intercellular bridge connecting the daughter cells through a large cytoplasmic channel. During spermatogenesis, proposed roles for the intercellular bridge include germ cell communication, synchronization, and chromosome dosage compensation in haploid cells. Although several essential components of the midbody have recently been identified, essential components of the vertebrate germ cell intercellular bridge have until now not been described. Herein, we show that testis-expressed gene 14 (TEX14) is a novel protein that localizes to germ cell intercellular bridges. In the absence of TEX14, intercellular bridges are not observed by using electron microscopy and other markers. Spermatogenesis in *Tex14*$^{-/-}$ mice progresses through the transit amplification of diploid spermatogonia and the expression of early meiotic markers but halts before the completion of the first meiotic division. Thus, TEX14 is required for intercellular bridges in vertebrate germ cells, and these studies provide evidence that the intercellular bridge is essential for spermatogenesis and fertility.

cytoplasmic bridges | knockout mouse | male infertility | male sterility | ring canals

Cytokinesis is the fundamental biological process by which one cell divides into two daughter cells (1–3). At the end of somatic cell cytokinesis, a transient intercellular bridge contained in the midbody connects the two daughter cells (3–5). This connection remains until the midbody is abscised through a syntaxin2 and endobrevin/VAMP8-dependent mechanism (5) separating the daughter cells. Germ cells, in contrast, do not abscise the midbody. Instead, the midbody intercellular bridge is altered both structurally and molecularly into a distinct, stable germ cell intercellular bridge that connects the daughter cells in a syncytium. More than 50 years of study have provided a detailed morphological and structural description of intercellular bridges and the resulting syncytium (6–13). Intercellular bridges are produced by both mitotic (6, 12) and meiotic (7, 8) divisions and create large, 0.5- to 3-μm connections linking the cytoplasm of generations of daughter cells. Because the intercellular bridges remain stable during development, germs cells sharing a common parent cell develop clonally in a syncytium (6–11), which can contain hundreds of synchronously developing germ cells (12, 13).

Intercellular bridges are evolutionarily conserved from invertebrates to humans and have been extensively studied in *Drosophila melanogaster*, where the structure is called a ring canal. Several genes important for ring canal function have been identified in female fruit flies (14–16). Mutations in these genes cause sterility and have shown that the major role of the ring canal in female *Drosophila* is to accommodate cytoplasmic transfer from nurse cells to the oocyte. Ring canals in male fruit flies are compositionally different from female ring canals (17). Mutations in conserved cytokinesis genes in male fruit flies prevent normal ring canal formation, creating multinucleated germ cells (18–20). These multinucleated cells complete meiosis and develop into multinucleated spermatids. Surprisingly, genes essential for cytokinesis and subsequent formation of male ring canals are not necessarily essential for female ring canals or fertility (18). Likewise, two kinases with similarity to the testis-expressed gene 14 (TEX14) kinase domain, Src64 and Tec29, are essential for female ring canals but have no reported defect in male *Drosophila* ring canals (21).

Several roles for mammalian intercellular bridges have been hypothesized (22). After meiosis, cytoplasmic sharing may be necessary for haploid germ cells to remain phenotypically diploid. Supporting this idea, mRNA (23, 24) and organelles (10) have been observed to move between haploid spermatids. Because premeiotic germ cells do not need to compensate for genetic imbalance, another possibility is that intercellular bridges permit cytoplasmic sharing of essential signals for the synchronous cell divisions seen in longitudinal segments of seminiferous tubules (6, 8, 13) or for "critical stages," such as coordinating the entry into meiosis (25, 26). It remains unknown whether any of these proposed functions of the intercellular bridge are essential for spermatogenesis. Although actin (27), heat-shock factor 2 (HSF2) (28), protocadherin α3 (29), cytokeratin 5 (30), δ-tubulin (31), and plectin (32) are believed to be components of the male intercellular bridge, no essential components of the mammalian intercellular bridge had previously been identified. Herein we provide the first evidence that TEX14 is an essential component of the intercellular bridge and that vertebrate intercellular bridges play an essential role in diploid germ cells before the completion of the first meiotic division.

Results and Discussion

Targeted Disruption of the *Tex14* Locus Results in Male Sterility. In an *in silico* search for germ cell-specific genes (33), we discovered several genes on mouse chromosome 11 that were preferentially expressed in the male testis (34–36), including testis-expressed gene 14 (*Tex14*) (37). To define the roles of TEX14 in mammalian development, a targeted deletion of *Tex14* exon 10 (*Tex14*^{tm1Zuk}; herein called *Tex14*$^{-}$) was produced in ES cells (Fig. 1*A*). Heterozygous (*Tex14*$^{+/-}$) mice from four independent ES cell lines were viable and fertile over 6 months of breeding (7.14 ± 0.56 pups per litter; 1.13 ± 0.04 litters per month; n = 20 mating pairs). χ^2 analysis of 630 F_2 offspring from these intercrosses (Fig. 1*B* and Table 1, which is published as support-

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Abbreviations: HSF2, heat-shock factor 2; SYCP3, synaptonemal complex protein 3; CCNA1, cyclin A1; TEX14, testis-expressed gene 14; PLZF, promyelocytic leukemia zinc finger.

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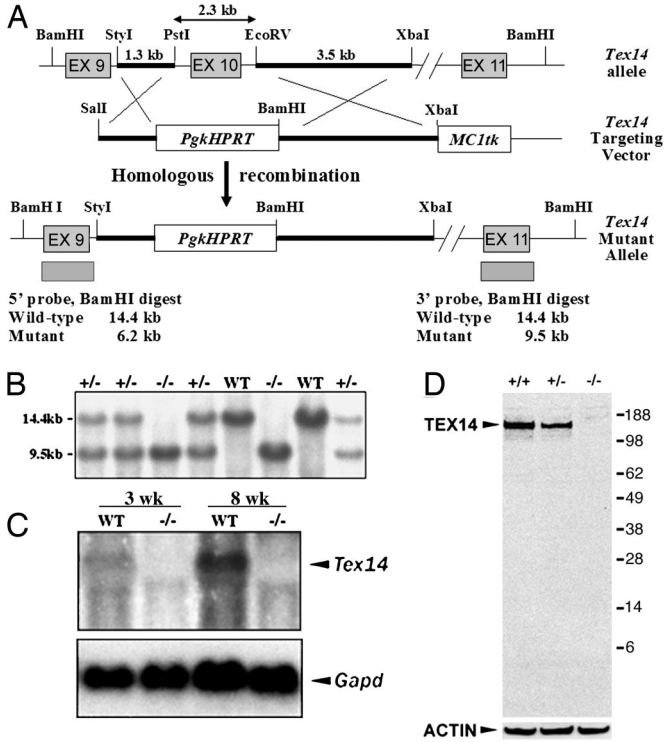

Fig. 1. Targeting of the *Tex14* allele and creation of *Tex14* mutant mice. (*A*) A targeting vector was constructed to replace exon 10 of the mouse *Tex14* gene with a *PgkHPRT* expression cassette. The MC1*tk* expression cassette was used for negative selection. Twenty-two of 70 (31.4%) of the ES cell clones analyzed were correctly targeted at the *Tex14* locus. Four targeted ES cell clones were injected into blastocysts to produce 23 chimeric male mice (51, 52), which were bred to produce F_1 $Tex14^{+/-}$ offspring. (*B*) Southern blot analysis of genomic DNA derived from a litter from $Tex14^{+/-}$ (+/−) intercrosses. Similar percentages of male and female mice were genotyped as *Tex14* homozygous null (−/−) (see Table 1). (*C*) Northern blot analysis of RNA derived from WT and *Tex14* mutant mice. RNA was probed with 5′ *Tex14* or *Gapd* cDNAs. (*D*) Western blot analysis of testis samples from 11-day-old *Tex14* WT, +/−, and −/− mice by using a polyclonal antibody to TEX14 (*Upper*) or an antibody to β-actin as a control for sample loading (*Lower*).

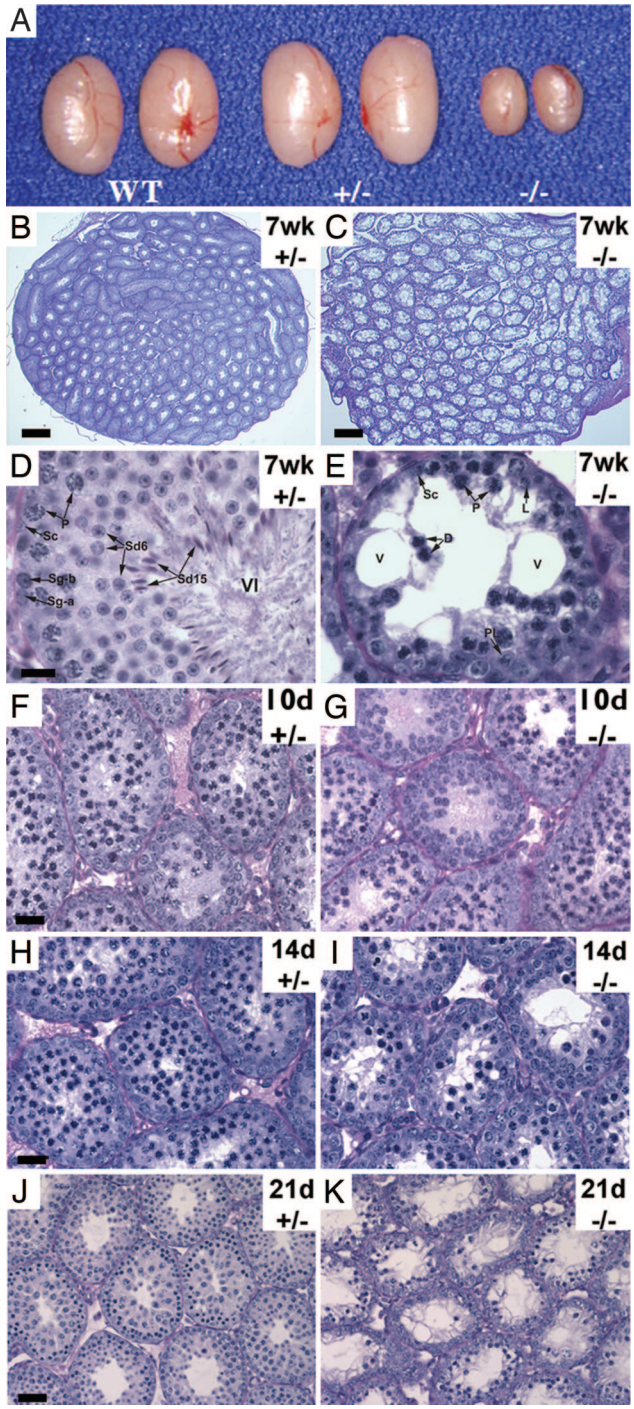

Fig. 2. Gross and histological analysis of postnatal testes. (*A*) Gross analysis of adult testes from 7-week-old littermate mice. (*B–I*) Histological analysis of testes of 49-day-old $Tex14^{+/-}$ (*B* and *D*) and $Tex14^{-/-}$ (*C* and *E*) mice, 10-day-old $Tex14^{+/-}$ (*F*) and $Tex14^{-/-}$ (*G*) mice, 14-day-old $Tex14^{+/-}$ (*H*) and $Tex14^{-/-}$ (*I*) mice, and 21-day-old $Tex14^{+/-}$ (*J*) and $Tex14^{-/-}$ (*K*) mice. D, dying spermatocytes; L, leptotene spermatocyte; P, pachytene spermatocyte; PL, preleptotene spermatocyte; Sc, Sertoli cells; Sd6, stage 6 spermatids; Sd15, stage 15 spermatids; Sg-a, type A spermatogonium; Sg-b, type B spermatogonium; V, vacuoles. VI in *D* designates a stage VI seminiferous tubule. [Scale bars: 200 μm (*B*), 100 μm (*C*), 15 μm (*D* and *E*), 20 μm (*F–I*), and 40 μm (*J* and *K*).]

ing information on the PNAS web site) demonstrated a statistically significant loss of homozygotes ($Tex14^{-/-}$) in the embryonic, perinatal, and postnatal periods [189 WT (30.0%), 328 heterozygotes (52.1%), and 113 homozygotes (17.9%); $P < 0.001$]. The cause of this loss is not clear at this time. Although *Tex14* is preferentially expressed in the testis (37), it is also expressed developmentally in other cell types (e.g., UniGene cluster Mm.103080 contains ESTs from neonatal brain and fetal pancreas) and must at times play some unknown extratesticular function. However, the majority of the *Tex14*-null mice were grossly indistinguishable from their littermates and lived to the adult stage. Whereas adult $Tex14^{-/-}$ females were normal and fertile, adult $Tex14^{-/-}$ 129S6/SvEv inbred males ($n = 5$) or C57Bl6/J/129S6SvEv hybrid males ($n = 12$) from all four ES cell lines were sterile when bred to control females over a 6- to 12-month period. Absence of the *Tex14* mRNA and protein in $Tex14^{-/-}$ mice (Fig. 1 *C* and *D*) confirmed that the *Tex14* mutation was null. Thus, we have generated *Tex14*-null mice and shown that TEX14 is required for male fertility.

Spermatogenesis Is Disrupted in $Tex14^{-/-}$ Males. Because $Tex14^{-/-}$ males were sterile, we determined whether they could mate with control females. $Tex14^{-/-}$ males appeared to have an intact neuroendocrine axis because they could produce copulation plugs, and there was no difference ($P > 0.05$) between serum testosterone levels of adult $Tex14^{-/-}$ (60.8 ± 7.2) and $Tex14^{+/-}$ (44.7 ± 11.2) males. To further define the causes of infertility in $Tex14^{-/-}$ males, postnatal testes were analyzed grossly and

histologically (Fig. 2). Testes from adult and juvenile *Tex14*$^{-/-}$ males were significantly smaller ($P < 0.01$) than *Tex14*$^{+/-}$ or WT littermates (Fig. 2*A*). Six week-old *Tex14*$^{-/-}$ testes (23.2 ± 1.1 mg; $n = 14$) were 26% the weight of WT (89.3 ± 2.7 mg; $n = 11$) and *Tex14*$^{+/-}$ (89.9 ± 2.7 mg; $n = 10$) testes. Whereas WT and *Tex14*$^{+/-}$ testes from adult males demonstrated robust spermatogenesis (Fig. 2 *B* and *D*), the seminiferous tubules of *Tex14*$^{-/-}$ males showed markedly reduced late meiotic (i.e., late pachytene and diplotene spermatocytes) and postmeiotic (i.e., spermatids and spermatozoa; Fig. 2 *C* and *E*) germ cells and contained significant vacuolization.

To define the onset of the disruption of spermatogenesis in the absence of TEX14, we analyzed testes of younger mice. *Tex14*$^{+/-}$ and *Tex14*$^{-/-}$ testes at postnatal day 10 were similar at the gross and histologic levels (Fig. 2 *F* and *G* and Fig. 6, which is published as supporting information on the PNAS web site). At 14 days, *Tex14*$^{-/-}$ testes (Fig. 2*I*) contained fewer spermatocytes, as compared with *Tex14*$^{+/-}$ testes (Fig. 2*H*), and early signs of Sertoli cell vacuolization (caused by severe germ cell depletion through endocytosis) was observed. By 21 days, *Tex14*$^{-/-}$ testes displayed severe degeneration and depletion of spermatocytes (Fig. 2*K*), and Sertoli cell vacuolization was more evident. Numerous round spermatids were present in the epithelium in *Tex14*$^{+/-}$ testis (Fig. 2 *D* and *J*), whereas no spermatids and drastically reduced numbers of spermatocytes (mainly preleptotene, leptotene, and few zygotene spermatocytes) were visible in the *Tex14*$^{-/-}$ testis. Thus, absence of TEX14 appears to lead to early meiotic death during the first cycle of spermatogenesis in postnatal testes. Examination of older animals revealed an increasingly severe phenotype (Fig. 6). At 1 year, haploid cells remain absent, whereas spermatogonia and rare, dying spermatocytes are still present in *Tex14*$^{-/-}$ testes (Fig. 6 *E* and *F*) despite the overt decrease in germ cells.

To further confirm our histological findings, we analyzed several genes that are expressed in spermatogonia or spermatocytes. *Ccna1* mRNA [encoding cyclin A1, a marker for pachytene spermatocytes (38, 39)] is abundant in *Tex14*$^{+/-}$ testes at 3 weeks and 8 weeks of age but is barely detectable in *Tex14*$^{-/-}$ testes of the same ages (Fig. 3*A*), showing that *Tex14*$^{-/-}$ testes contain very few pachytene spermatocytes as early as 3 weeks. By immunohistochemistry, we detected numerous cyclin A1-positive (late pachytene and diplotene) spermatocytes (38, 39) in *Tex14*$^{+/-}$ testes (Fig. 3*B*). In contrast, only rare cyclin A1-positive cells were observed in *Tex14*$^{-/-}$ testes (Fig. 3*B* and Fig. 7 *A* and *B*, which is published as supporting information on the PNAS web site), which is consistent with our Northern blot and histologic analyses. An antibody to synaptonemal complex protein 3 (SYCP3), a component of the synaptonemal complex and a marker for all primary spermatocytes (40, 41), labeled fewer SYCP3-positive spermatocytes in the *Tex14*$^{-/-}$ testes than in the *Tex14*$^{+/-}$ testes (Fig. 3*B*), confirming that *Tex14*$^{-/-}$ testes contain few pachytene spermatocytes and drastically reduced numbers of early spermatocytes including preleptotene, leptotene, and zygotene spermatocytes. In contrast, mRNA levels of *Kit* (Fig. 3*A*), a marker for differentiating spermatogonia (42), remained unchanged in 3 week-old *Tex14*$^{-/-}$ testes and were higher in 8 week-old *Tex14*$^{-/-}$ testes (caused by relative enrichment of spermatogonia because of severe depletion of spermatocytes and absence of haploid spermatids), supporting the observation that spermatogonia are maintained in *Tex14*$^{-/-}$ testes. No decrease was seen in the number of promyelocytic leukemia zinc finger (PLZF)-positive cells (Fig. 7 *C* and *D*), a marker of A single (A_s), A paired (A_{pr}), and A aligned (A_{al}) spermatogonia. Thus, at both the molecular and histological levels, at least some spermatogonial stem cells in *Tex14*$^{-/-}$ males are able to undergo transit amplification and enter meiosis I.

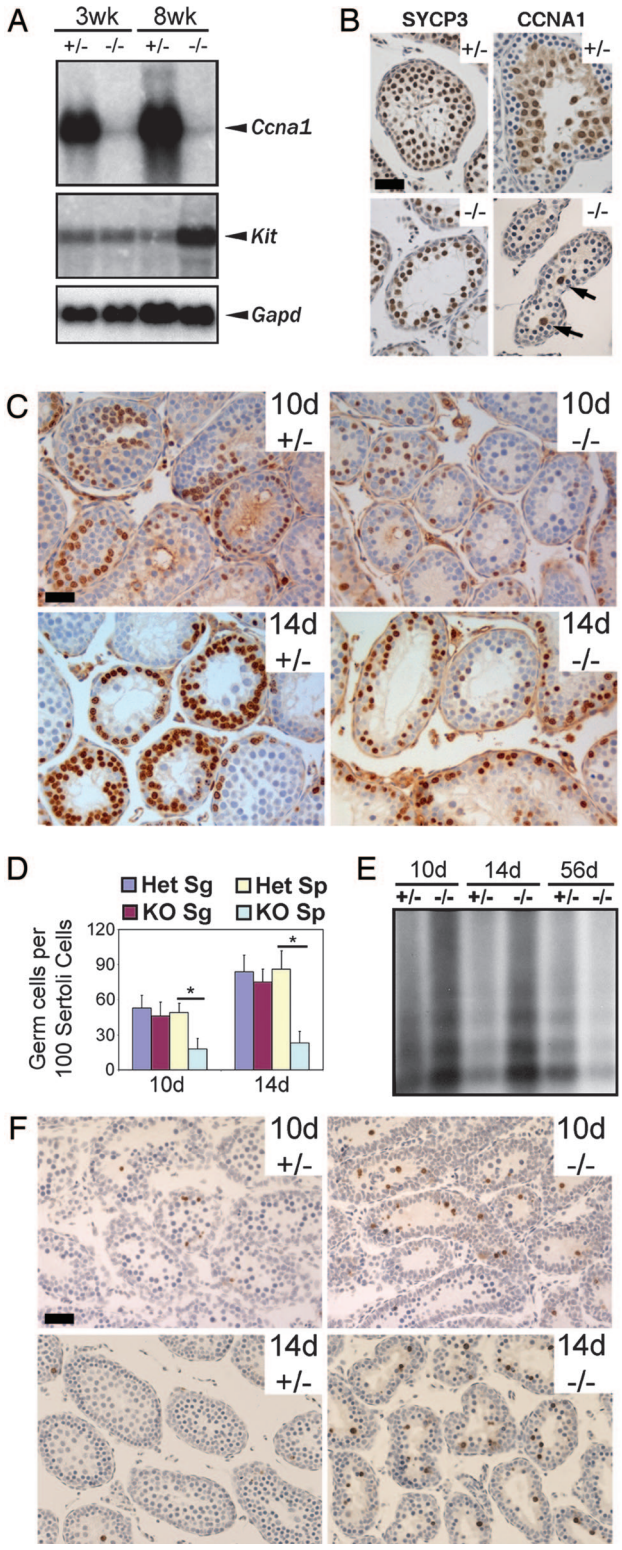

Fig. 3. Quantitative and qualitative analysis of *Tex*$^{+/-}$ and *Tex14*$^{-/-}$ testes. (*A*) Northern blot analysis of 3-week-old and 8-week-old *Tex14*$^{+/-}$ and *Tex14*$^{-/-}$ littermates. (*B*) Immunohistochemical analysis of 21-day-old *Tex14*$^{+/-}$ and *Tex14*$^{-/-}$ testes using antibodies to SYCP3 or cyclin A1 (CCNA1) (arrows point to rare pachytene spermatocytes). (*C* and *D*) BrdU labeling (*C*) and quantitative analysis (*D*) of BrdU-labeled spermatogonia (Sg) and spermatocytes (Sp) in *Tex14*$^{+/-}$ (Het) and *Tex14*$^{-/-}$ (KO) testes. (*E*) DNA laddering analysis of testes from 10-, 14-, and 56-day-old mice. (*F*) TUNEL analysis was performed on the testes of 10-day-old and 14-day-old *Tex14*$^{+/-}$ and *Tex14*$^{-/-}$ littermates. [Scale bars: 25 μm (*B* and *C*) and 50 μm (*F*).]

Decreased DNA Synthesis and Increased Apoptosis in *Tex14*$^{-/-}$ Males. To more precisely define the onset of the defects and potential causes of the disruption in the absence of TEX14, we used *in vivo* incorporation of BrdU (BrdU injection at 1 h before killing) in conjunction with immunohistochemical detection of BrdU-positive cells to quantify the number of S-phase spermatogonia and preleptotene spermatocytes (primary spermatocytes undergoing active meiotic DNA synthesis). By counting the number of BrdU-positive spermatogonia and spermatocytes in *Tex14*$^{-/-}$ and *Tex14*$^{+/-}$ testes at postnatal days 10 and 14, we found a significant reduction in the number of BrdU-positive preleptotene spermatocytes (Fig. 3 *C* and *D*). Consistent with our *Kit* mRNA analysis, spermatogonial populations are not statistically different in the absence of TEX14 (Fig. 3 *C* and *D*). The reduced number of DNA-synthesizing preleptotene spermatocytes may reflect decreased proliferative activity or enhanced apoptosis.

To determine whether the reduced number of early spermatocytes is due to enhanced apoptosis, we first performed a TUNEL assay (Fig. 3*F*). In *Tex14*$^{-/-}$ testes at postnatal days 10 and 14, there is enhanced spermatocyte apoptosis because TUNEL-positive spermatocytes are present in almost every tubule, in contrast to small clusters of TUNEL-positive spermatocytes that are occasionally seen in a few tubules in *Tex14*$^{+/-}$ testes. To confirm the TUNEL assay results, we isolated DNA from the *Tex14*$^{+/-}$ and *Tex14*$^{-/-}$ testes at postnatal days 10, 14, and 56 and performed an apoptosis DNA ladder assay (Fig. 3*E*). The internucleosomal cleavage of DNA is a hallmark of apoptosis and can be demonstrated as a ladder of discrete 185- to 200-bp multimeric bands after agarose gel electrophoresis. The *Tex14*$^{-/-}$ testes displayed quantitatively enhanced apoptotic DNA ladder patterns at both 10 and 14 days, as compared with *Tex14*$^{+/-}$ littermates (Fig. 3*E*). At 56 days, the *Tex14*$^{-/-}$ testes showed decreased levels of apoptotic DNA laddering, reflecting an absence of apoptotic germ cells due to a depletion of the degenerating germ cells. Thus, enhanced apoptosis of spermatocytes contributes to the decreased number of spermatocytes in the *Tex14*$^{-/-}$ testes.

TEX14 Is a Component of the Intercellular Bridge. TEX14 is a large protein (1,450 aa; predicted molecular mass of 162.5 kDa) with three N-terminal ankyrin repeats, a central kinase-like domain, and a C-terminal domain with limited homology to known proteins (37). To determine the subcellular localization of TEX14, we generated polyclonal antibodies to TEX14. Western blot analysis demonstrated that the antibody was specific, detecting an ≈160-kDa protein in WT testes but no protein in the testes of *Tex14*$^{-/-}$ mice (Fig. 1*D* and Fig. 8, which is published as supporting information on the PNAS web site). By immunohistochemistry, TEX14 was detected in a "ring" structure that bridged spermatids, spermatocytes, and spermatogonia (Fig. 4 *A* and *E*). TEX14 staining was rarely observed at postnatal day 5 but was frequently localized to spermatogonial intercellular bridges at postnatal day 7 (Fig. 8 *A* and *C*).

To confirm that TEX14 localized to the intercellular bridge, we compared the TEX14 expression pattern to a known bridge component. Of the six known or suspected components of the intercellular bridge, five are broadly expressed cytoskeletal components that localize to multiple structures (27, 29–32). HSF2 is unique because it is the only noncytoskeletal bridge component (28). Although HSF2 is broadly expressed, its strong localization to the intercellular bridge contrasts clearly with its diffuse cytoplasmic and nuclear staining. Additionally, HSF2 localization to the intercellular bridge has been confirmed by immunoelectron microscopy (28). TEX14 colocalizes with HSF2 (Fig. 4 *B–D*), confirming the subcellular localization of TEX14 to the intercellular bridge.

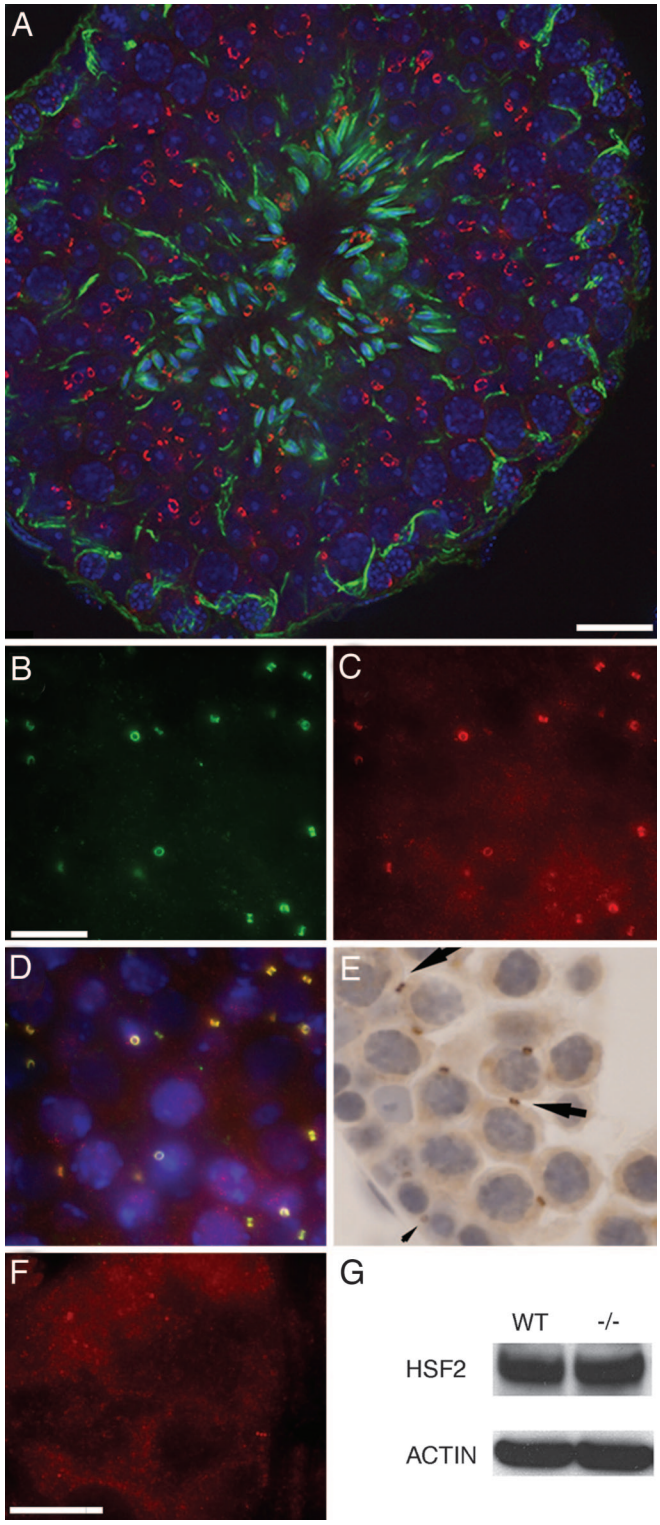

Fig. 4. Immunohistological localization of TEX14 to intercellular bridges. (*A*) Immunofluorescence of a 6-week-old WT seminiferous tubule in which staining for TEX14 (red), actin (green), and DNA (DAPI, blue) were merged. Note the presence of the TEX14-positive (red) rings throughout the tubule. (*B–D*) Immunofluorescence of 9-day-old WT intercellular bridges showing TEX14 (*B*) and HSF2 (*C*) and merged with DAPI (*D*). (*E*) Immunohistochemistry labeling of TEX14 at intercellular bridges between spermatocytes (black arrows) and spermatogonia (black arrowhead). (*F* and *G*) HSF2 immunofluorescence of 9-day-old *Tex14*-null testis lacking intercellular bridge localization (*F*) and the corresponding HSF2 protein levels in 9-day-old testes by Western blot analysis (*G*). [Scale bars: 25 μm (*A*) and 10 μm (*B–F*).]

Because TEX14 localizes to the intercellular bridges of germ cells, we examined the bridge structure in the *Tex14*$^{-/-}$ testes by electron microscopy. Intercellular bridges between spermatocytes were readily located by their classic bridge densities in 11-day-old control testes (Fig. 9 *A–C*, which is published as supporting information on the PNAS web site) but were not detected in age-matched *Tex14*$^{-/-}$ testes (Fig. 9*D*), suggesting that TEX14 may be essential for the intercellular bridge structure. To test this hypothesis with an independent assay, we examined the expression pattern of HSF2 in *Tex14*$^{-/-}$ testes. HSF2 localized to the intercellular bridge in control testes (Fig. 4*D*), whereas in *Tex14*$^{-/-}$ testes only the nuclear and cytoplasmic components of HSF2 expression were seen (Fig. 4*F*) despite unaltered HSF2 protein expression (Fig. 4*G*).

To further determine whether TEX14 is required in spermatogonial intercellular bridges, we looked at the clonal organization of the spermatogonial syncytia formed by intercellular bridges. We used PLZF again as a marker for spermatogonial stem cells and early dividing spermatogonia (i.e., A_s, A_{pr}, and A_{al}) (43, 44). We found that TEX14 is in the intercellular bridges connecting PLZF-positive germ cells in neonatal and adult testes (Fig. 5 *A* and *B*). We then examined the organization of these early syncytia using whole-mounted seminiferous tubules stained for PLZF. Whereas 35-day-old control tubules (Fig. 5*C*) contained single, paired, and aligned spermatogonia, 35-day-old *Tex14*$^{-/-}$ tubules (Fig. 5*D*) contained predominantly single spermatogonia with rare paired cells (possibly recently divided cells) and no aligned spermatogonia. We conclude from the absence of PLZF-positive syncytium in *Tex14*$^{-/-}$ tubules that TEX14 is required in early spermatogonial intercellular bridges. Intercellular bridges were abundant in other sterile knockout mouse models where spermatogenesis fails to progress beyond the first meiotic division (data not shown). Additionally, *Hsf2*-null male mice complete spermatogenesis, are fertile (45), and have intercellular bridges (data not shown). This finding further supports an essential function in the intercellular bridge specific for TEX14.

Tex14$^{-/-}$ mice demonstrate that spermatogenesis can progress without intercellular bridges through spermatogonial amplification and differentiation. However, spermatogenesis fails while the cells are still diploid but after the expression of early meiotic markers like SYCP3. Therefore, cytoplasmic contiguity is not essential for entry into meiosis. Whereas significant apoptosis and loss of proliferation at meiosis are consistent with the critical stage hypothesis (22, 25, 26), a shared cytoplasmic factor that would influence germ cell progression through meiosis is not yet known. Apoptosis is also a normal part of spermatogenesis, and there is evidence that apoptotic signals can be transmitted throughout the syncytium (46–48) or confined to one cell (48) by intercellular bridges. Further work to understand the mechanisms by which intercellular bridges regulate the transmission of apoptotic or other signals may explain the spermatogenic failure observed in *Tex14*$^{-/-}$ males.

Although our understanding of cytokinesis in somatic cells is rapidly advancing, little is known about the unique aspects of cytokinesis and intercellular bridge development in mammalian germ cells. In mammals, TEX14 is required for formation, maintenance, and/or stability of the intercellular bridge. In recruiting HSF2 and other bridge components, TEX14 most likely acts as a scaffold for intercellular bridge development rather than a kinase because there are unconventional amino acid replacements in the kinase-like domain of TEX14 (37, 49, 50), and TEX14 lacks kinase activity on generic substrates in standard tyrosine and serine/threonine kinase assays (Fig. 10 *A* and *B*, which is published as supporting information on the PNAS web site). That said, the TEX14 kinase-like domain may play a novel, nonstructural function in the intercellular bridge. It is interesting that the closest alignments for the kinase-like domain of mammalian TEX14 to the *Drosophila* genome are the kinase domains of Src64 and Tec29. It is tempting to speculate that an analogous function may exist for this domain. Further studies on TEX14 function and identification of other essential intercellular bridge components are necessary.

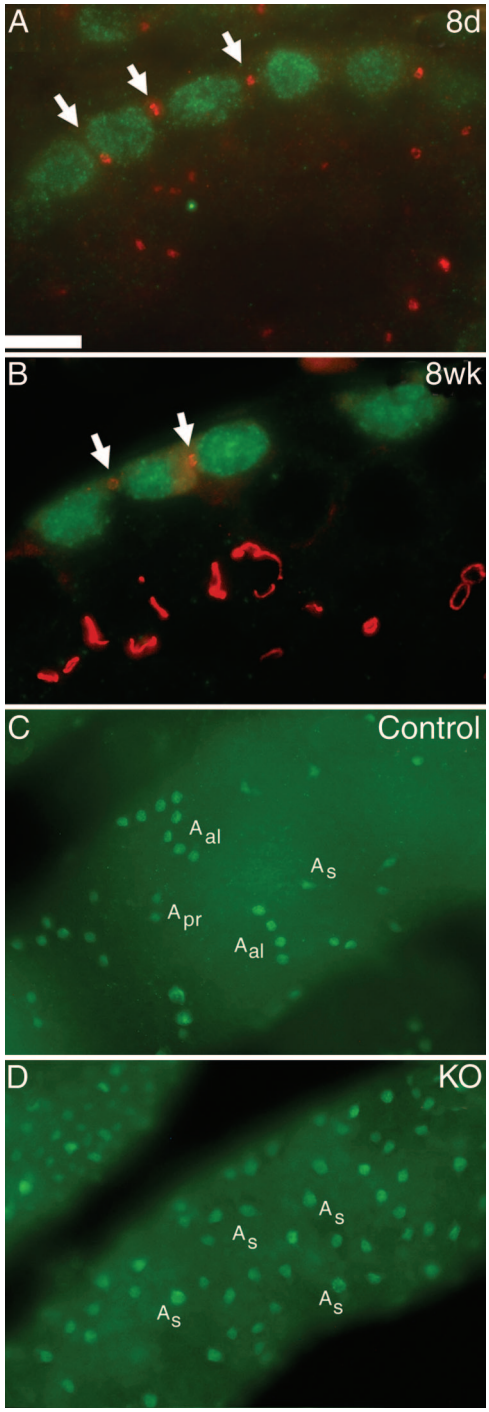

Fig. 5. Immunolocalization of TEX14 and PLZF in WT and *Tex14*$^{-/-}$ mice. TEX14 (red) localizes to the intercellular bridges between PLZF-positive spermatogonia (green) in 8-day-old, neonatal (*A*) and 8 week-old, mature (*B*) mice. PLZF labeling of whole-mount seminiferous tubules shows A_s, A_{pr}, and A_{al} spermatogonia in 35-day-old *Tex14*$^{+/-}$ tubules (*C*), whereas predominantly A_s spermatogonia appear to be present in 35-day-old *Tex14*$^{-/-}$ tubules (*D*). [Scale bar: 10 μm (*A* and *B*).]

Materials and Methods

Generation of *Tex14* Mutant Mice. We electroporated the linearized *Tex14* targeting vector (Fig. 1*A*) into the HPRT-negative AB2.2 ES cell line; selected clones in hypoxanthine, aminopterine, thymidine, and 1-(2′-deoxy-2′ fluoro-β-D-arabinofuranosyl)-5-iodouracil; and screened the ES cell DNA by Southern blot as described (51). Correctly targeted clones were identified by using 5′ and 3′ probes as shown (Fig. 1*A*). Mice were genotyped by Southern blot as described (51).

Production of Anti-TEX14 Antibody. A cDNA fragment containing sequences encoding amino acids 885-1301 of the mouse TEX14 protein was subcloned into pET23b (Novagen), His-tagged TEX14 was produced in BL21 (DE3) pLysS cells, and polyclonal antibodies were raised in guinea pigs (Cocalico Biologicals, Reamstown, PA).

For an extensive description of the materials and methods see *Supporting Materials and Methods*, which is published as supporting information on the PNAS web site.

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