The REV7 Subunit of DNA Polymerase ζ Is Essential for Primordial Germ Cell Maintenance in the Mouse

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Background: Biological significance of REV7 in mouse development has not been elucidated.

Results: REV7-deficient mice show germ cell aplasia at birth in both sexes, and primordial germ cells (PGCs) were lost owing to apoptosis during migration at an early embryonic stage.

Conclusion: REV7 is essential for PGC maintenance in the mouse.

Significance: REV7 is a novel regulator of PGC survival.

SUMMARY

REV7 (also known as MAD2L2 and MAD2B) is involved in DNA repair, cell cycle regulation, gene expression and carcinogenesis. In vitro studies show that REV7 interacts with several proteins and regulates their function. It has been reported that human REV7 is highly expressed in the adult testis by northern blot analysis. However, the significance of REV7 in mammalian development has not been elucidated. Here, we present analyses of REV7-deficient (Rev7−/−) mice to clarify the significance of Rev7 in mouse development. In WT mice (Rev7+/+), Rev7 expression was ubiquitously observed in the embryo and confined to germ cells in the testes after birth. Rev7−/− mice exhibited growth retardation and a partial embryonic lethal phenotype. Mice that survived to adulthood were infertile in both sexes and showed germ cell aplasia in the testes and ovaries. Analyses of Rev7−/− embryos revealed that primordial germ cells (PGCs) were present at embryonic day 8.5 (E8.5). However, progressive loss of PGCs was observed during migration, and PGCs were absent in the genital ridges at E13.5. An increase of apoptotic cells was detected not only among PGCs but also in the forebrain of the Rev7−/− embryo, while cell proliferation was
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unaffected. Moreover, DNA damage accumulation and increased levels of histone methylation were detected in Rev7−/− embryos, and expression of Oct4 and Nanog was deregulated by REV7 deficiency at E8.5. These findings indicate that Rev7 is essential for PGC maintenance by prevention of apoptotic cell death in the mouse.

Germ cell development in mice is regulated by specific genetic and epigenetic factors. Germ cells arise from pluripotent epiblast cells and are characterized as alkaline phosphatase (AP)-positive primordial germ cells (PGCs) that form a cluster of about 40–45 cells inside the extra-embryonic mesoderm at the posterior end of the primitive streak at around embryonic day 7.25 (E7.25). Subsequently, they migrate through the hindgut endoderm and reach the genital ridges by E11.5, where they proliferate and differentiate to spermatozoa or oocytes from E13.0 (1-4). By the time of sexual differentiation, the number of PGCs increases to approximately 25,000. Before germ cell specification, bone morphogenetic protein 4 (BMP4), BMP8b and BMP2 secreted from the extraembryonic ectoderm and visceral endoderm induce the formation of PGC precursor cells in the proximal epiblast. These PGC precursor cells express transcriptional regulators B lymphocyte maturation-induced protein 1 (Blimp1) and PR domain containing 14 (Prdm14). The former suppresses gene expression required for somatic cell differentiation, such as Snail and Hox genes, and the latter controls genome-wide epigenetic reprogramming in PGCs (2, 5-7). During PGC migration, expression of Nanos3 and c-Kit is essential for PGC maintenance. Nanos3 is an RNA binding protein expressed in PGCs after E7.5 and prevents apoptosis of PGCs during migration (8). c-Kit is a receptor tyrosine kinase, and the signal from the c-Kit ligand is required for proliferation and directed migration of PGCs (9). In addition, some knockout mouse studies have revealed essential factors for PGC survival between E8.5 and E13.5, including RNA binding protein dead end 1 (Dnd1), T cell-restricted intracellular antigen 1-related protein (TIAR), a homeodomain transcription factor of the POU family, Oct4, and a unique homeoprotein transcription factor, Nanog (10-13). Epigenetic modification also occurs during PGC migration. Reduction of H3K9 dimethylation (H3K9me2) and upregulation of H3K27 trimethylation (H3K27me3) are principal epigenetic modifications after E8.5, which control the chromatin state of the PGC genome (14, 15). PGCs proliferate for another 1 or 2 days after they colonize the genital ridges and then differentiate into spermatozoa or oocytes (16).

REV7 (also named MAD2L2 and MAD2B) is a protein involved in DNA repair, cell cycle regulation, gene expression, and carcinogenesis. In the yeast Saccharomyces cerevisiae, Rev7 and Rev3 bind to each other to form DNA polymerase ζ (Pol ζ), in which Rev3 is a catalytic subunit possessing a polymerase activity and Rev7 is an accessory subunit (17). Pol ζ is a member of the specialized low fidelity DNA polymerases, including Y-family polymerases, Pol η, Pol ι, Pol κ and Rev1, which are capable of synthesizing DNA at DNA lesions via a mechanism called translesion DNA synthesis (TLS) (18). Rev7 enhances the polymerase activity of Rev3, indicating the involvement of Rev7 in the DNA damage response (17). A human homolog of yeast Rev7 has been identified as an interacting partner of human REV3 (19). REV7 also interacts with human REV1, but the biological significance of REV7 association with REV3 and REV1 in humans is unclear (20-22). Human fibroblasts with siRNA-mediated depletion of REV7 expression show high sensitivity to UV-induced cytotoxicity and reduced sensitivity to UV-induced mutagenesis compared with those in control lines, indicating that REV7 is required for tolerance to UV-induced DNA damage (23). Recently, it has been reported that Pol ζ and REV1 are involved in homologous recombination repair of DNA double-strand breaks, although its mechanism is not fully understood (24, 25).

On the other hand, REV7 has been identified as the second human homolog of S. cerevisiae Mad2 (26). REV7 binds to CDH1 and CDC20, which are cell cycle regulating proteins, and inhibits the anaphase-promoting complex (APC) that degrades cyclin B1 (27, 28). When recombinant human REV7 protein is injected into Xenopus embryos, gastrulation is dramatically arrested (28). REV7 also interacts with the
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*Shigella* effector IpaB, and mediates cell cycle arrest of *Shigella*-infected epithelial cells (29). IpaB-bound REV7 is sequestered away from CDH1, causing unscheduled activation of the APC, which disrupts cell cycle-dependent regulation of cyclin B1 expression, resulting in cell cycle arrest at the G2/M phase in *Shigella*-infected epithelial cells. These findings indicate that REV7 is involved in cell cycle regulation. In addition, it has been reported that REV7 interacts with the transcription factor ELK-1 and regulates the expression of its target genes, *egr-1* and *c-fos*, after treatment with DNA-damaging agents (30). REV7 also interacts with TCF-4, a component of the WNT signaling pathway, and modulates TCF-4-mediated E-cadherin expression (31). Moreover, it has been reported that several proteins involved in carcinogenesis, such as PRCC (papillary renal cell carcinoma) and HCCA2 (hepatocellular carcinoma-associated gene 2), interact with REV7. Increased levels of REV7 mRNA in colon cancer are significantly correlated with reduced patient survival, suggesting that REV7 is associated with human cancer biology (32-34). However, the significance of REV7 in mammalian development has not yet been elucidated.

In the present study, we generated Rev7-deficient (*Rev7*^−/−^) mice and analyzed their phenotypes to understand the biological significance of REV7 in mouse development. We show that REV7 is involved in the prevention of apoptotic cell death of PGCs and is essential for PGC maintenance in the embryo. Our findings indicate the importance of REV7 expression in mouse development.

**EXPERIMENTAL PROCEDURES**

*Vector construction*—The targeting vector was constructed using pBlueScript II KS (Agilent Technologies) possessing a neomycin (*neo*) selection marker with a phosphoglycerine kinase (*PGK*) promoter. A 1330-bp genomic fragment upstream of the start codon of the mouse *Rev7* locus was synthesized by PCR and inserted before the *PGK-neo* cassette. A 5110-bp genomic sequence (containing *Rev7* exon 3–8) was synthesized by PCR and inserted after the *PGK-neo* cassette (Fig. 1A). PCR products were generated using PfuUltra™ High-Fidelity DNA polymerase (Agilent Technologies), and genomic DNA from the 129SvJ strain was used as a template. The targeting vector was verified by DNA sequencing and restriction mapping.

*Generation of Rev7-knockout mice*—The targeting vector was linearized and introduced by electroporation into ES cells derived from 129SvJ mice. After G418/diphtheria toxin A positive-negative selection, two ES cell clones with successful homologous recombination were identified by Southern blot screening of Nhe I-digested genomic DNA with a 5′ probe (Fig. 1A). The two clones were injected into C57BL/6J blastocysts and chimeric mice were generated by PhoenixBio. The genetic background of the mice used in this study was C57BL6J/129S6. All mice were housed in polycarbonate cages containing hardwood chip bedding at 25°C with a 12-h light/dark cycle. All animal protocols were approved by the Animal Care and Use Committee of Nagoya University Graduate School of Medicine.

*Mouse genotyping*—Genomic DNAs extracted from mouse tail biopsies were used for PCR genotyping. Sequences of the primer sets used for genotyping are listed in Table 1. PCR products of WT and targeted alleles were 230 bp and 750 bp, respectively. Templates were amplified with ExTaq polymerase (Takara).

*Southern blot screening*—Mouse genomic DNAs were digested with Nhe I. The 5′ probe indicated in Fig. 1A was radiolabeled with 32P-dCTP (PerkinElmer) using a High Prime kit (Roche). Southern hybridization was performed using a conventional protocol. A 4.66-kb fragment and a 3.96-kb fragment were detected for the WT and targeted alleles, respectively.

*Northern blot analysis*—A Mouse Multiple Tissue Northern Blot Membrane was purchased from Clontech. Total RNAs from mouse tissues were extracted using Trizol reagent (Invitrogen) according to the manufacturer’s instructions. Ten micrograms of total RNAs were separated by agarose gel electrophoresis and then transferred onto nylon membranes (GE healthcare). Northern hybridization was performed with radiolabeled mouse *Rev7* or β-actin cDNA probes using standard methodologies.

*Western blot analysis*—Small pieces of mouse tissue were homogenized by sonication in SDS...
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Sample buffer (62.5 mM Tris-HCl, pH 6.8, 2% SDS, 25% glycerol, and 20 μg/ml bromophenol blue). After measuring protein concentration using a DC protein Assay Kit (Bio-Rad Laboratories), lysates were boiled at 100°C for 2 min in the presence of 2% 2-mercaptoethanol. Lysates containing 50 μg protein were subjected to SDS-PAGE and then transferred onto PVDF membranes (Millipore Corporation). The membranes were blocked for 1 h at room temperature (RT) in Blocking One (Nacalai Tesque) with gentle agitation and then incubated with the primary antibody for 1 h at RT. After washing with TBST buffer (20 mM Tris-HCl, pH 7.6, 137 mM NaCl, and 0.1% Tween 20), membranes were incubated with the secondary antibody conjugated to HRP (Dako) for 1 h at RT. After washing the membranes, the reaction was detected using an ECL Detection Kit (GE Healthcare) and visualized by an ImageQuant LAS 4000 mini (GE Healthcare).

Antibodies—A rabbit polyclonal anti-REV7 antibody was produced by immunization with full-length human REV7 protein fused to GST. The antibody was affinity purified using full-length recombinant mouse REV7 protein. Anti-promyelocytic leukaemia zinc finger protein (PLZF), -GATA-4, -Oct4, -proliferating cell nuclear antigen (PCNA) and -GAPDH antibodies were purchased from Santa Cruz Biotechnology. An anti-cleaved caspase-3 antibody was purchased from Cell Signaling Technology, Japan. Anti-phospho-Histone H2A.X (Ser139), -dimethyl-Histone H3 (Lys9) and -trimethyl-Histone H3 (Lys27) antibodies were purchased from Millipore Corporation.

Histological analysis—Mouse tissues were fixed in 10% neutral-buffered formalin, dehydrated and embedded in paraffin. Four micrometer thick sections were prepared for H&E staining and immunohistochemistry. H&E staining was performed by a conventional method.

Immunohistochemistry—Sections were deparaffinized in xylene and rehydrated in a graded series of ethanol. For antigen retrieval, sections were immersed in Target Retrieval Solution, pH 9.0 (Dako) and heated for 30 min at 98°C in a water bath. Non-specific binding was blocked with 10% normal goat serum for 10 min at RT. Sections were then incubated with primary antibodies for 1 h at RT. Endogenous peroxidase was inhibited by incubation with 3% hydrogen peroxide in methanol for 15 min. Then, sections were incubated with the secondary antibody conjugated to HRP-labeled polymer (EnVision+ anti-rabbit; Dako) for 15 min at RT. The reaction products were visualized with diaminobenzidine (DAB) (Dako), and nuclei were counterstained with hematoxylin.

Whole-mount immunohistochemistry—Whole-mount immunohistochemistry was performed as described previously (35). Mouse embryos were fixed in 10% neutral-buffered formalin overnight, washed with PBST (10 mM phosphate-buffered saline, and 0.1% Tween 20, pH 7.4) for 10 min three times, washed with distilled water for 10 min three times, and then heated in Target Retrieval Solution for 30 min at 98°C. Embryos were then washed with PBST for 10 min three times and then immersed in 10% normal rabbit serum for 90 min at RT for blocking. Samples were incubated with an anti-Oct4 antibody (1:100 dilution) overnight at 4°C and then washed with PBST for 30 min 10 times. Endogenous peroxidase was inhibited by incubation with 2% hydrogen peroxide in TBST for 4 h at 4°C. Then, samples were incubated with an anti-mouse IgG antibody conjugated to HRP-labeled polymer (1:200 dilution) (Dako) overnight at 4°C, followed by washing with PBST for 30 min 12 times. The immunoreaction was visualized using DAB.

Real-time quantitative RT-PCR—To obtain embryos, pregnant mice were anesthetized with sevoflurane prior to being sacrificed. Dissected each organs (E17.5, approximately 30 mg), whole bodies (E8.5), or trunks (E13.5) were homogenized in RLT buffer of RNeasy Mini Kit (Qiagen) using BioMasher (Nippi). Total RNA was isolated from the homogenate using the RNeasy Mini Kit, followed by treatment with DNase (Qiagen) to minimize genomic DNA contamination according to the manufacturer's protocol. Purified RNA samples were reverse-transcribed using ReverTra Ace (Toyobo). Twelve ng RNA equivalent-cDNA was mixed with Thunderbird SYBR qPCR Mix (Toyobo) and amplified on a Mx3005P thermal cycler (Agilent Technologies) using gene specific primer sets as described in Table 1 (36,37).
In situ hybridization on frozen sections– Mice were perfused intravascularly with a 4% paraformaldehyde solution. The testes were dissected, embedded in OCT compound (Sakura Finetek) and then frozen on dry ice. Ten-micrometer-thick sections were prepared using a cryostat (Leica Microsystems). A mouse Rev7-specific riboprobe was designed to target the 207-bp region that was deleted in the Rev7–/– mouse. A PCR product containing the riboprobe target region and RNA polymerase binding sites (SP6 for the sense probe, and T7 for the anti-sense probe) was generated using the primers 5′-CCAAGCTATTTAGGTGACACTATAGAACACCCTCCAGCCTCCC-3′ and 5′-TGAATTGTAATACGACTCACTATAGGGAGATCCGGGTGACAGGACACTGAA-3′. Radiolabeled riboprobes were synthesized with SP6 and T7 RNA polymerases (Roche) and 33P-dUTP (Perkin Elmer). Sections were hybridized overnight at 60°C using 200 µl hybridization solution per section, consisting of 1 × 106 cpm radiolabeled probe and hybridization buffer (50% formamide, 10% dextran sulfate, 0.5 M NaCl, 1× Denhardt’s solution, 10 mM Tris, pH 8.0, 1 mM EDTA, 500 µg/ml yeast tRNA, and 10 mM DTT). Following hybridization, sections were immersed in 2× standard saline citrate for 15 min at RT and then in RNase buffer (20 µg/ml RNase A, 0.5 M NaCl, 10 mM Tris, pH 8.0, and 1 mM EDTA) for 30 min at 37°C. After extensive washing and dehydration, sections were dipped in twice-diluted Kodak Autoradiography Emulsion, Type NTB (Eastman Kodak) and then dried at RT for 30 min in a darkroom. Sections were stored at 4°C for approximately 2 weeks while protected from light and developed using Kodak D19 Developer and Fixer (Eastman Kodak).

Whole-mount in situ hybridization– Whole-mount in situ hybridization was performed with digoxigenin (DIG)-labeled RNA probes as described previously (38). Probes were synthesized using the same PCR product used for in situ hybridization on frozen sections and DIG RNA labeling mix (Roche). Mouse embryos were fixed overnight in 4% paraformaldehyde, treated with 10 µg/ml proteinase K in PBST at 37°C for 15 min and then treated with 2 mg/ml glycine at RT for 5 min. After re-fixation with 4% paraformaldehyde + 0.2% glutaraldehyde in PBST for 20 min at RT, embryos were immersed in pre-hybridization buffer (50% formamide, 5× SSC, 50 µg/ml heparin, 5 mg/ml trout RNA, 2% blocking reagent (Roche), and 0.1% Tween 20) for 90 min at 70°C, and then incubated with hybridization solution (0.2 µg DIG-labeled RNA probes in pre-hybridization buffer) at 70°C overnight. Following hybridization, samples were rinsed with washing buffer 1 (50% formamide, 5× SSC, and 0.1% Tween 20) at 70°C for 30 min three times, and then washing buffer 2 (50% formamide, 2× SSC, and 0.1% Tween 20) at 65°C for 30 min three times. After blocking with 5% normal sheep serum for 90 min, embryos were incubated with an anti-DIG antibody conjugated to AP (1:10,000 dilution) at 4°C overnight. Embryos were washed with PBST for 30 min 10 times, and the signals were visualized using BM purple AP substrate (Roche).

Fluorescence staining– For immunofluorescence staining, an Alexa Fluor 488- or 594-labeled secondary antibody (Invitrogen) was applied for 30 min at RT. The TUNEL assay was performed according to the manufacturer's instructions (In Situ Cell Death Detection Kit, Fluorescein; Roche). Images were obtained under a fluorescence microscope (Olympus).

Statistical analysis– Statistical significance was determined with the Student’s t-test. A value of p < 0.05 was considered significant.

RESULTS

Generation of Rev7-knockout mice– To generate mice lacking the Rev7 transcript, a targeting vector was designed to delete the start codon located in exon 1–2 of the Rev7 locus. This deletion was replaced by the PGK-neo cassette (Fig. 1A). ES cell clones with homologous recombination were injected into blastocysts. The resultant chimeric male mice were mated with C57BL/6 female mice to ascertain germ-line transmission of the targeted locus, and Rev7 heterozygous knockout (Rev7+/−) mice were produced. Rev7+/− siblings were then intercrossed to produce homozygous knockout (Rev7−/−) mice. Genotyping of Rev7+/+, Rev7+/− and Rev7−/− mice by Southern blotting using a 5’ probe showed a single band of 4.66 kb for Rev7−/− mice, a single band of 3.96 kb for Rev7+/− mice, and both bands for Rev7+/− mice (Fig. 1B). PCR genotyping using
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genomic DNAs and primers specific for WT and knockout alleles showed a 230-bp band, 750-bp band, and both bands for Rev7+/+, Rev7+/- and Rev7–/– mice, respectively (Fig. 1C). Northern blot analysis using RNAs extracted from the testes of Rev7+/+ and Rev7+/- mice showed a 1.3-kb band for Rev7+/+ mice and no band for Rev7–/– mice (Fig. 1D). In addition, western blot analysis with the anti-REV7 antibody using lysates prepared from the testes, kidneys, liver and spleen of Rev7+/+ and Rev7+/- mice showed high levels of REV7 protein in the testes and low levels in the liver of Rev7+/+ mice, and a complete absence of REV7 in all the tissues of Rev7–/– mice (Fig. 1E). These results confirmed the establishment of Rev7-knockout mice that were null for REV7 expression.

High level of REV7 expression in germ cells of the testis--The tissue distribution of REV7 expression was analyzed at mRNA and protein levels. Rev7 mRNA distribution was analyzed by northern blotting using a Multiple Tissue Northern Blot Membrane, revealing that Rev7 was expressed in the heart, liver, kidney, and most predominantly in the testis (Fig. 2A). REV7 protein distribution was analyzed by western blotting with the anti-REV7 antibody using lysates extracted from various organs of postnatal day 56 (P56) WT mice. A high level of REV7 protein was also detected in the testis, and a low level of REV7 protein was present in the ovary. However, REV7 was almost undetectable in other organs (Fig. 2B).

Next, we analyzed in detail the expression of REV7 in the testis. Rev7 mRNA was assessed by in situ hybridization using Rev7 anti-sense and sense probes and sections of P56 WT testis. The anti-sense Rev7 probe produced strong signals in the seminiferous tubules with stronger signals in the peripheral region (Fig. 2C). The sense Rev7 probe produced no signals, indicating that Rev7 is specifically expressed in the seminiferous tubules.

REV7 protein was analyzed by immunohistochemical and immunofluorescence staining using the anti-REV7 antibody. REV7 protein was clearly detected in a wide range of cells in the seminiferous tubules, and especially in spermatogonia and spermatocytes (Fig. 2D). However, its level was comparatively low in spermatozoa and almost undetectable in Sertoli cells. REV7 protein was localized mainly in the nucleus. These findings from in situ hybridization and immunostaining suggest that REV7 plays an important role in germ cell biology.

REV7 expression is ubiquitously detected in the mouse embryo and becomes confined to the testis after birth--Next, we evaluated REV7 expression during embryonic, newborn, and young adult periods. Using E9.5 WT mouse embryos, Rev7 mRNA was examined by whole-mount in situ hybridization using Rev7 anti-sense and sense probes. The anti-sense probe detected a positive signal throughout the entire body (purple signal), whereas no signal was detected using the sense probe (Fig. 2E, upper panels). In situ hybridization on tissue section of E9.5 WT embryos was also performed. A positive signal was detected throughout the body, especially in the periventricular area, by using the anti-sense probe (Fig. 2E, lower panels). We checked Rev7 expression in a late embryonic stage (E17.5) by real-time quantitative RT-PCR, in which Rev7 mRNA expression was detected in all the analyzed organs with high level expression in cerebrum and cerebellum (Fig. 2F). These results indicate that Rev7 is expressed not only in germ cells but also in somatic cells at an embryonic stage. On the other hand, during newborn and young adult periods, REV7 expression was clearly detected in the testis at all ages, whereas REV7 was gradually diminished with age in other organs analyzed by western blotting (Fig. 2G). These results indicate that REV7 is expressed in a wide range of cells during embryonic stages and its expression subsequently becomes confined to the testis after birth.

Rev7+/- mice are partially embryonic lethal--After Rev7+/- male and female mice were intercrossed to generate Rev7+/- mice, we observed that Rev7+/- mice were born but the number of Rev7+/- mice was fewer than expected according to the Mendelian ratio (Table 2). When we checked the numbers of Rev7+/-, Rev7+/-, and Rev7+/- embryos from E8.5 to E13.5, they were mostly compatible with the expected Mendelian ratios (Table 2). These findings indicate that Rev7+/- mice are partially embryonic lethal with some surviving to birth.

Rev7 deficiency results in a congenital spermatogonia defect in living male mice--Rev7+/- mice were fertile and showed no obvious
differences compared with that of WT mice. The Rev7−/− mice that were born looked feeble, and their body size was small compared with that of Rev7+/+ or Rev7+/− littermates (Fig. 3A). The body size of Rev7−/− mouse embryos was also small (Fig. 3B). The body weight of Rev7−/− male mice was significantly lower than that of Rev7+/+ or Rev7+/− mice (Fig. 3D). Rev7−/− male mice were infertile, and their testes were quite small compared with those of Rev7+/− siblings (Fig. 3C). The ratio of testis to body weight of Rev7+/− mice was not significantly different from that of Rev7+/+ mice (Fig. 3E). Histological analysis revealed that the seminiferous tubules of the testes of Rev7−/− mice were entirely atrophic at P14 and P56, which contained very few cells in the peripheral region and no spermatozoa, whereas numerous germ cells and spermatozoa were present in the seminiferous tubules of Rev7+/+ siblings (Fig. 3F). To understand why spermatogenesis did not occur in Rev7+/− testes, we performed immunohistochemical analyses using the anti-PLZF antibody that is specific for Sertoli cells, showed that all of the PLZF-positive spermatogonia were identified in the basal region of the seminiferous tubules of Rev7+/+ mice at P0 and P14. In contrast, no PLZF-positive spermatogonia were identified in the seminiferous tubules of Rev7−/− mice (Fig. 3G). Analysis using the anti-GATA-4 antibody, which is specific for Sertoli cells, showed that all of the cells in the seminiferous tubules of Rev7+/− mice were GATA-4-positive Sertoli cells (Fig. 3G). These findings indicate a congenital spermatogonial defect in Rev7+/− mice, which results in an absence of spermatogenesis, thereby retaining only Sertoli cells in the seminiferous tubules.

REV7 deficiency also impairs oogenesis in female mice– The body size of Rev7+/− female mice, which were also infertile, was significantly smaller, compared with that of Rev7+/+ and Rev7+/− siblings (Fig. 4A). Macroscopically, the ovaries of Rev7−/− female mice were quite small and atrophic compared with those of Rev7+/− siblings (Fig. 4B, left panels). Histopathologically, the ovaries of adult Rev7+/− mice at P28 had many follicles containing oocytes, whereas those of Rev7−/− mice consisted of stromal and epithelial cells without follicles (Fig. 4B, middle and right panels). We checked the Rev7 mRNA expression in WT mouse ovary by in situ hybridization, in which the anti-sense probe detected an elevated signal in the oocyte, demonstrating Rev7 expression in the oocyte (Fig. 4C). These results indicate that REV7 deficiency causes germ cell aplasia in both male and female mice.

Apoptotic cell death of PGCs during migration in REV7-deficient embryos– Because Rev7−/− germ cells were not present in either sex, we hypothesized that REV7 deficiency causes developmental abnormalities in PGCs during early embryonic stages before sexual differentiation. To ascertain the development of PGCs at early embryonic stages, we performed whole-mount immunohistochemistry with the anti-Oct4 antibody on Rev7+/+ and Rev7−/− mouse embryos to identify PGCs. At E8.5, PGCs were clearly identified at the base of the allantois in Rev7−/− mouse embryos, indicating no obvious difference in the number of PGCs from that in Rev7+/+ mouse embryos (Fig. 5A, left panels). During E9.5–10.5, migrating PGCs were detected in Rev7−/− mice, but the number of PGCs progressively decreased with time (Fig. 5A, middle and right panels). After E11.5, the presence of PGCs was assessed by immunohistochemical staining of tissue sections with the anti-Oct4 antibody. At E11.5, the majority of PGCs in Rev7−/− mice had disappeared and only a few PGCs were detected around the genital ridges (Fig. 5B, left panels). At E13.5, PGCs in Rev7−/− mice were completely undetectable at the genital ridges (Fig. 5B, middle panels). These results suggest that REV7 deficiency does not affect the development or migration of PGCs, but causes progressive loss of migrating PGCs during E9.5–13.5. Next, cleaved caspase-3 immunoreactivity was assessed using serial tissue sections of E11.5 embryos, in which cleaved caspase-3-positive cells were detected at a high frequency around the genital ridges of Rev7−/− mice compared with that of Rev7+/+ mice (Fig. 5B, right panels), and some cells were positive for both Oct4 and cleaved caspase-3, suggesting apoptosis of PGCs (Fig. 5B, arrows). We also performed double fluorescence staining for Oct4 and TUNEL using tissue sections around the hindgut of E9.5 embryos to confirm apoptosis of
PGCs (Fig. 5C). We found that some of the Oct4-positive PGCs were TUNEL positive in Rev7+/− embryos (Fig. 5C; arrowheads), whereas none of the Oct4-positive PGCs were TUNEL positive in Rev7+/+ mice, indicating that REV7 deficiency causes apoptotic cell death of PGCs during embryonic stages. We checked REV7 expression in PGCs by double immunostaining with anti-REV7 and anti-Oct4 antibodies using tissue sections of the genital ridge of E13.5 WT embryos. REV7 expression was detected in most of the cells in the genital ridge, and some of which were Oct4-positive PGCs (Fig. 5D). These results indicate that REV7 is expressed in PGCs and plays an essential role in the survival of PGCs in mouse embryos.

REV7 deficiency causes increased apoptotic cell death in the neuroblasts of mouse embryos but does not affect cell proliferation. We further evaluated cleaved caspase-3-positive apoptotic cells among neuroblasts in the forebrain of E11.5 embryos, in which the frequency of apoptotic cells significantly increased in Rev7+/− embryos (Fig. 6A, B), indicating that REV7 deficiency causes apoptotic cell death not only in PGCs but also in the somatic cells of embryos. We also assessed cell proliferation in the forebrain by immunohistochemical staining with the anti-PCNA antibody, but no significant difference was detected between Rev7+/− and Rev7+/+ embryos (Fig. 6C, D), suggesting that REV7 deficiency does not affect cell proliferation.

REV7 deficiency results in DNA damage accumulation and elevated levels of histone methylation. In order to investigate the cause of increased apoptotic cell death in Rev7+/− embryos, accumulation of DNA damage represented by double strand breaks, which can be detected by anti-phospho-H2AX (pH2AX) antibody, was assessed in Rev7+/− and Rev7+/+ embryos by immunohistochemical staining. Rev7+/− embryo at E9.5 displayed a number of cells positive for pH2AX, some of which were also positive for Oct4, whereas pH2AX-positive cells were undetectable in Rev7+/+ embryo (Fig. 7A). In Rev7−/− embryo at E13.5, when PGCs were completely disappeared in Rev7−/− embryos, many somatic cells around the genital ridge were appeared to be positive for pH2AX (Fig. 7B). These results indicate that REV7 deficiency causes DNA damage accumulation in both PGCs and somatic cells during embryonic period. In addition, histone methylation represented by H3K9me2 and H3K27me3 were assessed in Rev7+/+ and Rev7−/− embryos by immunofluorescence staining. Elevated levels of H3K9me2 and H3K27me3 were demonstrated in most cells of Rev7+/− embryo at E9.5 compared with Rev7+/+ embryo (Fig. 7A). However, in embryos at E13.5, no obvious difference was detected in the levels of H3K9me2 and H3K27me3 between Rev7+/+ and Rev7−/− embryos (Fig. 7B). These results indicate that REV7 deficiency also affects histone methylation levels at the early embryonic stage.

Deregulation of gene expression in REV7-deficient embryos at E8.5– To elucidate the effect of REV7-deficiency on gene expression important for PGC survival, real-time quantitative RT-PCR was performed using RNAs extracted from Rev7+/+ and Rev7−/− whole embryos at E8.5, when PGCs were present in Rev7+/− embryos like as in Rev7+/+ embryos (Fig. 5A), and the expression of Blimp1, Oct4, Nanog, c-Kit, and Tiar was analyzed. As shown in Fig. 8A, Oct4 and Nanog expression was upregulated in Rev7−/− embryos compared with those in Rev7+/+ embryos, whereas Blimp1, c-Kit and Tiar expression did not show obvious association with Rev7 status. We also analyzed the expression of Cdc20 and Rev3, whose products interact with REV7, in which no association was detected between their expression and the Rev7 status (Fig. 8B). It was revealed that Oct4 expression was downregulated in Rev7−/− embryos, whereas no obvious association was observed between the expression of other genes and the Rev7 status (Fig. 8B). It was speculated that downregulation of Oct4 expression in Rev7−/− embryos may be due to lack of PGCs. These findings suggest that REV7 is involved in the expression of several genes including Oct4 and Nanog in mouse embryos.

DISCUSSION

Infertility is a major problem for public health and approximately 15% of couples worldwide suffer from infertility (39, 40). Many genes involved in the regulation of male and
female reproduction have been identified in mice, and some genetic causes for male and female infertility have been identified in humans, including chromosomal aberrations and genetic alterations of genes involved in sex determination, endocrinopathies, and sperm production (41, 42). However, many cases are still diagnosed with idiopathic infertility, and most of these cases are thought to have undiscovered genetic and epigenetic alterations.

REV7 is involved in DNA damage tolerance, cell cycle regulation, gene expression, and carcinogenesis by regulating the function of its interaction partners (19-21, 23, 27-31). However, the significance of Rev7 in mouse development has not been investigated. In the present study, we have demonstrated that Rev7–/– mice display a loss of PGCs by apoptotic cell death during migration, and germ cell aplasia in both testes and ovaries after birth, resulting in infertility of both sexes. These findings indicate that REV7 is essential for PGC survival in the mouse embryo. We hypothesized that DNA damage accumulation due to impairment of DNA repair system or deregulation of the expression of specific genes necessary for PGC survival is likely to be the cause of PGC loss in Rev7–/– embryos. Consequently, we analyzed the frequency of cells with DNA damage of double strand breaks, and found that a number of cells with DNA damage were detectable not only in PGCs but also in somatic cells of Rev7–/– embryos, whereas they were almost undetectable in Rev7+/+ embryos (Fig. 7). This findings suggest that DNA damage accumulation and genetic instability may be a possible cause of apoptotic cell death of PGCs in Rev7–/– embryos. We also analyzed histone methylation of H3K9me2 and H3K27me3 and expression of some essential genes for PGC survival. We found that H3K9me2 and H3K27me3 were enhanced in Rev7–/– embryos at E9.5 (Fig. 7A) and Oct4 and Nanog expression was upregulated in Rev7–/– embryos at E8.5 (Fig. 8A). H3K9me2 and H3K27me3 are principal epigenetic modification in PGCs to control the chromatin state and gene expression (14,15). And both Oct4 and Nanog are essential transcription factors for PGCs during migration and required for the maintenance of PGC pluripotency (13, 43-45). Therefore, there is a possibility that deregulation of the expression of several genes in PGCs caused by Rev7 deficiency results in PGC apoptosis during migration, although more genes involved in PGC survival remain to be elucidated.

In this study, we found that Rev7–/– embryos exhibited a reduced body size, partial embryonic lethality, increased numbers of apoptotic somatic cells and a high frequency of DNA damaged somatic cells, suggesting that Rev7 is necessary for the maintenance of somatic cells as well as germ cells in the mouse embryo, and that partial embryonic lethality of Rev7–/– mice may possibly be due to numerous apoptotic cell death in somatic cells caused by DNA damage accumulation. It has been reported that Rev7–/– forming Pol ζ, a DNA polymerase involved in TLS, and suppression of REV7 impairs DNA damage tolerance in human fibroblasts (19, 20, 23). During embryogenesis, endogenous factors, such as metabolites, oxygen radicals and nitrogen species, induce various types of DNA damage, and an effective DNA repair system is very important for normal development of organisms (46-49). Pol ζ is a mispair extender polymerase that extends DNA synthesis from mispaired nucleotides and is necessary for TLS machinery at various DNA lesions (50). In addition, Pol ζ is involved in homologous recombination repair of DNA double-strand breaks (24, 25). Therefore, it has been suggested that Pol ζ plays a pivotal role in the DNA damage response system. Accordingly, the size of Rev3–/– mouse embryos is considerably smaller than that of WT or heterozygote littermates at E9.5-10.5 and abort at around E12.5 (51-54). Extensive apoptosis has been demonstrated in all cell lineages of Rev3–/– embryos (54). The phenotypes of Rev7–/– embryos in this study are similar, in part, to those of Rev3–/– embryos, suggesting the possibility that Rev7–/– phenotypes are associated with impairment of Pol ζ function. Some experiments using genetically modified mice indicate the importance of the DNA repair system for germ cell maintenance. Disruption of mouse Rad18, which is the main regulator of DNA damage tolerance, causes progressive loss of germ cells in the testis after 6 months postnatally, indicating its requirement for long-term maintenance of germ cells (55). Deficient expression of genes involved in nucleotide excision repair, such as HR23B, Ercc1...
and Xpa, also impairs normal spermatogenesis and oogenesis in mice (56-58). All of these findings suggest that the DNA repair system is important for germ cells to retain genetic integrity after birth. It is noteworthy that deficiency of REV3 and REV7 causes more severe phenotypes in mouse embryos compared with knockout phenotypes of other DNA repair genes, suggesting the importance of DNA Pol ζ in mouse embryogenesis.

On the other hand, REV7 is involved in cell cycle regulation by interacting with CDC20 and CDH1 that control the activity of the APC to degrade cyclin B1 (27, 28). Homozygous Cdh1 mutant mice cannot produce normal trophoblasts and die at E9.5–10.5, whereas Cdc20 knockout causes metaphase arrest at the two-cell stage and death at a very early embryonic stage (59, 60). Mice lacking the mitotic checkpoint protein MAD2, which is another mammalian homologue of S. cerevisiae Mad2, show extensive apoptosis at E7.0 and embryonic lethality during early embryonic stages (61). The Rev7−/− phenotypes in the present study were milder than those of Mad2−/−, Cdh1−/− and Cdc20−/− mice. The findings that REV7 deficiency caused apoptotic cell death of PGCs and somatic cells, but did not affect cell proliferation, suggest that the Rev7−/− phenotypes may not be a consequence of an impairment of cell cycle regulation.

Human REV7 is also involved in gene expression by interacting with transcription factors ELK-1 and TCF-4 (30, 31). REV7 depletion in human cells suppresses upregulation of the transcriptional activities of ELK-1 after DNA damage, whereas REV7 knockout blocks TCF-4-mediated E-cadherin expression and induces N-cadherin and vimentin expression, leading to epithelial-mesenchymal transdifferentiation (30, 31). Mice possessing an Elk-1-null mutation show normal development without any morphological abnormality and only mildly impaired neuronal gene expression (62). Tcf-4-null mice die shortly after birth without any gross abnormalities and possess no apparent proliferating cells in the crypt region of the small intestine, suggesting that TCF-4 is necessary for the maintenance of crypt stem cells in the small intestine (63). These mutant mice display phenotypes that are different from those of Rev7−/− mice. Interestingly, the phenotype of Rev7−/− mice shown in this study is almost the same as that of mice null for RNA-binding protein TIAR (9). TIAR binds to several mRNAs that encode translation factors and suppresses their translation in response to UV-C irradiation, thereby playing a role as a stress-responsive transcriptional repressor (64). Tiar−/− mice show embryonic growth retardation, partial embryonic lethality, reduced numbers of PGCs during migration and complete ablation of PGCs at E13.5 (9). In human cells, because REV7 is required for damage tolerance and TIAR is needed for stress-response transcriptional repression, it is possible that REV7 is functionally linked to TIAR via gene expression regulation and that the mouse Rev7−/− phenotype is associated with the loss-of-function of mouse Tiar, although expression of Tiar mRNA was unaffected by REV7 deficiency.

In the present study, the significance of REV7 in spermatogenesis after birth could not be analyzed because of germ cell aplasia at birth. Since REV7 is highly expressed in spermatogonia and spermatocytes, suggesting an important role in spermatogenesis after birth, inhibition of REV7 expression by epigenetic alterations after birth might possibly cause impairment of spermatogenesis.

In summary, we established REV7-deficient mice that show growth retardation and progressive loss of PGCs during migration. These results indicate that REV7 is necessary for PGC survival and prevention of apoptotic cell death of somatic cells in the mouse embryo. Further studies are required to elucidate the mechanism of apoptotic cell death that is induced by REV7 deficiency and to clarify the importance of REV7 in human infertility.
REFERENCES

1. Saga, Y. (2008) Mouse germ cell development during embryogenesis. Curr. Opin. Genet. Dev. 18, 337-341
2. Saitou, M. (2009) Germ cell specification in mice. Curr. Opin. Genet. Dev. 19, 386–395
3. Ewen, K. A. and Koopman, P. (2010) Mouse germ cell development: from specification to sex determination. Mol. Cell. Endocrinol. 323, 76-93
4. Matsui, Y. (2010) Epigenetic profiles in primordial germ cells: global modulation and fine tuning of the epigenome for acquisition of totipotency. J. Androl. 31, 61-65
5. Ohinata, Y., Payer, B., O’Carroll, D., Ancelin, K., Ono, Y., Sano, M., Barton, S. C., Obukhanych, T., Nussenzweig, M., Tarakhovsky, A., Saitou, M. and Surani, M. A. (2005) Blimp1 is a critical determinant of the germ cell lineage in mice. Nature 436, 207-213
6. Kurimoto, K., Yabuta, Y., Ohinata, Y., Shigeta, M., Yamanaka, K. and Saitou, M. (2008) Complex genome-wide transcription dynamics orchestrated by Blimp1 for the specification of the germ cell lineage in mice. Genes Dev. 22, 1617-1635
7. Yamaji, M., Seki, Y., Kurimoto, K., Yabuta, Y., Yuasa, M., Shigeta, M., Yamanaka, K., Ohinata, Y. and Saitou, M. (2008). Critical function of Prdm14 for the establishment of the germ cell lineage in mice. Nat. Genet. 40, 1016-1022
8. Tsuda, M., Sasaoka, Y., Kiso, M., Abe, K., Haraguchi, S., Kobayashi, S. and Saga, Y. (2003) Conserved role of nanos proteins in germ cell development. Science 301, 1239-1241
9. Runyan, C., Schaible, K., Molyneaux, K., Wang, Z., Levin, L. and Wylie, C. (2006) Steel factor controls midline cell death of primordial germ cells and is essential for their normal proliferation and migration. Development 133, 4861-4869
10. Beck, A. R., Miller, I. J., Anderson, P. and Streuli, M. (1998) RNA-binding protein TIAR is essential for primordial germ cell development. Proc. Natl. Acad. Sci. U. S. A. 95: 2331-2336
11. Kehler, J., Tolkunova, E., Koschorz, B., Pesce, M., Gentile, L., Boiani, M., Lomeli, H., Nagy, A., McLaughlin, K. J., Schöler, H. R. and Tomilin, A. (2004) Oct4 is required for primordial germ cell survival. EMBO Rep. 5, 1078-1083
12. Youngren, K. K., Coveney, D., Peng, X., Bhattacharya, C., Schmidt, L. S., Nickerson, M. L., Lamb, B. T., Deng, J. M., Behringer, R. R., Capel, B., Rubin, E. M., Nadeau, J. H. and Matin, A. (2005) The Ter mutation in the dead end gene causes germ cell loss and testicular germ cell tumours. Nature 435, 360-364
13. Mitsui, K., Tokuzawa, Y., Itoh, H., Segawa, K., Murakami, M., Takahashi, K., Maruyama, M., Maeda, M. and Yamanaka, S. (2003) The homeoprotein Nanog is required for maintenance of pluripotency in mouse epiblast and ES cells. Cell 113, 631-642
14. Seki, Y., Hayashi, K., Itoh, K., Mizugaki, M., Saitou, M. and Matsui, Y. (2005) Extensive and orderly reprogramming of genome-wide chromatin modifications associated with specification and early development of germ cells in mice. Dev. Biol. 278, 440-458
15. Seki, Y., Yamaji, M., Yabuta, Y., Sano, M., Shigeta, M., Matsui, Y., Saga, Y., Tachibana, M., Shinkai, Y. and Saitou, M. (2007) Cellular dynamics associated with the genome-wide epigenetic reprogramming in migrating primordial germ cells in mice. Development 134, 2627-2638
16. Schmahl, J., Eicher, E. M., Washburn, L. L. and Capel, B. (2000) Sry induces cell proliferation in the mouse gonad. Development 127, 65-73
17. Nelson, J. R., Lawrence, C. W. and Hinkle, D. C. (1996) Thymine-Thymine Dimer Bypass by Yeast DNA Polymerase ζ. Science 272, 1646-1649
18. Guo, C., Kosarek-Stancel, J. N., Tang, T. S. and Friedberg, E. C. (2009) Y-family DNA polymerases in mammalian cells. Cell. Mol. Life Sci. 66, 2363-2381
19. Murakumo, Y., Roth, T., Ishii, H., Rasio, D., Numata, S., Croce, C. M. and Fishel, R. (2000) A human REV7 homolog that interacts with the polymerase ζ catalytic subunit hREV3 and the spindle assembly
checkpoint protein hMAD2. *J. Biol. Chem.* **275**, 4391-4397
20. Murakumo, Y., Ogura, Y., Ishii, H., Numata, S., Ichihara, M., Croce, C. M., Fishel, R. and Takahashi, M. (2001) Interactions in the error-prone postreplication repair proteins hREV1, hREV3, and hREV7. *J. Biol. Chem.* **276**, 35644-35651
21. Masuda, Y., Ohmae, M., Masuda, K. and Kamiya, K. (2003) Structure and enzymatic properties of a stable complex of the human REV1 and REV7 proteins. *J. Biol. Chem.* **278**, 35644-35651
22. Hara, K., Hashimoto, H., Murakumo, Y., Kobayashi, S., Kagome, T., Unzai, S., Akashi, S., Takeda, S., Shimizu, T. and Sato, M. (2010) Crystal structure of human REV7 in complex with a human REV3 fragment and structural implication of the interaction between DNA polymerase ζ and REV1. *J. Biol. Chem.* **285**, 12299-12307
23. McNally, K., Neal, J. A., McManus, T. P., McCormick, J. J. and Maher, V. M. (2008) hRev7, putative subunit of hPolζ, plays a critical role in survival, induction of mutations, and progression through S-phase, of UV(254nm)-irradiated human fibroblasts. *DNA Repair (Amst)* **7**, 597-604
24. Okada, T., Sonoda, E., Yoshimura, M., Kawano, Y., Saya, H., Kohzaki, M. and Takeda, S. (2005) Multiple roles of vertebrate REV genes in DNA repair and recombination. *Mol. Cell. Biol.* **25**, 6103-6111
25. Sharma, S., Hicks, J. K., Chute, C. L., Brennan, J. R., Ahn, J.-Y., Glover, T. W. and Canman, C. E. (2012) REV1 and polymerase ζ facilitate homologous recombination repair. *Nucleic Acids Res.* **40**, 682-691
26. Cahill, D. P., da Costa, L. T., Carson-Walter, E. B., Kinzler, K. W., Vogelstein, B. and Lengauer, C. (1999) Characterization of MAD2B and other mitotic spindle checkpoint genes. *Genomics* **58**, 181-187
27. Chen, J. and Fang, G. (2001) MAD2B is an inhibitor of the anaphase-promoting complex. *Genes Dev.* **15**, 1765-1770
28. Pfleger, C. M., Salic, A., Lee, E. and Kirschner, M. W. (2001) Inhibition of Cdh1-APC by the MAD2-related protein MAD2L2: a novel mechanism for regulating Cdh1. *Genes Dev.* **15**, 1759-1764
29. Iwai, H., Kim, M., Yoshikawa, Y., Ashida, H., Ogawa, M., Fujita, Y., Muller, D., Kirikae, T., Jackson, P. K., Kotani, S. and Sasakawa, C. (2007) A bacterial effector targets Mad2L2, an APC inhibitor, to modulate host cell cycling. *Cell* **130**, 611-623
30. Zhang, L., Yang, S. H. and Sharrocks, A. D. (2007) Rev7/MAD2B links c-Jun N-terminal protein kinase pathway signaling to activation of the transcription factor Elk-1. *Mol. Cell. Biol.* **27**, 2861-2869
31. Hong, C. F., Chou, Y. T., Lin, Y. S. and Wu, C. W. (2009) MAD2B, a novel TCF4-binding protein, modulates TCF4-mediated epithelial-mesenchymal transdifferentiation. *J. Biol. Chem.* **284**, 19613-19622
32. Weterman, M. A., van Groningen, J. J., Tertoolen, L. and van Kessel, A. G. (2001) Impairment of MAD2B-PRCC interaction in mitotic checkpoint defective (t(X;1))-positive renal cell carcinomas. *Proc. Natl. Acad. Sci. U. S. A.* **98**, 13808-13813
33. Li, L., Shi, Y., Wu, H., Lan, B., Li, P., Zhou, L., Shi, H. and Huo, K. (2007) Hepatocellular carcinoma-associated gene 2 interacts with MAD2L2. *Mol. Cell. Biochem.* **304**, 297-304
34. Rimkus, C., Friederichs, J., Rosenberg, R., Holzmann, B., Siewert, J. R. and Janssen, K. P. (2007) Expression of the mitotic checkpoint gene MAD2L2 has prognostic significance in colon cancer. *Int. J. Cancer* **120**, 207-211
35. Matsunami, H. and Takeichi, M. (1995) Fetal brain subdivisions defined by R- and E-cadherin expressions: evidence for the role of cadherin activity in region-specific, cell-cell adhesion. *Dev. Biol.* **172**, 466-478
36. Anderson, R., Fässler, R., Georges-Labouesse, E., Hynes, R. O., Bader, B. L., Kreidberg, J. A., Scheible, K., Heasman, J. and Wylie, C. (1999) Mouse primordial germ cells lacking betal integrins enter the germline but fail to migrate normally to the gonads. *Development* **126**, 1655-1664
37. Wilkinson, D. G. and Nieto, M. A. (1993) Detection of messenger RNA by *in situ* hybridization to
tissue sections and whole mounts. Methods Enzymol. 225, 361-373
39. Matzuk, M. M. and Lamb, D. J. (2002) Genetic dissection of mammalian fertility pathways. Nat. Cell Biol. 4, s41-49
40. Ombelet, W., Cooke, I., Dyer, S., Gamal, S. and Devroey, P. (2008) Infertility and the provision of infertility medical services in developing countries. Hum. Reprod. Update 14, 605-621
41. Ferlin, A., Arredi, B. and Foresta, C. (2006) Genetic causes of male infertility. Reprod. Toxicol. 22, 133-141
42. Saitou, M. and Yamaji, M. (2010) Germ cell specification in mice: signaling, transcription regulation, and epigenetic consequences. Reproduction 139, 931-942
43. Schöler, H. R., Dressler, G. R., Balling, R., Rohdewohld, H. and Gruss, P. (1990) Oct-4: a germline-specific transcription factor mapping to the mouse t-complex. EMBO J. 9, 2185-2195
44. Yeom, Y. I., Fuhrmann, G., Ovitt, C. E., Brehm, A., Ohbo, K., Gross, M., Hübner, K. and Schöler, H. R. (1996) Germline regulatory element of Oct-4 specific for the totipotent cycle of embryonal cells. Development 122, 881-894
45. Chambers, I., Colby, D., Robertson, M., Nichols, J., Lee, S., Tweedie, S. and Smith, A. (2003) Functional expression cloning of Nanog, a pluripotency sustaining factor in embryonic stem cells. Cell 113, 643-655
46. Burcham, P. C. (1999) Internal hazards: baseline DNA damage by endogenous products of normal metabolism. Mutat. Res. 443, 11-36
47. Wink, D. A., Kasprzak, K. S., Maragos, C. M., Elespuru, R. K., Misra, M., Dunams, T. M., Cebula, T. A., Koch, W. H., Andrews, A. W., Allen, J. S. and Keefer, L. K. (1991) DNA deaminating ability and genotoxicity of nitric oxide and its progenitors. Science 254, 1001-1003
48. Jaroudi, S. and SenGupta, S. (2007) DNA repair in mammalian embryos. Mutat. Res. 635, 53-77
49. Vinson, R. K. and Hales, B. F. (2002) DNA repair during organogenesis. Mutat. Res. 509, 79-91
50. Waters, L. S., Minesinger, B. K., Wiltout, M. E., D’Souza, S., Woodruff, R. V. and Walker, G. C. (2009) Eukaryotic translesion polymerases and their roles and regulation in DNA damage tolerance. Microbiol. Mol. Biol. Rev. 73, 134-154
51. Bemark, M., Khamlichi, A. A., Davies, S. L. and Neuberger, M. S. (2000) Disruption of mouse polymerase ζ (Rev3) leads to embryonic lethality and impairs blastocyst development in vitro. Curr. Biol. 10, 1213-1216
52. Wittschieben, J., Shivji, M. K., Lalani, E., Jacobs, M. A., Marini, F., Gearhart, P. J., Rosewell, I., Stamp, G. and Wood, R. D. (2000) Disruption of the developmentally regulated Rev3l gene causes embryonic lethality Curr. Biol. 10, 1217-1220
53. Esposito, G., Godindagger, I., Klein, U., Yaspo, M. L., Cumano, A. and Rajewsky, K. (2000) Disruption of the Rev3l-encoded catalytic subunit of polymerase ζ in mice results in early embryonic lethality. Curr. Biol. 10, 1221-1224
54. Van Sloun, P. P., Varlet, I., Sonneveld, E., Boei, J. J., Romeijn, R. J., Eeken, J. C. and De Wind N. (2002) Involvement of mouse Rev3 in tolerance of endogenous and exogenous DNA damage. Mol. Cell. Biol. 22, 2159-2169
55. Sun, J., Yomogida, K., Sakao, S., Yamamoto, H., Yoshida, K., Watanabe, K., Morita, T., Araki, K., Yamamura, K. and Tateishi, S. (2009) Rad18 is required for long-term maintenance of spermatogenesis in mouse testes. Mech. Dev. 126, 173-183
56. Ng, J. M., Vrieling, H., Sugasawa, K., Ooms, M. P., Grootegoed, J. A., Vreeburg, J. T., Visser, P., Beems, R. B., Gorgels, T. G., Hanaoka, F., Hoeijmakers, J. H. J. and van der Horst, G. T. J. (2002) Developmental defects and male sterility in mice lacking the ubiquitin-like DNA repair gene mHR23B. Mol. Cell. Biol. 22, 1233-1245
57. Hsia, K. T., Millar, M. R., King, S., Selfridge, J., Redhead, N. J., Melton, D. W. and Saunders, P. T. (2003) DNA repair gene Ercc1 is essential for normal spermatogenesis and oogenesis and for functional integrity of germ cell DNA in the mouse. Development 130, 369-378
58. Nakane, H., Hirota, S., Brooks, P. J., Nakabeppu, Y., Nakatsu, Y., Nishimune, Y., Iino, A. and Tanaka,
K. (2008) Impaired spermatogenesis and elevated spontaneous tumorigenesis in xeroderma pigmentosum group A gene (Xpa)-deficient mice. DNA Repair (Amst) 7, 1938-1950
59. Li, M., York, J. P. and Zhang, P. (2007) Loss of Cdc20 causes a securin-dependent metaphase arrest in two-cell mouse embryos. Mol. Cell. Biol. 27, 3481-3488
60. García-Higuera, I., Manchado, E., Dubus, P., Cañamero, M., Méndez, J., Moreno, S. and Malumbres, M. (2008) Genomic stability and tumour suppression by the APC/C cofactor Cdh1. Nat. Cell Biol. 10, 802-811
61. Dobles, M., Liberal, V., Scott, M. L., Benezra, R. and Sorger, P. K. (2000) Chromosome missegregation and apoptosis in mice lacking the mitotic checkpoint protein Mad2. Cell 101, 635-645
62. Cesari, F., Brecht, S., Vintersten, K., Vuong, L.G., Hofmann, M., Klingel, K., Schnorr, J. J., Arsenian, S., Schild, H., Herdegen, T., Viebel, F. F. and Nordheim, A. (2004) Mice deficient for the Ets transcription factor Elk-1 show normal immune responses and mildly impaired neuronal gene activation. Mol. Cell. Biol. 24, 294-305
63. Korinek, V., Barker, N., Moerer, P., van Donselaar, E., Huls, G., Peters, P. J. and Clevers H. (1998) Depletion of epithelial stem-cell compartments in the small intestine of mice lacking Tcf-4. Nat. Genet. 19, 379-383
64. Mazan-Mamczarz, K., Lal, A., Martindale, J. L., Kawai, T. and Gorospe, M. (2006) Translational repression by RNA-binding protein TIAR. Mol. Cell. Biol. 26, 2716-2727

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FIGURE LEGENDS

Figure 1. Establishment of the Rev7-knockout mouse. (A) Homologous recombination strategy to generate Rev7–/– mice. The genomic sequence containing Rev7 exon 1 (including the start codon) and exon 2 was replaced by a neomycin cassette. Positions of the 5′ probe for Southern blot screening (grey bars) and PCR primers (arrows) for WT and targeted alleles to screen mouse genotypes are shown. Arrowhead indicates the position of the start codon. (B) Genotyping of Rev7+/+, Rev7+/– and Rev7–/– mice by Southern blotting. Genomic DNAs extracted from mouse tails were digested with Nhe I and Southern hybridization was performed using the 32P-labeled 5′ probe. The 4.66-kb and 3.96-kb bands represent WT and targeted alleles, respectively. (C) Genotyping of Rev7+/+, Rev7+/– and Rev7–/– mice by PCR. The 230-bp and 750-bp bands represent WT and targeted alleles, respectively. (D) Northern blot analysis of total RNA extracted from the testes of Rev7+/+ and Rev7–/– mice. 32P-labeled full-length Rev7 cDNA was used as the probe (upper panel). Blotting for β-actin expression is shown as the internal control (bottom panel). (E) Western blot analysis to detect REV7 protein in the tissues of Rev7+/+ and Rev7–/– mice (upper panel). Lysates were prepared from the testes, kidneys, liver, and spleen of Rev7+/+ and Rev7–/– mice. Detection of β-actin is shown as the internal control (bottom panel).

Figure 2. Rev7 expression in WT mice. (A) Northern blot analysis of Rev7 expression. A Mouse Multiple Tissue Northern Blot membrane was hybridized with the 32P-labeled Rev7 cDNA probe (upper panel). A blot hybridized with the β-actin cDNA probe is shown as the internal control (bottom panel). (B) Western blot analysis of REV7 expression. Lysates were prepared from P56 WT mice, and western blotting was performed using the anti-REV7 antibody. REV7 expression was quantitatively assessed using ImageQuant TL, which is indicated at the bottom. (C) In situ hybridization to detect the Rev7 transcript in the testis. 32P-labeled anti-sense and sense probes for the Rev7 transcript were used for in situ hybridization on testis sections of P56 WT mice. Scale bars: 500 μm. (D) Immunohistochemical staining for REV7 expression in the testis. Immunostaining was performed with the anti-REV7 antibody on testis sections from P56 WT mice. Positive signals were visualized with DAB or a fluorescently labeled secondary antibody. Immunofluorescence staining without the primary antibody is shown as the negative control (NC). Scale bars: 100 μm. (E) In situ hybridization using whole embryonic tissues (upper panels) and tissue sections (bottom panels) to detect Rev7 expression in E9.5 embryos. Whole-mount in situ hybridization was performed with DIG-labeled anti-sense and sense probes for the Rev7 transcript and in situ hybridization on tissue sections was performed with 32P-labeled probes as described in EXPERIMENTAL PROCEDURES. In the whole mount in situ hybridization, the purple color throughout the body represents specific signal (upper left panel). H&E-stained image of tissue section was also shown (bottom, left panel). Scale bar: 500 μm. (F) Real-time quantitative RT-PCR analysis of Rev7 expression in E17.5 embryos. Total RNAs were extracted from the indicated organs. Real-time quantitative RT-PCR was performed using gene-specific primers for Rev7 and GAPDH transcripts listed in Table 1. Relative expression of Rev7 transcript in each organ is graphically shown. Reactions for GAPDH are used as internal controls. (G) Western blot analysis of REV7 at postnatal stages. Lysates were prepared from the indicated organs, and western blot analysis was performed with anti-REV7 and anti-GAPDH antibodies. Relative expression of REV7 was quantitatively assessed using ImageQuant TL, which is indicated at the bottom of each panel. The bands indicated by asterisks are non-specific.

Figure 3. Growth retardation and germ cell aplasia of Rev7–/– male mice. (A, B) Gross appearance of Rev7+/+ and Rev7–/– mice at P28 (A) and E10.5 (B). Scale bar: 3 mm. (C) Gross appearance of the testes of Rev7+/+ and Rev7–/– mice at P7. Scale bar: 5 mm. (D) Comparison of the body weight of Rev7+/+, Rev7+/– and Rev7–/– male mice. Means and SDs are indicated. Asterisks indicate a significant difference (p < 0.005) between Rev7+/+ mice and Rev7+/– or Rev7–/– mice. (E) Comparison of
testis size between Rev7+/+ and Rev7−/− mice. Means and SDs of the ratios of heart to body weight and testis to body weight at P14 and P56 are indicated. Asterisks indicate a significant difference (p < 0.001) between Rev7+/+ and Rev7−/− mice. (F) Histological images of the testes of Rev7+/+ and Rev7−/− mice. Testis sections prepared from Rev7+/+ and Rev7−/− mice at P14 and P56 were stained with H&E. Scale bars: 200 µm. (G) Immunohistochemical analysis of testis sections from Rev7+/+ and Rev7−/− mice. Immunohistochemical staining with the anti-PLZF antibody for spermatogonia and the anti-GATA-4 antibody for Sertoli cells are indicated. Scale bars: 100 µm.

Figure 4. Growth retardation and germ cell aplasia of Rev7−/− female mice. (A) Comparison of the body weight of Rev7+/+, Rev7+/− and Rev7−/− female mice. Means and SDs are shown. Asterisks indicate a significant difference (p < 0.005) between Rev7+/+ mice and Rev7+/− or Rev7−/− mice. (B) Gross appearances and histological images of the ovaries from Rev7+/+ and Rev7−/− mice. Macroscopic images of the ovaries at P70 (left panels) and microscopic images of H&E stained sections of the ovaries at P28 were shown (middle panels). Right panels are high magnification images. Scale bars: 200 µm (middle panels) and 50 µm (right panels). (C) In situ hybridization to detect the Rev7 transcript in the ovary. 33P-labeled anti-sense and sense probes for the Rev7 transcript were used for in situ hybridization on ovary sections of WT mouse of 18 weeks old. Scale bar: 200 µm.

Figure 5. Apoptotic cell death of PGCs during migration in Rev7−/− embryos. (A) Immunohistochemical analysis of whole-mount mouse embryos to identify PGCs during migration. Rev7+/+ and Rev7−/− embryos at E8.5, E9.5 and E10.5 were whole-mount immunostained using the anti-Oct4 antibody as described in EXPERIMENTAL PROCEDURES. Images were obtained under a stereomicroscope. (B) Immunohistochemical analysis of paraffin sections to detect PGCs and apoptotic cells. Sections around the genital ridges of Rev7+/+ and Rev7−/− embryos at E11.5 and E13.5 were immunostained with the anti-Oct4 antibody to detect PGCs (left and middle panels). Those of Rev7+/+ and Rev7−/− embryos at E11.5 were immunostained with the anti-cleaved caspase-3 antibody to detect apoptotic cells (right panels). Sections of E11.5 embryos used for Oct4 and cleaved caspase-3 staining (left and right panels) were serial sections. Arrows indicate cells positive for both Oct4 and cleaved caspase-3. Scale bars: 100 µm (left and right panels), 200 µm (middle panels). (C) Fluorescence staining to demonstrate PGC apoptosis. Sections around the hindgut of Rev7+/+ and Rev7−/− mouse embryos at E9.5 were subjected to immunostaining with the anti-Oct4 antibody, TUNEL staining, and nuclear staining with DAPI. Merged images are also shown. Arrowheads indicate cells positive for both Oct4 and TUNEL. Scale bars: 25 µm. (D) REV7 expression in the PGCs of E13.5 embryos. Sections of genital ridges of E13.5 WT embryos were double immunostained with anti-Oct4 and anti-REV7 antibodies. Immunoreactivity of the anti-Oct4 antibody was visualized with DAB and that of the anti-REV7 antibody was visualized using an Alexa Fluor 488-labeled secondary antibody. The two images were merged after conversion of the DAB color to red. Immunofluorescence staining without the primary antibody is shown as the negative control (NC). Scale bars: 50 µm.

Figure 6. REV7 deficiency increases the number of apoptotic cells among forebrain neuroblasts but does not affect cell proliferation. (A) Detection of apoptotic cells in the forebrain. Sections of the forebrain of Rev7+/+ and Rev7−/− mouse embryos at E11.5 were immunostained with the anti-cleaved caspase-3 antibody. Scale bars: 200 µm. (B) Percentages of cleaved caspase-3-positive cells (means and SDs) among the forebrain neuroblasts of Rev7+/+ and Rev7−/− embryos shown in A. An asterisk indicates a significant difference between Rev7+/+ and Rev7−/− embryos (p < 0.05). (C) Analysis of cell proliferation in the forebrain. Sections of the forebrain of Rev7+/+ and Rev7−/− mouse embryos at E11.5 were immunostained with the anti-PCNA antibody. Scale bars: 200 µm. (D) Percentages of PCNA-positive cells (means and SDs) among the forebrain neuroblasts of Rev7+/+ and Rev7−/− embryos shown in C.
Figure 7. **REV7 deficiency results in DNA damage accumulation and increased levels of histone methylation in mouse embryos.**

**(A,B)** Tissue sections around the hindgut of *Rev7*+/+ and *Rev7*−/− mouse embryos at E9.5 (A) and around the genital ridges at E13.5 (B) were subjected to immunostaining with the anti-Oct4, -phospho-Histone H2A.X (Ser139) (pH2AX), -dimethyl-Histone H3 (Lys9) (H3K9me2) and -trimethyl-Histone H3 (Lys27) (H3K27me3) antibodies. Immunoreactivity of the anti-Oct4 and -pH2AX antibodies was visualized with DAB and that of the anti-H3K9me2 and -H3K27me3 antibodies was visualized using an Alexa Fluor 488-labeled secondary antibody. Serial sections were used for staining of E9.5 embryos (A). Open arrowheads in A indicate cells positive for pH2AX, and closed arrowheads in A indicate cells positive for both Oct4 and pH2AX. A number of pH2AX-positive cells were detected in *Rev7*−/− mouse embryos at E9.5 and E13.5. In addition, increased levels of H3K9me2 and H3K27me3 are demonstrated in *Rev7*−/− embryos at E9.5, but not in those at E13.5. Scale bars: 50 µm.

Figure 8. **Deregulation of gene expression in *Rev7*−/− embryos.**

**(A,B)** Total RNAs were extracted from whole bodies of *Rev7*+/+ and *Rev7*−/− embryos at E8.5 (A) and from trunks of embryos at E13.5 (B). Real-time quantitative RT-PCR was performed using gene-specific primers for *Oct4*, *Nanog*, *Blimp1*, *c-Kit*, *Tiar*, *Cdc20*, *Rev3*, *Rev7* and *GAPDH* listed in Table 1. The expression levels of each gene in *Rev7*+/+ and *Rev7*−/− embryos were determined as relative values to that in *Rev7*+/+ #1 (A) or *Rev7*+/+ #3 (B). Relative expression values of *Oct4* and *Nanog* in *Rev7*−/− #1 and #2 embryos are; *Oct4*, 8.2 and 40.5; *Nanog*, 10.1 and 38.9, respectively. Reactions for *GAPDH* are used as internal controls.
Table 1. List of primers used in this study

| allele or gene | direction | sequence (5’ → 3’) |
|---------------|-----------|--------------------|
| **For Mouse Genotyping** | | |
| wild-type allele | forward: | ACAAAGAGCTACTAAGCAGTGG |
| | reverse: | TCAAAGACAGGCACTCTGTCAG |
| targeted allele | forward: | GAATGAACGTCAGGAGACGAG |
| | reverse: | ACTAGAAGGCAAGTCAGGAG |
| **For real-time PCR** | | |
| Rev7 | forward: | GGGAAAGGATGACCACCCCTCACG |
| | reverse: | TCAGCTGTTCTTATGCGCTC |
| c-kit(37) | forward: | GCGTCCTGGTGTGCCTGCCTCGTG |
| | reverse: | CTGTCCAGGCTAGTATGTCAGGTC |
| Oct4(36) | forward: | TTGGGCTAGAGAAGGATGTGGTT |
| | reverse: | GGAAAGGCACTGAGATGAGTGTTG |
| Blimp1 | forward: | GTGAACGACCACCCCTGGA |
| | reverse: | ACCGATGAGGGGACCACACG |
| Nanog | forward: | AGGGGTCTGACTGAGATGCTCTG |
| | reverse: | CAACCACTGTTTCTGCTGCCACCG |
| Tiar | forward: | GGGTGCTAGGGTGGAGGAGGT |
| | reverse: | GCCCGGAAGCAATTCTCCAC |
| Cdc20 | forward: | TCAAGGCGCTGTCAGGGCTCTG |
| | reverse: | GCCCACACCAGTACAGTCTCA |
| Rev3 | forward: | ACCTGGCTGGTCAAGGTCTCAG |
| | reverse: | TGTCAGCAGCTACGGACATCCAC |
| GAPDH | forward: | TGCAACCAGCAACTGCTTAG |
| | reverse: | GAGGCAGGGATGATGTTC |
Table 2. Numbers (%) of mice obtained from Rev7+/− mice intercross

| stage        | genotype     | total     |
|--------------|--------------|-----------|
|              | Rev7+/+      | Rev7+/−   | Rev7−/−   | total |
| E8.5-E13.5   | 33 (26.0)    | 64 (50.4) | 30 (23.6) | 127 (100) |
| after birth  | 65 (34.4)    | 106 (56.1)| 18 (9.5)  | 189 (100)  |
**A**

Wild-type allele

```
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
```

Nhe I

```
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
```

Nhe I

```
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
```

5' probe

WT PCR primers

Targeting vector

```
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
```

Nhe I

```
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
```

1330 bp (short arm)

```
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
```

Nhe I

```
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
```

3960 bp

5' probe

KO PCR primers

Targeted allele

```
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
```

Nhe I

```
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
```

PGK-neo

```
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
```

1330 bp (short arm)

```
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
```

PGK-neo

```
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
```

5110 bp (long arm)

**B**

Rev7 genotype

| +/+ | +/- | -/- |

4.66 kb

3.96 kb

**C**

Rev7 genotype

| +/+ | +/- | -/- |

750 bp

230 bp

**D**

Rev7 genotype

| +/+ | +/- | -/- |

Rev7

1.0 kb

β-actin

2.0 kb

**E**

Rev7+/+

Rev7-/-

| testis | kidney | liver | spleen | testis | kidney | liver | spleen |

REV7

24 kDa

β-actin

42 kDa
A

Rev7 (1.35 kb)  
β-actin (2.4 kb)

B

REV7 (24 kDa)  
GAPDH (38 kDa)

C

anti-sense  
sense

D

REV7  
NC

E

anti-sense  
sense

H&E  
anti-sense  
sense

F

Relative expression

G

P7  
P14  
P21  
P28

REV7  
GAPDH

REV7  
GAPDH

REV7  
GAPDH

REV7  
GAPDH
**Figure 3**

A. Imaging of *Rev7*+/+ and *Rev7*−/− mice at postnatal days P14 and P56.

B. Imaging of *Rev7*+/+ and *Rev7*−/− mice at postnatal days P14 and P56.

C. Imaging of *Rev7*+/+ and *Rev7*−/− mice at postnatal days P14 and P56.

D. Graph showing body weight (g) over postnatal days (P) for *Rev7*+/+ (n=7) and *Rev7*−/− (n=3). Asterisks indicate significant differences.

E. Graph showing weight ratio (%) for heart and testis at P14 and P56 for *Rev7*+/+ (n=4) and *Rev7*−/− (n=3). Asterisks indicate significant differences.

F. Imaging of *Rev7*+/+ and *Rev7*−/− mice at P14 and P56 for PLZF (spermatogonia).

G. Imaging for GATA-4 (Sertoli cell) at P0 and P14 for *Rev7*+/+ and *Rev7*−/−.
A. Cleaved caspase-3

B. % of cleaved caspase-3-positive cells

C. PCNA

D. % of PCNA positive cells
A

Relative expression

Oct4 Nanog Blimp1 c-Kit Tiar Cdc20 Rev3 Rev7

Rev7+/+ #1
Rev7+/+ #2
Rev7+/+ #3
Rev7+/+ #4
Rev7–/– #1
Rev7–/– #2
Rev7–/– #3
Rev7–/– #4

8.2 40.5 10.1 38.9

B

Relative expression

Oct4 Nanog Blimp1 c-Kit Tiar Cdc20 Rev3 Rev7

Rev7+/+ #1
Rev7+/+ #2
Rev7+/+ #3
Rev7+/+ #4
Rev7–/– #1
Rev7–/– #2
Rev7–/– #3
Rev7–/– #4
The Rev7 Subunit of DNA Polymerase ζ Is Essential for Primordial Germ Cell Maintenance in the Mouse
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