Stem Cell Reports, Volume *15*

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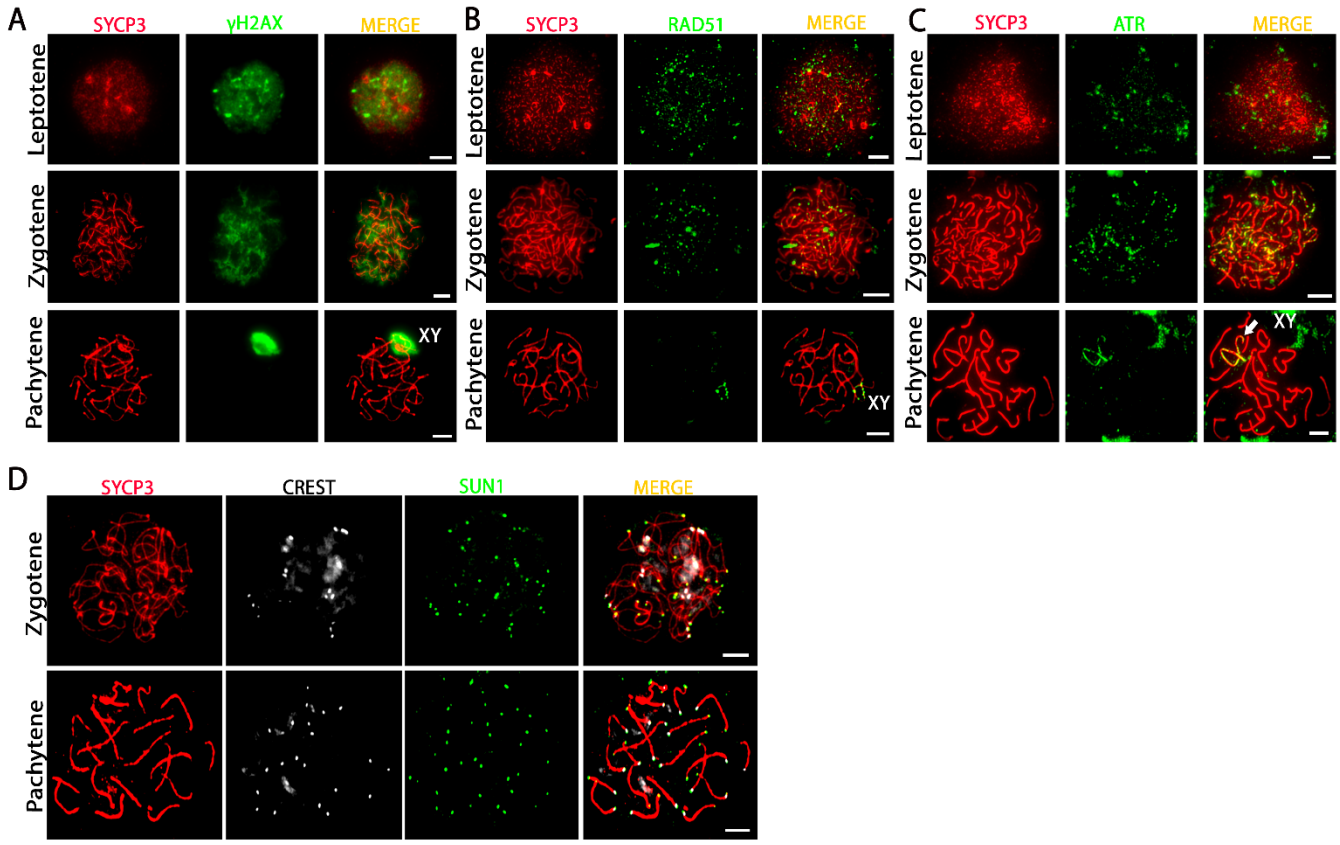

Figure S1. Positive control experiments using mouse testicular cells.

In vivo meiotic cells stained for SYCP3 (red) and **(A)** γH2AX (green), **(B)** RAD51 (green), **(C)** ATR (green), **(D)** SUN1 (green) and centromeres (CREST, white). Scale bar, 5µm.

Supplemental Experimental Procedures

Primary antibodies				
Target Protein	Host	Source	Cat. Number	IHC Dilution
γ-H2AX	Mouse	Merck Millipore	05–636	1:10,000
SYCP3	Mouse	Abcam	ab97672	1:600
SYCP3	Rabbit	NOVUS	NB300-231	1:2500
CREST-serum	Human	FitzGerald	90C-CS1058	1:600
ATR	Rabbit	Cell Signaling Technology	#2790	1:100
α-Tubulin	Mouse	Sigma	T9026	1:200
RAD51	Rabbit	Thermo Fisher Scientific	PA5-27195	1:100
MLH1	Mouse	BD Pharmingen	550838	1:50
SUN1	Guinea pig	Provided by Manfred Alsheimer	N/A	1:600
Secondary antibodies				
Fluorescence	Host	Source	Cat. Number	IHC Dilution
Alexa Fluor 488	Donkey anti-Mouse	Thermo Fisher Scientific	A21202	1:1000
Alexa Fluor 488	Donkey anti-Rabbit	Thermo Fisher Scientific	A21206	1:1000
Alexa Fluor 488	Goat anti-Guinea pig	Thermo Fisher Scientific	A11073	1:1000
Alexa Fluor 555	Goat anti-mouse	Thermo Fisher Scientific	A21424	1:1000
Alexa Fluor 555	Donkey anti-Rabbit	Thermo Fisher Scientific	A31572	1:1000
Alexa Fluor 647	Goat anti-Human	Thermo Fisher Scientific	A21445	1:1000

Table S1. Antibodies used in this study. Related to Figures 1, 3, 4, 5 and S1.

Supplemental Experimental Procedures

GS cells and Sertoli cell line culture

Mouse GS cells were cultured as previously reported (Kanatsu-Shinohara et al., 2003; Mulder et al., 2017; Zheng et al., 2017). After the third passage, the cells were cultured on mitotically inactivated mouse embryonic fibroblasts (MEFs; Gibco, A34962), using in a medium mainly composed of StemPro-34 SFM medium (Thermo Fisher Scientific), StemPro-34 Supplement (Thermo Fisher Scientific), 1% fetal bovine serum (FBS), recombinant human GDNF (10 ng/ml, 450-10, Peprotech), recombinant human bFGF (10 ng/ml, 100-18B, Peprotech), recombinant human EGF (20 ng/ml, AF-100-15, Peprotech), recombinant human LIF (10 ng/ml, CYT-644, Prospec), as well as other components as previously reported (Kanatsu-Shinohara et al., 2003). GS cells were refreshed every 2-3 days, dissociated by accutase (Thermo Fisher Scientific) and passaged every 5-7 days (doubling time, 3 days) at a ratio of 1:4-6 on fresh mitotically inactivated mouse embryonic fibroblasts. The cells were maintained at 37°C in 5% CO_2 in air. The cells used for this research were mostly at passage 20, with a maximum of 26 passages.

As a feeder cell, we used available Sertoli cell lines SK49 or TM4. SK49 was established by Walther et al., from 10-day-old male H-2Kb- tsA58 transgenic mice carrying an inducible temperature-sensitive SV40 T antigen (Walther et al., 1996). This cell line is able to express Sertoli cell-specific pattern markers and exhibit distinct Sertoli cell properties. TM4 was establishes by Matfier et al., from 11-13 days of age male BALB/c - nu/+ mice (Matfier, 1980). Both Sertoli cell lines were cultured at 37°C and 5% CO_2 in Dulbecco's Eagle's medium (DMEM; Thermo Fisher Scientific, Waltham, MA, USA) supplemented with 10% fetal bovine serum (FBS), penicillin (100 U/mL) and streptomycin (100 U/mL).

In vitro meiosis of GS cells

SK49 cells or TM4 Sertoli cells, inactivated by mitomycin (10µg/ml, M7949, Sigma), were grown on 12-well plates pre-coated with laminin (20 µg/ml, L2020, Sigma) to a density of 1 x10^5. Then GS cells were seeded on these Sertoli cells for two days to maintain GS cell proliferation as described previously (Kanatsu-Shinohara et al., 2003). To induce meiosis, day 0 to day 3 (Fig. 1A), the cells were cultured in a medium composed of StemPro-34 SFM medium and StemPro-34 Supplement, 10% KnockOut Serum Replacement (KSR), Retinoic acid (2µM, R2625, sigma), Recombinant Mouse BMP-4 Protein (20 ng/ml, 5020-BP, R&D Systems), Recombinant Mouse Activin A Protein (100 ng/ml, 338-AC, R&D Systems). From day 3 to day 9 after meiosis induction, the medium was composed of StemPro-34 SFM medium and StemPro-34 Supplement, 10% KSR, Bovine Pituitary Extract (BPE) (50µg/ml, 354123, Corning Life Sciences), Follicle-stimulating hormone (FSH) (200 ng/ml, F4021, Sigma), Testosterone (10 µM, 86500, Sigma) and refreshed daily.

Okadaic acid (OA)-induced generation of metaphase-like cells in vitro

For generation of metaphase-like cells *in vitro*, cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 5% FBS, 3 µM okadaic acid (OA; 459618, Millipore) at 34°C for 4 hours and fixed in 4% paraformaldehyde (PFA). For negative controls no OA was added.

Cytospins

The cells were detached from the culture dish using 0.25% trypsin and washed with 1x phosphate buffered saline (PBS) and diluted in 200 µL PBS/1% BSA containing 30,000 to 50,000 cells for each cytospin spot and spun for 7 minutes at 112g. The slides were air dried for 10min, fixed in 4% PFA and stored at 4°C in PBS or stored at -80°C after air drying.

Karyotyping

The Cells were dissociated into single cell suspension after 8 days after spermatogonial differentiation, subjected to hypotonic treatment with 75 mM KCl at 37 ◦C for 10 minutes and fixed in freshly prepared methanol/acetic acid (ratio: 3:1). Cells were dropped onto glass slides from 1 m distance and air dried. Chromosomes were stained with Hoechst 33342.

Flow Cytometry

For FACS analysis, after the cells were fixed and permeabilized in 70% Ethanol (EtOH). The cells were labeled with 1 µg/1 × 10^5 cells propidium iodide (PI, Sigma, P4964) in FACS/EDTA buffer (1× PBS with 1% FCS/0.1% NaN3/2 mM EDTA) containing DNAse-free RNAse A (v/v 1:20) for 5 min. The measurements and sorting were performed on a Sony SH800Z cell Sorter (Sony Biotechnology Inc. Japan). Data was analyzed using FlowJo software version 10. For acrosome detection, the sorted "haploid" peak (putative 1C-region) cells were incubated with lectin peanut agglutinin (PNA) conjugated with Alexa Fluor 488 conjugate (1:700, L21409, Life Technologies) for 15min.

Supplemental References:

Kanatsu-Shinohara, M., Ogonuki, N., Inoue, K., Miki, H., Ogura, A., Toyokuni, S., and Shinohara, T. (2003). Long-term proliferation in culture and germline transmission of mouse male germline stem cells. Biology of reproduction *69*, 612-616.

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