Supporting Information

Allard and Colaiácovo 10.1073/pnas.1010386107

SI Materials and Methods

Time Course Analysis for RAD-51 Foci. Quantitation of RAD-51 foci for all seven zones composing the germline was performed as described in ref. 1. Four germlines were scored for each exposure condition. The average numbers of nuclei scored per zone (n) for ethanol- and BPA-exposed wild-type worms were as follows: zone 1, $n = 182$; zone 2, $n = 198$; zone 3, $n = 197$; zone 4, $n = 193$; zone 5, $n = 177$; zone 6, $n = 160$; and zone 7, $n = 130$.

Quantitative Analysis of Germ Cell Apoptosis. Germ cell corpses were scored in adult hermaphrodites between 20 and 24 h post-L4, using acridine orange as described in ref. 2.

Statistical Analysis. All statistical analyses were performed using the two-tailed Mann–Whitney test with a 95% confidence interval.

DAPI Analysis and Immunostaining. Whole-mount preparation of dissected gonads, DAPI staining, immunostaining, and analysis of stained germline nuclei were performed as in ref. 1, except for ATL-1, AIR-2, and pCHK-1 antibodies, where gonads were fixed with 1% formaldehyde. Primary antibodies were used at the following dilutions: rabbit α-SYP-1, 1:100; mouse α-RAD-51, 1:100; rabbit α-HTP-3, 1:500; guinea pig α-HIM-8, 1:100; rabbit α-ATL-1, 1:50; rabbit α-AIR-2, 1:100; and rabbit α-pCHK-1, 1:50 (Santa Cruz). Secondary antibodies used were Cy3 anti-rabbit, FITC anti-guinea pig, and FITC anti-mouse (Jackson Immunochemicals), each at 1:100.

Imaging and Microscopy. Immunofluorescence images were collected at 0.2-μm intervals with an IX-70 microscope (Olympus) and a cooled CCD camera (model CH350; Roper Scientific) controlled by the DeltaVision system (Applied Precision). The images presented are projections approximately halfway through 3D data stacks of whole nuclei, except for diakinesis and HIM-8 immunostaining images, which encompass entire nuclei. Images were subjected to deconvolution analysis using the SoftWorx 3.0 program (Applied Precision) as in ref. 3. OD57, an integrated line expressing mCherry-H2B; GFP-γ-tubulin (4), was used for live imaging and worms were immobilized on 3% agarose pads. The OD57 strain was incubated at 25 °C for 24 h before imaging. Images were captured on a Leica DM5000B microscope (40× objective) every 10 s for 5 min.

Worm Lysis for Liquid Chromatography–Tandem Mass Spectrometry Analysis. Worms were grown on ten 100 × 15-mm plates until the adult stage. After collection of the worms in M9, worms were washed 10 times in M9 buffer and frozen in liquid nitrogen. The worm pellet was then resuspended in lysis buffer [25 mM Hepes (pH7.6), 5 mM EDTA, 0.5 M sucrose, 0.5% CHAPS, and 0.5% deoxycholic acid]. This solution was then sonicated 5 times and centrifuged at high speed for 5 min to remove nonsonicated/lysed fragments. BPA was detected by LC-MS/MS performed by Axys Analytical Services.

Quantitative RT-PCR Analysis. Three samples of 30 worms each were collected in TRIzol for four conditions: *glp-1* mutants exposed to either ethanol or BPA and maintained at either 15 °C or 25 °C. The RNA was extracted and reverse transcription was carried out using Transcriptor Reverse Transcriptase (Roche) according to the manufacturer's protocol. Quantitative PCR was performed using Brilliant II SYBR green QPCR master mix with low Rox (Stratagene), following the manufacturer's instructions. Each sample was run in duplicate. Sample values were normalized to *gpd-1* (GAPDH), taking into account primer amplification efficiency calculated from the standard curve. The average normalized values from ethanol-treated samples were then statistically compared with the average normalized values from BPA-treated samples. Primer sequence information is indicated in Table S1.

1. Colaiácovo MP, et al. (2003) Synaptonemal complex assembly in *C. elegans* is dispensable for loading strand-exchange proteins but critical for proper completion of recombination. *Dev Cell* 5:463–474.
2. Kelly KO, Dernburg AF, Stanfield GM, Villeneuve AM (2000) *Caenorhabditis elegans msh-5* is required for both normal and radiation-induced meiotic crossing over but not for completion of meiosis. *Genetics* 156:617–630.
3. de Carvalho CE, et al. (2008) LAB-1 antagonizes the Aurora B kinase in *C. elegans*. *Genes Dev* 22:2869–2885.
4. McNally K, Audhya A, Oegema K, McNally FJ (2006) Katanin controls mitotic and meiotic spindle length. *J Cell Biol* 175:881–891.

Fig. S1. BPA does not impair the initiation of synapsis. The assembly of both axial (HTP-3) and central region (SYP-1) components of the synaptonemal complex upon entrance into meiosis is indistinguishable between transition zone nuclei in either BPA- or ethanol-exposed gonads (progression into meiosis is from left to right). (Scale bars, 5 μm.)

Fig. S2. Exposure to BPA does not affect homologous chromosome pairing. Immunostaining for the X chromosome pairing center protein HIM-8 (green) in control (ethanol)- and BPA-exposed germlines is shown. Only one HIM-8 focus was detected per pachytene nucleus under both conditions, indicating proper pairing of the X chromosome. n = 7 gonads analyzed per condition. (Scale bars, 5 μm.)

Fig. S3. Impaired meiotic DSB repair progression. Histograms depict the quantitation of RAD-51 foci performed on control (ethanol)- and BPA-exposed gonads. The number of RAD-51 foci per nucleus is categorized by the color code shown on the right. The percentage of nuclei observed for each category (*y* axis) is depicted for each zone along the germline axis (*x* axis and diagram).

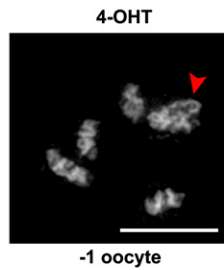


Fig. S4. 4-Hydroxytamoxifen exposure leads to chromosome defects at diakinesis. Worms exposed to 4-hydroxytamoxifen (4-OHT) show chromosome defects at diakinesis similar to those observed following either BPA or ICI 182780 exposure. These defects include a frayed appearance, aggregates, fragments, and the presence of chromatin bridges (red arrowhead). (Scale bar, 5 μm.)

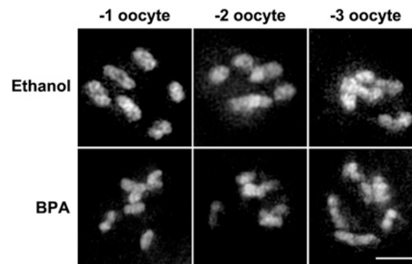


Fig. S5. *nhr-14* mutants do not show increased sensitivity to BPA. *nhr-14(tm1473)* worms were exposed to either ethanol or 0.5 mM BPA. This lower concentration of BPA allowed us to assess a possible modulation in the sensitivity of the *nhr-14* mutant worms. The mutants did not display an increased sensitivity to BPA and the chromosome morphogenesis defects were observed only in the −3 oocytes. Twenty worms were analyzed per condition. (Scale bar, 5 μm.)

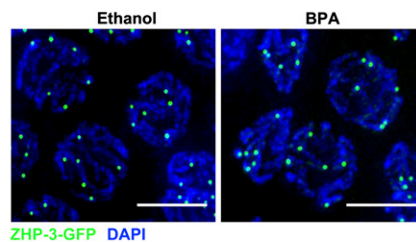


Fig. S6. Normal number of ZHP-3 foci following BPA exposure. ZHP-3 foci were visualized by using direct fluorescence from the ZHP-3::GFP integrated line (*Materials and Methods*). Six foci per nucleus were detected in late pachytene nuclei in both ethanol control- and BPA-exposed animals. $n > 400$ nuclei each. (Scale bars, 5 μm.)

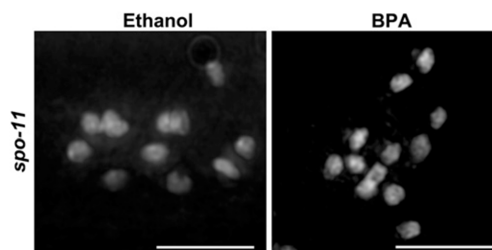
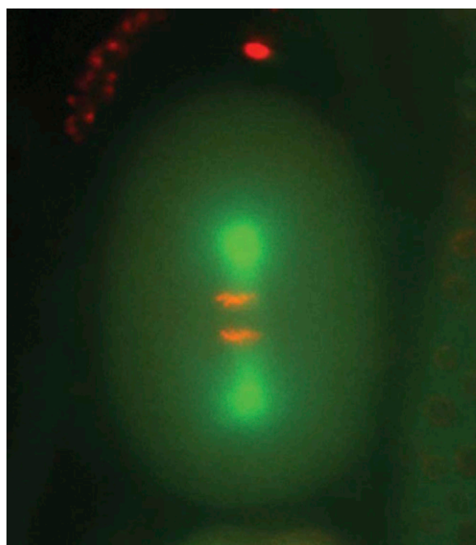


Fig. S7. BPA exposure does not rescue the *spo-11* diakinesis defects. *spo-11(ok79)* mutant worms were exposed to either ethanol or 1 mM BPA. Twelve DAPI-stained bodies were observed in diakinesis oocytes under both conditions. $n = 28$ and 24 oocytes at diakinesis for ethanol and BPA, respectively. (Scale bars, 5 μm.)

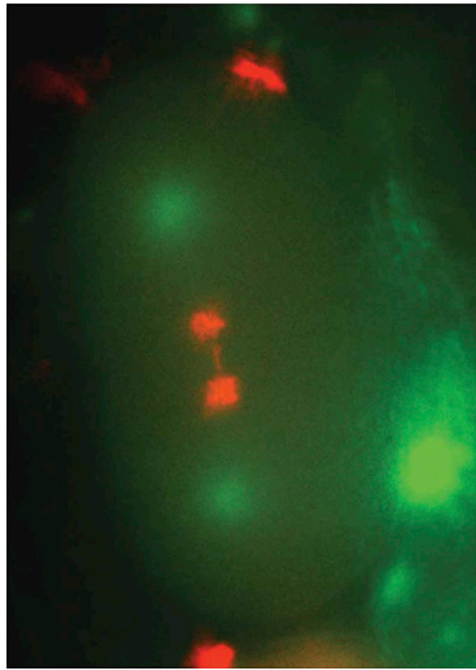
Table S1. Primers used for quantitative RT-PCR analysis

	Primer pair	Primer sequence
1	*cep-1*F	ttccgacgcaagtagtctcc
	*cep-1*R	ccgtttgcattgaacaacac
2	*atm-1*F	cccgattctgattgaaggaa
	*atm-1*R	ggcttctcggaaatttgtc
3	*spo-11*F	tggacctacgaaagaatttgc
	*spo-11*R	tgatcgatggtgaaacgatg
4	*rad-51*F	ccaggctgatgctaaaaagc
	*rad-51*R	ttcggcttctggtaaattgg
5	*rad-54*F	cgtcttcgaatgtggatcg
	*rad-54*R	gtcgttttcttcggcttcag
6	*mre-11*F	ctgtttggaaagcacagcaa
	*mre-11*R	ttgaatgctcgaacaagacg
7	*msh-5*F	ccccaaaacagctttccata
	*msh-5*R	ggcgtcttgaatggatcact
8	*clk-2*F	ccgaatcctccatcttcaaa
	*clk-2*R	gtgcaatgatcagacgcact
9	*hus-1*F	aagatactgcggcaatcgac
	*hus-1*R	tgaaccaacttccaccatca
10	*mrt-2*F	tagaaacgggtcaatgcaca
	*mrt-2*R	gtgccacgttcctgtatcct
11	*atl-1*F	gcattctcctgcgttttctc
	*atl-1*R	cgtcgaaccttcgtcttctc
12	*chk-1*F	gtctggtcgtctgggattgt
	*chk-1*R	ttgctgatccatcccatgta
13	*pmrt-5*F	aacttgtggaccgttggaag
	*pmrt-5*R	ggcacttggaattgatgctt
14	*gpd-1*F	actcgtccattttcgatgct
	*gpd-1*R	tcgacaacacggttcgagta



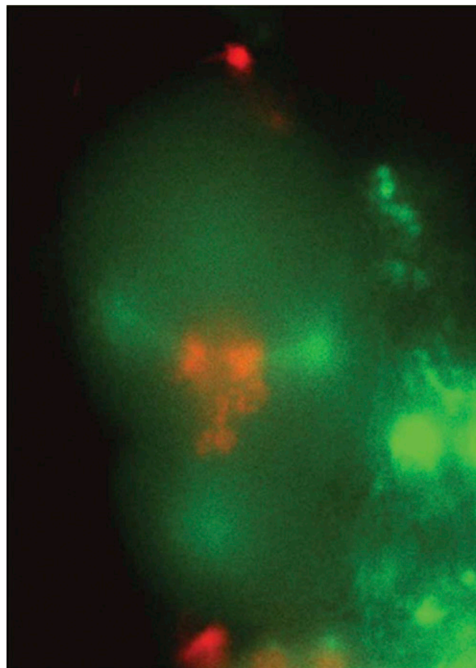
Movie S1. Ethanol-exposed embryos show normal chromosome dynamics at the first cell division. Time-lapse microscopy of the first mitotic division in H2B::mCherry; γ-tubulin::GFP transgenic embryos exposed to ethanol. Normal pronuclear fusion, chromosome congression at the metaphase plate, and subsequent segregation at anaphase were observed. Chromosomes are marked by H2B::mCherry (red) and spindle poles by γ-tubulin::GFP (green).

Movie S1



Movie S2. Chromosomes fail to congress and segregate properly during the first embryonic division following BPA exposure. A representative example is shown of congression failure and chromatin bridge observed in an H2B::mCherry; γ-tubulin::GFP transgenic embryo exposed to BPA.

Movie S2



Movie S3. Multiple spindle poles are observed following BPA exposure. The same embryo shown in Movie S2 was also followed over the next S phase (note that cytokinesis did not occur). Congression failure, as well as the presence of three spindle poles, was observed.

Movie S3