Supplemental Data

EGO-1, a Putative RNA-Dependent RNA Polymerase, Is Required for Heterochromatin Assembly on Unpaired DNA during *C. elegans* Meiosis

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Supplemental Experimental Procedures

Mutant Strains

Standard culture conditions were used [S1]. Wild-type strain *C. elegans* variant Bristol (N2) and mutations used are as described by Chen et al. [S2], as listed in Wormbase (http://www.wormbase.org), or as indicated in the text. Mutations used were: LG (linkage group) I: *ego-1(om54*, *om71*, *om84*, and *om97)*, *drsh-1(rm654)*, *rde-2 (ne221)*, *rrf-1(pk1417)*, *rrf-2(ok210)*, *unc-15(e73)*, *ccIs4251*; LGII: *rrf-3 (pk1426)*; LGIII: *dcr-1(ok247)*, *rde-4(ne301)*, *unc-32(e189)*, *sDp3(III:f), svDp1(III;f)*, *sDf134, unc-32(e189)*; LGIV: *him-8(e1489)*; LGV: *rde-1(ne219)*, *him-17(ok434)*. The following mutations are known to be null: *ego-1(om84* and *om97)* [S3], *dcr-1(ok247)*, *him-17(ok434)* [S4], and *drsh-1(tm654)* [S5]. The *ego-1(om84)* strain was used as the canonical null allele in our indirect immunofluorescence and genetic studies. Both *ego-1(om54)* and *ego-1(om71)* contain single amino acid substitutions at conserved residues in the RdRP domain [S6]. The full genotypes of double mutant strains are as follows: (1) *ego-1(om84)/ccIs4251 unc-15; him-17(ok434)*, (2) *ego-1(om84)/ccIs4251 unc-15; rrf-3(pk1426)*, (3) *ego-1(om84)/ccIs4251 unc-15; him-8 (e1489)*, (4) *ego-1(om84)/+; sDp3; dpy-17 sDf134 unc-32*. The *ccIs4251 unc-15* chromosome was used to balance *ego-1*. The integrated transgene, *ccIs4251*, carries nuclear targeted GFP under control of the *myo-3* promoter, and therefore expresses GFP in muscle cell nuclei [S7]. The following transgenic strains were used. (1) Strain PD7291 contains *ccEx7291*, an extrachromosomal repetitive array composed of plasmids pBK48 (*let-858::gfp)* and pRF4 [*rol-6(gf)*]. (2) Strain JH227 contains an integrated, complex array composed of pRF4, a plasmid carrying *pie-1::gfp*, and high sequence complexity DNA (kindly provided by G. Seydoux). (3) Strain BW2063 contains the chromosomal fragment, *svDp1*, which was made by fusing *sDp3* with a repetitive array that contains the *sur-5::gfp* marker gene [S8].

Indirect Immunofluorescence

Experiments were performed using published methods [S9, S10] and/or the following protocol, which gave similar results. Tissue was dissected and treated in deep well slides, and then mounted onto

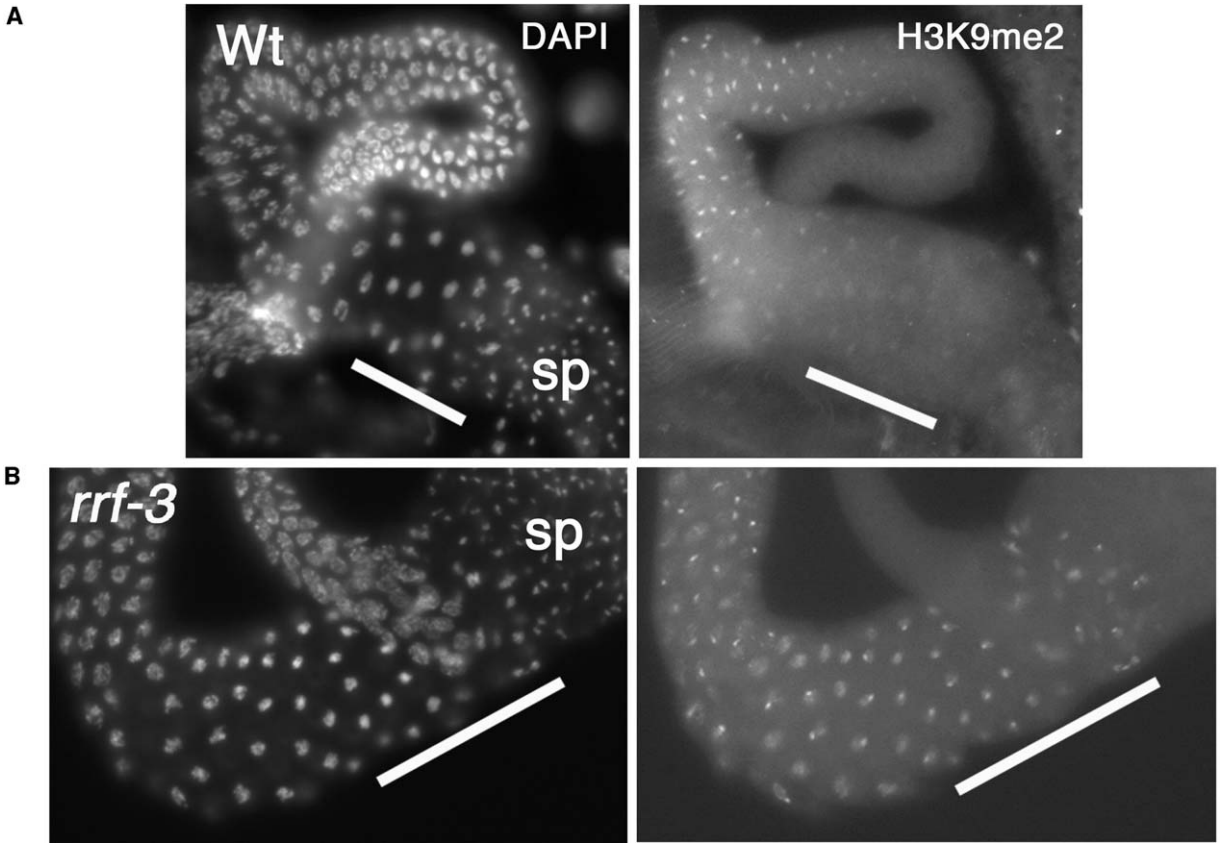

Figure S1. Elevated H3K9me2 in *rrf-3* Mutant Germlines

Each panel shows a portion of the male meiotic germline stained with polyclonal antibody against H3K9me2 and with DAPI to visualize DNA. Null alleles of *ego-1* and *rrf-3* were used; *rrf-3* animals were raised at 25°C (see text and Supplemental Experimental Procedures). Photographs are oriented with distal tissue to the left.

(A) In the wild-type (wt) germline, H3K9me2 staining rapidly decreases as germ cells become primary spermatocytes (bar).

(B) In the *rrf-3* germline, full H3K9me2 staining is retained in primary spermatocytes. The H3K9me2 level is very reduced in mature sperm (sp).

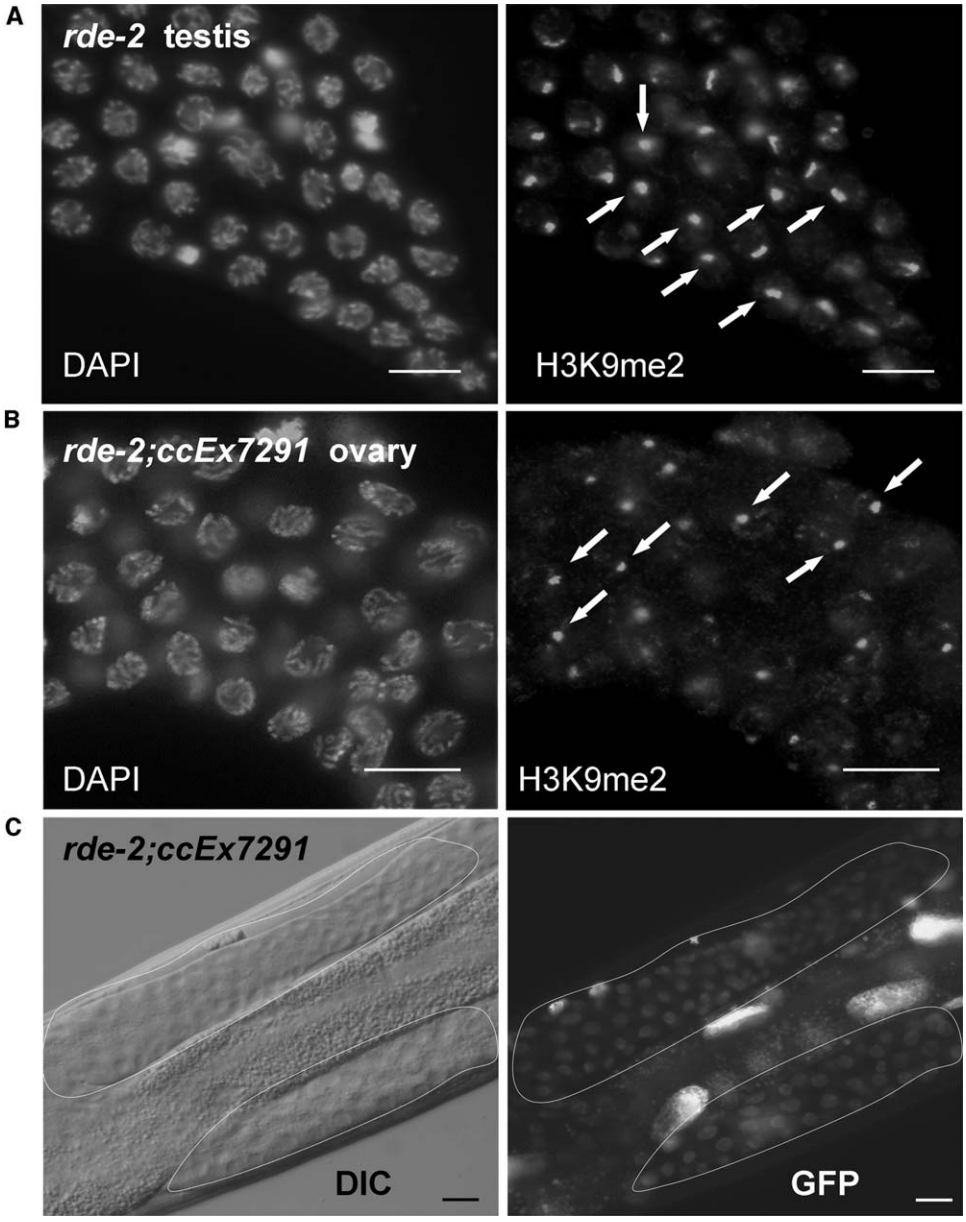

Figure S2. H3K9me2 Is Targeted Normally in the *rde-2* Mutant Germline

(A and B) A portion of the meiotic germline stained with DAPI to visualize DNA and polyclonal anti-H3K9me2 antibody.

(A) A bright focus of H3K9me2 staining is visible in *rde-2* male germ cells, presumably corresponding to the X chromosome.

(B) A bright focus of H3K9me2 staining is visible in *rde-2;ex7291* hermaphrodite germ cells, presumably corresponding to the *ex7291* extrachromosomal array.

(C) GFP is visible in germ cell nuclei, reflecting expression of the *let-858::gfp* reporter gene that is present on the array. Germline tissue is outlined in white and flanks intestinal tissue (with large, brightly staining nuclei). Scale bar equals 10 μm.

a flat slide for viewing. Washed animals were dissected in 1× PBS/1 mM levamisole to faciliate extrusion of gonads. Dissected gonads were fixed for 5 min in 2.5% paraformaldehyde/1× PBS and washed in 1× PBS/0.1%Tween-20 (PBST). Tissue was blocked for at least 1 hr in a solution containing 1× PBS, 0.5% Tween-20, and 30% goat serum (PBST/GS) and then incubated overnight at 15°C with primary antibody in fresh PBST/GS. Tissue was washed 3× in PBST, incubated with anti-rabbit secondary antibody (1/150 dilution; Pierce), and washed 3× in PBS. DAPI was added to the penultimate wash to visualize DNA.

Protein Analysis

Total protein extracts were prepared and EGO-1 protein blotting was done as described [S3]. Nuclear extract was prepared based on the method of Pazin [S11] with some modifications. A 100 μl volume of packed wild-type (N2) worms was used. Worms were washed in M9 medium, pelleted, and resuspended in 300 μl of homogenization buffer (HB; 1.5 ml 5 M HEPES [pH 7.6], 200 μl 2.5 M KCl, 250 μl 1 M $MgCl_2$, 10 ml 60% sucrose, 10 μl 0.5 M EDTA, 38 ml ddH_2O). 100 μl of glass beads were added, and tissue was disrupted by a 25 s treatment at speed 5.5 on a Thermo Savant FP120 Fast Prep. Microscopy was used to confirm that animals were disrupted and tissue was spun at 9000 rpm for 2 min to pellet membranes. The supernatant was removed to a clean tube, 0.5 μl NP-40 was added to a final concentration of 0.2%, and material was spun at 8000 rpm for 2 min at room temperature (RT). The pellet was resuspended in 500 μl HB. Cells were disrupted by grinding in a dounce homogenizer and repeated pipeting. Material was spun

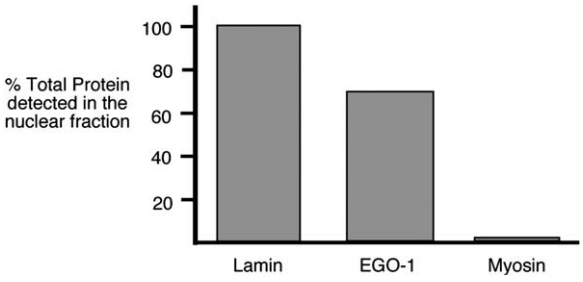

Figure S3. EGO-1 Is Present in Nuclear Extracts

Histogram shows the relative amount of EGO-1 protein detected in nuclear versus total protein extracts. Protein extracts were assayed by protein blot for the presence of EGO-1, lamin, and myosin. Lamin, a nuclear protein, was used to determine the efficiency of recovery of nuclei in the nuclear preparation. Myosin, a cytoplasmic protein, was used to determine the level of cytoplasmic contamination of the nuclear preparation (see Supplemental Experimental Procedures). Myosin contamination of the nuclear extract was negligible. Approximately 65% of the total EGO-1 pool was detected in nuclear extracts.

at 8000 rpm for 2 min at RT, and the nuclear pellet was resuspended in 100 μl HEMK buffer (2.5 ml 5 M HEPES [pH 7.6], 4 ml 2.5 M KCl, 625 μl 1 M $MgCl_2$, 10 μl 0.5 M EDTA, 5 ml glycerol, 50 μl NP-40 in 50 ml final volume; protease inhibitors were added immediately before use, to 5 ml of this solution as follows: 5 μl 1 M DTT, 5 μl aprotinin, 0.5 μl pefabloc SC). Material was sonicated to disrupt nuclei, and then spun at 14,000 rpm for 8 min at 4°C. Supernatant was removed, and an equal volume of 2× SDS buffer was added. Material was boiled 3 min and cooled prior to loading on a polyacrylamide gel. 15 μl of the preparation, 10% of the total extract, was added per lane. Whole protein and nuclear extracts were run in parallel. Equivalent blots were incubated with anti-EGO-1 [S3], anti-lamin [S12], or anti-myosin [S13]. Anti-lamin was used to assay the efficiency of recovery of nuclei in the nuclear preparation. Anti-myosin was used to assay for contamination of the nuclear preparation by cytoplasmic protein. Band intensity was quantified using Kodak Gel Documentation software, and data were normalized to determine the relative proportion of nuclear versus total EGO-1 protein based on recovery of lamin in nuclear versus total extracts and (very low) contamination of nuclear extract with cytoplasmic protein.

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