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me101 is a new allele of *rad-51*

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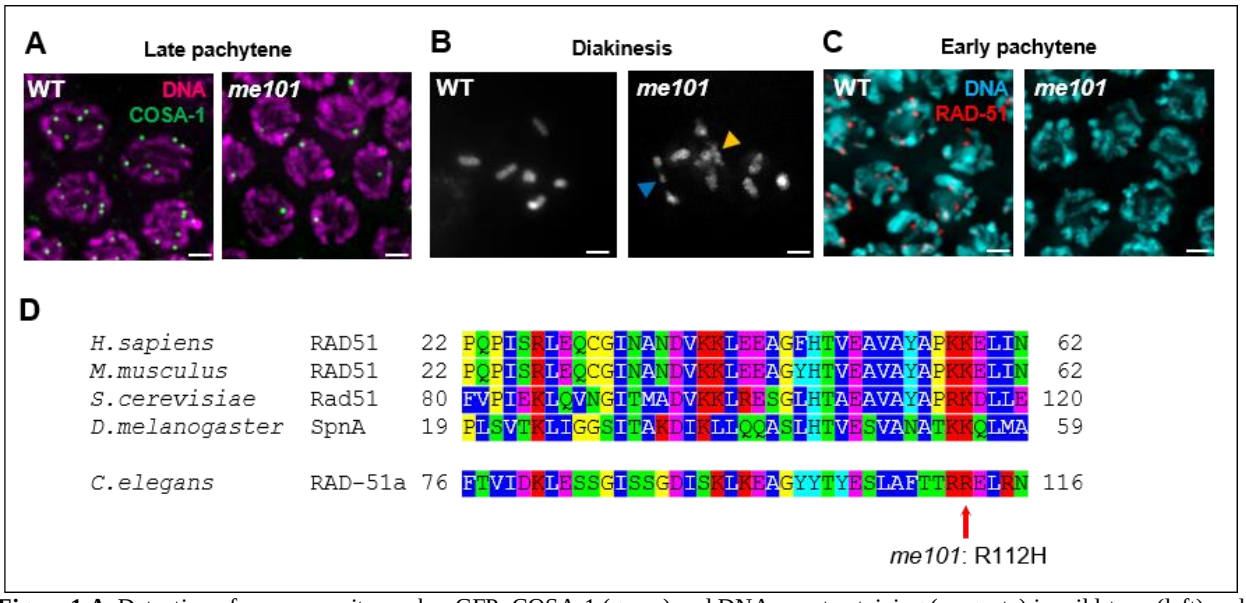

Figure 1 A. Detection of crossover site marker GFP::COSA-1 (green) and DNA counterstaining (magenta) in wild-type (left) and *me101* mutant (right) late pachytene nuclei. **B.** DAPI-stained chromosomes in oocytes of the indicated genotype at diakinesis, the last stage of meiotic prophase; while six pairs of attached homologs are consistently detected at this stage in wild-type oocytes, poorly condensed chromosomes, chromosome fragments (blue arrowhead) and/or chromosome aggregates (yellow arrowhead) are observed in me101 mutant meiocytes. **C.** The recombinase RAD-51 (red) is detected in wild-type but absent in *me101* mutant early pachytene nuclei. Scale bar in panels A-C represents 2µm. **D.** ClustalW alignment of protein sequences of RAD-51 orthologs from the indicated species. The *me101* mutation induces a substitution in a conserved acidic residue.

Description

The *me101* allele was isolated in a genetic screen for mutants with an altered number of GFP::COSA-1 foci, which mark the sites of crossovers in *C. elegans* germ cells (ROSU *et al.* 2013). After multiple rounds of outcrossing, we confirmed that *me101* mutants were defective in some aspects of meiotic prophase, as late pachytene *me101* mutant meiocytes failed to form the six GFP::COSA-1 foci observed in wild-type late pachytene meiocytes (Fig 1.A). We also observed structural defects ranging from chromosome fragmentation to the formation of chromosome aggregates in *me101* diakinesis-stage oocytes (Fig. 1B), suggesting a defect in some aspect of the DNA damage response. Further, 100% of eggs laid by *me101* mutant hermaphrodites are inviable. We then assessed the localization of the recombinase RAD-51, an essential component of the homologous recombination machinery that is required for the repair of DNA breaks and the maintenance of genome integrity during meiosis (RINALDO *et al.* 2002; ALPI *et al.* 2003); no RAD-51 foci were observed in the gonads of *me101* mutants (Fig 1C). Sequencing of the *rad-51* locus in the *me101* mutant revealed a single G to A substitution (IV:10283785 from WS269), leading to an Arginine to Histidine substitution in a conserved residue (Fig. 1D). Failure to detect RAD-51 foci in the *me101* mutant indicates that this residue is important for the loading and/or the stability of the RAD-51 protein.

Methods

Cytology: Immunofluorescent detection of GFP::COSA-1 and RAD-51 was performed as described in (Martinez-Perez and Villeneuve 2005) using a mouse anti-GFP antibody (Sigma-Aldrich #11814460001) and a rabbit anti-RAD-51 antibody (Colaiacovo *et al.* 2003).

Reagents

Strains:

AV727 *meIs8[pie-1p::gfp::cosa-1 + unc-119(+)] II ; ltIs37[pie-1p::mCherry::his-58 + unc-119(+)] IV ; ltIs38[pie-1p::gfp::ph(PLC1delta1) + unc-119(+)]*

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AV880 *meIs8[pie-1p::gfp::cosa-1 + unc-119(+)] II ; rad-51(me101) ltIs37[pie-1p::mCherry::his-58 + unc-119(+)] / nT1[qIs51] IV ; +/nT1 V; ltIs38[pie-1p::gfp::ph(PLC1delta1) + unc-119(+)]*

References

Alpi, A., P. Pasierbek, A. Gartner and J. Loidl, 2003 Genetic and cytological characterization of the recombination protein RAD-51 in *Caenorhabditis elegans*. Chromosoma 112: 6-16.

Colaiacovo, M. P., A. J. MacQueen, E. Martinez-Perez, K. McDonald, A. Adamo 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.

Martinez-Perez, E., and A. M. Villeneuve, 2005 HTP-1-dependent constraints coordinate homolog pairing and synapsis and promote chiasma formation during *C. elegans* meiosis. Genes Dev 19: 2727-2743.

Rinaldo, C., P. Bazzicalupo, S. Ederle, M. Hilliard and A. La Volpe, 2002 Roles for *Caenorhabditis elegans rad-51* in meiosis and in resistance to ionizing radiation during development. Genetics 160: 471-479.

Rosu, S., K. A. Zawadzki, E. L. Stamper, D. E. Libuda, A. L. Reese et al., 2013 The *C. elegans* DSB-2 protein reveals a regulatory network that controls competence for meiotic DSB formation and promotes crossover assurance. PLoS Genet 9: e1003674.

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