microPublication BIOLOGY

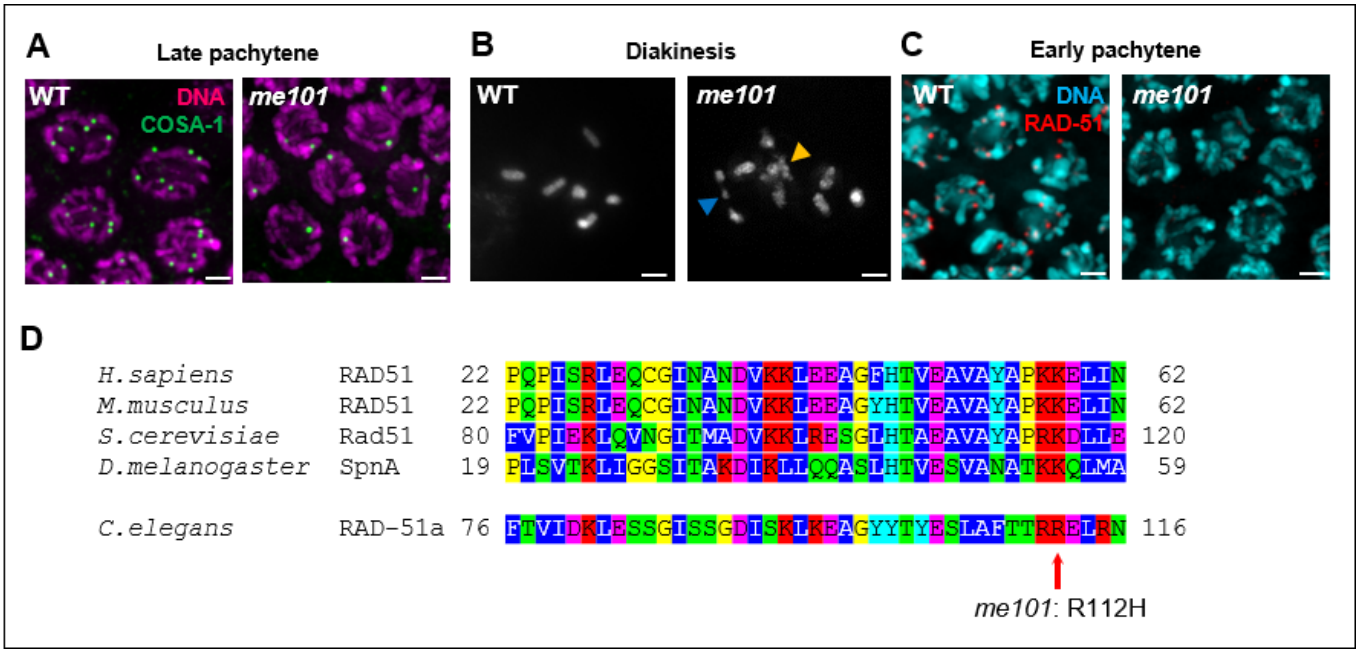
4/26/2019 - *Open Access*

me101 is a new allele of rad-51

Baptiste Roelens[1], Karl A Zawadzki[1] and Anne M Villeneuve[1§]

[1]Departments of Developmental Biology and Genetics, Stanford University School of Medicine

[§]To whom correspondence should be addressed: annev@stanford.edu



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

Request a detailed protocol

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(+)]*

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. PMID: 12684824.

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. PMID: 12967565.

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. PMID: 16291646.

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. PMID: 11861554.

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. PMID: 23950729.

Funding: This work was supported by NIH grants R01GM067268 and R35GM126964 to AMV.

Author Contributions: Baptiste Roelens: Writing - original draft, Writing - review and editing, Formal analysis. Karl A Zawadzki: Conceptualization, Formal analysis. Anne M Villeneuve: Conceptualization, Writing - original draft, Writing - review and editing.

Reviewed By: Cori Cahoon

History: Received April 11, 2019 **Accepted** May 24, 2019 **Published** April 26, 2019

Citation: Roelens, B; Zawadzki, KA; Villeneuve, AM (2019). me101 is a new allele of rad-51. microPublication Biology. https://doi.org/10.17912/micropub.biology.000107