Temperature increases cause transposon-associated DNA damage specifically in spermatocytes

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Introduction

Sexually-reproducing organisms use meiosis to generate haploid gametes, such as sperm and eggs, to transmit their genome to the next generation. All tissues are susceptible to dramatic increases in temperature; however, developing sperm in the testes are unusually sensitive to small temperature fluctuations. Failure to thermoregulate spermatogenetic tissue and prolonged exposure to elevated temperatures are linked to male infertility. Further, temperature increases are known to cause DNA damage in spermatocytes, but the molecular mechanisms underlying this damage are unclear. Here we show that upon a brief heat-shock, the spermatocytes (but not oocytes) of *Caenorhabditis elegans* exhibit an increase in double strand DNA breaks (DSBs), that these temperature-induced DSBs occur via a SPO-11 independent pathway, and occur concurrent with impaired male fertility and temperature-induced transposon activity within the genome.

Organization of the male *C. elegans* germline

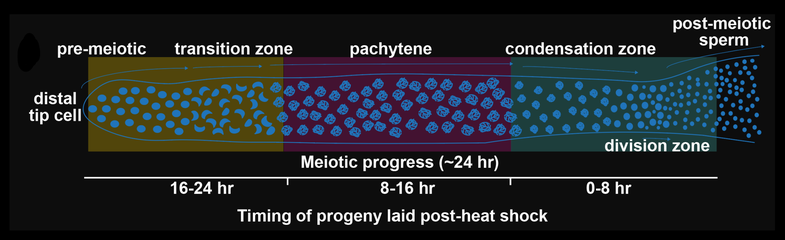


Figure 1. Illustration of a male *C. elegans* gonad. Nuclei in all germ lines are organized in a spatial-temporal gradient. Changes in chromosome morphology permit easy identification of the stages of meiotic prophase. Insets show discrete stages of meiotic prophase I and illustrate the zones in which RAD-51 foci are detectable. Progression of the spermatocyte from the distal tip of the gonad through meiosis to mature spermatid takes ~20-24 hours, thus progeny resulting from each 8-hour interval represent spermatids that were present in specific areas of the germline.

Double strand DNA break (DSB) formation in Meiosis

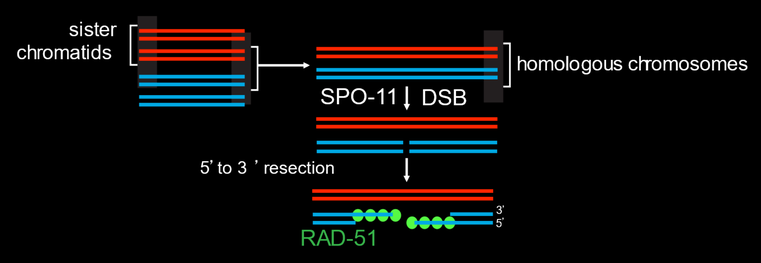


Figure 2. SPO-11 is a conserved endonuclease that induces double strand DNA breaks (DSBs) during meiosis. Soon after a DSB is formed, the recombinase RAD-51 binds to the 3' DNA overhangs surrounding the DSB site. RAD-51 is required for DSB repair during meiosis and serves as a marker for meiotic DSB formation. A subset of DSBs are repaired as crossover recombination events, which are required to form the chiasma between homologous chromosomes necessary for proper chromosome segregation during the meiosis I division.

Acute exposure to elevated temperature induces DSBs specifically in *C. elegans* spermatocytes

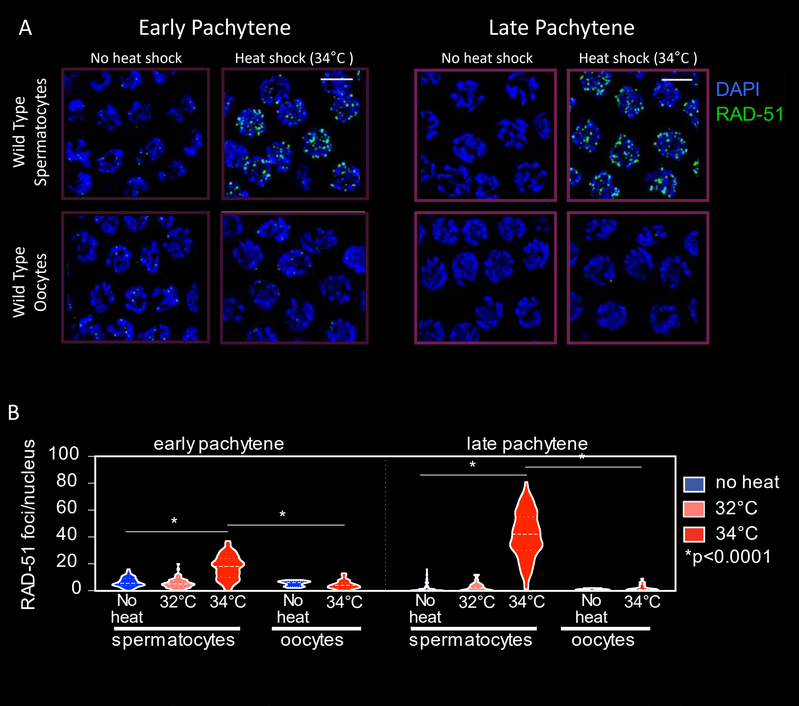


Figure 3. (A) Immunofluorescence for the recombinase RAD-51 (green) and DNA/DAPI (blue) reveals that a two-hour 34°C heat shock causes a dramatic increase in RAD-51 foci (green) per nucleus (DNA in blue) specifically in wild type spermatocytes throughout the meiotic region of the male germ line. (B) Wild type oocytes do not exhibit any temperature-induced RAD-51 foci in any part of the meiotic prophase I.

Temperature-induced DNA damage in spermatocytes is exacerbated at temperatures above 34°C

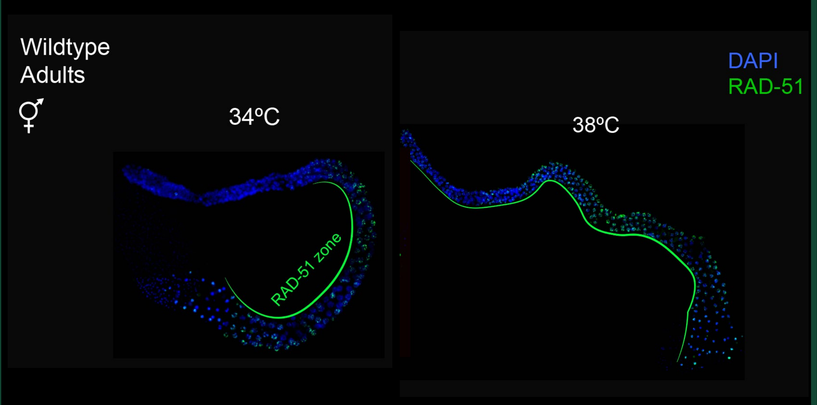
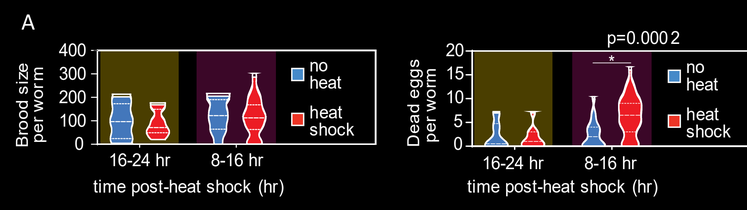


Figure 4. Immunofluorescence for DNA (blue) and the recombinase RAD-51 (green) was used to assess DNA damage throughout the germlines of wild-type male C. elegans with exposure to heat shock (34°C and 38°C for 2 hours are shown). The "RAD-51 zone" where RAD-51 foci are consistently present and associated with the nuclei of developing spermatocytes is indicated by a green line along the length of the gonad.

Temperature-induced DNA damage during meiotic prophase I is associated with reduced male fertility



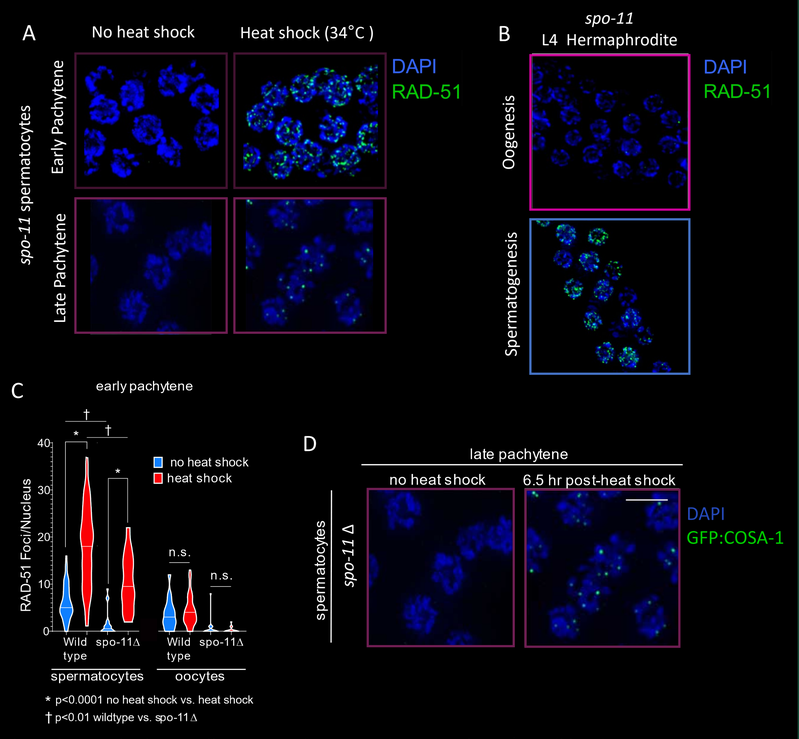
B 8-16 hours post-exposure: Total count (avg ± SD)

Treatment	Total progeny	Live progeny	Dead eggs	% Dead eggs	
No heat	2845 (121±59)	2789 (119±66)	56 (2.4±2.6)	1.9%	n=23 worms
Heat shock	4321 (107±71)	4084 (113±74)	237* (6.2±4.1)	5.8%	n=38 worms

*p > 0.0001 (Fishers exact)

Figure 5. Male fertility was assessed using wildtype male worms and *fog-2* null obligate females. (A) Quantification of dead-eggs and total brood size produced from each mating pair within subsequent 8-hour intervals. (C) Average ± standard deviation for total progeny, living progeny, and dead eggs per mated pair in the 8-16 hours post exposure window. There is a significant increase in the number of dead eggs laid by *fog-2* females mated to heat-shocked males.

Temperature-induced RAD-51 foci are SPO-11 independent DSBs that are able to form crossovers



* p<0.0001 no heat shock vs. heat shock
† p<0.01 wildtype vs. spo-11Δ

Figure 6. (A) Using immunofluorescence for RAD-51 (green) and DNA/DAPI (blue) in spo-11 mutants (which lack DSBs and crossovers), temperature-induced RAD-51 foci still form in *spo-11* mutants at a similar frequency found in wild-type (B) Immunofluorescence in the germ line of an L4 stage spo-11 mutant hermaphrodite reveals that despite sharing the same cytoplasm, only spermatocytes exhibit temperature-induced DSBs (C) Quantification of RAD-51 foci per nucleus during early pachytene for both wild-type and *spo-11* null strains; spermatocytes and oocytes. (D) Immunofluorescence for the crossover marker COSA-1 (green) shows that heat shock restores crossover formation in *spo-11* mutants.

Temperature-induced DSBs progress with repair less than 3 hours after formation in spermatocytes

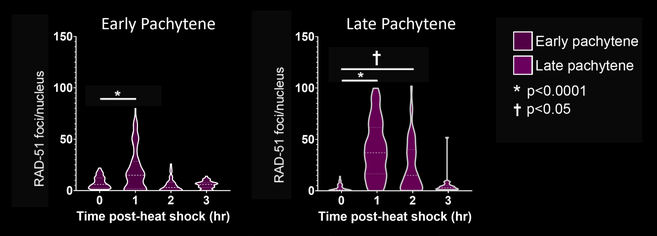


Figure 7. The appearance and disappearance of a RAD-51 focus indicate the initiation and subsequent progression of a DSB through a repair pathway. Measurement of RAD-51 foci counts before heat shock and at 1-hour time points following heat shock reveal that most, if not all, of the temperature-induced DSBs have unloaded RAD-51 and progressed down a repair pathway by 3 hours following heat shock.

A subset of spermatocyte nuclei show persistent DNA damage in late prophase I

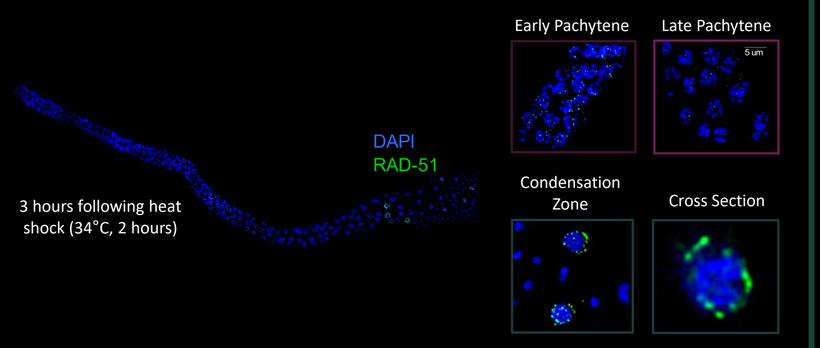
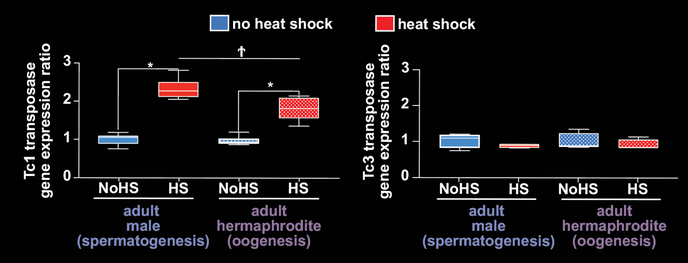


Figure 8. RAD-51 foci were visualized and quantified in the germline of wild-type male *C. elegans* with exposure to heat shock after 3-hours post-exposure. Magnified panels highlight the degree of heat-induced DNA damage in early and late pachytene. Notably, in the condensation region of heat shocked male germ lines at the 3-hour time point, a subset of nuclei had massive amounts of unrepaired DSBs that were largely localized to the periphery of the nucleus.

Increase in Tc1 transposon expression and insertion sites correspond with temperature-induced DSBs



*p<0.0001 heat shock vs. no heat shock
† p<0.005 male vs. hermaphrodite

Figure 9. Quantitative PCR for Tc1a and Tc3 transposase expression reveals that Tc1a transposase expression and not Tc3 transposase is increased significantly following heat shock. Expression levels were all normalized to *act-1* expression levels.

Tc1 transposon positions in the spermatocyte genome become altered following temperature increases

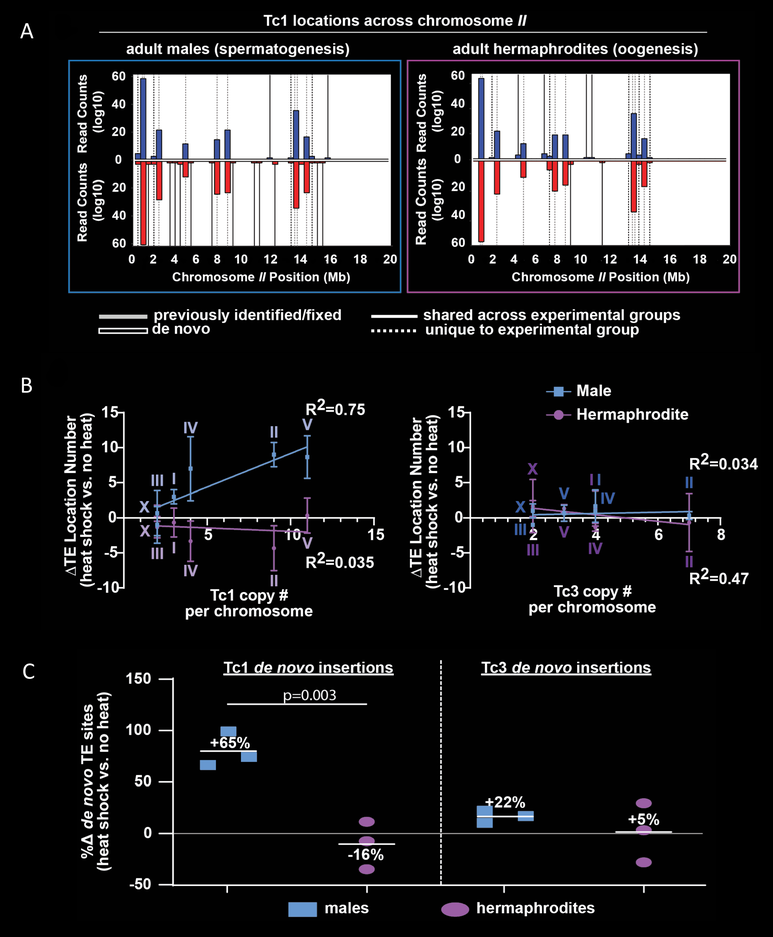


Figure 10. Tc1 mobility was assessed globally and across individual chromosomes. (A) Inverse PCR paired with amplicon sequencing was used to identify Tc1 locations genome-wide. Representative plots for Chromosome II from adult males and hermaphrodite populations are shown. (B) To evaluate transposon mobility across individual chromosomes, the change in Tc1 and Tc3 transposon location number upon heat shock was plotted against Tc1 or Tc3 copy number. (C) Global *de novo* Tc1 and Tc3 insertion site number was quantified and the total number of *de novo* insertion sites in heat shocked worms was compared with non-heat shocked worms.

Tc1 transposons exhibit copy-specific patterns of mobilization following heat-shock

Figure 11. Polymorphisms within individual copies of Tc1 were used to track their mobility throughout the genome following heat shock. (A) Schematic of Tc1 transposon structure with three unique Tc1 copies aligned below. Positions of previously reported polymorphisms unique to each individual Tc1 copy are indicated by solid boxes (Fischer et al. *Genetics* 2003). (B) Assessment of individual Tc1 copies across individual chromosomes indicate the relative percent of reads representing Tc1 transposons present at their previously reported home location, at other known Tc1 locations, and at de novo locations. (C) Comparison of percentage of polymorphism-containing reads at each type of location between each exposure group. Reads containing SNPs previously reported to be specific to Tc1.9 were found to increase at de-novo insertion sites only in the heat-shocked males.

Summary

1. **Increased temperatures cause RAD-51 marked DNA damage specific to spermatocytes during meiotic prophase I.**
2. **Temperature-induced DNA damage forms SPO-11 independent double strand DNA breaks that have the capacity to repaired as crossover recombination events.**
3. **Temperature-induced DNA damage during meiotic prophase I correlates with reduced male fertility**
4. **Tc1 transposons excise and insert into the genome upon temperature increases in wild-type spermatocytes.**

Funding Sources

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