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LEM-3 – A LEM Domain Containing Nuclease Involved in the DNA Damage Response in *C. elegans*

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Abstract

The small nematode *Caenorhabditis elegans* displays a spectrum of DNA damage responses similar to humans. In order to identify new DNA damage response genes, we isolated in a forward genetic screen 14 new mutations conferring hypersensitivity to ionizing radiation. We present here our characterization of *lem-3*, one of the genes identified in this screen. *LEM-3* contains a LEM domain and a GIY nuclease domain. We confirm that *LEM-3* has DNase activity in vitro. *lem-3(lf) mutants* are hypersensitive to various types of DNA damage, including ionizing radiation, UV-C light and crosslinking agents. Embryos from irradiated *lem-3* hermaphrodites displayed severe defects during cell division, including chromosome mis-segregation and anaphase bridges. The mitotic defects observed in irradiated *lem-3* mutant embryos are similar to those found in *bat-1* (barrier-to-autointegration factor) mutants. The *bat-1* gene codes for an essential and highly conserved protein known to interact with the other two *C. elegans* LEM domain proteins, *LEM-2* and *EMR-1*. We show that *bat-1*, *lem-2*, and *emr-1* mutants are also hypersensitive to DNA damage and that loss of *lem-3* sensitizes *bat-1* mutants even in the absence of DNA damage. Our data suggest that BAF-1, together with the LEM domain proteins, plays an important role following DNA damage – possibly by promoting the reorganization of damaged chromatin.

Introduction

Living organisms are continuously exposed to genotoxic stress, caused both endogenously by cellular metabolism and exogenously by e.g. UV light. To maintain genome integrity, cells possess a number of DNA damage response pathways, which ensure that lesions are removed from the DNA or that cells with irreparable damage are removed by apoptosis. Defects in DNA damage responses can lead to a number of diseases, including predisposition to cancer (for review see [1]).

We and others have shown that *Caenorhabditis elegans* displays a spectrum of DNA damage responses comparable to humans, and that it can be used with success to genetically and molecularly dissect DNA damage response pathways (for review see [2–4]). DNA damage results in two types of cellular responses in the adult *C. elegans* germ line: In the mitotically dividing germ stem cell compartment, DNA damage results in cell cycle arrest, whereas meiotic cells in the pachytene zone respond by activating apoptosis [5]. Both responses are induced via evolutionarily conserved signalling pathways (for review see [2–4]). Mutants in these pathways are often characterized by defects in either or both cell cycle arrest and apoptosis.

Failure to sense or signal DNA damage can also lead to defects in DNA repair. Indeed, many *C. elegans* DNA damage response mutants display a high level of embryonic lethality following DNA damage (for review see [3,4]), likely due to defects in the replication and segregation of the damaged genome during the rapid early cell cycles (in contrast to dividing germ cells, early embryos do not undergo cell cycle arrest following DNA damage [6]).

In 1982, Hartman and Herman [7] took advantage of this hypersensitivity phenotype to search for genes involved in DNA damage responses. In a screen for mutants which displayed high embryonic lethality upon treatment with UV-C light, they found nine non-allelic radiation-sensitive mutants named *rad-1* to *rad-9*. For example, *rad-3* corresponds to the *C. elegans* XPA homologue *xpa-1*, which is required to remove UV-induced 6-4 photoproducts and cyclobutane dimers [7–9]. *rad-51/clk-2*, another mutant identified in this screen, is sensitive to various DNA damaging agents and displays defects in cell cycle arrest and apoptosis [10]. Recent studies show that the role of *clk-2* is also conserved in humans, where HCLK2 plays a critical role in the S-phase checkpoint [11,12]. Taken together, these examples emphasize that forward genetic screens are powerful tools for identifying new key players in the complex processes following DNA damage.

As the original screen by Hartman and Herman [7] was not saturated, we conducted a similar screen for mutants displaying reduced survival upon X-radiation. Here we describe the identification and characterization of the novel evolutionarily conserved DNA damage response gene *lem-3*. *LEM-3* contains a LEM (LAP2 – emerin - MAN1) domain and a GIY-YIG nuclease domain. Biochemical analysis confirms that *LEM-3* acts as nuclease *in vitro*. Loss of *lem-3* function does not impair cell cycle arrest or apoptosis in
the germ line but leads to high embryonic lethality following treatment with X-rays, UV-C light and cisplatin. Following DNA damage \textit{lem-3} embryos display severe defects during cell division, including chromosome mis-segregation and anaphase bridges. These mitotic defects are similar to those found in \textit{baf-1} (barrier-to-autointegration factor) mutants [13]. The \textit{baf-1} gene encodes an essential and highly conserved protein known to interact with the other two \textit{C. elegans} LEM domain proteins, LEM-2 and EMR-1 [14–18]. Here we show that \textit{baf-1}, \textit{lem-2}, and \textit{emr-1} mutants are also hypersensitive to DNA damage and that loss of \textit{lem-3} sensitizes \textit{baf-1} mutants even in the absence of DNA damage. Our data uncover a novel function for \textit{baf-1} following DNA damage, which is dependent on the LEM domain proteins.

\section*{Results}

A forward genetic screen for mutants hypersensitive to DNA damage

To find novel components involved in DNA damage response pathways, we conducted a forward genetic screen for mutations that show increased embryonic lethality following exposure to sublethal doses of ionizing radiation. In a screen of approximately 2,000 genomes, we found 14 mutants with such a phenotype (Figure S1). Of these, we selected \textit{op444} for further analysis because it showed the highest increase in sensitivity to ionizing radiation but had no strong defect in the absence of exogenous DNA damage. Dose-response studies showed that \textit{op444} mutants were an order of magnitude more sensitive to ionizing radiation (IR) than wild type (LD\textsubscript{50} approx. 6 Gy vs. 60 Gy, Figure 1A).

\textbf{op444} mutants are also sensitive to UV-C and cisplatin

We next tested whether \textit{op444} mutants showed sensitivity to other types of DNA damage. UV-C light (<280 nm) induces cyclobutane pyrimidine dimers and 6-4 photoproducts, which are mainly repaired by the nucleotide excision repair (NER) pathway. To determine whether \textit{op444} mutants are also sensitive to UV-C light, we exposed adult hermaphrodites to 60 J/m\textsuperscript{2} and assessed embryonic lethality of their progeny (Figure 1B). We found that \textit{op444} mutants displayed a high lethality following UV-C exposure, similar to that observed in \textit{xpa-1(ok698)} mutants, which carry a...
deletion in the *C. elegans* homolog of human XPA, a component of the nucleotide excision repair (NER) pathway [19].

We also treated *op444* animals with cisplatin, a potent DNA damaging agent frequently used in chemotherapy, which generates intra- and interstrand DNA crosslinks. *op444* mutants displayed increased embryonic lethality at both concentrations tested (1 mM and 5 mM, Figure 1C). Taken together, these data indicate that a variety of genotoxic insults can impair the survival of *op444* mutant embryos.

**op444 is a mutation in lem-3**

We took advantage of fragment length polymorphisms (FLP) and single nucleotide polymorphisms (SNP) between the Hawaiian isolate CB4856 and the Bristol wild-type strain N2 [20,21] to map *op444* to a 20 kb interval containing six predicted genes on the right arm of LG I. Unfortunately, we could not phenocopy the radiation sensitivity of *op444* mutants by RNAi knockdown of any of these six genes. However, by sequencing genomic DNA from *op444* mutants, we identified a G to T point mutation at position +2363 in *lem-3* (F42H11.2) (Figure 2A), a gene not previously implicated in any DNA damage response pathway. This mutation results in the change of an evolutionarily conserved leucine to a phenylalanine in the C-terminus of the protein (Figure 2B and D).

We used bioinformatic transformation [22] to generate transgenic lines expressing *lem-3* under the control of the promoter of the ubiquitously expressed gene *npr-1*. Three independent transgenic lines fully rescued the radiation sensitivity of *op444* mutants; two of them are shown in Figure 2C. These results confirm that mutation of *lem-3* is the cause of the hypersensitivity phenotype observed in *op444* mutants. To determine the localization of *lem-3*, we built transgenic lines expressing N-terminally YFP tagged *LEM-3*. Two lines rescued the radiation sensitivity of *lem-3(op444)*, one of them is shown in Figure S2A. YFP::LEM-3 was found to be expressed only in embryos. It localised in a foci-like pattern but we could not observe it inside the nucleus (Figure S2B). The localisation pattern does not change following irradiation (data not shown). The exact nature of the embryonic foci is currently unclear (see discussion).

**rad-1 mutants carry a mutation in lem-3**

Hartman and Herman (1982) had, in a screen similar to ours, isolated and genetically characterized nine *rad* (radiation hypersensitive) genes. One of these, *rad-1* (n155), showed striking phenotypic similarities to *lem-3(op444)* and mapped within 2 cM of *lem-3* [23], thereby prompting us to test whether *rad-1* (n155) is allelic to *lem-3*. Indeed, *rad-1* (n155) failed to complement the radiation sensitivity phenotype of *lem-3(op444)* (Figure S3A). Sequencing revealed a C to T point mutation at position 671, which leads to a premature stop codon (Figure 2A). This R190STOP mutation leads to a truncated protein lacking the LEM domain and the GIY-YIG domain (*Figure 2B and see below*). Even though *rad-1* is the older name for *F42H11.2*, we propose *lem-3* as the common name for this gene, as the name *rad-1* has been previously used for different, non-homologous genes in various organisms.

*lem-3(op444)* also fails to complement *tm3468*, a 330 bp in-frame deletion generated by the Japanese National Bioresource Project (Figure 2A). This allele displays a weaker sensitivity to ionizing radiation (Figure S3B), possibly because the in-frame deletion does not affect any of the conserved domains of LEM-3.

**LEM-3 is a member of the GIY-YIG superfamily**

The LEM-3 protein contains two ankyrin repeats in its N-terminus, followed by a LEM (LAP2 Emerin Man1) domain and a GIY-YIG domain of the type COG3680 at the C-terminus (Figure 2B and 2D). The LEM domain is an approximately 40 amino acid motif which is found in both inner nuclear membrane proteins and nucleoplasmic proteins [24], whereas the GIY-YIG domain is a nuclease domain, which is found in a diverse set of proteins that interact with DNA, including phage T4 endonuclease, restriction enzymes, and recombination and repair enzymes such as the bacterial nucleotide excision repair proteins UvrC and Cho (UvrC homologue).

**LEM-3 has nuclease activity**

As LEM-3 contains a GIY-YIG domain, we wanted to test whether LEM-3 also has nuclease activity. We cloned the cDNAs of wild-type *lem-3* and *lem-3(op444)* and expressed the respective proteins in insect cells (Figure S4 and Materials & Methods). Incubation of supercoiled plasmid DNA with increasing amounts of wild-type LEM-3 protein converted the supercoiled plasmid to relaxed circular (nicked) and linear molecules (Figure 3B). This reaction was dependent on the concentration of protein added and was abolished in the presence of EDTA. In contrast, addition of the mutant protein only resulted in a minor, if at all, release of the supercoiled form. To further support these findings, we investigated the enzymatic activity of LEM-3 on DNA from *P. Chlamydia*, which is rich in secondary structures that can be cleaved by many structure-specific endonucleases. Incubation of wild-type LEM-3 with *P. Chlamydia* resulted in DNA cleavage (Figure 3C). Again, this endonuclease activity was significantly reduced when mutant LEM-3 was used. In conclusion, these results indicate that LEM-3 has nuclease activity and that the L to F mutation corresponding to *lem-3(op444)* greatly diminishes this activity.

**Cell cycle arrest is normal in op444 mutants**

DNA damage induces a transient cell cycle arrest in the mitotic stem cell compartment of the adult *C. elegans* germ line (Figure 4A), resulting in a temporary reduction in cell numbers. Because DNA damage arrests cell division but not cell growth, arrested cells eventually become much larger than in control animals (Figure 4C) [5]. Unlike many DNA damage checkpoint mutants and DNA repair mutants which are defective for cell cycle arrest [2], *lem-3(op444)* mutants showed a normal proliferation arrest and recovery following exposure to ionizing radiation (Figure 4B and C). We conclude that the pathways that sense DNA damage and signal cell cycle arrest are not impaired in *lem-3* mutants.

**Apoptotic cell death is not impaired in op444 animals**

During oocyte development (Figure 4A), approximately half of the germ cells die of apoptosis, through a developmental program called physiological germ cell death. Upon DNA damage, the number of apoptotic corpses increases significantly due to a CEP-1/p53-mediated up-regulation of the BH3 domain proteins EGL-1 and CED-13 [25–27]. Many DNA damage response mutants are defective in DNA damage induced germ cell apoptosis. By contrast, both basal and DNA damage induced apoptosis were normal in *lem-3(op444)* animals (Figure 4D). We conclude that regulation and execution of apoptosis is unimpaired in *lem-3(op444)* animals. Given that cell cycle arrest and apoptosis following DNA damage are both normal in *lem-3(op444)* mutants, we speculate that *lem-3(op444)* mutants are not impaired in DNA damage signalling but rather in another part of the DNA damage response system, most likely DNA repair.

**Analysis of DNA damage response in lem-3(op444) mutants**

Based on our data above, we next asked which repair pathway LEM-3 might participate in. We first investigated double strand break repair, as this process occurs extensively in the germ line as part
of meiotic recombination. Failure to resolve the double strand breaks induced by the SPO-11 endonuclease results in increased germ cell apoptosis due to activation of CEP-1/p53 and increased meiotic nondisjunction. The resulting aneuploidy leads to a high embryonic lethality and a high incidence of males. However, none of these phenotypes is apparent in lem-3(op444) mutants in the absence of exogenous DNA damage (Figure 1A, 4D, and data not shown). Thus, we conclude that lem-3 mutants are proficient in both meiosis and the resolution of dsDNA breaks induced during meiotic recombination.

Rad54 has been implicated in several steps during homologous recombination and repair [28]. Down-regulation of rad-54 by RNAi results in increased apoptosis and radiation-induced embryonic lethality [29]. We have already shown that RAD-54 forms foci following IR, likely marking sites of active repair [30]. To get further insights into the ability of lem-3(op444) mutants to repair exogenously generated dsDNA breaks, we analyzed the subcellular localisation of YFP::RAD-54. In the absence of exogenous damage, YFP::RAD-54(opIs257) localized to distinct foci in the meiotic zone of the germ line, but showed a diffuse nuclear staining pattern in the mitotic zone (Figure 5A). This is in line with the known role of RAD-54 during homologous recombination. Upon irradiation, foci also appeared in the mitotic zone (Figure 5B).
zone (Figure 5B). Quantitative analysis revealed that the number of RAD-54 foci per nucleus were similar in wild type and lem-3(op444) (Figure 5D). After 17 hours, most cells in the mitotic zone had successfully removed RAD-54 in both wild type and lem-3(op444). Only a few, enlarged nuclei still showed RAD-54 foci (Figure 5C). These likely represent cells with persistent DNA damage that failed to re-enter the cell cycle. We conclude from these data that the repair of exogenously generated dsDNA breaks is normal lem-3(op444).

**lem-3 and xpa-1 act in separate pathways**

Since lem-3 mutants are also hypersensitive to UV-C light, we investigated a possible involvement of lem-3 in the NER pathway. We built and analyzed a double mutant with xpa-1(ok698), a factor...
Figure 4. *lem-3(op444)* mutants show normal cell cycle arrest and apoptotic responses. (A) Schematic representation of a *C. elegans* gonad. The most distal part is capped by the somatic distal tip cell. The cells close to the DTC are in mitosis. As they travel down the gonad, they enter the pachytene stage of meiosis I. Near the gonadal bend, some cells die by apoptosis. Apoptotic bodies become visible as “refractive disks”. The mature oocytes are then pushed through the spermatheca and become fertilized. (B) Quantification of cell cycle arrest response following IR. The number of nuclei within 50 μm of the distant tip cell ± S.D was scored at various time points. n, number of germ lines analyzed. (C) Cell cycle arrest is normal in *lem-3*(*op444*) mutants. Representative DIC images of dissected gonads from wild-type and *lem-3*(*op444*) mutants are shown. Size bar: 10 μm. Black arrowheads indicate small (cycling) cell nuclei, white arrow heads indicate big (arrested) cells. (D) DNA damage-induced apoptosis is normal in *lem-3*(*op444*) mutants. Hermaphrodites were exposed to 60 Gy at L4/adult molt and apoptotic corpses were quantified at the indicated time points. Data shown represent the average of three independent experiments (10 germ lines per experiment) ± S.D.

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common to transcription-coupled repair and global genomic repair, the two branches of the NER pathway. Interestingly, we found that the double mutant showed a significant increase in embryonic lethality compared to both single mutants (Figure 3A). Thus, we conclude that \textit{lem-3} and \textit{xpa-1} likely do not act in a linear pathway.

\textbf{LEM-3 is required for proper chromosome segregation following DNA damage}

As we could not find any clear defects in the germ line of \textit{lem-3(op444)} mutants, we investigated in more detail the cause of the lethality observed following DNA damage. To get a first insight into the nature of the defect, we stained chromatin in early stage embryos derived from irradiated hermaphrodite worms. Whereas chromosome segregation was mostly normal in irradiated wild type embryos (91%, \(n = 67\)), 77% (\(n = 31\)) of irradiated \textit{lem-3(op444)} embryos showed chromosome mis-segregation starting at the second cell division, as evidenced by the generation of extra nuclear chromatin material and chromatin bridges following mitosis (Figure 6). We confirmed this result by using a GFP::H2B transgene to follow chromosome segregation in early embryos by time lapse microscopy (Movie S1 and Movie S2). To determine whether LEM-3 is also required for proper chromosome segregation during larval development, we irradiated freshly hatched L1 larvae and scored the animals as adults for locomotion defects or protruding vulvae – phenotypes arising from defective proliferation/division of the Pn.a neuroblast or Pn.p vulval precursor cells, respectively. These phenotypes can also be observed following irradiation of mutants of the non-homologous end joining pathway genes \textit{cku-70}, \textit{cku-80} and \textit{lig-4} [31]. Only 2% of irradiated \textit{lem-3} mutants developed to adults without any developmental defects (96% displayed an uncoordinated phenotype (Unc), 61% had a protruding vulva (P-vul)). In contrast, 86% of irradiated wild type L1 larvae developed to phenotypically normal adults (Figure 1D). These results suggest that \textit{lem-3} mutant embryos die after irradiation because of loss of genomic integrity due to chromosome segregation defects. Additionally, we conclude that \textit{lem-3} is also required during post-embryonic cell divisions after genotoxic stress to ensure normal cell proliferation.

\textbf{LEM-3 genetically interacts with BAF-1}

Three LEM-domain proteins, EMR-1 (Ce-emerin), LEM-2 (Ce-MAN1), and LEM-3 have been identified in \textit{C. elegans}. EMR-1 and LEM-2 have a transmembrane domain and an N-terminally located LEM domain [14], whereas LEM-3 lacks a transmembrane domain, and has a LEM-domain located in the middle of the protein [14]. EMR-1 and LEM-2 bind LMN-1, the \textit{C. elegans}
lem-3 mutants caused high embryonic lethality even in the absence of any genotoxic insult. Our bioinformatic analysis revealed the presence of a GIY-YIG domain in LEM-3, which can be found in various enzymes involved in DNA repair and recombination [37]. When tested in two different types of assays in vitro, wild-type LEM-3 was able to cleave DNA, while the activity of the L659F (op444) mutant was largely reduced (Figure 3B and C). Given these results, we postulate that LEM-3 is a novel endonuclease involved in DNA damage repair. The exact repair pathway (if any) LEM-3 might participate in remains however to be identified.

**LEM-3 is conserved in humans**

LEM-3 is evolutionary conserved. A report by Brachner et al. (co-submitted) demonstrates that the human LEM-3 homologue Ankle1 is capable of inducing DNA cleavage when forced to localize to the nucleus. A leucine to phenylalanine mutation corresponding to the mutation in lem-3(op444), however, failed to cause DNA damage under the same conditions. These observations suggest that the molecular function of LEM-3/Ankle1 might be conserved through evolution.

**Subcellular localization of LEM-3**

We found that YFP::LEM-3 localizes in a distinct foci-like structure clearly outside of the nucleus (Figure S2B). To date, we have no definitive explanation for this localization pattern, which is independent of DNA damage. We speculate that LEM-3 is present at low concentrations in the nucleus where it fulfills its function. At the onset of anaphase, microtubules pull the chromosomes into a poleward direction. In between, non-mitotic microtubules form a bundled structure and assemble as part of the central spindle complex, also referred to as the “midbody”. The observed foci could be remnants of the midbody – we were however unable to confirm this hypothesis.

**Interaction between LEM-3 and BAF-1**

Whereas lem-3 mutants do not show any defects in the adult germ line following DNA damage, embryos generated from irradiated germ lines show major defects in chromosome segregation starting at the second cell division. This phenotype is reminiscent of embryos depleted of bmn-1, baf-1 or co-depleted of lem-2 and emr-1. We found that a reduction of BAF-1 function in lem-3 mutants causes high embryonic lethality even in the absence of any genotoxic insult. Our data suggest a model in which LEM-3 is required to support BAF-1 function. We postulate that, for a currently unknown reason,
requirement for BAF-1 function increases following DNA damage. What exact function BAF-1 plays following DNA damage and how LEM domain proteins contribute to this function remains to be determined. Loss of \textit{lem}-3 function uncovers this requirement. This model is supported by the fact that also the other \textit{C. elegans} LEM domain protein mutants \textit{lem}-2 and \textit{emr}-1 show a moderate and a weak sensitivity to IR, respectively.

**Materials and Methods**

**Strains and general procedures**

N2 Bristol strain [38] was used as the wild-type strain in all experiments. For mapping experiments the polymorphic strain CB4856 was used. All strains were kept on NGM agar plates seeded with \textit{Escherichia coli} OP50 at 20°C unless otherwise stated.
**Mutant alleles.** *lem-3(op444)* (this study), *rad-1(mn155)* [7], *lem-3(tm 3460)* (this study), *rad-5(mn159)* [7], *txp-1(ok630)* [19], *cha-80(ok861)* [31], *lem-2(9n182)* (this study), *ems-1(qk119)* [39], *baf-1(t6539)* [40].

**Transgenes.** *opIs257 [P~rad-3 (: C~rad-54 : YFP : 3’ UTR~rad-54] [30],* *opIs309 [P~agg : C~lem-3 : 3’ UTR~lem-3] (this study),* *opIs384 [P~agg : C~lem-3 : 3’ UTR~lem-3] (this study),* *opIs83 [P~agg : C~YFP : 3’ UTR~rad-54] (this study),* *rad32 [unc-119(+)+ pie-1:GFP: H2B] [22].

**Ethyl methane sulfate (EMS) based screen**

Synchronized L4-stage wild-type worms [38] were subjected to 50 mM EMS (M0880 Sigma-Aldrich) and incubated at 20°C for 4 hours. The screening was performed according to the second scheme previously described [7]. But instead of UV-C light, a dose of 60 Gy was used for inflicting DNA damage.

**Irradiation**

An Isovolt Titan 160 with Isovolt 160 M2/0.4–3.0 (Seiffert) and a Stratalinker UV croslinker, model 1800 (Strategene) were used to deliver the indicated doses.

**Embryonic lethality (X-ray and UV-C)**

Animals were irradiated 24 h post L4/adult molt, individualized on plates and allowed to lay eggs for 4 hours. Eggs were quantified; unhatched eggs were counted 24 h later, and the percentage of embryonic lethality was calculated. All assays were performed on 20°C except otherwise stated.

**Late Rad phenotypes**

Animals were synchronized [freshly hatched L1s] and irradiated. Phenotypes of those animals that had reached adulthood were quantified 3 days later using a dissection microscope. The Uncoordinated (Unc) phenotype was scored on the basis of sluggish movement.

**Cisplatin treatment**

For embryonic survival assays synchronized animals (L4/adult molt) were exposed to different doses of cisplatin (Sigma-Aldrich) on agar plates by evenly distributing 1.2 ml of the corresponding concentration of cisplatin on a 10 cm plate. Animals were transferred to fresh plates 24 h post-treatment. Animals were allowed to lay eggs for 12 hr. Adults were then removed and eggs were counted. Unhatched eggs were quantified 24 h later, and the percentage of embryonic lethality was calculated.

**Germline apoptosis**

Staged animals (12 hours post L4/adult molt) were irradiated with 60 Gy. Apoptotic corpses were quantified in the meiotic zone of the germ line at indicated time points, as previously described [41].

**Cell cycle arrest**

Staged animals (L4/adult molt) were irradiated. Germ lines were dissected at the indicated time points. Images were taken using an ORCA-ER digital CCD camera. The diameters of 10 nuclei (in focus) were measured and the average diameter of nuclei per germ line was calculated using Image J 1.40 g software (Wayne Rasband, http://rsb.info.nih.gov/ij).

**FM4-64 staining**

Embryos were dissected from gravid adults in M9 containing 64 μM FM4-64 (Invitrogen). The eggshell was cracked as previously described [42].

**Time lapse microscopy**

For time lapse microscopy, an Olympus BX61 was used equipped with a Retiga 2000R camera. Dissected embryos were put on a 2% agarose pad. Images were taken every 20 seconds at 2× binning. Pictures were processed using Openlab software (Improvision).

**Alignments**

Alignments were ClustalW alignment done with the Jalview software (Version 11.0).

**Transgenic lines**

Transgenic lines were obtained by microparticle bombardment in a Biolistic PDS-1000 (Bio-Rad) transformation, as previously described [22].

**DNA constructs**

PCR amplification was done with Phusion High-Fidelity DNA Polymerase (Finnzymes). Oligonucleotide primers were synthesized by Microsynth.

**Primers**

*npp-1* promoter

GATCCCTGCAGTTATTTGGTCATTT-GGTTGATTATGATTG

Pnpp-1Asc_rev: CCGATGGCGCGCCCTTCGCTGAAAACAA-

CAGATTHTTAAGAGAAATG

lem-3 genomic plus 3’UTR

3lem3AscFw: GATCGGCGGCGCCATGCCTCAAAACGAGCAATCAC-

CAGCA

3lem3ApaRev: CTGGAGGCCCAGCATTACCCGACTAATTAGGT-

AGTTAG

**Expression and enrichment of LEM-3**

The cDNA of LEM-3 WT and L~R~F mutant was cloned into a modified pFastBac1 vector (a kind gift of Petr Cejka) that contains an N-terminal MBP-tag. Baculovirus stocks were generated by a modified pFastBac1 vector (a kind gift of Petr Cejka) that contains an N-terminal MBP-tag. Baculovirus stocks were generated by a modified pFastBac1 vector (a kind gift of Petr Cejka) that contains an N-terminal MBP-tag. Baculovirus stocks were generated by a modified pFastBac1 vector (a kind gift of Petr Cejka) that contains an N-terminal MBP-tag. Baculovirus stocks were generated by a modified pFastBac1 vector (a kind gift of Petr Cejka) that contains an N-terminal MBP-tag.

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GATCCCTGCAGTTATTTGGTCATTT-GGTTGATTATGATTG

Pnpp-1Asc_rev: CCGATGGCGCGCCCTTCGCTGAAAACAA-

CAGATTHTTAAGAGAAATG

lem-3 genomic plus 3’UTR

3lem3AscFw: GATCGGCGGCGCCATGCCTCAAAACGAGCAATCAC-

CAGCA

3lem3ApaRev: CTGGAGGCCCAGCATTACCCGACTAATTAGGT-

AGTTAG

**Expression and enrichment of LEM-3**

The cDNA of LEM-3 WT and L~R~F mutant was cloned into a modified pFastBac1 vector (a kind gift of Petr Cejka) that contains an N-terminal MBP-tag. Baculovirus stocks were generated by a modified pFastBac1 vector (a kind gift of Petr Cejka) that contains an N-terminal MBP-tag. Baculovirus stocks were generated by a modified pFastBac1 vector (a kind gift of Petr Cejka) that contains an N-terminal MBP-tag. Baculovirus stocks were generated by a modified pFastBac1 vector (a kind gift of Petr Cejka) that contains an N-terminal MBP-tag. Baculovirus stocks were generated by a modified pFastBac1 vector (a kind gift of Petr Cejka) that contains an N-terminal MBP-tag.
terminated by the addition of 0.1% SDS, 14 mM EDTA and 0.1 mg/ml Proteinase K and incubation at 35°C for 10 min. 10% glycerol was added and the samples were separated on a 0.8% agarose gel for 45 min at 80 V. [43].

**PhiX174 non-specific endonuclease assay**

PhiX174 circular virion ssDNA (NEB) was incubated with LEM-3 wild type and L→F mutant in endonuclease buffer (25 mM Hepes-KOH pH 7.4, 60 mM KCl, 1 mM MgCl2) for 1 h at 25°C (EcoRI, HinfI, NheI and MboI) were incubated for 4 h at 25°C; The reaction was stopped by addition of 0.1% SDS, 0.014 M EDTA and 0.1 mg/ml Proteinase K, and incubation at 35°C for 1 h. 10% glycerol was added and the DNA species were separated on a 0.8% agarose gel by 1 h at 70 V. Subsequently, the gel was stained with SYBR Gold and analyzed with an Imager (Typhoon).

**Supporting Information**

**Figure S1 Embryonic lethality of isolated mutants.**

Mutants were irradiated with the indicated doses and embryonic lethality was scored. Data shown represent the average number of dead embryos of five hermaphrodites ± S.D. (EPI)

**Figure S2 YFP::LEM-3 expression pattern.**

(A) The transgene op4383 [p opp—::YFP::lem-3::3UTR/lem-3] rescues the hypomorphic sensitivity of lem-3(op444) mutants. Data represent the average of three experiments ± S.D. The progeny of 10 worms were analyzed for each experiment. (B) Representative DIC and fluorescence images of embryos expressing YFP::LEM-3 in green; YFP::LEM-3, in red; membrane staining with the dye FM4-64. (EPI)

**Figure S3 Complementation tests.**

lem-3(op444) fails to complement (A) rad-1(nm355) and (B) lem-3(tm3468). F1 embryonic lethality after irradiation with 30 Gy was quantified. Data shown represent the average embryonic lethality of the progeny of 10 hermaphrodites. (EPI)

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**Movie S1 Chromatin segregation in wild-type embryos after irradiation.**

Wild-type hermaphrodites carrying the transgene GFP::H2B were irradiated with 30 Gy. Embryos were dissected 2 h after irradiation treatment. Images were taken every 20 sec (as described in Materials and Methods). (RAR)

**Movie S2 Chromatin segregation defects in lem-3(op444) embryos after irradiation.**

lem-3(op444) hermaphrodites carrying the transgene GFP::H2B were irradiated with 30 Gy. Embryos were dissected 2 h after irradiation treatment. Images were taken every 20 sec (as described in Materials and Methods). (RAR)

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**Author Contributions**

Conceived and designed the experiments: CMD KK YG JJ MOH. Performed the experiments: CMD KK AS. Analyzed the data: CMD MOH. Contributed reagents/materials/analysis tools: YG. Wrote the paper: CMD MOH.
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