Optogenetic mutagenesis in *Caenorhabditis elegans*

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Reactive oxygen species (ROS) can modify and damage DNA. Here we report an optogenetic mutagenesis approach that is free of toxic chemicals and easy to perform by taking advantage of a genetically encoded ROS generator. This method relies on the potency of ROS generation by His-mSOG, the mini singlet oxygen generator, miniSOG, fused to a histone. *Caenorhabditis elegans* expressing His-mSOG in the germline behave and reproduce normally, without photoinduction. Following exposure to blue light, the His-mSOG animals produce progeny with a wide range of heritable phenotypes. We show that optogenetic mutagenesis by His-mSOG induces a broad spectrum of mutations including single-nucleotide variants (SNVs), chromosomal deletions, as well as integration of extrachromosomal transgenes, which complements those derived from traditional chemical or radiation mutagenesis. The optogenetic mutagenesis expands the toolbox for forward genetic screening and also provides direct evidence that nuclear ROS can induce heritable and specific genetic mutations.

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Reactive oxygen species (ROS) are known to damage DNAs and proteins, induce oxidative stress, and trigger a plethora of protective responses in cells or organisms. In recent years, a number of genetically encoded photosensitizers, such as KillerRed, SuperNova, and mini singlet oxygen generator (miniSOG) have been developed to manipulate ROS in vivo for a variety of purposes. Among these, miniSOG, a 106 amino-acid monomeric protein, was originally reported to produce singlet oxygen upon blue light exposure, and may also produce ROS other than singlet oxygen. MiniSOG has been used for correlating light and electron microscopy to visualize tagged proteins within tens of nanometers of their subcellular residence. The ability of miniSOG to locally generate ROS has also been exploited to ablate cells and to inactivate proteins in a blue light dependent manner. Such applications have facilitated investigations of neural circuits and synaptic transmission.

ROS are also known to modify DNA. For example, chemically induced ROS was shown to cause a range of mutations in bacterial phase DNA. However, to our knowledge, it is not yet known whether genetically encoded photosensitizers can produce heritable DNA mutations in multicellular organisms. Here, we demonstrate that miniSOG fused to a histone (His-mSOG) induces heritable mutations in a light-dependent manner in C. elegans. We refer to the mutagenesis using photoactivation of genetically encoded proteins as ‘optogenetic mutagenesis’. This method is rapid, simple and free of toxic chemicals. Optogenetic mutagenesis produces a wide variety of changes in DNA such as single-nucleotide variants (SNVs), deletions, and insertion of extrachromosomal transgenes. The distinctive mutagenic spectrum of His-miniSOG makes it a valuable complement to traditional chemical or radiation mutagenesis. Additionally, our observations demonstrate that high levels of ROS in the nucleus can cause specific and genetically heritable mutations.

**Results**

**His-mSOG mutagenizes the genomic DNA.** We reasoned that activation of miniSOG in proximity to DNA could generate mutagenic ROS. To test this idea we fused miniSOG to the C-terminus of a histone H3.3 variant, C. elegans HIS-72 (ref. 10) (His-mSOG). To express His-mSOG in the germline we generated a single-copy integrated transgene ju5Xi164[Pmex-5-HIS-72:: miniSOG-3'UTR[bb-2]] using the mex-5 promoter and the 3' untranslated region of bb-2 (ref. 11) (Fig. 1a). Without blue light treatment, His-mSOG animals had normal growth rate and brood size (Fig. 1b, His-mSOG, 0 min light), displayed wild-type body shape and movement (Fig. 1c), and lived a normal lifespan (Fig. 1d, His-mSOG, 0 min light). We activated miniSOG by illuminating young adult hermaphrodites with blue light and examined if they produced mutant progeny. The blue light condition used was 2.0 mW/mm² for 30 min with a 460-nm wavelength (see Methods), which did not affect the brood size or lifespan of N2 wild-type or His-mSOG animals (Fig. 1b,d, 0 min light). Among the F₂ progeny from the light-treated His-mSOG parents, we found mutant animals with visible phenotypes such as uncoordinated and dumpy (Fig. 1c). We singled 87 F₂ hermaphrodites, and observed that 10% of them were sterile and 20% gave F₂ progeny with visible mutant phenotypes in the light-treated His-mSOG group (Fig. 1e). Most of these phenotypes were inherited in the following generations, suggesting that they were caused by genetic mutations in the germline or early embryos. Wild-type animals treated with blue light did not produce visible mutants, nor did His-mSOG animals without exposure to blue light (Fig. 1e), indicating that the observed mutagenesis is dependent on the combination of blue-light exposure and the His-mSOG transgene.

We used outcrossing and genetic mapping to determine whether the observed phenotypes were due to mutations in single genes. A dumpy mutation ju1157 was mapped to the left arm of chromosome III in the course of outcrossing, and failed to complement dpy-1(e1). We determined by Sanger sequencing that it contained a 442-bp deletion in dpy-1. An uncoordinated mutation ju1158 was mapped to the right arm of chromosome I. By whole-genome sequencing (WGS), we found a dinucleotide GA was mutated to C in the unc-122 gene, resulting in a frameshift. ju1158 failed to complement unc-122(e2520). Thus, optogenetically induced mutations can attribute to single genes.

**Frequency of optogenetically induced mutants.** We next investigated the frequency of optogenetically induced mutations using a genetic suppressor screen for the locomotion defects of rpm-1(∥f); syd-2(∥f) double mutants. This screen has three advantages: first, well-moving suppressor mutants are easily identified among the severely uncoordinated rpm-1(∥f); syd-2(∥f) animals; second, saturation EMS screens have identified only a small number of suppressor loci that are distributed in three chromosomes; third, it allows us to quantitatively compare the efficiency and spectrum of optogenetically induced mutations with those of previously characterized EMS-induced mutations. The frequency of mutations was calculated from the number of independent suppressors and of the mutagenized haploid genomes based on the average brood size (see Methods). In the rpm-1(∥f); syd-2(∥f) background, the brood size of His-mSOG strain was dramatically decreased when animals were exposed to blue light for >30 min (Fig. 2a). Therefore, we illuminated these animals with blue light for 0, 10 and 30 min, and found that the frequency of mutations increased at higher light dosage (Fig. 2b). The rpm-1(∥f); syd-2(∥f) strain lacking His-mSOG transgene displayed a much lower mutation rate even with 30-min blue light exposure (0.03 ± 0.03/1,000 haploid genomes, n = 8 independent experiments, total ~64,000 mutagenized haploid genomes, Fig. 2b). When rpm-1(∥f); syd-2(∥f) animals with His-mSOG were maintained continuously under ambient light, we found only one suppressor mutation among the progeny of ~70,000 F₁ animals, which is comparable to the spontaneous mutation rate in C. elegans. These data support a conclusion that optogenetic mutagenesis is specific to the interaction of His-mSOG and blue light.

In C. elegans it is highly recommended to mutagenize animals at fourth larval (L₄) stage when germline proliferation is at its peak. We examined the efficiency of optogenetic mutagenesis at different developmental stages, and found that gravid young adults showed a higher mutation frequency than L₄ under the 30-min blue light illumination protocol (Fig. 2c). Compared to chemical mutagens, which may not efficiently pass through adult cuticles and/or egg shells, blue light might efficiently affect the transparent eggs in optogenetic mutagenesis.

Based on nine independent suppressor screens of rpm-1(∥f); syd-2(∥f) using the 30-min illumination protocol, we determined the mutation frequency of His-mSOG mutagenesis to be 0.72 ± 0.14 per 1,000 haploid genomes (Fig. 2d, total ~78,000 mutagenized haploid genomes). This mutation rate did not change after maintaining the His-mSOG transgenic strain over ten generations, suggesting that the germline expression of His-mSOG is not silenced over time or generations. We further compared the mutagenesis efficiency of His-mSOG to that of EMS, the most commonly used chemical mutagen in C. elegans. We treated rpm-1(∥f); syd-2(∥f) animals without His-mSOG transgenes (CZ1338) with a standard 50 mM EMS protocol, which is known to give rise to one loss-of-function mutation of any particular gene out of 2,000 mutagenized haploid genomes.
Figure 1 | His-mSOG induces heritable mutations in a light-dependent manner. (a) Schematic of the HIS-72::miniSOG construct and optogenetic mutagenesis procedure. Parental (P₀) His-mSOG worms expressing a single copy transgene µS164 in the germline are treated with blue light. Mutants are isolated from the F₂ generation (Mut/Mut) after self-fertilizing F₁ (Mut/+). (b) The brood size of wild-type and His-mSOG animals treated with blue light for different durations at gravid young adult stage. The brood sizes of N2 and His-mSOG worms were comparable and were decreased by prolonged blue light treatment. Error bars indicate S.E.M. Statistics: One-way ANOVA with Bonferroni post-test for comparison of light duration, *P<0.05, **P<0.001. Two-tailed t-test for comparison between N2 and His-mSOG (ΔP>0.05). (c) Bright-field images of animals with genotype and condition indicated. (d) Survival curve of wild-type and His-mSOG worms with and without blue light treatment at young adult stage. n=4 experiments. (e) Frequency of miniSOG-induced mutants in F₁ progeny. Young adult parental worms were treated with or without blue light for 30 min and F₁ animals were isolated. 'Mut/+’ and ‘sterile’ indicate the fraction of F₁ animals segregating progeny with visible phenotypes and no progeny, respectively. n = number of F₁ animals.

(see Methods). We calculated the mutation rate of EMS in our screen to be 3.1 ± 0.52 (n = 4 independent experiments, total ~21,000 mutagenized haploid genomes), about 4.4 times higher than that of His-mSOG optogenetic mutagenesis using 30-min illumination protocol (Fig. 2d). It might be possible to increase the efficiency of optogenetic mutagenesis by increasing the duration of light exposure in a wild-type background since wild-type animals are more tolerant to blue light exposure than are rpm-1(²f); syd-2(²f) animals (Figs 1b and 2a).

Frequency of optogenetically induced SNVs. The mutation frequency described above is estimated from the specific suppressor loci. To determine the genome-wide frequency of optogenetically induced mutations, we carried out WGS analysis. We obtained >20-fold genome coverage for three groups of strains: (1) the parental His-mSOG strain (CZ20638 µS164; julS1; rpm-1(²f); syd-2(²f)); (2) three optogenetically-induced suppressor strains from the parental strain; (3) two isogenic siblings from CZ20638 that were not exposed to the blue light but were handled in parallel to the three suppressor strains. We determined the unique homozygous SNVs using Galaxy platform (see Methods)¹⁷. The non-mutagenized strains contained 2.0 ± 1.0 total SNVs (Fig. 3a), and none of them were in exons (Fig. 3b), compared to the parental strain. This is within the range of the previously reported rate of spontaneous mutations in wild-type C. elegans¹⁵. In conjunction with the phenotype-based mutation rate (Fig. 2b), we conclude that His-mSOG does not induce mutations without blue light exposure. In contrast, the three optogenetically induced suppressor strains had increased numbers of total SNVs and SNVs in exons (37.0 ± 8.1 and 9.7 ± 1.2, respectively, Fig. 3a,b). By comparison with previous studies¹⁹,²⁰, we estimate that His-mSOG mutagenesis induces approximately ten times fewer SNVs than 50 mM EMS mutagenesis. 26% of C. elegans genome is known to contain exon sequences²¹. Although histone might have different distribution between exons and non-exons, we did not see any bias toward exon regions (SNVs in exons/total
Figure 2 | Efficiency of His-mSOG mutagenesis based on the rpm-1(lf); syd-2(lf) suppressor screen. The mutation rate of His-mSOG mutagenesis was estimated by the number of suppressor mutations isolated from screening in the rpm-1(lf); syd-2(lf) background as described in Methods. (a) Brood size of CZ20638 juSi164 unc-119(ed3); juIs1; rpm-1(lf); syd-2(lf) after exposure to different duration of blue light at gravid young adult stage. n = 10 animals. (b) Effect of light dosage. The frequency of mutations of His-mSOG-mutagenesis at gravid young adult stage was determined for different durations of light exposure. Negative controls are non-transgenic rpm-1(lf); syd-2(lf) animals with and without light exposure. (c) Effect of developmental stage. His-mSOG-carrying animals were treated with blue light for 30 min at different developmental stages. (d) Comparison between the efficiency of EMS and His-mSOG. The mutation rate of EMS mutagenesis was determined from 50 mM EMS treatment of CZ1338 juIs1; rpm-1(lf); syd-2(lf) and compared to His-SOG-mutagenesis with 30-min light exposure at gravid young adult stage. Error bar indicates S.E.M. (a–d) n = number of independent experiments, 20 P0 per experiment. Statistics: (a,b) One-way ANOVA, ***P < 0.001. (d) Two-tailed t-test, ***P < 0.001.

Figure 3 | His-mSOG mutagenesis induces SNVs and deletions. (a–c) Two untreated non-suppressors and three light-induced suppressors from the rpm-1(lf); syd-2(lf) suppressor screen were analyzed by WGS. (a) Total unique homozygous SNVs. (b) Unique homozygous SNVs in exons. Statistics: Two-tailed t-test, (a) P = 0.061, (b) *P < 0.05. (c) Types of SNVs in the three light-induced suppressors. (d) Comparison of types of mutations generated in His-mSOG- and EMS- mutagenesis. The causative mutations of rpm-1(lf); syd-2(lf) suppressors were categorized. ‘Other’ includes possible large deletions and/or chromosomal rearrangements. n = number of suppressor mutants.
SNVs = 9.7/37 = 0.26). We found about eight non-synonymous SNVs in each optogenetically induced suppressor strain. Table 1 lists these nucleotide changes and predicted amino-acid changes of genes affected. Among non-synonymous SNVs, we found two SNVs in two suppressor strains. Complementation tests confirmed that these mutations caused loss of function in each gene. These analyses show that His-mSOG can modify functions of genes at a suitable frequency for mutagenesis.

Spectrum of optogenetically induced mutations. To determine the types of mutations of His-mSOG mutagenesis, we first examined the genome-wide landscape of optogenetically induced SNVs by analyzing WGS data for three rpm-1(lf); syd-2(lf) suppressors described above. We found that these SNVs showed a high ratio of G:C to T:A transversions (45.7 ± 5.6%, n = 3 suppressors, Fig. 3c), while G:C to C:G transversions and G:C to A:T transitions were also detected (17.4 ± 3.0% and 17.4 ± 3.0%, respectively, n = 3 suppressors, Fig. 3c). We further characterized causative mutations of eighteen additional rpm-1(lf); syd-2(lf) suppressors by complementation tests and Sanger sequencing (Table 2 and Methods). Optogenetically induced SNVs tended to be G:C to T:A and G:C to C:G transversions, accounting for 42.9% and 14.3% of causative suppressor mutations, respectively (Fig. 3d).

We next examined deletion mutations. Through analysis of WGS data, we found several uncovered regions of >200-bp in the optogenetically induced suppressors. We confirmed two of these uncovered regions to be a 437-bp deletion and a 1,427-bp deletion, Table 2. These results suggest that His-mSOG mutagenesis is not strongly biased with respect to chromosomes or positions on the chromosomes (Fig. 3a). The lack of bias was also observed in the analysis of the optogenetically induced loci of the rpm-1(lf); syd-2(lf) suppressors. Our optogenetic mutagenesis hit five out of six major known suppressors. Moreover, the relative frequency of the suppressors obtained from His-mSOG-mutagenesis matched that observed in our previous saturation EMS screens (Fig. 4b)\(^{14}\). Thus, we conclude that His-mSOG mutagenesis causes a broad spectrum of mutations.

Distribution of optogenetically induced mutations. We asked if His-mSOG mutagenesis displayed any chromosomal bias since X chromosome is known to become depleted in Hexosome 3.3 including HIS-72 during gametogenesis\(^{10}\). Figure 4a shows the chromosomal distribution of optogenetically induced SNVs based on WGS analysis of three rpm-1(lf); syd-2(lf) suppressors described above. There were more SNVs on chromosomes I and IV because of the linkage to the causative suppressor mutations in dkl-1 (LG I) and pmk-3 (LG IV), respectively. Nonetheless, optogenetically induced SNVs showed relatively equal distribution on all chromosomes including X, suggesting that His-mSOG mutagenesis is not strongly biased with respect to chromosomes or positions on the chromosomes (Fig. 4a).

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**Table 1 | Optogenetically induced homozygous non-synonymous SNVs.**

| Chr | Position (base) | Wild type | Mutant | Gene | WT/Mut AA |
|-----|----------------|-----------|--------|------|-----------|
| CZZ22336 | dkl-1(ju1290); juSi164 unc-119(ed3); juIs1; rpm-1(lf); syd-2(lf) | 4725957 | G | T | ugt-27 | G/C |
| | | 5711255 | G | C | H06D01.2 | D/E |
| | | 7219282 | A | G | nhr-23 | Y/C |
| | | 124570028 | C | A | dkl-1 | V/F |
| | | 12650161 | G | T | H28016.2 | R/S |
| | | 13657414 | G | T | W09C5.7 | G/V |
| | | 16073314 | G | T | col-187 | R/I |
| | | 16927569 | C | A | C30E1.2 | N/K |

| CZZ22337 | dkl-1(ju1291); juSi164 unc-119(ed3); juIs1; rpm-1(lf); syd-2(lf) | 2415913 | G | T | F23C8.13 | G/W |
| | | 10465587 | C | G | den-3 | C/S |
| | | 2817065 | C | G | KO718.10 | A/T |
| | | 8656791 | T | C | C49C8.1 | X/R |
| | | 11587778 | G | T | F286.1 | K/N |
| | | 16796857 | G | T | W06H3.3 | Q/H |
| | | 9552913 | C | A | F49E5.2 | A/D |
| | | 11760549 | C | A | lact-7 | P/T |
| | | 14091587 | C | A | dsl-4 | P/H |

| CZZ22338 | juSi164 unc-119(ed3); pmk-3(ju1292) juIs1; rpm-1(lf); syd-2(lf) | 9421262 | G | T | palp-2 | R/S |
| | | 2797146 | G | A | F08D12.1 | V/I |
| | | 132562 | G | A | C29F9.1 | G/R |
| | | 1754908 | A | C | Y46E12A.3 | L/W |
| | | 8141724 | G | T | pmk-3 | G/V |
| | | 4750824 | C | A | C18G1.6 | H/N |
| | | 10920642 | A | G | nas-3 | L/P |

*ju1291 is likely a chromosome deletion and/or rearrangement in dkl-1.*

**Optogenetic integration of multi-copy transgenes.** A common practice in the making of transgenic reporter lines is to integrate extrachromosomal high-copy transgenes into the genome to achieve stable and consistent expression. Methods such as UV irradiation with trimethylsulphonol (TMS) and gamma irradiation\(^{23}\) are widely used for transgene integration but require the use of toxic chemicals and/or special equipment. These methods are thought to induce chromosome breaks, which lead to transgene integration. His-mSOG mutagenesis also appears to create chromosome breaks because it produced deletions (Fig. 3d, see Discussion), suggesting that His-mSOG mutagenesis could be applied for integration of transgenes. To test this idea, we made transgenic worms expressing the rol-6(su1006) genomic fragment, which causes a dominant roller phenotype\(^{24}\), in the background of His-mSOG transgene. After blue light illumination for 30 min, we obtained an average of one integrated line from 200 F1 animals in three independent experiments (Supplementary Fig. 2a). These transgenes were confirmed to be chromosomal integrations based on Mendelian segregation after outcrossing (~75% roller animals in the F2 generations, Supplementary Fig. 2b). The efficiency of His-mSOG-based integration is comparable to the traditional UV/TMP integration in our background.
Discussion

We have established an optogenetic approach for genome-wide mutagenesis that avoids the use of toxic chemicals such as EMS, ENU or TMP. Unlike chemical or radiation mutagenesis, only dozens of parental (P₀) animals are needed for blue light illumination using a simple and inexpensive LED setup, and the mutagenesis process is complete within an hour. Since the mutagenic effect of His-mSOG is dependent on strong light, His-mSOG strains without blue light exposure are more stably maintained than mutator strains, which induce high frequency of spontaneous mutations by transposon activation in the germline. Furthermore, His-mSOG mutagenesis can complement other methods because it induces a different spectrum of mutations, compared to those induced by traditional chemical or radiation mutagenesis. For example, EMS-induced mutations are strongly biased toward G:C to A:T transitions, comprising 87.5% of the mutations induced in our screen (Fig. 3d). Therefore, some codons are rarely induced (CCX for Pro and GGX for Gly) or mutated (TTW for Phe, ATY and ATA for Ile, TAY for Tyr, AAY for Asp, and AAR for Lys) in EMS mutagenesis. In contrast, we found these substitution mutations in His-mSOG mutagenesis: Lys150Asn and Ala451Pro in PMK-3, and Tyr215Stop in CEBP-1 (Table 2). We further note that all the optogenetically induced SNVs in the rpm-1(lf); syd-2(lf) suppressor screen affect amino acid residues different from those affected in the suppressor mutations previously found after

Table 2 | Optogenetically induced rpm-1(lf); syd-2(lf) suppressors.

| Allele | Gene* | NT change (unspliced) | NT change (spliced) | AA change |
|--------|-------|----------------------|---------------------|-----------|
| ju1202 | dlk-1 | G2552T               | G788T               | C263F     |
| ju1209 | dlk-1 | G3665T               | G1570T              | G524X     |
| ju1211 | dlk-1 | G2442T               | G678T               | W226C     |
| ju1290 | dlk-1 | G2764T               | G904T               | V302F     |
| ju1203 | dlk-1 | 2314-T C3-2315       |                     |           |
| ju1210 | dlk-1 | 44-59 deletion       |                     |           |
| ju1218 | dlk-1 | ND                   |                     |           |
| ju1220 | dlk-1 | ND                   |                     |           |
| ju1291 | dlk-1 | ND                   |                     |           |
| ju1207 | pmk-3 | C2364A               | C506A               | T169K     |
| ju1213 | pmk-3 | G2010C               | G450C               | K150N     |
| ju1215 | pmk-3 | G3508C               | G1351C              | A451P     |
| ju1292 | pmk-3 | G2520T               | G617T               | G206V     |
| ju1208 | pmk-3 | 1180-1499 deletion   |                     |           |
| ju1222 | pmk-3 | 1248 deletion        |                     |           |
| ju1212 | mkk-4 | G135T                |                     |           |
| ju1221 | mkk-4 | C736A                |                     |           |
| ju1216 | mkk-4 | C3276G               |                     |           |
| ju1219 | mkk-4 | 1177-1274 deletion   |                     |           |
| ju1214 | cebp-1| C719A                |                     |           |

*Causative genes were determined by complementation tests.
†The strains were analyzed by WGS.
§Some PCR products were not obtained when amplified from dlk-1 locus using multiple pairs of primers, suggesting that they have a deletion and/or chromosomal rearrangement.
||No SNVs were found in the entire coding sequences of dlk-1, suggesting a mutation in regulatory regions.
EMS mutagenesis. These substitution mutations provide insights into protein functions. For instance, the PMK-3 (Lys150Asn) mutation affects the conserved ATP-binding site in the kinase domain, highlighting the functional importance of this residue in vivo. ENU has a similar mutagenic efficiency to EMS with a less biased spectrum of nucleotide changes. G:C to T:A transversions, which are common in optogenetic mutagenesis, are rare in ENU as well as EMS. UV/TMP is a less potent mutagen compared to EMS and ENU, and preferentially used to induce insertion and/or deletion mutations. Thus, the unique mutational spectrum of optogenetic mutagenesis complements those of traditional mutagenesis.

Optogenetically induced SNVs have preference towards G:C to T:A or G:C to A:T transversions. It is thought that singlet oxygen mainly reacts with guanine nucleoside as an electrophile and leads to G:C to T:A changes by creating 8-oxo-7,8-dihydro-2-deoxyguanosine (8-oxodG)28. Also, oxidative stress produces oxidized cytosine, 5-hydroxy uracil, which causes G:C to A:T mutations29.

The spectrum of optogenetically induced mutations is similar to that of singlet oxygen induced mutations using chemicals in bacteria28,29 and that of spontaneous mutations in wild-type C. elegans, which are most likely caused by oxidative DNA damage18. These observations support the model that ROS produced by miniSOG is mutagenic to genomic DNA. His-mSOG also induced deletions. ROS including singlet oxygen possibly causes DNA strand breaks31,32, which can result in deletions and chromosome rearrangements. Indeed, previous studies indicate that histone tagged with the genetically encoded photosensitizer KillerRed can induce double-strand breaks (DSBs) of genomic DNA in cultured cells31–33 as assayed by terminal deoxynucleotidyl transferase (TdT)-mediated dUTP Nick-End Labeling (TUNEL) method34. There are two major pathways to repair DSBs: error-free homologous recombination (HR) and error-prone non-homologous end joining (NHEJ), which can generate deletions35. A recent study also suggests that, instead of NHEJ, polymerase theta-mediated end joining is a major driver of mutations after DSB in C. elegans germline36. Different mechanisms have been proposed to create different sizes of deletions after DSB37. Therefore, it is possible that deletions caused by His-mSOG mutagenesis ultimately arise from mutagenic DSBs. However, we cannot exclude the possibility that some mutagenic effects of His-mSOG are indirect results of disruption of histone function, as miniSOG-generated ROS can inactivate nearby proteins7. In any case, the spectrum of optogenetically induced mutations provides important biological insights into the type of mutations induced by nuclear oxidative stress.

Since miniSOG is genetically encoded, we envisage possible applications of optogenetic mutagenesis in addition to genome-wide random mutagenesis using histone. For example, miniSOG could be fused to a sequence-specific DNA binding protein to specifically mutate its unknown targets because potent ROS production from miniSOG appears to be local3. Furthermore, optogenetic mutagenesis should be applicable to other organisms such as Drosophila and zebrafish.

Methods

**C. elegans maintenance.** We maintained C. elegans strains on NGM plates at 22.5 °C (ref. 12). Except for routine propagation and crosses using a standard stereomicroscope, we took caution to keep His-mSOG transgenic worms in ambient light.

**Plasmids and strains.** pMexc-5-HIS-72;miniSOG-3’UTR(ubb-2) was cloned into pCF150 backbone for Mos1-mediated single-copy insertion (MossCD)38 using Gibson assembly39. The resulting plasmid pCZ886 contains 488 bp of 5’ region of mex-5 gene plus his-72 coding region with a SRVPAT linker inserted between his-72 and miniSOG, and 330 bp of 3’ region ofubb-2 gene. pCZ886 plasmid was injected at 50 ng/μl into EGC8080 estrT444[115605 ÷ NeoΔ[+ ÷ unk-18(+)] unc-119(ed3)] to generate a single-copy-inserted transgene jusi164[pmEx-5-HIS-72;miniSOG-3’UTR(ubb-2), Cb-unc-119(+)] III using a standard Universal MosSCI insertion method39. We detected no visible fluorescence in the germline under the epifluorescence microscope with 488-nm light, generally consistent with weak fluorescence of miniSOG-generated ROS can inactivate nearby proteins7. nny any cannot exclude the possibility that some mutagenic effects of mutagenesis ultimately arise from mutagenic DSBs. However, we proposed to create different sizes of deletions after DSBs37. In our standard protocol, we recovered 20 worms after blue light or EMS treatment that were wrapped with foil without exposure to blue light. After light treatment single P0 worms were transferred to a new plate daily to count the number of F1 progeny. For lifespan measurement, 20 light-treated worms were transferred to a new plate every two days and the number of surviving worms was plotted as a function of days after egg-laying. For the screen in the wild-type background, CZ20310 jusi164 unc-119(ed3) worms were treated with blue light and F2 worms with viable mutant phenotypes were isolated. Phenotypes were scored as heritable mutants if they were propagated to the F3 or subsequent generations. For the suppressor screen of rpm-1[0]; syd-2[0]; CZ20683 jusi164 unc-119(ed3) worms were treated with blue light or 50 mM EMS (Sigma) following a standard protocol22, respectively. We recovered 20 worms after blue light or EMS treatment and transferred individual worms on single seeded plates. We scored the number of plates carrying at least a few moving F2 worms. From the number of the plates carrying suppressors and the average brood size in each condition, the frequency of the mutations was calculated as:

\[
\text{Frequency(mutations/1000 haploid genomes)} = \frac{\text{Number of plates with suppressors}}{\text{Average brood size}} \times 2 \times 1000
\]

The average mutation rate using 50 mM EMS is known to be about one loss-of-function mutation per gene in 2,000 haploid genomes22. Since there are six major loci for the rpm-1[0]; syd-2[0] suppressor screen, the predicted frequency is 3.0 suppressor mutations/1,000 haploid genomes. Our average mutation frequency (3.09 ± 0.52/1,000 haploid genomes, n = 4 experiments, total ~ 21,000 mutagenized haploid genomes) matched the estimated value, validating our method to calculate the frequency of mutations (Fig. 2d). It should be noted that equation (1) assumes all mutants from the same P0 are siblings because the chance to get more than one mutants should be reasonably low.

**Genetic mapping.** The Dumpy mutation jui157 was linked to jusi164 (III: -0.85) and unc-119 (ed3, III: + 5.59) during outcrossing (Fig. 4a). The Uncoordinated mutation jui158 was mapped to the right arm of the chromosome I by SNV mapping using Hawaiian CB4856 strain (Fig. 4a). The molecular lesions of jui157 and jui158 were found by WGS and confirmed by Sanger sequencing. The suppressors of rpm-1[0]; syd-2[0] were subjected to complementation tests with known alleles for the identification of loci24. The molecular lesions were then determined by PCR and Sanger sequencing.

**Whole-genome sequencing.** CZ20683 was treated with and without light using the standard protocol. Two non-suppressed worms and three suppressed worms were isolated from F2 generations of independent untreated and light-treated plates, respectively. To drive existing mutations to homozygosity single hermaphrodites from these strains and the parental CZ20683 strain were propagated by self-fertilization for five generations. The number of changes described below is likely an under-estimate because some mutations may be lost in the singling process. Genomic DNA

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was prepared from three 15-cm plates each using Puregene Cell and Tissue Kit (Qagen, Valencia, CA) according to the manufacturer’s instruction. Purified genomic DNA was sequenced using the 90-bp paired-end Illumina HiSeq 2000 at Beijing Genomics Institute (BDI Americas) with 20-fold coverage for 95% of nucleotides. The obtained raw sequences were mapped to the C. elegans reference genome (Wormbase release 220) using BWA40 in the Galaxy platform41. The threshold of the ‘quality score’ in Galaxy for reliable SNVs was set to 200 as previously suggested12. GATK tools42 were used for variant calling, and then by filtering the variants in the original strains and common variants among the strains in our laboratory, we determined unique homologous SNV variants for strains with and without light exposure. Unique uncovered regions were defined as those not overlapping with uncovered regions in other strains for which we had WGS data. Unique uncovered regions of >200-bp were analyzed using Microsoft Excel and confirmed by PCR and Sanger sequencing.

Chromosomal integration. We used C22318 juIs164 unc-119(ed3); juEx6771 [rol-6(u1006)] because of its low transgene transmission rate (~20%) and used it for chromosomal integration by optogenetic mutagenesis. C22318 worms were treated with the 30-min blue light protocol at 4 Hz. Two hundred F1 roller worms were single on the seeded plates (F0 plates). The F2 worms were singled from the F1 plates with >75% roller worms. Some F1 animals showed 100% transmission of the transgene in the following generation, suggesting that the transgene may have been integrated chromosomally. To distinguish chromosomal integrations from transgene stabilization, possible integrations were outcrossed to C22835 n1532[P::pexc-7-GFP] males to examine segregation in the F2 generation.

Statistical analysis. Two-tailed t-test and one-way ANOVA with Bonferroni’s multiple comparison were used for statistical analyses of one pair and more than one pair of comparisons, respectively, using GraphPad Prism 5.0 software (GraphPad Software, La Jolla, CA).

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Author contributions

K.N. designed and performed the experiments, and co-wrote the paper. Y.J. designed the experiments and co-wrote the paper.

Additional information

Accession codes: The whole genome sequencing reads from the mutagenised C. elegans have been submitted to the NCBI Sequence Read Archive with the accession codes
Supplementary Information accompanies this paper at http://www.nature.com/naturecommunications

Competing financial interests: The authors declare no competing financial interests.

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