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DNA damage in germ cells induces immune response triggering systemic stress resistance

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Abstract

DNA damage responses have been well characterized in their cell-autonomous checkpoint functions leading to cell cycle arrest, senescence, and apoptosis ¹. In contrast, systemic responses to tissue-specific genome instability remain poorly understood. In adult *C. elegans* worms germ cells undergo mitotic and meiotic cell divisions while somatic tissues are entirely postmitotic. Consequently, DNA damage checkpoints function specifically in the germline ², whereas somatic tissues in adult *C. elegans* are highly radio-resistant ³. Some DNA repair systems such as global-genome nucleotide excision repair (GG-NER) remove lesions specifically in germ cells ⁴. Here we investigated how genome instability in germ cells affects somatic tissues in *C. elegans*. We show that exogenous and endogenous DNA damage in germ cells evokes elevated resistance to heat and oxidative stress. The somatic stress resistance is mediated by the ERK MAP kinase MPK-1 in germ cells that triggers the induction of putative secreted peptides associated with innate immunity. The innate immune response leads to activation of the ubiquitin-proteasome system (UPS) in somatic tissues, which confers enhanced proteostasis and systemic stress resistance. We propose that elevated systemic stress resistance promotes endurance of somatic tissues to allow delay of progeny production when germ cells are genomically compromised.

GG-NER defective *xpc-1* mutants and wild type (wt) animals that were treated with low doses of UVB showed extended survival upon heat and oxidative stress (Figure 1A, B, Suppl. Fig 1) that are well known to impair somatic tissue functioning causing premature death ⁵. Similarly, IR and Hydroxyurea (HU) treatment evoked heat stress resistance in adult worms (Figure 1C). ATR checkpoint defective *atl-1* and *clk-2/rad-5* mutant strains, both of

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which carry endogenous DNA damage in germ cells^{6,7}, were highly resistant to heat stress (Figure 1D,E). We next tested whether meiotic double strand breaks (DSBs), which are induced by the SPO-11 endonuclease and persist into late pachytene stages in synaptonemal complex mutants *syp-2*⁸ might trigger somatic stress resistance. Indeed, *syp-2* mutants exhibit elevated heat stress resistance that is reverted in the absence of SPO-11 (Figure 1F). In fact, *spo-11* mutant worms are more heat sensitive than wt animals, suggesting that even physiologically induced meiotic DSBs are sufficient to confer somatic stress resistance (Figure 1F). To validate the requirement of the germline for somatic stress resistance upon DNA damage, we tested *glp-1* mutants that fail to develop a germline. Indeed, germline-less *glp-1* mutants did not elevate heat stress resistance upon UV or IR treatment (Figure 1G, Suppl. Figure 2). Together, these results indicate that DNA damage in germ cells causes somatic stress resistance.

glp-1 mutants are known to display a constitutively high stress resistance through activation of the DAF-16 and DAF-12 transcription factors^{9,10}. However, *daf-16* and *daf-12;daf-16* double mutants showed UV- and IR-induced heat stress resistance indicating that germline DNA damage-mediated stress resistance is exerted independently of the established “germline ablation pathway” (Suppl. Figure 3).

We wondered whether the elevated stress resistance might support somatic endurance to influence offspring generation upon DNA damage in germ cells. While UV treatment reduced initial offspring generation, progeny production increased from day three of adulthood thus resulting in a similar number of total offspring (Figure 1H). In analogy to cellular DNA damage checkpoints that allow time for DNA repair, the somatic stress resistance might act as systemic DNA damage checkpoint that preserves somatic functions when offspring generation is delayed as a consequence of germ cell DNA damage.

To address through which DNA damage response mechanisms systemic stress resistance is mediated, we tested the DNA damage checkpoint regulators HUS-1 and ATM-1, as well as the apoptosis inducing CEP-1/p53 and executing caspase CED-3, all of which were dispensable for DNA damage induced heat stress resistance (Suppl. Figure 4). The mitogen activated protein kinases (MAPK) JNK, p38 and ERK1/2 are also activated upon DNA damage in multiple species¹¹⁻¹³. While both JNK-defective *jnk-1*, and p38-defective *pmk-1* mutants exhibited elevated heat stress resistance upon genotoxic treatment, ERK-defective *mpk-1* mutant and *mpk-1(RNAi)* worms failed to exert enhanced heat stress resistance (Figure 2A, Suppl. Figure 5A). As both *mpk-1* and *pmk-1* mutants displayed elevated baseline heat stress resistance, we used *pmk-1(RNAi)* to revert the compensatory baseline resistance of the *mpk-1* mutant strain. Yet, *mpk-1* mutants with *pmk-1(RNAi)* failed to evoke heat stress resistance upon DNA damage (Suppl. Figure 5B). MPK-1 that is activated upon IR in meiotic pachytene cells¹¹ was phosphorylated upon IR treatment in wt but not *glp-1* mutants (Figure 2B). Together, these results suggest that DNA damage-induced MPK-1 activation in germ cells instigates the systemic stress resistance.

To identify mediators of the systemic stress resistance that are induced by distinct types of DNA damage, we analysed the transcriptomes upon IR¹⁴ and UV treatment. IR in wt and UV in *xpc-1* mutant worms led to significant induction of 51 genes (Suppl. Table 1), among

which the pathogen response C-type lectin protein domain ¹⁵ was overrepresented ($p < 0.001$). Consistently, MPK-1 signalling has been shown to function in the response to pathogen infection by *Microbacterium nematophilum* ¹⁶. To assess whether the DNA damage response overlaps with the innate immune response we conducted comparative correlation analyses between the transcriptomes upon DNA damage and infection with *M. nematophilum* ¹⁷ and *Pseudomonas aeruginosa* as well as *pmk-1* inactivation ¹⁸. PMK-1 is required for inducing the innate immune response to a variety of pathogens including *P. aeruginosa* ¹⁹, and MPK-1- and PMK-1-mediated defence responses are significantly correlated indicative of a common downstream effector gene expression program (Suppl. Figure 6). Strikingly, gene expression changes upon UV and IR ($p < 0.01$) correlate with pathogen infection transcriptomes (Figure 3A), suggesting similarities between DNA damage and innate immune responses.

The nematode's innate immune response is mediated through the secretion of peptides that can be recognized by the presence of secretory signal peptides ^{18,20}. 35 of the 51 UV and IR-induced genes carry signal peptides and 33 of those are predicted to localize extracellularly (Suppl. Table 1). Among the 26 *pmk-1* and 34 *M. nematophilum* response genes that were also induced by UV and IR, 24 and 15 genes carried signal peptides with 22 and 10, respectively, associated with extracellular secretion (Suppl. Table 2). While wt worms induced the established putative secreted immune factors C17H12.8, K08D8.5, and T24B8.5 ^{18,21}, *glp-1* and *mpk-1* mutants failed to induce their expression following UV or IR treatment (Figure 3B). Moreover, the expression of the immune factors was strongly induced in germlines isolated from UV and IR treated animals (Figure 3C, Suppl. Figure 7). These data suggest that DNA damage-induced MPK-1 activation in the germline leads to the induction of putative secreted immune peptides.

To evaluate whether DNA damage leads to a functional immune response we tested survival upon *P. aeruginosa* infection. Preconditioning with IR or immunogenic -but non-pathogenic- *Bacillus subtilis* (Suppl. Figure 8) led to enhanced survival in wt worms (Figure 3D). *B. subtilis* but not IR led to elevated pathogen resistance in *mpk-1* and *glp-1* mutant animals (Figure 3D), while *pmk-1* mutants developed enhanced pathogen resistance upon IR but not *B. subtilis* preconditioning (Suppl. Figure 9). Consistent with a pathogen defence response, the intestinal *agIs219* transgenic reporter that expresses GFP under the control of the T24B8.5 promoter ²² showed marked induction starting at 4 hours after UV or IR treatment in wt but not *glp-1(RNAi)* worms (Suppl. Figure 10). We conclude that DNA damage-induced MPK-1 signalling in germ cells evokes a functional pathogen defence response.

We next determined whether the innate immune response upon pathogen infection would provoke somatic stress resistance. Strikingly, pathogenic *P. aeruginosa* preconditioning resulted in elevated heat stress resistance (Figure 3E). In contrast, *pmk-1* mutant worms failed to develop enhanced heat stress resistance in response to infection, while they were proficient for IR-induced heat stress resistance (Figure 3E). Conversely, *mpk-1* and *glp-1* mutants that failed to induce heat stress resistance upon IR treatment did exhibit heat stress resistance upon *P. aeruginosa* infection (Figure 3E, Suppl. Figure 11). To address whether the innate immune response was required for inducing somatic stress resistance upon DNA

damage we employed *atf-7(qd22)* gain-of-function mutants that constitutively repress the transcription of innate immune genes²¹ (Suppl. Figure 12A). UV and IR-induced heat stress resistance was strongly reduced in *atf-7(qd22)* mutants and reconstituted in intragenic revertant mutants *atf-7(qd22 qd130)* (Suppl. Figure 12B). Together, these results indicate that the innate immune response is necessary and sufficient to mediate heat stress resistance upon germline DNA damage.

To address the effector pathway in somatic tissues, we assessed HSF-1-mediated chaperone induction by following the expression of the heat shock protein *hsp-70* promoter fused to GFP⁵. *hsp-70::GFP* was not induced upon UV or IR treatment; and HSF-1 was largely dispensable for DNA damage-induced somatic stress resistance (Suppl. Figure 13). To assess altered protein turnover in somatic tissues we employed a *sur-5::UbV-GFP* reporter strain that expresses ubiquitin fused to GFP under the control of the *sur-5* promoter in various somatic cell types and allows monitoring of UPS activity²³. Strikingly, protein levels of UbV-GFP were strongly reduced upon exposure to IR or UV, while both non-degradable UbV^{K29/48R}-GFP or GFP alone, and mRNA levels were not altered (Figure 4A, B; Suppl. Figure 14) thus indicating enhanced ubiquitin-mediated protein turnover. We next assessed whether UPS activity was required for germline DNA damage-induced stress resistance. Indeed, depletion of the 19S cap structure components *rpn-6*, *rpn-8*, or the 20S core particle component *pas-6* resulted in failure to degrade UbV-GFP and to evoke heat stress resistance upon genotoxic insult (Figure 4C, D). The immune reporter induction was not altered by *rpn-6* knockdown, suggesting that the immune response indeed functions upstream of UPS activation (Suppl. Figure 15). In contrast to non-degradable UbV^{K29/48R}-GFP, transient immunogenic *B. subtilis* exposure strongly decreased UbV-GFP protein levels (Figure 4E). However, constitutive feeding with *B. subtilis* resulted in accumulation of UbVGFP indicative of attenuated UPS activity after long immunogenic exposure (Figure 4E). Together, these data establish that the transient activation of the innate immune response triggers UPS activation, which in turn promotes elevated systemic stress resistance.

Our data link the MAPK-mediated innate immune response to DNA damage in germ cells to systemic stress resistance through activation of the UPS (Figure 4F). Immune reactions to DNA damage also occur in higher species. In human cells the inflammasome can recognize cytosolic DNA²⁴ and genotoxic stress leads to induction of natural killer cell receptor NKG2D ligands²⁵ and cytokine secretion²⁶. In addition to causing inflammation, the mammalian innate immune system has recently been implicated in the maintenance of tissue homeostasis and regeneration^{27,28}. It will be highly interesting to determine whether innate immune responses to DNA damage in humans might systemically enhance tissue maintenance before chronic inflammation is manifested. We have uncovered systemic stress responses to DNA damage in germ cells that confer systemic protection of somatic tissues against multiple stress factors that we propose to name “germline DNA damage-induced systemic stress resistance” (GDISR).

As the recognition of DNA lesions is highly sensitive with a low threshold for triggering DNA damage responses, activation of the UPS might ameliorate protein turnover already at low damage levels before massive protein misfolding ensues in a toxic environment. In addition, the UPS might facilitate the innate immune response by alleviating pressure from

the protein folding machinery similar to the unfolded protein response²⁹. We propose that GDISR comprises an ancestral somatic stress resistance program that prolongs somatic preservation through UPS-mediated enhanced proteostasis when genomically compromised germ cells require extended somatic endurance to ensure offspring generation.

Methods

Worm strains

All strains were cultured according to standard conditions³⁰. Strains used were N2 (Bristol; wild type), FX03886 *xpc-1(tm3886)*, DR26 *daf-16(m26)*, AA67 *daf-12(rh61; rh411); daf-16(mgDf50)*, DW101 *atl-1(tm853)*, SP506 *rad-5(mn159)*, PS3551 *hsf-1(sy441)*, *atl-1(tm853); atm-1(gk186)*, WS2277 *hus-1(op241)*, XY1054 *cep-1(lg12501)*, MT1522 *ced-3(n717)*, MT8186 *mpk-1(oz140)*, VC8 *jnk-1(gk7)*, KU25 *pmk-1(km25)*, CB4037 *glp-1(e2141)*, *syp-2(ok307)/nT1 [qIs51]* (V), *spo-11(ok79) IV/nT1; syp-2(ok307) V/nT1*, AU78 *agIs219* (carrying PT24B8.5::GFP::unc-54-3'UTR), ZD442 *agIs219 atf-7(qd22) III*, ZD318 *agIs219 atf-7(qd22 qd130) III*, ZD39 *agIs219 III*; BC10060 *dpy-5(e907)*; sEx884[*hsp-70::GFP + pCeh361*], PP563 *unc-119(ed4)*; hhIs64[*unc-119(+)*; *sur-5::UbV-GFP*]III, PP545 *unc-119(ed4)*; hhIs53[*unc-119(+)*; *sur-5::K29/48R-UbV-GFP*], PP556 *unc-119(ed4)*; hhIs57[*unc-119(+)*; *sur-5::GFP*].

Bacterial strains and growth conditions

E. coli (OP50) and *Pseudomonas aeruginosa* (UCBPP-PA14) were seeded on LB agar and kept at 4°C. *Bacillus subtilis* (ATCC9372) was grown on blood agar plates and kept at the same temperature. Overnight cultures were grown at 37°C in LB media without antibiotics in all cases. For feeding worms with *P. aeruginosa*, enriched peptone plates were used as previously described³¹, in all other cases feeding was performed on standard NGM agar.

Microarray analysis

Approximately 1000 age-synchronized young adult hermaphrodites (24h post the L4 larval stage) per sample were irradiated with 0mJ/cm² or 60mJ/cm² of UV (three different sample per condition). RNA was extracted 6h post treatment using TRIZOL (Invitrogen) according to the manufacturer's protocol. RNA was purified from TRIZOL using the RNeasy Mini Kit (Qiagen). RNA quality was assessed using a Nanodrop 1000 spectrophotometer (Thermo Scientific) and a Bioanalyzer (Agilent). Expression profiles were obtained using a GeneChip *C. elegans* Genome Array (Affymetrix) according to the manufacturers specifications by the CCG facility (Berlin, Germany). Hybridization signals were normalized by quantile normalization and differentially expressed genes were determined by two-way ANOVA analysis using the Partek software package. Data are deposited at ArrayExpress under the accession number E-MTAB-1689.

Gene Ontology Classification and Secreted peptide analysis

All significant gene entries were subjected to GO classification (<http://www.geneontology.org>). Significant over-representation of GO-classified biological processes was assessed by comparing the number of pertinent genes in a given biological process to the total number of the relevant genes printed on the array for that particular

biological process (Fisher exact test) using the publicly accessible software DAVID (<http://david.abcc.ncifcrf.gov/summary.jsp>). Due to the redundant nature of GO annotations, we employed Kappa statistics to measure the degree of the common genes between two annotations, and heuristic clustering to classify the groups of similar annotations according to kappa values (<http://david.abcc.ncifcrf.gov/summary.jsp>).

Putatively secreted peptides were analyzed for the presence of signal peptides using SignalP (<http://www.cbs.dtu.dk/services/SignalP>) as described in ³² and for target compartments using WoLF PSORT (<http://wolfsort.org/>) as described in ³³.

Real-time quantitative PCR analysis

For qPCR analysis, cDNA was generated using Superscript II (Invitrogen). Quantitative real-time PCR (qPCR) was done with Biorad MyIQ real-time PCR machines using SYBR Green I (Sigma) and Platinum Taq polymerase (Invitrogen). All qPCR reactions were done in duplicate. The generation of specific PCR products was confirmed by melting curve analysis. Each primer pair was tested with a logarithmic dilution of a cDNA mix to generate a linear standard curve, crossing point (CP) plotted versus *log* of template concentration, which was used to calculate the primer pair efficiency ($E = 10^{(-1/\text{slope})}$). For data analysis, the second derivative maximum method was applied, and induction of target cDNA was calculated: $[E_{\text{target}}^{\text{CP}(\text{cDNA}_{\text{untreated}} - \text{cDNA}_{\text{treated}})_{\text{target}}} / [E_{\text{control}}^{\text{CP}(\text{cDNA}_{\text{untreated}} - \text{cDNA}_{\text{treated}})_{\text{control}}}]$.

For GFP expression, worms were analyzed as described ³⁴, for the expression of immune genes the analysis was performed as described in ²¹. Expression levels were normalized to actin (*act-1*), lamin (*lmn-1*), and g-Tubulin (*tbg-1*).

Irradiation

For UV treatment, worms were irradiated with 310-315nm UVB light using narrow band TL01 36W bulbs in a Waldmann UV 181 BL irradiation device or were mock treated. Irradiance was measured using a UVX digital radiometer and a UVX-31 probe from UVP. For IR treatment, worms were exposed to X-ray irradiation using ISOVOLT, Titan E machine from GE with a 0.5mm Aluminum filter.

Fecundity

Worms were staged and L4 larvae were irradiated and allowed to recover for 24 hours at 20°C. Worms were transferred to new OP50-seeded plates and allowed to lay eggs. Adult worms were transferred to new OP50-seeded plates every 24 hours until the end of the experiment. Viable offspring on each plate was counted 48h later.

Heat stress treatment

Worms were placed on 35 mm dishes containing NGM agar and bacteria at a density of 35 worms per plate. For each condition, 3 independent plates were used. Plates were packed into closed carton boxes to avoid dehydration and put into the Sanyo MIR-154 incubator that was running at 35°C. Experiments involving *P. aeruginosa* were performed on 65 mm dishes; two dishes per condition, 55 worms per dish. This was done due to technical limitations of the S2 facility where pathogenic materials were handled.

Worms with protruding vulva were excluded from the survival experiments, which is a standard procedure in *C. elegans* survival studies.

Statistical analysis

Error bars represent standard deviations in all cases. Statistical significance was determined by using log rank analysis for all stress resistance and pathogen resistance experiments. The survival curves for the treatment conditions do not cross each other at any time point. For figure 1H paired t-test with two-tailed distribution was performed to evaluate statistical significance. *P*-values are presented as follows: * - $p < 0.05$; *** - $p < 0.0001$. Pearson correlation analysis was performed using the Prism software package.

Information about sample size is included in all figure legends, for most experiments *n* was over 100, all cases with different sample size are mentioned specifically. Randomization was not applied because the group allocation was guided by the genotype of the respective mutant worms. Worms of a given genotype were randomly selected from large strain populations for each experiment without any preconditioning. Blinding was not applied as the experiments were carried out under highly standardized and predefined conditions such that an investigator-induced bias can be excluded.

Microscopy

GFP expressing worms were analyzed and images were taken by using LEICA M165FC microscope and LEICA Application Suite V3.3.0 software.

Western blot

Immunoblot analysis was performed according to the previously described protocol²³. Antibodies used were anti-GFP Living Colors A.v. Monoclonal Antibody (JL-8), Clontech art-no 632381; Mouse Monoclonal anti- α Tubulin (Clone DM 1A), Sigma-Aldrich art-no T6199; Phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) Antibody 9101, Cell Signalling, NEB art-no 9101S.

Western blot quantification

Was performed by using fluorescently labeled IRDye secondary antibodies, Odyssey infrared scanner and Odyssey V3.0 software

RNAi treatment

HT115 bacteria containing specific RNAi constructs were grown on LB agar plates supplemented with ampicillin and tetracycline. Plates were kept at 4°C. Overnight cultures were grown in LB media containing ampicillin. RNAi expression was induced by adding 1mM IPTG and incubating the cultures at 37°C for 20 minutes prior to seeding bacteria on NGM agar supplemented with ampicillin and 3mM IPTG.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. DNA damage in the germline leads to somatic stress resistance

(A) Worms were UVB-treated at the L4 stage and 48 hours later exposed to heat shock (HS) at 35°C (A) or 5mM paraquat (B). (C) Wt worms were treated at L4 stage with UVB (520mJ/cm²), IR (90Gy) or HU (25mM) and exposed to heat shock as in (A). (D-F) Heat shock at 35°C was applied on day two of adulthood. (G) Wt and *glp-1* mutants were treated as described in (A). (H) L4 worms were treated with UVB (520mJ/cm²) and live progeny was monitored from the time of exposure until the indicated days. Total offspring: untreated 263 (SD±12), UV-treated 256 (SD±6) viable offspring per worm. (Error bars=SD. *n* = 100 for each experimental condition (A-G); *n*=10 (H). *=*P*<0.05; ***=*P*<0.0001; log rank analysis (A-G) and two-tailed t-test (H)).

Figure 2. Stress resistance induced by germline DNA damage is mediated through MPK-1
 (A) L4 larvae were exposed to 520mJ/cm² UVB or 90Gy IR and subjected to heat stress as described in Fig.1. (B) Worms were grown at 25°C and IR treated on day 1 of adulthood. Respective phospho-ERK signal was compared to tubulin. (Error bars=SD. In A, B *n* 100 for each experimental condition; ***=*P*<0.0001, log rank test).

Figure 3. Somatic stress resistance upon DNA damage in germ cells is mediated through MPK-1 induced functional innate immune response

(A) Pearson correlation analysis of significantly induced genes upon UV treatment with transcriptomes upon IR, pathogen infections (MN= *M. nematophilum*, PA= *P. aeruginosa*, BS= *B. subtilis*), and *pmk-1* mutants. (B) Gene expression was assayed by qPCR 6h post UV and IR treatment. (C) Germlines were dissected 1-2h post UVB (520mJ/cm²) or IR (90Gy) for qPCR analysis. (D) L4 larvae were treated with 90Gy IR or placed for 10h on *B. subtilis*, and 24h later exposed to *P. aeruginosa*. (E) Young adults were treated with 90Gy IR or *P. aeruginosa* for 4h, and 24h later exposed to heat stress. (Error bars=SD. *n* 100 for each experimental condition (D, E); ***=*P*<0.0001, log rank test).

Figure 4. Innate immune responses trigger UPS activation to confer systemic stress resistance (A, B) *sur-5::UbV-GFP* reporter expression was assessed 24h post 90Gy at L4 stage. (C) Early L4 *sur-5::UbV-GFP* reporter worms and *sur-5::UbV^{K29/48R}-GFP* transgenic worms were fed for 12h with RNAi against proteasomal subunits and after treated and analyzed as in (B). OP50 bacteria served as control. (D) Worms were fed RNAi as in (C), treated with UVB (520mJ/cm²) or IR (90Gy) and exposed to heat stress as described in Fig. 1. (Error bars=SD. *n* = 100 for each experimental condition (D); ***=*P*<0.0001, log rank analysis). (E) *sur-5::UbV-GFP* and *sur-5::UbV^{K29/48R}-GFP* reporter worms were treated with 520 mJ/cm² UVB or fed with *B. subtilis* at L4 stage for indicated times and protein levels analysed on day 1 of adulthood. (F) The “germline DNA damage-induced systemic stress response” (GDISR) is triggered by MPK-1 activity in germ cells while intestinal pathogen infection activates stress resistance through PMK-1. Both MAPKs induce putative secreted immune peptides that we suggest to mediate systemic stress resistance by activation of the UPS. We propose that UPS activity in turn enhances proteostasis to elevate somatic endurance in the presence of genomically compromised germ cells or pathogen infection.