

FUdR extends the lifespan of the short-lived AP endonuclease mutant in *Caenorhabditis elegans* in a fertility-dependent manner

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The anticancer drug 5-fluorouracil (5-FU) and its metabolite 5-fluoro-2'-deoxyuridine (FUdR) inhibit thymidylate synthase and induce uracil bases in DNA. FUdR is commonly used for inhibiting fertility when measuring the lifespan of the nematode *Caenorhabditis elegans*. However, it is not known whether DNA damage induced by FUdR affects lifespan. EXO-3 is an apurinic/apyrimidinic endonuclease in *C. elegans*, and we reported previously that deletion of the *exo-3* gene causes reproductive abnormalities and decreased lifespan. In this study, we found that FUdR extended the lifespan of *exo-3* mutants. We measured the lifespan of multiple germline mutants to examine whether this lifespan extension effect was dependent on fertility. In the presence of a *fem-1* mutation, which causes a deficiency in sperm production, FUdR did not extend the lifespan of the *exo-3* mutant. In *glp-1* mutants, which do not develop gonads, the *exo-3* mutant was not short-lived, and FUdR did not extend its lifespan. These results suggest that the lifespan extension effect of FUdR depends on fertility and the presence of gonads. *fem-3* mutants, which do not produce oocytes, had increased lifespan in the presence of FUdR, independent of the *exo-3* mutation. It is possible that the *fem-3* mutant was susceptible to the lifespan extension effect of FUdR. From these results, we suggest that FUdR affects the lifespan of *C. elegans* in two ways: by interfering with fertility, which extends lifespan, and by inducing DNA base damage, which reduces lifespan.

Key words: 5-fluoro-2'-deoxyuridine (FUdR), *Caenorhabditis elegans*, *exo-3*, fertility, lifespan

INTRODUCTION

5-fluorouracil (5-FU) is an antimetabolite drug used in cancer treatment (Longley et al., 2003; Kim et al., 2009). It induces base damage by being incorporated into DNA and RNA (McNeill et al., 2009). 5-fluoro-2'-deoxyuridine (FUdR) is a metabolite of 5-FU. It binds to thymidylate synthase and strongly inhibits its activity. Thymidylate synthase is an enzyme that converts dUMP to dTMP. This is the sole synthetic pathway for thymine in the cell. Therefore, DNA replication and cell growth are strongly inhibited in the presence of 5-FU and FUdR. In addition, FUdR and 5-FU treatment increase uracil misincorporation into DNA, because inhibition of thymidylate synthase causes accumulation of dUMP.

DNA base damage such as 5-FU and uracil is mainly processed by base excision repair (BER). In this pathway, DNA glycosylases recognize damaged bases in DNA, and excise them by cleaving the N-glycosidic bond to produce an apurinic/apyrimidinic (AP) site. AP endonuclease then incises DNA at the AP site, and the resulting single-strand break (SSB) is resynthesized by DNA polymerase and DNA ligase (Dalhus et al., 2009). Because misincorporated 5-FU and uracil are converted to AP sites by DNA glycosylases, the number of AP sites is also increased by treatment with 5-FU (McNeill et al., 2009). In the nematode *Caenorhabditis elegans*, AP endonucleases and mismatch repair process 5-FU to induce checkpoint-mediated autophagy (SenGupta et al., 2013).

FUdR is widely used when measuring the lifespan of *C. elegans* to suppress worm reproduction and the growth of progeny (Mitchell et al., 1979). It is believed that FUdR does not affect lifespan or cause severe side effects in adult worms because somatic cell division does not occur

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in the adult stage (Sulston and Horvitz, 1977). FUDR significantly reduces the time and effort required to measure lifespan. However, recent studies have revealed that FUDR extends the lifespan of some mutant worms, including *tub-1* and *gas-1* mutants, but the mechanism has not been elucidated (Aitlhadj and Stürzenbaum, 2010; Van Raamsdonk and Hekimi, 2011). In addition, it was reported that inhibition of DNA synthesis by FUDR improves protein homeostasis and increases stress resistance, extending healthspan via a fertility pathway that regulates sexual development (Angeli et al., 2013). Therefore, studies on the side effects of FUDR on worm lifespan are needed.

Here, we aimed to verify the effect of FUDR on lifespan from the viewpoint of DNA damage. Because 5-FU induces DNA base damage (McNeill et al., 2009), we first hypothesized that FUDR adversely affects the lifespan of BER gene mutants. To date, FUDR has not been shown to induce DNA damage in *C. elegans*. We previously reported that deletion of the *exo-3* gene, but not the *apn-1* gene, results in decreased lifespan in the absence of FUDR

in a *ung-1*-dependent manner (Kato et al., 2015). EXO-3 and APN-1 are AP endonucleases (Shatilla et al., 2005; Schlotterer et al., 2010; Zakaria et al., 2010; Yang et al., 2012), and UNG-1 is a uracil DNA glycosylase in *C. elegans* (Nakamura et al., 2008). In this study, we measured the lifespan of the *exo-3* mutant in the presence of FUDR, and found that FUDR extended the lifespan of the mutant. To investigate the mechanism of lifespan extension by FUDR, we conducted lifespan analyses using germline mutants. We focused on *fem-1*, *glp-1* and *fem-3* as genes involved in fertility (Nelson et al., 1978; Barton et al., 1987; Priess et al., 1987). In *fem-1* mutant worms, which do not produce sperm, lifespan extension by FUDR was not observed. In *glp-1* mutant worms, which do not develop gonads, the *exo-3* mutant was not short-lived, and FUDR treatment did not result in lifespan extension. FUDR extended the lifespan of *fem-3* mutant worms (which do not produce oocytes) independent of the *exo-3* mutation. We suggest that there are both positive and negative effects of FUDR on worm lifespan.

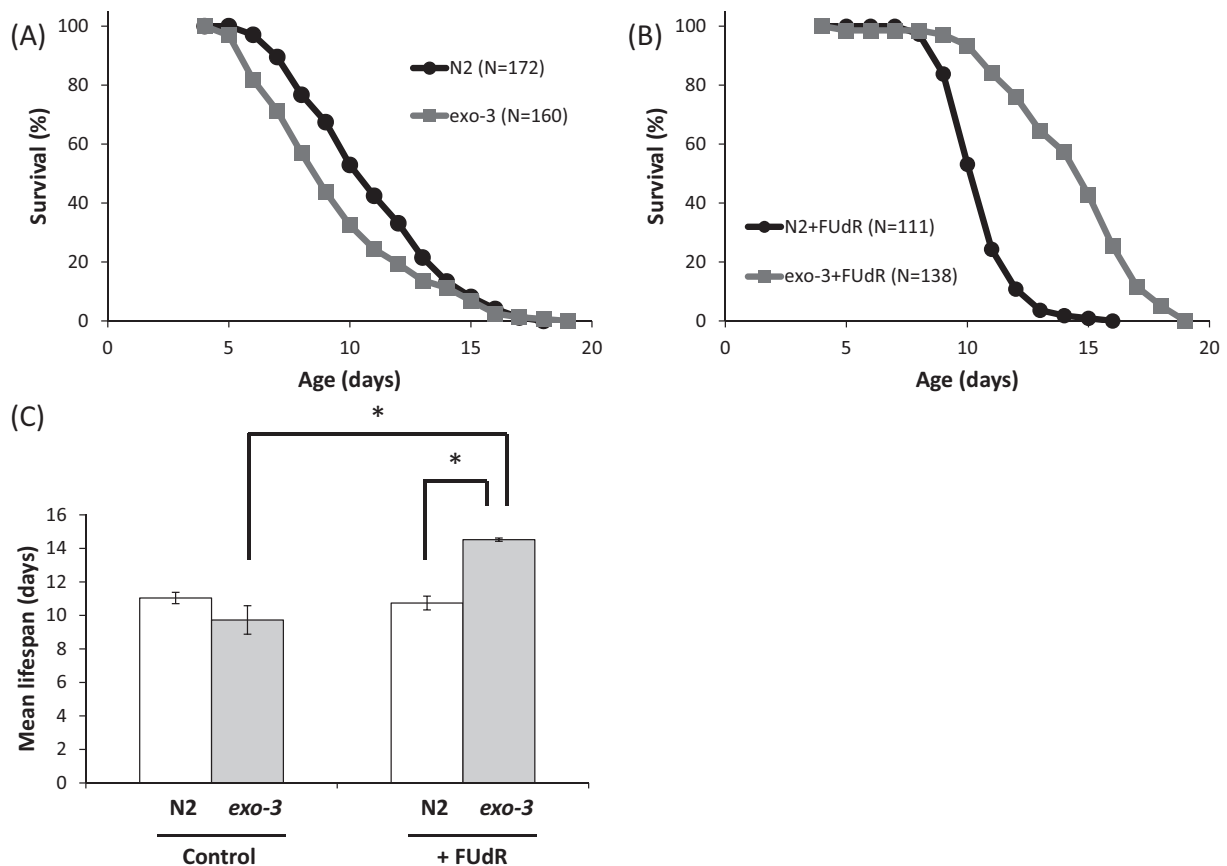


Fig. 1. Lifespan of the *exo-3* mutant in the presence of FUDR. (A and B) Survival plots of *exo-3* (*tm4374*) mutants in the absence (A) and presence (B) of 50 μ M FUDR at 25 $^{\circ}$ C. Worms were synchronized at the L1 stage and grown at 25 $^{\circ}$ C. Adult worms were placed on normal NGM plates, or NGM plates containing 50 μ M FUDR, and transferred to a fresh plate daily. (C) Mean lifespan of the *exo-3* mutant. Data shown are the mean $\pm$ SD from three independent experiments. * denotes a significant difference ($P < 0.05$) as determined by *t*-test.

MATERIALS AND METHODS

Strains and culture conditions The *C. elegans* strains used in this study were as follows: Bristol N2, BA17 [*fem-1* (*hc17*) IV] (Nelson et al., 1978), JK816 [*fem-3* (*q20*) IV] (Barton et al., 1987) and CF1903 [*glp-1* (*e2141*) III] (Priess et al., 1987) were obtained from the Caenorhabditis Genetics Center, University of Minnesota. TM4374 [*exo-3* (*tm4374*) I] (Kato et al., 2015) was kindly provided by Shohei Mitani (Tokyo Women's Medical College) of the National Bioresource Project for the Nematode. N2 and *exo-3* mutant worms were grown and maintained at 20 °C, while *fem-1*, *glp-1* and *fem-3* mutant worms were grown and maintained at 16 °C. Worms were cultured on standard NGM agar plates and fed *Escherichia coli* strain OP50 (Morinaga et al., 2009).

Lifespan measurement To synchronize the worms, embryos were prepared by the standard alkaline bleach method, and eggs were hatched overnight in M9 buffer without food. L1 worms were grown at 25 °C for 2.5

days. Adult worms were transferred to an NGM plate containing 50 μ M FUDR, and their lifespan was measured by transferring worms daily to fresh plates using a platinum pick. As it was assumed that the worms were damaged by picking, we conducted all measurements at 25 °C to finish the experiments in a short period. Worms that did not respond to touch were counted as dead (Kato et al., 2015). Mean lifespans were calculated from the results of three independent experiments, and are presented as means $\pm$ SD in figures. For statistical analysis, Student's *t*-test was carried out, and data were considered significantly different when the *P* value was < 0.05 .

RESULTS

FUDR extends the lifespan of the *exo-3* mutant worm To examine whether FUDR affected the lifespan of *C. elegans* via DNA damage induction, we measured the lifespan of *exo-3* mutant worms in the presence and absence of FUDR. We found that the lifespan of short-lived *exo-3* mutants was restored, and indeed extended,

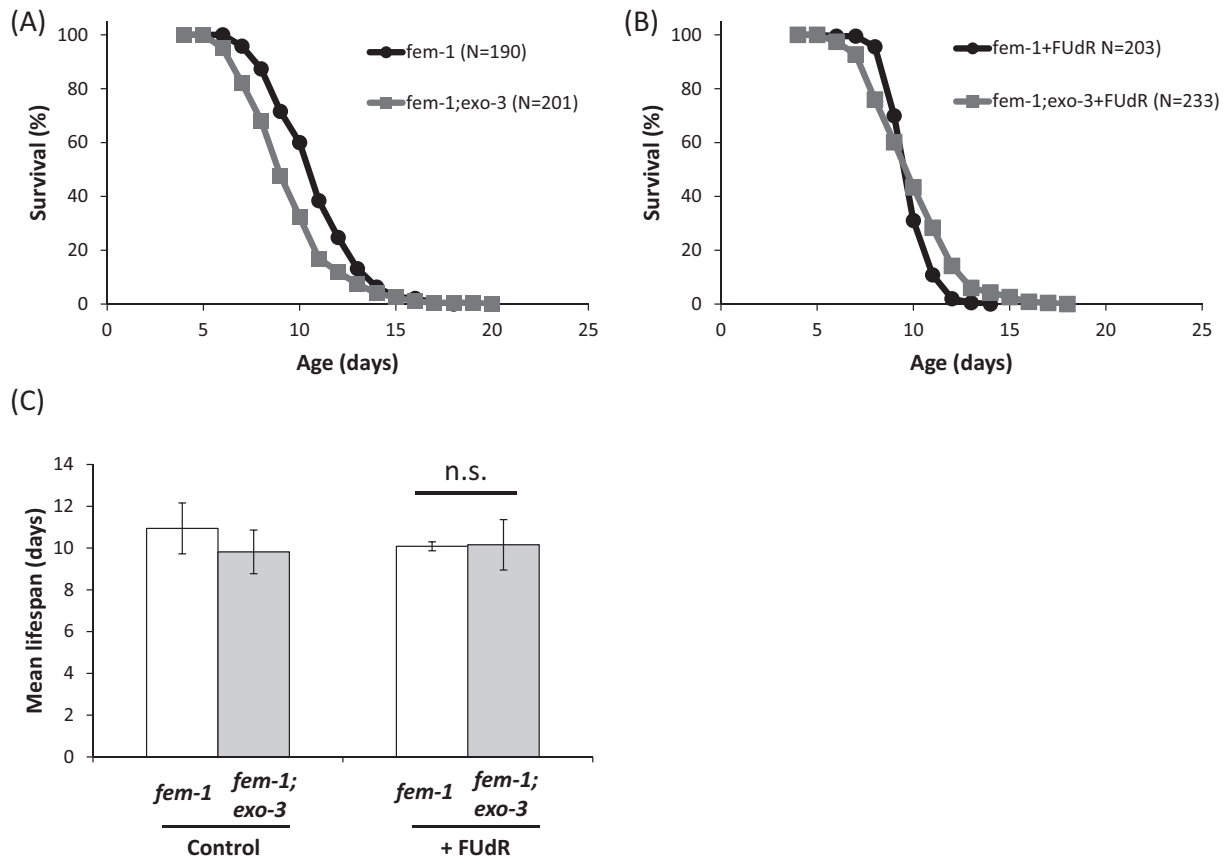


Fig. 2. Lifespan of *fem-1* mutants in the presence of FUDR. (A and B) Survival plots of *fem-1* (*hc17*) mutants in the absence (A) and presence (B) of 50 μ M FUDR at 25 °C. When grown at 25 °C, *fem-1* (*hc17*) mutants do not produce sperm. To measure lifespan, worms were synchronized at the L1 stage and grown at 25 °C. Adult worms were placed on normal NGM plates, or NGM plates containing 50 μ M FUDR, and transferred to a fresh plate every day. (C) Mean lifespan of the *fem-1* mutants. Data shown are the mean $\pm$ SD from three independent experiments. n.s., not significant.

by FUDr (Fig. 1, A and B). FUDr increased the mean lifespan of *exo-3* mutants by 50% (in the absence and presence of FUDr, the mean lifespan of *exo-3* mutants was 9.73 days and 14.52 days, respectively, and the *P* value from a *t*-test was < 0.05) (Fig. 1C). Furthermore, *exo-3* mutants had a significantly longer lifespan than N2 worms on FUDr-containing plates (mean lifespan was 14.52 days and 10.74 days, respectively, and the *P* value from the *t*-test was < 0.05).

FUDr extends the lifespan of the *exo-3* mutant in a sperm- and gonad-dependent manner Worms homozygous for mutations in *exo-3* show abnormalities in reproduction (Kato et al., 2015), and we assumed that FUDr extended the lifespan of *exo-3* mutant worms by interfering with fertility. To examine this hypothesis, we measured the lifespan of multiple germline mutants. *fem-1* (*hc17*) mutant worms do not produce sperm at 25 °C (Nelson et al., 1978). In the absence of FUDr, *fem-1;exo-3* double mutants had a decreased lifespan compared with *fem-1* mutants (Fig. 2A). The mean lifespan

of the *fem-1* and *fem-1;exo-3* mutants was 10.94 and 9.82 days, respectively (Fig. 2C). However, in the presence of FUDr, *fem-1;exo-3* mutants had a similar lifespan to *fem-1* mutants (Fig. 2B). In the presence of FUDr, the mean lifespans of *fem-1* and *fem-1;exo-3* mutants were 10.09 and 10.16 days, respectively, and the *P* value from the *t*-test was > 0.05 (Fig. 2C). Worms homozygous for the *glp-1* (*e2141*) allele do not develop gonads at 25 °C (Priess et al., 1987). *glp-1;exo-3* double mutant worms had a normal lifespan similar to *glp-1* mutants in the absence of FUDr (Fig. 3A). The mean lifespans of *glp-1* and *glp-1;exo-3* worms were 12.52 and 12.15 days, respectively, and the *P* value from the *t*-test was > 0.05 (Fig. 3C). In addition, FUDr did not extend the lifespan of *glp-1;exo-3* mutants (Fig. 3B). In the presence of FUDr, the mean lifespan of *glp-1* and *glp-1;exo-3* mutants was 12.70 and 13.57 days, respectively, and the *P* value from the *t*-test was > 0.05 (Fig. 3C).

Inhibition of oocyte production results in longevity in the presence of FUDr To further investigate the

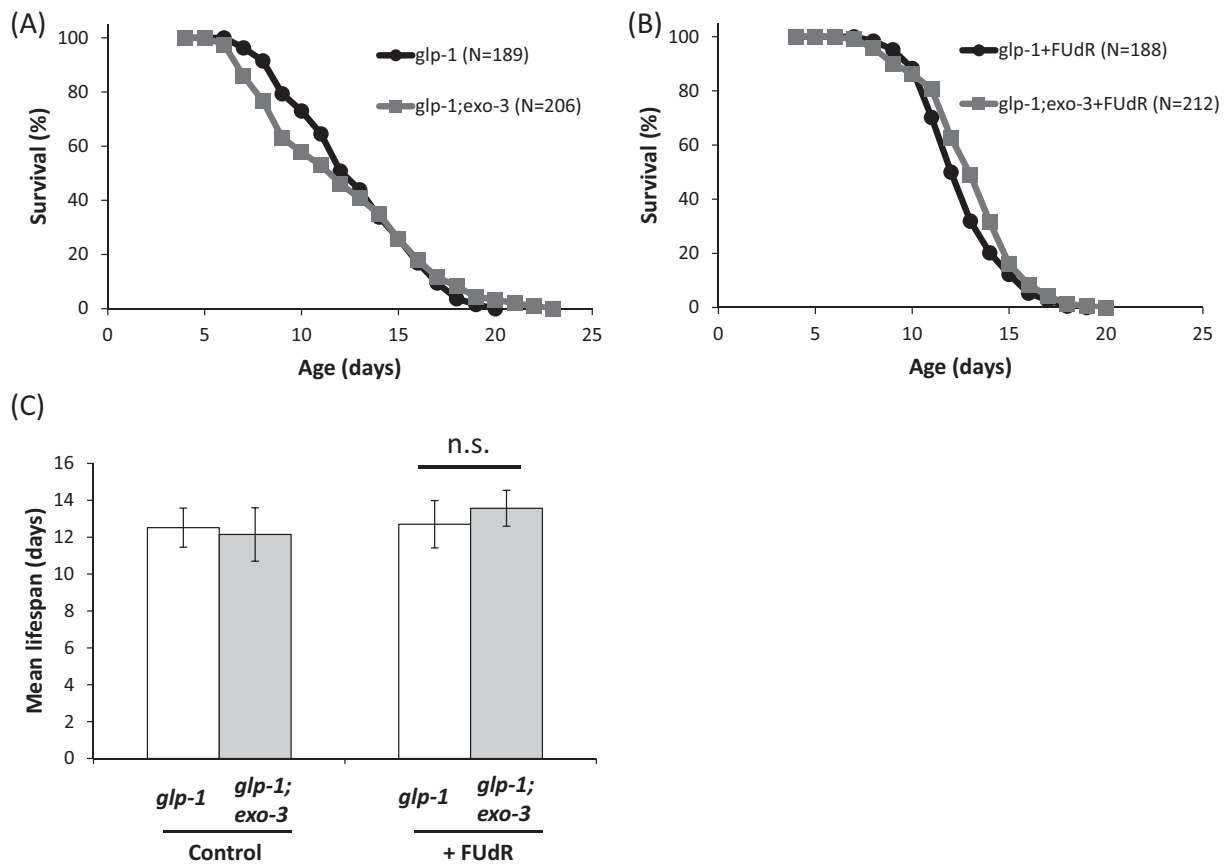


Fig. 3. Lifespan of *glp-1* mutants in the presence of FUDr. (A and B) Survival plots of *glp-1* (*e2141*) mutants in the absence (A) and presence (B) of 50 μ M FUDr at 25 °C. When grown at 25 °C, *glp-1* (*e2141*) mutants do not develop gonads. To measure lifespan, worms were synchronized at the L1 stage and grown at 25 °C. Adult worms were placed on normal NGM plates, or NGM plates containing 50 μ M FUDr, and transferred to a fresh plate every day. (C) Mean lifespan of *glp-1* mutants. Data shown are the mean $\pm$ SD from three independent experiments. n.s., not significant.

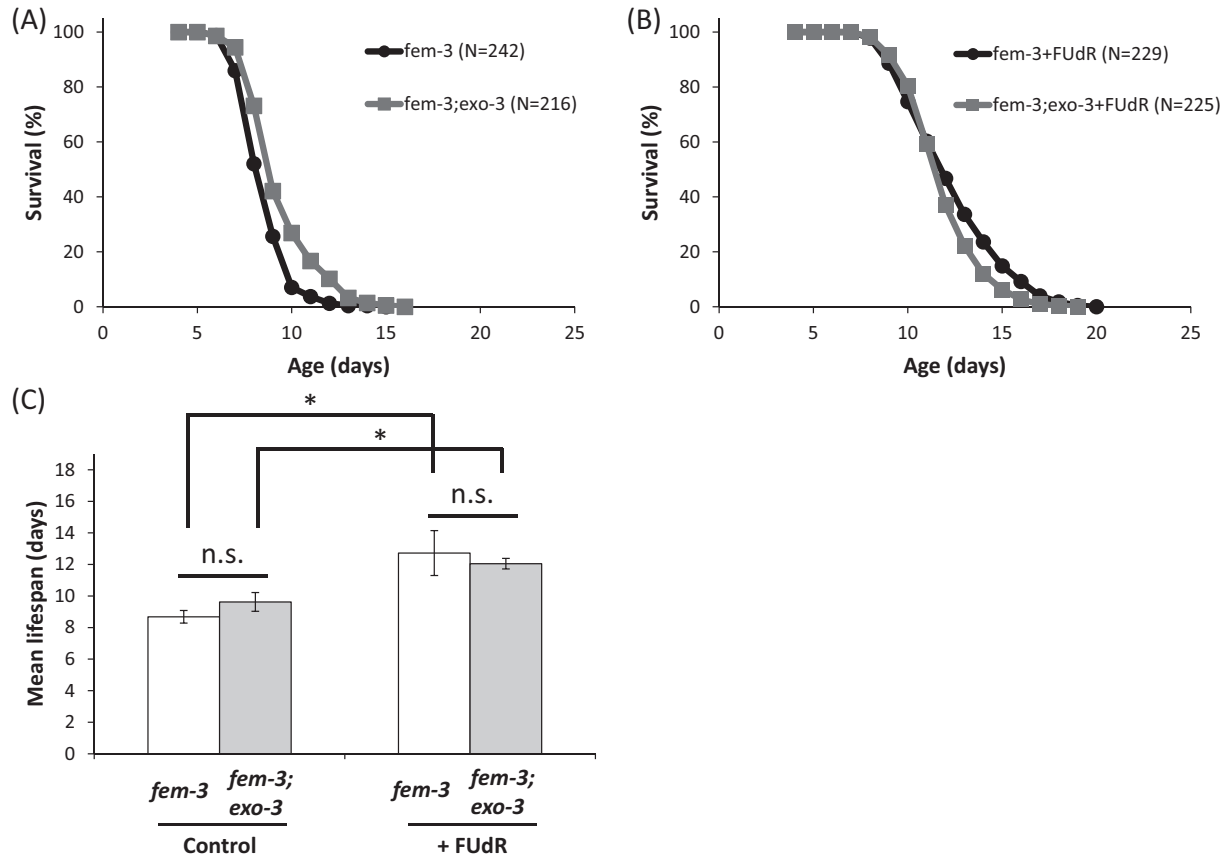


Fig. 4. Lifespan of *fem-3* mutants in the presence of FUDR. (A and B) Survival plots of *fem-3* (*q20*) mutants in the absence (A) and presence (B) of 50 μ M FUDR at 25 $^{\circ}$ C. The *fem-3* mutant does not produce oocytes when grown at 25 $^{\circ}$ C. To measure lifespan, worms were synchronized at the L1 stage and grown at 25 $^{\circ}$ C. Adult worms were placed on normal NGM plates, or NGM plates containing 50 μ M FUDR, and transferred to a fresh plate every day. (C) Mean lifespan of the *fem-3* mutants. Data shown are the mean $\pm$ SD from three independent experiments. * denotes a significant difference ($P < 0.05$) as determined by *t*-test; n.s., not significant.

relationship between worm fertility and lifespan extension by FUDR, we measured the lifespan of *fem-3* (*q20*) mutant worms, which do not produce oocytes at 25 $^{\circ}$ C (Barton et al., 1987). In the absence of FUDR, *fem-3;exo-3* mutants had a normal lifespan similar to *fem-3* mutants (Fig. 4A). Mean lifespans of the *fem-3* and *fem-3;exo-3* mutants were 8.68 and 9.62 days, respectively, and the *P* value from the *t*-test was > 0.05 (Fig. 4C). Also, in the presence of FUDR, *fem-3;exo-3* mutants had a similar lifespan to *fem-3* mutants (Fig. 4B). On FUDR-containing plates, the mean lifespans of *fem-3* and *fem-3;exo-3* mutants were 12.72 and 12.05 days, respectively, and the *P* value from the *t*-test was > 0.05 (Fig. 4C). Importantly, FUDR extended the lifespan of *fem-3* mutants independently of mutations in *exo-3* (*P* values from *t*-tests of *fem-3* vs. *fem-3* with FUDR and of *fem-3;exo-3* vs. *fem-3;exo-3* with FUDR were both < 0.05).

DISCUSSION

FUDR as a DNA damage inducer in *C. elegans*
Whether FUDR induces DNA base damage in *C. elegans*

is not known, and further investigation is needed to reveal if FUDR affects worm lifespan via induction of DNA base damage. Contrary to our initial expectation, FUDR extended the lifespan of the *exo-3* mutant (Fig. 1B). This result suggests that FUDR affects worm lifespan via the BER pathway. Our result conflicts with a previous report that 5-FU induces base damage (McNeill et al., 2009). We measured the level of AP sites in *C. elegans* by an aldehyde reactive probe assay, but found no significant increase in AP sites caused by FUDR treatment (data not shown). Inhibition of DNA synthesis and induction of base damage are conflicting effects of FUDR. It is possible that in the presence of a higher concentration of FUDR (for example, 50 μ M in this study), DNA synthesis is strongly inhibited so that misincorporation of 5-FU and uracil rarely occurs. Lower concentrations of FUDR might induce base damage in *C. elegans*. It has been reported that lower concentrations of 5-FU ($< 5 \mu$ M) induce AP endonuclease- and mismatch repair-mediated autophagy in *C. elegans* (SenGupta et al., 2013). Another possible reason why we did not detect any difference in the number of AP sites following FUDR

treatment is that FUDR-mediated DNA damage is dependent on DNA replication. In adult worms, only germline cells are dividing (Sulston and Horvitz, 1977). Since an involvement of *fem-1*, *glp-1* and *fem-3* in base damage has not been reported, we speculate that the sensitivities of these mutants to FUDR-induced base damage are nearly identical.

Effect of reproductive inhibition on lifespan In *C. elegans*, it is recognized that lifespan is related to reproduction. For example, killing the germline precursor cells Z2 and Z3 in larvae increases longevity (Arantes-Oliveira et al., 2002). The *glp-1* mutant, in which the formation of gonads is prevented, also lives longer (Berman and Kenyon, 2006). We previously reported that deletion of the *exo-3* gene causes abnormality in reproduction, including decreased self-brood size (Kato et al., 2015). In this study, we found that the *exo-3* mutant has a short lifespan dependent on production of oocytes and gonads. When worms lacked oocytes (*fem-3;exo-3*) or gonads (*glp-1;exo-3*), *exo-3* mutants were not short-lived (Figs. 3A and 4A). These results suggest that deletion of the *exo-3* gene caused decreased lifespan via reproduction (especially production of oocytes). Lifespan extension by FUDR was not observed when worms were deficient in sperm or gonads

(Figs. 2B and 3B). This suggests that lifespan extension by FUDR was related to worm reproduction and fertility. Because FUDR extended the lifespan of oocyte-less *fem-3* mutants independently of mutations in *exo-3* (Fig. 4B), it is possible that *fem-3* mutants were susceptible to the lifespan extension effect of FUDR. FUDR is very useful when measuring worm lifespan. However, it may not be suitable for measuring the lifespan of mutants that show abnormal reproduction.

FUDR affects lifespan in two ways In this study, we suggest that deletion of the *exo-3* gene caused a short lifespan via the fertility pathway, and that lifespan is restored by inhibiting oocyte production or gonad development. However, in the presence of FUDR, the lifespan of *exo-3* mutants was increased, not just restored, compared with N2 worms (Fig. 1B). This phenomenon cannot be explained by the hypothesis that FUDR extends the lifespan of *exo-3* mutants by interfering with fertility. Taking DNA base damage induction by FUDR into account might explain this phenomenon. We hypothesize that FUDR affects lifespan in two opposing ways (Fig. 5). By interfering with fertility in a specific manner, FUDR extends worm lifespan. However, FUDR also reduces worm lifespan by inducing DNA base damage, resulting in AP sites and SSBs. In N2 worms, it seems that FUDR does not affect lifespan because these two effects are almost equal and counteract each other. In *exo-3* mutant worms, AP sites are not efficiently converted into SSBs. SSBs are more harmful than DNA base damage and AP sites in the short term, and therefore deletion of the *exo-3* gene is advantageous for survival in the presence of FUDR. FUDR extends the lifespan of *exo-3* mutants because *exo-3* mutants were barely affected by the negative aspects of FUDR treatment compared with the N2 worms. FUDR may only influence specific aspects of nematode fertility. In worms lacking *fem-1* function, these aspects may still be present, which explains the slight decrease in lifespan observed in *fem-1* mutant worms treated with FUDR (Fig. 2C). The *glp-1* mutant tested does not possess gonads; therefore, lifespans were affected by neither the *exo-3* mutation nor FUDR (Fig. 3C). It is possible that the *fem-3* mutant was susceptible to the lifespan extension effect of FUDR because the mutant was long-lived independent of the *exo-3* mutation (Fig. 4C).

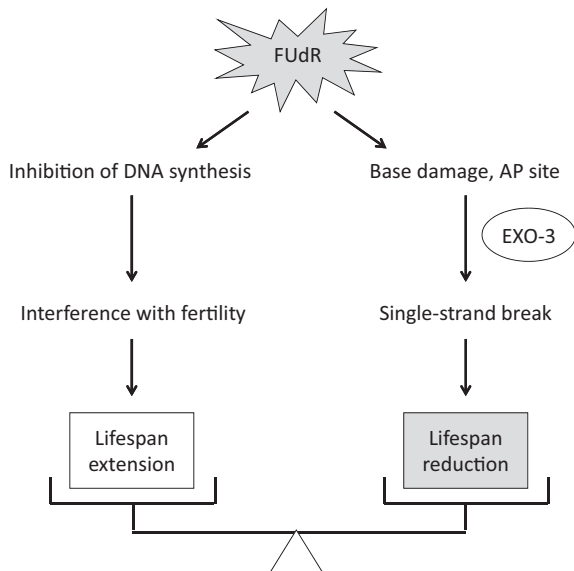


Fig. 5. Model of lifespan extension/reduction by FUDR. FUDR inhibits DNA replication by binding to thymidylate synthase. This interferes with fertility, and ultimately results in lifespan extension. FUDR also induces base damage including 5-FU and uracil. These are converted to AP sites by DNA glycosylase, and the AP sites are then processed by EXO-3. As SSBs are more harmful than base damage and AP sites, this pathway causes lifespan reduction in an *exo-3*-dependent manner. Thus, FUDR affects lifespan of *C. elegans* in two different ways, and lifespan is determined by the proportion of these two effects in the presence of FUDR.

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REFERENCES

- Aitlhadj, L., and Stürzenbaum, S. R. (2010) The use of FUDR can cause prolonged longevity in mutant nematodes. *Mech. Ageing Dev.* **131**, 364–365.
- Angeli, S., Klang, I., Sivapatham, R., Mark, K., Zucker, D., Bhaumik, D., Lithgow, G. J., and Andersen, J. K. (2013) A DNA synthesis inhibitor is protective against proteotoxic stressors via modulation of fertility pathways in *Caenorhabditis elegans*. *Aging (Albany NY)* **5**, 759–769.
- Arantes-Oliveira, N., Apfeld, J., Dillin, A., and Kenyon, C. (2002) Regulation of life-span by germ-line stem cells in *Caenorhabditis elegans*. *Science* **295**, 502–505.
- Barton, M. K., Schedl, T. B., and Kimble, J. (1987) Gain-of-function mutations of *fem-3*, a sex-determination gene in *Caenorhabditis elegans*. *Genetics* **115**, 107–119.
- Berman, J. R., and Kenyon, C. (2006) Germ-cell loss extends *C. elegans* life span through regulation of DAF-16 by *kri-1* and lipophilic-hormone signaling. *Cell* **124**, 1055–1068.
- Dalhus, B., Laerdahl, J. K., Backe, P. H., and Bjørås, M. (2009) DNA base repair—recognition and initiation of catalysis. *FEMS Microbiol. Rev.* **33**, 1044–1078.
- Kato, Y., Moriwaki, T., Funakoshi, M., and Zhang-Akiyama, Q. M. (2015) *Caenorhabditis elegans* EXO-3 contributes to longevity and reproduction: Differential roles in somatic cells and germ cells. *Mutat. Res.* **772**, 46–54.
- Kim, S., Park, D. H., Kim, T. H., Hwang, M., and Shim, J. (2009) Functional analysis of pyrimidine biosynthesis enzymes using the anticancer drug 5-fluorouracil in *Caenorhabditis elegans*. *FEBS J.* **276**, 4715–4726.
- Longley, D. B., Harkin, D. P., and Johnston, P. G. (2003) 5-fluorouracil: mechanisms of action and clinical strategies. *Nat. Rev. Cancer* **3**, 330–338.
- McNeill, D. R., Lam, W., DeWeese, T. L., Cheng, Y. C., and Wilson, D. M. (2009) Impairment of APE1 function enhances cellular sensitivity to clinically relevant alkylators and antimetabolites. *Mol. Cancer Res.* **7**, 897–906.
- Mitchell, D. H., Stiles, J. W., Santelli, J., and Sanadi, D. R. (1979) Synchronous growth and aging of *Caenorhabditis elegans* in the presence of fluorodeoxyuridine. *J. Gerontol.* **34**, 28–36.
- Morinaga, H., Yonekura, S., Nakamura, N., Sugiyama, H., Yonei, S., and Zhang-Akiyama, Q. M. (2009) Purification and characterization of *Caenorhabditis elegans* NTH, a homolog of human endonuclease III: essential role of N-terminal region. *DNA Repair (Amst)* **8**, 844–851.
- Nakamura, N., Morinaga, H., Kikuchi, M., Yonekura, S., Ishii, N., Yamamoto, K., Yonei, S., and Zhang, Q. M. (2008) Cloning and characterization of uracil-DNA glycosylase and the biological consequences of the loss of its function in the nematode *Caenorhabditis elegans*. *Mutagenesis* **23**, 407–413.
- Nelson, G. A., Lew, K. K., and Ward, S. (1978) Intersex, a temperature-sensitive mutant of the nematode *Caenorhabditis elegans*. *Dev. Biol.* **66**, 386–409.
- Priess, J. R., Schnabel, H., and Schnabel, R. (1987) The *glp-1* locus and cellular interactions in early *C. elegans* embryos. *Cell* **51**, 601–611.
- Schlotterer, A., Hamann, A., Kukudov, G., Ibrahim, Y., Heckmann, B., Bozorgmehr, F., Pfeiffer, M., Hutter, H., Stern, D., Du, X., et al. (2010) Apurinic/apyrimidinic endonuclease 1, p53, and thioredoxin are linked in control of aging in *C. elegans*. *Aging Cell* **9**, 420–432.
- SenGupta, T., Torgersen, M. L., Kassahun, H., Vellai, T., Simonsen, A., and Nilsen, H. (2013) Base excision repair AP endonucleases and mismatch repair act together to induce checkpoint-mediated autophagy. *Nat. Commun.* **4**, 2674.
- Shatilla, A., Ishchenko, A. A., Saparbaev, M., and Ramotar, D. (2005) Characterization of *Caenorhabditis elegans* exonuclease-3 and evidence that a Mg²⁺-dependent variant exhibits a distinct mode of action on damaged DNA. *Biochemistry* **44**, 12835–12848.
- Sulston, J. E., and Horvitz, H. R. (1977) Post-embryonic cell lineages of the nematode, *Caenorhabditis elegans*. *Dev. Biol.* **56**, 110–156.
- Van Raamsdonk, J. M., and Hekimi, S. (2011) FUDR causes a twofold increase in the lifespan of the mitochondrial mutant *gas-1*. *Mech. Ageing Dev.* **132**, 519–521.
- Yang, X., Fan, J., Ishchenko, A. A., Patel, D., Saparbaev, M. K., and Ramotar, D. (2012) Functional characterization of the *Caenorhabditis elegans* DNA repair enzyme APN-1. *DNA Repair (Amst)* **11**, 811–822.
- Zakaria, C., Kassahun, H., Yang, X., Labbé, J. C., Nilsen, H., and Ramotar, D. (2010) *Caenorhabditis elegans* APN-1 plays a vital role in maintaining genome stability. *DNA Repair (Amst)* **9**, 169–176.