

# Abrogation of the CLK-2 checkpoint leads to tolerance to base-excision repair intermediates

Marlene Dengg<sup>1\*</sup>, Tatiana Garcia-Muse<sup>2\*</sup>, Stephen G. Gill<sup>3</sup>, Neville Ashcroft<sup>3</sup>, Simon J. Boulton<sup>2+</sup>  
& Hilde Nilsen<sup>1++</sup>

<sup>1</sup>The Biotechnology Centre, University of Oslo, Oslo, Norway, <sup>2</sup>Cancer Research UK, London Research Institute, Clare Hall Laboratories, Hertfordshire, UK, and <sup>3</sup>Genome Damage and Stability Centre, University of Sussex, Falmer, Brighton, UK

**Incorporation of uracil during DNA synthesis is among the most common types of endogenously generated DNA damage. Depletion of *Caenorhabditis elegans* dUTPase by RNA interference allowed us to study the role of DNA damage response (DDR) pathways when responding to high levels of uracil in DNA. dUTPase depletion compromised development, caused embryonic lethality and led to activation of cell-cycle arrest and apoptosis. These phenotypes manifested as a result of processing misincorporated uracil by the uracil-DNA glycosylase UNG-1. Strikingly, abrogation of the *clk-2* checkpoint gene rescued lethality and developmental defects, and eliminated cell-cycle arrest and apoptosis after dUTPase depletion. These data show a genetic interaction between UNG-1 and activation of the CLK-2 DDR pathway after uracil incorporation into DNA. Our results indicate that persistent repair intermediates and/or single-stranded DNA formed during repair of misincorporated uracil are tolerated in the absence of the CLK-2 checkpoint in *C. elegans*.**

Keywords: base-excision repair; checkpoints; CLK-2; UNG; uracil  
EMBO reports (2006) 7, 1046–1051. doi:10.1038/sj.embor.7400782

## INTRODUCTION

Cellular responses to DNA damage include the activation of DNA repair and a highly conserved signal-transduction cascade, called the DNA damage response (DDR), which leads to cell-cycle arrest or apoptosis. Activation of the DDR by endogenously generated DNA base damage has not been studied extensively, partly

because methods to introduce such lesions above steady-state levels have not been readily available. Incorporation of uracil into DNA due to the use of dUMP instead of dTMP by DNA polymerases is the most common type of endogenous DNA damage (Guillet & Boiteux, 2003). Uracil misincorporation is counteracted by dUTP nucleotidohydrolase (dUTPase), which maintains a low intracellular pool of dUTP (Fig 1; Tye & Lehman, 1977). Hence, uracil incorporation into DNA can be promoted by depleting dUTPase (*dut-1*). Using RNA interference (RNAi) in *Caenorhabditis elegans* to deplete dUTPase allowed us to circumvent the lethality observed in *dut-1* loss-of-function mutants and to expand on previous studies in single-celled *dut-1* mutants. Thus, we could assess the activation of DDR pathways in response to misincorporation of uracil in DNA in an animal model.

*C. elegans* is the simplest animal that allows the study of the DDR (Stergiou & Hengartner, 2004). Genotoxic insult or replicative stress leads to two spatially distinct checkpoint responses in the *C. elegans* germ line. Cell-cycle arrest can be assessed by the presence of fewer but enlarged mitotic nuclei, whereas DNA-damage-induced apoptosis is restricted to the pachytene region of the meiotic compartment, in which dying cells can be monitored by their distinct morphological appearance. Both responses require DNA-damage-induced signalling through conserved pathways (Stergiou & Hengartner, 2004). In response to ionizing radiation (IR)- and hydroxyurea (HU)-induced replication stress, recruitment of replication factor A (RPA-1) and ATL-1 (*C. elegans* ATR) to chromatin is responsible for activation of the DDR checkpoint (Garcia-Muse & Boulton, 2005). The proliferating cell nuclear antigen-like complex (9-1-1 complex), consisting of a heterotrimer of the HPR-9/HUS-1/MRT-2 proteins (Hofmann *et al*, 2002), is required for IR-induced checkpoint responses but is dispensable for the checkpoint that follows replication stress. Checkpoint responses to IR- and HU-induced replication stress also require CLK-2 (Ahmed *et al*, 2001), but the precise molecular function of this protein is unknown.

Here, we identify a novel function for the CLK-2 checkpoint protein in the activation of cell-cycle arrest and apoptosis in response to *dut-1*(RNAi). Furthermore, we show a genetic interaction between the base-excision repair (BER) enzyme

<sup>1</sup>The Biotechnology Centre, University of Oslo, PO Box 1125 Blindern, Gaustadalleen 21, 0317 Oslo, Norway

<sup>2</sup>Cancer Research UK, London Research Institute, Clare Hall Laboratories, South Mimms, Hertfordshire EN6 3LD, UK

<sup>3</sup>Genome Damage and Stability Centre, University of Sussex, Falmer, Brighton BN1 9RR, UK

\*These authors contributed equally to this work

+Corresponding author. Tel: +44 1707 625774; Fax: +44 208 2693801; E-mail: simon.boulton@cancer.org.uk

++Corresponding author. Tel: +47 22840511; Fax: +47 22840555; E-mail: hilde.nilsen@biotek.uio.no

UNG-1 and activation of the DDR signalling pathway through CLK-2 after uracil excision from DNA.

## RESULTS AND DISCUSSION

### *dut-1(RNAi)* is lethal in *Caenorhabditis elegans*

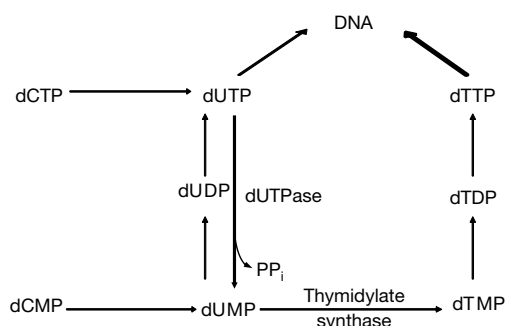
To increase uracil incorporation into DNA, dUTPase was depleted by RNAi feeding (*dut-1(RNAi)*). Larval stage 4 (L4) N2 wild-type worms subjected to *dut-1(RNAi)* resulted in embryonic lethality in the offspring (72% dead embryos of 1,147 inspected), indicating that dUTPase is essential for viability (Table 1). The progeny that hatched (28%) remained thin and elongated, consistent with developmental arrest, and only 0.8% grew to an L4-like size. A total of 26% of the offspring showed a severe protruding vulva (Pvl) phenotype (supplementary Fig 1 online). Reduction of the *dut-1(RNAi)* dose by lowering the IPTG (isopropyl-beta-D-thiogalactopyranoside) concentration gave fewer animals with a Pvl phenotype. However, the vulval structures still remained dysfunctional, resulting in egg-laying-defective (Egl) animals (supplementary Fig 1 online). Partial rescue of lethality and emergence of Egl rather than Pvl phenotypes with thymidine-supplemented growth medium (data not shown) strongly suggests that the observed defects are related directly to dUTPase depletion. Thus, *dut-1(RNAi)* interfered with germline and vulva development, the two proliferating organs of the adult worm.

### Cell-cycle arrest and apoptosis after *dut-1(RNAi)*

The incomplete penetrance of *dut-1(RNAi)* allows the observation of post-embryonic phenotypes resulting from dUTPase depletion. Detailed analysis of 4,6-diamidino-2-phenylindole (DAPI)-stained, dissected germ lines of *dut-1(RNAi)*-fed animals showed the presence of enlarged mitotic nuclei (Fig 2A). Also, the number of mitotic nuclei was significantly reduced from about 65 nuclei in worms fed the empty vector control RNAi to fewer than 40 fed on *dut-1(RNAi)* (Fig 2A). This reduction, combined with the increased nuclear size, is consistent with checkpoint activation and induction of cell-cycle arrest (Stergiou & Hengartner, 2004). Scoring of apoptotic corpses showed 1–2 apoptotic corpses per gonad arm in the control RNAi-fed animals, whereas 6–12 corpses were seen in *dut-1(RNAi)*-fed animals, consistent with activation of apoptosis. We conclude that the DDR checkpoint is activated in the *C. elegans* germ line in response to dUTPase depletion.

### *dut-1(RNAi)* lethality is suppressed by *ung-1(RNAi)*

The BER pathway is the principal repair pathway for endogenous DNA damage (Barnes & Lindahl, 2004). The lethality resulting from dUTPase depletion might therefore be caused by excision of uracil by UNG-1, the only uracil-DNA glycosylase encoded in the *C. elegans* genome. Indeed, co-depletion of *dut-1* and *ung-1* in the RNAi-sensitive strain *rrf-3* partly rescued germline development,



**Fig 1** | The *de novo* pathway for thymidine biosynthesis. A simplified scheme is shown. Use of dUTP for DNA synthesis in place of dTTP can be promoted by depletion of dUTPase.

allowing formation of oocytes and sperm in 3% of the animals inspected (Table 2). Rescue was never observed when *dut-1(RNAi)* was fed together with the empty vector control, although a fraction of the offspring (0.8%) grew to an adult size, later arresting with a Pvl phenotype (Table 2). The rescue of *dut-1(RNAi)* lethality by *ung-1(RNAi)* strongly suggests that the phenotype observed is a direct consequence of BER-dependent processing of misincorporated uracil.

### Screen for suppressors of *dut-1(RNAi)*

To identify genes required for cell-cycle arrest and apoptosis in response to *dut-1(RNAi)*, we co-depleted genes known to be involved in DDR pathways in *C. elegans*. Surprisingly, co-depletion of dUTPase with either *clk-2* or *chk-1* checkpoint genes resulted in 7% and 5% of the offspring, respectively, developing into adults (Table 2). Although the *clk-2(RNAi);dut-1(RNAi)* offspring could be studied further, the *chk-1(RNAi);dut-1(RNAi)* offspring were all sterile, preventing further analyses of germline phenotypes as with the phenotype observed for *chk-1(RNAi)* alone (Kalogeropoulos *et al*, 2004). Co-depletion of *hpr-17*, the *C. elegans* homologue of the human RAD17 (RFC1-like alternative clamp loader), or *hus-1*, failed to rescue the lethality. This indicates that signalling through the 9-1-1 complex is dispensable for the *dut-1(RNAi)* phenotypes (supplementary Table 1 online). This was supported by the lack of rescue in the *mrt-2(e2663)* mutant (data not shown). Moreover, a loss-of-function mutation in CEP-1 (*cep-1(gk138)*), the *C. elegans* homologue of p53, did not rescue *dut-1(RNAi)* lethality (data not shown), consistent with the fact that CEP-1 is dispensable for checkpoint-dependent cell-cycle arrest (Schumacher *et al*, 2001).

Although ATL-1 and CLK-2 are both required for the S-phase checkpoint (Garcia-Muse & Boulton, 2005), co-depletion of *atl-1*

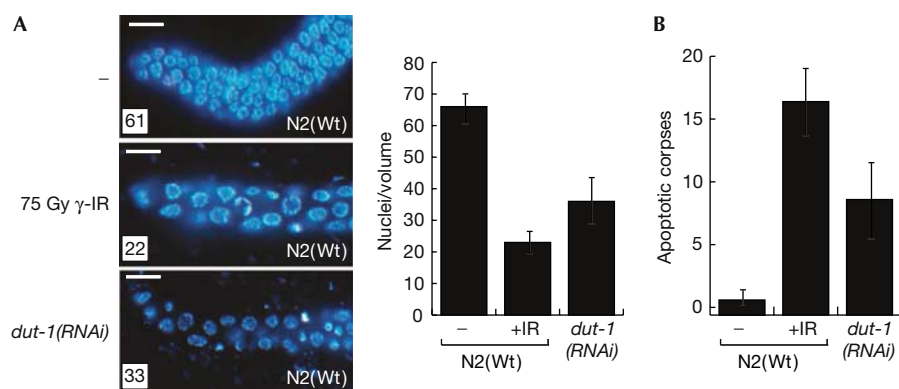
**Table 1** | *dut-1(RNAi)* is embryonic lethal in N2 wild-type *Caenorhabditis elegans*

RNAi	Animals inspected	Embryonic lethal (%)	Hatching (%)	Adult (%)	<i>t</i> -Test ( <i>P</i> )*
L4440	1,260	6 (0.5)	1,254 (99.5)	1,254 (99.5)	NA
<i>dut-1</i>	1,147	824 (72)	324 (28)	9 (0.8) <sup>‡</sup>	<0.001

NA, not applicable; RNAi, RNA interference.

\**P*-values calculated from two-tailed Student's *t*-test after *dut-1(RNAi)* compared with empty vector control.

<sup>‡</sup>L4-like phenotype characterized by adult-sized animals but no formation of oocytes or embryos.



**Fig 2** | *dut-1(RNAi)* induces cell-cycle arrest and apoptosis in wild-type animals. (A) The presence of fewer but enlarged 4,6-diamidino-2-phenylindole-stained nuclei in the mitotic region of dissected gonads shows induction of cell-cycle arrest in *dut-1(RNAi)*-fed N2 animals. Germ lines extruded from untreated (–) and  $\gamma$ -irradiated (75 Gy  $\gamma$ -IR) animals are shown for comparison. The number of nuclei was counted in a volume of  $54,000 \mu\text{m}^3$  16 h after the exposure of L4 larvae to the indicated treatments in 25 germ lines. Scale bars, 5  $\mu\text{m}$ . (B) Induction of apoptosis was scored by counting apoptotic corpses in the pachytene region of the germ line under differential interference contrast before (–) and after (+) ionizing radiation (IR), and after *dut-1(RNAi)*. Error bars indicate the standard error of the mean for at least 20 adult worms from three independent experiments. RNAi, RNA interference; Wt, wild type.

**Table 2** | Suppressors of (*dut-1*)RNAi lethality in *rrf-3*

RNAi	Animals inspected	Adults (% of total brood)	t-Test (P)*
<i>L4440</i>	1,399	1,385 (99)	NA
<i>dut-1</i>	1,965	7 (0.4) <sup>‡</sup>	NA
<i>L4440;dut-1</i>	1,593	13 (0.8) <sup>‡</sup>	NA
<i>ung-1</i>	1,227	1206 (98)	NA
<i>ung-1;dut-1</i>	1,993	52 (3)	0.08
<i>clk-2</i>	571	535 (95)	NA
<i>clk-2;dut-1</i>	489	34 (7)	0.013
<i>chk-1</i>	1,156	481 (42) <sup>§</sup>	NA
<i>chk-1;dut-1</i>	1,016	46 (5) <sup>§</sup>	0.011

NA, not applicable; RNAi, RNA interference.

\*P-values calculated from one-tailed Student's *t*-test between either *dut-1(RNAi)* in combination with empty vector or *dut-1(RNAi)* in combination with test constructs as indicated.

<sup>‡</sup>L4-like phenotype characterized by adult-sized animals but no formation of oocytes or embryos.

<sup>§</sup>Sterile offspring.

with dUTPase failed to rescue the *dut-1(RNAi)* phenotype. These different phenotypic outcomes might point to a function not shared by CLK-2 and ATL-1.

### Genetic mutants rescue *dut-1(RNAi)* lethality

The double-RNAi screen identified genes that provided an obvious, but not highly penetrant, rescue of the *dut-1(RNAi)* phenotype. Therefore, *clk-2* and *ung-1* mutants (supplementary Fig 2 online) were used to confirm our previous findings. Depletion of dUTPase in either *clk-2* or *ung-1* mutants robustly rescued the *dut-1(RNAi)* phenotype compared with the N2 control, with about 30% of the progeny developing into adults (Table 3). Germline development after *dut-1(RNAi)* was also rescued in both *clk-2* (Fig 3A,e) and *ung-1* (Fig 3A,f) mutants. Although the *clk-2* mutant completely rescued vulva development

**Table 3** | Rescue of *dut-1(RNAi)* lethality in genetic mutants

Strain	RNAi	Adults (% of total (n))*	t-Test <sup>§</sup>
N2	<i>L4440</i>	99.5 ± 0.4 (1260)	
	<i>dut-1</i>	0.8 ± 0.5 (1147) <sup>‡</sup>	NA
<i>clk-2</i>	<i>L4440</i>	98.4 ± 2.4 (502)	
	<i>dut-1</i>	24.5 ± 14 (1230)	P < 0.001
<i>ung-1</i>	<i>L4440</i>	99.8 ± 0.4 (535)	
	<i>dut-1</i>	30 ± 8 (686)	P = 0.001

NA, not applicable; RNAi, RNA interference.

\*Survival was scored as the number of offspring that developed into adults and is given ( $\pm$  s.e.m.) for at least five independent experiments. *n* is the total number of animals inspected.

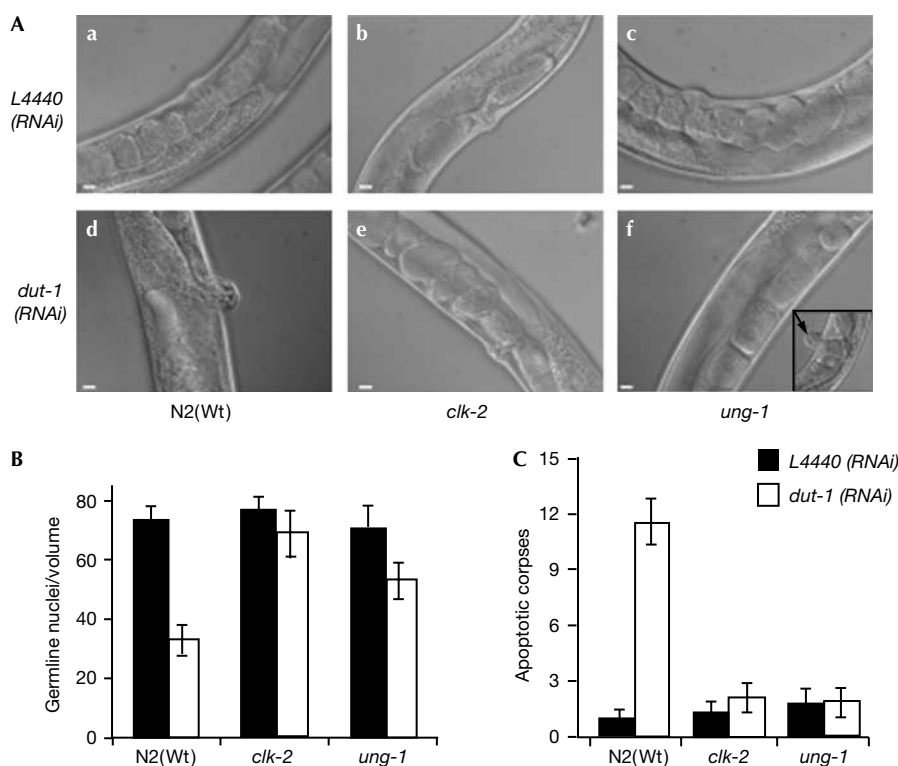
<sup>‡</sup>L4-like phenotype characterized by adult-sized animals but no formation of oocytes or embryos.

<sup>§</sup>P-values calculated from two-tailed Student's *t*-test after *dut-1(RNAi)* in N2 compared with *clk-2* or *ung-1*.

and functionality (Fig 3Ae), some *ung-1* mutants developed a Pvl phenotype (Fig 3Af, inset), suggesting incomplete rescue. Importantly, both mutants abrogated activation of cell-cycle arrest, as no significant reduction in the number of mitotic nuclei, or enlarged mitotic nuclei (data not shown), were seen in either mutant (Fig 3B). Similarly, no elevation in the number of apoptotic corpses was observed in response to *dut-1(RNAi)* in either mutant (Fig 3C). We conclude that both excision of uracil by UNG-1 and a functional CLK-2 checkpoint are required to activate cell-cycle arrest and apoptosis in response to uracil misincorporation into DNA in *C. elegans*.

### Repair intermediates are tolerated in *clk-2*

Consistent with the activation of cell-cycle arrest and apoptosis after *dut-1(RNAi)*, dUTPase depletion in the N2 strain resulted in the accumulation of RPA-1 (data not shown) and ATL-1 (Fig 4A,B) foci on chromatin, which indicates S-phase checkpoint activation



**Fig 3** | Rescue of *dut-1(RNAi)* in *ung-1* and *clk-2* mutants. (A) Differential interference contrast images of representative phenotypes observed in the progeny in empty vector *L4440(RNAi)* control-fed (a–c) compared with *dut-1(RNAi)*-fed (d–f) animals. N2 worms fed *dut-1(RNAi)* develop a strong protruding vulva (Pvl) phenotype (d), which was fully rescued in the *clk-2* mutant (e) but only partly rescued in *ung-1*, as some animals showed a Pvl phenotype (f, arrow pointing to Pvl in the inset). Scale bars, 10  $\mu$ m. (B) Cell-cycle arrest and (C) apoptotic corpses per germ line were counted 16 h after exposing L4 to *dut-1(RNAi)* (open bars) compared with animals fed empty vector control, *L4440(RNAi)* (filled bars). Error bars indicate the standard error of the mean for at least 20 adult worms from three independent experiments. RNAi, RNA interference; Wt, wild type.

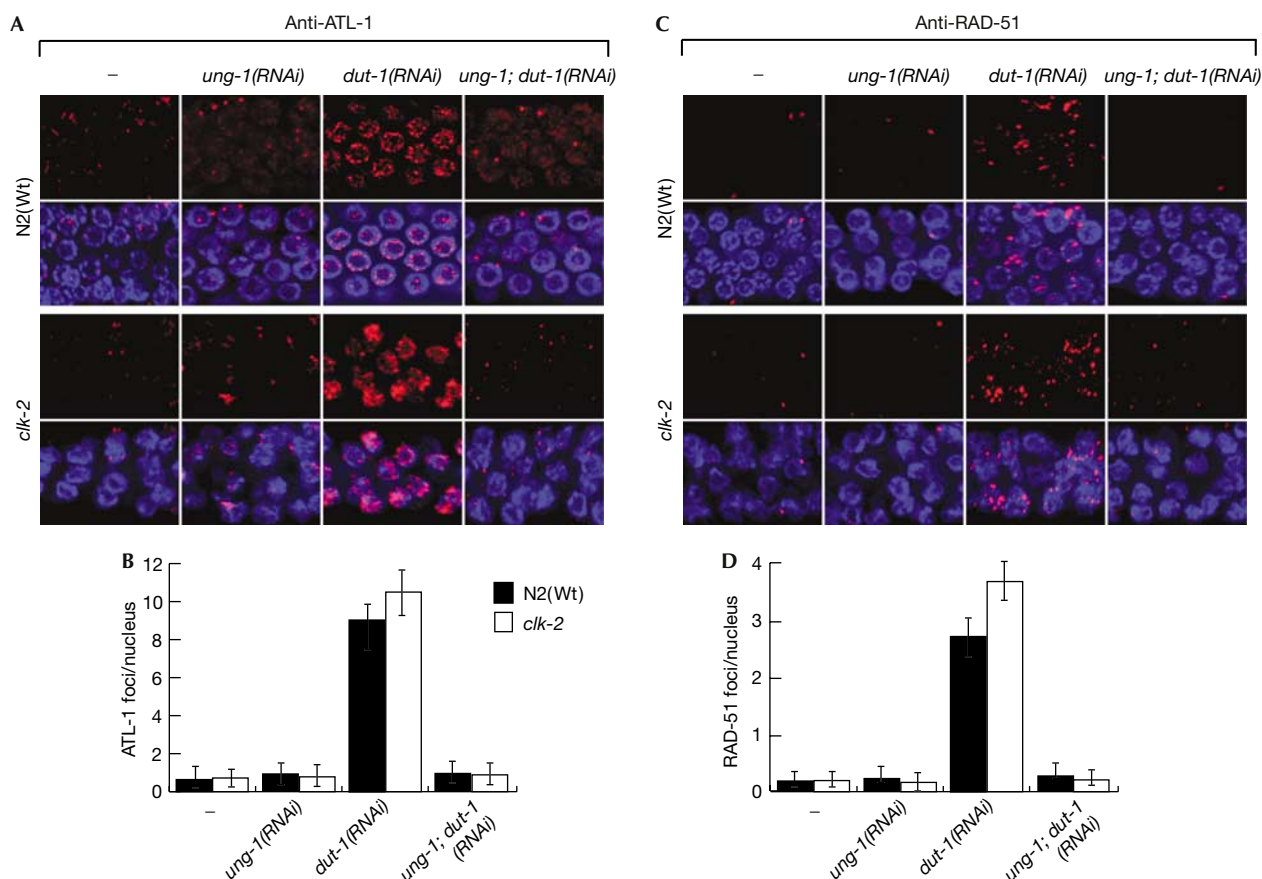
(Garcia-Muse & Boulton, 2005). Uracil-containing DNA is itself not sufficient to trigger the DNA damage checkpoint, as co-depletion of *ung-1* and *dut-1* abolished RPA-1 and ATL-1 focus formation. This result confirms that processing of misincorporated uracil by UNG-1 is required to trigger checkpoint activation. Furthermore, *clk-2* mutants under *dut-1(RNAi)* conditions also accumulate RPA-1 (data not shown) and ATL-1 foci, similar to that observed after *dut-1* depletion in the wild type. Immunostaining with anti-RAD-51 antibodies, the most accepted marker for double-strand break (DSB) formation in *C. elegans* (Alpi et al, 2003), showed a significant increase in RAD-51 foci after *dut-1(RNAi)* in both wild type and *clk-2* mutants. RAD-51 focus formation depended on the UNG-1 enzyme (Fig 4C,D). This result indicates that the different outcomes of *dut-1(RNAi)* in the wild-type and *clk-2* mutant strains are not due to a reduction in DSB formation in the mutant. Collectively, these results indicate that the rescue of *dut-1(RNAi)* in the *clk-2* mutants is not due to a defect in incorporation or excision of uracil, but that the DNA repair intermediates arising from the UNG-1-mediated excision of uracil are tolerated in the absence of CLK-2. These data further suggest a role for CLK-2 downstream from RPA-1 and ATL-1 in the S-phase checkpoint pathway (Garcia-Muse & Boulton, 2005).

In conclusion, depletion of dUTPase activity by RNAi enhances uracil misincorporation into DNA. This leads to checkpoint

activation only after processing of uracil by UNG-1. Uracil excision leads to recruitment of RPA-1 and ATL-1 to chromatin and subsequent activation of the CLK-2 checkpoint, which manifests as cell-cycle arrest and activation of apoptosis in the *C. elegans* germ line, independent of the 9-1-1 complex. Thus, the genetic requirements for activation of the DDR in response to *dut-1(RNAi)* differ from those primarily responding to ionizing radiation. Previous studies of molecular events leading to lethality in dUTPase mutants concentrated on the network of enzymes directly processing uracil in DNA (Guillet et al, 2006). However, we report a novel genetic interaction between UNG-1 and CLK-2 demonstrating that activation of the DDR in response to misincorporated uracil depends on DNA repair. Indeed, abrogation of the CLK-2 checkpoint leads to tolerance to DNA repair intermediates. Consequently, misincorporated uracil, a presumed innocuous lesion, can be processed by UNG-1 to yield repair intermediates that might be carried into the next generation as a mutagenic and cytotoxic lesion in the absence of CLK-2.

Recent reports demonstrated that DDR pathways are constitutively activated in pre-neoplastic human cells (Bartkova et al, 2005; Gorgoulis et al, 2005). It was suggested that inappropriate stimulation of replication could lead to replication stress and activation of the DNA damage checkpoint, and that this would provide selection pressure for the subsequent inactivation of





**Fig 4** | Recruitment of ATL-1 and RAD-51 to chromatin after *dut-1(RNAi)* depends on UNG-1. Representative images of anti-ATL-1 (top left, A) and anti-RAD-51 (top right, C) staining of fixed mitotic nuclei from N2 wild-type and *clk-2* mutant animals 16 h after exposure of L4 larvae to the indicated RNAi. (B,D) Quantification of the data is shown for N2 (filled) and *clk-2* (open) bars. Error bars indicate the standard error of the mean for at least 20 adult worms from two independent experiments. RNAi, RNA interference; Wt, wild type.

tumour suppressor genes. The results presented here confirm that endogenous DNA base damage might contribute to replication stress and activation of DDR pathways. Therefore, we speculate that elevated steady-state levels of uracil could contribute to the development of cancer, not only by increasing the mutation rate (An *et al*, 2005) but also by contributing to replication stress. Our data indicate that CLK-2 could function to prevent mutagenesis caused by propagation of repair intermediates arising from the processing of endogenous DNA damage.

## METHODS

**Strains and culture conditions.** *C. elegans* strains were cultured using standard procedures. All experiments were performed at 15 °C unless stated otherwise. The *Caenorhabditis* Genetics Centre (University of Minnesota, St Paul, MN, USA) provided the following strains: the reference strain Bristol N2, *clk-2(mn159)*, NL2099 *rrf-3(pk1426)*, VC172 *cep-1(gk138)* and CB5348 *mrt-2(e2663)*. A frozen trimethylpsoralen-UV (TMP-UV) deletion library was prepared essentially as described (Liu *et al*, 1999). The *ung-1(qa7600)* allele was identified by poison primer PCR screening (Edgley *et al*, 2002). The *ung-1* mutant was recovered by sibling selection and was backcrossed six times into the N2

wild-type strain. RNAi was performed by feeding, as described previously (Boulton *et al*, 2002). Double-feeding experiments were performed in the RNAi-sensitive strain *rrf-3(pk1426)* (Simmer *et al*, 2002) by mixing fresh overnight cultures of *Escherichia coli* strains harbouring *dut-1(RNAi)* feeding construct at 1:5 ratio with strains expressing either test plasmids or the empty vector control, pL4440, to establish baseline survival. Survival in RNAi feeding experiments was scored as the fraction of the progeny that developed past L4.

**Gateway recombination cloning.** Open-reading-frame-specific primers compatible with the Gateway system (Invitrogen, UK) were designed for *dut-1* (K07A1.2), *ung-1* (Y56A3A.29a), *clk-2* (C07H6.6), *chk-1* (Y39H10A.7), *rad-51* (Y43C5A.6), *chk-2* (Y60A3A.12), *atl-1* (T06E4.3), *hpr-17* (F32A11.2), *hus-1* (H26D21.1) and *cep-1* (F52B5.5), PCR amplified and cloned into pL4440-dest, as described previously (Boulton *et al*, 2002).

**Cytological preparation and immunostaining.** After L4 animals were subjected to RNAi for 16 h, the germ lines of gravid hermaphrodites were extruded and processed for immunostaining, as described previously (Garcia-Muse & Boulton, 2005). Primary antibodies were diluted in TBS-T (TBS + 0.5% BSA + 0.1% Triton X-100; 1:200 for anti-RAD-51, 1:500 for anti-RPA-1 and 1:500 for

anti-ATL-1) and incubated overnight at 4 °C in a humid chamber. The secondary antibody, anti-rabbit Cy3 (Sigma, UK), was used at a 1:10,000 dilution. Apoptotic corpses were scored using differential interference contrast (DIC) microscopy, and cell-cycle arrest was scored in extruded germ lines stained with DAPI, as described previously (Gartner *et al*, 2004).

**Microscopy.** DIC microscopy was performed on a Zeiss Axiovert 200M inverted microscope with ×20 Plan-Apochromat 0.8 NA or ×63 Plan-Apochromat 1.4 NA objectives. Deltavision microscopy was used to examine germ lines after immunostaining with ×40 or ×63 1.4 NA Planachromat objectives, as described previously (Garcia-Muse & Boulton, 2005).

**Supplementary information** is available at *EMBO reports* online (<http://www.emboreports.org>).

#### ACKNOWLEDGEMENTS

We thank the *C. elegans* Genetics Centre for providing *C. elegans* and bacterial strains, and Dr E. Boye and S.S. Salus for a critical reading of the manuscript. This work was supported by the University of Oslo (H.N., M.D.) and by Breast Cancer Campaign (GA3221) and Cancer Research UK (S.J.B. and T.G.-M.).

#### REFERENCES

- Ahmed S, Alpi A, Hengartner MO, Gartner A (2001) *C. elegans* RAD-5/CLK-2 defines a new DNA damage checkpoint protein. *Curr Biol* **11**: 1934–1944
- Alpi A, Pasierbek P, Gartner A, Loidl J (2003) Genetic and cytological characterization of the recombination protein RAD-51 in *Caenorhabditis elegans*. *Chromosoma* **112**: 6–16
- An Q, Robins P, Lindahl T, Barnes DE (2005) C→T mutagenesis and λ-radiation sensitivity due to deficiency in the Smug1 and Ung DNA glycosylases. *EMBO J* **24**: 2205–2213
- Barnes DE, Lindahl T (2004) Repair and genetic consequences of endogenous DNA base damage in mammalian cells. *Annu Rev Genet* **38**: 445–476
- Bartkova J *et al* (2005) DNA damage response as a candidate anti-cancer barrier in early human tumorigenesis. *Nature* **434**: 864–870
- Boulton SJ, Gartner A, Reboul J, Vaglio P, Dyson N, Hill DE, Vidal M (2002) Combined functional genomic maps of the *C. elegans* DNA damage response. *Science* **295**: 127–131
- Edgley M, D'Souza A, Moulder G, McKay S, Shen B, Gilchrist E, Moerman D, Barstead R (2002) Improved detection of small deletions in complex pools of DNA. *Nucleic Acids Res* **30**: e52
- Garcia-Muse T, Boulton SJ (2005) Distinct modes of ATR activation after replication stress and DNA double-strand breaks in *Caenorhabditis elegans*. *EMBO J* **24**: 4345–4355
- Gartner A, MacQueen AJ, Villeneuve AM (2004) Methods for analyzing checkpoint responses in *Caenorhabditis elegans*. *Methods Mol Biol* **280**: 257–274
- Gorgoulis VG *et al* (2005) Activation of the DNA damage checkpoint and genomic instability in human precancerous lesions. *Nature* **434**: 907–913
- Guillet M, Boiteux S (2003) Origin of endogenous DNA abasic sites in *Saccharomyces cerevisiae*. *Mol Cell Biol* **23**: 8386–8394
- Guillet M, Van Der Kemp PA, Boiteux S (2006) dUTPase activity is critical to maintain genetic stability in *Saccharomyces cerevisiae*. *Nucleic Acids Res* **34**: 2056–2066
- Hofmann ER, Milstein S, Boulton SJ, Ye M, Hofmann JJ, Stergiou L, Gartner A, Vidal M, Hengartner MO (2002) *Caenorhabditis elegans* HUS-1 is a DNA damage checkpoint protein required for genome stability and EGL-1-mediated apoptosis. *Curr Biol* **12**: 1908–1918
- Kalogeropoulos N, Christoforou C, Green AJ, Gill S, Ashcroft NR (2004) *chk-1* is an essential gene and is required for an S–M checkpoint during early embryogenesis. *Cell Cycle* **3**: 1196–1200
- Liu LX *et al* (1999) High-throughput isolation of *Caenorhabditis elegans* deletion mutants. *Genome Res* **9**: 859–867
- Schumacher B, Hofmann K, Boulton S, Gartner A (2001) The *C. elegans* homolog of the p53 tumor suppressor is required for DNA damage-induced apoptosis. *Curr Biol* **11**: 1722–1727
- Simmer F, Tijsterman M, Parrish S, Koushika SP, Nonet ML, Fire A, Ahringer J, Plasterk RH (2002) Loss of the putative RNA-directed RNA polymerase RRF-3 makes *C. elegans* hypersensitive to RNAi. *Curr Biol* **12**: 1317–1319
- Stergiou L, Hengartner MO (2004) Death and more: DNA damage response pathways in the nematode *C. elegans*. *Cell Death Differ* **11**: 21–28
- Tye BK, Lehman IR (1977) Excision repair of uracil incorporated in DNA as a result of a defect in dUTPase. *J Mol Biol* **117**: 293–306