Cellular responses to DNA damage involve distinct DNA repair pathways, such as mismatch repair (MMR) and base excision repair (BER). Using *Caenorhabditis elegans* as a model system, we present genetic and molecular evidence of a mechanistic link between processing of DNA damage and activation of autophagy. Here we show that the BER AP endonucleases APN-1 and EXO-3 function in the same pathway as MMR, to elicit DNA-directed toxicity in response to 5-fluorouracil, a mainstay of systemic adjuvant treatment of solid cancers. Immunohistochemical analyses suggest that EXO-3 generates the DNA nicks required for MMR activation. Processing of DNA damage via this pathway, in which both BER and MMR enzymes are required, leads to induction of autophagy in *C. elegans* and human cells. Hence, our data show that MMR- and AP endonuclease-dependent processing of 5-fluorouracil-induced DNA damage leads to checkpoint activation and induction of autophagy, whose hyperactivation contributes to cell death.

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5'-Fluorouracil (5-FU) remains a central component of systemic treatment of a wide range of solid cancers in the adjuvant setting. The active metabolite, 5-fluoro-2'-deoxyuridine monophosphate, inhibits thymidylate synthase, which leads to imbalanced nucleotide pools with subsequent incorporation of dUTP and 5-fluoro-2'-dUTP into DNA, and the corresponding ribonucleotides into RNA. As the resulting uracil and fluorouracil (FUra) bases in DNA do not lead to formation of strand breaks directly, it is thought that repair intermediates generated through incomplete or aberrant processing of the original lesions by DNA repair enzymes is the basis for DNA-directed toxicity. Removal of uracil or FUra bases from DNA is generally believed to be required for generating the repair intermediates leading to DNA-directed toxicity induced by 5-FU. Thus, we asked whether mutants of the lesion recognition enzymes in the BER or MMR (Fig. 1a) pathways led to 5-FU resistance in C. elegans.

A null mutant of the UNG-1 enzyme, ung-1(qa7600; ref. 11, also see Supplementary Fig. S1 for description of the mutant alleles) was as sensitive to 5-FU as the wild type (N2; Fig. 1b). In contrast, pronounced 5-FU resistance was observed after RNA interference (RNAi)-mediated depletion of the AP endonucleases EXO-3 and APN-1 (Fig. 1c). As the AP endonucleases act downstream of UNG-1 in the BER pathway (Fig. 1a), this surprising result indicates that DNA-mediated toxicity proceeds via an UNG-1-independent alternative pathway that also requires AP endonucleases.

An MSH-6 mutant, msh-6(pk2504) was remarkably resistant to 5-FU (Fig. 1d). After treating nematodes with 2 μM 5-FU, >80% of the msh-6(pk2504) mutant offspring developed into adults compared with <5% of the wild type (Fig. 1d). In contrast, only 50% of MSH-2 mutants, msh-2(ok2410), developed into adults (Fig. 1d) and msh-1(ok1917) mutants animals were as sensitive as msh-2(ok2410) mutants (Fig. 1d). This surprising intermediate phenotype of msh-2(ok2410) mutants would be consistent with aberrant processing of 5-FU-induced DNA damage initiated by MSH-6 without appropriate recruitment of downstream repair factors. This was unexpected given that MSH-2 acts together with MSH-6 in the MutS complex and the stability of human MSH-6 depends on MSH-2 (ref. 12). However, western blot analysis confirmed that in C. elegans, MSH-6 was expressed in msh-2(ok2410) mutants and in the wild type after RNAi-mediated depletion of MSH-2, but was not detected in msh-6(pk2504) mutants and msh-6(RNAi) animals (Fig. 1e). Moreover, expression of an MSH-2::GFP translational fusion protein could be detected after depleting MSH-6 but not MSH-2 (Fig. 1e). As depletion of MSH-6 or MSH-2 was epistatic to the msh-1(ok1917) mutation (Fig. 1f), we conclude that processing of 5-FU-induced DNA damage through the MMR pathway is the basis for DNA-directed toxicity, and that MSH-6 seems to have an upstream function in lesion processing (see Supplementary Fig. S2 for RNAi efficiency and confirmation that phenotypes induced by RNAi resemble mutant phenotypes).

To test whether the AP endonucleases act in the same pathway as MMR to mediate 5-FU-directed toxicity, we depleted APN-1 and EXO-3 in msh-6 and msh-2 mutant animals (Fig. 1g,h, respectively). In both cases epistasis was observed, as no statistically significant difference in median survival was found between the groups. Thus, the BER AP endonucleases act in the same pathway as MMR to mediate 5-FU toxicity. Further epistasis analyses in exo-3(tm4374) mutants suggested that MSH-6 operates upstream of EXO-3, whereas MSH-2, MLH-1 and APN-1 probably act downstream of EXO-3, as their depletion resulted in 5-FU sensitivity indistinguishable from the exo-3(tm4374) mutant-fed control RNAi (Fig. 1i). Analyses in exo-3;msh-6 and exo-3;mlh-1 double mutants (Fig. 1j,k, respectively) confirmed the epistasis between EXO-3, and both MSH-6 and MLH-1. Finally, the msh-6 single and exo-3;msh-6 double mutants showed most pronounced tolerance to 5-FU, supporting an upstream role of MSH-6 in eliciting 5-FU toxicity. Hence, the epistasis experiments support a model where the BER AP endonucleases function downstream of MutS to elicit DNA-directed toxicity in response to 5-FU.

Results

C. elegans MMR and BER mutants are resistant to 5-FU. Removal of uracil or FUra bases from DNA is generally believed to be a feature of DNA-directed toxicity in human cells. Thus, we used C. elegans as a model to investigate the function of the two DNA repair pathways in eliciting 5-FU toxicity. C. elegans has only one characterized UDG, the enzyme UNG-1 (ref. 10, and has significantly contributed to our understanding of the role of DNA repair pathways in initiating DNA damage response (DDR) signalling in response to misincorporated uracil. Furthermore, as C. elegans also has only one MutS complex, it allows genetic interrogation of the separate role of BER and MMR in eliciting DNA-mediated toxicity in response to 5-FU.

Here we show that the DNA damage recognition complex of MMR, but not BER, acts as a sensor of DNA damage induced by 5-FU. Furthermore, epistasis analyses show that the BER AP endonucleases APN-1 and EXO-3 function in the same pathway as MMR to induce toxicity in response to 5-FU. Immunohistochemical analyses suggest that EXO-3 generates DNA nicks required for MMR activation, whereas APN-1 is required for checkpoint activation. Processing of DNA damage via this pathway, in which both BER and MMR enzymes are required, leads to induction of autophagy in C. elegans and in human U2OS cells. This suggests that failure to process 5-FU-induced DNA damage via this pathway is a basis for resistance.

DNA repair-dependent checkpoint activation. Next, we asked whether the processing of 5-FU-induced DNA damage led to apoptosis. Despite the appearance of cytological markers for DDR activation (Supplementary Fig. S3a,b), apoptotic cell death was
survival curves (Supplementary Fig. S3c) nor in embryos (Supplementary Fig. S3d).

However, RPA-1-positive foci, which are early markers for DDR activation, accumulated in 5-FU-treated embryos (Fig. 2a). RPA-1 foci were observed in animals depleted for UNG-1 (Fig. 2b). In contrast, RPA-1 focus formation depended on MSH-6 (Fig. 2a,b and Supplementary Table S1) and, on further processing, through the MMR pathway, as RPA-1 foci did not form in the msh-6(ok1917) mutant and were suppressed in the exco-3(tm1842) mutant (Fig. 2c and Supplementary Table S2). Hence, we conclude that RPA-1 focus formation depends on processing 5-FU-induced DNA damage through the MMR pathway.

The epistasis of MSH-6 between EXO-3 and APN-1 (Fig. 1g,j) could be explained if these endonucleases provide the DNA nicks required for the loading of EXO-1. If so, we would expect that depletion of the BER AP endonucleases prevented RPA-1 focus formation. This was indeed the case for EXO-3 (Fig. 2b and Supplementary Table S1). In contrast, depletion of APN-1, which, according to our epistasis analysis, acts downstream of EXO-3, did not significantly suppress RPA-1 focus formation in the wild-type background (Fig. 2a,b). However, depletion of APN-1 in exco-3(tm1842) mutants completely abrogated RPA-1 focus formation (Fig. 2c and Supplementary Table S2). The different outcomes with respect to RPA-1 focus formation confirmed that APN-1 and EXO-3 have non-redundant functions. The finding that EXO-3 was required for RPA-1 filament formation suggested that it might contribute to generate nicks required for the activation of MMR.

RPA-1 filament formation in response to DNA damage is an indication of DNA damage checkpoint activation that, in 5-FU-treated human cells, involves CHK-1 phosphorylation\(^\text{14}\), 5-FU induced-phosphorylation of CHK-1 in wild-type C. elegans but not in msh-6(pk2504) mutant embryos (Fig. 2d,e). Interestingly, CHK-1 phosphorylation was not induced in APN-1-depleted embryos (Fig. 2d,e) despite the presence of RPA-1 foci. The immunohistochemical analysis therefore supports that checkpoint activation depends on DNA repair. Although MSH-6 function is required both for RPA-1 focus formation and CHK-1 phosphorylation, APN-1 is dispensable for RPA-1 filament formation.

Figure 1 | Epistatic interactions between MutS and the BER AP endonucleases with respect to 5-FU sensitivity. (a) Cartoons showing schemes of the (i) BER and (ii) MMR pathways. F1 survival (b) in N2 wild type (black diamonds) and ung-1 mutants (red squares), (c) following the depletion of EXO-3 (red triangles), APN-1 (blue circles) or control RNAi (L4440, black diamonds) in the N2 wild-type background, and (d) in msh-2(ok2410) (orange triangles), msh-6(pk2504) (green circles) and mlh-1(ok1917) (grey squares) mutants. (e) Western blottings showing expression of MSH-6 protein in the wild type strain grown on E. coli expressing control (N2) or the indicated RNAi, as well as in msh-6(pk2504) and msh-2(ok2410) mutants (top panel), and expression of MSH-2:GFP fusion protein in transgenic worms grown on E. coli expressing RNAi for MSH-2, MSH-6 or empty vector control as indicated (lower panel). Actin was used as the loading control. (f) F1 survival after depletion of MSH-2 and MSH-6 in the mlh-1(ok1917) mutant. (g,h) F1 survival after depletion of the AP endonucleases EXO-3 and APN-1 in (g) the msh-6(pk2504) or (h) msh-2(ok2410) mutant background. Kruskal–Wallis one-way analysis on ranks (P = 0.963 and P = 0.985 in h and g, respectively). Mann–Whitney rank-sum test failed to identify a difference in median survival after depleting EXO-3 in the msh-6 mutant (g, P = 0.929). (i) F1 survival following depletion of the indicated genes in exco-3(tm3744) mutants. (j) F1 survival in msh-6(pk2504), exco-3(tm3744) and exco-3;mlh-1 double mutants. (k) F1 survival in exco-3(tm3744), mlh-1(ok1917) and exco-3;mlh-1 double mutants. All survival curves (b–f–k) show the mean ± s.d. for each data point from three independent experiments.
The absence of RAD-51-positive foci (Supplementary Table S3) showed that checkpoint activation was not likely to be a result of DNA double-stranded break (DSB) formation. However, compared with untreated nuclei, 5-FU-treated nuclei were larger with faint DAPI (4',6-diamidino-2-phenylindole) staining already at the four- to six-cell stage embryo (Fig. 2a). Taken together, this suggested that 5-FU primarily leads to chromatin decompaction rather than DSB formation. Consistently, the maximum nuclear diameter, defined by the perimeter of lamin-1 (LMN-1) staining in the four-cell stage embryo, increased from 8.2 to 9.0 μm (Student’s t-test, P < 0.05) by 5-FU treatment concomitant with reduced DAPI staining intensity in an MSH-6-dependent manner (Supplementary Fig. S4). Chromatin decompaction was confirmed by the loss of linker histone H1X in 5-FU-treated embryos (Fig. 2f,g). H1X levels were not reduced upon 5-FU treatment in the msh-6(pk2504) mutant. This gives independent confirmation that chromatin decompaction and checkpoint activation depended on DNA-repair-mediated processing of 5-FU-induced DNA damage.

5-FU induces autophagy in C. elegans. As we recently showed that uracil incorporation induces cell death with both apoptotic and autophagic features15, we tested whether 5-FU may induce autophagy as an alternative cell death response16. During the process of macroautophagy (hereafter, it is referred to as autophagy), parts of the cytoplasm are sequestered by a double lipid layer that grows to form a small vesicle (autophagosome). The autophagosome then fuses with a lysosome to generate an autolysosome, in which the cargo is degraded by acidic hydrolases. To monitor the activation of autophagy, we used the transgenic reporter strain expressing LGG-1 (C. elegans Atg8/ LC3) in fusion with green fluorescent protein (GFP). LGG-1 remains associated with autophagic membranes from the early nucleation step until fusion with lysosomes. Increased LGG-1 expression and appearance of GFP::LGG-1-positive puncta indicated that autophagy was activated in the embryos of 5-FU-treated hermaphrodites (Fig. 3a). LGG-1 accumulation was dependent on the core autophagic machinery, as it was suppressed after depletion of UNC-51 (C. elegans beclin1/Atg6), which is a serine/threonine kinase that regulates autophagy induction; the class III phosphatidylinositol 3-kinase (PI3K) complex subunit BEC-1 (C. elegans beclin1/Atg6), which is required for vesicle nucleation; and ATG-7, an E1-like enzyme involved in conjugation of the ubiquitin-like proteins LGG-1 and ATG-12 to autophagic membranes (Fig. 3a). Depletion of LGG-1 itself suppressed puncta formation in a BEC-1::GFP reporter strain (Supplementary Fig. S5a–c). To exclude the possibility that 5-FU-induced autophagy could be an artefact of the transgenic reporter system used, we monitored the expression

Figure 2 | DNA damage checkpoint activation in C. elegans. (a) Immunofluorescence images showing anti-RPA-1-positive foci in response to 5-FU after depleting the indicated genes by RNAi or empty vector control RNAi (L4440; scale bars, 5 μm). (b) Quantification of the fraction (%) of 5-FU-treated embryos that contain RPA-1-positive foci. (c) Quantification of the fraction (%) of 5-FU-treated embryos that contain RPA-1-positive foci in N2, msh-6(ok1917) and exo-1(tm1842) mutants fed control (L4440) or RNAi targeting APN-1 (apn-1). (d) Immunofluorescence showing CHK-1 phosphorylation at Ser139 in response to 5-FU in N2 but not in msh-6(pk2504) mutants or in N2 after apn-1(RNAi) (scale bars, 5 μm). (e) Quantification of the fraction (%) of 5-FU-treated embryos with phospho-CHK-1 foci. (f) Western blottings showing H1X.101 levels in N2 and 5-FU-treated worms. (b,c,e,g) Bar graphs show the mean ± s.d. from three independent experiments.
of the endogenous VPS-34 protein (a homologue of human class III PI3K) that interacts with BEC-1 (ref. 17). Confirming autophagy activation in response to 5-FU treatment, an overall increase in VPS-34 expression was observed together with the appearance of VPS-34-positive puncta around the nuclear periphery and in the cytoplasm (Fig. 3b). Distinct VPS-34 foci were also observed in the nucleoli, indicating that VPS-34 shuttles between the nucleus and cytoplasm, similar to what was previously described for its interaction partner Beclin 1 (ref. 18). Furthermore, as LGG-1 itself is degraded as part of the autophagic process, the appearance of a 25-kDa band corresponding to GFP in 5-FU-treated embryos, which disappeared upon addition of the PI3K inhibitor 3-methyladenine (3-MA), confirmed increased autophagic flux upon 5-FU treatment (Fig. 3c). Although autophagy primarily acts as a prosurvival mechanism following various forms of stress, including starvation, excessive activation of autophagy can promote cell death \(^{15,16}\). The significant increase in progeny survival in animals depleted for \textit{bec-1} and \textit{atg-7} before 5-FU treatment showed that autophagy, in this case, contributes to toxicity (Fig. 3d).

A connection was recently described between the ATR (Ataxia telangiectasia and Rad3 related) DNA damage checkpoint and activation of autophagy in yeast\(^ {19}\). Depletion of ATL-1 (the \textit{C. elegans} ATR orthologue\(^ {20}\)) reduced autophagy induction by about 50% in \textit{C. elegans} (Fig. 3e and Supplementary Table S4), whereas no effect was seen upon loss of CEP-1 (the \textit{C. elegans} p53 orthologue; Fig. 3f and Supplementary Table S4). In \textit{atl-1} (\textit{tm853}) mutants, there was weaker cytoplasmic anti-VPS-34 staining and the fraction of embryos with VPS-34-positive puncta was halved compared with the N2 control (Fig. 3f), supporting a partial requirement of ATL-1 for 5-FU-induced autophagy. In contrast, the fraction of embryos with GFP::LGG-1-positive puncta was indistinguishable in the \textit{atm-1} (\textit{gk186}) mutant background and the wild-type background. (Fig. 3h).

DNA repair-dependent induction of autophagy. Next, we asked whether autophagy induction, similar to chromatin decompaction, RPA-1 focus formation, and CHK-1 phosphorylation also depended on DNA repair. This was addressed by counting embryos with GFP::LGG-1-positive foci in animals after RNAi-depletion of BEC-1 (red triangles) and ATG-7 (blue squares) as compared with worms fed control RNAi (black diamonds). The survival curve shows the mean ± s.d. for each data point from three independent experiments. (e) Induction of autophagy (% GFP-positive embryos) following depletion of the indicated genes. (f) Immunofluorescence showing anti-VPS-34 staining in dissected embryos in the absence or presence of 5-FU (scale bar, 5 μm). (g) The fraction (as % of untreated control) of embryos with VPS-34-positive foci following 5-FU treatment. (h) Induction of autophagy (% GFP-positive embryos) in control (GFP::LGG-1) and \textit{atm-1} (\textit{gk186}) mutants (\textit{atm-1}; GFP::LGG-1). (e-g,h) Bar graphs represent mean ± s.d. from three independent experiments.

Figure 3 | Activation of autophagy in 5-FU-treated \textit{C. elegans}. (a) Autophagy induction in response to 5-FU measured in a GFP::LGG-1 reporter strain fed RNAi (L4440) or RNAi targeting the indicated genes (scale bars, 10 μm). (b) Immunofluorescence showing anti-VPS-34 staining in dissected embryos in the absence or presence of 5-FU (scale bar, 5 μm). (c) Western blot analysis of embryonic extracts with and without the addition of 5-FU or the autophagy inhibitor 3-MA, detecting the ~40-kDa GFP::LGG-1 fusion protein and the cleaved ~25-kDa product. (d) F1 survival measured after depletion of BEC-1 (red triangles) and ATG-7 (blue squares) as compared with worms fed control RNAi (black diamonds). The survival curve shows the mean ± s.d. for each data point from three independent experiments.
mediated depletion of individual DNA-repair proteins. All embryos in 5-FU-treated control animals were GFP-positive and depletion of UNG-1 had a minor effect (Fig. 4a,b and Supplementary Table S4). Confirming the survival experiments (Fig. 1c,d), 51% of MSH-2-depleted embryos were GFP positive, whereas depletion of MSH-6, MLH-1, PMS-2, APN-1 and EXO-3 dramatically reduced the number of GFP-positive embryos (Fig. 4b and Supplementary Table S4). Similar results were obtained using accumulation of VPS-34 as a read-out for autophagy activation (Supplementary Fig. S5d,e). Thus, 5-FU-induced autophagy depends on DNA repair. As predicted, as depletion of core autophagy genes attenuated 5-FU toxicity (Fig. 3d), suppression of autophagy further improved survival in the already-tolerant msh-6(pk2504) mutant (Fig. 4c).

We also monitored the expression of the ATF-2 transcription factor, which negatively regulates the expression of autophagy genes such as lgg-1 and bec-1 in C. elegans. In line with an upstream function of the MutS complex, we found that depletion of msh-2 and msh-6 resulted in 41% and 36% (93 out of 225, or 54 out of 150 embryos scored) of embryos showing excessive ATF-2 expression, which was not seen in the control (1,825 embryos out of 150 embryos scored) or delayed nuclei showing excessive ATF-2 staining and failure to induce autophagy. This suggests that RNA- and DNA-mediated 5-FU toxicity have a common mechanistic component.

DNA repair-dependent ribosome biogenesis defect. The DNA repair-dependent accumulation of VPS-34 in the nucleoli indicated that processing of 5-FU-induced DNA damage led to specific problems in this compartment. It is known that 5-FU inhibits ribosome biogenesis at the level of late ribosomal RNA processing. These phenotypes are commonly interpreted to be resulting from incorporation of 5-fluorouridine triphosphate into RNA. As regulation of ribosome biogenesis is tightly linked to regulation of chromatin condensation state, we suspected that MMR-dependent chromatin decompaction in response to 5-FU might contribute to these phenotypes. In C. elegans, ribosome biogenesis defects can be monitored as the appearance of visible (enlarged) nucleoli during early embryonic development. The appearance of enlarged nucleoli in ~70% of the four-cell stage embryos treated with 5-FU (Fig. 5c) is therefore a phenotype consistent with an RNA biogenesis defect. The enlarged nucleoli phenotype is in this case, however, unlikely to be a consequence of incorporation of 5-fluorouridine triphosphate into RNA, as it was absent in the msh-6 mutant (Fig. 5c,d). Moreover, the enlarged nucleoli phenotype was not observed after depletion of MSH-2, MLH-1 and EXO-3, and was partially suppressed by APN-1 depletion. In contrast, enlarged nucleoli were seen at wild-type levels after depletion of UNG-1 (Fig. 5d). Hence, the appearance of enlarged nucleoli depended on DNA repair, and its suppression coincided with the suppression of nucleolar VPS-34 staining and failure to induce autophagy. This suggests that RNA- and DNA-mediated 5-FU toxicity have a common mechanistic component.

5-FU induces DNA repair-dependent autophagy in human cells. To confirm the relevance of our finding for human cells, we monitored induction of autophagy in human U2OS cells treated with 10 μM 5-FU. Upon induction of autophagy, cytosolic LC3 (orthologous to C. elegans LGG-1; LC3-I, 18 kDa band) is cleaved and bound to phosphatidylethanolamine in the autophagic membranes (LC3-II, 16 kDa band) and, as LC3-II remains bound to autophagic membranes throughout the pathway, it can be used as a marker for autophagy. A weak but consistent induction of autophagy was seen following 18 h of exposure to 5-FU, with maximum LC3-II expression between 24 and 48 h (Fig. 6a,e). Autophagy was not activated secondarily to apoptosis as neither poly (ADP-ribose) polymerase 1 nor caspase-3 cleavage was observed. Induction of autophagy was suppressed by inhibiting the major mammalian AP endonuclease APE1 by methoxyamine (Mx; Fig. 6a,b). Similarly, a reduction of 5-FU-induced LC3-positive puncta was seen in the presence of Mx in U2OS cells (Fig. 6c,d). Induction of autophagy by 5-FU also depended on MMR in human cells, as depletion of MSH-2 effectively suppressed LC3-II induction (Fig. 6e,f) and formation of LC3-positive puncta (Fig. 6g,h). Similar results were obtained after depletion of MSH-6 by short interfering RNA (siRNA) as expected, as the stability of MSH-2 protein depends on MSH-6 in mammalian cells (Supplementary Fig. S6a).

Finally, we asked whether nucleolar proteins or nucleic acids were degraded by autophagy as suggested by the nucleolar VPS-34-positive foci seen in 5-FU-treated C. elegans. Expression of the RPS3 subunit of the 40S small ribosomal particle, which shuttles between the nucleolus and cytoplasm, was gradually lost in 5-FU-treated U2OS cells (Fig. 6i and Supplementary Fig. S6b), although RPS3 mRNA levels were unaffected (Fig. 6i). Indeed, depletion of Ulk1, a kinase required for induction of autophagy, prevented the 5-FU-induced degradation of RPS3 (Supplementary Fig. S6c,d) confirming that autophagic activity contributed to RPS3 degradation.

In summary, we have identified a novel conserved mechanism for 5-FU-induced toxicity. MutS and the BER AP endonucleases are required to elicit DNA-directed toxicity, leading to checkpoint failure to induce autophagy.
activation and autophagy induction in response to 5-FU in C. elegans embryos and in human cell lines. Epistasis analyses in C. elegans are consistent with MutS and the BER AP endonucleases acting in the same pathway with EXO-3, providing a nick for activation of the MMR pathway in vivo.

**Discussion**

Preclinical and clinical evidence show that MMR deficiency is associated with poor therapeutic response to a wide range of cytotoxic drugs. It is not clear whether this effect is due to a classical DNA repair function of MMR or a role for MMR in signalling DNA damage. The DNA damages induced by 5-FU can be repaired by BER and MMR, but little is known about the nature of the crosstalk between these DNA repair pathways beyond the fact that they share substrates. Here we present genetic and molecular evidence to show that the MMR MutS complex is the upstream requirement for DNA-mediated toxicity. Importantly, we show that the BER AP endonucleases EXO-3 and APN-1 act in the same pathway as classical MMR in eliciting DNA-directed toxicity in response to 5-FU in C. elegans and in a human cell line. We present evidence that EXO-3, the C. elegans orthologue of human APN-1, generates the nicks required for MMR activation in vivo. Processing of 5-FU-induced DNA damage via this pathway led to autophagy activation, which coincided CHK-1 phosphorylation, showing that autophagy is activated as a consequence of DNA damage processing and checkpoint activation. Failure to induce autophagy correlated with resistance to 5-FU. Autophagy was, as such, a determinant for sensitivity.

We identified a specific requirement for MMR and BER to elicit 5-FU toxicity. Depletion of genes participating in other DNA repair pathways did not result in similar resistance (Supplementary Fig. S7). Genetic studies in Saccharomyces cerevisiae suggest that the nucleotide excision repair (NER) pathway may contribute to repair of AP sites in the absence of BER, which might explain the somewhat increased 5-FU sensitivity of a mutant in XPA-1, a DNA damage binding protein required for NER (Supplementary Fig. S7a). In contrast, depletion of ERCC-1 or XPF-1, two proteins that function together as a structure-specific endonuclease in the NER pathway, resulted in a mild tolerance (Supplementary Fig. S7b), but this effect might be due to the role of ERCC-1/XPF-1 in repair of DSB rather than in NER. Similar tolerance was also observed after depleting proteins acting in the two main DSB repair pathways, homologous recombination and non-homologous end joining (Supplementary Fig. S7c,d, respectively). These results suggest that both NER and DSB repair pathways may process DNA-repair intermediates generated by MMR and BER in C. elegans, but that they have modest effects on overall toxicity. Moreover, our results revealed that the MutS complex, in particular the MSH-6 protein, acts as the initial sensor of 5-FU-induced DNA damage, as none of the cellular effects induced by 5-FU in the wild-type background was seen in MSH-6-deficient animals; msh-6 mutants showed no chromatin decompaction, failed to activate the DNA damage checkpoint as measured by RPA-1 focus formation and CHK-1 phosphorylation, and failed to induce autophagy. The nature of the in vivo substrates for cytotoxic MMR processing remains to be demonstrated. However, as only BER is expected to be able to process 5-FUra/Ura base paired with adenine, these lesions probably do not contribute much to DNA-mediated toxicity, as lack of UNG-1 does not affect overall survival. Nevertheless, UNG-1-initiated BER probably repairs 5-FU-induced DNA damage, as depletion of

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**Figure 5 | Loss of MutS induces ATF-2 expression and attenuates nucleolar stress phenotypes.** (a) Expression of ATF-2::GFP in 5-FU-treated hermaphrodites fed control or RNAi targeting the indicated genes (scale bars, 20 μm). (b) The number of animals harbouring embryos with excessive ATF-2::GFP expression was scored in at least 150 animals and given as the fraction (%) of the total. (c) N2 or msh-6(pk2504) embryos were continuously monitored under differential interference contrast microscopy (scale bars, 5 μm) from the three-cell stage until completion of the four-cell stage. Magnified images of representative nuclei are shown. (d) The fraction of embryos with visible, enlarged nucleoli (arrows) after 5-FU treatment was scored. Bar graphs represent mean ± s.d. from three independent experiments.
Figure 6 | DNA repair-dependent autophagy induction upon 5-FU treatment in human cells. (a) Western blots showing autophagy induction by monitoring LC3 levels in whole-cell extracts from U2OS cells treated or not with 5-FU alone or in combination with Mx for the indicated time. Staurosporin was used as an apoptosis induction shown by the appearance of cleaved fragments of caspase-3 (17 and 19 kDa) and poly (ADP-ribose) polymerase 1 (PARP1; ΔPARP, 85 kDa). α–Actin was used as loading control. (b) Quantification of LC3-II levels in cells treated as in a. Intensities of the lower LC3 band (LC3-II) was normalized to those of actin. (c) Autophagy induction demonstrated by accumulation of LC3-positive puncta in U2OS cells treated or not with 5-FU alone or in combination with Mx for 48 h. The cells were fixed and prepared for immunofluorescence of LC3 (green). DAPI staining is shown in blue. Scale bar, 20 μm. (d) Quantification of LC3-positive puncta in cells treated as in c. In each separate experiment, 200–1,600 cells were scored per condition. (e) Western blots showing absence of LC3-II accumulation in U2OS cells transfected with siRNA against MSH-2. The same blot probed with anti-MSH-2 antibodies confirm efficient knockdown. (f) Quantification of LC3-II levels in cells transfected with control or MSH-2 siRNA with or without FU treatment for 24 h. (g) Immunofluorescence showing reduced accumulation of LC3 puncta in U2OS cells transfected with siRNA against MSH-2. (h) Quantification of LC3-positive puncta in cells treated as in g. (i) Quantification of normalized RPS3 protein level (black bars) or RPS3 mRNA level (white bars) in U2OS cells after 48 h 5-FU treatment. RPS3 mRNA levels were determined by quantitative real-time reverse transcriptase–PCR. Graphs (b,d,f,h,i) show mean values ± s.e.m. quantified from at least three independent experiments. Student’s t-tests were performed to assess the significance between treatment groups in all panels as indicated; *P<0.05; **P<0.01.
exo-3 and apn-1 gave less-effective suppression of toxicity in ung-1 mutants than in the wild type (Supplementary Fig. S2b), and depletion of UNG-1 improved survival in the exo-3 mutant (Supplementary Fig. S2d). Interestingly, the sharp reduction in F1 survival in exo-3-depleted animals treated with higher concentrations of 5-FU (Fig. 1c) was lost in ung-1 mutants (Supplementary Fig. S2b) and, conversely, upon depletion of UNG-1 in exo-3 mutants (Supplementary Fig. S2d). This suggests that BER depletion of UNG-1 improved survival in the exo-3 mutant of low-fidelity DNA polymerase activity of APN-1 (ref. 31) contributes little to this function. RPA-1 functions downstream of RPA-1 filament formation to allow recruitment of the downstream factors required for checkpoint activation. Further experiments are required to pinpoint which activity of the multifaceted APN-1 enzyme is required for checkpoint activation, but the observation that some APN-1-depleted cells had extended RPA-1-positive tracks could point to a possible function of APN-1 in limiting MMR-mediated resection.

The observed APN-1-dependent CHK-1 phosphorylation further argues that autophagy was primarily induced as a consequence of DNA damage checkpoint activation resulting from processing the 5-FU-induced DNA damage via the pathway described. As autophagy is a cytoplasmic degradation pathway, it was surprising how it was activated by DNA damage, but two observations allow us to present a model for autophagy activation in response to 5-FU. First, the appearance of VPS-34 foci in the nucleoli suggested that cargo in this organelle might be collected and targeted for degradation in 5-FU-treated cells. The degradation of RPS3 by autophagy in human cells supported this interpretation. Second, we showed that 5-FU primarily leads to global chromatin decompaction rather than DNA strand breaks. Our data therefore allow us to speculate that a relaxed chromatin landscape induced by MSH-6-initiated processing of 5-FU-induced DNA damage interferes with rDNA organization in the nucleoli leading to autophagy induction. Consistent with this model, the specific degradation of RPS3 by autophagy in human cells indicated that autophagy contributes to remove faulty or excess nucleolar proteins.

In summary, the data presented here allow us to propose a model for crosstalk between the BER and MMR pathways in eliciting DDR activation in response to 5-FU where the BER enzyme EXO-3 generates a nicks required for MMR activation. Our data suggest that MSH-6-dependent processing of 5-FU-induced DNA damage interferes with rDNA organization in the nucleoli leading to autophagy induction. Together, our data strongly suggest that the role of autophagy in mediating DNA damage-induced cell death is more significant than previously anticipated.

Methods
C. elegans strains and culture conditions. C. elegans strains N2 Bristol, msh-2(kc2410) I, msh-1(ok1917) III and msh-6(pk2504) I were obtained from Caenorhabditis Genetics Center. The ung-1(qa7600) III strain was generated previously11. The exo-3 (tm437) and exo-1 (tm1842) were obtained from Shohai Mitani (Tokyo Women’s Medical University School of Medicine, Japan). All these strains were expected to be loss-of-function mutants, with the possible exception of the msh-6(ok1917) mutant, which, if producing a stable protein, would give rise to a truncated protein of 521 aa (Supplementary Fig. S1). The exo-3 msh-6 and exo-3 msh-1 double mutants were generated for this study. Reporter strains has the following genotypes: buEx070[plgg-1::GFP::LGG-1 þ rol-6(su1006)] and bee1(ok91); zen-5[exo-1::BEC-1::GFP þ rol-6(su1006)] and Ex[apf-2::GFP þ unc-119( þ)]; unc-119(c297), him-5(c490) V, adh-22[gg-1::GFP þ rol-6(su1006)] and atm-1(k186); adh-22[gg-1::GFP þ rol-6(su1006)], which were kind gifts from Katsuta (Tokyo University). The msh-2[pc120::GFP::LCG-1 þ rol-6(su1006)] and msh-2[plgg-1::GFP::LCG-1 þ rol-6(su1006)] strains were obtained from C. elegans Genetics Center. The following genotypes were used: let-56; w1118; rol-6(su1006); and Ex[apf-2::GFP þ unc-119( þ)]; unc-119(c297) generated for this study. Brieﬂy, the msh-2 coding region was ampliﬁed using the reverse primer 5′-TTTCCGGACGACAGTGAAGCAGTC-3′ and forward primer 5′-ccagcacttcgcttgAAACCGGC-G-3′, and cloned into the pDF575 vector into the SmaI and Xhol sites. The construct was co-copied with a plasmid containing unc-119( þ) into unc-119( –) mutant animals. The non-Unc progenies were selected and screened for GFP expression. Worms were cultured and
maintained at 20 °C using standard procedures, using OP50 as the food source. For experiments using the att-l1(m853) IV r Tcl1(unc-75m74) let-2 q50fer (IV; strain 7189), unc-119(e168) and unc-159 young adults were exposed to 5-FU, embryos fixed and stained with anti-unc-34 antibodies. Images of non-unc GFP-negative homozygous att-l1(m853) mutants are presented. For RNAi experiments, worms were maintained for three generations on Nematode Growth Medium (NGM) plates containing 2% agarose (isopyrid A [6-1-thiogalactopyranoside] seeded with Escherichia coli HT115(DE3) expressing RNAi constructs in the maintainer at 20°C.

C. elegans and antibodies. 5-FU, 5-fluoro-2-deoxyuridine, methylnethanolsulphonate, Mx and 3-MA were from Sigma (Oslo, Norway). The following commercially available antibodies were used: LG1, UK1, poly (ADP-ribose) polymerase, Caspase-3 and MSH-6 (Cell Signalling Technology); RPS3 and actin (Abcam); β-actin (Sigma-Aldrich); MSH2, P-Histone 3 (pSer10), P-CHK1 (pSer345) (Santa Cruz Inc.); anti-Cdk1 (pTyr15) (VWR); and GFP (Roche). Antibodies directed against the following C. elegans proteins were kindly gifts: VPS-34, Alexa 488-conjugated anti-rabbit and anti-rat (Invitrogen), RPA-1 from Hyeon Sook Koo (Yonsei University, Institute for Biophysical Chemistry, Goettingen, Germany). Secondary antibodies were used at the following dilutions: RAD-51 (1/200), RPA-1 (1/200), Histone 3 (pSer10) (1/400) and Cdk1 (pTyr15) (1/100). The following secondary antibodies were used for detection: Alexa Fluor 555-conjugated anti-rabbit at 1/1,500 for detection of VPS-34, Rad-51, LNM-1 and pCHK1. Alexa Fluor 555 anti-rabbit at 1/1,500 to detect RPA-1 (Gartner), and Alexa Fluor 488-conjugated anti-mouse at 1/1,000 to detect RPA-1 (Koo). The slides were imaged under a Zeiss LSM-510 META14 Konfocal microscope with × 63 Plan-Apochromat 1.4 NA objective.

C. elegans nuclear stress. Induction of nuclear stress was monitored as previously described25. Briefly, embryos were dissected out of 5-FU-treated hermaphrodites and observed continuously from the three-cell through the four-cell stage under differential interference contrast in a Zeiss Axiovert200M inverted microscope with × 100 Plan-Neofluar 1.45 NA objective. A fraction of four-cell stage embryos with visible nucleoli was scored in three independent experiments comprising a minimum of eight embryos per experiment.

C. elegans charinoti decomposition. To examine the charinoti decomposition state after 5-FU treatment, dissected embryos were immunostained with α-LNM-1 as described above to visualize the nuclear membrane. The maximum nuclear diameter was measured in 4-cell embryos in 15–32 embryos per condition after identification of the appropriate focal plane from a stack under a Zeiss LSM-510 META14 Konfocal microscope with × 63 Plan-Apochromat 1.4 NA objective.

Human cells and transfection. Human osteosarcoma U2OS cells were grown under 5% CO2 in DMEM media supplemented with 10% FCS, 100 U/ml penicillin and 100 µg/ml streptomycin (all from Gibco). Cells were transfected with siRNA oligo targets in the absence of nucleofection reagents. Cells were transfected into six-well plates (1.5 × 10^5 cells per well), using 1 ml transfection solution containing 0.64 µl Lipofectamine RNAiMAX (Invitrogen) and a final concentration of 10 nm siRNA according to the manufacturer’s instructions. After 5 h, the cells received complete growth medium and were cultured for 24 h before addition of the drugs indicated in the figure legend. For siRNA oligos, the following sequences (sense strand): UK1-a, 5′-CCGCAGAGGAGCCAGAC-3′ (Drmacon); UB1-b, 5′-UCAGUGACGCGUCUCAGAC-3′ (Drmacon); MSH-2, 5′-CGUGAGUCCCGAUCUCA-3′ (GE Healthcare Ambion). Control cell transfections were performed with a non-targeting control duplex (Drmacon).

Human cell immunofluorescence. U2OS cells were seeded in Lab-Tek II Chambered Coverglass (Nunc) 1 day before the experiments were performed and treated with 5-FU (10 µM) or Mx (1 µM) for the indicated times. The cells were then fixed in 100% methanol for 10 min and blocked in 5% FCS before incubation with anti-LC3 antibody (Clone 5F10, Nanotools), followed by incubation with Alexa488 labelled donkey anti-mouse antibody (Molecular Probes). The nuclei were stained with DAPI (1 µg ml^-1) in PBS. Pictures were taken using an Cell Observer microscope (Zeiss) equipped with × 40 objective. The number of LC3 spots per cell was quantified from 200 to 1,600 cells per condition in each experiment. Four independent experiments were performed and the mean number of LC3 spots per cell was normalized to that of untreated control cells.

Quantitative real-time reverse transcriptase—PCR. Transcriptional activation of C. elegans ced-13 and egl-1 was measured in synchronized L4 hermaphrodites treated with 100 µM 5-FU for 6 h and harvested upon complete breakdown of the exocyst under a Zeiss LSM-510 META14 Konfocal microscope with × 63 Plan-Apochromat 1.45 NA objective. A fraction of four-cell stage embryos with visible nucleoli was scored in three independent experiments comprising a minimum of eight embryos per experiment.
dDNA Synthesis Kit (Bio-Rad). The real-time PCR analysis was run on a CFX96 Real-time PCR Detection System (Bio-Rad) using SsoFast EvaGreen Supermix (Bio-Rad) and prevalidated Quanti-Tect Primer Assays (QIAGEN). The cycling conditions were 95 °C for 1 min, followed by 40 cycles of 95 °C for 5 s, 55 °C for 5 s and 72 °C for 7 s. Relative quantities of Exo-1 or RP53 transcripts were determined using the regression analysis method provided in the CFX Manager Software (Bio-Rad). Transcript quantities were normalized to the relative quantity of the housekeeping genes TBP (TATA-box binding protein) and SDHA (succinate dehydrogenase complex subunit A, flavoprotein) for each condition. The following prevalidated Quanti-Tect Primer Assays were used: Hs_RP53_2.5G, Hs_EXO_1.1G, Hs_TBP_1.1G and Hs_SDHA_2.5G.

Western blot analyses. Synchronized young GFP-LGG-1-expressing adults were cultured for 24 h on 10 cm OP50-seeded plates with no treatment or in the presence of 100 μM 5-FU. 3-MA (10 mM) was added after 20 h to inhibit autophagy. Embryos were collected by bleeding, washed and resuspended in worm lysin buffer (40 mM Tris-HCl, pH 7.4, 150 mM NaCl, 0.05%, NP-40, 2 mM EDTA, 1 tablet protease inhibitor from Roche) from N2, msh-6(kc2504), msh-2(ok2410) and MSH-2:GFP. After addition of 1.0 mm zirconia beads (BioSpec product), the embryos were disrupted in a Mini-Beadbeater 8 (BioSpec Products) as described previously.33 Protein concentrations were determined using the Bradford Reagent (Bio-Rad) and the extracts were separated on criterion 12.5% and 7.5% polyacrylamide gels (Bio-Rad), followed by electroblotting to Immobilon-P membranes (Millipore). The blots were probed with primary antibodies (GFP (1:1,000), actin (1:1000) and MSH-6 (1:1,000)) for 24–48 h at 4 °C overnight, washed, detected with anti-mouse IgG HRP-linked (Amersham) and anti-rabbit IgG HRP (Santa Cruz), and visualized using Supersignal west pico and west femto (Thermo Scientific).

For detection of histone 1 γ expression, protein extracts were prepared by boiling embryos in 1% SDS solubilizing buffer (0.3% ethanalamine, 2 mM EDTA, 1 mM phenethylmethylsulfonyl fluoride, 5 mM dithiothreitol and 1 × protease inhibitor for 25–50 s and immediately added SDS buffer along with reducing agent from the Nu-Page system (Invitrogen). The crude extract was separated in 12% Novex bis tris gel (Invitrogen). The blots were probed with anti H1X (1:1,000) antibodies, detected with ECL anti-rabbit IgG HRP (GE Healthcare) antibody at 1/2,500 dilution and visualized using Supersignal west pico and west femto (Thermo Scientific).

For preparation of human whole-cell extracts, U2OS cells were washed in cold PBS, lysed in RIPA buffer supplemented with a mixture of protease inhibitors (Roche) and 1 mM of MG132 (Enzo Life Sciences) for 25–50 s and immediately added SDS buffer along with reducing agent from the Nu-Page system (Invitrogen). The crude extract was separated in 12% Novex bis tris gel (Invitrogen). The blots were probed with specific antibodies, which were detected using standard ECL procedures. The band signal intensities were quantified by Image J (National Institute of Health).

Statistical analysis. Mean values ± s.e.m. were calculated for each condition. The statistical significance of the differences was determined by paired Student’s t-test. *P < 0.05, **P < 0.01, ***P < 0.001 were considered to be statistically significant.

References
1. Longley, D. B., Harkin, D. P. & Johnston, P. G. 5-fluorouracil: mechanisms of action and clinical strategies. Nat. Rev. Cancer. 3, 330–338 (2003).
2. Pettersen, H. S. et al. UNG-initiated base excision repair is the major repair pathway for 5-fluorouracil in DNA, but 5-fluorouracil cytotoxicity depends mainly on RNA incorporation. Nucleic Acids Res. 39, 8430–8444 (2011).
3. Lindahl, T. & Barnes, D. E. Repair of endogenous DNA damage. J. Cell. Biochem. 105, 245–254 (2008).
4. Wyatt, M. D. & Wilson, 3rd D. M. Participation of DNA repair in the response to double-strand breaks and autophagy. Nature 471, 74–79 (2011).
5. Holway, A. H., Kim, S. H., La Volpe, A. & Michael, W. M. Checkpoint silencing during the DNA damage response in Caenorhabditis elegans embryos. J. Cell Biol. 172, 999–1008 (2006).
6. Jiricny, J. The multifaceted mismatch-repair system. Nature 385, 389–396 (2000).
7. Deng, M. et al. Abrogation of the CLK-2 checkpoint leads to tolerance to base-excision repair intermediates. EMBO Rep. 7, 1046–1051 (2006).
8. Curtain, D. K., Rietveld, L., Goel, A., Chang, C. L. & Boland, C. R. Steady-state regulation of the human DNA mismatch repair system. J. Biol. Chem. 275, 1842–18431 (2000).
9. Kim, S., Park, D. H. & Shim, J. Thymidylate synthase and dihydroxyiminodéhydrogenase levels are associated with response to 5-fluorouracil in Caenorhabditis elegans. Mol. Cell. 26, 349–348 (2007).
10. Kourtis, N. & Tavernarakis, N. Autophagy and cell death in model organisms. Cell Death Differ. 16, 21–30 (2009).
11. Takacs-Vellai, K. et al. Inactivation of the autophagy gene bec-1 triggers apoptotic cell death in C. elegans. Curr. Biol. 15, 1513–1517 (2005).
12. Shatilla, A., Leduc, A., Yang, X. & Ramotar, D. Identification of two apurinic/apyrimidinic endonucleases from Caenorhabditis elegans by cross-species complementation. DNA Repair 4, 655–670 (2005).
13. Shatilla, A., Leduc, A., Yang, X. & Ramotar, D. Identification of two apurinic/apyrimidinic endonucleases from Caenorhabditis elegans by cross-species complementation. DNA Repair 4, 655–670 (2005).
14. Yang, X. et al. Functional characterization of the Caenorhabditis elegans DNA repair enzyme APN-1. DNA Repair 11, 811–822 (2012).
15. Zlatanov, A. et al. The human mismatch complex acts in concert with monoubiquitinated PCNA and Pol eta in response to oxidative DNA damage in human cells. Mol. Cell 43, 649–662 (2011).
16. Arczewska, K. D. et al. Caenorhabditis elegans NDX-4 is a MutT-type enzyme that contributes to genomic stability. DNA Repair 10, 176–187 (2011).
17. Duerer, J. S. in Immunohistochemistry. (ed. WormBook) (The C. elegans Research Community, WormBook 2006), doi/10.1895/wormbook.1.105.1 http://www.wormbook.org.
18. Beddington, R. D. & Scambler, P. Cell cycle checkpoint repair as a source of genomic instability in human cells. Mol. Cell 47, 669–680 (2012).
19. Chasan, S., Castor, D., Fischer, F. & Jiricny, J. Interference of mismatch and base excision repair during the processing of adjacent U/G mispairs may play a key role in somatic hypermutation. Proc. Natl Acad. Sci. USA 106, 5593–5598 (2009).
20. Shatilla, A., Leduc, A., Yang, X. & Ramotar, D. Identification of two apurinic/apyrimidinic endonucleases from Caenorhabditis elegans by cross-species complementation. DNA Repair 4, 655–670 (2005).
21. Yang, X. et al. Functional characterization of the Caenorhabditis elegans DNA repair enzyme APN-1. DNA Repair 11, 811–822 (2012).
22. Zlatanov, A. et al. The human mismatch complex acts in concert with monoubiquitinated PCNA and Pol eta in response to oxidative DNA damage in human cells. Mol. Cell 43, 649–662 (2011).
23. Arczewska, K. D. et al. Caenorhabditis elegans NDX-4 is a MutT-type enzyme that contributes to genomic stability. DNA Repair 10, 176–187 (2011).
24. Duerer, J. S. in Immunohistochemistry. (ed. WormBook) (The C. elegans Research Community, WormBook 2006), doi/10.1895/wormbook.1.105.1 http://www.wormbook.org.
25. Jiricny, J. The multifaceted mismatch-repair system. Nature reviews. Mol. Cell. Biol. 6, 335–345 (2006).
26. Skjeldam, H. K. et al. Loss of Caenorhabditis elegans UNG-1 uracil-DNA glycosylase affects apoptosis in response to DNA damaging agents. Repair 9, 861–870 (2010).
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Author contributions
H.N. conceived and designed the study; T.S.G., M.L.T., H.K. and T.V. performed experiments; T.V., A.S. and H.N. wrote the manuscript.

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Publisher Correction: Base excision repair AP endonucleases and mismatch repair act together to induce checkpoint-mediated autophagy

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