

# MRT-2 checkpoint protein is required for germline immortality and telomere replication in *C. elegans*

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The germ line is an immortal cell lineage that is passed indefinitely from one generation to the next. To identify the genes that are required for germline immortality, we isolated *Caenorhabditis elegans* mutants with mortal germ lines—worms that can reproduce for several healthy generations but eventually become sterile. One of these *mortal germline* (*mrt*) mutants, *mrt-2*, exhibits progressive telomere shortening and accumulates end-to-end chromosome fusions in later generations, indicating that the MRT-2 protein is required for telomere replication. In addition, the germ line of *mrt-2* is hypersensitive to X-rays and to transposon activity. Therefore, *mrt-2* has defects in responding both to damaged DNA and to normal double-strand breaks present at telomeres. *mrt-2* encodes a homologue of a checkpoint gene that is required to sense DNA damage in yeast. These results indicate that telomeres may be identified as a type of DNA damage and then repaired by the telomere-replication enzyme telomerase.

In most higher organisms, the germ line is responsible for perpetuation of the species. Differentiated germ cells fuse together to form a zygote, which then develops into an organism containing a soma and a new germ line, and this cycle repeats until a species becomes extinct. Thus, the germ line has the ability to proliferate indefinitely and can be thought of as an immortal cell lineage<sup>1</sup>.

We decided to study the problem of germline immortality in the nematode *C. elegans*. *C. elegans* worms clearly demarcate their germ cells from the very first zygotic cell division<sup>2</sup>. In addition, *C. elegans* populations are normally composed of self-fertilizing hermaphrodites that are homozygous at most or all genetic loci<sup>3</sup>. Therefore, a single worm can give rise to generations of descendants that are essentially genetically identical and whose germ lines represent a single continuous immortal cell lineage.

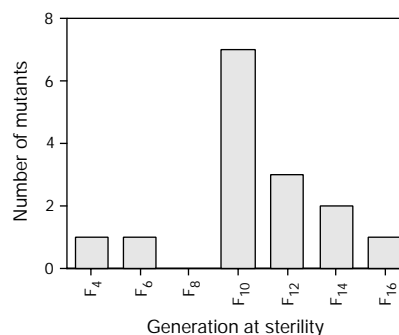
We have identified a number of *C. elegans* mutants with mortal germ lines. The *mrt-2* mutant displays the telomere shortening and late-onset chromosome fusion phenotypes that are seen in 'telomerase-negative' mouse and yeast mutants<sup>4–6</sup>. Telomerase is a reverse transcriptase that adds repeats to the ends of chromosomes<sup>7</sup>. Five genes are known to be required for telomerase activity *in vivo*<sup>8</sup>. Mutations in these genes result in late-onset sterility/senescence, and end-to-end chromosome fusions that can be explained by progressive telomere shortening<sup>4–6,8–10</sup>. However, the telomerase-defective *C. elegans mrt-2* mutant has additional phenotypes: it displays weak chromosome loss at all times and is also hypersensitive to agents that damage DNA. These results suggest that *mrt-2* may have a defect in its response to DNA damage (a defect in DNA repair, in a DNA damage checkpoint, or in both).

Some yeast mutants with defects in double-strand break DNA repair (*ku*, *sir* and the *mre11/xrs2/rad50* nuclease mutants) have short telomeres<sup>11–15</sup>. In addition, a number of mutants that have defects in sensing DNA damage (DNA damage checkpoint mutants) also exhibit telomere defects. For example, deficiencies in the human ATM checkpoint protein result in aberrant telomere shortening<sup>16,17</sup>, and a number of yeast checkpoint mutants have short but stable telomeres<sup>18–20</sup>. *mrt-2* encodes the *C. elegans* homologue of the *Schizosaccharomyces pombe rad1*<sup>+</sup> and *Saccharomyces cerevisiae RAD17* checkpoint genes. Although the *S. pombe rad1* mutant has shorter telomeres than wild type<sup>19</sup>, it does not exhibit the telomere catastrophe that we find in the higher eukaryote *C. elegans*.

## A screen for mortal germline mutants

To investigate how the germ line achieves immortality, a screen was conducted for *C. elegans* mutants with mortal germ lines. Four hundred lines were established from single F<sub>2</sub> progeny of ethylmethanesulphonate (EMS) mutagenized worms and grown clonally for 16 generations at 25 °C. Sixteen independent mortal germline mutants were identified that become effectively sterile (<2 progeny per worm) between generations F<sub>4</sub> and F<sub>16</sub> (Fig. 1). All of these *mrt* mutants behaved as expected for single recessive mutations when they were outcrossed (data not shown). The unexpectedly large number of *mrt* mutants that were identified in our small pilot screen suggests a conservative estimate of ~50 genes that are specifically required for germline immortality in *C. elegans* (the forward mutation frequency per gene is, on average, 1 in every 4,000 F<sub>2</sub> progeny from EMS-mutagenized worms)<sup>21</sup>.

Of these 16 *mrt* mutants, 12 are temperature-sensitive and will grow indefinitely at 15 °C or 20 °C, but will become sterile if they are shifted to 25 °C for several generations (data not shown). There are two possible explanations for the large number of temperature-sensitive mortal germline mutants that we uncovered. First, these



**Figure 1** The generation at sterility for 16 mortal germline mutants. Clonal strains were grown on petri dishes at 25 °C and could grow for two generations per dish before starvation. Strains were passaged by transferring six L1 larvae to a fresh dish, which ensured that zygotic or maternal sterile mutations that may have been segregating in the mutant strains would not be mistakenly identified as *mrt* mutations. Lines were scored as 'sterile' when there were less than two progeny per worm per plate.

temperature-sensitive mutations may identify genes that are required to protect the germ line against damage that accumulates only at high temperature. Second, there may be some redundancy in the genes that control germline immortality, such that one only observes a mutant phenotype at high temperature when one branch of a pathway has been knocked out by temperature and the other branch by mutation.

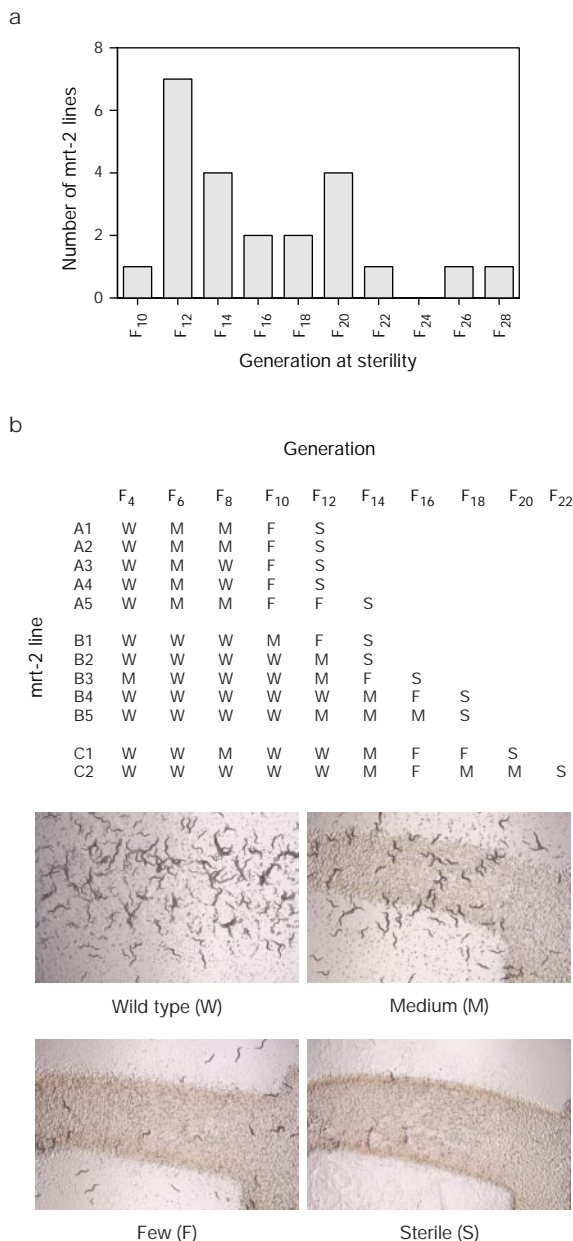
Four of the *mortal germline* mutants that we isolated became sterile at all temperatures (data not shown). These non-temperature-sensitive *mrt* mutants must be outcrossed periodically to

maintain them. One of these mutants, *mrt-2* (e2663), became sterile at generation F<sub>14</sub> when it was first identified in our *mortal germline* screen. *mrt-2* was outcrossed several times, and homozygous *mrt-2* lines were re-established. The generation at sterility for 23 different *mrt-2* lines varied widely, from generation F<sub>10</sub> to generation F<sub>28</sub> (Fig. 2a). However, different F<sub>2</sub> lines from the same F<sub>1</sub> heterozygote all became sterile at about the same time (Fig. 2b; and data not shown). These results suggest that *mrt-2* accumulates some kind of damage that is inherited and segregates evenly amongst the progeny of an outcross. In addition, *mrt-2* produces normal brood sizes at generation F<sub>2</sub> (about 300 progeny per worm), but brood size gradually drops to the point of sterility (Fig. 2b). This gradual drop in brood size indicates that the germ line of *mrt-2* accumulates damage slowly over many generations. *mrt-2* has additional phenotypes, described below, which led us to focus attention on it.

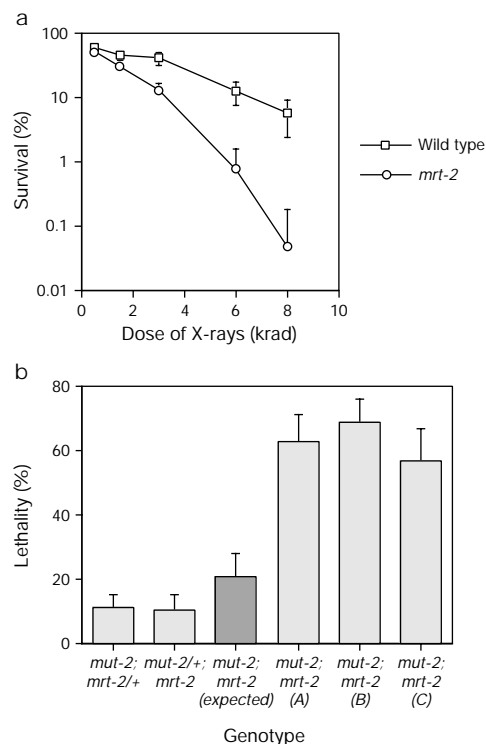
### Genome instability in the *mrt-2* mutant

*C. elegans* populations are normally composed of XX hermaphrodites, but XO males occasionally arise as a result of X-chromosome loss<sup>22</sup>. *mrt-2* displays a weak high incidence of males (Him) phenotype and produces  $0.9 \pm 0.6\%$  males ( $n = 25$ , F<sub>3</sub> broods) as opposed to the 0.2% normally observed in wild type. This increase in X-chromosome loss suggests a defect in genome stability.

Although the *mrt-2* germ line is not significantly hypersensitive to ultraviolet light (data not shown), it is hypersensitive to X-rays (Fig. 3a) which can damage DNA by inducing double-strand breaks. In another test of the ability of *mrt-2* to withstand double-strand breaks, we used a *C. elegans* mutator strain, *mut-2*(r459), which has high levels of transposon activity in its germ line that are likely to result in double-strand breaks<sup>23</sup>. The germ lines of *mrt-2*;*mut-2*(r459) double mutants gave rise to progeny with two to three times the level of lethality expected for the sum of the single mutants



**Figure 2** The Mortal Germline phenotype of *mrt-2*. **a**, The generation at sterility for 23 different *mrt-2*F<sub>2</sub> lines. **b**, Brood size drops for 12 *mrt-2* lines. Three different *mrt-2*+ F<sub>1</sub> lines (A, B and C) gave rise to the *mrt-2* sibling lines that are shown. Brood size was determined by examining plates seeded with 6 *mrt-2*L1 larvae after 7 days of growth at 20 °C. Plates were scored as wild type (W), medium (M), few (F) and sterile (S). A light-brown *Escherichia coli* lawn used to feed *C. elegans* worms is visible on M, F and S plates. Brood sizes of individual worms at these stages were wild type, 262 ± 115 (4 ± 4 dead eggs); medium, 70 ± 52 (13 ± 12 dead eggs); few, 20 ± 11 (55 ± 31 dead eggs); and sterile, 4 ± 5 (47 ± 43 dead eggs) (10 broods scored at each level of fecundity; 5 each from 2 different lines).



**Figure 3** *mrt-2* has defects in responding to DNA damage. **a**, *mrt-2* germ lines are more sensitive to X-rays than are N2 wild-type germ lines (10 broods scored at each dose). **b**, Levels of embryonic lethality in three independent lines (A, B and C) of *mut-2*; *mrt-2* double mutants and their *mut-2*;*mrt-2*+ and *mut-2*+;*mrt-2* siblings (9–10 broods scored for each column). The expected additive lethality for *mut-2* and *mrt-2* is indicated (dark grey).

(Fig. 3b). The simplest explanation for this synergistic *mrt-2*;*mut-2(r459)* lethality is that *mrt-2* is defective in responding to double-strand breaks caused either by transposition or by X-rays.

### Chromosome fusions and telomere shortening

To gain further insight into the genome stability defect of *mrt-2*, *mrt-2* worms were stained with diamidinophenolindole (DAPI) and their chromosomes were examined. Early generation *mrt-2* worms had the six pairs of chromosomes expected for wild-type, whereas late generation *mrt-2* worms contained only three, four or five DAPI-staining chromosome pairs per worm, suggesting the occurrence of chromosome fusions (Fig. 4a). In addition, the number of visible chromosome pairs decreased over the course of eight generations in late-generation *mrt-2* lines (Fig. 4b).

When late-generation *mrt-2* worms were outcrossed, we frequently observed a dominant Him phenotype. This unusual phenotype is known to occur in *C. elegans* as a result of X-autosome

chromosome fusions<sup>24</sup>. Although in most organisms dicentric chromosome fusions are frequently torn apart during mitosis<sup>25</sup>, *C. elegans* has holocentric chromosomes<sup>26</sup>, therefore chromosome fusions are stable and can be mapped genetically<sup>24</sup>. The dominant Him phenotype from 12 independent *mrt-2* lines consistently mapped to one end of the X chromosome (data not shown). In three out of three cases examined, this phenotype also mapped to the end of an autosome (different in each case) (Fig. 4c), confirming the presence of X-autosome chromosome fusions in late-generation *mrt-2* worms. All chromosome fusions tested were homozygous viable ( $n = 10$ ; data not shown), indicating that they were not missing any essential genes and were therefore probably end-to-end chromosome fusions.

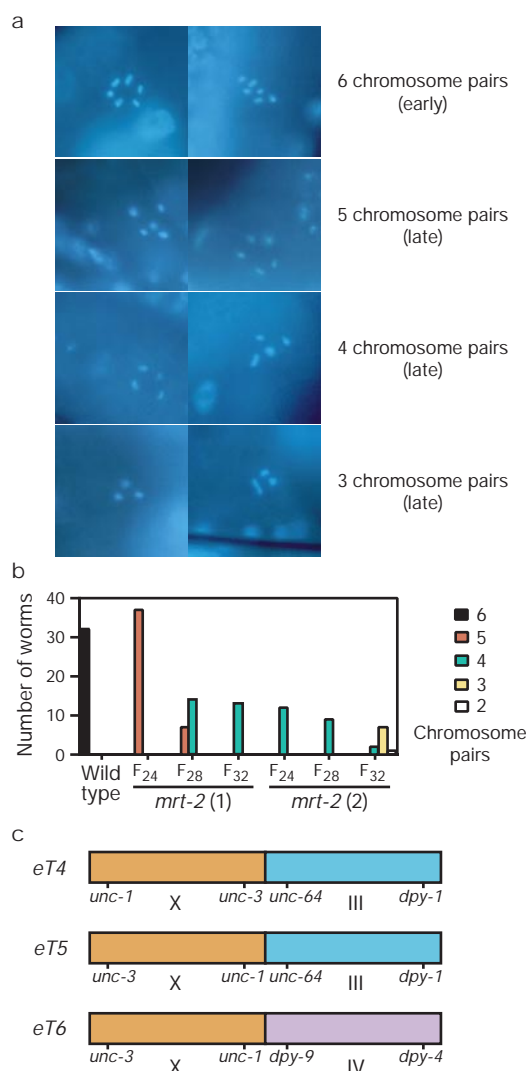
The late-onset end-to-end chromosome fusion phenotype of *mrt-2* suggested a defect in telomere replication<sup>4-6</sup>. *C. elegans* has telomeres consisting of simple tandem TTAGGC repeats similar to those found in most other eukaryotes<sup>27</sup>. In Southern blots, the telomeres of wild-type worms appear as a smear that runs from 2 to 7 kilobases (kb) and either stays fairly constant in length at 20 °C (Fig. 5a), or increases in length if worms have short telomeres to begin with (Fig. 5b). In contrast, the telomeres of *mrt-2* worms shorten at a rate of about 12 base pairs (bp) per cell division (about 125 bp per generation and an estimated 10 cell divisions per generation) (Fig. 5). *mrt-2* telomeres shorten progressively at least until generation F<sub>18</sub> (Fig. 5c), by which time most *mrt-2* lines have become sterile (Fig. 2a). Occasional telomere signals disappear as *mrt-2* worms are passaged (Fig. 5a, arrow), which may be the consequence of a telomere fusion event, a recombination event, or perhaps the segregation of long and short alleles of a particular telomere.

In initial experiments, *mrt-2* lines always produced X-autosome chromosome fusions that had fused at the right end of the X chromosome ( $n = 5$ ). This result indicates that the right telomere of the X chromosome may be prone to fusion events, perhaps because it is shorter than the left. To test this possibility, we identified several *mrt-2* lines that were homozygous for a short telomere at the left end of the X chromosome. Four independent X-autosome chromosome fusions were recovered from these lines, of which two had fused at the right end and two had fused at the left end of the X chromosome (Fig. 2d, *eT5*; and data not shown). Thus, the left end of the X chromosome will undergo late-generation chromosome fusion events if its initial length is short. These results suggest that telomere shortening causes the late on-set chromosome fusions observed in *mrt-2* (Fig. 2).

A dominant-negative allele of the telomere-binding protein TRF2 can induce end-to-end chromosome fusions in mammalian cells<sup>28</sup>. This phenotype occurs within a few cell divisions, however, and the resulting telomere–telomere fusions produce an increase in telomere length as observed by Southern blot<sup>28</sup>. End-to-end chromosome fusions have also been observed in mice mutant for poly(ADP-ribose) polymerase, and telomere length in these mice is short but stable<sup>29</sup>. These chromosome-fusion phenotypes are distinct from the late-onset chromosome fusion and progressive telomere shortening phenotypes observed in *mrt-2*. Instead, *mrt-2* is similar to the telomerase-negative mouse and yeast mutants, which also experience progressive telomere shortening, late-onset chromosome fusions and late-onset sterility/senescence<sup>4-6,9</sup>. Although yeast telomerase-negative mutants can use recombination to periodically recover from senescence<sup>5,30</sup>, we have never observed a *mrt-2* line to recover from its Mortal Germline phenotype (Fig. 2). The late-onset sterility of *mrt-2* lines probably results from increasing numbers of chromosome fusions, which will lead to meiotic non-disjunction and massive aneuploidy.

### *mrt-2* is a checkpoint gene

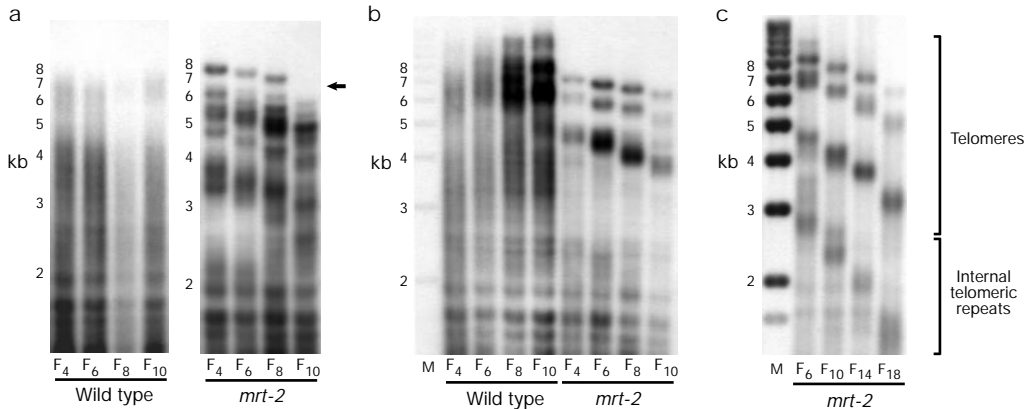
The *mrt-2* mutant is defective in responding both to normal double-strand breaks present at telomeres and to abnormal DNA damage



**Figure 4** *mrt-2* exhibits late-onset end-to-end chromosome fusions. **a**, DAPI-stained early and late generation *mrt-2* oocyte nuclei arrested at metaphase I in diakinesis (when homologous chromosomes are synapsed together). To ensure that chromosome numbers were accurately determined, three or more oocytes with clearly separated bivalents were scored per worm. These are photographs of representative nuclei in which all chromosome pairs happen to lie in the same plane of focus. **b**, Number of worms containing a particular number of visible chromosome pairs. Wild-type control and successive generations of two late generation *mrt-2* strains (1 and 2) are shown. **c**, X-autosome chromosome fusions isolated from *mrt-2* display pseudo-linkage between an end of the X chromosome and an end of an autosome.

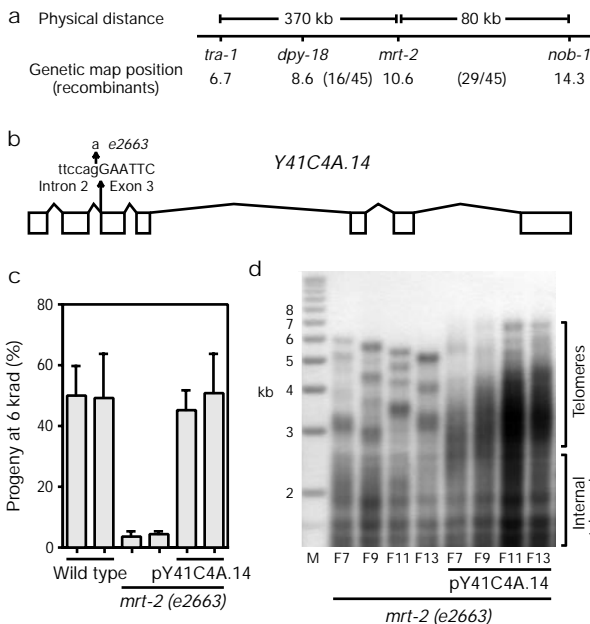
caused by X-rays and transposition. We mapped *mrt-2* between *dpy-18* and *nob-1* on the right arm of chromosome III (Fig. 6a). Blast analysis of this region of the genome revealed a gene *Y41C4A.14*, also known as *hpr-1*, a homologue of the *S. pombe rad1*<sup>+</sup> checkpoint gene<sup>31,32</sup>. The *S. pombe rad1* mutant is hypersensitive to X-rays<sup>33</sup> and has short telomeres<sup>19</sup>. This candidate gene was sequenced in *mrt-2*(*e2663*) and contained a mutation in the 3' splice junction of the second intron (Fig. 6b). This mutation occurs in a highly conserved AG dinucleotide that is found at the 3' ends of most *C. elegans*

introns. Five independent extrachromosomal arrays containing the wild type *Y41C4A.14* gene were able to rescue the X-ray hypersensitivity, telomere shortening and Mortal Germline phenotypes of *mrt-2* (Fig. 6c, d; and data not shown). In contrast, when *green fluorescent protein* was inserted in-frame between exons 1 and 2 of *Y41C4A.14*, the fusion construct failed to rescue *mrt-2* (data not shown), confirming that *mrt-2* is the *Y41C4A.14* checkpoint gene. *mrt-2* is the *C. elegans* homologue of the *S. pombe rad1*<sup>+</sup> and *S. cerevisiae RAD17* checkpoint genes that are conserved from yeast

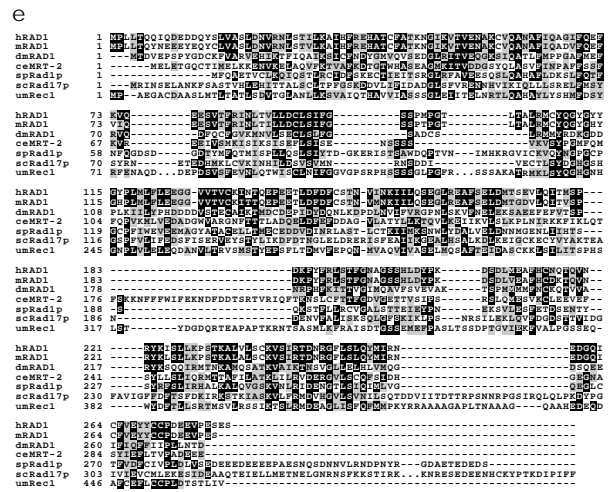


**Figure 5** *mrt-2* has a progressive telomere-shortening phenotype. Southern blots were probed with the *C. elegans* telomere sequence (TTAGGC)<sub>26</sub>. **a**, *mrt-2* telomeres shorten progressively. An arrow marks an *mrt-2* telomere that vanishes in this lineage. **b**, If a *C. elegans* strain with short telomeres, such as *mrt-2*, is crossed with a wild-type strain and

wild-type F<sub>2</sub> are picked, telomeres from the parent with short telomeres will elongate as they equilibrate back to normal lengths. In contrast, telomeres of a sibling *mrt-2* strain all shorten progressively. **c**, Telomere length of a *mrt-2* strain grown until generation F<sub>1a</sub>.



**Figure 6** *mrt-2* encodes a homologue of the *S. pombe rad1*<sup>+</sup> and *S. cerevisiae RAD17* checkpoint genes. **a**, *mrt-2* was mapped between *dpy-18* and *nob-1* using three-factor crosses<sup>50</sup>, by picking Dpy-non-Nob and Nob-non-Dpy F<sub>2</sub> progeny from *dpy-18nob-1/mrt-2* F<sub>1</sub> and scoring the X-ray hypersensitivity phenotype in the F<sub>3</sub> generation. The number of recombinants picked between these genes is indicated in parentheses. Although *mrt-2* maps closer to *dpy-18* than to *nob-1*, *mrt-2* is only 80 kb from *nob-1* indicating that there may be some distortion of genetic distance near *nob-1*. **b**, *mrt-2* (*e2663*) contains a G to A mutation in the splice acceptor of intron 2 of the *Y41C4A.14/hpr-1* checkpoint gene. **c**, Two extrachromosomal arrays containing a single 7-kb gene—the wild type *Y41C4A.14* gene (pY41C4.14)—rescue the X-ray hyper-



sensitivity of *mrt-2*(*e2663*) strains. Non-rescued *mrt-2* sibs and N2 wild type controls are shown (5 broods scored for each column). **d**, Telomere length in a *mrt-2* strain carrying an extrachromosomal array containing pY41C4A.14. A non-rescued *mrt-2*(*e2663*) sibling strain is shown for comparison. **e**, MRT-2 homologues. Identities are highlighted in black and conservative substitutions in grey. The proteins are human RAD1 (hRAD1), mouse RAD1 (mRAD1), *D. melanogaster* RAD1 (dmRAD1), *C. elegans* MRT-2 (ceMRT-2), *S. pombe* Rad1p (spRad1p), *S. cerevisiae* Rad1p (scRad1p) and *U. maydis* Rec1 (umRec1). Long stretches of non-homologous sequences were omitted (dotted line) for ScRad1p and umRec1. Sequences were aligned using clustalW1.7 and then manually adjusted.

to mammals (Fig. 6e)<sup>31,32,34,35</sup>. In yeast, these genes are required to delay cell-cycle progression in response to DNA damage or in response to a block in DNA replication<sup>33,36,37</sup>. As might be expected for a *C. elegans* checkpoint mutant, the germ line of *mrt-2* is defective in responding to DNA damage caused by X-rays and transposition (Fig. 3). In addition, *mrt-2* exhibits the progressive telomere shortening, end-to-end chromosome fusion, and late-onset sterility phenotypes that are seen in 'telomerase-negative' mouse and yeast mutants<sup>4-6</sup>. Thus, the *mrt-2* checkpoint gene is required for telomere replication. Although several yeast checkpoint mutants including *S. pombe rad1* have telomeres that equilibrate to lengths that are shorter than wild type, they do not exhibit the progressive telomere shortening and senescence phenotypes characteristic of yeast telomerase mutants<sup>19</sup>. Curiously, *S. pombe rad1*<sup>+</sup> is in the same epistasis group as *rad3*<sup>+</sup> (ref. 33), and a *rad3,tel1* double mutant that is mutant for two related phosphatidylinositol-3-like kinases has a 'telomerase-negative' phenotype *in vivo*<sup>6</sup>. The homologous *S. cerevisiae* double mutant, *mec1,tel1*, also appears to lack telomerase activity *in vivo*<sup>20</sup>. Although *tel1* mutants do not have major defects in checkpoint function<sup>38,39</sup>, these results argue that the *rad1*<sup>+</sup>/*RAD17*/*mrt-2* checkpoint may be required for telomere replication in yeast, and that genetic redundancy may mask this function in single checkpoint mutants.

How might checkpoint proteins recognize telomeres? In *S. cerevisiae*, the *rad1*<sup>+</sup>/*RAD17*/*mrt-2* checkpoint can delay the cell cycle in response to a single double-strand break<sup>40</sup>. Telomeres are double-strand breaks with short 3' overhangs<sup>41</sup>, and these ends fold back and the 3' overhangs are buried in double-stranded telomeric DNA in human cells<sup>42</sup>. These telomeric loops may unfold during S phase to reveal double-strand breaks that could evoke a checkpoint response. Alternatively, a checkpoint response can be triggered in yeast as a result of a block in DNA replication<sup>36,37</sup>, and it is possible that replication forks stall at telomeres and that these structures are recognized by checkpoint proteins.

Which checkpoint function might be required for telomere replication? Loss of the *S. cerevisiae* telomere-binding protein Cdc13p results in *RAD17*-dependent degradation of the C-rich strand of telomeres<sup>43</sup>, suggesting that the *RAD17* checkpoint may be involved in the C-strand degradation that is normally observed in late S phase and may be required for telomerase activity<sup>44</sup>. In this context, it is relevant that an allele of *CDC13* is telomerase negative<sup>45</sup>. It is also possible that checkpoint proteins recognize telomeres as a special kind of DNA damage and actively recruit telomerase to initiate repair. In *S. cerevisiae*, the Ku and Sir double-strand-break repair proteins are stored in reservoirs at telomeres but then migrate to double-strand breaks elsewhere in the genome in a checkpoint-dependent manner<sup>46,47</sup>. In addition, work in the ciliate *Oxytricha* has shown that the telomerase RNA is sequestered away from telomeres during most of the cell cycle, but colocalizes with replicating DNA during S phase<sup>48</sup>.

Our study shows that the problems of telomere maintenance and germline immortality can be addressed using an unbiased forward genetic approach in *C. elegans*. Our main conclusion is that a deficiency for a single DNA damage checkpoint protein can result in a telomerase-negative phenotype *in vivo*. This finding was unanticipated from studies of yeast checkpoint mutants<sup>19</sup> and has implications for genome stability in germline and somatic cells of other higher eukaryotes such as mammals. For example, mice that are deficient in both telomerase and the p53 checkpoint protein display high levels of cancer<sup>49</sup>; thus, the genome instability resulting from a telomerase deficiency has serious consequences in the absence of a response to DNA damage. The checkpoint protein described here, MRT-2, is required both for telomere replication and for response to DNA damage. If the human MRT-2 homologue, RAD1, shares these characteristics, then its loss would result in a double-edged sword: in this context, we note the presence of a tumour suppressor gene near the human RAD1 locus<sup>31,32,34</sup>. □

## Methods

### Strains

*C. elegans* strains were mutagenized, cultured and crossed as described<sup>50</sup>. All experiments were carried out at 20 °C unless otherwise stated.

### X-ray hypersensitivity

Young L4 larvae were irradiated in a Torrex X-ray machine at 143kV, and larvae were picked to separate plates and transferred after 48 h and again after 24 h. Plates were scored for adult progeny 36 h after transfer. The number of progeny from irradiated worms was compared with that of unirradiated siblings to give the percentage of survival after irradiation, providing a measure of how sensitive a worm's germ line is to X-rays.

### *mrt-2* molecular genetics

The *Y41C4A.14* gene was amplified by polymerase chain reaction (PCR) from multiple wild-type and *mrt-2*(*e2663*) strains and sequenced directly to ascertain the presence of the *mrt-2*(*e2663*) splice-junction mutation. The wild-type *Y41C4A.14* gene including its 4-kb promoter was PCR-amplified from a *Y41C4* yeast artificial chromosome (YAC) miniprep for subcloning. Plasmid DNA was injected at 0.5 ng μl<sup>-1</sup> pY41C4A.14 (*FspI*-linearized), 0.5 ng μl<sup>-1</sup> pCes1943 (*rol-6*, gift of Diana Janke) (*NruI*-linearized) and 50 ng μl<sup>-1</sup> N2 genomic DNA (*PvuII*-linearized).

### Telomere length

Genomic DNA was prepared using a Puregene DNA isolation kit (Gentra). *HinFI*-digested genomic DNA was separated on 0.6% agarose gels at 1.5V cm<sup>-1</sup>, and Southern blotting was carried out using a digoxigenin-dUTP-labelled PCR probe according to manufacturer's protocols (Boehringer Mannheim). The probe was made using T3 and Te12 (5'-GAATAATGAGAATTTTCAGGC-3') primers to amplify telomeric repeats from the cTel5X plasmid<sup>27</sup> using PCR.

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1. Wylie, C. Germ cells. *Cell* **96**, 165–174 (1999).
2. Strome, S. & Wood, W. B. Immunofluorescence visualization of germ-line-specific cytoplasmic granules in embryos, larvae, and adults of *Caenorhabditis elegans*. *Proc. Natl Acad. Sci. USA* **79**, 1558–1562 (1982).
3. Hodgkin, J. & Doniach, T. Natural variation and copulatory plug formation in *Caenorhabditis elegans*. *Genetics* **146**, 149–164 (1997).
4. Blasco, M. A. *et al.* Telomere shortening and tumor formation by mouse cells lacking telomerase RNA. *Cell* **91**, 25–34 (1997).
5. Nakamura, T. M., Cooper, J. P. & Cech, T. R. Two modes of survival of fission yeast without telomerase. *Science* **282**, 493–496 (1998).
6. Naito, T., Matsuura, A. & Ishikawa, F. Circular chromosome formation in a fission yeast mutant defective in two ATM homologues. *Nature Genet.* **20**, 203–206 (1998).
7. Greider, C. W. & Blackburn, E. H. The telomere terminal transferase of *Tetrahymena* is a ribonucleoprotein enzyme with two kinds of primer specificity. *Cell* **51**, 887–898 (1987).
8. Nugent, C. I. & Lundblad, V. The telomerase reverse transcriptase: components and regulation. *Genes Dev.* **12**, 1073–1085 (1998).
9. Lundblad, V. & Szostak, J. W. A mutant with a defect in telomere elongation leads to senescence in yeast. *Cell* **57**, 633–643 (1989).
10. Lee, H. W. *et al.* Essential role of mouse telomerase in highly proliferative organs. *Nature* **392**, 569–574 (1998).
11. Palladino, F. SIR3 and SIR4 proteins are required for the positioning and integrity of yeast telomeres. *Cell* **75**, 543–555 (1993).
12. Porter, S. E., Greenwell, P. W., Ritchie, K. B. & Petes, T. D. The DNA-binding protein Hdf1p (a putative Ku homologue) is required for maintaining normal telomere length in *Saccharomyces cerevisiae*. *Nucleic Acids Res.* **24**, 582–585 (1996).
13. Boulton, S. J. & Jackson, S. P. Identification of a *Saccharomyces cerevisiae* Ku80 homologue: roles in DNA double strand break rejoining and in telomeric maintenance. *Nucleic Acids Res.* **24**, 4639–4648 (1996).
14. Boulton, S. J. & Jackson, S. P. Components of the Ku-dependent non-homologous end-joining pathway are involved in telomeric length maintenance and telomeric silencing. *EMBO J.* **17**, 1819–1828 (1998).
15. Nugent, C. I. *et al.* Telomere maintenance is dependent on activities required for end repair of double-strand breaks. *Curr. Biol.* **8**, 657–660 (1998).
16. Vaziri, H. *et al.* ATM-dependent telomere loss in aging human diploid fibroblasts and DNA damage lead to the post-translational activation of p53 protein involving poly(ADP-ribose) polymerase. *EMBO J.* **16**, 6018–6033 (1997).
17. Sprung, C. N., Bryan, T. M., Reddel, R. R. & Murnane, J. P. Normal telomere maintenance in immortal ataxia telangiectasia cell lines. *Mutat. Res.* **379**, 177–184 (1997).
18. Greenwell, P. W. *et al.* TEL1, a gene involved in controlling telomere length in *S. cerevisiae*, is homologous to the human ataxia telangiectasia gene. *Cell* **82**, 823–829 (1995).
19. Dahlen, M., Olsson, T., Kanter-Smoler, G., Ramne, A. & Sunnerhagen, P. Regulation of telomere length by checkpoint genes in *Schizosaccharomyces pombe*. *Mol. Biol. Cell.* **9**, 611–621 (1998).
20. Ritchie, K. B., Mallory, J. C. & Petes, T. D. Interactions of TLC1 (which encodes the RNA subunit of telomerase), TEL1, and MEC1 in regulating telomere length in the yeast *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **19**, 6065–6075 (1999).
21. Anderson, P. Mutagenesis. *Methods Cell Biol.* **48**, 31–58 (1995).
22. Hodgkin, J. A., Horvitz, H. R. & Brenner, S. Nondisjunction mutants of the nematode *C. elegans*. *Genetics* **91**, 67–94 (1979).
23. Plasterk, R. H. A. & van Leunen, H. G. A. M. *Transposons in C. elegans II* (eds Riddle, D. L., Blumenthal, T., Meyer, B. J. & Priess, J. R.) 97–116 (Cold Spring Harbor Laboratory Press, Plainview, 1997).
24. Herman, R. K., Kari, C. K. & Hartman, P. S. Dominant X-chromosome nondisjunction mutants of *Caenorhabditis elegans*. *Genetics* **102**, 379–400 (1982).

25. McClintock, B. The stability of broken ends of chromosomes of *Zea mays*. *Genetics* **26**, 234–282 (1941).
26. Albertson, D. G. & Thomson, J. N. Segregation of holocentric chromosomes at meiosis in the nematode, *Caenorhabditis elegans*. *Chromosome Res.* **1**, 15–26 (1993).
27. Wicky, C. *et al.* Telomeric repeats (TTAGGC)<sub>n</sub> are sufficient for chromosome capping function in *Caenorhabditis elegans*. *Proc. Natl Acad. Sci. USA* **93**, 8983–8988 (1996).
28. van Steensel, B., Smogorzewska, A. & de Lange, T. TRF2 protects human telomeres from end-to-end fusions. *Cell* **92**, 401–413 (1998).
29. d'Adda di Fagagna, F. *et al.* Functions of poly(ADP-ribose) polymerase in controlling telomere length and chromosomal stability. *Nature Genet.* **23**, 76–80 (1999).
30. Lundblad, V. & Blackburn, E. H. An alternative pathway for yeast telomere maintenance rescues *est1*–senescence. *Cell* **73**, 347–360 (1993).
31. Dean, F. B., Lian, L. & O'Donnell, M. cDNA cloning and gene mapping of human homologs for *Schizosaccharomyces pombe rad17*, *rad1*, and *hus1* and cloning of homologs from mouse, *Caenorhabditis elegans*, and *Drosophila melanogaster*. *Genomics* **54**, 424–436 (1998).
32. Bluysen, H. A. *et al.* A human and mouse homolog of the *Schizosaccharomyces pombe rad1+* cell cycle checkpoint control gene. *Genomics* **54**, 331–337 (1998).
33. al-Khodairy, F. & Carr, A. M. DNA repair mutants defining G2 checkpoint pathways in *Schizosaccharomyces pombe*. *EMBO J.* **11**, 1343–1350 (1992).
34. Marathi, U. K. *et al.* RAD1, a human structural homolog of the *Schizosaccharomyces pombe* RAD1 cell cycle checkpoint gene. *Genomics* **54**, 344–347 (1998).
35. Freire, R. *et al.* Human and mouse homologs of *Schizosaccharomyces pombe rad1(+)* and *Saccharomyces cerevisiae RAD17*: linkage to checkpoint control and mammalian meiosis. *Genes Dev.* **12**, 2560–2573 (1998).
36. Weinert, T. A. & Hartwell, L. H. Cell cycle arrest of cdc mutants and specificity of the RAD9 checkpoint. *Genetics* **134**, 63–80 (1993).
37. Enoch, T., Carr, A. M. & Nurse, P. Fission yeast genes involved in coupling mitosis to completion of DNA replication. *Genes Dev.* **6**, 2035–2046 (1992).
38. Morrow, D. M., Tagle, D. A., Shiloh, Y., Collins, F. S. & Hieter, P. *TEL1*, an *S. cerevisiae* homolog of the human gene mutated in ataxia telangiectasia, is functionally related to the yeast checkpoint gene *MEC1*. *Cell* **82**, 831–840 (1995).
39. Matsuura, A., Naito, T. & Ishikawa, F. Genetic control of telomere integrity in *Schizosaccharomyces pombe*, *rad3(+)* and *tel1(+)* are parts of two regulatory networks independent of the downstream protein kinases *chk1(+)* and *cds1(+)*. *Genetics* **152**, 1501–1512 (1999).
40. Sandell, L. L. & Zakian, V. A. Loss of a yeast telomere: arrest, recovery, and chromosome loss. *Cell* **75**, 729–739 (1993).
41. Henderson, E. in *Telomeres* (eds Blackburn, E. H. & Greider, C. W.) 35–68 (Cold Spring Harbor Laboratory Press, Plainview, 1995).
42. Griffith, J. D. *et al.* Mammalian telomeres end in a large duplex loop. *Cell* **97**, 503–514 (1999).
43. Lydall, D. & Weinert, T. Yeast checkpoint genes in DNA damage processing: implications for repair and arrest. *Science* **270**, 1488–1491 (1995).
44. Wellinger, R. J., Wolf, A. J. & Zakian, V. A. *Saccharomyces* telomeres acquire single-strand TGI-3 tails late in S phase. *Cell* **72**, 51–60 (1993).
45. Nugent, C. L., Hughes, T. R., Lue, N. F. & Lundblad, V. Cdc13p: a single-strand telomeric DNA-binding protein with a dual role in yeast telomere maintenance. *Science* **274**, 249–252 (1996).
46. Mills, K. D., Sinclair, D. A. & Guarente, L. *MEC1*-dependent redistribution of the Sir3 silencing protein from telomeres to DNA double-strand breaks. *Cell* **97**, 609–620 (1999).
47. Martin, S. G., Laroche, T., Suka, N., Grunstein, M. & Gasser, S. M. Relocalization of telomeric Ku and SIR proteins in response to DNA strand breaks in yeast. *Cell* **97**, 621–633 (1999).
48. Fang, G. & Cech, T. R. Telomerase RNA localized in the replication band and spherical subnuclear organelles in hypotrichous ciliates. *J. Cell Biol.* **130**, 243–253 (1995).
49. Chin, L. *et al.* p53 deficiency rescues the adverse effects of telomere loss and cooperates with telomere dysfunction to accelerate carcinogenesis. *Cell* **97**, 527–538 (1999).
50. Sulston, J. & Hodgkin, J. in *The Nematode Caenorhabditis elegans* (ed. Wood, W. B.) 587–606 (Cold Spring Harbor Laboratory Press, Plainview, 1988).

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