# Checkpoint silencing during the DNA damage response in *Caenorhabditis elegans* embryos

Antonia H. Holway,[1] Seung-Hwan Kim,[1] Adriana La Volpe,[2] and W. Matthew Michael[1]

[1]The Biological Laboratories, Department of Molecular and Cellular Biology, Harvard University, Cambridge, MA 02138
[2]Istituto di Genetica e Biofisica "Adriano Buzzati-Traverso," Consiglio Nazionale delle Ricerche, 80125 Naples, Italy

In most cells, the DNA damage checkpoint delays cell division when replication is stalled by DNA damage. In early *Caenorhabditis elegans* embryos, however, the checkpoint responds to developmental signals that control the timing of cell division, and checkpoint activation by nondevelopmental inputs disrupts cell cycle timing and causes embryonic lethality. Given this sensitivity to inappropriate checkpoint activation, we were interested in how embryos respond to DNA damage. We demonstrate that the checkpoint response to DNA damage is actively silenced in embryos but not in the germ line. Silencing requires *rad-2*, *gei-17*, and the *polh-1* translesion DNA polymerase, which suppress replication fork stalling and thereby eliminate the checkpoint-activating signal. These results explain how checkpoint activation is restricted to developmental signals during embryogenesis and insulated from DNA damage. They also show that checkpoint activation is not an obligatory response to DNA damage and that pathways exist to bypass the checkpoint when survival depends on uninterrupted progression through the cell cycle.

## Introduction

Early embryogenesis in many organisms, including *Xenopus laevis*, *Drosophila melanogaster*, and *Caenorhabditis elegans,* is characterized by rapid progression through the cell cycle (for review see O'Farrell et al., 2004). Features of early embryonic cell cycles that distinguish them from somatic cycles include cell division in the absence of cell growth and a lack of Gap phases. Another important difference between somatic and embryonic cell cycles concerns the utilization of S phase checkpoint pathways. In somatic cells, the S phase checkpoint senses DNA damage and responds by delaying progression into mitosis (for reviews see Bartek et al., 2004; Sancar et al., 2004). The protein kinases ATR and Chk1 are central to S phase checkpoint signaling. DNA damage causes replication fork stalling, which in turn activates ATR and promotes the ATR-directed phosphorylation of Chk1. Activated Chk1 delays cell cycle progression through attenuation of core cell cycle regulators such as the Cdc25 protein phosphatase. Thus, in somatic cells, a major function of the ATR checkpoint is to delay cell cycle progression in response to DNA damage until replication can finish.

In embryonic cells, the ATR checkpoint is activated by endogenous, developmentally programmed cues. The nature of these signals is not defined, but it is clear that developmental activation of the checkpoint is important for regulating the timing of cell division during early embryogenesis. Two examples highlight this importance. In *D. melanogaster*, the *mei-41* (ATR) and *grapes* (Chk1) genes affect a developmentally programmed slowing of the cell cycle that occurs at the midblastula transition (Sibon et al., 1997, 1999; Su et al., 1999; Yu et al., 2000). Fly embryos perform 13 rounds of rapid and synchronous cell division before the midblastula transition. After cycle 13, the *mei41/grapes* checkpoint is activated by an endogenous signal, and this slows the cell cycle down. Slowing of the cell cycle in turn allows for zygotic transcription to begin, and the control of cell division is thereby transferred from maternal to zygotic regulators. In *mei-41* or *grapes* mutants, the cell cycle does not slow down, zygotic control of the cell cycle does not happen on schedule, and the embryo dies. Therefore, in *D. melanogaster*, the checkpoint plays an important role in remodeling the cell cycle so that zygotic transcription can begin on schedule.

Another example of DNA damage–independent utilization of the ATR checkpoint is found in *C. elegans.* The one-cell embryo, or P0 cell, divides asymmetrically to produce the smaller (P1) and the larger (AB) daughter cells. The next round of cell division is asynchronous: AB divides first, followed by

Correspondence to W. Matthew Michael: mmichael@fas.harvard.edu

Abbreviations used in this paper: HU, hydroxyurea; MMS, methanesulphonate; PCNA, proliferating cell nuclear antigen; RNAi, RNA interference.

The online version of this article contains supplemental material.


The Journal of Cell Biology, Vol. 172, No. 7, March 27, 2006 999–1008
http://www.jcb.org/cgi/doi/10.1083/jcb.200512136

P1 about 2 min later. This 2-min delay is controlled in part through differential activation of the S phase checkpoint in the P1 cell (Brauchle et al., 2003). Developmental checkpoint activation in the early embryo requires the *C. elegans* homologues of ATR (*atl-1*) and Chk1 (*chk-1*). Checkpoint-mediated asynchrony in cell division is extremely important to embryonic patterning in *C. elegans*. When asynchrony is reduced, through loss of *chk-1*, the germ line fails to develop and the animal is sterilized (Brauchle et al., 2003; Kalogeropoulos et al., 2004). Extending the asynchrony also has deleterious consequences. Hypomorphic mutations in *div-1*, a gene encoding DNA polymerase α, cause replication problems that result in inappropriate activation of the *chk-1* pathway (Encalada et al., 2000; Brauchle et al., 2003). The *div-1*–mediated activation of *chk-1* extends the asynchrony in cell division, and this results in mislocalization of developmental regulators, embryonic patterning defects, and lethality (Encalada et al., 2000).

From these examples it is clear that, although checkpoint activation is important for development, it must only occur in response to developmental signals and not in response to unscheduled events such as replication problems. A common source of replication problems in wild-type cells is DNA damage, and thus it would seem that early embryogenesis in *C. elegans* would be particularly sensitive to DNA damage because of the deleterious consequences of unscheduled checkpoint activation. Paradoxically, this is not so, as previous work has shown that wild-type embryos are resistant to relatively high amounts of both UV light and the alkylating agent methyl methanesulphonate (MMS; Hartman and Herman, 1982; Holway et al., 2005), two DNA-damaging agents that are known to cause replication problems and subsequent checkpoint activation (Lupardus et al., 2002; Stokes et al., 2002; Tercero et al., 2003). We resolve this paradox by showing that the checkpoint is actively silenced during the DNA damage response in early embryos. We go on to define genetic requirements and the basis for checkpoint silencing. Our results identify a novel developmental mechanism that ensures that cell cycle progression is not attenuated by DNA damage, thus providing embryos with a chance of survival even when their chromosomes are heavily damaged.

## Results

### Levels of DNA-damaging agents that trigger a checkpoint arrest in germ cells do not activate the checkpoint in embryos

It was not known whether the *C. elegans* checkpoint pathway can sense the types of DNA damage that cause replication stress, such as alkylation or UV light–induced damage. Previous work has shown that nuclei in the mitotic zone of the hermaphrodite gonad, a nonembryonic tissue, undergo checkpoint-dependent cell cycle arrest in response to replication blocks and ionizing radiation (Gartner et al., 2000; MacQueen and Villeneuve, 2001). This arrest is reflected by a reduction in nuclei number and an increase in nuclear size. To see whether MMS and/or UV light induced checkpoint activation in the gonad, animals were exposed to 0.005% MMS and then fixed and stained with Hoechst 33258 to visualize nuclei. MMS reduced the number of nuclei within the mitotic zone from a mean of 35 to a mean of 22 (Fig. 1, A and B; and Table I). The effect of MMS in the germ line was reversed when the checkpoint gene *atl-1*, the worm orthologue of ATR (Brauchle et al., 2003), was

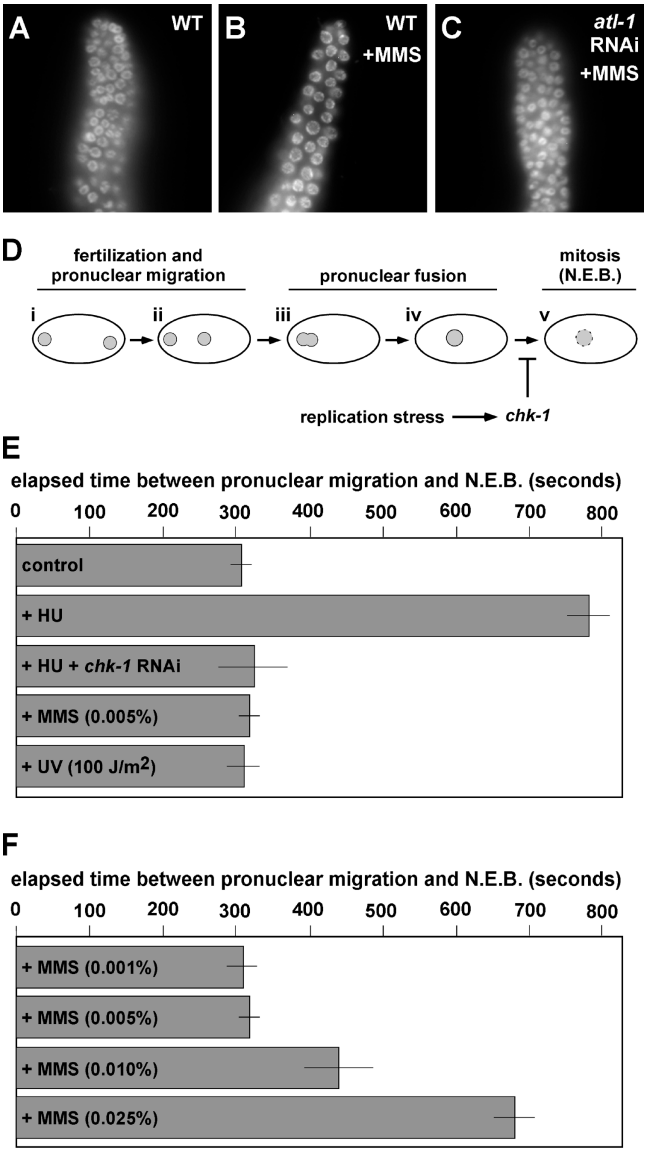


Figure 1. **Differential checkpoint responses to DNA damage in the germ line and early embryo.** (A–C) Gonads were dissected from adult hermaphrodites, fixed, and stained with Hoechst 33258 to visualize the nuclei. Where indicated, the worms had been exposed to MMS (B and C; 0.005% MMS) or *atl-1* RNAi (C) before fixation. Nuclei were then visualized and photographed by fluorescence microscopy. (D) Schematic depiction of the first cell cycle during *C. elegans* embryogenesis. (E) Wild-type (N2) embryos were cultured on regular media (control), media containing 75 mM HU (HU), media containing HU and *E. coli* expressing double-stranded RNA against *chk-1* (+ HU + *chk-1* RNAi), or media containing 0.005% MMS (+ MMS) or were exposed to 100 J/$m^2$ of UV light (+ UV). The timing of the first embryonic cell cycle was then determined by microscopic examination of living embryos, and the mean time, from a minimum of 10 samples per data point, for P0 S phase progression is displayed. P0 S phase progression is defined as the elapsed time required to progress from step ii to v in the diagram in D. Error bars represent one standard deviation from the mean. (F) Same as E, except that the media contained the indicated concentrations of MMS.

Table I. **Nuclei in the mitotic zone of the hermaphrodite gonad**

| Genotype | Condition | Nuclei count |
|---|---|---|
| Wild type | Control | 35 ± 2.6 |
| Wild type | MMS | 22.5 ± 2.0 |
| Wild type | UV | 20.9 ± 3.5 |
| *atl-1* RNAi | Control | 34.1 ± 2.9 |
| *atl-1* RNAi | MMS | 33.4 ± 4.0 |
| *atl-1* RNAi | UV | 34.8 ± 4.3 |
| *gei-17* RNAi | Control | 34.6 ± 3.9 |
| *gei-17* RNAi | MMS | 22.6 ± 3.1 |
| *gei-17* RNAi | UV | 21.0 ± 2.4 |

Young adult hermaphrodites of the indicated genotype were fixed and stained with Hoechst 33258 to visualize nuclei in the mitotic zone of the gonad. The nuclei within a fixed volume were then counted for a minimum of 10 samples per data point. Shown are these counts with the standard deviation. Condition refers to animals that were not exposed to DNA-damaging agents (control), animals that were exposed to 0.005% MMS (MMS), or animals that were exposed to 100 J/m$^2$ of UV light (UV).

Table II. **Embryonic lethality after chronic MMS exposure**

| MMS | Time | Emb |
|---|---|---|
| % | *h* | % |
| − | 16 | 0 |
| − | 32 | 0 |
| 0.001 | 16 | 0 |
| 0.001 | 32 | 0 |
| 0.005 | 16 | 1.3 |
| 0.005 | 32 | 2.4 |
| 0.01 | 16 | 73 |
| 0.01 | 32 | 94 |
| 0.025 | 16 | 96 |
| 0.025 | 32 | 98 |

Young adult hermaphrodites were transferred to media containing the indicated concentration of MMS. After 16 h, the animals were transferred to fresh MMS plates, and incubation was continued for an additional 16 h. At the end of each 16-h incubation, the eggs that had been laid were counted and then counted again 20 h later. Emb refers to the percentage of embryonic lethality or the percentage of eggs that failed to hatch during the 20 h after removal of the adults.

depleted by RNA interference (RNAi; Fig. 1 C and Table I). Similar results were obtained when animals were irradiated with 100 J/m$^2$ of UV light (Table I). We conclude that germ cells undergo checkpoint-dependent cell cycle arrest upon exposure to either MMS or UV light and that, therefore, the *C. elegans* checkpoint can indeed sense MMS- and UV light–induced damage.

The effect of MMS and UV light on cell cycle progression in the early embryo was examined next. Fig. 1 D shows the major events of the first mitotic interphase in the early embryo. After fertilization (step i), the female pronucleus migrates across the embryo, or P0 cell, where it meets and apposes the male pronucleus (steps ii–iv). DNA replication then finishes, and mitosis is initiated by nuclear envelope breakdown (step v). Previous work has shown that replication stress–induced checkpoint activation, as triggered by the replication inhibitor hydroxyurea (HU), occurs at the one-cell stage (Brauchle et al., 2003). This checkpoint requires the *chk-1* gene (Brauchle et al., 2003) and prevents the transition from step iv to v in Fig. 1 D. Animals were exposed to MMS, UV light, or, as a positive control, HU, and the timing of the first cell cycle was determined by direct microscopic visualization of living embryos. As shown in Fig. 1 E, and as previously reported (Brauchle et al., 2003), when embryos were treated with HU, there was a significant delay in progression through the P0 cell cycle. This delay was checkpoint dependent, as it was reversed after depletion of *chk-1* by RNAi. In contrast to HU, we did not detect a significant P0 cell cycle delay when the embryos were exposed to MMS or UV light (Fig. 1 E). We conclude that the amounts of MMS or UV light that are sufficient to activate a checkpoint response in the germ line (0.005% and 100 J/m$^2$, respectively) cannot do so in early embryos. We refer to this phenomenon as early embryonic checkpoint silencing.

We next tested how much MMS embryos could endure before a delay in cell division was detected. For this, we timed the first cell division after exposure to a range of MMS concentrations and found that concentrations >0.005% caused both a delay in progression through S phase (Fig. 1 F) and high levels of embryonic lethality (Table II). These data show that the ability of embryos to avoid a checkpoint response to MMS is saturable. They also indicate that checkpoint silencing and survival of DNA damage are linked, and this is consistent with previous work showing that even modest perturbations in the timing of cell division are lethal to the developing embryo (Encalada et al., 2000).

## Embryonic checkpoint silencing is independent of lesion repair yet under genetic control

A simple explanation for checkpoint silencing is that embryos rapidly repair damaged DNA. However, extensive analysis of the kinetics of DNA repair in *C. elegans* has been reported (Hartman and Herman, 1982; Hartman, 1984; Hartman et al., 1989; Jones and Hartman, 1996), and these studies demonstrate that repair is unlikely to account for checkpoint silencing in the embryo. For example, >80% of (6-4) photoproducts remain in embryos 3 h after a dose of 50 J/m$^2$ of UV light is delivered (Hartman et al., 1989). The data in Fig. 1 E were collected 1 h after a dose of 100 J/m$^2$ was delivered, and thus the embryo could not possibly have repaired even a modest percentage of the damage in that short a period of time. We conclude that cell cycle progression occurs unimpeded even when the level of damage present greatly exceeds the capacity of the embryo to repair it.

If embryonic cell cycle progression is truly independent of repair, then mutant embryos that are deficient in DNA repair would nonetheless exhibit normal cell cycles after DNA damage. To test this, we examined cell cycle progression in early *rad-3* embryos. *rad-3* mutant embryos have a defect in excision repair and are consequently very sensitive to both MMS (Fig. 2 A) and UV light (Hartman and Herman, 1982; Hartman et al., 1989). The rate of repair in *rad-3* embryos has been determined and is threefold lower than wild type (Hartman et al., 1989). Despite this reduced capacity for repair, however, the timing of cell division in *rad-3* mutant embryos was indistinguishable from wild type after exposure to either UV light or MMS (Fig. 2 B). The dose of UV light used in the experiment is sufficient to kill 100% of the *rad-3* mutant embryos and <10% of wild-type

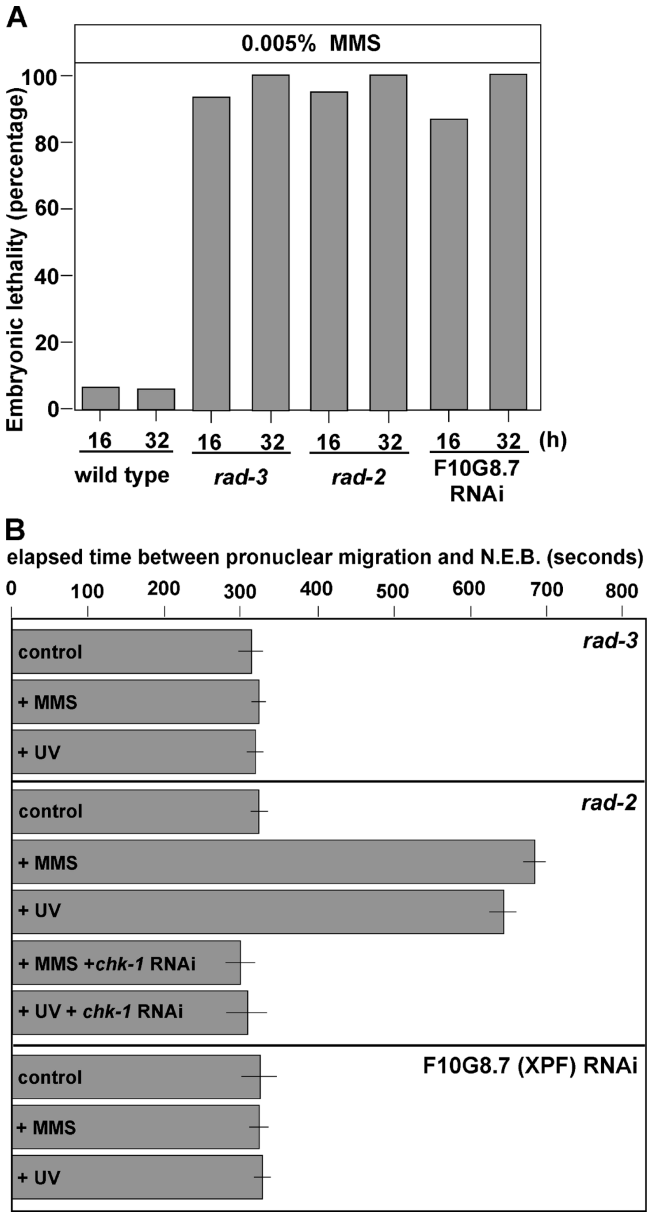


Figure 2. **Normal progression through the early cell cycles in excision repair–deficient embryos exposed to DNA damage.** (A) MMS sensitivity of embryos of the indicated genotype. Details on the MMS sensitivity assay can be found in Materials and methods and in Holway et al. (2005). (B) Timing of the P0 S phase was determined as in Fig. 1 E for embryos of the indicated genotype. Control refers to regular media, + MMS refers to media containing 0.005% MMS, and + UV refers to exposure to 100 J/m$^2$ of UV light.

embryos (unpublished data; see Hartman and Herman [1982] for UV sensitivity of *rad-3* mutants). Interestingly, another radiation- and MMS-sensitive mutant, *rad-2*, showed altered progression through the first cell cycle after exposure to UV or MMS (Fig. 2, A and B). *rad-2* mutant embryos delayed progression through the P0 S phase in a manner that was dependent on DNA damage and similar to wild-type embryos exposed to HU. The damage-induced delay in *rad-2* embryos was checkpoint dependent, as it was reversed when *chk-1* was depleted by RNAi (Fig. 2 B). Importantly, the rate of repair in *rad-2* mutants is indistinguishable from wild type (Hartman, 1984). Thus repair-deficient *rad-3* mutants have normal cell cycles after DNA damage, whereas repair-proficient *rad-2* mutants do not. This shows that a process that is independent of DNA repair is responsible for preventing checkpoint activation during the early embryonic cell cycle. Consistent with this, we also found that RNAi-mediated depletion of another excision repair gene, the *C. elegans* homologue of the human XPF endonuclease (F10G8.7), renders embryos extremely sensitive to both UV light (Fig. S1, available at http://www.jcb.org/cgi/content/full/jcb.200512136) and MMS (Fig. 2 A) yet had no affect on cell cycle progression in the early embryo (Fig. 2 B). We conclude that checkpoint silencing is independent of lesion repair (based on the results with *rad-3* and F10G8.7) yet nonetheless under genetic control (based on the results with *rad-2*).

### *gei-17* suppresses the checkpoint response to DNA damage but not developmental signals or stalled replication forks in early embryos

The finding that checkpoint silencing is under genetic control prompted a search for genes that silence the checkpoint when DNA damage is present. The *rad-2* gene has not yet been cloned, and we are currently working toward accomplishing this. Recent work from our laboratory has shown that the *gei-17* gene, which encodes an E3 SUMO ligase related to yeast *SIZ1* and human PIAS1, is an important participant in the embryonic DNA damage response in *C. elegans* (Holway et al., 2005). Depletion of *gei-17* by RNAi renders embryos sensitive to both MMS (Holway et al., 2005) and UV light (Fig. S1). MMS-exposed *gei-17* RNAi early embryos display abnormal nuclear morphology, characterized by fused nuclei and anaphase bridging (Holway et al., 2005). These results suggested that *gei-17* is important for early embryonic cell cycle progression when damage is present and prompted us to examine the kinetics of cell division in *gei-17* RNAi early embryos. MMS exposure delayed progression through the P0 S phase in *gei-17* RNAi embryos. At 0.005% MMS, we observed an ∼440-s delay (Fig. 3 A) and at 0.001% MMS the delay was ∼300 s (not depicted). These data demonstrate that S phase takes longer in *gei-17* RNAi embryos exposed to 0.001% MMS than it does in wild-type embryos exposed to 10-fold more MMS (the wild-type delay at 0.01% MMS was ∼120 s; Fig. 1 F). To determine whether these MMS-induced effects were caused by activation of the checkpoint, we codepleted *gei-17* with *chk-1*. As was the case with *rad-2*, codepletion of *gei-17* with *chk-1* reversed the MMS-induced delay in progression through the P0 cell cycle (Fig. 3 A). This result demonstrates that *gei-17* activity suppresses checkpoint activation in response to DNA damage in the early embryo.

To see whether the effect of *gei-17* on checkpoint activation was specific for DNA damage, we next examined checkpoint activation in *gei-17* RNAi embryos in response to both HU and developmental signals. For the HU experiment, we used a lower concentration of HU than that used in Fig. 1 (25 as opposed to 75 mM), and this resulted in a more modest delay in cell division in wild-type embryos (∼160 s delay after 25 mM HU in contrast to the ∼475-s delay after 75 mM; Fig. 1 E

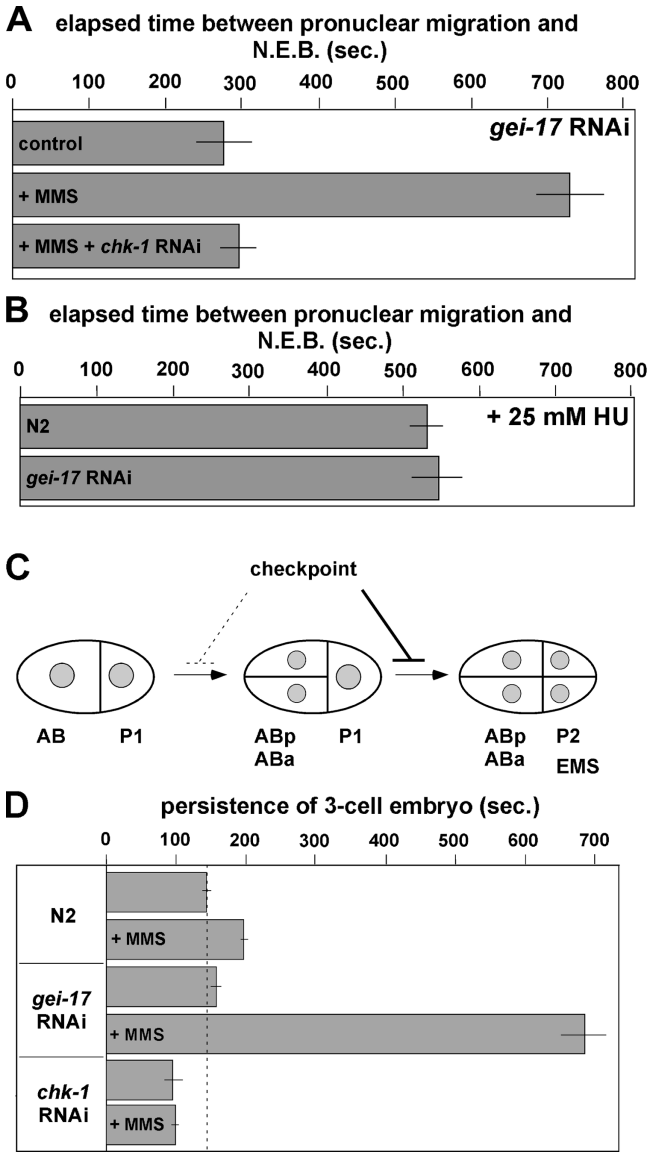


Figure 3. ***gei-17* attenuates checkpoint activation in response to DNA damage but not HU or developmental signals.** (A) Bar graph displaying the amount of time required for P0 S phase in *gei-17* RNAi embryos exposed to control or MMS media and *gei-17/chk-1* codepleted embryos exposed to MMS media. The analysis was performed as described in Fig. 1. (B) Bar graph displaying the amount of time required for P0 S phase in N2 or *gei-17* RNAi embryos exposed to media containing 25 mM HU. (C) Schematic depiction of the effect of checkpoint activation by developmental signals on cell division in the early embryo. During S phase of the second round of cell division, a checkpoint is activated preferentially in the P1 cell (Brauchle et al., 2003). The result is that transition from a two- to three-cell embryo is only briefly delayed (dotted line), whereas transition from the three- to four-cell embryo is more robustly delayed (solid line). The P1-specific delay therefore results in persistence of a three-cell embryo. (D) Cell division in living embryos was monitored microscopically, and the time in seconds that the three-cell embryo persisted was recorded. Persistence was defined as the elapsed time between division of AB relative to P1 and was assessed for wild-type (N2), *gei-17* RNAi, or *chk-1* RNAi embryos. + MMS indicates inclusion of 0.005% MMS. The dotted line represents the endogenous delay that results in part through activation of the checkpoint by developmental signals. $n = 15$.

and Fig. 3 B). If *gei-17* functions to suppress HU-induced checkpoint activity, we would expect this modest delay to be extended in *gei-17* RNAi embryos, but this did not occur (Fig. 3 B). Similar results (i.e., no difference between wild-type and *gei-17* RNAi embryos) were obtained when 75 mM HU was used to trigger a stronger checkpoint response (unpublished data). We conclude that although *gei-17* activity reduces the checkpoint response to DNA damage, it has no effect on checkpoint activation by HU.

To examine the effect of loss of *gei-17* on checkpoint activation in response to developmental signals, we analyzed the second round of cell division in early embryos. As described in the Introduction, there is a checkpoint-dependent delay in division of the P1 cell relative to the AB cell during normal development (Fig. 3 C). The delay normally lasts 2 min; however, when *chk-1* is depleted by RNAi, it is reduced to ~1 min (Brauchle et al., 2003; Fig. 3 D). If *gei-17* negatively controlled the checkpoint response to developmental signals, we would expect the delay to be extended in *gei-17* RNAi embryos, but this was not the case, as *gei-17* RNAi embryos showed the same delay as wild type (Fig. 3 D). When MMS was included, however, the lag was significantly extended in *gei-17* RNAi embryos and only very modestly extended in wild type (Fig. 3 D). We conclude that *gei-17* functions to suppress checkpoint activity specifically in response to DNA damage and not in response to HU-induced stalled replication forks or developmental signals.

### *gei-17* promotes replication fork progression through damaged DNA

One explanation for the ability of *gei-17* to suppress damage-induced checkpoint activation is that it promotes the rapid replication of damaged DNA. In both *X. laevis* and yeast, the checkpoint response to MMS-induced damage is known to require the stalling of replication forks (Stokes et al., 2002; Tercero et al., 2003); thus, if *gei-17* prevents damage-induced fork stalling, then checkpoint activation would not be expected to occur. To directly assess a role for *gei-17* in the replication of damaged DNA, a previously described assay system was used to monitor DNA replication in the early embryo (Edgar and McGhee, 1988; Holway et al., 2005). Egg shells from four-cell embryos were permeabilized and the samples treated with cytochalasin B to block cytokinesis. The embryos were then cultured for 1 h before fixation and DNA staining. Despite the block to cell division, the DNA replication cycle continues unabated, and after 1 h this results in embryos that contain multiple nuclei in each of the four cells (Fig. 4 A). The appearance of multiple nuclei is dependent on DNA synthesis because it does not occur in the presence of the replication inhibitors aphidicolin or HU (Edgar and McGhee, 1988; Holway et al., 2005). MMS did not affect the appearance of multinucleated cells in wild-type embryos (Fig. 4 B, compare panels II and III). In contrast, the combination of MMS and *gei-17* RNAi caused a defect in DNA replication, as these embryos failed to produce multinucleated cells (Fig. 4 B, panel VI). This was not observed in undamaged *gei-17* RNAi embryos (Fig. 4 B, panel V), demonstrating that *gei-17* is required for the replication of damaged, but not undamaged, chromosomes. We also note that the replication defect in MMS-exposed *gei-17* RNAi embryos was uniform and occurred in all four cells of the embryo. As the checkpoint is only highly active in one of these cells (the

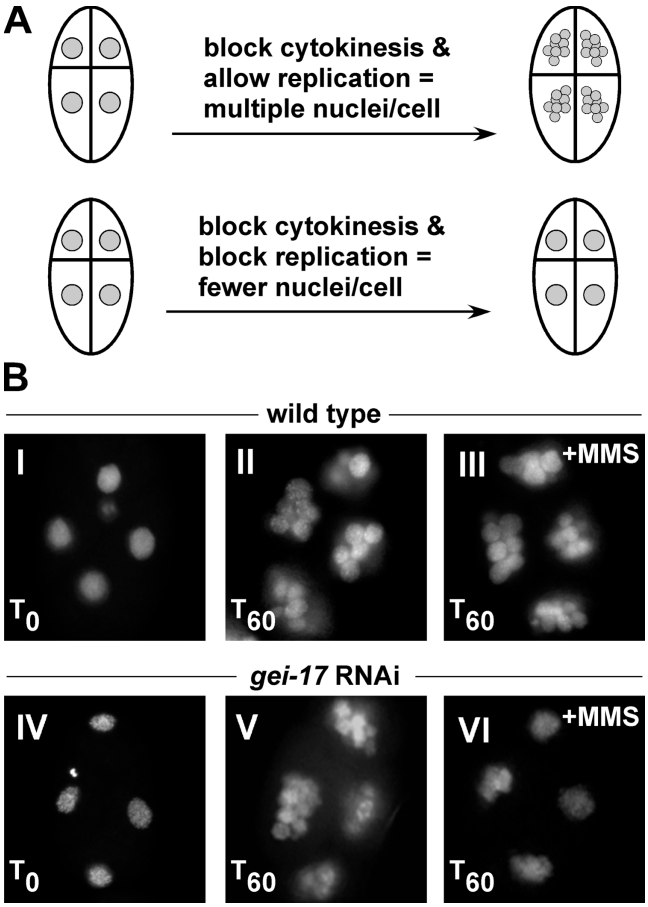


Figure 4. ***gei-17* is required for the replication of damaged but not undamaged DNA.** (A) Schematic depiction of the assay used to monitor DNA synthesis in early embryos. (B) Four-cell wild-type (panels I–III) or *gei-17* RNAi (panels IV–VI) embryos were prepared and cultured as described in the text. The samples were then fixed and stained with Hoechst 33258 after 0 (I and IV) or 60 min, in either the absence (II and V) or presence (III and VI) of MMS. The images displayed are representative of a group of 20 or more embryos that were examined per sample.

P lineage cell; Brauchle et al., 2003) in intact embryos, this result suggests that the requirement for *gei-17* in the replication of damaged DNA is independent of the checkpoint status of the cell. It is possible, however, that permeabilization perturbs the asymmetric distribution of the checkpoint within the four-cell embryo.

The double-strand break repair protein RAD-51 is known to accumulate in immunologically detectable foci when replication forks are stalled by DNA damage (Haaf et al., 1995; Scully et al., 1997). The results in Figs. 3 and 4 show that *gei-17* is required for S phase progression (Fig. 3) and for DNA replication (Fig. 4), specifically when chromosomes are damaged. To determine whether loss of *gei-17* induces RAD-51 foci, we stained early embryos (<100 cells) with anti–RAD-51 antibodies (Colaiacovo et al., 2003). In the absence of MMS, we did not detect RAD-51 foci in either wild-type or *gei-17* RNAi early embryos or in the mitotic zone of the hermaphrodite gonad (Fig. 5, panels I–III). In MMS-exposed animals, we could detect robust RAD-51 foci formation within the mitotic zone of the hermaphrodite gonad (Fig. 5, panel VI) but not in wild-type early embryos (Fig. 5, panel IV). In contrast to wild type, *gei-17* RNAi

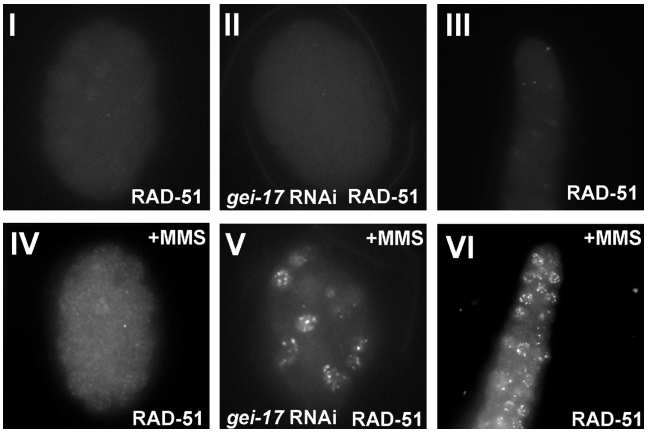


Figure 5. ***gei-17* prevents replication fork stalling on damaged chromosomes.** RAD-51 immunostaining of *C. elegans* embryos (I, II, IV, and V) or gonads (III and VI) in the presence (IV–VI) or absence (I–III) of MMS (0.005%). Panels I, III, IV, and VI are wild-type embryos or animals, whereas panels II and V are *gei-17* RNAi embryos.

embryos readily formed RAD-51 foci in response to MMS (Fig. 5, panel V). These data indicate that stalled replication forks, as inferred by the presence of MMS-induced RAD-51 foci, form in cells where MMS triggers the checkpoint (wild-type germ lines and *gei-17* RNAi embryos) but not in cells where the checkpoint is silenced (wild-type embryos). RAD-51 foci were not observed in MMS-exposed *chk-1* RNAi embryos (unpublished data), indicating that attenuation of the checkpoint alone is not sufficient to explain damage-induced foci formation. These results provide further evidence that loss of *gei-17* causes replication fork stalling in MMS-exposed embryos.

### Checkpoint silencing requires the *C. elegans* orthologue of DNA polymerase eta but not Rad6 or homologous recombination

All organisms contain mechanisms for promoting the replication of damaged DNA in a manner that does not rely on physical repair of the lesion. These pathways, termed postreplication repair or lesion bypass, rely on either translesion synthesis or homologous recombination to rescue replication forks that stall at sites of damage (for review see Barbour and Xiao, 2003). To explore an involvement of lesion bypass pathways in embryonic checkpoint silencing, we determined the effect of inactivation of known lesion bypass components on P0 cell cycle progression after DNA damage. The role of homologous recombination was assessed by studying embryos derived from adults carrying homozygous deletion mutations in the essential recombination genes *rad-51* and *-54*. Neither mutant displayed a defect in P0 cell cycle progression after MMS exposure, showing that homologous recombination is not essential for checkpoint silencing (Fig. 6 A). This is consistent with a lack of RAD-51 foci in MMS-exposed wild-type embryos (Fig. 5).

The other major lesion bypass pathway in eukaryotes is translesion synthesis, where specialized DNA polymerases are recruited to the replication fork to synthesize DNA across damaged bases on the template strand (for review see Prakash et al., 2005). In yeast and human cells, access of translesion polymerases to

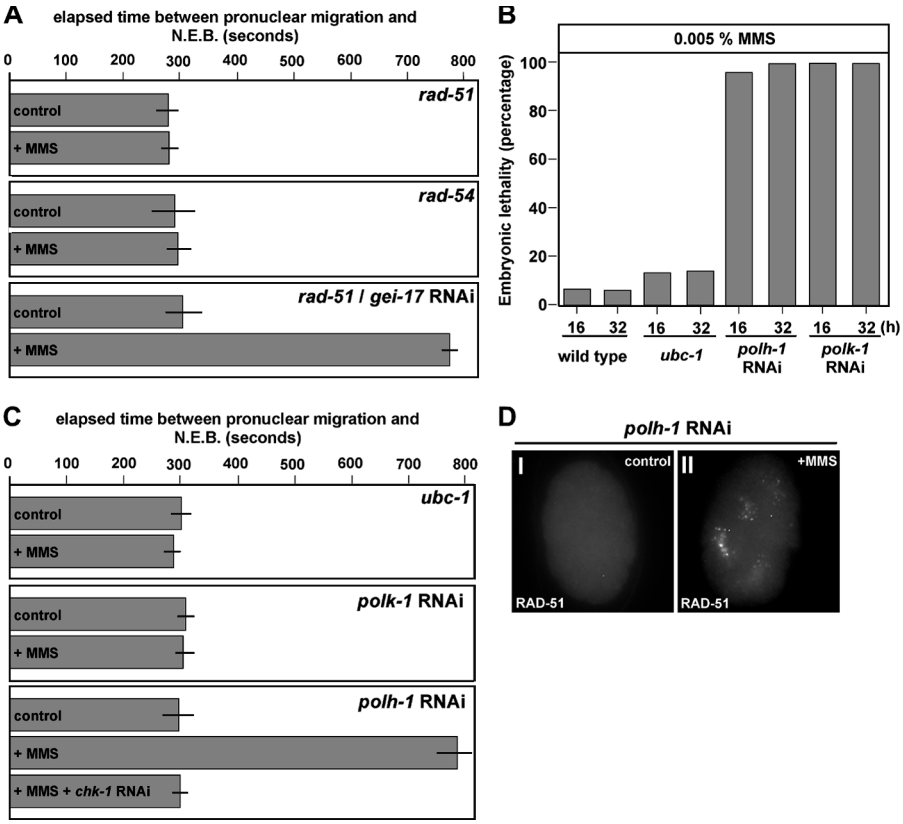


Figure 6. **Translesion synthesis allows checkpoint bypass during the early embryonic DNA damage response.** (A) The timing of the first cell cycle for embryos of the indicated genotype was determined as in Fig. 1 E. $n = 15$. (B) MMS sensitivity was performed as described in Materials and methods for embryos of the indicated genotype. $n = 200$. $n = 15$. (C) The timing of the first cell cycle for embryos of the indicated genotype was determined as in Fig. 1 E. (D) *polh-1* RNAi embryos were fixed and stained with anti–RAD-51 antibodies after incubation on either regular (panel I) or MMS-containing (panel II; 0.005% MMS) media.

sites of damage is thought to occur through *RAD6*-mediated ubiquitination of proliferating cell nuclear antigen (PCNA), a DNA replication clamp protein (Hoege et al., 2002; Kannouche et al., 2004; Watanabe et al., 2004). Yeast *rad6* mutants are sensitive to MMS and UV light and do not show DNA damage–induced mutagenesis, a hallmark of translesion synthesis (for review see Barbour and Xiao, 2003). To determine whether the Rad6 pathway is responsible for checkpoint silencing in early embryos, we examined the MMS response in *ubc-1* mutants. The *ubc-1* gene represents the sole *C. elegans* orthologue of budding yeast *RAD6*, and expression of the *ubc-1* gene in yeast is sufficient to rescue the *rad6* translesion synthesis defect (Leggett et al., 1995). Surprisingly, embryos derived from adults carrying a homozygous deletion of the *ubc-1* gene did not display MMS sensitivity (Fig. 6 B) and progressed normally through the P0 cell cycle after MMS exposure (Fig. 6 C). Thus, in *C. elegans* embryos, the Rad6 orthologue *ubc-1* is not important for the response to MMS-induced damage.

It was possible that in *C. elegans* translesions polymerases can access sites of damage in a Rad6/*ubc-1*–independent manner. To pursue this hypothesis, we screened all five of the identifiable translesion polymerases present in *C. elegans* by RNAi for MMS sensitivity in embryos. The genes that we screened included putative orthologues of human Polθ (W03A3.2), Polη (F53A3.2), Polκ (F22B7.6), Polζ (Y37B11A.2), and Rev1 (ZK675.2). The assignment of these *C. elegans* genes to their putative human counterparts is based purely on sequence conservation, as information on the biochemical properties of the encoded proteins is not available. Of the five, we found that RNAi-mediated depletion of both the Polη orthologue *polh-1* and the Polκ orthologue *polk-1* caused MMS sensitivity in embryos (Fig. 6 B). Only *polh-1* RNAi, however, delayed progression through P0 S phase (Fig. 6 C). This delay was dependent on MMS and was reversed upon codepletion of *chk-1* (Fig. 6 C), demonstrating that like *gei-17* and *rad-2*, loss of *polh-1* allows checkpoint activation in the early embryo. Consistent with this, we observed that RAD-51 foci could be detected in *polh-1* RNAi embryos, in an MMS-dependent manner (Fig. 6 D). RAD-51 foci were not observed in MMS-exposed early *polk-1* RNAi embryos (unpublished data). These data indicate that *polh-1*–mediated translesion synthesis is the lesion bypass mechanism used by early embryos to silence the checkpoint during the DNA damage response.

## Discussion

Fig. 7 summarizes the findings reported here and integrates them with previous work on cell cycle control in the early embryo. Previous studies have shown that developmental signals, the nature of which are unknown, trigger checkpoint activation and that this contributes to the asynchrony in cell division that is required for developmental patterning and germ line formation (Fig. 7, shaded portion). Thus, developmental signals represent one class of input into the embryonic checkpoint pathway. Another type of input is stalled replication (Fig. 7, unshaded portion). Stalled replication has been induced in early embryos through mutations in *div-1* (Encalada et al., 2000) or through the use of HU (Brauchle et al., 2003; this study). Embryonic sensitivity to stalled replication has been documented; it causes checkpoint activation and extends the natural

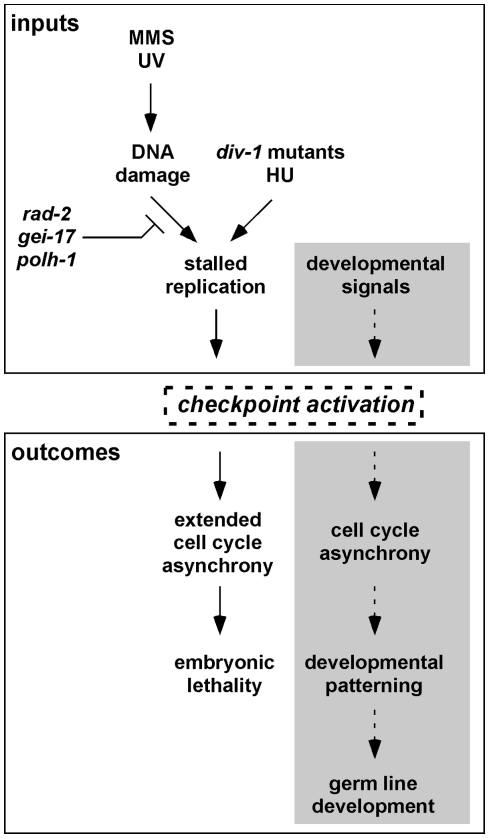


Figure 7. **Input/outcome diagram for early embryonic checkpoint responses.** Two types of inputs into the embryonic checkpoint are considered: stalled replication and developmental signals. The shaded portion represents developmentally programmed events, whereas the unshaded portion summarizes how stalled replication can occur and how the embryo prevents it. Arrows represent positive regulation, and the line with a bar on it represents suppression. Please see Discussion for more details.

asynchrony of cell division (Encalada et al., 2000; Brauchle et al., 2003). This in turn perturbs development and kills the embryo. The focus of the work presented here was on another inducer of stalled replication, DNA damage. We have found that early embryos do not stall replication when their chromosomes are damaged and that protection against damaged-induced stalled replication is conferred by *rad-2*, *gei-17*, and *polh-1*. These results explain how the checkpoint can be accessed by developmental signal–based inputs and insulated from DNA damage–based inputs. The checkpoint is not insulated from *div-1* mutant or HU-based inputs, but these are conditions that are irrelevant to wild-type worms in their natural environments.

Embryonic checkpoint control has been studied in other organisms, most notably *D. melanogaster* and *X. laevis*. In both of these organisms, checkpoints that respond to DNA damage are not evident until after the rapid cleavage cycles have ended, at the midblastula transition (Dasso and Newport, 1990; Sibon et al., 1997). In *X. laevis*, the lack of DNA damage checkpoint activation in early embryos is likely due to a low DNA/cytoplasm ratio, as it has been recently demonstrated that increasing the amount of damaged DNA in younger frog embryos results in a checkpoint-dependent delay in cell division before the midblastula transition (Conn et al., 2004). The interpretation of this is that the checkpoint signal is not strong enough to neutralize the mitosis-promoting capacity of the cytoplasm until the proper ratio is achieved. Thus, in frog and fly embryos checkpoint avoidance occurs passively. This is in contrast to the active mechanism that we have discovered in *C. elegans*, and the difference is likely due to when the checkpoint functions during development. In frogs and flies the checkpoint is not needed until the midblastula transition, whereas in worms it is used from the first division onward.

Rapid embryonic cell cycles occur in all major animal phyla (for review see O'Farrell et al., 2004). *C. elegans* is no exception, as the early cycles last only 10–40 min. It is possible that the rapid cycling allows no time for lesion repair, and therefore lesion bypass may be the only viable option for *C. elegans* embryos exposed to DNA-damaging agents. This is in contrast to the mitotic cells of the *C. elegans* gonad that can survive delays in cell division and go on to divide normally. Indeed, we have shown that mitotic gonad cells arrest in a checkpoint-dependent manner upon MMS or UV exposure and RAD-51 foci are clearly evident (Figs. 1 and 5 and Table I). Additionally, we were unable to detect any cell cycle arrest phenotype in the germ lines of *gei-17* RNAi animals after either high (Table I) or low (not depicted) MMS or UV exposure, suggesting that this pathway is not a major component of the germ line DNA damage response. Our results therefore demonstrate a distinct difference between embryonic and germ line responses to DNA damage that could be explained by embryonic sensitivity to the timing of cell division. The molecular basis for this difference is not yet known but likely involves differential expression and/or regulation of members of the *gei-17–polh-1* pathway.

Both *gei-17* and *polh-1* are components of an embryonic checkpoint silencing pathway that bypasses MMS-induced lesions. The identification of *polh-1* as the primary polymerase required for progression through S phase in MMS-exposed early embryos is somewhat surprising, as Polη in yeast and human cells is primarily associated with UV light–induced damage (for review see Prakash et al., 2005). However, budding yeast Polη (*RAD30*) efficiently bypasses abasic sites (a major MMS-induced lesion) when coupled to PCNA in vitro (Haracska et al., 2001) and is required for maximal abasic site bypass in vivo (Zhao et al., 2004). *rad30* mutants are accordingly MMS sensitive (Zhao et al., 2004). These findings therefore suggest that a role for *polh-1* in responding to MMS-induced damage in *C. elegans* could be explained by the ability of the enzyme to bypass abasic sites.

Although the role of *polh-1* as a translesion polymerase is directly related to replicating damaged DNA, it is not clear what role the E3 SUMO ligase, *gei-17*, actually plays in this process. Recent work has shown that *SIZ1*, which sumoylates PCNA, functions to ward off homologous recombination during lesion bypass through recruitment of the *RAD51* antagonist *SRS2* to the replication fork (Papouli et al., 2005; Pfander et al., 2005). It is therefore possible that *gei-17* promotes translesion synthesis in embryos through negative regulation of recombination, although we do not favor this model, as loss of *gei-17* still negatively affects S phase progression in *rad-51* mutant embryos (Fig. 6 A). Thus, the elimination of recombination in *rad-51* mutants

does not suppress the *gei-17* RNAi phenotype, and this argues against a role for *gei-17* in preventing recombination. One possibility is that *gei-17* functions in polymerase switching at sites of DNA damage, and biochemical analysis of the polymerase switch reaction in *C. elegans* embryos will be required to determine whether this is so. We note that in yeast and mammalian cells polymerase switching is controlled by the Rad6 E2 ubiquitin-conjugating enzyme and the Rad18 E3 ubiquitin ligase; however, we have shown here that the Rad6 orthologue *ubc-1* is not required for translesion synthesis in *C. elegans* embryos (Fig. 6, B and C), and there is no recognizable Rad18 homologue present in the worm genome.

Our results also shed light on the relationship between checkpoint activation and translesion synthesis, as they suggest that in the early embryo translesion synthesis trumps checkpoint activation to ensure that DNA damage does not slow the cell cycle down. How decisions are made at stalled replication forks to activate one pathway over another is not understood and is an active area of research. Our data show that, in the early embryo, translesion synthesis is so efficient that checkpoint activation fails to occur, even when relatively high levels of damage are present. This may reveal a general principle, in that during the DNA damage response the default response is to access the translesion synthesis pathway and that checkpoint activation can only occur at levels of damage that saturate translesion synthesis. Alternatively, embryo-specific factors may exist that allow translesion synthesis to supersede checkpoint activation.

The use of the POLH-1 translesion polymerase to prevent fork stalling during the early embryonic cell cycles answers the question of how *C. elegans* embryos bypass checkpoint activation and so survive exposure to DNA-damaging agents. Although this pathway allows embryonic cells to divide on schedule, translesion polymerases are notoriously error prone, and use of this pathway predicts that embryos likely trade survival for an increase in mutation frequency. This is especially true when abasic sites, which are noncoding forms of damage, are considered. Thus, it appears that during evolution there has been stronger selection for adherence to the schedule of cell division than for error-free replication during early embryogenesis, and understanding the basis for this preference will be the goal of future studies in this system.

## Materials and methods

### *C. elegans* strains and culturing

The N2 Bristol strain was used as wild type in all control experiments and for all RNAi experiments. SP482 (*rad-3[mn15]*), SP488 (*rad-2[mn156]*), TG5 (*rad-51[lg8701]*), VC531 (*rad-54[ok615]*), and VC18 (*ubc-1[gk14]*) strains were obtained from the Caenorhabditis Genetics Center. Animals were maintained as described previously (Brenner, 1974).

### RNAi

RNAi by feeding was performed for F10G8.7, W03A3.2, Y37B11A.2, ZK675.2, *atl-1*, *gei-17*, and *chk-1* as described previously (Timmons and Fire, 1998). All bacteria were cultured for 24 h at 37°C in Terrific Broth containing 50 μg/ml ampicillin, seeded onto nematode growth media (Brenner, 1974) plates containing 5 mM IPTG, and allowed to dry overnight. With the exception of *chk-1* RNAi, worms were grown for two generations on RNAi bacteria. F1 progeny of *chk-1* RNAi worms are sterile; therefore, *chk-1* RNAi was fed for one generation and analysis was performed on F-1 embryos. For *gei-17/chk-1* codepletions, worms were first grown on *gei-17* RNAi bacteria for one generation and then moved as F1 L1s onto a plate containing a 1:1 mixture of the feeding vectors. *polh-1* and *polk-1* RNAi was accomplished by soaking (Maeda et al., 2001). *polh-1/chk-1* codepletions were accomplished by first feeding worms *chk-1* bacteria and then soaking P0 L4s in *polh-1* double-stranded RNA. Worms were then plated onto regular media or media containing 0.05 mg/ml MMS (Sigma-Aldrich), both seeded with *chk-1* RNAi bacteria, and analysis was performed on their progeny.

### Analysis of the timing of cell division within living embryos

Worms were collected and placed in a drop of M9 buffer for dissection. Released embryos were then transferred to agarose pads (2% SeaKem Gold agarose in water) in a small volume of M9 and visualized under Nomarski optics on a microscope (BX51 TF; Olympus). Embryos exposed to MMS were timed after 20 h of exposure to plates containing 0.05 mg/ml MMS. Embryos exposed to HU (Calbiochem) were timed after 5 h of exposure to plates containing 75 mM HU. Embryos exposed to UV light were timed 1 h after irradiation. Irradiation was performed by placing an open dish of worms in a Stratalinker (Stratagene). To measure the P0 S phase, timing started when the female pronucleus passed the midline of the embryo. Timing continued until nuclear envelope breakdown had occurred, just before first mitosis. Because it is unclear when replication initiates, this represents the timing of a partial S phase (Brauchle et al., 2003). The persistence of three-cell embryos was determined by timing the interval between cytokinesis of the AB cell and cytokinesis of the P1 cell.

### DNA and antibody staining of embryos and germ lines

Worms were dissected on glass microscope slides and permeabilized by freeze cracking. Slides were fixed for 10 min in methanol/formaldehyde fixative at −20°C and washed in PBS Tween 20. Slides were then incubated with anti–RAD-51 antibody (Colaiacovo et al., 2003) at 1:200 overnight followed by a 2-h incubation in FITC-tagged anti-rabbit secondary antibody. DNA staining was accomplished by adding 10 μl of 10 μg/μl Hoechst 33258. To count nuclei in the mitotic zone of the gonad, adult worms were fixed and stained with Hoechst 33258. The distal tip of the gonad was then visualized using fluorescence microscopy, and the number of nuclei within a constant volume was counted.

### Embryo culture assays

Embryos were prepared for culturing as described previously (Holway et al., 2005). MMS exposure was accomplished by culturing worms for 20 h on 0.05 mg/ml MMS plates and then exposing permeabilized embryos to 0.2 mg/ml MMS in egg growth media. After incubation, embryos were stained with Hoechst 33258 and visualized on a microscope. Pictures were captured using a monochrome camera (SPOT RT; Diagnostic Instruments).

### MMS sensitivity assays

L4 F1 worms grown on plates containing the appropriate bacterial expression vectors were transferred to plates containing 0.05 mg/ml MMS. Eggs laid by these worms were collected over time and scored for survival as described previously (Holway et al., 2005).

### Image acquisition

The images shown in Fig. 1 (A–C), Fig. 4 B, Fig. 5, and Fig. 6 D were obtained as follows. All images were collected on a microscope. The type, magnification, and NA of the objective lenses were UPlanAPO, 60× oil, and NA 1.40, respectively. The experiments were performed at room temperature using Hoechst 33258 and FITC-labeled secondary antibodies as fluorochromes. Images were captured on a camera (model 2.1.1; Diagnostic Instruments) and processed using SPOT Advanced version 3.2.4 software (Diagnostic Instruments).

### Online supplemental material

Fig. S1 shows that F10G8.7 and *gei-17* embryos are sensitive to UV light. The supplemental text describes UV sensitivity assays and the observation that *gei-17* RNAi causes in UV sensitivity in embryos. Online supplemental material is available at http://www.jcb.org/cgi/content/full/jcb.200512136/DC1.

We dedicate this work to John Newport, a pioneer in the study of early embryonic cell cycles.

We thank Craig Hunter and Tim Schedl for advice.

Some of the strains used in this work were provided by the Caenorhabditis Genetics Center, which is funded by the National Institutes of Health National Center for Research Resources. A. La Volpe was supported by a

Telethon-Italy grant (GGP04010). A.H. Holway was supported by a National Institute of General Medical Sciences (NIGMS) Genetics and Genomics training grant (T32GM007620) and a Don Wiley Award for Excellence in Graduate Studies (funded by Merck). Support for this work was provided by an NIGMS research grant (R01GM67735) to W.M. Michael.

Submitted: 23 December 2005
Accepted: 17 February 2006

## References

Barbour, L., and W. Xiao. 2003. Regulation of alternative replication bypass pathways at stalled replication forks and its effects on genome stability: a yeast model. *Mutat. Res.* 532:137–155.

Bartek, J., C. Lukas, and J. Lukas. 2004. Checking on DNA damage in S phase. *Nat. Rev. Mol. Cell Biol.* 5:792–804.

Brauchle, M., K. Baumer, and P. Gonczy. 2003. Differential activation of the DNA replication checkpoint contributes to asynchrony of cell division in *C. elegans* embryos. *Curr. Biol.* 13:819–827.

Brenner, S. 1974. The genetics of *Caenorhabditis elegans*. *Genetics.* 77:71–94.

Colaiacovo, M.P., A.J. MacQueen, E. Martinez-Perez, K. McDonald, A. Adamo, A. La Volpe, and A.M. Villeneuve. 2003. Synaptonemal complex assembly in *C. elegans* is dispensable for loading strand-exchange proteins but critical for proper completion of recombination. *Dev. Cell.* 5:463–474.

Conn, C.W., A.L. Lewellyn, and J.L. Maller. 2004. The DNA damage checkpoint in embryonic cell cycles is dependent on the DNA-to-cytoplasmic ratio. *Dev. Cell.* 7:275–281.

Dasso, M., and J.W. Newport. 1990. Completion of DNA replication is monitored by a feedback system that controls the initiation of mitosis in vitro: studies in *Xenopus*. *Cell.* 61:811–823.

Edgar, L.G., and J.D. McGhee. 1988. DNA synthesis and the control of embryonic gene expression in *C. elegans*. *Cell.* 53:589–599.

Encalada, S.E., P.R. Martin, J.B. Phillips, R. Lyczak, D.R. Hamill, K.A. Swan, and B. Bowerman. 2000. DNA replication defects delay cell division and disrupt cell polarity in early *Caenorhabditis elegans* embryos. *Dev. Biol.* 228:225–238.

Gartner, A., S. Milstein, S. Ahmed, J. Hodgkin, and M.O. Hengartner. 2000. A conserved checkpoint pathway mediates DNA damage–induced apoptosis and cell cycle arrest in *C. elegans*. *Mol. Cell.* 5:435–443.

Haaf, T., E.I. Golub, G. Reddy, C.M. Radding, and D.C. Ward. 1995. Nuclear foci of mammalian Rad51 recombination protein in somatic cells after DNA damage and its localization in synaptonemal complexes. *Proc. Natl. Acad. Sci. USA.* 92:2298–2302.

Haracska, L., C.M. Kondratick, I. Unk, S. Prakash, and L. Prakash. 2001. Interaction with PCNA is essential for yeast DNA polymerase eta function. *Mol. Cell.* 8:407–415.

Hartman, P.S. 1984. UV irradiation of wild type and radiation-sensitive mutants of the nematode *Caenorhabditis elegans*: fertilities, survival, and parental effects. *Photochem. Photobiol.* 39:169–175.

Hartman, P.S., and R.K. Herman. 1982. Radiation-sensitive mutants of *Caenorhabditis elegans*. *Genetics.* 102:159–178.

Hartman, P.S., J. Hevelone, V. Dwarakanath, and D.L. Mitchell. 1989. Excision repair of UV radiation-induced DNA damage in *Caenorhabditis elegans*. *Genetics.* 122:379–385.

Hoege, C., B. Pfander, G.L. Moldovan, G. Pyrowolakis, and S. Jentsch. 2002. RAD6-dependent DNA repair is linked to modification of PCNA by ubiquitin and SUMO. *Nature.* 419:135–141.

Holway, A.H., C. Hung, and W.M. Michael. 2005. Systematic, RNA-interference-mediated identification of *mus-101* modifier menes in *Caenorhabditis elegans*. *Genetics.* 169:1451–1460.

Jones, C.A., and P.S. Hartman. 1996. Replication in UV-irradiated *Caenorhabditis elegans* embryos. *Photochem. Photobiol.* 63:187–192.

Kalogeropoulos, N., C. Christoforou, A.J. Green, S. Gill, and N.R. Ashcroft. 2004. chk-1 is an essential gene and is required for an S-M checkpoint during early embryogenesis. *Cell Cycle.* 3:1196–1200.

Kannouche, P.L., J. Wing, and A.R. Lehmann. 2004. Interaction of human DNA polymerase eta with monoubiquitinated PCNA: a possible mechanism for the polymerase switch in response to DNA damage. *Mol. Cell.* 14:491–500.

Leggett, D.S., D. Jones, and E.P. Candido. 1995. *Caenorhabditis elegans* UBC-1, a ubiquitin-conjugating enzyme homologous to yeast RAD6/UBC2, contains a novel carboxy-terminal extension that is conserved in nematodes. *DNA Cell Biol.* 14:883–891.

Lupardus, P.J., T. Byun, M.C. Yee, M. Hekmat-Nejad, and K.A. Cimprich. 2002. A requirement for replication in activation of the ATR-dependent DNA damage checkpoint. *Genes Dev.* 16:2327–2332.

MacQueen, A.J., and A.M. Villeneuve. 2001. Nuclear reorganization and homologous chromosome pairing during meiotic prophase require *C. elegans* chk-2. *Genes Dev.* 15:1674–1687.

Maeda, I., Y. Kohara, M. Yamamoto, and A. Sugimoto. 2001. Large-scale analysis of gene function in *Caenorhabditis elegans* by high-throughput RNAi. *Curr. Biol.* 11:171–176.

O'Farrell, P.H., J. Stumpff, and T.T. Su. 2004. Embryonic cleavage cycles: how is a mouse like a fly? *Curr. Biol.* 14:R35–R45.

Papouli, E., S. Chen, A.A. Davies, D. Huttner, L. Krejci, P. Sung, and H.D. Ulrich. 2005. Crosstalk between SUMO and ubiquitin on PCNA is mediated by recruitment of the helicase Srs2p. *Mol. Cell.* 19:123–133.

Pfander, B., G.L. Moldovan, M. Sacher, C. Hoege, and S. Jentsch. 2005. SUMO-modified PCNA recruits Srs2 to prevent recombination during S phase. *Nature.* 436:428–433.

Prakash, S., R.E. Johnson, and L. Prakash. 2005. Eukaryotic translesion synthesis DNA polymerases: specificity of structure and function. *Annu. Rev. Biochem.* 74:317–353.

Sancar, A., L.A. Lindsey-Boltz, K. Unsal-Kacmaz, and S. Linn. 2004. Molecular mechanisms of mammalian DNA repair and the DNA damage checkpoints. *Annu. Rev. Biochem.* 73:39–85.

Scully, R., J. Chen, R.L. Ochs, K. Keegan, M. Hoekstra, J. Feunteun, and D.M. Livingston. 1997. Dynamic changes of BRCA1 subnuclear location and phosphorylation state are initiated by DNA damage. *Cell.* 90:425–435.

Sibon, O.C., V.A. Stevenson, and W.E. Theurkauf. 1997. DNA-replication checkpoint control at the *Drosophila* midblastula transition. *Nature.* 388:93–97.

Sibon, O.C., A. Laurencon, R. Hawley, and W.E. Theurkauf. 1999. The *Drosophila* ATM homologue Mei-41 has an essential checkpoint function at the midblastula transition. *Curr. Biol.* 9:302–312.

Stokes, M.P., R. Van Hatten, H.D. Lindsay, and W.M. Michael. 2002. DNA replication is required for the checkpoint response to damaged DNA in *Xenopus* egg extracts. *J. Cell Biol.* 158:863–872.

Su, T.T., S.D. Campbell, and P.H. O'Farrell. 1999. *Drosophila* grapes/CHK1 mutants are defective in cyclin proteolysis and coordination of mitotic events. *Curr. Biol.* 9:919–922.

Tercero, J.A., M.P. Longhese, and J.F. Diffley. 2003. A central role for DNA replication forks in checkpoint activation and response. *Mol. Cell.* 11:1323–1336.

Timmons, L., and A. Fire. 1998. Specific interference by ingested dsRNA. *Nature.* 395:854.

Watanabe, K., S. Tateishi, M. Kawasuji, T. Tsurimoto, H. Inoue, and M. Yamaizumi. 2004. Rad18 guides poleta to replication stalling sites through physical interaction and PCNA monoubiquitination. *EMBO J.* 23:3886–3896.

Yu, K.R., R.B. Saint, and W. Sullivan. 2000. The Grapes checkpoint coordinates nuclear envelope breakdown and chromosome condensation. *Nat. Cell Biol.* 2:609–615.

Zhao, B., Z. Xie, H. Shen, and Z. Wang. 2004. Role of DNA polymerase eta in the bypass of abasic sites in yeast cells. *Nucleic Acids Res.* 32:3984–3994.