Current Biology 17, 286–292, February 6, 2007 ©2007 Elsevier Ltd All rights reserved DOI 10.1016/j.cub.2006.12.038

Report

AKT-1 Regulates DNA-Damage-Induced Germline Apoptosis in *C. elegans*

Celia Quevedo,[1] David R. Kaplan,[1] and W. Brent Derry[1,*]
[1] Cancer Research Program
Hospital for Sick Children
Toronto, Ontario M5G 1X8
Canada

Summary

The cellular response to genotoxic stress involves the integration of multiple prosurvival and proapoptotic signals that dictate whether a cell lives or dies. In mammals, AKT/PKB regulates cell survival by modulating the activity of several apoptotic proteins, including p53 [1]. In *Caenorhabditis elegans*, *akt-1* and *akt-2* regulate development in response to environmental cues by controlling the FOXO transcription factor *daf-16* [2], but the role of these genes in regulating p53-dependent apoptosis is not known. In this study, we show that *akt-1* and *akt-2* negatively regulate DNA-damage-induced apoptosis in the *C. elegans* germline. The antiapoptotic activity of *akt-1* is independent of its target gene *daf-16* but dependent on *cep-1*/p53. Although only *akt-1* regulates the apoptotic activity of *cep-1*, both *akt-1* and *akt-2* modulate the intensity of the apoptotic response independently of the transcriptional activity of CEP-1. Finally, we show that AKT-1 regulates apoptosis but not cell-cycle progression downstream of the HUS-1/MRT-2 branch of the DNA damage checkpoint.

Results and Discussion

To assess whether AKT regulates germline apoptosis in *C. elegans*, we examined the effects of DNA-damaging agents in worms homozygous for *akt-1(mg144)* gain-of-function and *akt-1(ok525)* and *akt-2(ok393)* loss-of-function mutants. We observed a proportional increase in the number of germ-cell corpses after exposure to increasing levels of ionizing radiation (IR) at all time points analyzed, and we observed this in all strains used. *akt-1* loss-of-function mutants exhibited increased sensitivity to DNA-damage-induced germ-cell apoptosis 12, 24, and 36 hr after irradiation, whereas *akt-1* gain-of-function mutants were more resistant to apoptosis than wild-type worms at the same time points (Figures 1A–1E). We also observed an increase in germline apoptosis in two different *akt-2* loss-of-function mutants treated with IR, although the effect was less pronounced than with *akt-1(ok525)* mutants (Figure 1F and Figures S1A, S1B, and S1D in the Supplemental Data available with this article online). Wild-type worms treated with *akt-1(RNAi)* were also hypersensitive to IR-induced germline apoptosis (Figure S1C). Similar to IR, the DNA-alkylating agent N-ethyl-N-nitrosourea (ENU) caused a significant increase in germline apoptosis in *akt-1* and *akt-2* loss-of-function mutants, whereas *akt-1* gain-of-function mutants showed a significant decrease in the number of germ-cell corpses (Figure 1G). None of the *akt* mutants affected developmental apoptosis (Table S1), and the engulfment rates of the germ-cell corpse were similar in all of them (Table S2). Taken together, these data indicate that AKT-1 and AKT-2 act as antiapoptotic proteins to modulate DNA-damage-induced apoptosis in the *C. elegans* germline. Consistent with our findings, human cancer cells that express constitutively active Akt are resistant to radiation [3–6], whereas thymocytes and embryo fibroblasts from mice with homozygous disruption of the Akt-1 gene are more susceptible to apoptosis induced by DNA-damaging agents [7]. Thus, the ability of Akt to prevent apoptosis in response to DNA damage is conserved through evolution.

To investigate whether AKT-1 and AKT-2 function redundantly as antiapoptotic proteins, we generated a double-mutant strain containing both *akt-1(ok525)* and *akt-2(ok393)* loss-of-function alleles. Although these worms arrest at the dauer stage of development, they are able to develop into adults when grown on *daf-16(RNAi)* [8]. However, we could not reliably score apoptosis because this strain exhibits an Egl (EGg-laying-defective) phenotype, in which eggs obstructed the germline and prevented accurate quantification of germ-cell corpses. In addition, *daf-16(RNAi)* itself has an effect on germ-cell apoptosis in response to ionizing radiation (see below).

Activation of germ-cell apoptosis in response to DNA-damaging agents requires the core apoptotic signaling pathway, which includes the *ced-3*, *ced-4*, and *ced-9* genes [9]. To evaluate whether the enhanced IR-induced germ-cell death observed in *akt-1(ok525)* loss-of-function mutants is dependent on the core cell death pathway, we created double-mutant strains between *akt-1(ok525)* and loss-of-function alleles of *ced-3* and *ced-4*, as well as a *ced-9* gain-of-function allele that reduces the binding of CED-9 to EGL-1 [10]. We found that the *ced-3(n717)*, *ced-4(n1162)*, and *ced-9(n1950)* alleles suppressed germline apoptosis in *akt-1(ok525)* mutants (Figure S2A), indicating that the regulation of germ-cell apoptosis by *akt-1* requires the core cell death pathway.

The forkhead family transcription factor DAF-16, the only confirmed target of AKT-1/2 in *C. elegans*, is phosphorylated by AKT-1 and AKT-2, and this causes it to be retained in the cytoplasm to prevent entry into the dauer stage [11]. Therefore, we reasoned that if DAF-16 also mediates the antiapoptotic activity of AKT-1, we would expect *daf-16* loss-of-function mutants to be resistant to germ-cell apoptosis in response to DNA damage, as recently described for the *daf-16(mu86)* allele [12]. Surprisingly, we observed no effect on IR-induced apoptosis with the *mu86* allele and increased levels of apoptosis in four different *daf-16* loss-of-function mutants treated with IR (Figures 2A and 2B). Inhibiting

*Correspondence: brent.derry@sickkids.ca

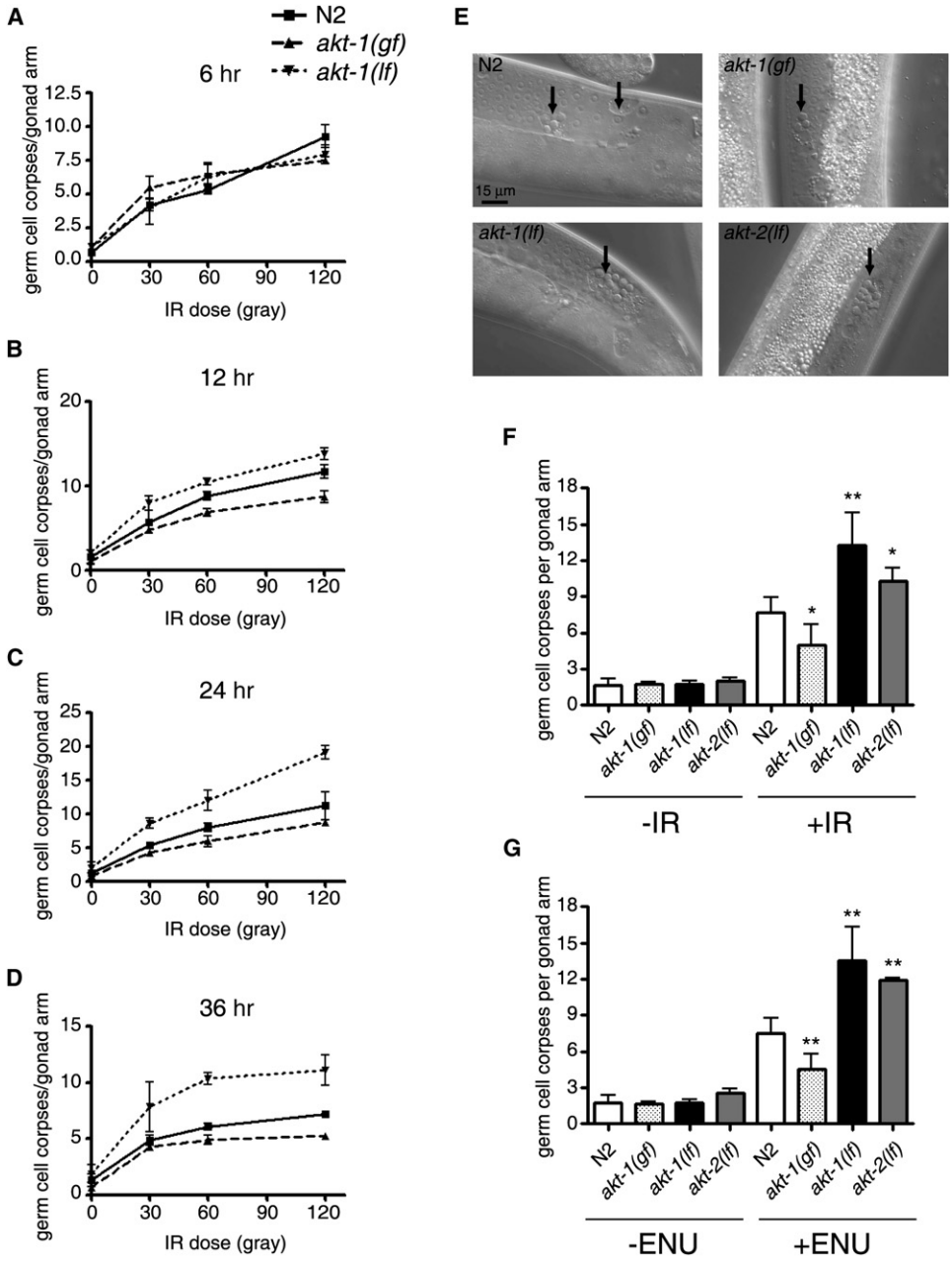

Figure 1. AKT-1 Regulates DNA-Damage-Induced Apoptosis

(A–D) The wild-type (N2) and *akt-1* mutant hermaphrodites were treated at the young-adult stage with increasing doses (30, 60, and 120 Gy) of IR, and germ-cell apoptosis was scored 6 (A), 12 (B), 24 (C), and 36 hr (D) after treatment. Three hours before counting, worms were treated with acridine orange (AO) as described in the Supplemental Experimental Procedures. Germ-cell corpses were quantified in the pachytene region of one gonad arm with Nomarski optics and fluorescence microscopy for detecting AO staining nuclei to confirm that cell corpses were apoptotic. For each time point, between 15 and 20 worms were scored per experiment and data represent the average of the mean of two to three independent experiments. Error bars represent the standard deviation (SD).

(E) N2 controls and *akt* mutants were treated with 60 Gy of IR. Panels show representative images of germlines 24 hr after IR treatment. Arrows indicate areas with apoptotic nuclei.

(F and G) N2 controls and *akt* mutant worms were treated with 60 Gy of IR (F) or with 5 mM ENU (G), and germ-cell corpses were quantified 24 hr after treatment as described above. Data represent the average of the mean of five to eight different experiments (15 to 20 worms were scored per experiment). Error bars represent the SD. *$p < 0.05$ and **$p < 0.01$ versus treated N2 worms. *akt-1(gf) = akt-1(mg144)*, *akt-1(lf) = akt-1(ok525)*, and *akt-2(lf) = akt-2(ok393)*.

daf-16 by RNAi also significantly increased the number of apoptotic germ cells in irradiated worms relative to controls (Figure 2C), suggesting that DAF-16 acts as an antiapoptotic protein in the *C. elegans* germline. The fact that *daf-16(RNAi)* also increased apoptosis in *akt-1* loss-of-function mutants treated with IR, and did not significantly enhance apoptosis in *akt-1* gain-of-function mutants (Figure 2C), suggests that *daf-16*

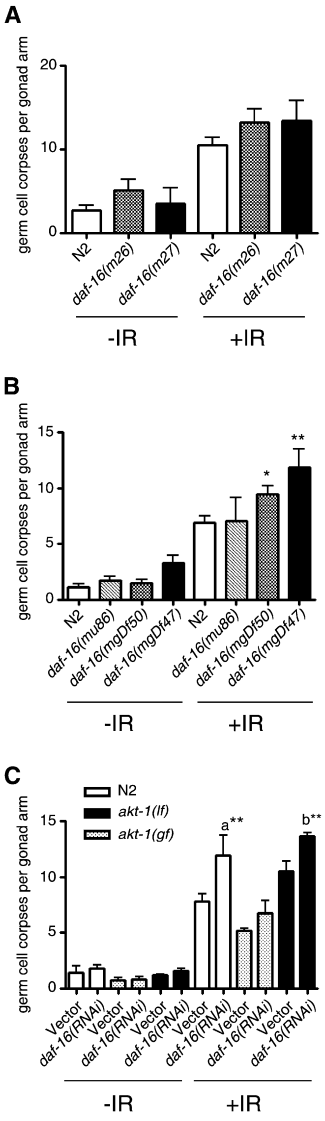

Figure 2. *daf-16* Mutant Worms Are Hypersensitive to DNA-Damage-Induced Apoptosis

(A and B) N2 controls, *daf-16* mutants (*m26* and *m27*) (A), and the deletion alleles (*mu86*, *mgDf50*, *mgDf47*) (B) were treated with 60 Gy of IR at the young-adult stage, and germ-cell apoptosis was quantified 24 hr later as described in Figure 1. Between 15 and 20 worms were scored per experiment, and data represent the average of the mean of four to eight independent experiments. Error bars represent the SD. *p < 0.05 and **p < 0.01 versus treated wild-type worms.

(C) N2 worms, *akt-1(mg144)*, and *akt-1(ok525)* worms grown on bacteria containing an empty RNAi control vector (L4440) or *daf-16(RNAi)* for one generation were treated as young adults with IR (60 Gy), and germ-cell corpses were quantified 24 hr later as described in Figure 1. Between 15 and 20 worms were scored per experiment, and data represent the average of the mean of three to six independent experiments. Error bars represent the SD. a**p < 0.01 versus IR-treated wild-type worms grown on RNAi control vector. b**p < 0.01 versus treated *akt-1(ok525)* worms grown on RNAi control vector. *akt-1(gf)* = *akt-1(mg144)*, and *akt-1(lf)* = *akt-1(ok525)*.

acts independently of *akt-1* to modulate DNA-damage-induced germline apoptosis. Although mammalian FOXO transcription factors are often thought of as apoptotic proteins, an antiapoptotic role for FOXO3 has also been reported [13–15].

The *C. elegans* p53 homolog *cep-1* is required for activation of germ-cell apoptosis in response to DNA damage [16, 17]. The elevated germ-cell apoptosis in *akt-1(ok525)* mutants exposed to IR was completely blocked by the deletion allele *cep-1(gk138)* (Figure 3A) and by *cep-1(RNAi)* (Figure S2B), and such a finding indicates that AKT-1 functions upstream of, or in parallel to, CEP-1/p53. Previous studies have shown that CEP-1/p53 transactivates the proapoptotic BH3-only proteins EGL-1 and CED-13 [18, 19]. Therefore, we assessed CEP-1/p53 activity in Akt mutants by measuring the transcript levels of *egl-1* and *ced-13* by quantitative real-time PCR (qPCR). In response to IR, we observed that the levels of both *egl-1* and *ced-13* transcripts were higher in *akt-1* loss-of-function mutants than in wild-type worms at all doses tested (Figures 3B and 3C). We also observed a clear decrease in *egl-1* (−1.9-fold) and *ced-13* (−1.6-fold) expression in *akt-1* gain-of-function mutants relative to wild-type controls at 120 Gy (Figures 3B and 3C). These results indicate that AKT-1 negatively regulates CEP-1/p53 transcriptional activity in response to DNA damage. However, in *akt-2* loss-of-function mutants, we did not observe a significant difference in the induction of these CEP-1 target genes compared with wild-type controls (Figures 3B and 3C). Thus, although both AKT-1 and AKT-2 regulate DNA-damage-induced apoptosis, only AKT-1 modulates CEP-1/p53 activity as assessed by *egl-1* and *ced-13* transcriptional activation.

In mammalian cells, p53 protein is activated by numerous posttranslational modifications in response to stress signals [20]. To understand how AKT-1 modulates CEP-1 activity, we examined the status of endogenous CEP-1 protein in the wild-type and *akt-1* mutants by western-blot analysis using a CEP-1 antibody (a generous gift from Dr. Anton Gartner). In the absence of IR, a specific band at 70 kDa was detected in the wild-type and *akt-1* mutants, whereas no band was detected in *cep-1(gk138)* deletion mutants (Figure 3D). After IR treatment, we detected a slower migrating band that likely represents a posttranslationally modified form of CEP-1/p53. To determine whether this corresponds to phosphorylated CEP-1, we dialyzed protein extracts in order to treat the sample with phosphatase and noticed that the modified CEP-1 band decreased after alkaline phosphatase treatment (Figure S3B). We also found that this modified band decreased in abundance after dialysis, presumably because of endogenous phosphatases in the extract. Furthermore, the presence of phosphatase inhibitors in the dialysis buffer prevented the decrease of this modified form of CEP-1 (Figure S3C). These experiments indicate that CEP-1 is phosphorylated in response to IR. The levels of phosphorylated CEP-1 were lower in *akt-1(mg144)* than in wild-type worms at all doses of IR tested (Figures 3D and 3E, left). In these mutants, the unmodified 70 kDa band was also significantly lower in abundance after treatment with 60 and 120 Gy IR, whereas in wild-type worms, the levels remained unchanged in response to IR. This suggests that CEP-1 is not only phosphorylated but also stabilized in response to DNA damage in wild-type animals, but in *akt-1* gain-of-function mutants, its stability is reduced. A third higher band of CEP-1 was always detectable in *akt-1* loss-of-function mutants

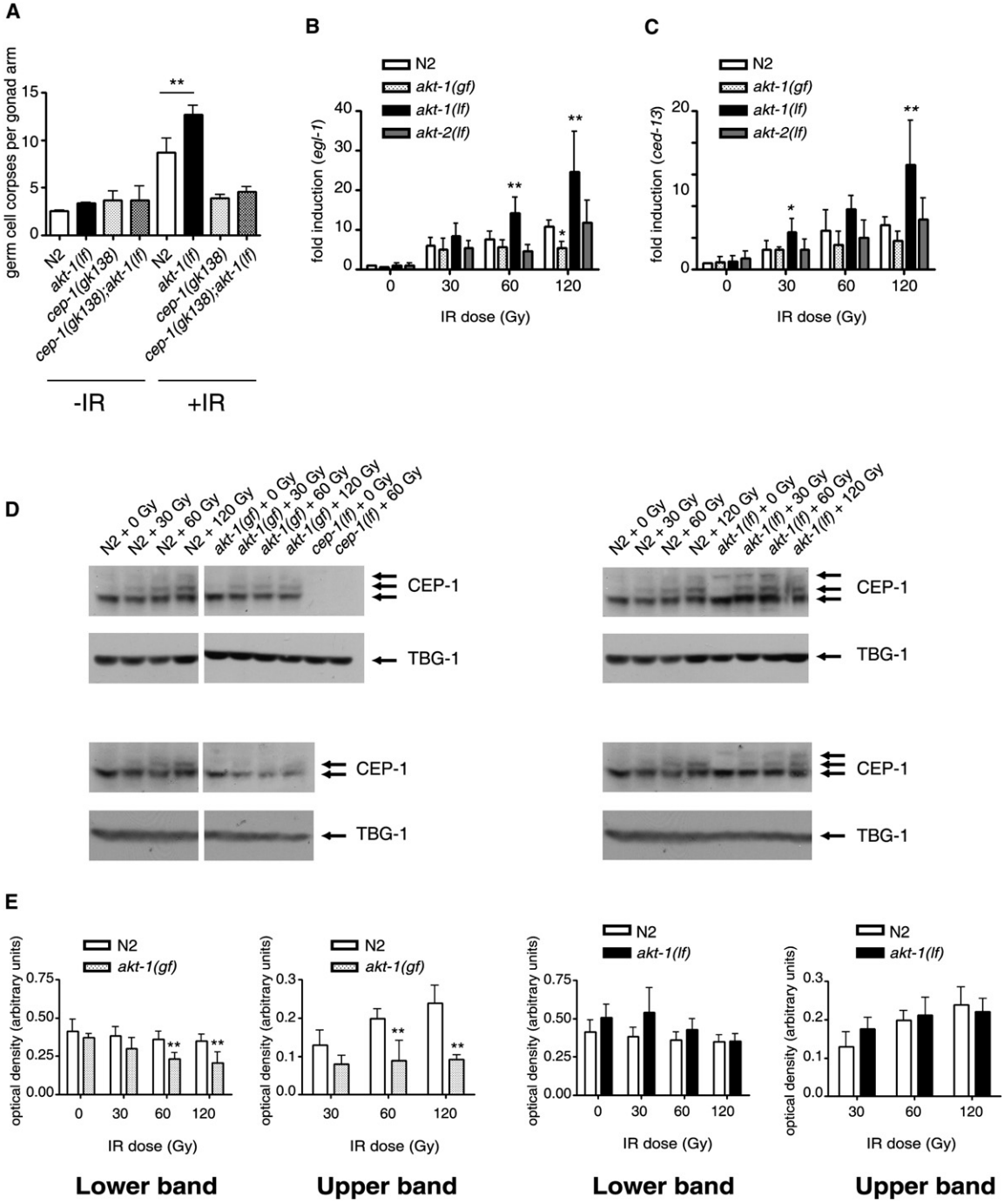

Figure 3. AKT-1 Regulates CEP-1/p53 Activity and Protein Levels after DNA Damage

(A) N2 worms, *akt-1(ok525)* and *cep-1(gk138)* single mutants, and *cep-1(gk138);akt-1(ok525)* double mutants were treated at the young-adult stage with 60 Gy of IR, and germ-cell apoptosis was quantified after 24 hr as described in Figure 1. Data represent the average of the mean of four independent experiments (n = 15–20 worms per experiment). Error bars represent the SD. **p < 0.01 versus treated N2 worms.

(B and C) N2 controls and *akt-1(mg144)*, *akt-1(ok525)*, and *akt-2(ok393)* mutant worms were synchronized and treated at the young-adult stage with the indicated doses of IR. Total RNA was isolated after 22 hr, and quantitative real-time PCR (qPCR) was carried out as described in the Supplemental Experimental Procedures so that *egl-1* (B) and *ced-13* (C) mRNA levels could be measured. Transcript levels were normalized with γ-tubulin mRNA in both experiments. Fold induction was calculated relative to levels in untreated N2 controls. Data represent the mean of six to ten independent experiments, each run in duplicate. Error bars represent the SD. *p < 0.05 and **p < 0.01 versus wild-type worms treated with the same dose of IR.

(D and E) N2 worms and *akt-1(mg144)*, *akt-1(ok525)*, and *cep-1(gk138)* mutant worms were synchronized and treated at the young-adult stage with the indicated dose of IR. Animals were lysed 22 hr after treatment as described in the Supplemental Experimental Procedures, and proteins were separated by SDS-PAGE and immunoblotted with CEP-1 (D, upper panel) and α-tubulin (D, lower panel) antibodies. Figure 3D depicts two representative experiments comparing wild-type worms with *akt-1(mg144)* gain-of-function mutants (left) and the wild-type with *akt-1(ok525)* loss-of-function mutants (right). All samples were run on the same gel, although they are shown separately so that differences compared

although similar levels of this CEP-1 isoform were only detected in wild-type worms treated with the highest dose of IR (120 Gy) (quantification in Figure S3A). This third band might represent further phosphorylation of CEP-1 or a different posttranslational modification that mainly appears when the DNA-damage signal is high or when *akt*-1 is absent (Figure 3D). Taken together, these results support a model whereby the posttranslational modifications and stability of CEP-1 are modulated by AKT-1, which controls the apoptotic activity of CEP-1, in response to DNA damage.

The precise mechanism by which AKT-1 regulates CEP-1 is not known. Promising candidates from mammalian systems, such as the Akt substrates Mdm-2 [21] and Chk-1 [20, 22], either have not been identified in the *C. elegans* genome (Mdm-2) or do not contain AKT-1 consensus phosphorylation sites (Chk-1). Because CEP-1 protein levels are modified in *akt-1* mutants, we screened all of the E3 ubiquitin ligases containing RING domains [23] and Akt consensus phosphorylation sites in the *C. elegans* genome for defects in DNA-damage-induced germ-cell apoptosis. We also examined the *C. elegans* homolog of the HECT domain containing E3 ligase ARF-BP1, which contains Akt consensus phosphorylation sites and is the only p53-specific E3 ligase that is conserved between mammals and invertebrates [24, 25]. We found that several RING finger ubiquitin ligase genes, as well as ARF-BP1, caused an increase in germ-cell apoptosis when inhibited in wild-type animals treated with IR (Table S3). However, none of these RNA interferences altered the transcriptional activity of CEP-1 or rescued the decreased germ-cell apoptosis in *akt-1* gain-of-function mutants (Figures S4A and S4B), suggesting that none of these E3 ligases are responsible for the regulation of CEP-1 by AKT-1. We also examined epistatic interactions between *akt-1*, *abl-1*, and *gld-1*, which regulate germline apoptosis in response to DNA damage via CEP-1. As previously described [26], inhibiting *abl-1* by RNAi increased IR-induced apoptosis in wild-type animals compared with controls, but there was no increase in *akt-1(mg144)* gain-of-function mutants (Figure S4C). This suggests that *akt-1* is downstream of *abl-1* or in a parallel signaling pathway. Recently, a hypomorphic mutation in the *gld-1* translational repressor gene was found to increase the translation of CEP-1 in the germline and lead to increased apoptosis in the absence and presence of DNA damage [27]. We created double mutants between *akt-1(mg144)* gain-of-function and *gld-1(op236)* loss-of-function alleles and found that the levels of apoptosis in the double mutant were no different than in the *gld-1(op236)* single mutant (Figure S4D), suggesting that *gld-1* acts downstream of *akt-1* or in an independent pathway to control *cep-1*-dependent apoptosis.

In *C. elegans*, *mrt-2*, *hus-1*, and *clk-2* encode checkpoint proteins that transmit DNA-damage signals to the core apoptotic pathway through CEP-1/p53. HUS-1 and MRT-2 form part of the 9:1:1 complex, whereas CLK-2 functions in parallel to the 9:1:1 complex [28, 29]. In addition to activating apoptosis, these checkpoint genes also promote cell-cycle arrest in the mitotic region of the germline in response to DNA damage independently of *cep-1* [16–18]. Checkpoint mutants also produce inviable embryos after treatment with IR because they are unable to repair damaged DNA [29]. Because AKT-1 appears to act upstream of CEP-1/p53, we asked whether *akt-1* also has a role in the checkpoint response. We found that germline cell-cycle arrest was not altered in either *akt-1* gain-of-function or loss-of-function mutants (Figures S5A and S5B), and the survival of progeny from *akt-1(mg144)* and *akt-1(ok525)* worms were no more sensitive to IR than wild-type worms (Table S4). Therefore, these results indicate that AKT-1 does not act as a checkpoint protein but likely lies downstream of the DNA damage checkpoint to regulate the apoptotic activity of CEP-1/p53. To test this, we generated double mutants between *akt-1(ok525)* and loss-of-function alleles in the *clk-2*, *mrt-2*, and *hus-1* checkpoint genes. We found that *clk-2(qm37);akt-1(ok525)* double mutants were as resistant to damage-induced apoptosis as *clk-2(qm37)* single mutants (Figure 4A), indicating that *akt-1* does not act downstream of *clk-2*. However, irradiated *mrt-2(e2663);akt-1(ok525)* or *hus-1(op244);akt-1(ok525)* double mutants exhibited similar levels of apoptosis as irradiated wild-type controls, indicating that AKT-1 acts downstream of, or in parallel to, the 9:1:1 checkpoint (Figure 4A). This suggests that inhibition of AKT-1 is part of the mechanism by which the HUS-1/MRT-2 complex signals to activate CEP-1/p53-dependent apoptosis in response to DNA damage. To assess this, we measured CEP-1/p53 transcriptional activity in *hus-1(op244);akt-1(ok525)* and *mrt-2(e2663);akt-1(ok525)* double-mutant animals. Although CEP-1/p53 is modestly activated in *hus-1(op244)* and *mrt-2(e2663)* single mutants treated with IR, presumably because the CLK-2 checkpoint is active, this activation was not enhanced by the *akt-1(ok525)* allele (Figure 4B). Therefore, the increased germ-cell apoptosis observed in *mrt-2(e2663);akt-1(ok525)* and *hus-1(op244);akt-1(ok525)* double mutants treated with IR was not due to an increase in CEP-1/p53 transcriptional activity. Because AKT-2 is able to regulate apoptosis without affecting CEP-1/p53 transcriptional activity (Figures 1F, 3B, and 3C), we also created *hus-1(op244);akt-2(ok393)* double mutants and observed similar levels of apoptosis in these mutants as with the *hus-1(op244);akt-1(ok525)* strain (Figure 4C). Because the increased IR-induced apoptosis observed in *akt-1(ok525)* mutants requires functional *cep-1* (Figure 3A), these results suggest that CEP-1 may also regulate apoptosis independently of its transcriptional activity, as described in mammalian cells [30]. We cannot rule out the possibility that CEP-1 regulates the transcription of genes, other than *egl-1*, that also regulate germline apoptosis. A third possibility is that AKT-1/2 can modulate the magnitude of the apoptotic response independently of CEP-1, perhaps by regulating components of the core apoptotic pathway.

with wild-type controls could be better appreciated. Graphs represent CEP-1 quantification from four to five independent experiments (E). Error bars represent the SD. **$p < 0.01$ versus wild-type worms treated with the same dose of IR. *akt-1(gf)* = *akt-1(mg144)*, *akt-1(lf)* = *akt-1(ok525)*, and *cep-1* = *cep-1(gk138)*.

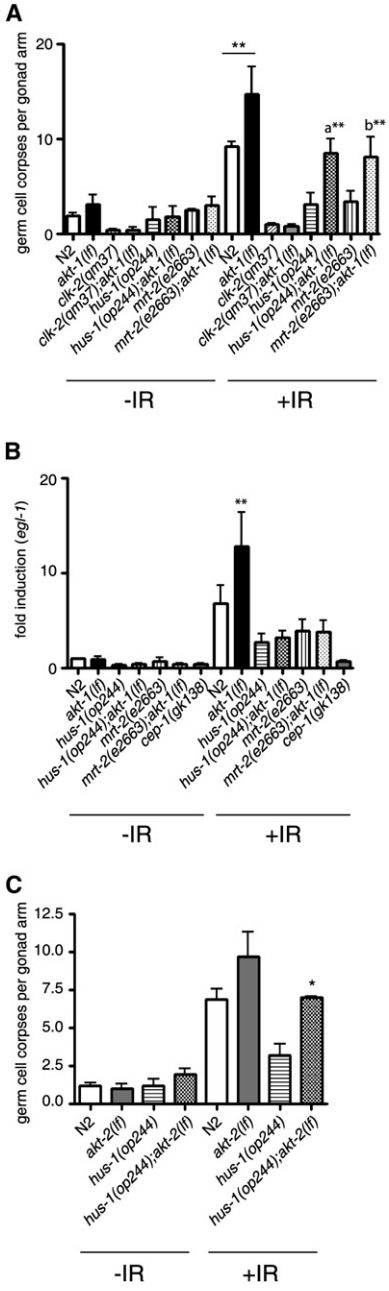

Figure 4. *akt-1* Is Epistatic to the *hus-1/mrt-2* Checkpoint

(A) N2 worms, *akt-1(ok525)*, *clk-2(qm37)*, *hus-1(op244)*, and *mrt-2(e2663)* single mutants, and *clk-2(qm37);akt-1(ok525)*, *hus-1(op244);akt-1(ok525)*, and *mrt-2(e2663);akt-1(ok525)* double-mutant animals were irradiated with 60 Gy of IR and scored for germ-cell apoptosis after 24 hr as described in Figure 1. Between 15 and 20 worms were scored per experiment, and data represent the average of the mean of three to five independent experiments. Error bars represent the SD. **p < 0.01 versus irradiated wild-type worms; a**p < 0.01 versus irradiated *hus-1(op244)*; b**p < 0.01 versus irradiated *mrt-2(e2663)*.

(B) N2 worms, *akt-1(ok525)*, *hus-1(op244)*, and *mrt-2(e2663)* single-mutant worms, and *hus-1(op244);akt-1(ok525)* and *mrt-2(e2663); akt-1(ok525)* double mutants were synchronized and treated as young adults with IR (60 Gy). Total RNA was isolated 22 hr after IR treatment, and qPCR was carried out as described in Figure 4 for quantifying *egl-1* mRNA levels. Data represent the mean of five to six independent experiments, each run in duplicate. Error bars represent the SD. **p < 0.01 versus irradiated N2 worms.

(C) N2 worms, *akt-2(ok393)* and *hus-1(op244)* single mutants, and *hus-1(op244);akt-2(ok393)* double mutants were irradiated (60 Gy) and scored for germ-cell apoptosis after 24 hr as described in Figure 1. Between 15 and 20 worms were scored per experiment, and data represent the average of the mean of two independent experiments. Error bars represent the SD. *p < 0.05 versus irradiated *hus-1(op244)* single mutants. *akt-1(lf)* = *akt-1(ok525)*, and *akt-2(lf)* = *akt-2(ok393)*.

In conclusion, we show that AKT-1 and AKT-2 regulate DNA-damaged-induced apoptosis in the *C. elegans* germline by acting downstream or in parallel to the HUS-1/MRT-2 branch of the checkpoint signaling pathway (Figure S6). AKT-1 regulates the CEP-1-dependent transcriptional activation of *egl-1* and *ced-13* only when both the 9:1:1 and CLK-2 checkpoint pathways are present. In addition, both AKT-1 and AKT-2 modulate the amplitude of the apoptotic response independently of CEP-1 transcriptional activity. Neither DAF-16, the only known substrate of AKT in worms, nor any of the E3 ubiquitin ligases with Akt consensus phosphorylation sites mediate the antiapoptotic activity of AKT-1/2.

Supplemental Data

Supplemental Data include Experimental Procedures, six figures, and four tables and can be found with this article online at http://www.current-biology.com/cgi/content/full/17/3/286/DC1/.

Acknowledgments

We thank the *C. elegans* Genetic Center and Dr. Shohei Mitani and the National Bioresource Project for the Nematode (Japan) for worm strains. We are grateful to Dr. Peter Roy (University of Toronto) for bacterial RNAi strains, Dr. Anton Gartner (University of Dundee) for CEP-1 antibody, and Jean-François Lavoie and Dr. Daniel Durocher for helpful comments on the manuscript. This work was supported by a postdoctoral fellowship from the Ministry of Education and Science of Spain to C.Q., CIHR operating grants to W.B.D. and D.R.K., and awards to W.B.D. from the Canada Foundation for Innovation and Ontario Innovation Trust. D.R.K. is a recipient of a Canada Research Chair. C.Q. was jointly supervised by D.R.K. and W.B.D.

Received: June 27, 2006
Revised: December 12, 2006
Accepted: December 18, 2006
Published: February 5, 2007

References

1. Song, G., Ouyang, G., and Bao, S. (2005). The activation of Akt/PKB signaling pathway and cell survival. J. Cell. Mol. Med. *9*, 59–71.
2. Paradis, S., and Ruvkun, G. (1998). Caenorhabditis elegans Akt/PKB transduces insulin receptor-like signals from AGE-1 PI3 kinase to the DAF-16 transcription factor. Genes Dev. *12*, 2488–2498.
3. Brognard, J., Clark, A.S., Ni, Y., and Dennis, P.A. (2001). Akt/protein kinase B is constitutively active in non-small cell lung cancer cells and promotes cellular survival and resistance to chemotherapy and radiation. Cancer Res. *61*, 3986–3997.
4. Tanno, S., Yanagawa, N., Habiro, A., Koizumi, K., Nakano, Y., Osanai, M., Mizukami, Y., Okumura, T., Testa, J.R., and Kohgo, Y. (2004). Serine/threonine kinase AKT is frequently activated in human bile duct cancer and is associated with increased radioresistance. Cancer Res. *64*, 3486–3490.
5. Altomare, D.A., and Testa, J.R. (2005). Perturbations of the AKT signaling pathway in human cancer. Oncogene *24*, 7455–7464.

6. Yang, X., Fraser, M., Moll, U.M., Basak, A., and Tsang, B.K. (2006). Akt-mediated cisplatin resistance in ovarian cancer: Modulation of p53 action on caspase-dependent mitochondrial death pathway. Cancer Res. *66*, 3126–3136.
7. Chen, W.S., Xu, P.Z., Gottlob, K., Chen, M.L., Sokol, K., Shiyanova, T., Roninson, I., Weng, W., Suzuki, R., Tobe, K., et al. (2001). Growth retardation and increased apoptosis in mice with homozygous disruption of the Akt1 gene. Genes Dev. *15*, 2203–2208.
8. Oh, S.W., Mukhopadhyay, A., Svrzikapa, N., Jiang, F., Davis, R.J., and Tissenbaum, H.A. (2005). JNK regulates lifespan in Caenorhabditis elegans by modulating nuclear translocation of forkhead transcription factor/DAF-16. Proc. Natl. Acad. Sci. USA *102*, 4494–4499.
9. Gartner, A., Milstein, S., Ahmed, S., Hodgkin, J., and Hengartner, M.O. (2000). A conserved checkpoint pathway mediates DNA damage–induced apoptosis and cell cycle arrest in *C. elegans*. Mol. Cell *5*, 435–443.
10. Parrish, J., Metters, H., Chen, L., and Xue, D. (2000). Demonstration of the in vivo interaction of key cell death regulators by structure-based design of second-site suppressors. Proc. Natl. Acad. Sci. USA *97*, 11916–11921.
11. Ogg, S., Paradis, S., Gottlieb, S., Patterson, G.I., Lee, L., Tissenbaum, H.A., and Ruvkun, G. (1997). The Fork head transcription factor DAF-16 transduces insulin-like metabolic and longevity signals in C. elegans. Nature *389*, 994–999.
12. Pinkston, J.M., Garigan, D., Hansen, M., and Kenyon, C. (2006). Mutations that increase the life span of C. elegans inhibit tumor growth. Science *313*, 971–975.
13. Kops, G.J., Dansen, T.B., Polderman, P.E., Saarloos, I., Wirtz, K.W., Coffer, P.J., Huang, T.T., Bos, J.L., Medema, R.H., and Burgering, B.M. (2002). Forkhead transcription factor FOXO3a protects quiescent cells from oxidative stress. Nature *419*, 316–321.
14. Nemoto, S., and Finkel, T. (2002). Redox regulation of forkhead proteins through a p66shc-dependent signaling pathway. Science *295*, 2450–2452.
15. Jonsson, H., Allen, P., and Peng, S.L. (2005). Inflammatory arthritis requires Foxo3a to prevent Fas ligand-induced neutrophil apoptosis. Nat. Med. *11*, 666–671.
16. Derry, W.B., Putzke, A.P., and Rothman, J.H. (2001). Caenorhabditis elegans p53: Role in apoptosis, meiosis, and stress resistance. Science *294*, 591–595.
17. Schumacher, B., Hofmann, K., Boulton, S., and Gartner, A. (2001). The *C. elegans* homolog of the p53 tumor suppressor is required for DNA damage-induced apoptosis. Curr. Biol. *11*, 1722–1727.
18. Hofmann, E.R., Milstein, S., Boulton, S.J., Ye, M., Hofmann, J.J., Stergiou, L., Gartner, A., Vidal, M., and Hengartner, M.O. (2002). *Caenorhabditis elegans* HUS-1 is a DNA damage checkpoint protein required for genome stability and EGL-1-mediated apoptosis. Curr. Biol. *12*, 1908–1918.
19. Schumacher, B., Schertel, C., Wittenburg, N., Tuck, S., Mitani, S., Gartner, A., Conradt, B., and Shaham, S. (2005b). C. elegans ced-13 can promote apoptosis and is induced in response to DNA damage. Cell Death Differ. *12*, 153–161.
20. Harris, S.L., and Levine, A.J. (2005). The p53 pathway: Positive and negative feedback loops. Oncogene *24*, 2899–2908.
21. Levav-Cohen, Y., Haupt, S., and Haupt, Y. (2005). Mdm2 in growth signaling and cancer. Growth Factors *23*, 183–192.
22. King, F.W., Skeen, J., Hay, N., and Shtivelman, E. (2004). Inhibition of Chk1 by activated PKB/Akt. Cell Cycle *3*, 634–637.
23. Moore, R., and Boyd, L. (2004). Analysis of RING finger genes required for embryogenesis in C. elegans. Genesis *38*, 1–12.
24. Chen, D., Kon, N., Li, M., Zhang, W., Qin, J., and Gu, W. (2005). ARF-BP1/Mule is a critical mediator of the ARF tumor suppressor. Cell *121*, 1071–1083.
25. Brooks, C.L., and Gu, W. (2006). p53 ubiquitination: Mdm2 and beyond. Mol. Cell *21*, 307–315.
26. Deng, X., Hofmann, E.R., Villanueva, A., Hobert, O., Capodieci, P., Veach, D.R., Yin, X., Campodonico, L., Glekas, A., Cordon-Cardo, C., et al. (2004). Caenorhabditis elegans ABL-1 antagonizes p53-mediated germline apoptosis after ionizing irradiation. Nat. Genet. *36*, 906–912.
27. Schumacher, B., Hanazawa, M., Lee, M.H., Nayak, S., Volkmann, K., Hofmann, E.R., Hengartner, M., Schedl, T., and Gartner, A. (2005). Translational repression of *C. elegans* p53 by GLD-1 regulates DNA damage-induced apoptosis. Cell *120*, 357–368.
28. Ahmed, S., Alpi, A., Hengartner, M.O., and Gartner, A. (2001). *C. elegans* RAD-5/CLK-2 defines a new DNA damage checkpoint protein. Curr. Biol. *11*, 1934–1944.
29. Stergiou, L., and Hengartner, M.O. (2004). Death and more: DNA damage response pathways in the nematode C. elegans. Cell Death Differ. *11*, 21–28.
30. Kohn, K.W., and Pommier, Y. (2005). Molecular interaction map of the p53 and Mdm2 logic elements, which control the Off-On switch of p53 in response to DNA damage. Biochem. Biophys. Res. Commun. *331*, 816–827.