Caenorhabditis elegans Chk2-like gene is essential for meiosis but dispensable for DNA repair

Atsushi Higashitani*, Hidetoshi Aoki, Akiyuki Mori, Yohei Sasagawa, Takako Takanami, Hideyuki Takahashi

Institute of Genetic Ecology, Tohoku University, 2-1-1 Katahira, Sendai 980-8577, Japan

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Abstract A *Chk2*-like gene was identified in the genome of *Caenorhabditis elegans*. The putative gene product, termed *Ce-chk-2* consists of 450 amino acid residues, and shows good homology with the Chk2/Cds1 gene family. The results of RNA-mediated interference (RNAi) indicated that the F1 generation from dsRNA injected animals grew to adulthood, but approximately 95% of their eggs (F2) died during early embryogenesis. Among the few surviving progeny, males (XO animals) arose at an abnormally high frequency (30%). In addition, 12 univalents were observed in full grown oocytes of the F1, while six bivalents were normally observed in wild-type oocytes. *Ce-chk-2* gene expression increased in the adult stage, and their expression level decreased in the *glp-4* mutant, which is defective in germ line proliferation. The radiation sensitivity of F1 embryos carrying *Ce-chk-2* RNAi was not significantly affected. **© 2000 Federation of European Biochemical Societies. Published by Elsevier Science B.V. All rights reserved.**

Key words: Chiasma; Pairing; RNA-mediated interference; Protein kinase; Forkhead homology-associated domain; Chk2/Cds1

1. Introduction

Meiosis consists of two specialized cell divisions that produce haploid gametes from diploid parental cells. The chromosome number is reduced during meiosis because a single round of DNA replication is followed by two divisions, anaphase I and II. The nematode *Caenorhabditis elegans* is a useful experimental organism for studying the processes of development and differentiation, including meiosis [1–3]. In the adult *C. elegans* hermaphrodite, germ cell differentiation switches to oogenesis after approximately 150 sperm have been produced in each of two gonadal arms at the L4 larval stage. The germ line nuclei of the distal arm divide mitotically and then progress into meiosis, from leptotene distally to diplotene of meiotic prophase I proximally. Full grown oocytes arrest at diakinesis during meiotic prophase I and enter the spermatheca where they become fertilized. After fertilization, meiotic divisions are completed and embryogenesis ensues in the uterus. Several mutants of meiosis and germ line development of the nematode have been isolated and reported [4].

Protein phosphorylation plays a central role in many regulatory functions of eukaryotes such as DNA replication, transcription, translation, cell-cycle control, stress responses, basic metabolism, cell proliferation, cell differentiation, cell–cell communication and several meiotic processes. In the nematode *C. elegans*, whose genome has been completely sequenced, approximately 500 protein kinases among the 19 000 predicted proteins have been identified [5].

The mammalian Chk2 kinase is a homolog of the *Saccharomyces cerevisiae* Rad53 gene and the *Schizosaccharomyces pombe* Cds1 kinase [6], which function in cell-cycle checkpoint control during DNA replication and following DNA damage [6–11]. These kinases contain the forkhead homology-associated (FHA) domain, which serves as a motif for modular phosphopeptide recognition [12]. In addition, mammalian Chk2 is most closely related to *Drosophila melanogaster* maternal nuclear protein kinase Dm-nk, an abundantly expressed protein of ovaries that may function in meiosis [13]. However, the molecular role of the Chk2 kinase in meiotic function is not yet fully understood. In this study, we describe a *C. elegans* Chk2-like kinase gene, *Ce-chk-2*, that is essential for the meiotic process but dispensable for the DNA repair process.

2. Materials and methods

2.1. C. elegans strains and general methods

A hermaphrodite of the wild-type N2 Bristol stain was used for RNA interference (RNAi) experiments. The *glp-4* temperature sensitive (ts) mutant [14], which is defective in germ line proliferation, was generously supplied by the *C. elegans* Genetic Center. The general methods used to culture and handle *C. elegans* have been described [15]. The nematode experiments were performed at 20°C unless otherwise noted.

2.2. Isolation and characterization of the Ce-chk-2 gene

A BLAST search with the human Chk2 kinase revealed good homology with the product of the predicted gene Y60A3A.12 in the *C. elegans* (DNA database accession number AL117207). This genomic sequence and an EST sequence of yk523b3 derived from this gene (accession numbers AV177078 and AV188394 isolated by Dr. Y. Kohara) were used to make primers for 5′- and 3′-RACE to isolate the native cDNA. The RACE products were subcloned with pGEM-T vector (Promega) and were sequenced with Big Dye terminator cycle sequencing kit (Applied Biosystems). The 1430 bp cDNA sequence of *Ce-chk-2* gene was registered as accession number AB049441 in DDBJ.

2.3. Expression analyses of the Ce-chk-2 gene

Total RNA was isolated by using Trizol Reagent (Gibco BRL Co., Ltd) at each of the developmental stages of the wild-type N2 strain of *C. elegans* and *glp-4* ts mutant. RT-PCR was performed with approximately 0.2 μg of each RNA sample and the following primer sets, which specifically amplify the *Ce-chk-2*, *gld-1* and *act-1* genes, respec-

*Corresponding author. Fax: (81)-22-263 9845.
E-mail: ahigashi@ige.tohoku.ac.jp

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tively: *Ce-chk-2*-ah1 (forward) 5′-AGCCGAGCAATCGCTAAAA-CTCGAG, *Ce-chk-2*-ah2 (reverse) 5′-GCCTTTTTCACAGGTC-CAGCTTGTG, *gld-1*-ah1 (forward) 5′-TGGGAGCATCTCGAA-GACGATCTGCACGTTCTTGTGC, *gld-1*-ah2 (reverse) 5′-GAA-AGAGGTGTTGTTGACTGAAGAAGCCGAGGGACTTG, *act-1*-ah1 (forward) 5′-CGTGGTTACTCTTTCACCACCACCGCTG, and *act-1*-ah2 (reverse) 5′-CATTTAGAAGCACTTGCGGTGAAC-GATGG. These primers sets were designed to amplify approximately 500–800 bp of the sequence from the C-terminal region of each gene. RT-PCR was performed for 22–26 cycles with all primer combinations.

2.4. RNAi experiments

To investigate the null phenotype of the *Ce-chk-2* gene, RNAi was carried out as described previously [16]. For the preparation of double stranded (ds) RNA (about 800 bp: corresponding to the nucleotide number 601-1394 in AB049441) containing kinase domain, above RT-PCR fragment of *Ce-chk-2* was phosphorylated at both of its 5′ ends

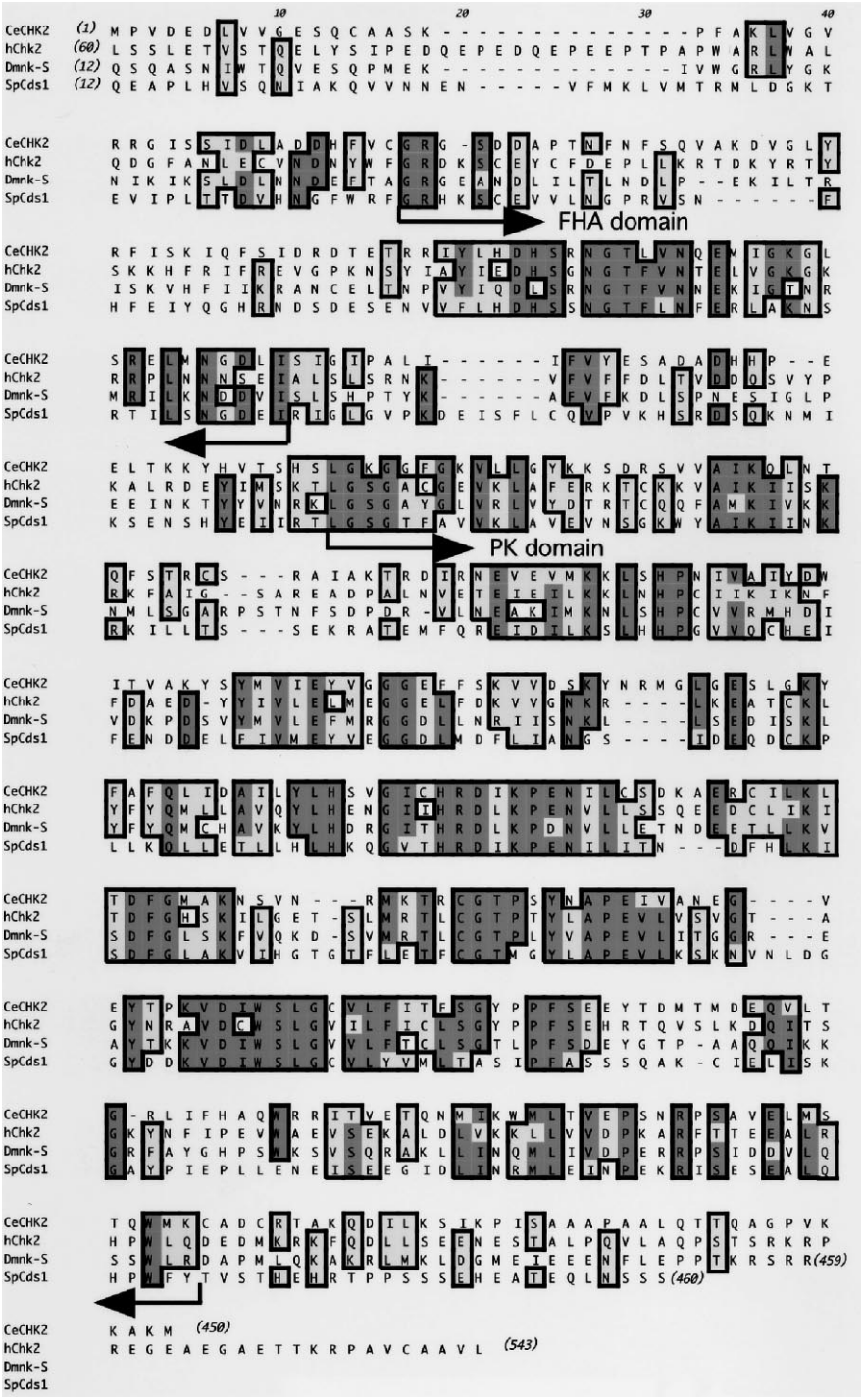

Fig. 1. Comparison of the amino acid sequences of the putative products of *Ce-chk-2* and other eukaryotic Chk2/Cds1 family. The amino acid sequences of the putative gene products of *Ce-chk-2* (accession number AB049441), human Chk2 (AF174135), *Drosophila* Dm-nk (AB007822) and *S. pombe* Cds1 (X85040) are aligned. The positions of identical amino acids and similar residues between Chk2/Cds1 family are indicated by black boxes and gray boxes.

with T4 polynucleotide kinase, and the sample was ligated to the T7 promoter linker (5′-GCGCGTAATACGACTCACTATAGGGC-GAAT and 5′-(p)TTCGCCCTATAGTGAGTCGTATTACGCGC). The ligated sample was re-amplified by PCR using the former non-phosphorylated T7 primer. The PCR product was subcloned with pGEM-T vector, and its sequence was determined. Subcloned *Ce-chk-2* cDNA fragment was re-amplified with the T7 primer, and then it was used as a template for in vitro dsRNA synthesis. Preparation and purification of dsRNA was carried out as described [17]. We also prepared dsRNA (about 570 bp: corresponding to the nucleotide numbers 52–625 in AB049441) containing FHA domain of *Ce-chk-2*. Young adult hermaphrodites were injected with each dsRNA (approximately 2 μg/μl) into the body-cavity tail or the gonads, and were transferred to fresh culture plates. The eggs (F1 generation) laid by the injected animals (I0) were collected 16–32 h after injection, which corresponded to the period of maximum expression of the interference phenotype.

The RNAi effects were examined in these F1 progeny as well as in the progeny of the next generation (F2). The eggs laid in every breed were counted, and the number of males in the surviving progeny was scored 3 days later. The germ line nuclei of the micro-dissected gonads in the young adult F1 progeny and age-matched wild-type controls were fixed, and stained with DAPI as described previously [18].

For X-ray irradiation, four young gravid hermaphrodites and four *Ce-chk-2* RNAi hermaphrodites were irradiated with X-rays (1.6 Gy/min: Hitachi Co, Ltd model MBR1520R) 24 h after injection (I0). Following irradiation, the animals were immediately transferred to new culture plates and egg survival (F1 generation) was measured.

3. Results and discussion

3.1. Primary structure and expression of the Ce-chk-2 (Y60A3A.12) gene

From a BLAST search analysis of a *C. elegans* database (ACeDB) using both the FHA domain and the protein kinase domain of human *CHK2* gene, significant homology to these domains was observed in a *C. elegans* gene, Y60A3A.12. The primary structure of the Y60A3A.12 cDNA was determined by cycle sequencing of the RACE products. The total length of the cDNA is 1430 bp and the putative gene product, termed *Ce-chk-2* consists of 450 amino acid residues (DNA database accession number AB049441 in DDBJ). The sequence shows 34.3% identity over 449 residues to human Chk2 and 35.9% identity over 426 residues to Dmnk (Fig. 1).

We studied the expression of the *Ce-chk-2* gene by RT-PCR analyses. The mRNA was detected in adult hermaphrodites. However, the levels were significantly reduced in eggs and L2 larvae (Fig. 2A). As a control, RT-PCR reactions were carried out for *gld-1* involved in germ line development [19], and for the actin gene *act-1* [20]. The *act-1* gene was constantly expressed at all stages (Fig. 2A). When the adult *glp-4* ts mutant, which has a defect in germ line proliferation [14], was cultured at the restrictive temperature (25°C), *Ce-chk-2* mRNA levels were significantly lower compared to those of the mutant cultured at the permissive temperature (15°C), in a similar manner as the *gld-1* gene expression (Fig. 2B). These results indicate that the *Ce-chk-2* gene is highly expressed in germ line tissue like the *Drosophila* Dmnk gene [13].

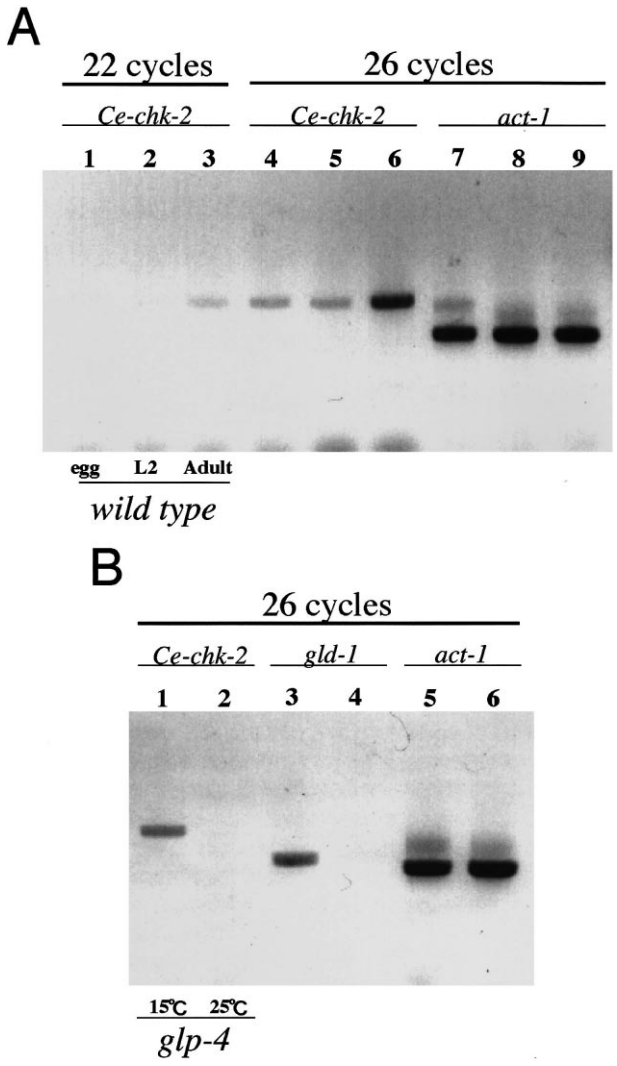

Fig. 2. Expression analysis of the *Ce-chk-2* gene by RT-PCR. A: Total RNA was isolated from eggs (lanes 1, 4 and 7), L2 larvae (lanes 2, 5 and 8), and adult hermaphrodites (lanes 3, 6, and 9). B: Total RNA was isolated from *glp-4* mutant adults cultured at either a permissive temperature (15°C: lanes 1, 3 and 5) or restrictive temperature (25°C: lanes 2, 4 and 6). RT-PCR was performed with primers specific for *Ce-chk-2*, *gld-1*, and *act-1*. DNA fragments were run on a 1.5% agarose gel, stained with ethidium bromide and quantified by densitometric scanning of the Polaroid 665 negative film.

3.2. Effect of Ce-chk-2 RNAi

To study the function of the *Ce-chk-2* gene, we carried out RNAi experiments in order to repress its expression [16]. Here, *Ce-chk-2* dsRNA (about 800 bp containing kinase domain) synthesized in vitro was micro-injected into six wild-type N2 animals at young adult stage and the eggs (F1 generation) laid between 16 and 32 h after injection were pooled and investigated. The hatching rate of these F1 eggs was not significantly different from that of eggs from mock-injected

Table 1
Effects of *Ce-chk-2* RNAi on the hatching rate of F2 eggs, and the Him phenotype

Individuals scored	Eggs laid	Dead eggs	Male progeny	Frequency of dead eggs	Frequency of males
12 F1 'escaper'	1641	1503	43	0.92	0.31
4 control (mock-injected)	783	21	0	0.03	<0.005

animals, and these F1 progeny grew to adulthood. However, approximately 95% of the eggs from the F1 progeny (F2 generation) died at early embryogenesis (Table 1). Moreover, among the few surviving F2 progeny, males were present at high frequency (approximately 30%). These results suggest that the F1 progeny grew to adults escaped the effect of RNAi by using the CeCHK2 protein synthesized before dsRNA injection. It has been reported that the effect of RNAi occurs at the post-transcriptional stage and causes degradation of specific (homologous) mRNA with dsRNA injected into gonad, body-cavity tail or head [16]. If this is the case, then it is not surprising that almost all the F2 eggs died, because the F1 'escapers' carrying injected dsRNA could not synthesize CeCHK2 protein due to RNAi. Thus, repression of the expression of the *Ce-chk-2* gene resulted in a maternal effect lethal phenotype. Males normally arise among the self-progeny of hermaphrodites at a low frequency (0.1–0.2%) due to spontaneous non-disjunction of the X chromosome during meiosis. Half of the progeny from crosses between the F2 generation males and wild-type hermaphrodites were also males, indicating that these males were not *tra* mutants, which arise as a result of sexual transformation of *C. elegans* [21], but normal XO animals. This phenotype was equally observed in animals injected into either the body-cavity tail or the gonads, and also equally observed with injection of dsRNA derived from either the 800 bp from the C-terminal end (containing kinase domain) or the 570 bp from the N-terminal end (containing FHA domain) of the *Ce-chk-2* gene.

The high frequency of arrested embryos and the high incidence of males (Him) suggested faulty crossing over or defective chromosome segregation during meiosis. To investigate the defect, the meiotic nuclei in the gonads of the F1 'escapers' were observed after DAPI staining. In the full grown oocytes of F1 'escapers' of *Ce-chk-2* RNAi, 12 univalents were observed (Fig. 3). This is in contrast to the six bivalents that are normally observed at the diakinesis stage of wild-type oocyte nuclei (Fig. 3). Moreover, in the diplotene nuclei of the F1, just before the diakinesis, abnormal and unpaired chromosomes without chiasma formation were observed (Fig. 3D). This indicated that *Ce-chk-2* gene is probably essential for chiasma formation. Oishi, I. et al. have presently described that this gene product is necessary for meiotic recombination, and also suggests that the phenotype of achiasmata by RNAi is due to the inhibition of meiotic crossing over [22].

These phenotypes mentioned above (arrested early embryos, Him and 12 univalents in oocyte) are similar to the repression phenotypes of the *C. elegans spo-11* and *him-3* gene, which encodes an enzyme required for the initiation of meiotic recombination and a meiosis specific component of chromosome cores, respectively [18,23]. It is also known that in the pachytene region of the gonad of *him-3* RNAi, synapses of meiotic chromosomes were disrupted [18]. On the other hand, synapses were normally formed in that of *spo-11* mutant and radiation-induced breaks partially alleviated its mutant phenotype [23]. Then we observed the pachytene region

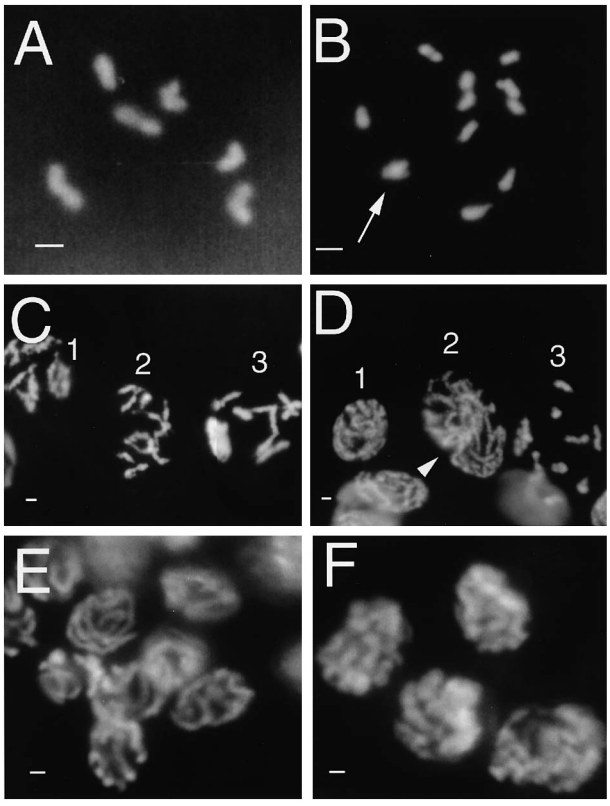

Fig. 3. Effects of *Ce-chk-2* RNAi on structure of meiotic chromosomes. Oocyte chromosomes were stained with DAPI and observed by fluorescence microscopy. A: Diakinesis chromosomes (six condensed bivalents) in a full grown oocyte of a wild-type adult hermaphrodite (control). B: Condensed, but 12 univalents of oocyte chromosomes in an F1 'escaper' of a *Ce-chk-2* RNAi injected animal. White arrow shows a signal from two overlapping univalents that are located on different focal planes. C and D: Gonad nuclei at the diplotene stage of oogenesis in wild-type hermaphrodite and the F1 'escaper', respectively. The progression of the meiotic diplotene nuclei was numbered. Arrowhead shows an overlapping region of two nuclei at diplotene stage. E: Gonad nuclei at the pachytene stage from wild-type hermaphrodites showing meiotic synapsed chromosomes. F: Abnormal nuclei from the pachytene zone of an adult F1 'escaper' of *Ce-chk-2* RNAi. In this picture, the focus was adjusted in the same level as that of the control panel E. However, tight alignment of chromosomal synapses was not observed in the pachytene nuclei. Scale bars represent 1 μm.

Table 2
Effects of *Ce-chk-2* RNAi on the X-ray sensitivity of F1 embryos

Individuals scored	X-ray (40 Gy)	0–8 h			8–24 h		
		Eggs laid	Dead eggs	Frequency of dead eggs	Eggs laid	Dead eggs	Frequency of dead eggs
4 control (mock-injected)	−	114	0	<0.01	247	0	<0.01
	+	113	37	0.33	136	4	0.03
4 I0 (*Ce-chk-2*)	−	121	0	<0.01	175	0	<0.01
	+	95	36	0.38	136	4	0.03
4 I0 (*Ce-rdh-1: rad-51*)	−	110	0	<0.01	230	62	0.27
	+	153	89	0.58	217	202	0.93

of the gonad and investigated the alleviation by radiation in the F1 'escapers' with *Ce-chk-2* RNAi. The result in Fig. 3 indicated that tight alignment of chromosomal synapses was absent in the pachytene nuclei of the F1. And radiation-induced breaks neither alleviated the poor hatchability of F2 eggs of the F1 'escapers' nor the achiasmata phenotype (data not shown). This indicated that the repression phenotypes of the *Ce-chk-2* gene are much similar to the RNAi phenotype of the *C. elegans him-3* gene. However, we still need to investigate the effects of the *Ce-chk-2* gene repression on the synapses using FISH analyses and also electron microscopic analyses as previously described [23].

3.3. No effect of Ce-chk-2 RNAi on radiation sensitivity

We also measured the radiation sensitivity of the F1 'escapers' carrying *Ce-chk-2* RNAi, since mammalian Chk2 kinase, *S. cerevisiae* Rad53 and *S. pombe* Cds1 kinase have been shown to function in cell-cycle checkpoint control during DNA replication and following DNA damage [6–11]. Young adult hermaphrodites were injected with *Ce-chk-2* dsRNA (injected animals: I0) and irradiated with 40 Gy X-rays 24 h later. The hatching rate of the eggs (F1 'escapers') laid between 0 and 8 and between 8 and 24 h after irradiation was scored. The eggs laid between 0 and 8 h received irradiation either during embryogenesis or during the diakinesis stage of full grown oocytes, while the eggs laid between 8 and 24 h received irradiation at the pachytene stage of meiotic division I. As a positive control, the radiation sensitivity of F1 'escapers' carrying *Ce-rdh-1 (rad51)* RNAi was also examined. The *Ce-rdh-1 (rad51)* gene is the only *recA*-like gene of the nematode, and is essential for meiotic recombination [24,25]. F1 'escapers' carrying *Ce-rdh-1* RNAi grow to adulthood, but all of their eggs (F2 generation) die at early embryogenesis due to a maternal lethal effect [24], which resembles that of the F2 progeny of *Ce-chk-2* RNAi injected animals. The results shown in Table 2 indicate that the hatching rates of the F1 eggs from the *Ce-chk-2* RNAi animals irradiated with 40 Gy X-rays were not significantly different from those of mock-injected irradiated control. Their rates between 0 and 8 h and between 8 and 24 h were decreased to about 60 and 90%, respectively, compared to those of the unirradiated controls. On the other hand, the hatching rate of the F1 eggs from the I0 animals with the *Ce-rdh-1 (rad-51)* RNAi was drastically reduced by X-ray irradiation.

The *Ce-atl-1* gene is the nematode ortholog of the mammalian ATM/ATR-like gene [17]. In yeast and mammals, ATM family functions as an upstream kinase of Chk2 kinase in checkpoint control [6,9]. We have found that the null phenotype induced by the *Ce-atl-1* RNAi resulted in a high frequency (approximately 50%) of early embryonic lethality, Him, increased somatic mutations, aneuploidy of oocyte chromosomes without achiasmata [17], and increased sensitivity to genotoxic stress (our unpublished data). The relative resistance of *Ce-chk-2* RNAi carrying F1 eggs to irradiation and the contrasting phenotypes of *Ce-atl-1* RNAi and *Ce-chk-2* RNAi animals suggest that the *Ce-chk-2* gene does not function in the DNA damage control checkpoint. However, as embryos of *C. elegans* appear to elicit very little or no checkpoint control in response to genotoxic stress [26], a checkpoint function for the *Ce-chk-2* gene cannot be completely ruled out. Gartner et al. [27] recently reported that checkpoints mediate DNA damage-induced apoptosis at the pachytene stage in *C. elegans*. Therefore, it will be necessary to study the function of the *Ce-chk-2* gene in the checkpoint that mediates DNA damage-induced apoptosis at the pachytene stage in this model organism.

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References

[1] Schedl, T. (1997) in: Developmental Genetics of the Germ Line (Riddle, D.L., Blumenthal, T., Meyer, B.J. and Priess, J.R., Eds.), *C. elegans* II, pp. 241–270, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
[2] Hall, D.H., Winfrey, V.P., Blaeuer, G., Hoffman, L.H., Furuta, T., Rose, K.L., Hobert, O. and Greenstein, D. (1999) Dev. Biol. 212, 101–123.
[3] Hubbard, E.J.A. and Greenstein, D. (2000) Dev. Dyn. 218, 2–22.
[4] Riddle, D.L., Blumenthal, T., Meyer, B.J. and Priess, J.R. (1997) in: *C. elegans* II, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
[5] Plowman, G.D., Sudarsanam, S., Bingham, J., Whyte, D. and Hunter, T. (1999) Proc. Natl. Acad. Sci. USA 96, 13602–13610.
[6] Matsuoka, S., Huang, M. and Elledge, S.J. (1998) Science 282, 1893–1897.
[7] Allen, J.B., Zhou, Z., Siede, W., Friedberg, E.C. and Elledge, S.J. (1994) Genes Dev. 8, 2401–2415.
[8] Sanchez, Y., Desany, B.A., Jones, W.J., Liu, Q., Wang, B. and Elledge, S.J. (1996) Science 271, 357–360.
[9] Weinert, T.A., Kiser, G.L. and Hartwell, L.H. (1994) Genes Dev. 8, 652–665.
[10] Murakami, H. and Okayama, H. (1995) Nature 374, 817–819.
[11] Brondello, J.M., Boddy, M.N., Furnari, B. and Russell, P. (1999) Mol. Cell. Biol. 19, 4262–4269.
[12] Durocher, D., Henckel, J., Fersht, A. and Jackson, S.P. (1999) Mol. Cell 4, 387–394.
[13] Oishi, I., Sugiyama, S., Otani, H., Yamamura, H., Nishida, Y. and Minami, Y. (1998) Mech. Dev. 71, 49–63.
[14] Beanan, M.J. and Strome, S. (1992) Development 116, 755–766.
[15] Lewis, J.A. and Fleming, J.T. (1995) Methods Cell Biol. 48, 3–29.
[16] Fire, A., Xu, S., Montgomery, M.K., Kostas, S.A., Driver, S.E. and Mello, C.C. (1998) Nature 391, 806–811.
[17] Aoki, H., Sato, S., Takanami, T., Ishihara, T., Katsura, I., Takahashi, H. and Higashitani, A. (2000) Mol. Gen. Genet., in press.
[18] Zetka, M.C., Kawasaki, I., Strome, S. and Muller, F. (1999) Genes Dev. 13, 2258–2270.
[19] Jones, A.R. and Schedl, T. (1995) Genes Dev. 9, 1491–1504.
[20] Files, J.G., Carr, S. and Hirsh, D. (1983) J. Mol. Biol. 164, 355–375.
[21] Meyer, B.J. (1997) in: sex determination and X chromosome dosage compensation (Riddle, D.L., Blumenthal, T., Meyer, B.J. and Priess, J.R., Eds.), *C. elegans* II, pp. 209–240, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
[22] Oishi, I., Iwai, K., Kagohashi, Y., Fujimoto, H., Kariya, K-I., Kataoka, T., Sawa, H., Okano, H., Otani, H., Yamamura, H. and Minami, Y. (manuscript in preparation) or (revised version just submitted to Mol. Cell. Biol.).
[23] Dernburg, A.F., McDonald, K., Moulder, G., Barstead, R., Dresser, M. and Villeneuve, A.M. (1998) Cell 94, 387–398.
[24] Takanami, T., Sato, S., Ishihara, T., Katsura, I., Takahashi, H. and Higashitani, A. (1998) DNA Res. 5, 373–377.
[25] Takanami, T., Mori, A., Takahashi, H. and Higashitani, A. (2000) Nucleic Acids Res., in press.
[26] Jones, C.A. and Hartman, P.S. (1996) Photochem. Photobiol. 63, 187–192.
[27] Gartner, A., Milstein, S., Ahmed, S., Hodgkin, J. and Hengartner, M.O. (2000) Mol. Cell 5, 435–443.