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Characterization of a *Caenorhabditis elegans recA*-like Gene *Ce-rdh-1* Involved in Meiotic Recombination

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Abstract

A recA-like gene was identified in the *Caenorhabditis elegans* genome project database. The putative product of the gene, termed *Ce-rdh-1* (*C. elegans RAD51* and *DMC1/LIM15* homolog 1), consists of 357 amino acid residues. The predicted amino acid sequence of *Ce-rdh-1* showed 46–60% identity to both *RAD51* type and *DMC1/LIM15* type genes in several eukaryote species. The results of RNAi (RNA-mediated interference) indicated that repression of *Ce-rdh-1* blocked chromosome condensation of six bivalents and dissociation of chiasmata in oocytes of F_1 progeny. Oogenesis did not proceed to the diakinesis stage. Accordingly, all the eggs produced (F_2) died in early stages. These results suggest that *Ce-rdh-1* participates in meiotic recombination.

Key words: *recA*-like gene; *DMC1*; *LIM15*; *RAD51*; meiosis

Genetic recombination is an essential process for both recombinational repair of damaged DNA and variability of the genome in sexual reproduction. In *Escherichia coli*, the RecA protein plays key roles in the genetic recombination by finding homologous stretches of nucleotide sequences between two DNA molecules and promoting strand exchange.[1,2] In several species of eukaryote, two types of RecA-like proteins, Dmc1/Lim15 and Rad51, have been identified (ref. 3–12 and see Fig. 1b). The *DMC1/LIM15* type genes are expressed exclusively in meiotic cells, participating in the formation of synaptonemal complexes and the repair of double-strand (ds) breaks at hotspots of meiotic recombination.[6,7,13] Null mutants of *dmc1* cause arrest of the meiotic cell cycle in both yeast and mouse, but not the mitotic cell cycle.[6,14,15] The *RAD51* type genes are expressed in both mitotic and meiotic cells, participating in recombinational repair of double-strand breaks.[3,12,16] Yeast *rad51* mutants are viable, but extremely sensitive to DNA-damaging agents such as ionizing radiation and methyl methane sulfonate (MMS).[17] In contrast, disruption of a murine *rad51* homologue causes early embryonic death,[18,19] and *rad51*-deficient vertebrate cells accumulate chromosomal breaks before cell death.[19,20]

Here, we describe identification of a *C. elegans* gene, *Ce-rdh-1*, which is homologous to *RAD51* and *DMC1/LIM15*. Primary structure analysis of the corresponding cDNA as well as phylogenetic analysis of the putative gene product were carried out. The RNAi (RNA-mediated interference) experiment with dsRNA of *Ce-rdh-1* was also carried out.

Blast search analysis of the *C. elegans* genome project database revealed only one homolog of the *recA*-like gene. It was located in the cosmid clone H36F17, corresponding to map position 4.6 on chromosome IV. An EST cDNA clone yk401c3 of this gene has already been isolated by Yuji Kohara (National Institute of Genetics, Japan). We determined the nucleotide sequence of the cDNA clone yk401c3 by chain-terminator sequencing. The total length of the cDNA was 1380 bp and the putative gene product consisted of 357 amino acid residues (Fig. 1a; the accession number of the nucleotide sequences is AB011382). The putative product showed a high degree of sequence similarity to both the *RAD51* type and the *DMC1/LIM15* type genes previously reported: *MmRAD51*, 59.2% identical; *ScRAD51*, 51.4%; *MmDMC1*, 50.7%; and *LiLIM15*, 48.7%. Two nucleotide binding motifs have apparently been conserved among these genes (see Fig. 1a). The *C. elegans* gene corresponding to the cDNA was termed *Ce-rdh-1* (*C. elegans RAD51* and *DMC1/LIM15* homolog 1). On the phylogenetic tree shown in Fig. 1b, the putative product of *Ce-rdh-1* gene is located between those of several *RAD51* and *DMC1/LIM15* homologs.

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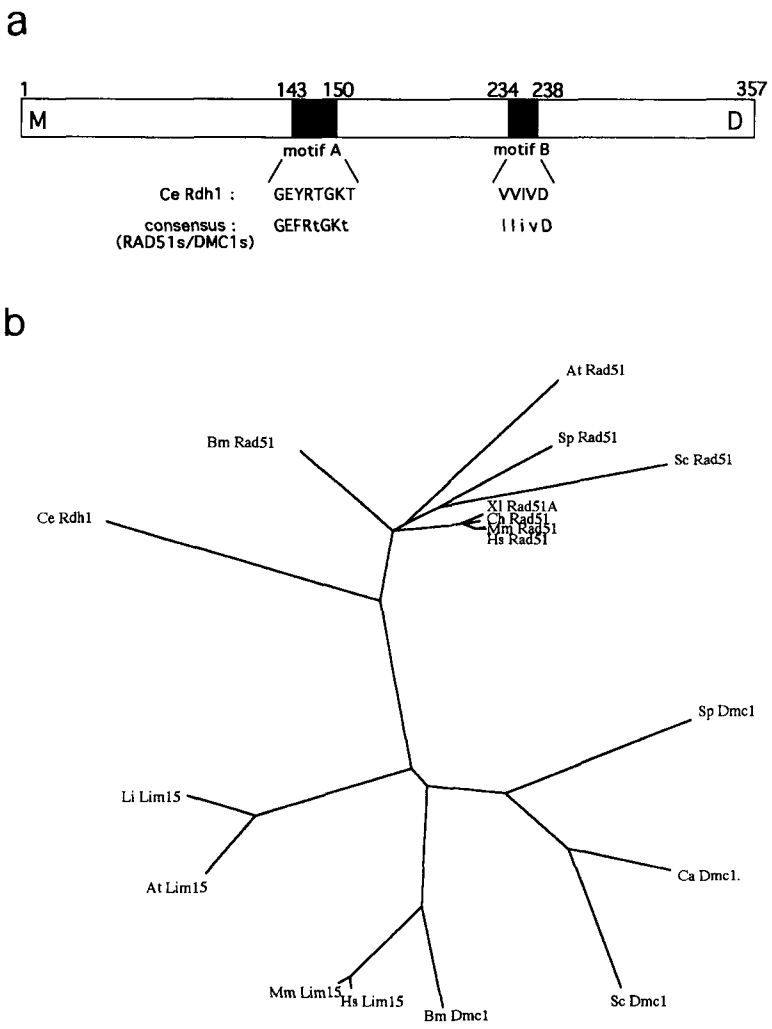

Figure 1. Structure of *Ce-rdh-1* cDNA product and phylogenetic tree of the eukaryotic *recA*-like proteins. (a) The nucleotide sequence of *Ce-rdh-1* was determined by sequencing of the yk401c3 clone (see DDBJ data base accession no. AB011382). The consensus sequences of nucleotide binding motifs A and B are indicated. (b) The phylogenetic tree of the eukaryotic *recA*-like genes. The tree was produced for entire regions of the putative product of eukaryotic *recA*-like genes; *ArLIM15* (At_Lim15) (*Arabidopsis thaliana*, DNA data base accession no. D45415); *AtRAD51* (At_Rad51) (*A. thaliana*, U43528); *BmDMC1* (Bm_Dmc1) (*Bombyx mori*, U94994); *BmRAD51* (Bm_Rad51) (*B. mori*, U94993); *DLH1* (Ca_Dmc1) (*Candida albicans*, U39808); *ChRAD51* (Ch_Rad51) (chicken, L09655); *HsLIM15*, a human homologue of *LIM15*, (D63882); *HsRAD51* (Hs_Lim15) (human, D13804); *LIM15* (Li_Lim15) (*Lilium longiflorum*, D21821); *MmLim15* (Mm_Lim15) (mouse, D58419); *MmRAD51* (Mm_Rad51) (mouse, D13803); *DMC1* (Sc_Dmc1) (*Saccharomyces cerevisiae*, M87549); *RAD51* (Sc_Rad51) (*S. cerevisiae*, M88470); *SpDMC1* (Sp_Dmc1) (*Schizosaccharomyces pombe*, D64035); *SpRAD51* (Sp_Rad51) (*S. pombe*, D13805); and *XRAD51* (Xl_Rad51A) (*Xenopus laevis*, D38488); according to the neighbor-joining method.[28]

To study the function of the *Ce-rdh-1* gene, we carried out RNAi to repress its expression.[21] The dsRNA whose length was about 1.4 kbp was synthesized *in vitro* with T3 and T7 RNA polymerases using the PCR-amplified fragment of the yk401c3 clone as the template. dsRNA (2 mg/ml) thus obtained was injected into gonads or intestinal cytoplasm of young-adult hermaphrodite of the wild-type N2 Bristol strain. The injected worms (I_0) were transferred to individual plates and grown at 20°C. Each worm laid about 50–100 eggs during its reproductive life span, while uninjected hermaphrodite that has not mated normally lays about 300 eggs. About 20% of the eggs developed to adult stage (F_1 progeny), and 80% died during early embryogenesis. The number of eggs laid by each F_1 progeny was as low as 20–50 eggs. Most eggs were variable in shape, and their proliferation was arrested in early stages (Fig. 2). Some eggs did not have a hard shell and hence were probably unfertilized (Fig. 2). These results suggest that the F_1 progenies that grew to adults escaped the effect of RNAi by using the CeRDH1 protein

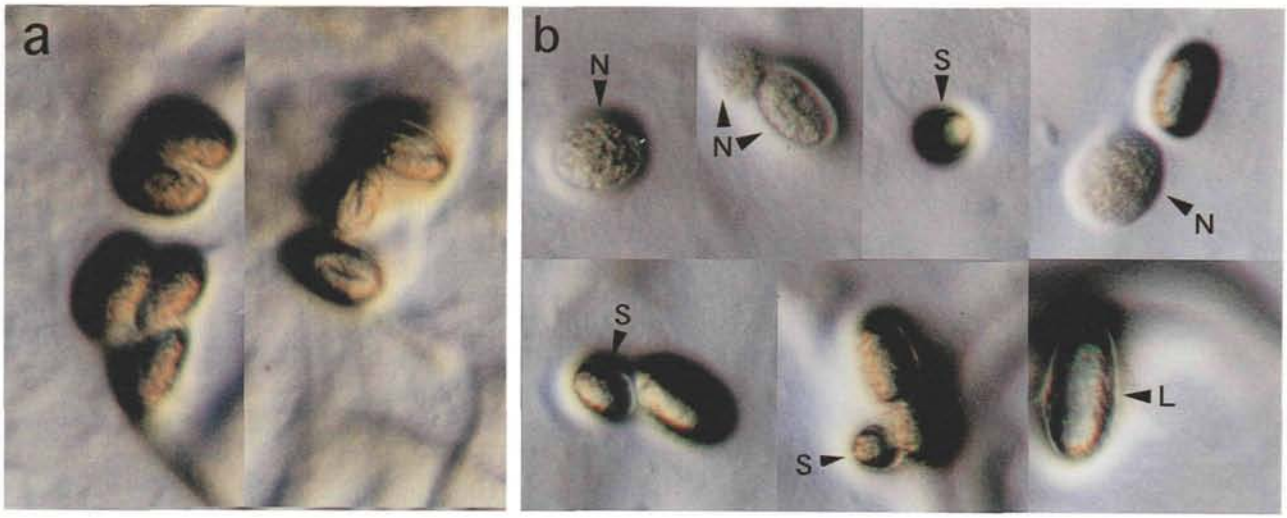

Figure 2. Effects of *Ce-rdh-1* RNAi on eggs. Eggs of wild-type and of F_1 progeny of worms injected with double-stranded *Ce-rdh-1* RNA are shown in panels (a) and (b), respectively. Smaller eggs (S); larger eggs (L); and non-shelled eggs (N) are denoted.

synthesized before the dsRNA injection. If this is the case, then it is not surprising that all the F_2 eggs died, because the F_1 "escapers" could not synthesize CeRDH1 protein due to RNAi. This phenotype was equally observed in worms injected into either the gonads or the intestinal cytoplasms.

The chromosome structure and general morphology of oocytes in I_0 worms (18 hr after injection) and in F_1 escapers were studied under microscope after DAPI staining. The shape of some oocytes of both I_0 and F_1 were irregular and variable compared with oocytes of control hermaphrodite (Fig. 3). The variability in the roundness of the eggs that died in the early stages due to RNAi with *Ce-rdh-1* (see Fig. 2) may be a result of the heterogeneous shape of oocytes in gonads. Moreover, the chromosome structure of oocytes was drastically changed in F_1 escapers. On the other hand, no significant difference in chromosome structure was observed between the F_1 and control worms during mitosis or in the early prophase of meiosis I (see Fig. 3). In all the oocytes of F_1 escapers, the chromosome condensation of six bivalents and the dissociation of chiasmata were blocked (Fig. 3f, g, and h). Meiosis in normal oocytes pauses in the diakinesis stage and the six condensed bivalents are visualized and counted (Fig. 3a and e). The oocyte nuclei do not go on to complete meiotic divisions I and II until ovulation and fertilization have occurred.[22] These results indicate that oogenesis in the F_1 escapers could not enter the diakinesis stage.

The *dmc1* knockout mice are sterile due to the arrest of gametogenesis in the early prophase of meiosis I.[14,15] After the arrest, the germ cells disappear by apoptosis. On the other hand, the oocytes of F_1 worms subjected to *Ce-rdh-1* RNAi did not disappear, even though their chromosome structure was irregular and they did not normally enter diakinesis (Fig. 3). Accordingly, the dead eggs of F_2 were produced (Fig. 2). In the mutants of *C. elegans* related to meiosis (e.g., *mei-1*, *mei-2* and several *him* mutants), the defective mutant oocytes do not disappear by apoptosis, and dead eggs are commonly produced. This mutant phenotype[23,24,25] makes *C. elegans* a useful experimental organisms for the study of meiotic processes.

The RNAi of *Ce-rdh-1* affected meiosis but not mitosis (Fig. 3). If the gene of *Ce-rdh-1* is essential for both mitosis and meiosis, as the vertebrate *RAD51* type genes are, germ line proliferation and somatic tissue proliferation of the F_1 escapers must be defective. *Ce-rdh-1* appears to be similar to the *DMC1/LIM15* type genes which participate specifically in the meiotic recombination. However, full characterization of *Ce-rdh-1* gene function must await isolation of a null mutation of the gene, since some of the somatic cells (e.g., nervous system cells) are not affected by RNAi (Fire, A. and Fleenor, J. personal communication).

The nucleotide sequence of *C. elegans* genomic DNA has been mostly determined. The unfinished regions, not more than 20% of the genome, are small gaps and heterochromatin regions containing few genes. The entire sequencing will be completed this year. Still, we have not been able to identify any additional *recA*-like gene in the current *C. elegans* genome project database. Moreover, we have tried to isolate another *recA*-like gene either from *C. elegans* genomic DNA or from cDNA libraries by PCR using several sets of primers, which were designed on the basis of the amino acid sequences conserved among the products of *RAD51* type and/or *DMC1/LIM15* type genes. Volpe, A. L. and Rinaldo, C. have isolated a homolog of *RAD51* in *C. eleagans*

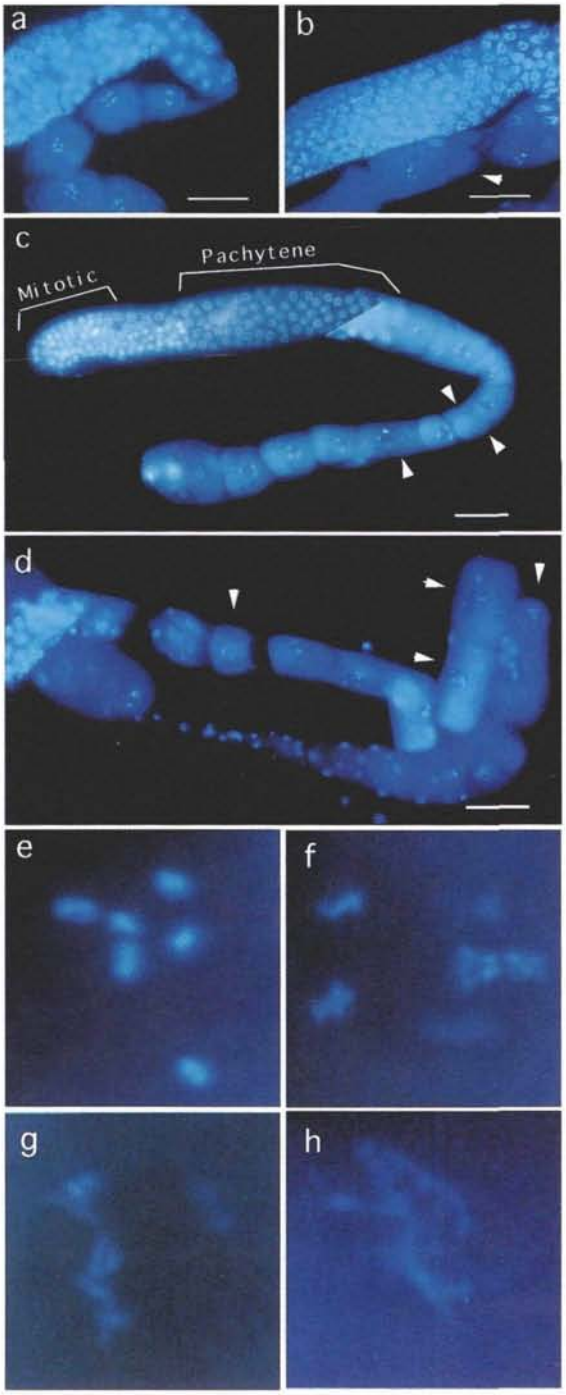

Figure 3. Analysis of *Ce-rdh-1* RNAi effects during oogenesis. Gonads were dissected from uninjected wild-type (N2) adult hermaphrodites, injected worms (I_0) with double-stranded *Ce-rdh-1* RNA and F_1 escapers. To visualize DNA, the gonads were fixed in 5% ethanol, 5% acetic acid, 0.1 M NaCl and 0.05 M potassium phosphate (pH 6), and were stained with DAPI (diaminophenoylindole). Microscopy was performed with a fluorescence microscope. (a) N2 wild-type gonad (control). (b) Gonad of I_0 worm at 18 hr after micro-injection. (c and d) Gonads of F_1 escapers. (e) Diakinesis chromosomes in a normal oocyte (control). (f, g, and h) Abnormal chromosomes in oocyte of F_1 escapers. Arrowheads indicate irregularly shaped oocytes. Scale bars represent 10 μm.

(personal communication). It is probably identical to *Ce-rdh-1* in this paper, since both genes are located on the same map position. They hypothesize that duplication of the original eukaryotic *recA*-like gene to yield *RAD51* and *DMC1/LIM15* genes did not occur in the evolution of *C. elegans* (Volpe, A. L. and Rinaldo, C. unpublished data). In the current *C. elegans* database, we could not find any homolog of *RAD52*, whose product forms a stable complex with Rad51. A homolog of *MRE11*,[26] whose function is the repair of double-strand breaks prior to the DNA strand exchange by Rad51 and Rad52, has been identified in the database. In *C. elegans*, it is known that gene targeting by homologous recombination is very difficult. This phenomenon may be, at least in part, a result of either loss of *RAD51/52* or failure in duplicating *RAD51* during its evolution. How is damaged DNA repaired without Rad51/52 proteins? It has been shown that the chromosomes of *C. elegans* are holocentrically organized and that the end-joining activity of broken DNA is relatively strong.[22,27] Therefore, the two types of chromosome rearrangements, small chromosome fragments called free duplications and translocation chromosomes, are stably propagated in *C. elegans*.

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