Deletion of lec-10, a Galectin-Encoding Gene, Increases Susceptibility to Oxidative Stress in Caenorhabditis elegans

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Galectins are a family of β-galactoside-binding lectins. They are involved in the regulation of a variety of biological phenomena in mammals. However, little is known about their roles in invertebrates. Caenorhabditis elegans is a well-characterized model organism whose complete genome has been sequenced. C. elegans is now being studied extensively in various fields of medical sciences. In this study, we examined the phenotypes of a mutant strain of C. elegans (tm1262) lacking lec-10, a galectin-encoding gene. We observed no difference in the rates of embryonic lethality and larval arrest/slow growth between this mutant strain and the wild-type strain. No apparent morphological defect was observed in the lec-10-deletion mutant (tm1262). Moreover, the life-spans of this mutant and the wild-type strain were equivalent. However, this mutant showed significantly greater susceptibility to paraquat and hydrogen peroxide than the wild type did. The lec-10-deletion mutants (tm1262) were as susceptible as the daf-16-deletion mutants (mu86) to paraquat and hydrogen peroxide. These results suggest that the deletion of lec-10 does not have a notable effect on the worm’s survival under laboratory conditions. However, this study indicates that lec-10 does confer some protection against oxidative stress.

Key words galectin; oxidative stress; Caenorhabditis elegans; life-span; lectin

Lectins are a class of proteins that specifically bind to certain carbohydrate structures. Galectins are a large family of β-galactoside-binding lectins, which are found in a wide variety of species. Intensive studies on mammalian galectins have revealed their protein structures, carbohydrate-binding properties, and biological functions. In mammals, galectins are known to be involved in various cellular events such as cell adhesion, migration, apoptosis, cell growth, and RNA splicing. Galectins are categorized into three structural types on the basis of their global architecture (not sequence similarity). In mammals, galectin-1,-2,-5,-7,-10,-13,-14, and -15 are proto-type galectins that contain a single carbohydrate recognition domain (CRD), whereas galectin-4,-6,-8,-9, and -12 are tandem-repeat-type galectins that contain two distinct CRDs in a polypeptide. Galectin-3 is the only chimera-type galectin identified in mammals; it contains a proline- and glycine-rich domain at the N-terminal region and a CRD at the C-terminal region. The term galectin-11 is presently not in use.

Caenorhabditis elegans is a well-characterized model organism whose complete genome was sequenced in 1998. C. elegans is now being extensively studied in various fields of medical sciences such as those related to aging and innate immunity. Moreover, C. elegans is also used in drug research. Its genome contains multiple galectin and galectin-like genes. Thus far, 10 galectin or galectin-like cDNAs (lec-1—lec-6 and lec-8—lec-11) have been cloned. In our earlier studies, we reported the sugar-binding ability of the recombinant proteins LEC-1, -2, -3, -4, -6, and -10 and analyzed their oligosaccharide-binding properties in detail. Among the previously cloned galectin and galectin-like genes of C. elegans, lec-8, -9, -10, and -11 are of the chimera type. However, these genes cannot be considered as orthologues of galectin-3 because they have a CRD at their N-terminal region, and the function of their C-terminal region is unknown.

Although galectins are widely distributed in multicellular organisms, little is known about the significance of these proteins in invertebrates. In the present study, we examined the phenotypes of a mutant worm lacking the lec-10 gene as the first step toward determining the biological functions of galectins in C. elegans. No obvious developmental and morphological abnormalities were found under standard laboratory conditions. Further, we hypothesized that lec-10 plays a role in the defense against cellular stresses and in the regulation of life-span of this organism.

C. elegans has a rapid life cycle and a short life-span, and its entire genome sequence has been determined; therefore, C. elegans is a powerful model for the study of the mechanism underlying longevity. The age-1 gene of C. elegans was the first gerontogene to be identified in multicellular organisms. This gene encodes a homologue of mammalian phosphatidylinositol-3-OH kinase, which acts downstream of a homologue of insulin/insulin-like growth factor I receptor (DAF-2). C. elegans with mutations in the genes related to the DAF-2 signaling pathway has been extensively studied. It has been found that age-1 mutants showed an increased life-span and hyper-resistance to oxidative stress. The daf-2 mutants also showed an increased life-span and hyper-resistance to oxidative stress. The DAF-2 signaling pathway negatively regulates the activity of DAF-16, a transcription factor that belongs to the Forkhead box, class O (FOXO) family and induces the expressions of genes countering oxidative stress. It was also observed that a daf-16 mutant (m26) showed a decreased life-span and increased susceptibility to oxidative stress. These are important findings in support of the oxidative stress theory of aging.

Moreover, various lines of evidence, including those involving C. elegans and mice, have revealed that oxidative stress is a major factor promoting aging. It is known that oxidative stress is triggered by reactive oxygen species and that it is essentially generated as part of the normal cellular metabolism during aging. In the present study, we induced ox-
idative stress in *C. elegans* by using paraquat, a herbicide, and hydrogen peroxide (H$_2$O$_2$); we found that a *lec-10*-deletion mutant, *tm1262*, was significantly susceptible to oxidative stress. On the basis of this result, we expected that this mutant (*tm1262*) would have a shorter life-span than wild-type *C. elegans*. However, we found that the life-span of this organism was not affected by the deletion of the *lec-10* gene.

**MATERIALS AND METHODS**

**Materials** In this study, we used Bristol N2 (the wild-type strain of *C. elegans*), a *lec-10*-deletion mutant (*tm1262*), and a *daf-16*-deletion mutant (*mu86*). The mutant *tm1262* was provided by Dr. Shohei Mitani (National Bioresource Project, Japan). The other strains were provided by the Caenorhabditis Genetics Center, which is funded by the National Center for Research Resources (NCRR) of the National Institutes of Health (NIH). Paraquat (methyl viologen hydrate) was purchased from Nacalai Tesque Inc. (Kyoto, Japan). H$_2$O$_2$ was purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan).

**Worm Culture and Genotyping** All the strains were grown on nematode growth medium agar plates seeded with *Escherichia coli* strain OP50 at 20°C, as described previously. The hermaphrodites of *lec-10* (*tm1262*) were backcrossed five times with wild-type N2 males before they were used for the analyses. The following primers were used for polymerase chain reaction (PCR) genotyping of the deletion allele:

\[
\text{tm1262-F1, 5'}-\text{GTGACATTGAACTGACCT-3'; tm1262-R1, 5'}-\text{CCAGATGTGCTATGTCGC-3'; tm1262-R2, 5'}-\text{AATTCACTCGTGCCTGTA-3'}; \mu u86-F1, 5'}-\text{CAAGAAGGCGGTATCCAT-3'; \mu u86-R1, 5'}-\text{GAGCCCATCTATGCTCCTC-3'; and \mu u86-R2, 5'}-\text{AACCATTTGTCGTAAC-3'}.
\]

PCR was carried out under the following cycling conditions: initial denaturation at 94°C for 5 min; followed by 40 cycles (for *tm1262*) or 35 cycles (for *mu86*) at 94°C for 30 s, at 56°C (for *tm1262*) or 60°C (for *mu86*) for 30 s, and at 72°C for 2 min. This was followed by final extension at 72°C for 7 min. Before the experiments, the worms were maintained for at least two generations in the presence of sufficient food.

**Analyses of Embryonic Lethality and Larval Arrest/Slow Growth** Adult hermaphrodites were transferred to new plates and allowed to lay eggs for 2h at 20°C. The worms were subsequently removed from the plates, and the eggs were counted. The plates were then incubated at 20°C. After 24 h, the unhatched eggs were counted to determine embryonic lethality. We determined the number of worms that did not reach the L4/adult stage even after 62 h in order to assess larval arrest/slow growth.

**Oxidative Stress Assay** Adult hermaphrodites were transferred to new plates and allowed to lay eggs overnight at 20°C. The adult worms were subsequently removed from the plates, and the plates were incubated for 3 d. Adult hermaphrodites (24 worms/group) were transferred from the plates to a 96-well plate containing M9 buffer with 200 mM paraquat or 3 mM H$_2$O$_2$. After incubation at 20°C for the specified durations, the number of dead worms was determined. Worms that did not respond to repeated touching with a platinum wire were considered to be dead. At least three independent experiments were performed. The unpaired two-tailed t-test was used for statistical analysis.

**Life-Span Assay** Adult hermaphrodites were transferred to new plates and allowed to lay eggs overnight at 20°C. They were subsequently removed from the plates, and the plates were incubated for 2 d. After this incubation, L4 hermaphrodites were transferred to new plates. The life-span of the worms was measured from the L4 stage when grown at 20°C. During the egg-laying stage, the worms were transferred to new plates every day. Worms that did not respond to repeated touching with a platinum wire were considered to be dead. Worms that crawled off the plate or died with hatched embryos in the uterus were censored at the time of the event. Three such independent experiments were performed. The log-rank (Mantel-Cox) test was used for statistical analysis.

**RESULTS**

**The *lec-10*-Deletion Mutant (*tm1262*) Was Developmentally Normal and Fertile** *C. elegans* has at least 10 galectin or galectin-like genes. However, the biological functions of the galectin proteins in this organism are virtually unknown. In the present study, we focused on *lec-10*, which is a chimera-type galectin gene, and investigated the phenotypes of a homozygous *lec-10*-deletion mutant (*tm1262*). This mutant is considered to be a null mutant on the basis of its deletion site, which is shown in Fig. 1a. Homozygous deletion of the *lec-10* gene in this mutant was verified on the basis of the PCR product size (Fig. 1b).

![Fig. 1. PCR Genotyping of the Deletion Mutants Used in This Study](image)

(a) Structure of *lec-10* and the deletion site of *tm1262*. Exons and introns are represented by boxes and lines, respectively. The Roman numerals in the boxes indicate the number of exons. Exon sizes (in base pairs) are indicated above the boxes, and the numbers in parentheses above the lines indicate the sizes of the introns. The shaded boxes represent the 5′- or 3′-untranslated regions. The thick bar indicates the deletion site of *tm1262*. The arrows indicate the position of the primers used for PCR genotyping. (b) PCR genotyping of the *lec-10*-deletion mutant (*tm1262*). Genomic DNAs from wild-type worms (N2) and the *lec-10*-deletion mutant (*tm1262*) were subjected to PCR genotyping using two sets of primer pairs (tm1262-F1/R1 or tm1262-F1/R2). The sizes of the PCR products are indicated at the right. (c) PCR genotyping of the *daf-16*-deletion mutant (*mu86*). The structure of *daf-16* and the deletion site of *mu86* have been described previously. Genomic DNAs from wild-type N2 and *daf-16*-deletion mutant (*mu86*) were subjected to PCR genotyping using two sets of primer pairs (mu86-F1/R1 or mu86-F1/R2). The sizes of the PCR products are indicated at the right. *Note that the PCR product from the wild-type genome was not detected during PCR with the primers mu86-F1/R1 because the PCR cycling conditions used were inappropriate for such a long amplicon (11391 bp).
The **lec-10**-deletion mutant (**tm1262**) was found to be viable and fertile under standard laboratory conditions at 20 °C. Further, we did not detect any morphological defect in the adult hermaphrodites under a light microscope (data not shown). Next, we examined the incidence of embryonic lethality and larval arrest/slow growth in the deletion mutants and the wild-type worms in order to confirm whether the mutants showed any abnormality. No difference was observed between the **lec-10**-deletion mutants (**tm1262**) and the wild-type worms in terms of embryonic lethality and the larval arrest/slow growth phenotypes (Table 1). These results indicate that the deletion of **lec-10** does not affect embryogenesis or morphogenesis.

**The **lec-10**-Deletion Mutant (**tm1262**) Showed Increased Susceptibility to Oxidative Stress** Since embryogenesis, morphogenesis, and growth appeared normal in the **lec-10**-deletion mutants (**tm1262**), we hypothesized that **lec-10** plays a protective role against certain cellular stresses during adulthood. To test this hypothesis, we performed an oxidative stress assay using paraquat. We found that in the presence of 200 mM paraquat, the incidence of rapid death among the **lec-10**-deletion mutants (**tm1262**) was significantly higher than that among the wild-type worms (Fig. 2a). The toxicity of paraquat is thought to be mediated by superoxide radical (**O_2^-***) production in cells. By using exogenous H_2O_2_, we also confirmed that compared to the wild-type worms, the **lec-10**-deletion mutants (**tm1262**) showed increased susceptibility to oxidative stress (Fig. 2b). We also performed the above oxidative stress assay using a **daf-16**-deletion mutant (**mu86**) under the same conditions and compared the susceptibilities of the **daf-16**- and **lec-10**-deletion mutants. The structure of **daf-16** and the deletion site of **mu86** have been described previously. The **daf-16**-deletion mutant (**mu86**) is considered to be a null mutant on the basis of its deletion site. Homozygous deletion of the **daf-16** gene in this mutant was confirmed on the basis of the PCR product size (Fig. 1c).

**Table 1.** Embryonic Lethality and Larval Arrest/Slow Growth Phenotypes of the **lec-10**-Deletion Mutant (**tm1262**)

<table>
<thead>
<tr>
<th>Strain</th>
<th>Embryonic lethality ( % )</th>
<th>n</th>
<th>Larval arrest/ slow growth ( % )</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type (N2)</td>
<td>0.92</td>
<td>545</td>
<td>4.38</td>
<td>365</td>
</tr>
<tr>
<td><strong>lec-10</strong> (tm1262)</td>
<td>0.84</td>
<td>594</td>
<td>5.20</td>
<td>404</td>
</tr>
</tbody>
</table>

a) Data were combined from three independent experiments. b) Data were combined from two independent experiments. c) The percentage provided excludes dead embryos.

**Table 2.** Summary of the Results of the Life-Span Assay of the **lec-10** Deletion Mutant (**tm1262**)

<table>
<thead>
<tr>
<th>Experiment number</th>
<th>Strain</th>
<th>Maximum life-span (d)</th>
<th>Median life-span (d)</th>
<th>Mean life-span±S.E.M. (d)</th>
<th>p value</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Wild type (N2)</td>
<td>34</td>
<td>18</td>
<td>19.8±1.1</td>
<td>0.271</td>
<td>30</td>
</tr>
<tr>
<td>2</td>
<td><strong>lec-10</strong> (tm1262)</td>
<td>28</td>
<td>18</td>
<td>18.0±0.9</td>
<td>0.545</td>
<td>60</td>
</tr>
<tr>
<td>3</td>
<td>Wild type (N2)</td>
<td>35</td>
<td>21</td>
<td>21.4±0.8</td>
<td>0.545</td>
<td>60</td>
</tr>
</tbody>
</table>

**Fig. 2. The **lec-10**-Deletion Mutant (**tm1262**) Showed Increased Susceptibility to Oxidative Stress**

Adult hermaphrodites were incubated in M9 buffer with 200 mM paraquat (a) or 3 mM H_2O_2_ (b). After incubation at 20 °C for the specified durations, the dead worms were counted as described in Materials and Methods. The average of the values from at least three independent experiments was calculated. The error bars indicate standard deviation. *p<0.05, **p<0.01.
mice. For instance, the deletions have been made in mice; for instance, p66 shc-deficient family members. This may be because of mutual compensation among other stressors such as those experienced during ischemia–reperfusion injury, rather than the gradually increasing oxidative stress experienced during the experiments (Fig. 2). To clarify this point, we need to determine the behavior of other galectin family members both in lec-10-deletion mutants (tm1262) and the wild-type worms. However, resistance to oxidative stress may not necessarily be linked to life-span. For instance, in the case of some mutants, including C. elegans superoxide dismutase-deletion mutants, it has been reported that increased susceptibility to oxidative stress does not result in a decreased life-span. Few reports are available on the relationship between lectins and oxidative stress response both in vertebrates and invertebrates. The mechanism underlying increased susceptibility of lec-10-deletion mutants (tm1262) to oxidative stress should finally be explained on the basis of the characteristics of LEC-10. The possible glycoprotein ligands of LEC-6 have been previously identified and include some lysosomal proteins such as F23B2.11 (PCP-3; lysosomal carboxypeptidase-like protein) and Y16B4A.2 (lysosomal protective protein). Although the functions of these glycoproteins are currently unknown, certain lysosomal functions such as the degradation of oxidized macromolecules and oxidatively damaged mitochondria through autophagy are responsible for oxidative stress responses. We have previously compared the oligosaccharide-binding property of recombinant LEC-10 with that of LEC-6 in detail by using frontal affinity chromatography. Although we noted a distinct feature of LEC-10, its high affinity for A-hexasaccharide (GalNAcβ1-3(Fucα1-2)Galβ1-3GlcNAcβ1-3Galβ1-4Glc), the overall oligosaccharide-binding profile of LEC-10 was very similar to that of LEC-6. If LEC-10 also binds to PCP-3 and Y16B4A.2, LEC-10 may regulate the function of lysosomes through interaction with these lysosomal glycoproteins. In a previous study, we identified some other unique properties of LEC-10: the presence of some histidine clusters in its polypeptide chain and its ability to strongly bind metal ions such as Ni²⁺ and Cu²⁺ (Hayama K., unpublished results). Thus, LEC-10 can interact with both glycoconjugates and metal ions, and this may be relevant to its function.

In our preliminary experiments, expression of lec-10 mRNA was detected in the wild-type adult worms but did not increase on treatment with paraquat. According to the Nematode Expression Pattern DataBase (NEXTDB) (http://nematode.lab.nig.ac.jp/), lec-10 mRNA is expressed in the intestine of larva and adult worms. In the future, the precise cellular localization of the LEC-10 protein should be determined and endogenous glycoconjugate ligands for LEC-10 should be identified.

It has been reported that human galectin-3 protects breast carcinoma cells against oxidative stress-induced apoptosis. In contrast, in another report, it was shown that galectin-3 induces oxidative stress that results in mast cell apoptosis. These results show that mammalian galectin-3 is involved in the regulation of cell death under oxidative stress conditions. These contrasting results suggest that the regulatory effect of mammalian galectins on apoptosis is largely dependent on the cell type. It will be interesting to examine whether C. elegans LEC-10 regulates cell death under oxidative stress.

This is the first report describing the phenotypes of a C. elegans lec-10-deletion mutant. In the present study, we found that the deletion of lec-10, a galectin-encoding gene, affects the organism’s susceptibility to oxidative stress. This finding
is important since it will help understand the relationship between oxidative stress and galectin-regulated cellular homeostasis. To elucidate the relationship between the carbohydrate-binding properties of each galectin and protective effect against oxidative stress, the phenotypes of mutants lacking other galectin family members are currently being examined.

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REFERENCES
