Cloning, Sequencing and Mapping of a Manganese Superoxide Dismutase Gene of the Nematode Caenorhabditis elegans

Norio SUZUKI,† Kaoru INOKUMA, Kayo YASUDA, and Naoaki ISHII*
Department of Molecular Life Science, Tokai University School of Medicine, Isehara, Kanagawa 259-11, Japan
(Received 1 June, 1996)

Abstract
We have cloned, sequenced and mapped a gene (sod-2) encoding manganese superoxide dismutase [EC 1.15.1.1] from the nematode Caenorhabditis elegans. The sod-2 was mapped to chromosome I by hybridization with a YAC polytene filter. The protein-coding region spans 1129 base pairs including 4 introns and encodes a protein of 221 amino acids (aa) (Mr = 24536) of which the first 24 aa are the presumed mitochondrial-targeting signal peptide. The gene sequence of sod-2 was slightly different from an isoform, sod-3.

Key words: Manganese superoxide dismutase; sod-2; C. elegans; cDNA sequence; genomic sequence

The reduction of molecular oxygen in all aerobic cells results in intermediates such as superoxide radicals, hydrogen peroxide and hydroxy ions which are highly toxic. Superoxide dismutase is an important metalloenzyme which eliminates, through a dismutation reaction, superoxide radicals produced within cells.1 It is known that the enzyme is induced when cells are placed under oxidative stress.2,3 There are two distinct types of SOD in eukaryotic cells. The copper- and zinc-containing enzyme (Cu/Zn-SOD) is found principally in the cytosole and the manganese-containing enzyme (Mn-SOD) is found exclusively in the mitochondrial matrix.

Recently, much attention has focused on the role of oxidative stress in aging.4,5 Caenorhabditis elegans has received much attention for the study of the genetic basis aging.5,6 There are currently published reports of mutants which are both abnormal in their responses to oxidative stress as well as age at abnormal rates.7,8 Investigation of these SOD genes and mutants in C. elegans should serve to illuminate the relationship between oxidative damage and aging.

To facilitate this process, we have cloned, sequenced and mapped a Mn-SOD gene, sod-2, in C. elegans.

1. Nucleotide and Deduced Amino Acid Sequence of sod-2 and Mapping

As detailed in the legend to Fig. 1, a clone was identified from a C. elegans cDNA library via its hybridization to an oligonucleotide corresponding to a conserved region of previously sequenced SOD genes. This clone was sequenced and found to contain a cDNA insert of 839 nucleotides (nt) which contained an open reading frame predicting a protein of 221 amino acids (aa) (Mr = 24536) of which the first 24 aa are the presumed mitochondrial-targeting signal peptide. The gene sequence of sod-2 was slightly different from an isoform, sod-3.

2. Nucleic Acid and Amino Acid Comparisons of sod-2 to another Mn-SOD (sod-3) from C. elegans and Mn-SOD from Human Liver

The sod-2 was mapped to chromosome I, which distinguishes it from another Mn-SOD gene, sod-3 (accession number X85790 in EMBL nucleotide sequence database), as G. Hunter mapped sod-3 to chromosome X (personal communication). By comparison, the exons and introns of sod-2 were similar in size and position to those in sod-3. These Mn-SOD genes were extensively homologous
Manganese Superoxide Dismutase Gene of *C. elegans* [Vol. 3.]

---

```
172 Manganese Superoxide Dismutase Gene of *C. elegans*

---

**Figure 1.** *A. elegans* cDNA library in λZAP vector was obtained from Robert Barstead (Oklahoma Medical Research Foundation) and was screened with the synthetic oligonucleotide which contains the conserved aa residues WEHAYYLQY. Eight positive clones were isolated from a total of approximately 300,000. The largest one of these clones was sequenced. A genomic clone was isolated for subsequent sequencing using the following strategy. The cDNA clone was used to probe a grid filter, obtained from Alan Coulson at the Medical Research Council (Cambridge, England), which contained an ordered set of YAC's corresponding to the majority of the *C. elegans* genome. This identified YAC clone Y37F9, which corresponds to a portion of linkage group I. The same cDNA clone was then used to probe an EcoKl digest of cosmid C05D8, which overlaps with Y37F9. Subsequently a 4.4-kb EcoKl fragment including the sod-2 gene was identified, subcloned into pUC18 and sequenced. Sequence analysis was performed either manually using Sequences Analysis Software (SDC Software Co., Japan). The nucleotide sequence was numbered from the N-terminal methionine of the predicted protein. The major promoter consensus sequences are underlined and poly(A) signals are double-underlined.

---

Downloaded from https://academic.oup.com/dnaresearch/article-abstract/3/3/171/342351 by guest on 09 March 2018
as the aa identity of the predicted sod-2 mature protein sequence was 88% to sod-3 and 69% to human liver Mn-SOD\(^9\) (accession number P04179 in SWISS-PROT protein sequence database) (Fig. 2). These data indicate that Mn-SOD in C. elegans contains at least two isoforms. Northern hybridization using sod-2 genomic DNA revealed only a single species of mRNA (data not shown).

**Acknowledgements:** The nematode strains used in this work were obtained from the Caenorhabditis Genetics Center, which is supported by contract NO1-AG-9-2113 between the NIH and the curators of the University of Missouri. We are indebted to Dr. Philip Hartman, Department of Biology, Texas Christian University for his kind advice and for reading the manuscript. This work was in part supported by a grant from International Core System for Basic Research (Science and Technology Agency, Japan), a Grant-in-Aid from the Ministry of Education, Science, Sports and Culture, Japan (Project No. 05834015) and a Health Sciences Research Grant by the Ministry of Health and Welfare, Japan to Naoaki Ishii.
References