FOUR ACETYLCHOLINESTERASE GENES IN THE NEMATODES CAENORHABDITIS ELEGANS AND CAENORHABDITIS BRIGGSAEA

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ABSTRACT

It was reported that three genes ace-1, ace-2 and ace-3 located respectively on chromosomes X, I and II, encode three pharmacological classes of acetylcholinesterase (A, B and C) in the nematode Caenorhabditis elegans. We have cloned these genes and studied the expression of ace-1 and ace-2 using GFP reporter constructs. We found that ace-1 is prominently expressed in muscle cells whereas ace-2 is mainly expressed in neurons. In addition we cloned two other ace genes (ace-x and ace-y) that are closely linked on chromosome II, the position expected for ace-3. Because of the close proximity of these two genes, we cannot identify, for the moment, which of these two genes encodes the class C AChE (and should be named ace-3). The sequence and structure of the four ace genes in C. elegans are compared in relation with homologous data on C. briggsae, a closely-related species.

INTRODUCTION

Plant parasitic nematodes are devastating pathogens, responsible for an estimated annual $100 billion loss in crops worldwide (1). Chemical nematicide treatments include

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carbamates and organophosphates that inhibit acetylcholinesterase and lead to inhibition of the neuromuscular transmission and to death. *Caenorhabditis elegans* is a free living nematode which constitutes however a fruitful model of parasitic species (2). Whereas a single gene encodes AChE in vertebrates (3) and in the majority of insects (4), the situation is more complex in *Caenorhabditis elegans*. Initial studies clearly identified two kinetically distinct major classes of AChE, A and B, encoded by two genes: *ace-l* on chromosome X and *ace-2* on chromosome I (5-7). Later, a third class of AChE (class C, accounting for only 5% of total AChE activity) was characterized by its very low *Km* for ACh and its high resistance to eserine. Class C AChE was shown to be encoded by *ace-3*, a locus on chromosome II (8). Finally, a careful examination of class C mutants suggested the existence of a fourth class (D) of AChE that represented less than 0.1% of the total AChE activity (9). Since no mutation in class D AChE was isolated, the corresponding *ace-4* locus was neither identified nor chromosome-mapped.

The complete coding sequences of *ace-l* in *C. elegans* (10) and in the related nematode species *C. briggsae* (11) have been reported. We now present additional data on the tissue specific expression of *ace-l* and *ace-2* and on the sequence of all *ace* genes (12). In particular we report the genomic organization of *ace-x* and *ace-y*, two *ace* genes located in close association on chromosome II. Because of the close proximity of these two genes, we cannot identify for the moment which of these two genes encode the class CAChE (and should be named *ace-3*), the other being *ace-4*.

**Ace-1**

*Ace-1* was initially cloned by PCR with degenerate oligonucleotides deduced from the sequences EDCLYLN and FGESAG that are conserved in the whole cholinesterase family. The deduced aminoacid sequence possessed all characteristic features of an acetylcholinesterase. The cDNA was expressed in Sf9 cells transfected by a recombinant baculovirus: the secreted enzyme had catalytic properties compatible with those of class A AChE (10). Finally, using the strain p1000 of *C. elegans* that harbors a null mutation in *ace-l*, we found a single nucleotide change introducing a stop codon TGA in place of codon TGQ encoding W84 (13). We have compared the sequence of *ace-l* in *C. elegans* and in the closely-related species *C. briggsae*. The coding sequence of *ace-l* in the two species is highly conserved, but not intronic sequences nor 3'UTR (11). We cloned a 2.4 kb fragment of the 5'UTR of *ace-l* in the two *Caenorhabditis* species, and found that transfection of *ace-l* gene including this upstream region was sufficient to restore a normal movement to the uncoordinated double mutant *ace-l/ace-2* of *C. elegans*, and a normal distribution of histochemically-detectable AChE activity (14). In order to study the tissue-specific expression of *ace-l* during development and in the adult, we transfected translational fusions of 2.4 kb of *ace-l* 5'UTR and the reporter gene GFP. This construct was expressed in all body-wall muscle cells, in the pharyngeal muscle cells pm5 and in a few neurons of the head and retrovesicular ganglia (14). A comparison of conserved sequences in the 5'UTR in *C. elegans* and *C. briggsae*, and GFP expression driven by different deletions of this region, show that a distal conserved block is responsible for the expression in body-wall muscle cells, and that another block is responsible for the expression in pharyngeal muscle cells (E. Culetto, unpublished results).

**Ace-2**

*Ace-2* (and the additional *ace-x* and *ace-y* genes) were not amplified by PCR using the initial sense primer EDCLYLN used for *ace-l*. We used RT-PCR and degenerate prim-