Genetic phenotypic and molecular aspects of the Caenorhabditis elegans habituation

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学位の種類	博士（理学）
学位記番号	博乙第252号
学位授与の日付	2002年9月30日
学位授与の要件	論文博士（学位規則第4条第2項）
学位授与の題目	Genetic Phenotypic and Molecular Aspects of the Caenorhabditis elegans Habituation （C.elegans 慣れ学習の遺伝学，行動学及び分子生物学的概観）
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学位論文要旨

ABSTRACT

The acquisition and retention of information by the nervous system are major processes for learning. Habituation is a simple learning process that occurs during repeated exposure to harmless stimuli. *C.elegans* is habituated when given mechanical stimuli repeatedly and recovers from the habituation when the stimuli are stopped. A mutation in *hab-1* results in a delay in habituation , but the normal habituation response resumes within one hour, although wild-type animals are necessary more than two hours. The chemotaxis and adaptation to odorants in a test were normal.

INTRODUCTION

Learning behavior is a useful indicator for the study of neural integration, as shown with *Aplysia* , *Drosophila* and mouse. Learning behavior is classified into associative and nonassociative learning. Nonassociative learning consists primarily of habituation and sensitization. *C. elegans* is a desirable subject for this kind of study. Habituation is a form of nonassociative learning in which an animal's responsiveness to weak repetitive stimuli decreases. This form of learning has important advantages for studying the molecular mechanisms underlying behavioral modifications. The nervous system of the nematode *C. elegans* is very simple, and its complete morphology and cell lineage are known. The behavior of *C. elegans* has been extensively studied in relation to the nervous system. The learning behavior of *C. elegans* accompanying mechanical stimuli was originally studied by Rankin *et al.*,. They found a neural circuit for tap-mediated stimuli. They also found that sensitization and habituation are accompanied by repeated tapping. One approach to clarifying the mechanism of habituation behavior is to analyze mutations that perturb regular response patterns.

RESULTS AND DISCUSSION

To understand the details of the mechanisms underlying learning behavior, the genes contributing to habituation have to be identified. We therefore established a method for isolating the abnormal mutants based on their tap- response- mediated habituation. These mutants are called *hab* mutants (for habituation mutation). As far as we know, habituation-abnormal mutants in *C. elegans* have not been reported. Genetic and phenotypic analyses of such mutants are reported in this paper.

We screened the ca 3×10^4 F_2 progeny of EMS-treated animals and isolated five potential mutants that showed abnormal responses to repeated tap stimuli. One of them, the *hab-1(cn308)* mutation was assigned to the linkage group I (LG I)(Fig.1).

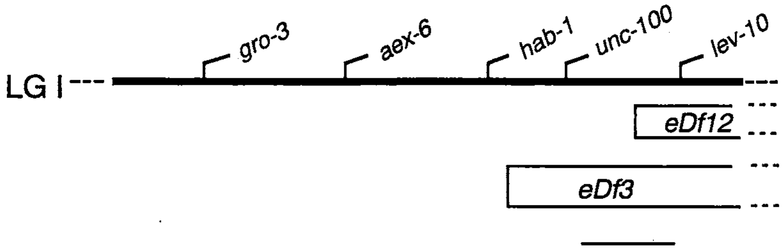

Figure 1 A simplified genetic map of *C.elegans* showing the position of *hab-1* in relation to markers used in the this study.

Mutant phenotypes for the tap stimuli applied to the *hab-1* mutant were compared with the wild-type phenotypes (Fig.2 and Fig.3). Wild-type *C. elegans* locomoted backward by more than one body length in 3.5 seconds when a tap stimulus was applied once. When the tap stimulus was repeated at 2-s ISI, the animal population entered a habituated state within 22 repetitions of the tap stimuli (Fig.2).

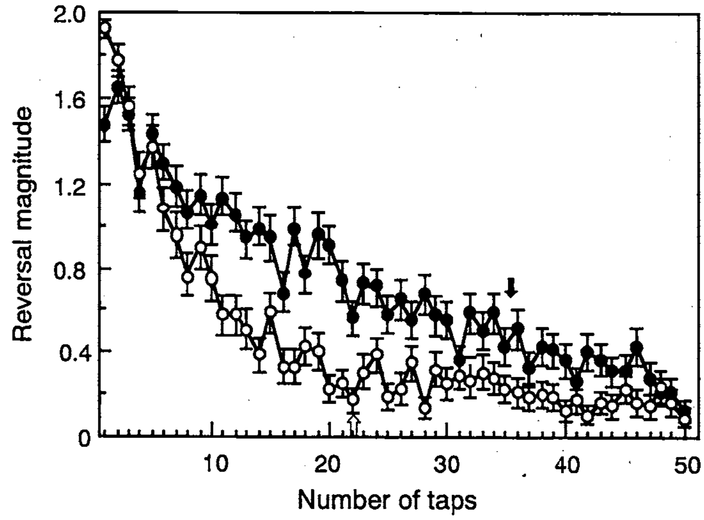

Figure 2 Habituation produced by 2-s ISI tap stimuli
Wild type (○)(n=75); *hab-1* (*cn308*)(●)(n=80).

Animals given 50 repetitions of the tap stimuli gradually recovered from the habituation. However, a period of more than two hours was necessary for complete recovery from habituation (Fig.3).

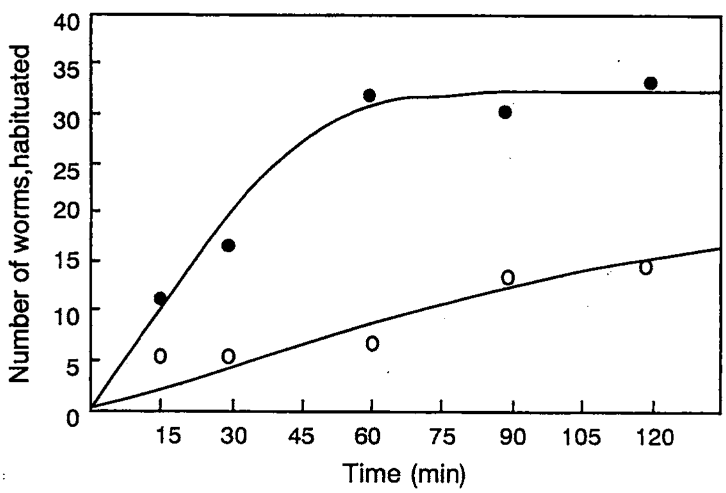

Figure 3 Time course of retention after 50 serial 2-s ISI taps.
Wild type (○); *hab-1* (*cn308*)(●).

The *hab-1(cn308)* mutant responded normally to the single tap stimulus. The mutant was habituated more slowly than wild-type but recovered rapidly from the habituated state (Fig.2 andFig.3).

In *C.elegans* transgenic animals are generated by injecting a cloned genome (usually cosmid clones) into the germ line syncytium, usually with a dominant co-expressed marker. The transgenes form extra chromosomal arrays that are transmitted to a certain fraction of the population and, in rare cases, become integrated into the genome. To verify that the gene responsible for the mutant phenotype has been properly identified, mutant sequences are analyzed for differences from the wild-type gene. To clone the gene we carried out cosmid rescue experiments with cosmid and YAC clones covering the gene. We inspected the *C.elegans* genomic sequence of the region to which the *hab-1* allele map is implicated(Fig.4).

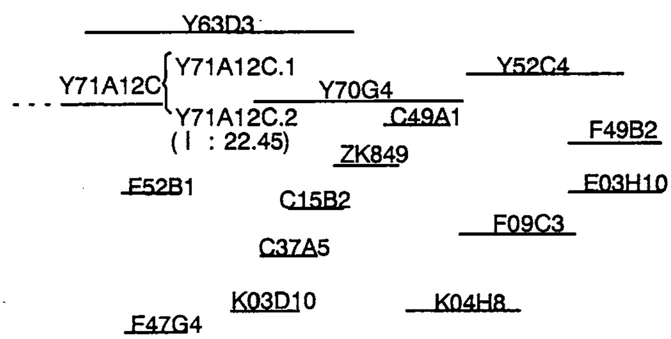

Figure 4 Cosmid maps covering the *hab-1* gene region.

We found two clones F52B1 and Y71A12C rescuing phenotype of *cn308 hab-1* allele. These clones encode three potential genes. the genes were amplified by PCR and again carried out rescue experiments by microinjection.

In summary we identified a novel gene *hab-1* whose mutation affects on habituation induced by mechanical stimuli. Usually the habituation memory consists of two phases, acquisition and retention. In the *hab-1* mutant, both phases are abnormal; that is, their acquisition is slower and retention is shorter. It is interesting that a single gene regulates both phases because distinct genes control the two phases for the *Drosophila* olfactory learning.

What is the function of *hab-1* gene? The neural circuit for touch reflexes has been identified. We compared the habituation of the *hab-1* mutants and wild-type animals, both of which were ablated of these neurons. Of the *hab-1* mutant had the target neurons of ablation, the animals were rapidly habituated, but the habituation rate was slower than that of wild-type animals. These results suggest that the *hab-1* mutant does not have defects in specific neurons, but is generally defective in habituation. It is sure that habituation occur at the synaptic levels. At the habituated state, the release of neurotransmitter is decreased irrespective of repeated stimulations. We identified the region encoding the *hab-1* gene. We therefore are in progress of cloning the *hab-1* gene.

学位論文審査結果の要旨

神経高次機能の一つである学習に伴う記憶形成とその維持機構は複雑さ故に研究はあまり進んでいない。申請者　許錫振は分子遺伝学的手法でこの課題に取り組み下記の成果をえた。

神経系が簡単なC elegansを用い機械的刺激慣れ学習に対し異常な突然変異体（cn308）を分離した。得られた変異体は野生株に比べ慣れ記憶獲得能が劣り，記憶維持能も劣っていた。本学習に関与する神経回路網を構成する神経細胞を焼却（neuron ablation）して学習行動を調べたが特定ニューロンの異常は観察されなかった。更に本遺伝子を同定するため遺伝子座決定を行った。その結果第一常染色体に位置していた。さらに詳細なマッピングを行い。22。74m. u. 内に同定し，hab-1と命名した。本遺伝子をカバーするcosmid及びYACクローンを用いinjectionによるrescueでクローニングを行った。

以上の知見は生命科学のみならず神経系疾患診断，治療に寄与することが期待される。よって本論文は博士（理学）論文に値すると判定された。