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学 位 論 文 題 目	合成dauer構成性表現型を指標としたC.elegansの神経系変異の分離と解析
論 文 審 査 委 員	主　査　教　授　廣瀬 進 教　授　小原 雄治 助 教 授　林 茂生 助 教 授　城石 俊彦 教　授　細野　隆次（金沢大学）

論文内容の要旨

The nematode *Caenorhabditis elegans* has several advantages as a model organism for studying the nervous system. First, its nervous system is very simple. Second, the complete structure of the nervous system has been determined by electron microscopy of serial sections. Third, genetic methods are available. Fourth, it is possible to kill specific neurons by laser ablation under a light microscope.

The functions of nervous systems can be studied genetically by isolating and characterizing mutants that are abnormal in various behaviors. Mutants of *C. elegans* that are abnormal in locomotion, egg-laying, pharyngeal pumping, chemotaxis, defecation *etc.* have been isolated and characterized in the past over 20 years. Functions of the nervous system can be studied also by a specific aspect of post-embryonic development, i.e., regulation of dauer larva formation.

The dauer larva is a special third-stage larva produced under harsh conditions. After hatching, *C. elegans* usually passes through 4 larval stages called L1, L2, L3 and L4, before becoming an adult worm. If there are little food and much pheromone (due to overcrowding) around the worm, it becomes a dauer larva instead of an L3 larva. The dauer larva does not feed, because its mouth is closed. When it encounters food, it molts and goes into the L4 larval stage.

The two main environmental cues for the dauer/L3 decision, namely food and pheromone, seem to be sensed by the nervous system. *C. elegans* has a pair of sensory organs called amphids in the head. It is known that if we kill two types of sensory neurons (ADF and ASI) in the amphids of will type worms, they become dauer larvae at a certain probability even under non-dauer-forming conditions. The probability increases to nearly 100%, if we also kill another type of amphid sensory neurons (ASG).

Many mutants that are abnormal in the dauer larva formation have been isolated and named *daf* mutants. They are classified into two groups. One is called dauer-constitutive (*daf-c*) mutants, which form dauer larvae even under non-dauer-forming conditions. The other is called dauer-defective (*daf-d*) mutants, which do not form dauer larvae even under dauer-forming conditions. Some of the *daf-d* mutants have defects in the structure of amphids. This is another piece of evidence showing that dauer formation is controlled by the nervous system.

Although about 30 *daf* genes and about 20 other genes are known to affect dauer formation, the number is still much less than the expected number of genes that affect neurons. There may be many neural mutations that show the synthetic Daf phenotype, namely, exhibit abnormality in dauer formation only if two or more of them are combined to form double- or multiple-mutations. Two examples of the synthetic dauer formation (Sdf) phenotype were known when Mr. Suzuki started this study: *unc-31;unc-3* and *unc-31;aex-3*.

He isolated mutants that exhibit the Sdf phenotype on *unc-31* black-ground. He used

unc-31 (*e169*);*utEx*[*unc-31*(+)], an *unc-31* strain in which a clone of the wild-type *unc-31* gene exists as an extrachromosomal array. He mutagenized *unc-31*(*e169*);*Ex*[*unc-31*(+)] with ethyl methanesulfonate and screened 5539 of F1 Unc+ progeny for those which segregate Unc dauer larvae but not Unc+ dauer larvae under non-dauer-forming conditions. Those which segregate both Unc and Unc+ dauer larvae were discarded, because they were *daf-c* rather than s*df* mutants. He obtained 44 mutants that showed the Sdf phenotype on the *unc-31* background.

He mapped 42 of the 44 mutants by using STS (Sequence-Tagged site) markers and known mutations, while two of them had too low penetrance to be mapped. Eight of the mapped mutations were found to be alleles of four known genes by complementation tests (one allele of *tax-2,* two alleles of *che-11,* two alleles of *osm-6* and three alleles of *aex-3*) The rest 34 mutations, which were most probably alleles of new genes, were classified into 18 complementation groups.

In the meantime, it was found in our laboratory that known *daf-d* mutations that are abnormal in dye-filling (Dyf phenotype) into amphids and phasmids have the Sdf phenotype if they were combined with the *unc-31* (*e169*) mutation. Therefore, he tested the 44 new mutations for the Dyf phenotype. Besides the each two alleles of *che-11* and *osm-6*, two mutations in two new genes (*sdf-3* (*utl60*) and *sdf-13* (*utl82*)) had the Dyf phenotype. In these mutants, environmental cues cannot reach the ASI neurons due to the structural abnormalities of amphids. In other mutants they probably can reach ASI but cannot be sensed by ASI, or the transmission of the signal may be blocked in ASI or downstream neurons.

Since the new mutations may cause abnormalities in neurons other than ASI, he checked some behaviors to detect such abnormalities. Some of the mutants were abnormal in osmotic avoidance (defect in ASH), while others were abnormal in chemotaxis to benzaldehyde (defect in AWC) or to diacetyl (defect in AWA). Especially interesting was *sdf-1* (*utl61*), which avoids benzaldehyde at a concentration that attracts wild-type worms.

In conclusion, this study shows that by isolating synthetic dauer-constitutive mutations, we can identify many new neural genes that probably control reception, signal transmission, and signal processing of the environmental cues (pheromone and food) for dauer formation. The mutants obtained in this study will be useful for analyzing functions of ASI neurons in the future.

論文の審査結果の要旨

線虫 C. elegans のL3幼虫は環境条件に応じて生育型か dauer かの二型を示す。この応答は神経支配を受けており高次な神経機能の表現型の一つである。dauer 幼虫形成に関わる変異としては、構成的に dauer 幼虫となる Daf-c 変異と、dauer 幼虫になれないDaf-d 変異が知られている。また、神経系の細胞で発現しているunc-31の変異と、unc-3 または aex-3 の二重変異体では Daf-c の表現型を示す。

鈴木教郎君は、神経機能に関わる新しい遺伝子を同定することを目的として unc-31 変異と組み合わせたときに、構成的に dauer 表現型を示す変異を多数分離し、その解析を行った。これらの変異は、４つの既知の遺伝子座と少なくとも１８の未知の遺伝子座にマップされた。多数の未知遺伝子を見出したことは、この方法の有効性を示している。得られた変異株のうち、６株は蛍光色素 DiO の取り込みに異常が見られ、化学受容器の構造に欠損があると予想される。残りの３８株は DiO を正常に取り込み、DiO で染色された神経細胞の数、位置や形態も正常であるため、神経の機能などに異常があると思われる。後者のうち、ut161 変異株は、野生型で正の走化性を示すベンズアルデヒドとイソアミルアルコールのどちらに対しても負の走化性を示す興味深い変異であった。以上のように、鈴木君は神経系の解析に必要な新しい変異株を多数分離し、今後の研究に重要な基礎を構築した。これらの成果は発生学と神経科学、特に臭いを介した神経系情報伝達の研究発展に寄与すると期待される。その内容は遺伝学専攻の博士論文としての条件を満たすものであることを、審査委員全員が認めた。