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学 位 記 番 号	総研大甲第770号
学位授与の日付	平成１６年3月２４日
学位授与の要件	生命科学研究科　遺伝学専攻 学位規則第４条第１項該当
学 位 論 文 題 目	Isolation and analysis of mutants defective in olfactory learning behavior in *C.elegans*
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論文内容の要旨

Animals, including humans, sense environmental cues and select appropriate behaviors depending upon the environment in which they live. This plasticity, called learning, is based on changes in their neural functions at cellular and molecular levels. Until now, many investigators have studied the molecular mechanism of neural plasticity using various species of animals. Because of the complexity of most nervous systems controlling this plasticity, its studies have often focused on relatively simple nervous systems and on reduced preparations.

The nematode *Caenorhabditis elegans* (*C.elegans*) is a good model organism for studying behavioral and neural science because of its simple multicellular structure consisting of only 959 somatic cells including 302 neurons (Brenner S, 1974, Sulston JE, Horvitz HR, 1977, White JG et al, 1986). Despite its simple structure, *C.elegans* shows the diversity of behavioral plasticity in response to environmental stimuli. For instance, animals conditioned with NaCl and food migrate toward NaCl, while animals conditioned with NaCl and starvation do not migrate toward NaCl (Saeki S et al, 2001). This learning behavior is probably based on modulation by the integration of paired stimuli, food and chemical stimuli. However, only limited results have been obtained on the cellular and molecular events in the sensing of food and the integration of paired signals.

Food and starvation also modulate sensory adaptation to an AWC-sensed odorants. Food inhibits sensory adaptation (Ishihara T and Katsura I, unpublished results; Bargmann CI, pers. comm.; Nuttley WM et al, 2002), while starvation enhances sensory adaptation (Colbert HA and Bargmann CI, 1997). On the basis of these observations, we developed an assay system to isolate mutants defective in olfactory learning behavior. In the assay system, wild-type animals were exposed to AWC-sensed odorants in the presence or absence of food, and then tested for their response to the same odorants. The results showed that animals conditioned with butanone and food exhibited enhanced chemotaxis to butanone, while those conditioned with benzaldehyde or isoamyl alcohol and food did not change the efficiency of chemotaxis to the same odorants. This phenomenon cannot be explained by the inhibition of olfactory adaptation by food, but can be explained by learning through the association of butanone and food.

Using this assay system, we screened 5,000 genomes and obtained mutants defective in the olfactory learning behavior induced by butanone and food. Among these mutants, *ut305* and *ut306* showed defects in olfactory learning behavior induced by butanone and food. Namely, these mutants exhibited weaker chemotaxis to butanone after conditioning with butanone and food. Interestingly, *ut305* also showed defects in adaptation to benzaldehyde or isoamyl alcohol, while *ut306* showed normal adaptation to these odorants. This observation may indicate that a specific pathway for butanone exists in olfactory learning. Furthermore, *ut305* also showed weak defects in chemotaxis to butanone.

Serotonin (5-HT) mediates some of the effects of food on many behaviors: it stimulates pharyngeal pumping (Avery L and Horvitz HR, 1990) and egg laying (Trent C et al, 1983), but inhibits locomotion (Horvitz HR et al, 1982). Exogenous 5-HT also inhibits the effect of starvation in sensory adaptation (Colbert HA, Bargmann CI, 1997, Nuttley WM et al, 2002). Analysis of mutants defective in 5-HT and catecholamine synthesis indicated that neither 5-HT nor catecholamine is required for the olfactory learning behavior induced by butanone and food. Furthermore, the experiments of exogenous 5-HT on *ut305* olfactory learning behavior revealed that *ut305* gene and 5-HT control distinct pathways: one for the olfactory learning (increase of chemotaxis index induced by butanone and food) and the other for the inhibition of olfactory adaptation.

I mapped *ut305* between R03A10 and C02C6 on chromosome X and *ut306* between F44C4 and VC5 on chromosome V. *ut305* was mapped to the region containing a single candidate gene C02C6.2. This protein was predicted to have 3 transmembrane domains and showed partial homology to the fruitfly raw protein. Raw protein is required for restriction of JNK signaling in embryogenesis. *ut305*::GFP fusion gene, which rescued the behavioral defects, was expressed in some neurons in pharynx and a pair of head neurons (AIA interneurons). Furthermore, killing of AIA neurons resulted in abnormality in the olfactory learning. AIA neurons are interneurons and receive synaptic inputs from many amphid sensory neurons including AWC. On the other hand, the experiment of extopic expression was shown that the expression of the wild type *ut305* gene in AWC and AWB sensory neurons (using *gcy-10* promoter) was sufficient for normal olfactory learning induced by butanone and food. Here, I would like to propose a hypothesis for the mechanism of action of *ut305* protein. Wild type *ut305* gene is thought to be involved in the sensitization of the olfaction of butanone

because *ut305* animals show weak defects in response to butanone. If this sensitization system is regulated by the signals of butanone, wild type animals may show olfactory learning behavior. Therefore, the connection between AWC sensory neurons and AIA interneurons would be important for this sensitization and the olfactory learning behavior by wild type *ut305* gene, when the gene is expressed in AIA neurons.

論文の審査結果の要旨

動物は様々な感覚入力情報を統合し，遺伝的に規定された神経回路の機能を変化させる．このような可塑性の分子的・生理的基盤を明らかにすることは神経生物学の最重要課題の一つである．虎山一郎君は，線虫 C. elegans を用いて連合学習の新しいパラダイムを築いた．

線虫では，走化性行動の adaptation が餌によって抑制されるという可塑性現象があることが知られていた．虎山君は，まず butanone に対する走化性行動が餌によって増強されることをみつけ，この現象が「におい」と「餌」という二つの刺激の連合学習であることを示した．そして，この現象に基づいて巧妙な"selection assay"を開発し，連合学習に異常がある個体を選別するための突然変異分離実験を行った．selection assay での行動異常を指標にして得られた10変異系統の内，ut305，ut306 の2系統は，餌が存在しても butanone に adaptation してしまうかのように振る舞った．また，ut305 は butanone 同様 AWC 神経で検出される benzaldehyde に対する adaptation も異常であった．つまり，ut305 変異の原因遺伝子は，AWC ニューロンを介して行われる2種類の可塑性現象に関与していることがわかった．

虎山君は ut305 変異のポジショナルクローニングを行い，その原因遺伝子が3つの膜貫通ドメイン様の配列を持つ新規蛋白質をコードすることを示した．この遺伝子を GFP でタグした系統では，AIA 介在ニューロンで GFP の発現がみられた．AIA ニューロンを破壊すると selection assay での行動が異常になったため，確かにこのニューロンが今回取り上げた学習に関与していることが示された．一方，ut305 系統の症状は，原因遺伝子を AIA で発現したときだけでなく，感覚神経である AWC，AWB で発現したときにもレスキューできた．AWC は感覚ニューロンとして AIA にシナプス出力を出すだけでなく，AIA からの入力も受ける．したがって，この遺伝子は感覚神経 AWC と介在神経 AIA を含む神経回路の複数の細胞で機能しうる可能性が高い．

以上のように，虎山君は，新たな学習実験系を開発し，それを用いてこれまで知られていなかった新たな症状を呈する突然変異系統を単離し，その原因遺伝子を同定した．この研究は学習の新規プロセスの発見につながる先駆的発見として高く評価できる．したがって，虎山一郎君の論文は博士号授与の要件を満たすと審査員全員一致で判断した．