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学位論文題目	G protein alpha subunit gene *odr-3* mediates olfactory learning in *C. elegans.*
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論文内容の要旨

The nematode *C. elegans* senses many attractive odorants in two pairs of head sensory neurons AWA and AWC. Molecular mechanism of sensory transduction, adaptation and asymmetrical development in AWC neurons has been intensively investigated.

The animal enhances chemotaxis to an AWC-sensed odorant butanone after pre-exposure of butanone with food, which we call olfactory learning. Olfactory learning draws attention because it shows some interesting properties: (1) olfactory learning is independent of serotonin or dopamine, which mediate food signals in other food-related behavioral plasticity, (2) olfactory learning is normal in adaptation defective mutants, and (3) the sensory cilium of AWC^{ON} cell, which expresses STR-2 in one of the two AWC neurons, is important for olfactory learning. Although this behavioral plasticity showed many interesting properties, this kind of plasticity was observed only after conditioning with butanone and food. In addition, signal transduction pathway that mediates this olfactory learning is almost unknown

To learn whether olfactory learning occurs in other odorants, I changed the parameters in olfactory learning assay. For conditioning, I tested four AWC-sensed odorants: butanone (Bu), isoamyl alcohol (Iaa), benzaldehyde (Bz), and 2,3-pentanedione (Pd) as well as an AWA-sensed odorant diacetyl (Di). I also changed the amount of odorants in conditioning. The initial amount of odorant used in conditioning was determined by that in adaptation studies. I also tested the amount of conditioning odorant for 1/10 and 1/100 of the initial amount. In the selection assay,

When the animals were conditioned with food and butanone, olfactory learning was observed for a wide range of conditioning and odorant concentrations
In contrast, conditioning by food with Di or Bz resulted in no change or decrease (i.e., adaptation). Interestingly, Iaa causes olfactory learning under limited conditions. Conditioning with Pd and food also enhanced chemotaxis to the odorant, although only a few parameters were tested. These results clearly indicate that olfactory learning occurs with odorants other than butanone and hence suggest that it may be important for the survival of *C. elegans* in nature.

Then I carried out mutant analysis to investigate the molecular mechanism of olfactory learning. I chose ut311 from 8 candidates because it showed abnormality specifically in

Bu olfactory learning and to belonged to new class of mutation.

I cloned the responsible gene of *ut311*. I revealed that cause of learning defective in *ut311* was due to the missense mutation in *odr-3* gene. *odr-3* encodes G protein alpha subunit and is important for odorant signal transduction. Although odr-3 null mutants showed strong defect, *ut311* showed normal responses to AWC-sensed odorants.

To learn whether *odr-3(ut311)* is functional in other sensory neurons, I tested the response to the AWA-sensed Di and the osmotic avoidance behavior mediated by ASH neurons, which were also known to be mediated by *odr-3*. *ut311* showed virtually normal response to Di at high concentrations, but weaker responses at a low concentration (10^{-4} dilution). Surprisingly, ut311 exhibited a strong defect in osmotic avoidance, which was comparable to one of the null mutants *odr-3(n2150)*. These results suggest that ODR-3(*ut311*) does not function or functions only very weakly in ASH neurons.

To learn more information about the molecular mechanism, I tested the phenotype of Bu learning in mutants in downstream of *odr-3* and sensory adaptation. In summary, I could not find genes that participate in Bu learning among those involved in downstream of *odr-3* and sensory adaptation.

Then I tested upstream gene of G protein signaling. I tested RGS (Regulator of G protein Signaling), GRK(G protein coupled receptor kinase) and arrestin mutants, where RGS is thought to interact with G protein alpha subunits, GRK interact with both G alpha and GPCRs and arrestin interact GPCRs.

I tested *grk-1(ok1239), grk-2(rt97), grk-2(gk268), arr-1(ok401)*, and *rgs-3(vs19)* mutants. *grk-1, arr-1 and rgs-3* mutants exhibited normal Bu learning. On the other hand, the *grk-2(rt97)* mutant, which has a missense mutation in the kinase domain, showed abnormality in Bu learning. Another mutant (*grk-2(gk268)*), which has a deletion mutation, showed slight enhancement after conditioning, but the change was very small. These data indicate that Bu learning was mediated by *grk-2*

In this study, I revealed some aspects of olfactory learning in *C. elegans*. I showed that some of AWC-sensed odorants cause olfactory learning, suggesting that olfactory learning is important for *C. elegans* food seeking. Then, I showed *odr-3* and *grk-2* pathway is involved in olfactory learning. This is the first example of the G protein signal transduction that involves olfactory learning including integration of two sensory signals.

論文の審査結果の要旨

動物は内外からの様々な情報を神経系で統合処理して行動として出力する。一條宏君は神経細胞における情報の統合化のメカニズム解明をめざして、モデル生物の線虫C. elegansの匂い学習の研究を行った。

C. elegansは302個の個々の神経細胞の配線図が同定されており、頭部感覚神経AWAとAWCで多くの誘引匂い物質を感知している。そのひとつブタノンはAWCによって感知され、線虫はブタノンへの走性を示すが、ブタノン存在下に餌を与えて（条件付け）飼育すると、その後はブタノンへの走性は強化される。これがブタノンによる匂い学習である。神経細胞での情報統合化のモデルとして非常に興味深い系である。

一條君はまず、ブタノン以外で匂い学習が起こる物質があるかどうか探索し、イソアミルアルコールや2,3ペンタンジオンが限られた条件ではあるが、匂い学習を引き起こすことを明らかにした。この中でやはりブタノンが最も広い範囲の濃度で匂い学習を引き起こすことなどから、一條君はブタノンによる匂い学習のメカニズム研究をさらに進めた。

一條君は所属研究室で得られていたブタノン匂い学習に特異的に異常があるut311変異に着目しその遺伝子クローニングを行った。その結果、ut311はodr-3遺伝子のミスセンス変異であることを明らかにした。odr-3はG蛋白質αサブユニットをコードし、AWCを含む感覚神経で発現する。ut311変異はAWA, AWC感知匂い物質への走性はほぼ正常であるが、odr-3のヌル変異はこれらへの走性は異常になる。そのヌル変異でブタノン匂い学習解析をおこなったところ、予想外のことであるが、ブタノンへの走性が失われているにもかかわらず餌との共存により匂い学習の効果が見られた。一條君は、匂い学習に複数の重複した経路が絡んでいるのではないかと議論しているが、ブタノンが広い濃度範囲で匂い学習を示すことや、学習濃度に強く反応する傾向があることなど温度学習と似た現象を観察しており、異なる濃度領域では別のシグナルが働いている可能性など、全く新しい局面の糸口である可能性がある。

一條君はさらにodr-3の上流下流遺伝子経路との関係を調べた。その結果、odr-3の下流、adaptationの変異体では顕著な関与は見られなかったが、Gタンパクシグナル伝達での上流の遺伝子に関係が見られた。具体的にはRGS（Regulator of G protein Signaling：Gタンパクαサブユニットと相互作用）、GRK（G protein coupled Receptor Kinase：GαとGPCRの両方と相互作用）、arrestin(GPCRと相互作用)の各変異体におけるブタノン学習を解析した結果、grk-1, arr-1, rgs-3は正常であり、grk-2で異常が見られた。

以上の研究で一條君はブタノン匂い学習の中核遺伝子のひとつodr-3を見出し、その働きについて濃度範囲などに応じた機構の可能性について、今後の研究が必要であるが新しい局面を拓いたと評価できる。また、Gタンパク経路が２つの感覚シグナルを統合する学習に含まれることを最初に示したことになり、この点でも高く評価できる。本研究は匂い学習という困難な課題に積極的に取り組み、様々な工夫と膨大な実験を積み重ね、興味深い結果を得たものであり、学位授与の水準に十分に達していると審査員一同判断した。