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論文内容の要旨

The last common ancestor of bilateria and cnidaria is considered to be the first animal to obtain nervous system over 700 million years ago. After that, animals have developed their own nervous systems that are seen now. During a long course of evolution traits of nervous systems in these animals are vastly diverged and different from each other. Some of the traits, however, seem to be shared between higher animals (vertebrates and/or arthropods) and cnidaria. These seemingly similar traits can be classified into two groups: analogous traits and homologous traits. The analogous traits are defined as traits currently shared by these animals but might have been different in the past or may be different in the future. The homologous traits are long conserved among animals during their evolution. Therefore, they should shed light on what prototypical nervous system was like. The question is how to distinguish them. Generally to say, the homologous traits share common underlying mechanisms to realize them.

This study and previous studies demonstrated interesting new aspects of *Hydra* nervous system. First, the nerve net of *Hydra* is divided into subpopulations. Second, each neuron subset expresses specific combination of neuropeptide genes. Third, each neuron subset is localized in a restricted region(s) along the oral-aboral axis. Fourth, some neighboring subsets of neurons are separated from each other with clear boundaries between them. And finally one of the possible functions of neuron subsets is local action by a localized neural neurotransmitter(s). The last two aspects are pointed out for the first time in this study. All these features imply that the neuron subsets in *Hydra* are neural compartments and they behave as sort of functional units like those of higher organisms. Are these seemingly similar traits analogous or homologous? In order to address this issue, I attempted to elucidate the mechanisms for generating neuron subsets of *Hydra* and compared them to that of neural compartments of higher animals.

A compartment is generally defined as a subdivided tissue that consists of lineage-restricted non-intermingling sets of cells between neighboring compartments. According to this definition, neuron subsets in *Hydra* may not be the neural compartments equivalent to higher organisms, because there is no lineage-restriction in the formation of neuron subsets in *Hydra*. The tissue displacement in *Hydra* continuously moves neurons in a subset into a neighboring subset. Despite this, however, each neuron subset keeps its location and size of population constant, maintaining clear boundaries between subsets.

There are two possible mechanisms to supply neurons for balancing a loss of neurons caused by the tissue displacement: new neuron differentiation from precursors and phenotypic conversion of preexisting neurons. By comparing tissue displacement rate and labeling kinetics of BrdU in Hym-176A+ neurons in the lower peduncle of adult *Hydra*, I estimated that about 70% of neuron turnover in the neuron subset was accounted for by new differentiation and the remaining 30% appeared to be accounted for by phenotypic conversion. I also found another example for phenotypic conversion in the middle of this neuron subset. These results suggest that both of the two mechanisms are involved in the formation of neuron subsets.

New differentiation always occurred near the upper boundary of the neuron subset

although neuron precursors could penetrate further down in the subset. When the situation was created where no preexisting neurons were present such as during foot regeneration or replacement of the normal foot with the nerve-free epithelial foot, essentially all the neurons produced were newly differentiated ones and distributed within the subset, not restricted at its upper boundary. These results suggest that new differentiation appears to be regulated by lateral inhibition of preexisting Hym-176A+ neurons and that more rapid new differentiation prevails in case of emergency in which new neurons are required. At the moment, the fate of neurons is not known when they leave the subset by tissue displacement. Cell death might be involved in addition to phenotypic conversion. Taken together, in *Hydra* although lineage-restriction may not be involved in maintaining clear boundaries and keeping the size of subsets constant, these are regulated positively by both of new differentiation and phenotypic conversion, and negatively by lateral inhibition and possibly cell death.

Next, I addressed the issue as to what determine the position of neuron subsets in *Hydra*. Prepattern genes, pairs of mutually repressing homeobox genes, such as Otx, Pax and Hox, determine the region where neural compartments are formed in higher animals. These genes are regulated by a few secreting molecules, such as Wnts, FGFs and retinoic acid. In *Hydra*, counterparts for some of these molecules are identified but only Wnts appear to be involved in axis formation. In this study I have shown that activation of the Wnt signaling pathway with LiCl and/or ALP, both of which inhibit GSK-3β as their common target, altered positional information and therefore localization of neuron subsets in *Hydra*. This suggests that the Wnt signaling pathway is conserved between neural compartments and neuron subsets in determining their localization.

How does this positional information direct region-specific differentiation of neuron subsets in *Hydra*? There must be a transcriptional control involved in it. I compared 5'-flanking genomic regions among Hym-176 paralogous genes. Some of them are expressed in different neuron subsets located in different axial regions while the others are in the same axial region. It is expected that the same or similar region-specific cis-regulatory elements may be shared by genes expressed in subsets of neurons located in the same axial region. I have found several conserved motifs. One of them was similar to the STATx binding motif that was shared by subsets of paralogues expressed in the lower peduncle. Although this one might be conserved between neuron subsets and neural compartments, others showed no homology to known motifs. These results seem to indicate that the mechanism of region-specific gene regulation in neuron subsets and neural compartments is not well conserved. In other words, cnidarians might have invented cis-regulatory elements of their own.

Comparison of the involved mechanisms between neuron subsets in *Hydra* and neural compartments in higher animals showed both conservation and divergence. This may be taken for granted, because it has been enormously long time since they were separated from the last common ancestor. However, a crucial point was that one of the most fundamental signaling systems all through the animal evolution, the Wnt pathway was conserved as one of the underlying mechanisms to determine the position of compartments between *Hydra* and higher

organisms. Downstream genes activated by the Wnt pathway appear to be different. More evidence should be accumulated. But I would like to temporarily conclude that it is too early to give up the idea that neuron subsets in *Hydra* and neural compartments in higher animals are homologous structure reminiscent of the ancient nervous system. I would further pursue my studies along this line.

論文の審査結果の要旨

動物の神経系構築の特徴は，体軸に沿った領域化（区画化）である．体軸の位置情報に従って神経細胞が異なる性質を獲得することにより，動物は高度な神経機能を発揮する．高等動物におけるこのような位置異存的な遺伝子発現パターンは，「特定の区画特異的運命を獲得した細胞が他の区画の細胞とまじりあわない」という機構によって維持されている．単純な体制を持つ腔腸動物ヒドラでも，口-反口方向の体軸に沿って位置情報があることが知られていた．しかし，網状構造をとるヒドラの神経組織も高等動物同様に領域化されているかどうかはわかっていなかった．野呂君はヒドラの Hym-176 神経ペプチド遺伝子ファミリーが，口-反口軸の位置情報に従って領域的に発現していることを見いだした．５つのペプチド遺伝子の発現パターンの組み合わせを指標にすると，ヒドラの神経系は口-反口軸に沿って４つの領域に分けられることがわかった．これは，ヒドラにおいても神経系が区画化されていることを示唆する結果である．

この結果を基に，野呂君はヒドラの神経系の区画化がいかにして維持されるかを解析した．「維持」の機構が特に重要なのは，ヒドラの神経細胞は誕生後一定の位置を保つのではなく，常に移動しているからである．ヒドラの幹細胞や神経前駆細胞は体内を上方または下方に向かってたえず移動して組織代謝をおこなっているし，上皮組織に組み込まれた神経細胞も上皮細胞と共にゆっくりと移動している．従って，細胞が時間経過と共に体軸上の場所を変えるにもかかわらず，位置特異的な神経細胞運命に基づく区画を維持する機構が必要である．野呂君は，（１）区画特異的な細胞運命獲得は神経細胞誕生時（最終分裂が起きた時期）だけ起こる（new differentiation）という可能性と，（２）一旦特定の運命を獲得した神経細胞が位置の移動と共に運命を変える（phenotypic conversion）という可能性を検討した．神経前駆細胞から産み出された神経細胞を BrdU で標識する実験の結果，柄部の上・下二つの区画の境界では new differentiation は起こっておらず，柄部下方の区画に属する細胞は，この区画への移動と共に phenotypic conversion によって別の神経細胞運命から運命転換することによって生じていることが明らかになった．また，胃腔部と柄部上方の区画境界においても，柄部上方の区画に属する細胞の約３０％は phenotypic conversion を起こしていることを示した．これは，組織構築がきわめてダイナミックに行われているヒドラにおいては，神経細胞は誕生後も絶えず位置情報にあわせて運命を変更しているということを示す興味深い結果である．また，野呂君は区画化の「確立」の過程についても解析を行っている．Hym-176 遺伝子ファミリーメンバーの 5′隣接領域の比較を行い，発現場所に相関した転写因子候補結合サイトを多数同定した．トランスジェニックヒドラ作成の技術を導入し，遺伝子発現調節の研究を進めている．

神経細胞が環境に応じて性質を変えるという知見は高等動物でも見いだされているが，これまで環境要因として考慮されてきたのは物理・化学的な体外環境や神経活動・ホルモンなどの生理的環境であった．野呂君の仕事は，ヒドラの神経細胞は「体軸の位置情報」という生体の基本情報の変化にも適応して細胞運命を調節することができるということを示した点で画期的である．この知見がきっかけとなり，これまで lineage restriction によって区画が維持されると考えられてきた高等動物においても，区画間の細胞移動や phenotypic conversion が発見される可能性もある．以上の理由で野呂行彦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．