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学 位 論 文 題 目 Evolutionary features of the central nervous system
revealed by the comparative approach of the gene
expression profiles

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論文内容の要旨

The understanding of the evolutionary process of the central nervous system (CNS), in particular a brain, is one of the challenging tasks in modern biology. It is easily imaginable that biological complexity of the CNS is mainly due to not only structural complexity but also complicated and elaborated network of function. There is no doubt that functional network must be maintained by a network of multiple gene interaction that are intriguingly maintained by gene expression control. The extreme difficulty of studying the CNS might be due to a lack of definite approach to understand multiple interaction of gene expression. However, it is fortunate that the recent advancement of genome projects and cDNA projects provided us with the molecular biological methods for identifying gene sets and the degree of gene expression in a particular organ of a given species. When we define a gene expression profile as an occurrence frequency of the expressed gene species in a given organ, I thought that comparative studies of gene expression profiles in the CNS among various organisms might give profound insight into the understanding of evolutionary processes of the CNS. Here in my thesis, I proposed a novel approach for the evolutionary study of CNS, focusing on the gene expression profiles. Since an expression profile results from the transcriptional activities of all genes involved in the networks, the expression profile should reflect the outcome of transcriptional regulations of all genes expressed in a given organ. Thus, the purpose of my thesis is to answer the question whether the evolutionary process of CNSs can be understood from the gene expression profiles.

To attain the purpose, I examined the gene expression profiles at the following levels: (1) a conservation pattern of the nervous system-related genes, (2) the divergence of the gene expression profiles at the cell level, and (3) the divergence of the gene expression profiles at the level of the organs such as a brain. In particular, I examined if the correspondence is good between the species tree conventionally obtained and the tree of gene expression profile invented in my study.

This thesis is composed of five chapters. In **chapter 1**, I described the outline of the present thesis, placing particular emphasis on the motivation and purpose of my study. I also noted that the evolutionary study of the CNS, in particular a brain, is of biological significance. In **chapter 2**, taking the planarian CNS as an example, I, first, examined the evolutionary divergence of the genes that were related to the nervous system, because the planarian is known to possess one of the most primitive brains. To elucidate the evolutionary process of CNS, I then conducted the comparative genomics studies of gene expression profiles in the CNS among different organisms. We sequenced 5,433 5'-ESTs from the cDNA library that was derived from the head portions of planarians (*Dugesia japonica*), obtaining

a total of 3,101 non-redundant EST clones.

To deal with the large amount of EST data, I have developed a computer software package, FinEST, in which an information analysis of EST sequence data including homology search can be done automatically. Conducting the homology search in my software package, I found that 44% of the 3,101 clones had significant similarity of amino acid sequences of gene products whose functions were known. Among these genes, at least 116 genes were found to be homologous to the CNS-related genes. I compared these 116 planarian gene sequences with all ORFs of the complete genome sequences of human, fruit fly and nematode. I then found that 110 genes were evolutionarily shared among all the bilateral animals examined, although only the remaining six genes were shared among a limited number of species. This feature of gene conservation can be considered as strong reflection of the selective constraints against CNS-related genes, suggesting that these shared genes are a part of the basic gene set of CNS which might have existed in the common ancestral CNS of bilateral animals. Based on these findings, I proposed a model of the evolutionary process of the CNS.

In chapter 3, with the aim of studying the diversity of the genes expressed in different cell types, we took a comparative approach using the gene expression profiles of single cells of ascidians (*Ciona intestinalis*). The swimming larval stage of ascidian has two different sensory organs, called "ocellus" and "otolith". These organs exist in a cerebral vesicle, which is often called as a brain. It has been reported that there are only two pigment cells in a total of about 2,600 cells that form the swimming larva. One pigment cell is found in the ocellus and the other is otolith. Thus, in this study, these pigment cells were called as ocellus cell and otolith cell. To attain our purpose, we examined the expression profiles of the ocellus cell and the otolith cell, and compared the expression profiles between these two different types of cells. First, we sequenced 964 and 774 ESTs from the cDNA libraries of the pigment cells of ocellus and otolith, respectively. As a result, we obtained 485 and 505 non-redundant clones from the ocellus and otolith cells, respectively. The composition of the highly expressed clones illustrated clear difference from that of planarian head ESTs, showing that one of characteristic features of the gene expression profiles in these single cells is less amount of the cytoskeletal genes expressed. Comparing the gene expression profiles between ocellus and otolith cells, we found that 60 clones were commonly expressed between two pigment cells. The relative frequencies of these 60 clones showed obviously distinct patterns between these two cells. This is the first report about the gene expression profiles of the single cells that compose an organ, showing clear characteristic features of the expression profiles at the single cell level.

In chapter 4, I made an attempt to understand the evolutionary process of a brain from the viewpoint of gene expression profiles. To attain the purpose, I

raised a question of whether the degree of the differences in the gene expression profiles of a particular organ between different species corresponds proportionally to the degree of the evolutionary divergence between the species. In practice, we sequenced EST clones from the cDNA libraries of brains of chickens (*Gallus gallus*) and lampreys (*Lampetra japonica*), the head of planarians (see above), and the whole body of the jellyfish (*Aurelia aurita*), obtaining over 2,000 clones from each library. If difference of the gene expression profiles corresponds to the evolutionary divergence of species, a topology of the tree based on the difference in the gene expression profiles (designated as a gene expression tree) might correspond to a topology of the species tree. To examine this statement, I quantified the differences in the gene expression profiles between different species by the Euclidean distance, and then constructed a gene expression tree. As a result, the topology of the gene expression tree showed correspondence to that of the species tree, though the current number of clones sequenced was still relatively small. Thus, I concluded that the gene expression profiles of brains might reflect the evolutionary process of the brain.

Finally, in chapter 5, I described the summary and conclusion of the present study. I also discussed the future perspectives of this study. In conclusion, this is the first attempt to conduct an evolutionary study by use of the gene expression profiles, successfully showing that the evolutionary process of the CNS can be traceable by use of the gene expression profiles.

論文の審査結果の要旨

峯田氏は、遺伝子発現プロフィールに着目し、中枢神経系の進化過程を遺伝子発現のパターンやゲノム配列データから推定することが可能なかを探索する研究を行った。まず、扁形動物のプラナリアが、系統進化において旧口動物と新口動物の分岐付近に出現し、起原の古い三胚葉性動物のひとつでありながら原始的な脳を持つと考えられていることに注目している。プラナリアの頭部で発現している遺伝子群を EST として 8000 本以上単離し、冗長性を除いて 3,101 種類の遺伝子を同定したのち、これらについて予測したアミノ酸配列をもとに、利用可能な多様な生物種の全配列データベースを対象に相同性検索を行っている。次に、神経系に関与すると報告のある遺伝子群と統計的に有意な相同性を有する配列を、プラナリアの EST から 116 個抽出した。峯田氏はこれらの遺伝子はプラナリアの神経系に関与する遺伝子群の候補であると推定したが、彼の所属する研究グループでは、この予測に基づく遺伝子のうち約 30 個について既に *in situ* hybridization 実験を行っており、すべてがプラナリアの中枢神経系である脳と VNC (Ventral Nerve Cord) で発現していることを確認している。

峯田氏が機能予測したプラナリアの 116 個の遺伝子には、N-CAM や arrestin など中枢神経を有する他の動物類の神経系で発現している遺伝子が多く含まれていることから、これらの遺伝子が旧口動物と新口動物の分岐付近かあるいはそれより以前に存在していた可能性の高いことを指摘している。さらに、相同性検索により神経系に関係すると推定したプラナリアの 116 個の遺伝子について、全ゲノム配列が解読されたヒト、ショウジョウバエ、線虫、シロイヌナズナ、酵母の全遺伝子と比較している。116 遺伝子のうちの約 95% が中枢神経系を持つ生物種のヒト、ショウジョウバエ、線虫と相同遺伝子を共有しており、また約 40% が神経系を持たない生物種の酵母、シロイヌナズナと相同遺伝子を共有していることを明らかにした。このことから、現在中枢神経系に関与している遺伝子群には、動物と植物の分岐よりも起源が古い相同遺伝子グループが存在することを明らかにした。峯田氏の得た成果は、プラナリアにおける世界初の大量 EST 解析を行った業績だけでなく、神経系に関与する遺伝子群にその起源が旧口動物と新口動物の分岐より古いものが多く存在するという可能性を指摘しており、分子進化学や関係分野における貢献は大きい。

さらに、プラナリアの頭部の遺伝子発現パターンをニワトリやカワヤツメなどの脳の遺伝子発現パターンと比較し、またホヤの幼生頭部に存在する異なった感覚器官を代表する単一細胞 (ocellus cell と otolith cell) の EST 解析から遺伝子発現プロフィールの明瞭な差を検出する試みを行なった。これらの試みは、EST データの少なさから現時点では確定的な結果は出せていないものの、その研究手法や解釈のあり方を提案した彼の積極性は、急速な進展を見せているゲノム科学や生命科学において、大量データを対象にしたこの分野の研究に貢献する上での能力の高さを示すものとして評価した。

本学位論文は、すべて英語で書かれており、本研究の主要部分は、峯田氏を筆頭著者として、国際誌に投稿中である。さらに現在までに国際誌に出版された他の原著論文は 2 編存在し、英語に関する能力も充分であると判断した。

以上のことから、審査員一同、峯田氏の業績と学位論文は、本学の博士の要件と基準を十分に満たしており、学位授与に値すると判定した。