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論文内容の要旨

A remarkable feature of the mammalian evolution is the expansion of the neocortex and emergence of the specific internal cytoarchitecture, the layer structure. All of the mammalian species share the neocortical layer structure, in which a similar type of neurons are arranged in a particular layer parallel to the brain surface. During the neocortical development, the neuronal subtypes in the neocortical layers are produced from neural progenitor cells in a stereotyped temporal sequence from deep to upper layers. This stereotyped sequence of neuronal production is attributed to the temporal restriction of the competence of neural progenitors. The progenitors initially possess the multipotency to generate the neuronal subtypes in all neocortical layers, but gradually loose the potency during the development, and eventually become only able to produce the upper layer neurons.

The dorsal region of the telencephalon called the pallium is the non-mamalian homologue of the neocortex, because the same developmentally important genes are commonly expressed in the mammalian neocortex and the non-mammalian pallium. Although the pallium is completely conserved among the vertebrates, the internal structure is variable. For example, the bird, one of the closest relatives of the mammals, possesses the well-developed pallium packed with distinct subtypes of neurons that are arranged in particular domains. Such observations suggest that the common ancestor of the mammals and the birds had already acquired the pallium, and that the pallial structure has been modified in an animal group-specific manner through alterations in the developmental processes. Therefore, a key event contributing to the evolutionary emergence of the neocortical layer structure could be found through comparison of the development between the mammalian neocortex and the non-mammalian pallium.

The chick pallium is a good model to approach the problem, because of the closest phylogenetic position to the mammals as well as the convenience of experimental manipulations. For over a century, it has been argued whether the avian pallium has a comparable neuronal repertory to the mammalian neocortex. Therefore, I first checked expression patterns of marker genes for the mammalian neocortical layers in the chick pallium. This analysis revealed that both the deep (layer V) and upper layer (layer II/III) marker genes were expressed in the chick pallium, suggesting that the chick pallium possesses a neuronal repertory similar to the mammalian neocortex. In addition to the molecular expressions, the axon projections were also found to be partially similar between the chick pallial neurons and the corresponding neuronal subtypes of the mammalian neocortex. In spite of the remarkable conservation in the neuronal repertory, spatial distribution patterns of the deep and upper layer neurons were entirely different from the layer arrangement of the mammalian neocortex; in the chick pallium, the deep and upper layer neurons were not arranged in parallel, but distantly located in the medial and lateral side, respectively.

The development of the deep and upper layer neurons in the chick pallium was investigated in detail. First, the birthdate analysis by BrdU pulse-labeling demonstrated that the deep layer neurons were generated earlier than the upper layer neurons in the chick pallium, suggesting that the temporal sequence of the neuron production is evolutionarily conserved between the mammals and the birds. Second, the fate mapping analysis revealed that the deep and upper layer neurons originated from the distinct neural progenitors on the medial and lateral sides in the chick pallium, respectively. This spatially separate production of the neurons is the critical difference from the mammalian neocortical development, in which the deep and upper layer neurons are uniformly produced across the entire neocortex. Probably related to this difference, I found that the late neurogenesis in the chick pallium predominantly occurs on the lateral side. This spatiotemporally biased neuronal production can explain the selective generation of the late-born upper layer neurons only from the lateral side in the chick pallium. Taken together, the distinct neurogenetic properties between the medial and lateral progenitors appeared to be the key to construct the non-layered domain-like cytoarchiteture in the chick pallium.

How then is the medio-lateral difference of neurogenetic properties is instructed in the chick pallium? I cultured neural progenitor cells from the medial and lateral sides of the chick pallium in a clonal density, and surprisingly found that most of the clones derived from a single progenitor cell contained both deep and upper layer neurons, regardless of its origin. This remarkable observation clearly demonstrated that the neural progenitor cells in the medial and lateral sides of the chick pallium intrinsically possess a similar neurogenic competence, and the neurocompetency is extrinsically regulated by the surrounding tissues according to the spatial positions.

On the basis on the results obtained, I propose the following model for the avian pallial development. The avian neural progenitors are intrinsically equivalent to those of mammals and capable of sequentially generating a full repertory of neuronal subtypes. However, the neurogenesis in the avian pallium is extrinsically regulated by two potential mechanisms. First, the deep layer fate in the early-born neurons on the lateral side is suppressed by environmental factors, and thereby, the lateral neural progenitor cells produce only the upper layer neurons in the later phase of neurogenesis. Second, the medial neural progenitors terminate the neurogenesis precociously before producing the upper layer neurons leading to the preferential generation of deep layer neurons from the medial side.

Lastly, the emergence of the layered neocortex in mammals has been a long-standing mystery in evolutionary biology. The present discovery of the evolutionary conservation in the neural progenitor competence between the mammals and the avian suggests that the common ancestor of the amniotes has already possessed the developmental potential to sequentially produce the multiple neuronal subtypes. During the evolutionary diversification into each lineage of animal groups, alterations in the spatial regulation of the neurogenetic program may have contributed to the emergence of animal group-specific brain structures, such as the layer structure in the mammalian neocortex and the domain structure in the avian pallium.

博士論文の審査結果の要旨

脊椎動物の脳の構造は体の骨格形態と同等あるいはそれ以上の多様性を示す．骨格とは異なり脳は化石記録に残らないため，脳構造の進化研究は現存する生物の脳の比較解剖学的解析に限られていた．鈴木君は，脳の発生過程に着目し，神経細胞の誕生や移動などの過程の時間的・空間的要素を比較解析の対象とするという新しいアプローチを採用した．その結果，系統間で共通の過程と違いがある過程を同定し，脳構造の進化の新たなモデルを提唱することに成功した．

多様な脳構造の中でこれまで最も注目を浴びてきたのは終脳背側領域の構造である．ほ乳類で大脳新皮質とよばれるこの領域は「層構造」という特徴的な構造を取り，同じ種類の神経細胞が層状に分布している．この層構造は神経幹細胞から産み出される多様な細胞が誕生順に次々と積み重なっていくことによって形成される．一方，哺乳類以外では対応する領域（外套）に明白な層構造が存在しないため，脳構造の多様性が発生プロセスのどのような違いによるのかはわかっていなかった．鈴木君は，層構造を取らない生物の代表例としてニワトリをえらび，その大脳皮質の発生過程を解析した．

まず神経細胞の多様性の進化的保存を調べるために，マウス大脳皮質で深層（DL），上層（UL）特異的に発現している複数の遺伝子を神経細胞多様性のマーカーとして用い，そのニワトリホモログの発現を解析した．その結果，ニワトリ脳にもマウス同様の神経細胞多様性があることがわかった．また，DL -> UL（初期：DL 細胞，後期：UL 細胞）という誕生の順序も保存されていた．しかし，ニワトリ脳では DL 細胞と UL 細胞は層状には配置しておらず，DL 細胞は内側に，UL 細胞は外側に存在していた．このことから，マウスとニワトリでは特定の神経細胞が産生される場所に違いがあることが示唆された．

そこで，神経細胞が神経幹細胞から産み出される過程をしらべたところ，ニワトリの神経幹細胞の性質はマウスには見られない顕著な領域特異的性を示すことを見いだした（表 1）．

表 1

神経幹細胞の位置	神経幹細胞から産み出される細胞の誕生順序	
	マウス	ニワトリ
内側	DL -> UL	DL -> -
外側	DL -> UL	？ -> UL

-：分裂停止
？：未同定の神経細胞

次に，神経幹細胞の細胞自律的な性質を探るために培養下での神経細胞生成能力を調べた．その結果，ニワトリの神経幹細胞もマウス同様，内側・外側どちらに位置するものも DL・UL 両種の神経細胞を産み出す能力を持っていることを発見した．この結果はマウスとニワトリの共通祖先がすでに「1 個の神経幹細胞が時間と共に多様な神経細胞を産み出す」というプロセスを保有していたことを強く示唆している．鈴木君は，ニワトリではこのプログラムに細胞非自律的シグナルにより修正が加えられ，領域特異的な性質が付加されることによって，マウスとは異なった神経細胞の配置や脳構造が生じていると結論した．

鈴木君は分子発生生物学と古典発生生物学を見事に組み合わせ，脳の発生過程における神経幹細胞の性質に羊膜類の共通祖先にまで遡る深い相同性があることをみいだした．また，この共通プログラムに系統特異的な変更が加えられたことにより，脳構造の大きな変化を産み出したという仮説を提出することができた．これは進化発生学への重要な貢献であるとともに，発生生物学の分野でも新たな研究の方向性を指し示すものである．以上のことから，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．