氏　　名	富木　毅
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論文審査委員	主　査　教授　徳永　万喜洋 教授　岡村　康司 教授　桂　勲 教授　西川　建 助教授　平田　たつみ

論文内容の要旨

Combinations of various functional systems are used in organisms for living, and each system is composed of various proteins. I predicted how functional systems had been evolved and how protein functions in each functional system had changed through molecular phylogenetic analyses.

In this study, I tried to predict the evolution of functional systems by superimposition of phylogenetic trees constructed by as many homologous proteins in the functional systems as possible. Under the assumption that the divergences of proteins in functional systems correspond to the divergences of functional systems, I superimposed phylogenetic trees of proteins in functional systems, and inferred the deduced phylogenetic tree of the functional systems. I did phylogenetic analysis at system level to predict phylogeny of functional systems.

Some of proteins are composed of multiple functional domains, and each domain might be inserted or deleted through evolution. Insertions or deletions of functional domains have changed protein functions. I tried to predict how domain compositions had changed through evolution and how protein functions had diversified by domain insertions and deletions through molecular phylogenetic analyses. I did phylogenetic analysis at the domain level by constructing composite gene trees so as to predict protein function changes in functional systems.

It is possible that protein functions are different even if domain compositions of proteins are the same. I tried to predict the essential amino acid substitutions for changing protein functions through evolution referring to phylogenetic trees and amino acid sequences which cannot be detected by domain level analysis. I did phylogenetic analysis at the amino acid level to predict protein function changes in functional systems.

As I described above, I predicted the evolution of functional systems and protein function changes in the functional systems at the three levels; system level, domain level and amino acid level. I applied the three level predictions to the two functional systems; electron transfer energy metabolism system (chapter 2) and neurotransmission system (chapter 3). The four electron transfer energy metabolism systems (photosynthesis, aerobic respiration, denitrification and sulfur respiration) share common characteristic features. Furthermore, homologous proteins exist among the four systems. They suggest that the four systems are evolutionarily related. But the exhaustive phylogenetic analysis of proteins among the four systems has not been tried yet. Therefore, I decided to try the three level predictions in the four energy metabolism systems to infer the phylogeny of the four systems and the protein function changes in the four systems. I constructed molecular phylogenetic trees by using amino acid sequences of functional domains. These trees and amino acid compositions of proteins suggest that domain insertions and deletions in the four systems made functions of electron transfer in proteins change. I tried to predict ligand binging specificities of catalytic proteins at the amino acid level, but experimental

data about ligand binding functions are not enough for doing such predictions. Therefore, I did not predict protein function changes at amino acid level. Most of proteins in the four systems are not homologous each other. Only some important proteins for generating energy are homologous among the four systems. It means that most of proteins have evolved independently, and few of proteins are conserved among the four systems. I tried to superimpose phylogenetic trees of homologous proteins in the four energy metabolism systems to predict the phylogeny of the four energy metabolisms, and the phylogeny of aerobic respiration, denitrification and sulfur respiration was predicted.

The three level predictions were also applied to chemical neurotransmission system. Neurotransmitters except for neuropeptides are produced by synthases in presynaptic cells, and the neurotransmitters are released to synapse. The neurotransmitters in the synapse are captured by receptors in postsynaptic cells, and the postsynaptic cells are activated. The neurotransmitters in the synapse are uptaken by transporters in the presynaptic cells or degradated in the synapse, and the chemical neurotransmission is inactivated. There are various kinds of neurotransmitters, and synthases, receptors and transporters for each neurotransmitter exist in chemical neurotransmission system. Therefore, each functional system for chemical neurotransmission can be defined as a system composed of synthases, receptors and transporters for each neurotransmission. I tried to predict how the chemical neurotransmission systems have been diversified by means of superimposing the phylogenetic trees of synthases, receptors and transporters. The phylogenetic trees of some synthases and some receptors are possible to superimpose, and the phylogenetic trees of some receptors and some transporters are also possible to superimpose. These proteins might evolve together. But most of other proteins seem to have evolved independently from this study. Therefore, the unit of evolution is not system in chemical neurotransmission systems. I also did phylogenetic analyses of receptors at the domain level and the amino acid level. I inferred domain composition changes which had changed protein functions from the domain level analyses. Some domain changes seem to be essential for generating some receptors. The essential amino acid substitutions for changing ligand specificities were also predicted from the amino acid level analysis.

Based on the phylogenetic analyses of energy metabolism and chemical neurotransmission system, I did phylogenetic analysis of voltage-gated potassium channels at two levels; domain level and amino acid level (chapter 4). Voltage-gated ion channels are important for generating action potentials in postsynaptic neurons after chemical neurotransmission. I focused on inactivation, one of the major electrophysiological features of voltage-gated ion channels, and predicted how the diversification of the inactivation had occurred. There are two kinds of inactivation in voltage-gated potassium channels. One is N-type inactivation which is sudden inactivation immediately after activation, the other one is C-type inactivation which is slow inactivation. Reffering to phylogenetic trees and domain compositions of voltage-gated potassium channels, domain composition changes seem not to affect inactivation differences. Previous studies suggest

that the specific chemical features of 20 N-terminal amino acids are important for generating N-type inactivation. Therefore, I investigated the specific chemical features of 20 N-terminal amino acid sequences in voltage-gated potassium channels. The specific chemical features of the 20 amino acids which induce N-type inactivation are found in the three subtypes out of the 21 subtypes. A small number of amino acid substitutions might produce the three N-type inactivation subtypes. The specific chemical features are not found in 20 N-terminal amino acids in the other five subclasses which can generate N-type inactivation. The amino acid substitutions which produce the five subtypes may different from those of the three subtypes which have the specific chemical features in the 20 N-terminal amino acids.

I did phylogenetic analyses at system, domain and amino acid levels. From system level analysis, most of proteins in functional systems seem to be evolved independently. Although functions have been conserved in energy metabolism systems and chemical neurotransmission system, most of proteins in these systems have not been conserved. Domain composition changes seem to be slower than a small number of amino acid substitutions referring to phylogenetic analyses in this study. The combinations of the slower domain composition changes and the faster amino acid substitutions may have changed protein functions and these combinations might produce divergence of protein functions. But the combinations might happen independent of evolution of functional systems. Functional systems may have conserved their functions by the combinations and been diversified also by the combinations.

論文の審査結果の要旨

本研究は、エネルギー代謝系・神経伝達系・イオンチャネルファミリーにおいて、各系を構成しているタンパク質がそれぞれどのように進化し、さらに各系がシステムとしてどのように進化したか、分子進化学的解析を行い、システムと機能いう観点から分子進化の特徴を明らかにした。

生命は、多様な系を組み合わせることにより営まれている。その進化の特徴を明らかにするために、アミノ酸、分子構造の基本単位としてのドメイン、機能ごとの分子システムとしての系の、３つのレベルに注目し、分子進化学的解析を行った。系の分岐が系に属するタンパク質の分岐に対応するか否かという点に注目しながら、系に属するタンパク質の系統樹を重ね合わせ、系の系統樹を推定し、系レベルの解析を行った。また、ドメインレベルでの系統樹を作成し、ドメイン構成の変化とタンパク質機能の多様化が、進化の過程でどのように関係しているかを解析した。さらに、進化の過程でのタンパク質の機能を変えるのに必須であったアミノ酸置換の推定を行い、アミノ酸レベルで進化とタンパク質機能との関係を解析した。

以上３つのレベルの解析を、電子伝達を伴うエネルギー代謝系、神経伝達系、イオンチャネルファミリーに関し行った。膨大なデータを解析し、独自にデータベースを構築した。

解析の結果、エネルギー代謝系では大部分のタンパク質は独立に進化していた。系を単位としては進化していないものの、系にとって重要なタンパク質は保存されてきたことがわかった。この保存されてきたタンパク質の系統樹を重ね合わせることにより、光合成、酸素呼吸、脱窒、硫黄呼吸の系統関係を推定した。ドメインレベルとアミノ酸レベルの解析から、ドメイン構成の変化により各タンパク質内の電子伝達機能が変化し、アミノ酸単位の置換により各タンパク質と反応する分子が変化したことが示された。

神経伝達系においても、系を構成する大部分のタンパク質は独立に進化しており、系を単位として進化していないことが分かった。神経伝達物質のレセプターに関して、ドメインレベルとアミノ酸レベルの解析を行ったところ、レセプターの誕生にとって重要なドメイン構成の変化と、レセプターのリガンド特異性の変化にとって重要なアミノ酸置換を推定することに成功した。

イオンチャネルファミリーにおいては、電位作動性カリウムチャネルに関し、チャネルの不活性化メカニズムの多様性と進化との関係を解析した。その結果、Ｎタイプの不活性化機構をもたらしたアミノ酸置換を明らかにした。

一連の結果から、ドメイン構成の変化とアミノ酸置換は系の進化とは独立に起きたこと、系としての機能自体は保存しながらも、アミノ酸置換とドメイン構成の変化によってタンパク質の機能が多様化してきた様子を解明した。

以上、富木は、独自の視点から分子進化学的解析を行い、新しい知見を見いだしており、関連分野への寄与は大きい。従って、審査委員会は、本研究が学位授与の要件を十分に満たすものと判断した。

公開発表会とそれに続く審査委員会の質疑応答における議論と、提出された論文を基に審議し、専門および関連分野の知識や理解力に関し学位にふさわしいと判断した。英文で書かれた博士論文により、英語に関して十分な実力を有していると判断した。