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学位論文題目 The early evolution of eukaryotes with special reference to  
ribosome export factors

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## 論文内容の要旨

It is believed that primordial eukaryotes were derived from prokaryotes, acquiring nucleus. A number of attempts have been made to reveal the early evolution of eukaryotes, and some hypotheses for the emergence of the early eukaryotes are proposed so far. However, the evolutionary process of early eukaryotes is still a controversial issue and remains one of the biggest questions in current biology. In this study, with the eventual goal toward elucidation of the evolutionary origin and process of early eukaryotes, I conducted molecular evolutionary analyses of transporter proteins of ribosomes between the nucleus and the cytoplasm, called ribosome export factors (REFs).

This thesis consists of four chapters and an appendix. In **Chapter 1**, I described the research background for this study, with particular emphasis on the molecular function of the REFs. The ribosome, one of the largest complexes in eukaryotic cells, is to be exported from the nucleus to the cytoplasm through nuclear pores. As discovered in recent years, the kinetic steps in this nucleocytoplasmic transport pathway are stimulated by the REFs. The REFs would be worth focusing on because they can be considered as one of the components in the eukaryotic core system, translation, and as one of the key genes in the evolutionary process of early eukaryotes for maintaining the mobility of the ribosomes under the existence of nuclear membrane in the then-emerging eukaryotic cells.

In **Chapter 2**, with the aim of revealing the functional significance of the REFs in the process of eukaryotic evolution, I examined the functional constraints of the entire translation system, the ribosomal proteins and the REF proteins. Estimating the relative evolutionary rates of the yeast REF proteins, I found that, although not as much as the ribosomal proteins, the REF proteins do slowly evolve. More interestingly, the evolutionary rates of the REFs can be classified into two groups. In order to explain this difference in evolutionary rates between the two groups, I considered two subcategories for the REFs, according to the steps in which the REFs are involved. Those two subcategories are non-membranous REFs (non-mREFs) and membranous REFs (mREFs). Interestingly, this categorization was coincided with the evolutionary rate difference: Namely, the rapidly evolving REFs were the non-mREFs while the slowly evolving REFs were the mREFs. These results show that the mREF proteins evolve slower than the non-mREF proteins, suggesting the functional importance of mREFs in the evolutionary process of eukaryotes.

In **Chapter 3**, I examined the evolutionary origin of the eukaryotic nucleus by

conducting the ortholog detection analysis of the REFs in prokaryotic lineages. The evolutionary origin of the nucleus is still unclear, although a number of hypotheses have been proposed so far. I searched for the origin of the REFs in archaeal and eubacterial lineages by the method of PSI-BLAST. The results obtained showed that the non-mREFs originated exclusively from eubacterial proteins whereas the mREFs were from both archaeal and eubacterial proteins. Thus, the REFs working inside the nuclear membrane (*i.e.* non-mREFs) are derived only from eubacteria, while alternatively, the REFs shuttling between the nucleus and the cytoplasm (*i.e.* mREFs) are from both archaea and eubacteria. If we assume that the early nucleus has parsimoniously employed intranuclear proteins as the intranuclear transporters (*i.e.* non-mREFs), these data suggest that the structure of the nucleus may be a descendant of the eubacterial cell. At least, it is suggested that the nucleus arose in a cell that contained chromosomes possessing a substantial fraction of eubacterial genes. Therefore, from the viewpoint of ribosome transport, it is plausible that the nuclear structure is not originated from archaea, but from eubacteria.

Lastly, in **Chapter 4**, I provided a summary and conclusions for the present study. I have shown that the REFs evolve slowly, in addition, the mREFs evolve more slowly, suggesting that the entire eukaryotic translation system is under the functional constraints, and in particular, that the mREFs are functionally important in the process of eukaryotic evolution. Moreover, from the prokaryotic origin of the REFs, it is suggested that the nucleus is rather a descendant of the eubacterial cell, not the archaeal cell.

In **Appendix**, I made particular mention to the biological database projects for eukaryotes, in which I have been involved. Comprehensive annotations of model eukaryotes and integrated databases for such annotations are becoming more and more important in the current post-genome era. Moreover, such databases are useful for the study of early evolution of eukaryotes that is the main aim of the present study. Such databases are also invaluable for comprehensive access to the information resources, and will stimulate the comparative evolutionary genomics. With the eventual goal to know the early evolution of eukaryotes, here I refer to three eukaryotic database projects in which I have been involved, the Molecular Database of *Hydra* Cells, the Rice Annotation Project Database (RAP-DB), and the H-Invitational Database (H-InvDB).

The Molecular Database of *Hydra* Cells includes the invaluable data of expression patterns of cell type-specific genes in *Hydra*, a member of phylum Cnidaria, which branched more than 500 million years ago from the main stem leading to all bilaterian animals. The database framework was developed by myself, and it serves a unique

opportunity for graphically browsing more than 100 cell type-specific genes in *Hydra*. All of the resources can be accessed through <http://hydra.lab.nig.ac.jp/hydra/>.

The RAP-DB is a database for *Oryza sativa* ssp. *Japonica*, one of the model eukaryotes, and has been developed in order to comprehensively house all the annotations produced by the RAP (Rice Annotation Project), which is internationally organized with the aim of providing standardized and highly accurate annotations of the rice genome. The latest version of the RAP-DB contains 31,439 genes validated by cDNAs. The RAP-DB has been also developed by myself, and employed in the analyses within **Chapter 2**. The RAP-DB is available at <http://rapdb.lab.nig.ac.jp/>.

The H-Invitational Database (H-InvDB) was originally developed as an integrated database of the human transcriptome that was based on extensive annotation of large sets of full-length cDNA (FLcDNA) clone. I participated in the Annotation Meeting of Genome Information Integration Project for the further development of the human genome annotations. Now, the database provides annotation for 175,537 human transcripts and 120,558 human mRNAs extracted from the public DNA databank, in addition to 54,978 human FLcDNA, in the latest release, H-InvDB\_4.3. The H-InvDB is available at <http://www.h-invitational.jp/>.

The three projects in which I have been involved produced comprehensive information for the model eukaryotes. Each database provides a nice implementation for each biological resource and will stimulate the further exploration in the early evolution of eukaryotes.

## 論文の審査結果の要旨

申請者の学位論文は、ribosome export factors (REF) 遺伝子の進化的解析から核の起源を考察するという内容である。申請者はまず「核移行のトラフィックタンパク質から、細胞のコンパートメント構造の起源と進化についてどのような示唆が得られるか」という観点からREF遺伝子に注目して研究をスタートさせた。酵母に存在する8個のREF遺伝子を取り上げ、核膜タイプと非核膜タイプに分類した後、検索手法を駆使して広く動・植物および菌類からオーソログを拾いだした。これらのオーソログを用い、進化速度の遅い多数のリボソームタンパク質や、そのほかの平均的な進化速度のタンパク質群などと比較することにより、核膜タイプの進化速度は非核膜タイプよりも遅く、リボソームタンパク質並みの強い制約を受けていることを示した。

次に、500以上の真正細菌、古細菌ゲノム配列データベースに対して、REF遺伝子の相対性探索を行った。その結果、非核膜タイプの5つのREFsは真正細菌のゲノムにのみ分布する事が明らかとなり、非核膜タイプはすべて真正細菌由来であると結論した。一方、3つの核膜タイプのREFsについては真正細菌と古細菌の両方から由来する事を見いだした。このように染色体構造の一部として核内に存在するnucleolusから核質を経て細胞質への輸送に必須なREFsのうち、核内に存在する非核膜タイプのREFsはすべて真正細菌由来であったことから、核の構造が真正細菌に起源を持つ可能性を示したことが、この論文の成果である。

核の起源に関しては、これまで幾つかの研究が発表されており、古細菌由来説が有力であるが、REFの起源から真正細菌由来を論ずる説は、今後の核の起源の議論に取り上げられるべき新たな知見である。ミトコンドリアの共生とは別の次元から真核生物の起源を考える上で、今後の研究の展開が待たれる。核の起源については、他説との比較と、より多くの視点からの展開が必要と思われるが、これまでの説だけでは解釈が難しいことを示す新たな知見を提示したことは、学位論文として価値のある内容であると考えられた。

発表論文のうち直接に博士論文に関わる内容がすでに2報出版されている。その他ヒドラ、ヒト、イネ等のゲノムアノテーションにも深く関わり、これらの生物種のデータベース構築を牽引し、かつそれらのデータを用いた内容が、博士論文のデータとして使われており、博士論文構成要素の一部をなしている。以上、新しい知見をもたらした研究内容と、それに貢献したデータ生産とデータベース構築の内容は、学位論文として十分な内容であると判断し、審査員一同学位論文として認める事を同意した。