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学 位 論 文 題 目 Evolutionary features of RNA viruses with special
reference to mutation rates and transmission modes

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論文内容の要旨

It is known that many kinds of diseases are caused by viruses having RNA as their genetic materials. In general, RNA viruses evolve by evolutionary factors including mutation and selection. Selection against RNA viruses is mainly caused by the interaction with the host species, because RNA viruses can survive only as parasites of the host species. Therefore, it is of particular importance to investigate the interactions between RNA viruses and the host for studying the evolution of RNA viruses. In this thesis, I focused on the following three interacting features with the host; 1) modes of viral infection to the host, 2) viral adaptation to a single host and 3) exchanging genomic regions between RNA viruses and the host.

In chapter 1, first, I defined the virus as an organism that could survive and grow only in the living cell, and that contained a protein coat surrounding a nucleic acid core but having no semipermeable membrane. In addition to the definitions, I outlined the taxonomy and evolutionary mechanisms of RNA viruses.

In chapter 2, I estimated the rates of synonymous substitution for 46 species of RNA viruses and found a large amount of variation in the rates (the difference in the 3rd orders of magnitude). On the other hand, through constancy in the rate of replication error among RNA viruses examined, I concluded that the main factor for the variation of the substitution rates was the differences in the replication frequency. This is because we can assume that the rate of synonymous substitution is determined by the rate of replication error and the replication frequency. Moreover, I examined relationships between the rates of synonymous substitution and several modes of viral infections to the host including the transmission modes. The results obtained indicate that the rate of synonymous substitution was strongly related to the difference in the

modes of viral infection to the host. The reason was speculated as that the modes of viral infection to the host altered the replication frequency.

In chapter 3, using porcine reproductive and respiratory syndrome virus (PRRSV) whose synonymous substitution rate was the highest among the 46 species of RNA viruses, I conducted evolutionary analyses in order to understand the evolutionary process of PRRSV. The virus is a recently emerged pathogen in domesticated swines. Epidemiological data suggest that the divergence time of PRRSV is about 15 years ago. For confirming the rapidness of the synonymous substitution rate in PRRSV, I first estimated the divergence time of PRRSV by molecular evolutionary analysis, and compared it with that inferred from the epidemiological data. As a result, the divergence time estimated by the evolutionary analysis well corresponded to that estimated by the epidemiological data. This correspondence ensured the rapidness of the rate in PRRSV. Second, I studied the envelope regions as an important element for viral adaptation to the host. In particular, positively selected sites were detected in the envelope gene by my computer analysis. Interestingly, the sites were located not only in the regions attacked by the host immune system but also in the transmembrane regions including a signal peptide. The positively selected sites in the transmembrane regions were considered to be irrelevant for escaping the immune system, because no amino acid substitutions were observed in the transmembrane regions of the sequences isolated from piglets that were experimentally infected by PRRSV. In other words, the transmembrane regions and the signal peptide are thought to be specific to a given membrane. Therefore, I think that the positively selected sites of the membrane regions are important not for the viral adaptation to the host immune system but for the viral attachment to the membrane of the new host cell, because PRRSV emerged

recently as mentioned above.

In chapter 4, I searched for eukaryotic genomic regions homologous to RNA viruses to find how often the exchange of a genomic sequence has occurred between RNA viruses including retro and non-retro viruses and 6 eukaryotic genomes such as *Homo sapiens*, *Mus musculus*, *Drosophila melanogaster*, *Caenorhabditis elegans*, *Arabidopsis thaliana*, and *Saccharomyces cerevisiae*. The evolutionary origin of the homologous regions was studied by phylogenetic analysis.

For the non-retrovirus RNA viruses, I obtained two major results: First, a part of the Borna virus genome (nucleocapsid protein gene) was shown for the first time to be derived from mammalian genomes. Second, the 6 eukaryotic genomes did not have any part of the virus genome.

In the case of the retroviruses and the two mammalian species, *Homo sapiens* and *Mus musculus*, I obtained four results. First, retrovirus-like regions occupied about 0.1 % of each of the whole genomes of the two species. Second, physical maps indicating the locations of the retrovirus-like regions were constructed for both genomes. Third, the retrovirus-like regions were not randomly distributed in both complete genomes at a significant level ($P < 0.01$). Forth, there exists a positive correlation between the GC content of retrovirus-like regions and that of the flanking regions for both species. From these results, I have concluded that retroviruses have been integrated into the host genome where the GC content was similar to each other.

The present study will give a insight not only into the evolutionary origin and process of RNA viruses but also the interacting features between RNA viruses and their hosts.

論文の審査結果の要旨

花田君は、RNA ウイルスの1種である PRRSV (Porcine reproductive and respiratory syndrome virus) の進化速度が異常に速い点に注目した。その点を確認するために、データベース中の (15 科 39 属に分類される) 46 種の RNA ウイルスについて、塩基配列の同義置換の解析を行い、同義置換速度を求めた。その結果、予想どおり進化速度は PRRSV でもっとも速く (年当り塩基サイト当り 10^{-2})、もっとも遅いもの (Simian foamy virus) に比べて数千倍も速いことがわかった。突然変異率は複製時のエラー率と複製頻度の積で表されるが、エラー率は各種のウイルスでほぼ変わらないことが知られているので、進化速度の大きな違いは主に複製頻度に起因すると考えられた。さらに、複製頻度はウイルスの感染様式に依存してきまり、空気感染や経口感染で持続的感染様式を示すものは複製頻度が高く、血液を経由して感染し潜伏的感染様式を示すものは低い複製頻度を示す、という一般的傾向を明らかにした。

次に花田君は研究対象を PRRSV に絞り、経年的に採取されたウイルスの塩基配列データを解析して系統樹を作成した。系統樹と採取年との関係から PRRSV は 1980 年代半ばに新種として登場したことを明らかにした。この結果は PRRSV が 1987 年にアメリカで初めて報告されたという疫学的事実とも良く符合する。また、PRRSV の envelope 遺伝子について同義/非同義置換を解析し、遺伝子上で positive selection (PS) が起きているサイトを同定した。PS の位置は遺伝子上の 16 サイトに限られ、エピトープ領域と transmembrane 領域、シグナル配列領域にマップされた。エピトープ領域は予想どおりの結果であるが、後二者の変異はこのウイルスが新種として生じた際に感染宿主を他の動物からブタに変更した結果であろうと推定した。さらに、花田君はこれらのデータ解析の結果を実験的に確かめるために、ブタ胎児に PRRSV ウイルスを感染させる実験を行った。感染後 1? 5 週間増殖したウイルスは、上記データ解析によって示された PS と同じ位置でのみ非同義置換を起すことを見出した。

第 3 の研究テーマとして、宿主動物のゲノムとウイルスとの関係に注目し、ヒト/マウスゲノム中に RNA ウイルスの塩基配列を探索した。その結果、ヒト (マウス) ゲノム中には retrovirus 以外の RNA ウイルスの配列はまったく見出されないこと、retrovirus が宿主ゲノムに侵入同化するときはほぼ等しい GC 含量をもつゲノム領域に入ることを明らかにした。

以上のように、花田君は RNA ウイルスについての広汎な研究を行い、基礎研究および疫学的観点から見て、いくつもの有益かつ新規な知見を明らかにしたので、博士論文としてふさわしい論文であると判断した。