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論文内容の要旨

This dissertation addresses the 4.9 kb (kilobases) nucleotide sequences of mitochondrial (mt) DNAs from five hominoid species (common and pygmy chimpanzees, gorilla, orangutan and simang), and presents their detailed analyses, together with the known human whole sequence, to assess the tempo and mode of hominoid mtDNA evolution. Particular attention was paid to the rate of synonymous substitutions in protein coding region as well as of silent substitutions in other regions. This work was further extended to the whole mitochondrial genomes of four hominoid species (human, common chimpanzee, gorilla and orangutan) with additionally determined 10 to 12 kb mtDNAs from common chimpanzee, gorilla and orangutan. These hominoid mtDNAs revealed several functionally and evolutionarily characteristic features and provided useful information on the history of hominoid species.

Most significant observations drawn from the present data are summarized as follows. First, comparison of the base compositions in any specified region of hominoid mtDNAs showed a strong base composition bias, as observed in other vertebrate mtDNAs. The L-strand of hominoid mtDNAs is rich in A (adenine) and C (cytosine) contents, but low in G (guanine) content. Base composition biases are strongest at the third codon positions and are evident along the whole genome, independent of the genomic regions. Both codon usage and amino acid preference of mitochondrial protein genes are in agreement with the base composition biases. These observations suggested that there is a biased mutation pressure in mtDNA. A possible cause may be differential deaminations of C residues owing to the asymmetric replication of both L- and H-strands of mtDNA. It is possible that differential deamination has resulted in the reduced number of C residues in the H-strand, although there has been no clear evidence for this possibility in hominoid mtDNAs.

Second, there exist functionally important nucleotide sites over the genome. Together with information on tertiary structures of proteins, as well as on secondary structures of transfer (t) RNAs, ribosomal (r) RNA genes and noncoding regions, the distribution of variable sites among hominoid mtDNAs suggested that some nucleotide sites have been playing important roles in peptide folding, assembly of proteins, or interaction to some other proteins and regulatory elements. Noteworthy are two functionally distinct regions in the major noncoding region (D-loop). One is concerned with promoter sequences for transcription and the other is with three conserved blocks. Oranguan mtDNA sequence revealed unusual substitutions at both of these regions. This suggested that the replication and transcription machinery in orangutan mtDNA may differ from that of other hominoid mtDNAs.

Third, comparison of nucleotide differences observed among closely related hominoids revealed a remarkably biased mode of changes. Between human and chimpanzee, 70% of the observed nucleotide differences are silent changes that occur mostly in the small noncoding regions or at the third codon positions of protein genes. Extensive deletions and additions are observed, but they are found only in the noncoding regions. Such observations suggested a conserved mode of the evolution of hominoid mtDNA genomes. There is also a strong preference to transitions over transversions. Out of 852 variable third positions of codons between the human and common chimpanzee mtDNAs, 93% account for transitions of which 66% are TC transitions (in the L-strand). Within the remaining 7% transversions, CA differences are most frequent while GT are least. These substitution biases correlate well with biased base compositions, particularly the low G content of the L-strand.

Fourth, owing to the outnumbered transitions and strong biases in the base compositions, synonymous substitutions reach rapidly a rather low saturation level. AG transitions attain a saturation level lower than TC transitions (in the L-strand), and such a low ceiling is observed even between the human and chimpanzee pair that diverged around five million years ago. At present, it seems inevitable to select appropriate regions that have experienced theoretically tractable numbers of substitutions. In the case of hominoid mtDNAs, candidates are all types of changes in the tRNA and rRNA regions, transversions in the noncoding regions, and nonsynonymous changes and synonymous transversions in the protein coding regions.

Fifth, rapidly evolving mtDNAs are potentially useful for addressing classical issues in taxonomy, provided that each nucletide site has not undergone extensive multiple-hit substitutions. From the whole 16209 sites of mtDNAs compared among the four hominoid species, it appears that 12137 such sites are suitable to phylogenetic use. The analysis strengthened the pattern and dating in hominoid diversification inferred from the previous analysis of 4.9 kb region in six hominoid species (among African apes, gorilla diverged first about 7.7 million years ago and then chimpanzee and human became distinct about 4.7 million years ago).

Finally, the synonymous and nonsynonymous substitution rates were examined under the assumption of the gorilla divergence being 7.7 million years ago. The extent of the compositional biases differs from gene to gene. Such differences in base compositions, even if small, can bring about considerable variations in observed synonymous differences, and may result in the region-dependent estimate of the synonymous substitution rate. A care should be taken for heterogeneous transition and base composition biases as well as different saturation levels of transition changes. The synonymous substitution rate estimated with this caution showed the uniformity over genes ($2.37 \pm 0.11 \times 10^{-8}$ per

site per year) and the high transition rate, about 17 times faster than the transversion rate. These synonymous and transition rates are comparable to the silent substitution rate in the noncoding segments dispersed between genes. On the other hand, the rate of nonsynonymous substitutions differs considerably from gene to gene as expected under the neutral theory of molecular evolution. The average differences in the gorilla - human and gorilla - chimpanzee comparisons indicated that the lowest rate is 0.7×10^{-9} per site per year for *COI* and that the highest rate is 5.7×10^{-9} for *ATPase 8*. The degree of functional constraints (measured by the ratio of the nonsynonymous to the synonymous substitution rate) is 0.03 for *COI* and 0.24 for *ATPase 8*. tRNA genes also showed variability in the base content and thus in the extent of nucleotide differences as well. The substitution rate averaged over 22 *tRNAs* is 5.6×10^{-9} per site per year. The rate for *12S rRNA* and *16S rRNA* is 4.1×10^{-9} and 6.9×10^{-9} per site per year, respectively. All of these observations strongly suggested that mutations themselves occur more or less with the same rate and compositional biases.

論文の審査結果の要旨

ミトコンドリアＤＮＡ（ｍｔＤＮＡ）は独自の複製・転写・翻訳機構とＡＴＰ生産に関する遺伝子群を持ち、真核生物の生命維持に欠かせない重要な働きを担っている。申請者はヒト上科６種（ｈｕｍａｎ，　ｃｏｍｍｏｎ　ｃｈｉｍｐａｎｚｅｅ，ｐｙｇｍｙ　ｃｈｉｍｐａｎｚｅｅ，　ｇｏｒｉｌｌａ，　ｏｒａｎｇｕｔａｎ，ｓｉｍａｎｇ）のｍｔＤＮＡの約５ｋｂの領域について、比較ＤＮＡ領域の塩基組成と塩基置換過程に関する詳細な解析を行い、各領域に適すると想定される統計的手法を用い、これら生物種の系統関係を明かにし、分岐年代を高い精度で推定した。また、この領域に含まれる５つの蛋白質遺伝子について同義置換速度と非同義置換速度の推定を行い、同義置換速度が遺伝子によらず一定であることを示した。ミトコンドリアゲノムにおける突然変異率の推定値に知見を与えると考えられ、その分子進化学的な意義は深い。

ｍｔＤＮＡの場合、２本鎖の間で特徴的な塩基組成の偏りがあり、哺乳動物の場合は、Ｌ鎖は顕著にＡ（ａｄｅｎｉｎｅ）とＣ（ｃｙｔｏｓｉｎｅ）に富む。このような異常な偏りの存在する場合の塩基置換速度推定法について、注意深い検証を行っており、ミトコンドリアのみならず塩基組成の偏りの顕著なゲノムの進化を解析する有力な手法を確立したと言える。さらに、３種（ｃｏｍｍｏｎ　ｃｈｉｍｐａｎｚｅｅ，ｇｏｒｉｌｌａ，　ｏｒａｎｇｕｔａｎ）で合計約３２ｋｂの塩基配列を決定し、ヒトを加えたヒト上科４種のｍｔＤＮＡの全塩基配列の比較をも行った。これらの成果の一部は分子進化学の国際学術雑誌Ｊｏｕｒｎａｌ　ｏｆ　Ｍｏｌｅｃｕｌａｒ　Ｅｖｏｌｕｔｉｏｎに発表され、既に評価を得ている。以上の内容は学位論文として充分であると判断する。