氏　　名	角 山 和 久
学位（専攻分野）	博士(理学)
学 位 記 番 号	総研大甲第405号
学位授与の日付	平成１１年３月２４日
学位授与の要件	生命科学研究科　遺伝学専攻 学位規則第４条第１項該当
学 位 論 文 題 目	Intragenic Variation of Synonymous Substitution Rates
論 文 審 査 委 員	主　査　教　授　舘野 義男 教　授　池村 淑道 教　授　西川　建 教　授　安永　照雄（大阪大学） 教　授　石和　貞男（お茶の水女子大学）

論文内容の要旨

Intragenic Variation of Synonymous Substitution Rates

In the protein-coding gene, nucleotide substitutions are classified into the synonymous and nonsynonymous substitutions. The synonymous substitution in a gene is defined as those not causing amino acid changes in the encoding protein, while the nonsynonymous substitution is defined as those causing amino acid changes in it. The synonymous substitution, by definition, is free from functional constraints of a protein contrary to the nonsynonymous substitution that is essentially constrained by protein function. Therefore, it is expected that for a given gene, the rate of synonymous substitution is constant over the nucleotide sites as long as mutation rate does not vary with the sites. It is also anticipated that synonymous substitutions take place more frequently than nonsynonymous substitutions. It follows that the difference between the numbers of synonymous and nonsynonymous substitutions reflects the degree of functional importance for a protein, meaning that the difference is larger as the degree is greater. I find these properties of synonymous and nonsynonymous substitutions useful for evaluating the functional importance for subunits as well as domains of a protein. Moreover, I could successfully show that the rate of synonymous substitution is variable with site not only among genes but also within a gene. I also find that for mammalian species, the intragenic variation of synonymous substitutions is mainly caused by mutation that preferentially occurs in non-randomly distributed CpG dinucleotides in a gene.

In Chapter I, I outline my thesis, placing particular emphasis on the motivation and purpose of my study. In Chapter II, for nicotinic acetylcholine receptor subunit genes of different species, I examined the degree of functional importance of subunits by conducting comparative analysis of the numbers of synonymous and nonsynonymous substitutions. It is known that nicotinic acetylcholine receptor is divided into two types, the muscular and nervous. The muscular type is composed of five subunits, a1, a1, b1, g and e. There are four trans-membrane regions, M1 – M4, in the receptor molecule. The structure of the nervous type is not well elucidated, but is known to be composed of a(2-9) and b(2-4) subunits. In particular, by computing the ratio (*f*) of the number of nonsynonymous substitutions to that of synonymous substitutions, I showed that the a1 subunit gene was the lowest in *f* value among the subunit genes in the muscle system, and so was a7 subunit gene in the nervous system. This result indicates that the two subunit genes in the two tissues have been subject to strong functional constraints in evolution. In fact, it is known that the two subunits of the receptor protein have crucial functions; a1 subunit has binding sites to the ligand of the receptor, and a7-containing receptor regulates releasing the

transmitter, acetylcholine. Moreover, the window analysis of f values shows that strong functional constraints have operated on M2 region in all the five muscle subunits. It is noted that M2 region is a part of the ion channel structure in the receptor molecule. Therefore, f value is shown to be useful for evaluating the degree of functional importance of not only a gene but also subregions within a gene.

In Chapter III, I conducted a statistical test to examine whether the rate of synonymous substitution varies within a gene, by using 418 homologous gene pairs from *Rattus norvegicus* and *Mus musculus* and 84 orthologous gene pairs from the whole bacterial genomes of *Mycoplasma genitalium* and *Mycoplasma pneumoniae*. As a result, more than 90% of gene pairs for both comparisons are demonstrated to show a significant intragenic variation of synonymous substitution rate. By examining all conceivable possibilities for the cause of the intragenic variation of synonymous substitution rates, I finally found a significant correlation between synonymous substitution rates and the frequency of CpG dinucleotides in rodents. These findings suggest that intragenic variation of synonymous substitutions in mammals is caused mainly by a mutation due to methylation of CpG dinucleotides which are unevenly distributed in the genome. In Chapter IV, I described the summary and conclusion of the present study, and I also discussed the future development of this line of study.

論文の審査結果の要旨

角山君の学位論文は３章から成り、第１章は序論、第２章はアセチルコリン受容体遺伝子における非同義置換率と同義置換率の比（f）の解析、そして第３章は遺伝子内の同義置換率の変異についての研究という内容になっている。この学位論文の主要課題は、進化の原動力である突然変異の意義を同義置換と非同義置換を通して探ることにあると言えよう。

特に彼は種々の創意工夫をもとに大量のデータ解析の結果、同義置換率は遺伝子内塩基座間で有意に異なるという結論を得た。そこで、この原因を探るため、彼はいくつかの仮説を立て検証した。その結果、同義置換はCpG塩基組成のところに起こり易く、CpGが遺伝子中に偏在しているので、遺伝子内塩基座間で変異があるという結論に至った。この結論は、突然変異がCpGに起こりやすいことを考え合わせると、同義置換は、コドン使用頻度や機能的制約に余り左右されずに起こり、突然変異をより直接的に反映しているという考察を導いた。この考察は、進化の実質的原動力である塩基置換の発生機構についての議論に一石を投じることになると思われる。

審査員全員は、角山君の学位論文は以上に述べたように、問題設定の有意性、そしてその解決における遂行能力と研究内容の独創性をよく表していると判断し、当大学院の学位を授与するのに相応しいと判定した。

さらに、角山君の学位論文の発表ならびに質疑応答をもとに審査員一同審査を行った。まず、発表内容であるが、これは基本的に論文の内容（主に３章）と同じなので、合格と判断した。次に、質疑応答であるが、応答の態度は相手の質問を注意深く聴き、自分の中で正しい解答を探そうという態度がよく出ていた。そして、常に自分の考えにもとづいて応答していた。

また、学位論文の第２章の内容は、角山君が筆頭著者で既に国際誌に発表されており、第３章の内容は、国際誌に投稿すべく準備中である。

次に、角山君の英語力であるが、百ページに近い学位論文は英語で書かれており、書く力は、当大学院の過去の学位授与者に比べて水準以上と判断できる。また、会話力は、彼が研究集会などでも英語で口頭発表をしていることや、審査員を交えた外国からの訪問者との種々の話し合いでの様子などから判断して、これもやはり水準以上と判断できる。

以上を総合判断して、合格とした。