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学位論文題目	Molecular Coevolution of Urate Oxidase(Uox) and Xanthine Oxidoreductase(Xor) Genes and Its Biological Implications
論文審査委員	主　査　教授　長谷川　政美 教授　高畑　尚之 助教授　今井　弘民 教授　竹中　修（京都大学）

Urate oxidase (Uox) and xanthine oxidoreductase (Xor) are purine metabolic enzymes. It is known that the final product of purine metabolism has been alterd from allantoin to uric acid due to loss of Uox activity during hominoid evolution. I have focused on the *Uox* and *Xor* genes, which are directly involved in uric acid metabolism. I have investigated molecular evolutionary changes of *Uox* and *Xor* genes, and their biological implical implications.

Firstly, I have determined and compared the promoter, coding and intronic sequence of the *Uox* gene of various primate species. Although I have confirmed the previous observation that inactivation of the gene in the clade of humans and great apes has resulted from a single CGA to TGA nonsense mutation in exon 2, I have found that the inactivation in the gibbon lineage has resulted from an independent nonsense mutation at a different CGA codon in exon 2, or from either one base deletion in exon 3 or one base insertion in exon 5. This is contrary to a previous claim that suggests that the cause is a 13 bp deletion in exon 2. I have also found that, compared to other organisims, the primate functional *Uox* gene is exceptional in terms of usage of CGA codons which are prone to TGA nonsense mutations. Nevertheless, I have demonstrated a rather strong selective constraint against nonsynonymous sites of the functional *Uox* gene and argued that this observation is consistent with the fact that the *Uox* gene is unique in the genome and is evolutionarily conserved not only among animals but also among eukaryotes. A further observation has indicated that there are a few substitutions in the *cis*-acting element or CAAT-box (or both) of primate functional Uox genes, which may explain the lowered transcriptional activity. I have therefore suggested that although the inactivation of the hominoid Uox gene is caused by independent nonsense or frameshift mutations, the gene has taken a two-step deterioration process, firstly in the promoter region and secondly in the coding region during primate evolution.

Secondly, I have examined molecular evolution of the *Xor* gene in relation to *Uox* inactivation. It is known that Xor activity is lower in humans than in other mammals, including rats and mice. The coding sequences (around 4 kb) of one human, six mammals and one chicken were retrieved from GenBank. From these sequences I have examined lineage-specific amino acid substitutions and estimated the degree of functional constraints by the maximum parsimony method. There are no amino acid substitutions that are responsible for the lowered Xor activity in hominoids. I have therefore determined and analyzed the promoter sequences (around 900 bp to 3.7kb) of the Xor gene of one human, one chimpanzee, one pygmy chimpanzee, one gorilla, one orangutan, one white-
handed gibbon, siamang, three Old World monkeys, New World monkeys, two prosimians, one tupaia and four other other mammals. It is found that a promoter module of activator protein 1 (AP-1) and glucocorticoid receptor (GR), predicted by an *in silico* approach, is present in most of the hominoids examined but absent in other species. The promoter module may render the *Xor* gene down modulated by interacting AP-1 and GR. In addition, I have found two independent substitutions caused by T to G transversions in repressor elements of the *Xor* gene, an E-box

with the consensus sequence GTTTC. One substitution is in the white-handed gibbon lineage and the other is in the lineage leading to the great ape clade. The presence of these repressor elements correlates well with reduced transcriptional activity of the *Xor* gene.

Finally, I have found that *Xor* gene repression may have occurred at least once in mammals and twice in hominoids The TATA-like element that appeared at the ancestral node of the tupaia, pig and cow is conserved. This observation indicates that the initial downregulation of the hominoid *Xor* gene occurred in the ancestral species of mammals. In hominoids, repression of the *Xor* gene may be directly related to Uox inactivation since nucleotide substitutions of the *Xor* gene, which form new TF binding sites, were observed just before (AP-1 and GR) and after (E-box and GTTTC) the time when Uox lost its enzymatic activity. These results suggest that molecular coevolution of the Uox and Xor genes began during mammalian evolution by lowering the expression level rather than by functional change of the protein. The molecular coevolution successively occurred due to loss of enzymatic activity and repressed transcriptional activity in hominoids.

From these findings, I have proposed the following biological implications of coevoluving the *Uox* and *Xor* genes (1) regulation of uric acid concentrations in blood (uric acid homeostasis) and (2) Contribution of molecular coevolution of antioxidant defense system in hominoids. Moreover this kind of molecular coevolution explains how metabolic pathways evolve among closely related species. Because the protein-coding region of genes which involve in various metabolic pathways is very well conserved for humans and mice, species-specific features of metabolism are unlikely to be found by comparing only the protein-coding regions. Thus, (3) molecular coevolution at the level of expression may play an important role as an evolutionary force in the metabolic pathway.

Such molecular coevolution in the purine metabolic pathway stems from evolutionary changes in protein and gene expression through mutation in the genome. This pattern may be generalized in other metabolic systems and provide a clue of comprehending hitherto unrevealed phenotypic evolution. I believe that molecular coevolution is a reflection of organismal history in changing environments.

論文審査結果の要旨

本学位論文は、代謝系の歴史的変容とその機構の解明を主眼として行ったものである。代謝系の変容過程とその進化機構については未知な点が多い。このことを改名するには、代謝系の表現型が異なる生物種間の比較が有効であるが、その一つとしてプリン代謝系が挙げられる。DNA や RNA の基本骨格となるプリン体の代謝関連酵素は、生物種により独立に欠損し、最終代謝物が異なっている。

本研究では、類人猿において尿酸酸化酵素（Uox）が不活性化したため、プリン体の最終代謝物は尿酸であるという表現型に焦点を当てた。類人猿以外の哺乳類は Uox の活性を有するので最終代謝産物はアラントインである。このような表現型の違いをもたらした要因や意義を明らかにするために、尿酸をアラントインに分解する Uox 及びヒポキサンチン、キサンチンから尿酸を産生するキサンチン酸化還元酵素（Xor）に注目した。ヒトの Xor 遺伝子の発現レベルはマウスと比較して 100 倍低いという観察事実に基づき、ヒトを含む類人猿における Uox の不活性化との関連性を予想して、塩基配列決定・比較及び分子進化学的解析を行った。

その結果、類人猿における Uox の不活性化過程は段階的であることが明らかとなった。具体的には、9 種の類人猿の共通祖先におけるプロモーター領域の変異に伴う遺伝子発現の低下と、大型類人猿と小型類人猿の進化過程においてコーディング領域に独立に生じた変異による酵素の不活性化である。次に、Xor 遺伝子の進化過程を Uox 遺伝子の不活性過程との関連で究明した。Xor 遺伝子の場合、タンパクコーディング領域には類人猿における機能変化に関わる変異は観察されない。しかし、プロモーター領域の研究から、Xor 遺伝子の発現低下が Uox 遺伝子と同様に段階的に生じたことが明らかになり、霊長類の進化の過程における Uox 遺伝子と Xor 遺伝子の分子共進化は、タンパクの機能変化よりもむしろ遺伝子発現レベルの変化に起因することが示された。

本論文では、これらの知見を元にして、遺伝子レベルの進化と表現型レベルの進化を結ぶメカニズムについて考察されている。ここで得られた多くの知見は新しいものであり、今後もこれを元にした研究の更なる発展が期待できる。

以上の評価より、本論文の内容は博士（理学）に十分に値するものであると判定した。なお、本学位申請論文の内容に関する 1 篇の原著論文（申請者は筆頭著者）が、国際学術誌である Mol.Biol.Eyol.に既に掲載されている。