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The tempo and mode of mammalian sex chromosome evolution

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Abstract

Humans and other mammals have a chromosomal system of sex determination. Although the X and Y are quite different in size and gene content, they do share some homologous genes. Nineteen X-Y homologous gene pairs could be categorized into four distinct groups (strata 1 to 4) based on the extent of synonymous sequence divergence (k_s). The mammalian amelogenin (*AMEL*) genes are shared by X and Y chromosomes. They are gametologous and are designated as *AMELX* and *AMELY*, respectively. It is also known that the human *AMELX* is located near the boundary between strata 3 and 4.

I confirmed that both *AMELX* and *AMELY* are single copy genes in non-human primates and located on the X and Y chromosome, respectively, by using fluorescence *in situ* hybridization (FISH). Furthermore, I compared 20 kb human BAC clones that encompass the *AMELX* and *AMELY* loci. I found that although the downstream region from intron 2 exhibits about 10 % sequence differences per site (*p*-distance), the upstream region exhibits higher level (p > 20%). This observation suggests that the human *AMEL*s span the boundary of strata 3 and 4. I determined the genomic sequences of *AMELs* in five primates, two Artiodactyla and one Perissodactyla to examine their boundary region. Comparisons of these genomic *AMEL* sequences reveal that the *p*-distances (the numbers of nucleotide differences per site) are significantly greater in the 5' portion from intron 2 than those in the 3' portion. The observation is the same as that in the case of the human *AMEL*s. Furthermore, the phylogenetic analysis of gametologous *AMEL*s shows their contrasting clustering patterns in different regions. I therefore concluded that the boundary between strata 3 and 4 in the mammalian X chromosome also lies within *AMEL* intron 2.

To investigate causes and mechanisms of creating evolutionary strata, I examined the human X and Y chromosome sequences which encompass strata 3 and 4 as well as the pseudoautosomal region 1 (PAR1). To this end, I retrieved the sequences of the short

arm of the human X chromosome (22 Mb) and aligned them with the Y homologous sequences. Because of extensive sequence changes in the Y chromosome, I could align only one fifth (544 Kb) of the total X chromosome sequences. It turned out that the X chromosome sequences could be divided into eleven segments (region I to XI) that ranges from the PAR1 to the region containing *ZFX* in stratum 3 on the X chromosome. These eleven segments correspond to fourteen segments dispersed on the Y chromosome. For each of these homologous regions, I examined the pattern and degree of nucleotide differences. Results revealed that the *p*-distances are < 0.1% in the PAR1 and increase abruptly to 10% in the middle of the *XG* locus that encompasses the pseudoautosomal boundary 1 (PAB1). The *p*-distances remain around 10% in region II through the distal part of region IX, and then abruptly increase again to >20% at intron 2 of *AMEL* and in regions X and XI in stratum 3.

To elucidate any features common to the PAB1 and the boundary between strata 3 and 4, I investigated if there are any sequence motifs that are shared by these boundaries. To this end, I analyzed the above eleven X chromosome segments in terms of GC content, AT and GC skews, and the resolution sequences for Holliday intermediates. Although in both regions I and IX, the *p*-distances increase sharply, no common sequence motifs were found. It therefore seems difficult to attribute these sequence motifs or the primary nucleotide sequence information to any signal for the formation of stratum boundaries.

Based on these results, I discussed the dating of sex chromosome differentiation, the roles of chromosomal inversion, the origin of evolutionary strata on the human sex chromosomes, and the tight linkages of male-determining genes as causes of the recombination suppression.

To date the emergence of strata 3 and 4, Jukes-Cantor distances (*d*-distances) were used to reconstruct the NJ tree. I estimated that the emergence of stratum 3 occurred at

88-117 million years ago (mya) based on *AMELX* and 76-102 mya based on *AMELY*. It therefore appeared that the mammalian *AMELX*s and *AMELY*s began to differentiate from each other almost immediately after they were translocated from an autosomal region. The boundary spanning the *AMEL* locus is an ancient PAB and was formed in the stem lineage of ancestral mammals. Furthermore, FISH results indicated that both X and Y chromosomal rearrangements have also occurred in the primate lineage. Moreover, it was apparent that the order of eleven human X chromosomal regions is substantially different from that of fourteen human Y chromosomal regions. Nevertheless, there were several evidences suggesting that inversions could not be a cause of strata.

On the other hand, for the stratum 4 differentiation, I showed that genes or regions containing PAB1 are different from species to species. Those genes straddle evolutionary strata 4 and PAR1. It thus seems that the PAB1 was determined independently in diverse mammalian lineages. This relatively recent and independent emergence of stratum 4 is reflected in a wide range of sequence divergences (8 to 15 %) exhibited in *AMEL* 3' region. In fact, I came to the conclusion that the emergence of stratum 4 initiated 27~70 mya, depending on different mammalian lineages.

I have found that the ancient PAB between strata 3 and 4 is the same among different mammals and interpreted this finding as the common ancestry of stratum 3. In other words, it was formed in the common ancestor of eutherian mammals. On the other hand, the present PAB was determined independently in diverse mammals. I therefore argued that both ancient and present PABs were determined by chance events during the evolution of mammals and primates.

However, I have failed to find molecular mechanisms that were responsible for stepwise suppression of homologous recombination in the mammalian sex chromosomes. It is likely that the suppression was caused by some mechanisms

working at the higher level than at the molecular level. Population genetics theory suggested that one such cause is the necessity of tight linkage between male-determining genes on the Y chromosome.

ヒトの染色体は２２対の常染色体と１対の性染色体からなる。男の場合、性染色体はＸとＹからなり、その大きさや遺伝子組成は大きく異なる。しかしＸとＹ染色体が一対の常染色体から進化してきたことは、相同遺伝子を共有することからも明らかである。ただ、常染色体と異なるのは、それら相同遺伝子同士の配列に違いがあることである。Lahn and Page(1999)は、ＸとＹの相同遺伝子間における同義置換の程度から、それらを４つの階層（領域）に分けた。それによると同義置換率が高い順に４つの領域に区分でき、それらは減数分裂期における相同遺伝子間の組み換えの抑制が起こった時期によって説明できるとした。つまり、最も古く抑制された領域では置換が多く蓄積され、順次段階的にその程度が低下するとした。またこの抑制の原因がＹ染色体で起こった逆位にあると推察した。

第４階層に属する遺伝子の中で amilogenin 遺伝子（AMEL：歯のエナメル質構築タンパク）は最も第３階層寄りにある。本論文は、AMEL のゲノミック配列をＸとＹとで比較したところ、遺伝子の中に階層間の境界点があることを見いだした。つまり、第２イントロンの上流側は第３階層に属し、下流側は第４階層に属していた。その境界点のシャープさは、塩基配列レベルでピンポイントできる程であった。このことは、もし組み換えの抑制が逆位だとしても、この境界点そのものが逆位した点であるとは考えにくいことを示している。この発見が本論文の出発点である。

この境界部が哺乳類共通であることは５種の霊長目、２種の偶蹄目と１種の奇蹄目の AMEL のゲノミック配列を決定・解析した結果から明らかになった。さらに、ヒトゲノムで決まった配列を利用して、現在もＸとＹが対合し、組み換えを行う偽常染色体領域（PAR1 と呼ぶ）から ZFX までの広い領域（22MB）を解析した。その結果、性染色体分化過程において段階的に相同組み換えの抑制が起きており、その境界部が AMEL の intron2 と PAB1 であることが明らかになった。しかし、１次配列レベルにおいて AMEL の intron2 内に位置する境界と PAB1 に共通点を検出できなかった。

以上の結果より、第３階層の組み換えの抑制は哺乳類が分岐する以前に起こったと考えられた。一方、第４階層と PAR1 との境界線（PAB1）は哺乳類の種によってそれぞれ異なっており、比較的最近それぞれの系統において組み換えの抑制が起こり、X、Y に分化したと考えられた。さらに集団遺伝学的考察から、組み換え抑制の末端（境界）は偶然によって決定され、段階的な抑制は清家って遺伝子の連鎖強度により生じたことが示唆された。

最後に、ヒトゲノムで決まった配列を利用して、PAR1 から PAB1、さらに AMEL より３階層領域までの広い領域について解析し、Ｘ と Ｙ とのジェノミック相同性部分においても階層が成り立つことを明らかにした。ただこのような抑制と領域との整然とした関係はヒトだけに成り立つらしいことは、近縁のサルの AMEL のＸ染色体上での部位がヒトの部位と異なる事から示唆された。

以上のように、この論文の内容は性染色体の組み換え抑制に関する新たな構造的特徴とその進化的位置づけをしたものであること、またこれらの成果は、１報はすでに国際誌に発表され、２報目もすでに国際誌に受理されていることなどから、総合的に見て生命体科学専攻の博士論文として十分値する内容を有していると審査員全員が認めた。