氏　　名	磯部　拓
学位（専攻分野）	博士(理学)
学位記番号	総研大甲第463号
学位授与の日付	平成１２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	マウスＭＨＣクラスII領域における減数分裂期組換えのホットスポットの遺伝学的解析
論文審査委員	主　査　教　授　小川　智子 教　授　池村　淑道 教　授　佐々木　裕之 教　授　松田　洋一（北海道大学） 助教授　今井　弘民（国立遺伝学研究所）

Abstract

In mammals, the mouse major histocompatibility complex (MHC) is the only region where breakpoints of meiotic recombination are systematically studied at the molecular level. In the class II region of the MHC, meiotic recombination does not occur at random but are clustered in limited regions known as hotspots. Outside of the hotspots, where recombination scarcely occurs, is called cold regions.

Thus far, four hotspots have been identified in the MHC class II region. The presence or absence of a hotspot depends on the MHC haplotype involved in genetic crosses used to detect recombination. Among the four hotspots, three hotspots have been well characterized at molecular level and their nucleotide sequences were determined. Comparison of the sequences indicated several molecular motifs, such as middle repetitive sequence of MT-family, a sequence similar to a transcription factor binding motif and TCTG or CCTG tetramer repetitive sequence, are commonly observed in the three hotspots. It was also reported that the hotspot regions are not always hypersensitive sites to endonuclease DnaseI (Mizuno et al. 1996), suggesting that chromatin structure of the hotspots is not open. Despite studies over almost 15 yeas since the discovery of the hotspots and findings mentioned above, the mechanism by which the recombinations in this region are restricted to the hotspot is not still clear.

In this study, I attempted to address this question by combining of molecular genetical approach and a recently developed cytogenetical approach. First, I carried out the molecular genetical analysis to reveal the structure of the hotspot in the vicinity of *Pb* gene, which has been left untouched for a long time. The result of this study indicated that the breakpoints of the recombinations are confined to a very short segment of DNA as seen in other three hotspots. Subsequently, comparing the sequences of all four hotspots, I tried to find general features of their molecular structure. From this analysis, I found that all four hotspots were located either in introns or at 3' end of genes, but not at 5' end of genes.

In addition, I searched molecular motifs shared by the hotspots in more systematic way. To do this, I surveyed a 1.3Mb nucleotide sequence of the whole MHC class II region, which was recently deposited to database, as well as sequences of the all four hotspots. As a result, it appeared that *Eb*, *Lmp2*, *Pb* hotspots have MT family sequences, and that all four hotspots have a octamer

transcription factor binding motif designated as O motif, B motif-like sequences that is similar to another transcription factor biding motif and the tetramar repetitive sequence. The direction of the O motif and the B motif-like sequences is opposite. The distance between these two motifs is within 300bp in all four hotspots. The tetramer repetitive sequences are located in DNA segments less than 1.2kb in length, which contain the O motif and the B motif-like sequences as well. It is notable that these motifs are also found in a hotspot identified in the mouse MHC clasIII region, as well as a hotspot identified in the vicinity of *TAP2* gene in the human MHC class II region. All these findings contrast the well established characterizations of the hotspots in budding yeast, *Saccharomyces cerevisiae*. In budding yeast, their hotspots are located in promoter regions at the 5' end of genes, which are overlapped with hypersensitive sites to endonuclease DNase I and MNase. Any consensus sequencec shared by the yeast hotspots are known.

In meiosis, chromatin forms special structure, which shows dynamic changes during the stages of meiosis. In the early stage, sister chromatids remain associated with each other (zygotene to pachytene stage). Then, two copies of homologous chromosome behave in a coordinated fashion. Homologous chromosomes must find their proper partners and physically pair with them along their length. In most eukaryotic organisms, this pairing is accompanied by formation of a proteinaceous structure known as the synaptonemal complex (SC). SC consists of the core region and chromatin loops that are anchored to the core region.

It is established that RecA homologs, Rad 51 and Dmc1 proteins, play central roles in the meiotic recombination. In mouse, both of Rad51 and Dmc1 are observed on the chromatin from the leptotene to the zygotene stage (Ikeya et al. 1997). In the zygotene stage, the protein complex known as early recombination nodule is observed on the inside of the two axial elements. In the pachytene stage, Rad51 protein is localized along the core of SC, and the late recombination nodule, which is protein complex larger than the early recombination nodule, is observed exclusively along the core of the SC. At the stage of segregation of the SC (diplotene to diakinesis stage), chiasmata that are stable connections between homologs and are thought to be cross over points can be seen. Then chiasmata are disassembled and recombination intermediates are resolved. Number of the late recombination nodules is almost equal to chiasmata, implying that the sites of the late recombination nodule are cross over points. This is also supported by the observation that the Rad51 proteins localized on the late recombination nodules along the core of SC.

Previous reports suggested that special sequences play a role in pairing homologous chromosomes in meiosis. For example, pairing of the X and Y chromosomes in *Drosophila melanogaster* is mediated by the rDNA repeats, which are present in two tandem clusters, one in the centromeric X heterochromatin and the other near the teromere of the short arm of the Y chromosome. A block of the tandem repeats on the X chromosome interacts with a similar block on the Y chromosome. Analysis of flies containing transgenic insertions of the rDNA has demonstrated that a 240bp sequence located in the intergenic spacer region of the rDNA is necessary and sufficient for the pairing (Ren et al. 1997). This report indicates that special sequences act to determine the pairing sites of homologous chromosomes.

If, in deed, these special sequences are able to initiate chromosome pairing, it is possible that regions containing such sequences become recombination hotspots. The hotspots in the mouse MHC are not found in promotor regions of 5' end of genes, where in budding yeast double-strand breaks (DSBs) of DNA occurs and recombination reaction initiates. This fact also supports the idea that unlike budding yeast, the locations of the mouse hotspots are determined by the specificity of chromosome pairing sites but not of initiation sites of recombination reaction by DSBs. If this is the case, it is expected that the mouse hotspots DNA tends to be localized closer to the SC core than cold region DNA in the pachyten stage. In this study, in order to examine this possibility, I carried out cytogenetical analysis to observe topological locations of the hotspot and the cold region in relative to the SC structure. Fluorescence in situ hybridization (FISH) with DNA probes for the *Lmp2* hotspot and the cold region and immuno-staining with antibody for the SC core demonstrated that the hotspot signals localize closer to the SC core than the signals of the cold region. This result indicates that mouse chromatin loop is not randomly attached to the SC core and the possibility that the hotspot region is specifically used as a landmark in the chromosome pairing, which determines the site of recombination.

論文の審査結果の要旨

遺伝的組換えの研究は、組換えが決まった領域で高頻度で起こる事を示してきた。その領域を組換えのホット・スポットと呼ぶ。このホットスポットが形成される理由として、次の2点が考えられている。その一つは、組換え酵素が、DNA や染色体の特定の構造を認識して結合し、組換えを開始する場合で、組換えは決まった位置から始まる。この機構は、大腸菌の RecBC や出芽酵母の Mre11 の研究で証明されている。他方は、減数分裂期のディプロテン期の染色体上で観察されるキアズマ構造である。キアズマは、相同染色体の対合が、まさに分離する点であるが、その分離が組換え中間体の解離と考えられている。

マウスの組換えのホット・スポットで、最も良く研究されているのは、MHC クラス II 領域にある4ヶ所のホット・スポット(Lim2、Eb、Ea と Pb)であるが、前記の3ホット・スポットについては塩基配列が決められている。

磯部拓君は、先ず、両親の Pb 領域とこの領域の組換え体6個の DNA 塩基配列を決定して組換え点を明らかにした。その結果と他の3ヶ所のホット・スポット領域の塩基配列を、ホット・スポット以外の配列と比較した。その結果、ホット・スポット領域には、オクタマー転写因子結合配列、B-モチーフ様配列、4塩基の繰り返し配列が共通に存在し、その位置関係にも規則性のあることを見いだした。しかし、いずれの組換え位置も、遺伝子のイントロンの内部か3'側に位置していた。また、ホット・スポット領域に含まれる ORF の転写産物は Northern 解析で検出できなかったので、この領域内の ORF は転写されず、転写と組換え体形成は、直接関係しないと結論した。

次に、減数分裂期染色体のコアを蛍光抗体法で染色した後、Lim2 ホット・スポット領域をプローブとして FISH を行った。　その結果、コントロールとして用いたホット・スポット領域以外の DNA は、染色体のコアから離れて位置するクロマチンループ上にあったが、Lim2 領域は、シナプトネマ複合体のコアの上か、離れてもその近傍に存在することを示した。磯部君は、以上の結果をまとめて次の様に考察した。

マウス減数分裂期の相同染色体の対合は、オクタマー配列、B-モチーフ様配列、4塩基からなる繰り返し配列などに、特定な蛋白質が結合し、それらの蛋白質を介して相同染色体の検索が行われる。これらの結合サイトは組換え開始前は、染色体の Axial element 上にあるが、相同染色体が全領域で対合したシナプトネマ複合体では、そのコア上に位置するので、相同性検索に用いられた対合部位が組換えのホット・スポットとして観察されるのではないかと考えた。

磯部君は、両親由来の MHC クラス II 領域の塩基配列を決定し、組換え体マウス染色体上の数塩基の配列の違いを検出した。また、染色体の蛍光抗体染色と FISH を同時に行って、組換え部位を染色体上に決定した。これらの結果は、高等真核生物のこの分野の理解に多大な貢献をすると判断され、審査委員全員の一致で、理学博士の学位論文として、充分な内容を持つと結論した。