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学位論文題目	Molecular structure of recombinational hotspots in the mouse ＭＨＣ
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論文内容の要旨

It was believed that genetic lengths deduced from recombination frequencies are proportional to the physical length between two marker genes. However, a recent advance in linkage analysis has shown that recombination frequencies along the human and mouse chromosomes are by no means constant. For example, meiotic recombinations in the proximal region of the mouse major histocompatibility complex (MHC) on chromosome 17 are clustered at certain segments, termed hotspots. At present, it is known that there are four hotspots in this region. Recombination at these hotspots is dependent on the MHC haplotypes of mice used in crosses. Of the four hotspots, recombination at a hotspot, which is located between the *Pb* and *Ob* genes, occurs in crosses including *wm7* or *cas3* haplotype, derived from Asian wild mice. Recombination frequency as high as 2.0% was observed at this hotspot and the breakpoints of the recombinants were clustered in a short DNA segment of 2.0 kb. However, it is poorly understood why meiotic recombinations take place in the restricted regions and in crosses including limited MHC haplotypes.

In order to reveal the molecular basis of site-specificity of hotspots in the proximal region of the mouse MHC, the author first characterized the fine molecular structure of a hotspot located in the *Pb/Ob* interval. The author constructed a fine restriction map of a 20 kb segment containing the hotspot and determined the sequence of a 7.25 kb segment from the hotspot toward the distal end. The hotspot was found to be located very close to the 3' end of the gene for low molecular mass polypeptide-2 (*Lmp2*) , a subunit of a proteolytic proteasome, and was designated as the *Lmp2* hotspot. In a lower eukaryote such as budding yeast, *Saccharomyces cerevisiae*, most of meiotic recombinational hotspots are known to be located at the 5' end of genes, which are potential promoters. It was notable that two mouse hotspots characterized at molecular level are located outside of potential promoter regions.

There are a number of reports suggesting that transcription potentiates homologous recombination. These reports imply that transcription remodels the chromatin into a form that is more accessible to the recombinational machinery, with resultant initiation of recombinational events. However, there are also conflicting reports that indicate the absence of direct correlation between recombinational activity and level of transcription. To determine whether the transcriptional level of the *Lmp2* gene has any influence on recombination activity at the *Lmp2* hotspot, the author examined the transcription of this gene. It was found that the gene is transcribed in spleen cells but not in testicular cells where meiotic recombination occurs, indicating that the level of the transcript has little significance for recombination at the *Lmp2* hotspot.

In *S. cerevisiae*, there is a close correlation between the location of DNase I-hypersensitive sites (DHSSs) and the distribution of recombinational hotspots.

DHSSs are colocalized with double-strand DNA breaks (DSBs) which initiate most meiotic recombination. It has been reported that DSBs are established before entry of cells into meiosis and DHSSs are a prerequisite for formation of DSBs. A recent report indicates that DHSSs associated with hotspots are preserved constitutively both in somatic and meiotic cells in *S. cerevisiae*. It is likely that DSBs occur at sites at which nucleosomes are disrupted and the DNA is accessible to the enzymes involved in the recombination. In this context, the chromatin structure around the hotspots in the mouse MHC was analyzed. Although it has been reported that there are DHSSs within the *Eb* hotspot which is located in the second intron of the MHC class II *Eb* gene during meiotic cells, the contribution of chromatin structure to hotspot activity is still controversial in the mouse.

The author extended the study in more detail to reexamine the correlation between the chromatin structure and hotspot activity, and therefore focused on two hotspots, *Lmp2* hotspot, and the *Eb* hotspot. The author analyzed the chromatin structure around the two hotspots, by monitoring DNase I-hypersensitivity of chromatin prepared from both somatic cells and testicular cells in meiotic prophase I. As results, DNase I-hypersensitive sites (DHSSs) were detected at both hotspots in the somatic cells, but not in the meiotic cells, although prominent DHSSs in meiotic cells were observed at the proximal region of the *Lmp2* hotspot. These results contrast with those obtained in *S. cerevisiae*, in which meiotic recombinational hotspot are predominantly associated with constitutive DHSSs.

In these experiments, the author used two different crosses, one is associated with a high frequency of recombination at the *Lmp2* hotspot, and another is not associated with recombination at the same hotspot. If the structure of the chromatin influences recombinational activity at the hotspot, one might expect a difference in patterns of DHSSs between these two strains. It was found, however, that the pattern of the DHSSs in two different crosses was almost identical. This result confirmed that chromatin structure, as monitored by hypersensitivity to DNase I, is not responsible for determining recombinational activity at the hotspots.

Thus, the present study suggests that neither level of transcription nor the state of DNase I-hypersensitivity of chromatin plays a key role in determining the sites at which meiotic recombination is initiated in the proximal region of the mouse MHC, unlike the case in the lower eukaryote, *S. cerevisiae*.

論文の審査結果の要旨

マウス主要組織適合抗原複合体（ＭＨＣ）のクラスII領域には、減数分裂期の組換え高発部位（ホットスポット）が４つ存在し、それら以外の領域では、ほとんど組換えを起こさない。水野君はまずPb-Ob 遺伝子間（約１５０kb）にあるホットスポットの高次構造の解析を行い、このホットスポットがLmp2 遺伝子の３’側に位置し、組換えは約２kbの非常に狭い範囲に限定されることを示した。このホットスポットをLmp2 ホットスポットと命名し、これを中心的対象に、組換えの部位特異性を決定する分子メカニズムの研究を行っている。

酵母では、ほとんどのホットスポットが遺伝子の５’側にあり、DNase I 高感受性部位に集中して存在している。遺伝子の転写活性が上がることとホットスポットの組換え頻度に密接な関係が存在することも報告されている。水野君は、これらの酵母における知見に基づき、Lmp2 遺伝子の発現とLmp2 ホットスポットの関連性を調べたところ、Lmp2 の発現は直接的にはLmp2 ホットスポットの組換えに関与していないとの結論に達した。DNase I 高感受性部位についての解析も行なっており、体細胞においてはLmp2 ホットスポット内にDNase I 高感受性部位が検出されたが、組換えが起こっている減数分裂前期細胞ではホットスポット内にDNase I 高感受性部位は検出されなかった。興味深い点として、Lmp2 ホットスポットよりセントロメア側に複数の強いDNase I 高感受性部位を検出している。Lmp2 ホットスポットで高頻度組換えを起こす系統と起こさない系統でDNase I 高感受性部位のパターンに有意な差は見いだされていない。これらのマウスの解析結果は、酵母で得られていた結果とは大きく異なっており、単細胞生物である酵母と多細胞生物である哺乳類とでは減数分裂期における組換え機構が異っているとの結論を得ている。哺乳類のみならず、高等植物を含む高等多細胞生物での減数分裂における組換え機構を解明する上で貴重な知見と言える。

本論文の研究は指導者の指導の下に、申請者自身が主体的に計画を立て実施されたものであり、その成果は学術的に価値のあるものと判断された。また、申請者の研究者の独自性と学識及び語学力を認めることができた。したがって、本論文は学位の授与に適切なものと判断した。