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Meiosis is a specialized cell division essential for sexual reproduction. It consists of two successive rounds of chromosome segregation following a single round of DNA replication. Prophase of the first meiotic division (prophase I) involves highly complex series of events to establish the exchanges of genetic materials between homologous chromosomes, so-called crossovers (COs). Synapsis is one of characteristic events of prophase I, in which homologous chromosomes are tightly connected along their entire length via a zipper-like proteinaceous structure, the synaptonemal complex (SC). Transverse filament (TF) protein is a major component of SC central region, which bridges the axes of homologous chromosomes. TF protein is structurally conserved in various organisms and suggested to be important for synapsis and CO formation, but the exact role and functional conservation of TF proteins are still not completely understood. In most organisms, meiotic COs are non-randomly distributed along chromosomes, such that they are more widely spaced than predicted for a random distribution (so-called positive CO interference), but it is poorly understood how this CO distribution is established. SC has long been speculated to be involved in positive CO interference, but such function of SC has yet been demonstrated

At the beginning of this study, none of the structural component of SC central region was identified in monocot plants. Moreover, the *Arabidopsis* TF protein ZYP1 was the only the component of SC central region identified in plant kingdom, but its functions have been limitedly studied. In this thesis, I describe the identification and functional characterization of *OsZYP1*, the rice (*Oryza sativa*) ortholog of *Arabidopsis ZYP1*. *OsZYP1* gene encodes a long coiled-coil protein showing a structural similarity to TF proteins of other organisms and is strongly expressed in meiotic flowers. Immuno-localization study showed that OsZYP1 exhibits a thread-like localization along the length of synapsed chromosomes in prophase I, consistent with a role as a structural component of SC. These results strongly support the idea that OsZYP1 functions as the TF protein of rice. Analysis of *oszyp1* null mutants revealed that OsZYP1 is essential for normal meiosis. In the absence of OsZYP1, SC is not formed and homologous chromosomes are not synapsed. Despite the lack of synapsis in *oszyp1*, homologs are still loosely aligned and COs are successfully formed between them, resulting in a normal number of bivalents. Nevertheless, some chromosomes are abnormally segregated in the following meiotic divisions, thereby resulting in partial sterility. I also found that

OsZYP1 regulates the localization of a conserved HORMA domain protein PAIR2. PAIR2 is localized along the length of chromosome axes before synapsis initiation but is depleted from the axes where OsZYP1 is loaded as synapsis progresses. On the contrary, PAIR2 is not depleted from chromosome axes in the *oszyp1* mutant, indicating that OsZYP1 directly or indirectly removes PAIR2 from the axes. A mutation analysis of the gene for meiotic recombination protein OsMSH4 suggested that COs in *oszyp1* are formed through normal recombination pathway. Bivalent number is severely reduced oszyp1 *osmsh4* double mutant as in *osmsh4* mutant, indicating that many of COs in *oszyp1* is OsMSH4-dependent COs as in wild-type. I further analyzed the recombination in OsZYP1 by a genetic method using polymorphic markers between two rice cultivars. This confirmed that OsZYP1 is dispensable for CO formation, but intriguingly revealed that it is essential for normal CO distribution. In the *oszyp1* mutant, positive CO interference seen in wild-type is completely abolished and instead COs tend to show negative interference (a tendency of clustering of COs). This result suggests that OsZYP1 regulates CO distribution so that adjacent COs do not occur close to each other. The findings of this study provide evidence for a link between SC and positive CO interference.

博士論文の審査結果の要旨

減数分裂の過程で、相同染色体は synaptonemal complex（SC）と呼ばれる構造を介して結合する。SC は種間で保存されており、多くの種で、SC 内の transverse filament (TF) と呼ばれるタンパク質が交叉現象に必要であることが示されていた。一方、減数分裂期における染色体の交叉はランダムにおこらず、ある部位での交叉が隣接する領域の交叉を抑制するように働く（干渉）ことが多くの生物で知られている。SC が干渉に関与すると推察されてきたが、直接示した例はなかった。

米田君は、シロイヌナズナの TF タンパク質 ZYP1 との類似性からイネのホモログ OsZYP1 を同定し、抗体作成やイネの遺伝子破壊系統作成を通じて、その機能を解析した。OsZYP1 タンパク質の局在を免疫染色法によって調べた結果、対合した染色体にそって分布することが明らかになった。また、*OsZYP1* 遺伝子の破壊系統では、相同染色体は対合しなかった。ただし、相同染色体どうしが少し離れた状態で緩く並んでおり、交叉も起こっていた。その後の減数分裂では、染色体の分離がしばしば異常となり、稔性が低下していた。また、対合期の染色体からの PAIR2 タンパク質の消失が、*OsZYP1* 破壊系統では起こらなかった。減数分裂期の組換えに関与するタンパク質である OsMSH4 の遺伝子機能が欠損した条件下でも、*OsZYP1* の変異は交叉頻度に影響しなかった。

他の生物の TF 関連遺伝子の欠損変異体では交叉が起こらないのに対して、*OsZYP1* 破壊イネでは交叉が起こったのは興味深い。さらに、SC の組換え干渉に対する効果を知るため、イネ系統間の多型を用いて、組換え頻度を調べた。その結果、野生型で見られるような正の組換え干渉が *OsZYP1* の変異体では見られなくなっていた。

これらの研究は、SC 形成と交叉、およびその分布に関して新たな知見をもたらすものである。特に、イネの TF 変異体が稔性を持つことを利用し、交叉と SC の関係という大きな問題の理解に貢献した。審査員全員で審査した結果、本大学院における学位授与の水準を十分に満たす論文であると判断した。