（別紙様式第３号）

学 位 論 文 要 旨

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題目: The roles of homeodomain proteins during the clamp cell formation in a bipolar mushroom, *Pholiota nameko*
(二極性担子菌 *Pholiota nameko* のクランプ形成におけるホメオドメイン蛋白質の役割に関する研究)

Mating is an essential step in the life cycle of sexually reproducing organisms. In the basidiomycete mushrooms, mating compatibility is controlled by one or two sets of multiple allelomorphic genes known as bipolar or tetrapolar mating systems, respectively. The mushroom *Pholiota nameko* (Strophariaceae) is known to carry a bipolar *A* incompatibility locus. In the previous study, the *P. nameko* genes encoding the homeodomain protein *hox1* were sequenced and characterized. Restriction fragment length polymorphism (RFLP) and linkage analyses indicated that only *hox1* is involved in regulating incompatibility in *P. nameko*. However, the composition of *A* mating type locus and how the homeodomain protein genes control clamp cell formation were still unknown before this study. In this study, chapter 2 described the genomic structure of *A* mating type in *P. nameko*; chapter 3 showed the exploit of the transformation system in *P. nameko*; in chapter 4 the roles of homeodomain proteins during the clamp cell formation in vivo were investigated; chapter 5 showed the conclusions.

In chapter 2, using gene walking technique, the flanking DNA sequence of *hox1* including upstream sequence and downstream sequence was investigated in *P. nameko*. A second homeodomain protein *hox2* was discovered upstream of *hox1*. Although a 39,882 bp nucleotide region containing *hox1* was amplified and sequenced, only a pair of homeodomain protein genes exist in the *A* mating type locus. Thirteen genes flanking around the *A* locus of *P. nameko* were discovered. Using GenBank similarity searches to find homologues gene in other mushrooms, eight genes around *A* locus of *P. nameko* (including *β-fg*, *mip, up11, up10, up2, up8, sec61, glydh*) have homologues genes around *A* locus in the bipolar mushroom *Coprinellus disseminatus* and the tetrapolar mushrooms *Coprinopsis. cinerea* and *Laccaria bicolor*. Moreover, the order and transcription of these genes were very similar in these four species. Although four other genes (including *mmsd*, *lmwppp*, *hp1*, *amtp)* in *P. nameko* were not discovered around *A* locus in other three species, they were found upstream of *A* locus both in *C. cinerea* and *L. bicolor*. Analysis of the deduced protein sequences of the homeodomain protein genes from two strains of *P. nameko* show that the putative functional domains differ from those of the homeodomain proteins of the tetrapolar mushrooms, *C. cinerea* and *L. bicolor*.

In chapter 3, to investigate whether only homeodomain proteins control mating

and clamp cell formation in *P. nameko*, a high efficient DNA-mediated transformation system was needed to constructed in this species. To further transformation of two mating types genes, two selective markers, the homologous selective marker gene (carboxin resistant gene) and a heterologous drug selective marker gene [hygromycin B phosphotransferase gene (*hph*)], were used to construct transformation system, respectively. Both of these two transformation systems we constructed have high transformation efficiency; efficiency of carboxin resistant transformation was about 88.8 transformants per μg pMBsip2 DNA using 5×10^6 protoplasts in regeneration plates containing 1.0 μg/ ml carboxin; efficiency of hygromycin B resistant transformation was about 122.4 transformants per μg pMBhph1 DNA using 5×10^6 protoplasts in regeneration plates containing 150 μg/ ml hygromycin B. The transformation efficiency is believed to do the further transformation of mating type genes.

In chapter 4, using the DNA-mediated transformation system, the functions of homeodomain proteins genes were investigated. When a single homeodomain protein gene (*A3-hox1* or *A3-hox2*) from the *A3* monokaryon strain was transformed into the *A4* monokaryon strain, the transformants produced many pseudo-clamps but very few clamps. When two homeodomain protein genes (*A3-hox1* and *A3-hox2*) were transformed either separately or together into the *A4* monokaryon, the ratio of clamps to the clamp-like cells in the transformants was significantly increased to approximately 50%. We, therefore, concluded that the gene dosage of homeodomain protein genes is important for clamp formation. When the *sip* promoter was connected to the coding region of *A3-hox1* and *A3-hox2* and the fused fragments were introduced into NGW19-6 (*A4*), the transformants achieved more than 85% clamp formation and exhibited two nuclei per cell, similar to the dikaryon (NGW12-163 × NGW19-6). The results of real-time RT-PCR confirmed that *sip* promoter activity is greater than that of the native promoter of homeodomain protein genes in *P. microspora.* So, we concluded that nearly 100% clamp formation requires high expression levels of homeodomain protein genes and that altered expression of the *A* mating-type genes alone is sufficient to drive true clamp formation.

In chapter 5, we concluded that in *P. nameko* the *A* mating-type locus comprises only a pair of homeodomain protein genes, and to drive nearly 100% true clamp formation in this species, both homeodomain proteins participated in the clamp cell formation and high expression levels of homeodomain protein genes is required. In all, in bipolar mushroom, the regulation mechanism of the homeodomain proteins existing in *A* mating-type locus became clear on genetics and molecular biology.