# 学位論文内容の要旨

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学位論文題名

**Analysis of the DNA damage signal transducer ortholog *Mop53BP1* in *Pyricularia oryzae***

（イネいもち病菌のDNA損傷トランスデューサーオーソログMop53BP1の解析）

*Pyricularia oryzae* (teleomorph: *Magnaporthe oryzae*) is the causal agent of the rice blast, the most important disease that affects rice production worldwide. To gain entry into host plant, the fungus develops a specialized structure called appressorium, which is an important step in the pathogenesis of this disease. Recently, a strong relationship between appressorium differentiation and cell cycle had been found in *P. oryzae*. An ortholog gene for p53BP1, a signal transducer protein that participates in G2-M cell cycle checkpoint in higher eukaryotes, had been identified in the genome of *P. oryzae.* Also, the deletion mutants of Mop53BP1 formed abnormal multiple appressoria per conidia and were unable to develop pathogenicity. Therefore, the main objective of this work is to clarify the importance of *Mop53BP1* during appressorium formation by means of gene expression analysis and studying the relationship of Mop53BP1 with proteins related to cell cycle progression.

1. **Expression analysis of Mop5BP1 in the presence of DNA damage agents**

To test the response to DNA damage agents and for better understanding of the physiological function of *Mop53BP1*, the expression of this gene was evaluated in wild-type strains cultured in liquid media with DNA damaging agents. qRT-PCR analyzes showed that the expression of *Mop53BP1* was low for all treatments suggesting that this gene does not have crucial role in vegetative growth and DNA double-strand break repair of *P. oryzae*.

2. **Cellular localization and expression of Mop53BP1 in *P. oryzae***

In order to visualize the location and study the expression of Mop53BP1 during the appressorium differentiation, we fused the green fluorescent protein (eGFP) to

Mop53BP1 and conducted a microscopic observation of different stages of appressorium development. Fluorescence signals were detected in nuclei regions of conidia and in the initial germ tube stage. Also, qRT-PCR analyzes revealed that *Mop53BP1* expression was highest at initial point and decreased according to appressorium development. These results suggest that *Mop53BP1* expression occurs during the first hour of appressorium formation.

**3. Overexpression of *Mop53BP1***

To study the influence of *Mop53BP1* overexpression during the appressorium formation, we replaced the native promoter by the constitutive TEF1 gene promoter region generating a TEF1-GFP-Mop5BP1 mutant. Thus, a strong fluorescence was observed in the nuclei during all the stages of appressorium formation. Also, these mutants produced normal appressorium and infection structures, suggesting that Mop53BP1 overexpression did not affect the appressorium development and that the protein localizes to nuclei during all steps of plant infection.

**4. Role of Mop53BP1 in cell cycle control**

In order to clarify whether Mop53BP1 is related to G1/S, G2/M checkpoints, we observed the appressorium formation of wild-type, *ΔMop53BP1* and overexpression mutants in the presence of DNA synthesis inhibitor hydroxyurea (HU) and microtubule inhibitor benomyl. In the presence of HU, the cell cycle progression was arrested in wild-type and deletion mutants, showing that Mop53BP1 is not participating in the DNA replication checkpoint. However, *Mop53BP1* null mutants displayed hypersensitivity to benomyl, while overexpression mutants showed some resistance to the microtubule inhibitor. These results suggested the relationship between Mop53BP1 and microtubule.

Taken together, this thesis revealed that Mop53BP1 has an important role in nuclear division and distribution in *P. oryzae*, via interaction with microtubules. In addition, this thesis first uncovered the role of microtubule in the initiation of appresorium formation. These knowledges should contribute to further understanding of appressorium formation in *P. oryzae*, which is an important target for the disease control.