

## 主 論 文 の 要 約

論文題目

Functional analysis of MADS box protein McmA, a combinatorial transcription factor involved in cellulase production as well as other multiple cellular functions

(セルラーゼ生産や様々な細胞機能に関わる MADS box コンビナトリアル転写因子 McmA の機能解析)

氏 名 李 諾

MADS box proteins are transcription factors that are widely distributed among eukaryotes and are involved in the regulation of a wide range of cellular functions through interaction with their cofactors, however their physiological roles are still not well understood in filamentous fungi. The aim of this thesis is to study the roles of MADS box protein McmA in a model filamentous fungus *Aspergillus nidulans* with a special interest in its involvement in regulation of cellulase genes.

### ***McmA-dependent and -independent regulatory systems governing expression of ClrB-regulated cellulase and hemicellulase genes in *Aspergillus nidulans****

Fungal cellulolytic and hemicellulolytic enzymes are promising tools for industrial hydrolysis of cellulosic biomass; however, the regulatory network underlying their production is not well understood. The recent discovery of the transcriptional activators ClrB and McmA in *A. nidulans* implied a novel regulatory mechanism driven by their interaction, experimental evidences for which were obtained by transcriptional and DNA binding analyses. ClrB was essential for inductive expression of all the genes examined in this study, while McmA dependency of their expression was gene-dependent. DNA-binding studies with recombinant ClrB and McmA derivatives revealed that McmA assisted the recruitment of ClrB to the cellulose-responsive element (CeRE) in the promoters of *egIA* and *egIB*, expression of which was significantly reduced in the *mcmA* mutant. The CCG triplet within the CeRE served as the ClrB recognition sequence. By contrast, ClrB did not require McmA for binding to the CGGN<sub>8</sub>CCG sequences in the promoter of *mndB*, expression of which was least affected by the *mcmA* mutation among all the examined genes. Thus, there are two types of ClrB-mediated regulation: McmA-assisted and McmA-independent. This novel McmA–ClrB synergetic system provides new insights into the complex regulatory

network involved in cellulase and hemicellulase production.

### ***McmA-independent regulation of mannanase genes by ClrB and ManS in A. nidulans***

*Aspergillus* species are able to degrade  $\beta$ -mannan by secreting endo- $\beta$ -mannanase and  $\beta$ -mannosidase, and the enzymes have been widely used in food industries. Transcriptional regulation of mannanase genes in *A. oryzae* is solely controlled by a transcription factor ManR. However, due to the presence of two ManR homologs (ClrB and ManS), regulation of mannanase genes in *A. nidulans* is more complex. While ClrB is essential to cellulase production, ClrB and ManS both regulated mannanase production with ManS as a main transcriptional activator. Double deletion of *clrB* and *manS* abolished mannanase production, confirming the involvement of both factors in regulation of mannanase genes. Dependency on ClrB and ManS of their expression could be categorized into several types. In the 1<sup>st</sup> type, ManS works as the transcriptional activator in response to mannobiose; in the 2<sup>nd</sup> type, ClrB and ManS compete for binding to the same promoter region so that the former represses and the latter activates the gene expression; in the 3<sup>rd</sup> type, ClrB and ManS recognize different binding sites and activate the gene expression separately in response to cellobiose and mannobiose in the former and to mannobiose in the latter. In the last type, ClrB and ManS form a heterodimer to activate the gene expression when being induced by mannobiose. Clarification of the complex system mediated by ClrB and ManS deepened the understanding of the regulatory network involved in hemicellulase production in filamentous fungi.

### ***Involvement of McmA in regulation of extracellular enzyme production and asexual/sexual development in A. nidulans***

Effects of the *mcmA* mutation were examined by RNA sequencing. Sequencing data revealed that expression of cellulase genes were significantly decreased by the mutation as reported previously. Furthermore, expression of various hemicellulolytic enzyme genes, several extracellular protease genes, the *nosA* and *rosA* genes involved in sexual development, and AN4394 encoding an ortholog of EcdR involved in *A. oryzae* conidiation, were significantly decreased by the mutation. As expected from the RNA sequencing data, the *mcmA* mutant had reduced protease production, cleistothecial development, and conidiation. This is the first discovery of the involvement of SRF-MADS proteins in protease production in fungi and of asexual and sexual development in *Aspergillus*.