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Molecular Studies on Cytoplasmic Male Sterility and Shoot Apical Meristem in Rice

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Summary

Understanding the mysteries of pollen and shoot apical meristem (SAM) hold the key to a high yielding crop. We are focusing on pollen sterility caused by incompatibility between mitochondria and nucleus, which is called cytoplasmic male sterility (CMS). The CMS and restoration of a fertility (Rf) system is commercially important for hybrid seed production. Apart from its agronomic importance, the CMS/*Rf* system provides a clue to elucidate conflict/reconciliation between the mitochondrial and the nuclear genomes. We are also studying SAM-specific *KNOX* genes. Formation and maintenance of SAM is a fundamental process for plant development, since it forms lateral organs such as leaves and flowers. Here we report our recent advances on genes related to CMS and SAM in rice.

Cytoplasmic Male Sterility in Rice

We are focusing on pollen sterility caused by incompatibility between mitochondria and nucleus, which is called cytoplasmic male sterility (CMS). CMS has been widely utilized for F_1 hybrid rice breeding in Southeastern Asia. F_1 hybrid plants, in general, exhibit hybrid vigor or heterosis, which promise a high yielding crop. F_1 hybrid rice has an average 15% to 20% yield advantage over inbred lines. In China, it is planted on approximately 16 million hectares —more than half of China's total rice area of 28 million hectares. Most of the commercial rice hybrids are based on a single CMS source, the WA cytoplasm. To avoid the potential threat of genetic vulnerability of rice hybrids, the development of several CMS/*Rf* systems is desirable. We have been studying three types of CMS in rice: BT-CMS originating from Chinsurah Boro II (Kazama *et al.*, 2008), LD-CMS from Lead Rice (Itabashi *et al.*, 2009) and CW-CMS from Chinese Wild rice (Fujii and Toriyama, 2009).

BT-CMS originates from an *indica* variety of *Oryza sativa* L., Chinsurah Boro

II. The restorer gene is called *Rf1*, and the race to uncover its properties has reached global proportions. We are the first to uncover the secrets of its nucleotide sequences. We have revealed that *Rf1* encodes a pentatricopeptide repeat (PPR)-containing protein. A chimeric ORF, *orf79*, is reported as a CMS-associated mitochondrial gene. We have recently demonstrated that the RF1 protein binds to co-transcribed *atp6-orf79* transcripts and promotes processing of the transcripts (Kazama *et al.*, 2008). As a result, transcripts of *orf79* were degraded and were not translated into protein. The study of PPR in mitochondria is a frontier field unique to higher plant species (Fujii and Toriyama, 2008 for a review).

CW-CMS was first discovered by Professor Emeritus Usaburo Mizushima, who was the first professor of the Laboratory of Plant Breeding, Faculty of Agriculture, Tohoku University. Although the CW-type cytoplasm was the first CMS system discovered in rice, it has not been utilized for hybrid rice breeding, due to the lack of any restorer genes among cultivars thus far tested. We found that pollen fertility is restored gametophytically by a single nuclear gene, *RF17*. Cloning of *RF17* for CW-CMS revealed that *RF17* encoded an unknown protein containing acyl-carrier protein synthase-like domain (Fujii and Toriyama, 2009). We found that a reduced expression allele of the *RF17* gene restored fertility in haploid pollen, while a normal expressing allele caused pollen to die in CW-CMS. The mRNA expression level of *RF17* in mature anthers was cytoplasmic genotype-dependent, suggesting that *RF17* is a candidate gene to be regulated via retrograde signaling from mitochondria to the nucleus. We therefore designated *RF17* as *RETROGRADE-REGULATED MALE STERILITY (RMS)*. Our study has highlighted the unique features of the CW-CMS/*Rf17* system, and these features provide novel insights into retrograde signaling and CMS. The CMS/*Rf* system provides a clue to elucidate conflict/reconciliation between the mitochondrial and the nuclear genomes. We are also studying the function of genes that were down-regulated in the CW-CMS line to elucidate the mechanism of pollen abortion and retrograde signaling.

What we are starting to understand more about how CMS of rice can be applied to other plants as well. Studying the mysteries of pollen can lead to many different avenues. In the growing concern over food shortages, we are hoping our research can feed a billion people in the future.

Shoot Development in Rice

In plants, the shoot apical meristem (SAM) is generated during embryogenesis and maintained throughout its life cycle. Lateral organs such as leaves and flowers are successively formed from a flank of the SAM. Thus, formation and maintenance of the SAM and generation of lateral organs from the SAM are

fundamental for plant development.

KNOX genes are closely associated with these events. The expression of *KNOX* genes is SAM-specific, and lateral organs arise where *KNOX* genes are down-regulated. Loss-of-function mutations of *KNOX* genes show a shoot meristemless phenotype. This indicates that *KNOX* genes are essential for the formation and/or maintenance of the SAM (Scofield and Murray, 2006). Gain-of-function mutations of *KNOX* genes, in which corresponding genes were ectopically expressed in leaves, resulted in abnormal lateral organ development (Scofield and Murray, 2006). These analyses clearly demonstrate the importance of SAM-specific expression of *KNOX* genes for proper shoot development.

To understand the mechanisms underlying the SAM-specific expression of *KNOX* genes, we examined a promoter activity of *OSH1*, a *KNOX* gene in rice (Ito and Kurata, 2008). We generated transgenic rice plants containing an *OSH1* promoter-GUS construct or *OSH1* promoter-GFP construct. These reporter gene activities were observed in leaves in addition to the SAM. Thus, the promoter region used in this experiment was not sufficient to confer the SAM-specific expression of *OSH1*, and other regions contained a cis-element necessary to repress *OSH1* expression in leaves. Next, we generated transgenic plants containing *OSH1* cDNA without a promoter sequence. Surprisingly, these plants showed overexpressor-like phenotypes, and both endogenous *OSH1* and transgene (cDNA) were expressed in leaves. These results suggest that exons contain a cis-regulatory sequence, and an extra copy of the regulatory sequence disrupts the repression mechanism of *OSH1* in leaves (Ito and Kurata, 2008).

To identify the factor which is involved in repression of *KNOX* gene expression in leaves and confers the SAM-specific expression of *KNOX* genes, we screened loss-of-function mutants which resembled the morphology of *KNOX* overexpressors (Tsuda *et al.*, 2009). We identified 12 mutant lines derived from three loci. These mutants were named *onion1* (*oni1*), *oni2* and *oni3*. Expression analysis of *KNOX* genes showed that four *KNOX* genes out of five were ectopically expressed in mutant leaves, whereas in the wild type no expression of any of the *KNOX* genes was observed in leaves. Although some genes which are required for repression of *KNOX* genes in leaves have been identified in *Arabidopsis* and maize, none of their orthologues was mapped in the regions where *ONI* genes locate. Thus, *ONI* genes are different from previously known *KNOX* repressor genes and are novel regulators of *KNOX* gene expression (Tsuda *et al.*, 2009). Detailed analyses of these mutants will uncover complex mechanisms of the SAM-specific expression of *KNOX* genes.

References

Fujii, S., and Toriyama, K., *Plant Cell Physiol.*, **49**, 1484–1494 (2008)

Fujii, S., and Toriyama, K., *Proc. Natl. Acad. Sci. USA*, **106**, 9513-9518 (2009)
Itabashi, E., Kazama, T., and Toriyama, K., *Plant Cell Rep.*, **28**, 233-239 (2009)
Ito, Y., and Kurata, N., *Plant Sci.*, **174**, 357-365 (2008)
Kazama, T., Nakamura, T., Watanabe, M., Sugita, M., and Toriyama, K., *Plant J.*, **55**, 619-628 (2008)
Scofield, S., and Murray, A.H., *Plant Mol. Biol.*, **60**, 929-946 (2006)
Tsuda, K., Ito, Y., Yamaki, S., Miyao, A., Hirochika, H., and Kurata, N., *Plant Sci.*, **177**, 131-135 (2009)