Genetic Control of Flowering Time in Rice, a Short-Day Plant

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Endogenous genetic factors and environmental signals control the time of flowering in plants. One of the environmental signals is photoperiod. Genetic control mechanisms for the photoperiodic response of flowering of long-day plants (LDPs) have been extensively analyzed through the use of Arabidopsis as a model plant (for review, see Coupland, 1998; Levy and Dean, 1998; Samach and Coupland, 2000). In contrast, mechanisms in short-day plants (SDPs) remain unclear, although many physiological studies have been performed on SDPs, such as Pharbitis nil (for review, see Lumsden, 1998). Recent progress in genome analysis has provided a new strategy for analyzing the genetic control of flowering in rice (Oryza sativa; SDP). Several studies have demonstrated that the structure of genes involved in the photoperiodic response of flowering in rice show remarkable similarity to those in Arabidopsis.

NATURAL VARIATIONS: A NEW RESOURCE FOR GENETIC ANALYSIS OF FLOWERING IN RICE

In rice, genetic analyses of flowering time (often called heading date) have been performed on mutants and natural variants. Several genes involved in the photoperiodic response (photoperiod sensitivity) have been identified (Yokoo et al., 1980; Yamagata et al., 1986; Yokoo and Okuno, 1993; Okumoto and Tanisaka, 1997). A series of nearly isogenic lines (NILs) for several photoperiod sensitivity genes have been developed to facilitate genetic analysis of flowering time in rice (Yamagata et al., 1986). However, the nature of the quantitative inheritance of flowering time has prevented us from performing more detailed analyses, including analysis of epistatic interactions and determination of chromosomal locations of genes. In the last decade, the progress in DNA markers made quantitative trait locus (QTL) analysis possible to clarify the number and nature of the genes controlling flowering time in rice (Yano and Sasaki, 1997).

We have performed a QTL analysis of heading date using several types of progeny derived from a single cross between rice cv Nipponbare (japonica) and rice cv Kasalath (indica) and have identified 14 QTLs controlling flowering time in rice (Fig. 1). Five QTLs, Hd1 through Hd5, have been mapped based on analysis of the F2 population (Yano et al., 1997), and an additional three QTLs, Hd7, Hd8, and Hd11, have been detected by using BC1F3 lines (Lin et al., 1998). In addition, other loci, Hd6, Hd9, Hd10, Hd12, Hd13, and Hd14, have been detected only when we used advanced backcross progeny, such as BC3F2 or BC4F2, but not F2 or BC1F3 (Yamamoto et al., 2000; Lin et al., 2002; M. Yano, unpublished data).

The development of NILs by marker-assisted selection, in which a small chromosomal segment including the detected QTL of donor variety Kasalath was substituted into the Nipponbare genetic background, has provided many advantages for the genetic analysis of flowering time in rice (for review, see Yano and Sasaki, 1997). For example, the QTL-NILs can be used in the characterization of the photoperiodic response, epistatic interaction analysis, and fine genetic linkage mapping for target QTLs. The QTLs were classified into two groups based on the response of the QTL-NILs to photoperiod. Five QTLs, Hd1, Hd2, Hd3, Hd5, and Hd6, were found to confer the photoperiod sensitivity (Lin et al., 2000; Yamamoto et al., 2000; M. Yano, unpublished data). By the genetic analysis using QTL-NILs, the existence of an epistatic interaction between Hd1 and Hd3 was clarified. It was also suggested that the Kasalath allele of Hd3 itself does not affect photoperiod sensitivity, but that it is involved in enhancement of expression of the Nipponbare alleles of photoperiod sensitivity QTLs, Hd1 and Hd2 (Lin et al., 2000). In addition, epistatic interaction between Hd2 and Hd6 was clearly detected in the analysis of the advanced progeny. The
effect of the Kasalath allele of Hd6 could be observed only in the presence of the Nipponbare allele of Hd2 (Yamamoto et al., 2000).

QTL-NILs could also be used for the fine mapping of target QTLs. Five QTLs, Hd1, Hd2, Hd3, Hd6, and Hd9, were mapped precisely on the genetic linkage map as single Mendelian factors (Yamamoto et al., 1998, 2000; Lin et al., 2002; H.X. Lin and M. Yano, unpublished data). Moreover, high-resolution mapping enabled us to dissect two tightly linked loci, Hd3a and Hd3b, in the Hd3 region (Fig. 1; Monna et al., 2002). Analysis of the photoperiodic response in NILs of Hd3a and Hd3b revealed that the Kasalath allele of Hd3a promotes flowering under short-day (SD) conditions, and that the Kasalath allele of Hd3b delays late flowering under long-day (LD) and natural field conditions (Monna et al., 2002). Together, it is clearly demonstrated that genetic control mechanisms of flowering in rice could be dissected into each component by a series of genetic analyses of flowering date based on the QTL analysis.

MOLECULAR ANALYSIS OF GENES INVOLVED IN PHOTOPERIODIC RESPONSE

A major QTL, Hd1, which controls response to photoperiod, was cloned by means of a map-based cloning strategy (Yano et al., 2000). Hd1 is an orthologue of CO (constans) in Arabidopsis (Putterill et al., 1995) and encodes a protein with the structure of a zinc finger domain and a nuclear localization signal. In addition, structural analysis demonstrated that the major gene controlling the response to photoperiod, photoperiod sensitivity 1 (Se1), is allelic to Hd1. The genetic study demonstrated that Hd1 may function differently under SD and LD conditions to promote flowering in the SD condition and inhibit it in the LD condition (Table I; Lin et al., 2000). Genetic linkage mapping and transgenic analysis clearly proved this bifunctional nature of Hd1 expression (Yano et al., 2000). It is noteworthy that, under LD conditions, Hd1 inhibits flowering of rice, whereas CO promotes flowering of Arabidopsis. This suggests that those genes may regulate the target genes in an opposite

<table>
<thead>
<tr>
<th>Gene</th>
<th>Effect on Flowering Time</th>
<th>Putative Function</th>
<th>Arabidopsis Orthologue</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hd1</td>
<td>Early flowering in SD and late flowering in LD</td>
<td>Transcription factor</td>
<td>CO</td>
<td>Yano et al. (2000)</td>
</tr>
<tr>
<td>Hd6</td>
<td>Late flowering in LD</td>
<td>Protein kinase CK2α</td>
<td>CK2</td>
<td>Takahashi et al. (2001)</td>
</tr>
<tr>
<td>Hd3a</td>
<td>Early flowering in SD</td>
<td>Not clarified</td>
<td>FT</td>
<td>Kojima et al. (2001)</td>
</tr>
<tr>
<td>SE5</td>
<td>Late flowering in LD</td>
<td>Heme-oxygenase</td>
<td>HY1</td>
<td>Izawa et al. (2000)</td>
</tr>
</tbody>
</table>

* Effect of wild-type allele on the phenotype.
manner in LD. Because CO positively regulates the FT (flowering time T) and SOC1 (suppressor of over-expression of CO 1) genes (Kobayashi et al., 1999; Onouchi et al., 2000; Samach et al., 2000) of Arabidopsis, Hd1 may positively regulate the counterpart genes of rice in SD conditions and negatively in LD conditions. Recently, PnCO, with similarity to the Arabidopsis CO gene, was isolated by a differential display method in P. nil (Liu et al., 2001). The expression of PnCO was found to be photoperiodically regulated, and the Arabidopsis co mutant was complemented with PnCO cDNA. In addition to Hd1 in rice, this result clearly supports the concept that a CO-like protein promotes flowering in different inductive photoperiods, SD and LD.

Another QTL, Hd6, located on the long arm of chromosome 3, is involved in rice photoperiod sensitivity (Yamamoto et al., 2000). The Kasalath allele inhibits flowering under natural and LD conditions but not under the SD condition. High-resolution and fine-scale genetic mapping of Hd6 delimited the candidate for Hd6 to a 26.4-kb genomic region; finally, it was proved by complementation analysis that Hd6 encodes the α-subunit of protein kinase CK2 (CK2α; Takahashi et al., 2001; Table I). This result indicates that CK2α plays an important role in the photoperiodic response of flowering in rice. In Arabidopsis, CK2 interacts with and phosphorylates the Arabidopsis circadian clock-associated 1 protein (CCA1) in vitro (Sugano et al., 1998). Overexpression of the β-subunit of CK2 shortened periods of rhythmic expression of CCA1 and caused early flowering in both LD and SD conditions (Sugano et al., 1999). This suggests that CK2 is involved in the control of flowering in Arabidopsis as well. These results demonstrate that a common mechanism may exist in the photoperiodic response of flowering in both SDPs and LDPs. It remains to be analyzed whether the alteration in circadian phenotypes, such as a daily rhythmic expression of a reporter gene, occurs in the NILs for Hd6. Recently, a good monitoring system of gene expression regulated by the circadian clock has been developed based on cab1::luc transgenic plants (Sugiyama et al., 2001). It should be possible to analyze the alteration in circadian phenotypes using this system.

Hd3a, located on the short arm of chromosome 6, and involved in the photoperiodic response and in promoting flowering in the SD condition, was also identified by a map-based strategy (Kojima et al., 2001). Hd3a showed a high level of similarity with the FT gene (Kobayashi et al., 1999) that promotes flowering in LD conditions (Table I). Transgenic analysis revealed that the introduction of Hd3a resulted in early flowering in SD and LD conditions (Kojima et al., 2001). In addition, Hd3a mRNA is up-regulated in the SD conditions, which induces flowering in rice. These results suggest that Hd3a plays an important role for promotion of flowering in SD conditions.

Through the analysis of artificial mutants in rice, Izawa et al. (2000) demonstrated that phytochromes confer the photoperiodic control of flowering. They cloned the gene corresponding to the photoperiodic sensitivity 5 (se5) mutant (Yokoo and Okuno, 1993) that shows complete loss of the photoperiodic response of flowering. SE5 encodes a putative heme oxygenase (HY1 in Arabidopsis) involved in phytochrome chromophore biosynthesis. Light-stable phytochromes may play a major role in measuring the day length in rice, because se5 mutants flowered early even under constant light conditions, in which the wild-type rice did not flower. It is noteworthy that rice phyA mutations did not affect flowering time in rice (Takano et al., 2001). It was also reported that a photoperiod sensitivity gene, Ma6, encodes a phytochrome B in sorghum (Sorghum bicolor [L.] Moench; Childs et al., 1997). These results suggest that light-stable phytochromes play an important role in the photoperiodic induction of flowering in SDPs, although light-stable phytochromes generally inhibit flowering regardless of the photoperiodic responses in both LDPs and SDPs (Thomas, 1998; Lin, 2000). On the other hands, PHY A and CRY2, which are light-labile photoreceptors, are major players in the photoperiodic control of flowering in Arabidopsis (LPD; Guo et al., 1998; Johnson et al., 1994). Therefore, it would be interesting to examine the role of cryptochromes in the control of flowering time in rice.

In Arabidopsis, the signals from light/dark cycle received by phytochromes and cryptochromes are transmitted to the circadian clock (for review, see Levy and Dean, 1998). The circadian clock regulates the transcription factor gene CO (Suarez-Lopez et al., 2001). Then CO activates the FT, SOC1, and LFY (leafy) genes (Kobayashi et al., 1999; Samach et al., 2000), which in turn activate the ABC floral organ identity genes. In rice, expression profiles of Hd1 and Hd3a remained to be analyzed with regards to photoperiods. However, based on the molecular structure of those genes and epistatic interactions, it is possible that Hd1 acts like CO in Arabidopsis to mediate flowering signals from the environmental changes. Therefore, in rice, it can be speculated that Hd1 mediates a signal from the circadian clock to the Hd3a gene.

**FUTURE PROSPECTS**

To understand the photoperiodic control of flowering in rice more comprehensively, other QTLs, such as Hd2, Hd3b, and Hd5, should be isolated. New genetic factors that control flowering must also be explored. To exploit a wide range of allelic variations in the genes controlling flowering in rice, wild relatives, which are adapted to specific environmental conditions, can be used as donor parents to develop
mapping populations. Chromosome segment substitution lines covering whole rice chromosomes have been developed through the use of wild relatives as donor parents and have been used for QTL analysis (for review, see Yano, 2001). In fact, new QTL for flowering time have been identified by using such wide cross combinations (Doi et al., 1998). There are also unique varieties within cultivated species. Rice varieties adapted to Hokkaido, the northernmost island in Japan, have a functional allele at the Se1 (Hd1) locus (Ichitani et al., 1997, 1998). However, those varieties show complete loss of photoperiodic response of flowering, suggesting that some other genetic cofactor might be required to express photoperiodic response with Hd1. Those varieties should be used as parental lines in the QTL analysis of flowering time to detect such putative factors.

Arabidopsis flowering mutants often exhibit altered circadian clock phenotypes. Several genes involved in the circadian behavior of leaf movement of Arabidopsis have been identified through the QTL analysis of natural variation (Swarup et al., 1999). This approach detected some flowering-time genes, which have been reported in the previous studies, and new members of genes for the circadian system. The analysis of natural variation in circadian clock-related traits is an alternative strategy for finding new components of flowering time regulation in rice.

In addition to the phenotype-based approach mentioned above, the microarray system and differential display methods will contribute to identifying new components of the flowering time control system. In P. nil, the differential display method was used to isolate other candidate factors involved in the photoperiodic response of flowering (Sage-Ono et al., 1998). A cDNA, PnC401, which accumulated during the inductive dark period, was isolated. Fluctuations in PnC401 mRNA abundance with regard to circadian rhythm and the day/night cycle suggested that PnC401 might be involved in the photoperiodic response of flowering. PnC401 showed no distinct similarity to known proteins but showed significant similarity to Arabidopsis expressed sequence tag. It will be interesting to learn the biological function of PnC401 with regard to the photoperiodic response of flowering.

Several genes have been molecularly identified in rice. Although biochemical functions of Arabidopsis CO and FT seem to be conserved in rice Hd1 and Hd3a, the inductive photoperiod for flowering is different between rice and Arabidopsis. This raises a simple question: What kind of gene(s) or mechanism(s) are involved in generating the completely opposite reaction to the photoperiod between SDPs and LDs? Further comparative studies between Arabidopsis and rice will allow us to clarify conserved and/or diverse features in such an important and complex developmental system as flowering.

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