Plant Biotechnology 24, 83–86 (2007)

Review

Day-neutral response of photoperiodic flowering in tomatoes: possible implications based on recent molecular genetics of *Arabidopsis* and rice

Tsuyoshi Mizoguchi*, Kanae Niinuma, Riichiro Yoshida

Institute of Biological Sciences, University of Tsukuba, Tennodai 1-1-1, Tsukuba, Ibaraki 305-8572
* E-mail: mizoguchi@gene.tsukuba.ac.jp Tel: +81-298-53-6005 Fax: +81-298-53-7723

Received November 26, 2006; accepted December 28, 2006 (Edited by M. Umeda)

Abstract Photoperiodic flowering responses are classified into three major types: long day (LD), short day (SD), and day neutral (DN). *Arabidopsis* is one of the best-characterized LD plants and flowers much earlier under LD than SD conditions. In contrast, SD conditions promote flowering of SD plants such as rice and *Pharbitis nil*. Recently, molecular genetics of *Arabidopsis* (LD) and rice (SD) have identified genes that play key roles in the photoperiodic flowering of these plants. A common role of the *GIGANTEA* (*GI*)-*CONSTANS* (*CO*)-*FLOWERING LOCUS T* (*FT*) pathway in *Arabidopsis* and rice, and a specific role of *EARLY HEADING DATE 1* (*EHD1*) in rice, but not in *Arabidopsis*, have been demonstrated. The DN response is the most poorly characterized among the three types of photoperiodic flowering responses. In this short review, we discuss how the DN flowering response is achieved in tomato based on recent progress in the analysis of *Arabidopsis* mutations in the photoperiodic flowering pathway and circadian rhythms.

Key words: *Arabidopsis*, circadian rhythms, day-neutral plant, long-day plant, photoperiodic flowering, short-day plant.

Fluctuations in day length affect the developmental processes and behaviors of many organisms. This phenomenon is called photoperiodism and allows the detection of seasonal changes and anticipation of future environmental conditions (Garner and Allard 1920). Garner and Allard (1920) found that many plants flower in response to changes in day length. The photoperiodic control of flowering time is tightly linked to the circadian clock (Mizoguchi et al. 2002, 2005). In *Arabidopsis*, *LATE ELONGATED HYPOCOTYL* (*LHY*) and *CIRCADIAN CLOCK ASSOCIATED 1* (*CCA1*) each encode a myb protein essential for clock function (Schaffer et al. 1998; Mizoguchi et al. 2002; Wang and Tobin 1998;). Two floral activator genes, *GIGANTEA* (*GI*) and *CONSTANS* (*CO*), play key roles in photoperiodic flowering (Fowler et al. 1999; Park et al. 1999; Putterill et al. 1995). We recently proposed that GI plays dual roles, acting within the circadian clock to regulate period length and circadian phase while also more directly promoting the expression of a circadian clock output pathway that includes *CO* and *FLOWERING LOCUS T* (*FT*) and promotes flowering in *Arabidopsis* (Mizoguchi et al. 2005). *FT* has been proposed as an important component of the elusive “florigen” signal (Abe et al. 2005; Huang et al. 2005; Wigge et al. 2005).

The photoperiodic flowering responses of plants are classified into 3 major types, long-day (LD), short-day (SD) and day-neutral (DN) (Thomas and Vince-Prue 1997). The LD plants only flower (an absolute LD plant) or flower most rapidly (a facultative LD plant) with more than a certain number of hours of light in each 24 h period. By contrast, the SD plants only flower or flower most rapidly with fewer than a certain number of hours of light in each 24 h period. The DN plants flower at the same time irrespective of the photoperiodic conditions. *Arabidopsis* is a facultative LD plant and flowers much earlier in a photoperiod with a long light period and a short dark period (e.g. 16 h light/8 h dark) than that with a short light period and a long dark period (e.g. 8 h light/16 h dark or 10 h light/14 h dark).

The reverse response to day length observed between *Arabidopsis* (LD plant) and rice (*Oryza sativa* L.; SD plant) is partly explained by the difference in the function of CO in *Arabidopsis* and the rice homolog HEADING DATE 1 (Hd1; Hayama et al. 2003; Izawa et al. 2002). However, the molecular mechanisms underlying the photoperiodic responses of other LD, SD, and day-neutral (DN) plants are largely unknown. Among these, the DN response is the most poorly characterized. Finding novel mechanisms involved in photoperiodism and switching one type to another (e.g.,

---

Abbreviations: *CCA1*, *CIRCADIAN CLOCK ASSOCIATED 1*; *CO*, *CONSTANS*; *DN*, day neutral; *FT*, *FLOWERING LOCUS T*; *GI*, *GIGANTEA; LD*, long day; *LHY*, *LATE ELONGATED HYPOCOTYL*; *SD*, short day; *SFT*, *SINGLE FLOWER TRUSS*.

LD to SD or DN plants) remain important topics of investigation. In the first section of this short review, we summarize recent progress in comparisons of molecular mechanisms of photoperiodic flowering in *Arabidopsis* and rice. In the second section, we summarize how to create DN *Arabidopsis* based on recent genetic analyses of genes involved in photoperiodic flowering and circadian rhythms. Finally, we discuss potential molecular mechanisms of the DN response in tomatoes.

Why does photoperiod not affect flowering time in tomato?

Lessons from Arabidopsis research

Photoperiods affect the developmental processes not only of plants but also of insects and animals. Diapause induction and termination of certain kinds of insects are under photoperiodic control. For examples, the production of elusive signal, pigment dispersing factor (pdf), is controlled by circadian clock, and this signal mediates between the clock and the photoperiodic response, diapause in flies (Figure 1).

In the past decade, photoperiodic flowering of the LD plant *Arabidopsis* (Figure 1) and the SD plant rice has been well characterized using molecular genetics. Loss-of-function or over-expression of certain genes changes the photoperiodic response type of *Arabidopsis* from the LD-type to the DN-type. DN (or day-length insensitive) responses can be further classified into several groups: types I to III (Figure 1).

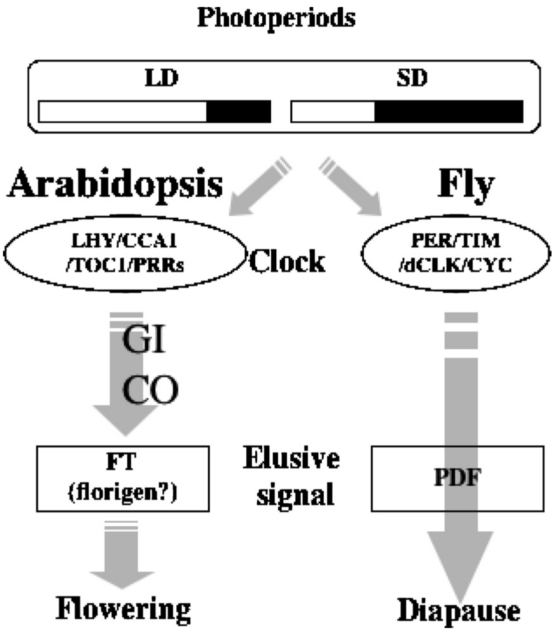

Figure 1. Connection between the photoperiodic responses and circadian clocks in *Arabidopsis* and fly. Circadian clocks play key roles in photoperiodic responses such as flowering in *Arabidopsis* and diapause in flies. In both cases, the production of elusive signals, i.e., FT and pigment dispersing factor (pdf), is controlled by the clocks, and these signals mediate between the clocks and the photoperiodic responses. The *Arabidopsis* clock not only affects the gene expression of *GI–CO*, but may also play important roles in distinct pathway(s) to control *FT* expression.

Arabidopsis is a facultative LD plant that flowers much earlier under LD than SD conditions. Loss-of-function of floral activators in the photoperiod pathway such as *gi*, *co*, and *ft* largely delay flowering under LD, but not SD conditions (Koornneef et al. 1991). The *gi* mutation has the most severe effect among these mutations. The *gi* mutant plants produce more leaves than do wild-type plants in the inductive condition (LD), but the flowering times of *gi* and wild-type plants are almost the same in the noninductive condition (SD; type I; Figure 2).

In contrast, over-expression of *CO* (*CO-ox*) accelerates flowering even under SD conditions (Simon et al. 1996). The *CO-ox* plants flower earlier than wild-type plants under LD conditions (type II; Figure 2). Photoperiodic flowering is affected by the circadian clock (Mizoguchi and Coupland 2000; Mizoguchi et al. 2006). For example, *LHY* and *CCA1* encode myb proteins and play key roles in the control of circadian rhythms and flowering time. The *lhy cca1* double loss-of-function causes day-length insensitive early flowering (Mizoguchi et al. 2002) and is classified as type II (Figure 2).

The third class of the DN response of flowering can be obtained by over-expression of either *LHY* or *CCA1*. These plants flower later than wild-type plants under LD conditions, but slightly earlier under SD conditions (Mizoguchi et al. 2005; Súarez-López et al. 2001).

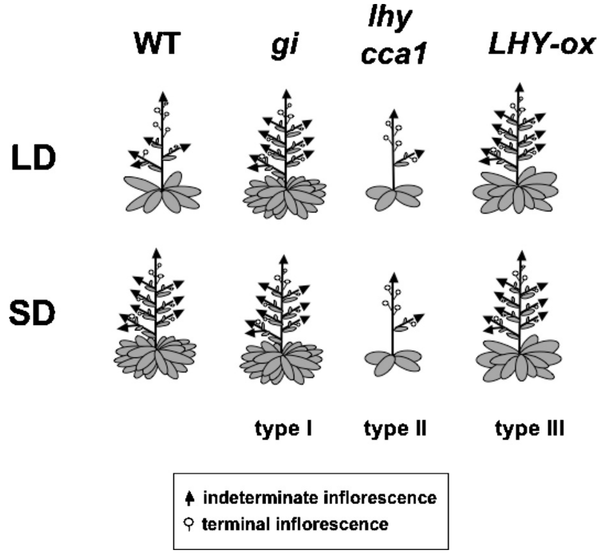

Figure 2. Three types of day-neutral flowering response in *Arabidopsis*. Delay of flowering in the inductive condition (long day [LD]; type I, e.g., *gi*) and acceleration of flowering in the noninductive condition (short day [SD]; type II, e.g., *CO-ox* and *lhy cca1*) cause day-neutral flowering responses in *Arabidopsis*. Over-expression of either LHY or CCA1 has a unique effect on flowering; it delays and accelerates flowering under LD and SD conditions, respectively (type III). WT, wild type. See text for details.

Almost all of the late and early flowering patterns described above are well correlated with levels of *FT* expression in *Arabidopsis* (Calvino et al. 2005; Fujiwara et al. 2005a, b, c; Mizoguchi et al. 2005).

Can the tomato day-neutral response type be explained by simple loss- or gain-of-function of *GI*, *CO*, or *FT*?

Current tomato cultivars with the DN response have been chosen by breeders using long-term selection. The modern tomato has a mild short day response that is sometimes unnoticed due to other environmental inductions (high light or mild temperatures) (Samach and Lotan 2006, this issue). This allows for the harvest of tomato fruits over the entire year if temperatures in the growth environment are high enough. How did the ancestral tomato with the strong/mild photoperiodic response type become day-neutral? Or did the ancestral tomato has DN response type?

Day length affects a number of secondary compounds in tomato cultivars. For example, in *syn*. *Solanum hirstum*, levels of the steroidal glycoalkaloid tomatine are different under LD than SD conditions at the same irradiance (Sinden et al. 1978). The levels of tomatine are correlated with the susceptibility to attacks by the Colorado potato beetle (Sinden et al. 1978).

Trichome density is also affected by day length in tomatoes and *Arabidopsis* (Chien and Sussex 1996; Gianfagna et al. 1992). Tight connections between photoperiodic responses and circadian rhythms have been outlined in detail in recent reviews (Mizoguchi et al. 2000, 2006). Some biological processes in tomatoes are controlled by the circadian clock (Samach and Lotan 2007, this issue), although core clock components in tomatoes have not been identified to date.

These results suggest that tomatoes have a sense of time measurement and that the connection between the circadian clock and one of the output pathways, flowering, may be impaired at a certain point. Below, we show several examples of how to make an LD plant, *Arabidopsis*, day neutral (Figure 1).

The loss-of-function of an *FT* homolog in tomato (*SINGLE FLOWER TRUSS*; *SFT*) delays flowering, which indicates that SFT in tomato actually functions as a floral activator in a manner similar to those in other plant species such as *Arabidopsis* and rice (Lifschitz and Eshed 2006; Lifschitz et al. 2006). What about *GI* and *CO* homologs? Tomatoes possess *GI* and *CO*-related genes, and the *GI*-homolog shows diurnal and circadian expression patterns (Niinuma et al. 2007, this issue), suggesting that at least the GI homolog may have similar functions to those of *Arabidopsis* and rice. This possibility can be tested by analyzing both loss- and gain-of-function of the *GI* and *CO* homologs in tomatoes.

Although the GI–CO–FT pathway is well conserved in *Arabidopsis*, rice, and other plant species, a unique molecule that seems to be specific to rice has also been reported. The *EARLY HEADING DATE 1* (*EHD1*) encodes a B-type response regulator and plays key roles in the photoperiodic flowering of rice (Doi et al. 2004). The analysis of approximately 36,000 expressed sequence tags (ESTs) from the one of the tomato cultivars ‘Micro-Tom’ revealed that these ESTs included more than 10,000 unigenes, approximately 30% of which have not been found in *Arabidopsis* and rice and appear to be unique to tomato (Yamamoto et al. 2005). Novel players may have key roles in the control of photoperiodic flowering in tomato. The identification of the genetic network for photoperiodic flowering in tomatoes and a comparison of the genetic networks among *Arabidopsis* (LD plant), rice (SD plant), and tomato (DN plant) are important topics for future investigations. The molecular mechanisms underlying the DN flowering response in tomato have not been elucidated. EMS-mutagenesis and generation of T-DNA insertional lines of ‘Micro-Tom,’ which are currently underway in our laboratory, will be useful in determining these mechanisms.

Acknowledgments

This work was supported in part by a grant from PROBRAIN (to T.M.). The authors thank Ms. Midori Moro-oka for technical assistance.

References

Abe M, Kobayashi Y, Yamamoto S, Daimon Y, Yamaguchi A, Ikeda Y, Ichinoki H, Notaguchi M, Goto K, Araki T (2005) FD, a bZIP protein mediating signals from the floral pathway integrator FT at the shoot apex. *Science* 309: 1052–1056

Calvino M, Kamada H, Mizoguchi T (2005) Is the role of the short-day solely to switch off the CONSTANS in *Arabidopsis*? *Plant Biotechnol* 22: 179–183

Chien JC, Sussex IM (1996) Differential regulation of trichome formation on the adaxial and abaxial leaf surfaces by gibberellins and photoperiod in *Arabidopsis thaliana* (L.) Heynh. *Plant Physiol* 111: 1321–1328

Doi K, Izawa T, Fuse T, Yamanouchi U, Kubo T, Shimatani Z, Yano M, Yoshimura A (2004) Ehd1, a B-type response regulator in rice, confers short-day promotion of flowering and controls FT-like gene expression independently of Hd1. *Genes Dev* 18: 926-936

Fowler S, Lee K, Onouchi H, Samach A, Richardson K, Morris B, Coupland G, Putterill J (1999) *GIGANTEA*: a circadian clock-controlled gene that regulates photoperiodic flowering in *Arabidopsis* and encodes a protein with several possible membrane-spanning domains. *EMBO J* 18: 4679–4688

Fujiwara S, Nakagawa M, Kamada H, Coupland G, Mizoguchi T (2005) Circadian clock components in *Arabidopsis* I. The *terminal flower 1* enhances the early flowering phenotype of a circadian clock mutant, *lhy cca1*. *Plant Biotechnol* 22: 311–317

Fujiwara S, Oda A, Kamada H, Coupland G, Mizoguchi T (2005) Circadian clock components in *Arabidopsis* II. The circadian clock components LHY/CCA1 regulate the floral integrator gene *SOC1* in both GI-dependent and -independent pathways. *Plant Biotechnol* 22: 319–325

Fujiwara S, Nakagawa M, Kamada H, Mizoguchi T (2005) Circadian clock components in *Arabidopsis* III. Roles of the circadian clock components LHY/CCA1/GI in regulating the floral integrator genes *LFY*/*SOC1*/*FT* to control flowering time and shoot architecture. *Plant Biotechnol* 22: 327–331

Garner WW, Allard HA (1920) Effect of the relative length of day and night and other factors of the environment on growth and reproduction in plants. *J Agric Res* 23: 871–920

Gianfagna TJ, Carter CD, Sacalis JN (1992) Temperature and photoperiod influence trichome density and sesquiterpene content of *Lycopersicon hirsutum* f. *hirsutum*. *Plant Physiol* 100: 1403–1405

Hayama R, Yokoi S, Tamaki S, Yano M, Shimamoto K (2003) Adaptation of photoperiodic control pathways produces short-day flowering in rice. *Nature* 422: 719–722

Huang T, Bohlenius H, Eriksson S, Parcy F, Nilsson O (2005) The mRNA of the *Arabidopsis* gene *FT* moves from leaf to shoot apex and induces flowering. *Science* 309: 1694–1696

Izawa T, Oikawa T, Sugiyama N, Tanisaka T, Yano M, Shimamoto K (2002) Phytochrome mediates the external light signal to repress FT orthologs in photoperiodic flowering of rice. *Genes Dev* 16: 2006–2020

Koornneef M, Hanhart CJ, van der Veen JH (1991) A genetic and physiological analysis of late flowering mutants in *Arabidopsis thaliana*. *Mol Gen Genet* 229: 57–66

Lifschitz E, Eviatar T, Rozman A, Shalit A, Goldshmidt A, Amsellem Z, Alvarez JP, Eshed Y (2006) The tomato FT ortholog triggers systemic signals that regulate growth and flowering and substitute for diverse environmental stimuli. *Proc Natl Acad Sci USA* 103: 6398–6403

Lifschitz E, Eshed Y (2006) Universal florigenic signals triggered by FT homologues regulate growth and flowering cycles in perennial day-neutral tomato. *J Exp Bot* 57: 3405–3414

Mizoguchi T, Coupland G (2000) ZEITLUPE and FKF1: novel connections between flowering time and circadian clock control. *Trends Plant Sci* 5: 409–411

Mizoguchi T, Wheatley K, Hanzawa Y, Wright L, Mizoguchi M, Song HR, Carre IA, Coupland G (2002) *LHY* and *CCA1* are partially redundant genes required to maintain circadian rhythms in *Arabidopsis*. *Developmental Cell* 2: 629–641

Mizoguchi T, Wright L, Fujiwara S, Cremer F, Lee K, Onouchi H, Mouradov A, Fowler S, Kamada H, Putterill J, Coupland G (2005) Distinct roles of *GIGANTEA* in promoting flowering and regulating circadian rhythms in *Arabidopsis*. *Plant Cell* 17: 2255–2270

Mizoguchi T, Putterill J, Ohkoshi Y (2006) Kinase and phosphatase: the cog and spring of the circadian clock. *Int Rev Cytol* 250: 47–72

Niinuma et al. 2007

Park DH, Somers DE, Kim YS, Choy YH, Lim HK, Soh MS, Kim HJ, Kay SA, Nam HG (1999) Control of circadian rhythms and photoperiodic flowering by the *Arabidopsis GIGANTEA* gene. *Science* 285: 1579–1582

Putterill J, Robson F, Lee K, Simon R, Coupland G (1995) The *CONSTANS* gene of *Arabidopsis* promotes flowering and encodes a protein showing similarities to zinc finger transcription factors. *Cell* 80: 847–857

Samach A, Lotan H (2007) The transition to flowering in tomato. *Plant Biotechnol* 24: 71–82

Schaffer R, Ramsay N, Samach A, Corden S, Putterill J, Carre IA, Coupland G (1998) The late elongated hypocotyl mutation of *Arabidopsis* disrupts circadian rhythms and the photoperiodic control of flowering. *Cell* 93: 1219–1229

Simon R, Igeno MI, Coupland G (1996) Activation of floral meristem identity genes in *Arabidopsis*. *Nature* 384: 59–62

Sinden SL, Schalk JM, Stoner AK (1978) Effects of daylength and maturity of tomato plants on tomatine content and resistance to the Colorado Potato Beetle. *J Amer Soc Hort Sci* 103: 596–600

Súarez-López P, Wheatley K, Robson F, Onouchi H, Valverde F, Coupland G (2001) CONSTANS mediates between the circadian clock and the control of flowering in *Arabidopsis*. *Nature* 410: 1116–1120

Thomas B, Vince-Prue D (1997) Photoperiodism in plants. Academic Press

Wang ZY, Tobin EM (1998) Constitutive expression of the *CIRCADIAN CLOCK ASSOCIATED 1* (*CCA1*) gene disrupts circadian rhythms and suppresses its own expression. *Cell* 93: 1207–1217

Wigge PA, Kim MC, Jaeger KE, Busch W, Schmid M, Lohmann JU, Weigel D (2005) Integration of spatial and temporal information during floral induction in *Arabidopsis*. *Science* 309: 1056–1059

Yamamoto N, Tsugane T, Watanabe M, Yano K, Maeda F, Kuwata C, Torki M, Ban Y, Nishimura S, Shibata D (2005) Expressed sequence tags from the laboratory-grown miniature tomato (*Lycopersicon esculentum*) cultivar Micro-Tom and mining for single nucleotide polymorphisms and insertions/deletions in tomato cultivars. *Gene* 356: 127–134