CRYPTIC PRECOCIOUS/MED12 is a Novel Flowering Regulator with Multiple Target Steps in Arabidopsis

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The proper timing of flowering is of crucial importance for reproductive success of plants. Regulation of flowering is orchestrated by inputs from both environmental and endogenous signals such as daylength, light quality, temperature and hormones, and key flowering regulators construct several parallel and interactive genetic pathways. This integrative regulatory network has been proposed to create robustness as well as plasticity of the regulation. Although knowledge of key genes and their regulation has been accumulated, there still remains much to learn about how they are organized into an integrative regulatory network. Here, we have analyzed the CRYPTIC PRECOCIOUS (CRP) gene for the Arabidopsis counterpart of the MED12 subunit of the Mediator. A novel dominant mutant, crp-1D, which causes up-regulation of SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1 (SOC1), FRUITFULL (FUL) and APETALA1 (API) expression in a FLOWERING LOCUS T (FT)-dependent manner, was identified in an enhancer screen of the early-flowering phenotype of 35S::FT. Genetic and molecular analysis of both crp-1D and crp loss-of-function alleles showed that MED12/CRP is required not only for proper regulation of SOC1, FUL and API, but also for up-regulation of FT, TWIN SISTER OF FT (TSF) and FD, and down-regulation of FLOWERING LOCUS C (FLC). These observations suggest that MED12/CRP is a novel flowering regulator with multiple regulatory target steps both upstream and downstream of the key flowering regulators including FT florigen. Our work, taken together with recent studies of other Mediator subunit genes, supports an emerging view that the Mediator plays multiple roles in the regulation of flowering.

Keywords: Arabidopsis • Flowering • FLOWERING LOCUS C • FLOWERING LOCUS T • Mediator • MED12.

Abbreviations: AP1, APETALA1; bZIP, basic region/leucine zipper; CCT, CENTER CITY; CDK, cyclin-dependent kinase; CycC, cyclin C; CO, CONSTANS; CRP, CRYPTIC PRECOCIOUS; EMS, ethylmethane sulfonate; FLC, FLOWERING LOCUS C; FT, FLOWERING LOCUS T; FUL, FRUITFULL; GCC, GRAND CENTRAL; GFP, green fluorescent protein; GUS, β-glucuronidase; HEN, HUA ENHANCER; LFY, LEAFY; MAR, MACCHI-BOU; MED, Mediator complex subunit; MS, Murashige and Skoog; NRT1.7, NITRATE TRANSPORTER 1.7; PFT, PHYTOCHROME AND FLOWERING TIME; PID, PINOID; qRT–PCR, quantitative reverse transcription–PCR; RT–PCR, reverse transcription–PCR; 35S, Cauliflower mosaic virus 35S RNA promoter; SAM, shoot apical meristem; SOCI, SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1; STM, SHOOTMERISTEMLESS; SULTR2;1, SULFATE TRANSPORTER2;1 promoter; TSF, TWIN SISTER OF FT; ZT, Zeitgeber time.

The nucleotide sequences reported in this paper have been submitted to DDBJ under accession numbers: MED12/CCT/CRP locus (AB690343) and MED12/CCT/CRP cDNA (AB690341 and AB690342).

Introduction

The plant life cycle is divided into distinct developmental phases by the morphological and functional features of the organs formed at the flank of the shoot apical meristem (SAM) (Araki 2001, Poethig 2003, Baurle and Dean 2006). The proper timing of the transition from leaf-forming vegetative phase to flower-forming reproductive phase, called flowering, is especially important for reproductive success. Studies of Arabidopsis (Arabidopsis thaliana) have shown that regulation of flowering is orchestrated by inputs of multiple
environmental and endogenous signals such as daylength, light quality, temperature and hormones, and that flowering time genes construct several parallel and interactive genetic pathways (Simpson and Dean 2002, Boss et al. 2004, Fornara et al. 2010). This integrative regulatory network has been proposed to create robustness as well as plasticity to increase fitness under genetic and environmental perturbation (Wilczek et al. 2009).

Several genes represent key nodes in the regulatory network. FLOWERING LOCUS T (FT) encodes a small 20 kDa protein, which belongs to the phosphatidylethanolamine-binding protein (PEBP)/Raf kinase inhibitor protein (RKIP) family, and plays a pivotal role in promoting flowering, integrating signals from converging pathways (Kardailsky et al. 1999, Kobayashi et al. 1999). Expression of FT is strictly controlled by transcription factors and chromatin-associated proteins in the leaf vasculature (Takada and Goto 2003, Kobayashi and Weigel 2007, Adrian et al. 2010, Imaizumi, 2010). A B-box zinc-finger protein CONSTANS (CO) plays a central role in FT regulation in the photoperiod pathway (Kardailsky et al. 1999, Kobayashi et al. 1999, Samach et al. 2000). The transcriptional and post-translational controls of CO are important for monitoring the seasonal daylength changes (Suárez-López et al. 2001, Yanovsky and Kay 2002, Valverde et al. 2004, Kobayashi and Weigel 2007). As the florigen, FT protein is translated in the leaf, moves via phloem to the SAM, and activates several positive floral regulators to initiate the formation of flowers (Corbesier et al. 2007, Jaeger and Wigge 2007, Mathieu et al. 2007, Notaguchi et al. 2008). FT forms a protein complex involving a bZIP (basic region/leucine zipper) transcription factor FD, and directly activates APETALA1 (API) expression (Abe et al. 2005, Wigge et al. 2005), and either directly or indirectly activates FRUITFULL (FUL) expression (Teper-Bammolker and Samach 2005). FT also promotes expression of LEAFY (LFY) via up-regulation of SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1 (SOC1) (Searle et al. 2006, Liu et al. 2008). A MADS box transcription factor, FLOWERING LOCUS C (FLC), is a potent repressor of FT, TWIN SISTER OF FT (TSF), FD and SOC1, and thereby negatively regulates both florigen production and response (Searle et al. 2006). FLC expression is regulated by histone modification in response to vernalization and autonomous pathways (Bäurle and Dean 2006, Schmitz and Amasino 2007). Although knowledge of the regulation of key genes has been accumulated, there still remains much to learn about how they are organized into an integrative regulatory network.

Mediator is an evolutionarily conserved multiprotein complex in eukaryotes, whose biochemistry and functional roles have been extensively studied in yeast and animals. Mediator plays numerous roles in controlling the function of RNA polymerase II pre-initiation complex, and the structural plasticity of the multisubunit complex enables Mediator to regulate virtually all protein-coding genes (Malik and Roeder 2005). Genetic and molecular studies collectively suggest that the Mediator complex is essential for the proper development of the organism. Mediator complex consists of a ‘core module’ and ‘CDK8 module’. The CDK8 module contains cyclin-dependent kinase 8 (CDK8), cyclin C (CycC), Mediator complex subunit (MED) 12 (MED12) and MED13. Of the four subunits, MED12 and MED13 have been shown to be important for proper control of developmental programs in animals such as zebrafish, Drosophila and Caenorhabditis elegans (Treisman 2001, Janody et al. 2003, Hong et al. 2005, Yoda et al. 2005, Rau et al. 2006, Henteges 2011). Recently, plant Mediator subunits have been identified (Bäckström et al. 2007, Bourbon 2008), and the physiological roles of some subunits have been revealed (Auran et al. 2002, Kidd et al. 2009, Gillmor et al. 2010, Ito et al. 2011, Kidd et al. 2011, Kim et al. 2011). For example, a subunit of the core module, MED25, which was originally described as PHYTOCHROME AND FLOWERING TIME 1 (PFT1), was shown to act in jasmonate signaling and in the light quality pathway of flowering acting downstream of phytochrome B (phyB) to regulate expression of FT (Cerdán and Chory 2003, Wollenberg et al. 2008, Elving et al. 2011).

In this study, we identified and investigated the CRYPTIC PRECOCIOUS (CRP) gene for the Arabidopsis counterpart of MED12. We isolated a novel dominant mutant, crp-1D, as a genetic modifier of the precocious-flowering phenotype in 35S::FT. Genetic and molecular analyses of both dominant crp-1D and recessive crp loss-of-function alleles suggest that MED12/CRP is a novel flowering regulator with multiple regulatory target steps both upstream and downstream of the key flowering regulators including FT florigen. Furthermore, our present work and previous works (Gillmor et al. 2010, Ito et al. 2011) strongly suggest that MED12/CENTER CITY (CCT)/CRP and MED13/GRAND CENTRAL (GCT)/MACCHIBOU 2 (MAB2) proteins act closely together in the same pathways to regulate various aspects of plant development from embryogenesis to flowering and floral morphogenesis.

**Results**

**cryptic precocious-1D, a dominant enhancer mutation of 35S::FT**

To understand the regulatory mechanisms acting downstream of the floral integrator FT, we carried out ethylmethane sulfonate (EMS) mutagenesis of a weak line of 35S::FT and screened for mutations that affect its precocious-flowering phenotype. We isolated one dominant mutation, *cryptic precocious-1D (crp-1D)*, which strongly enhanced the precocious-flowering phenotype to skip the vegetative phase (Fig. 1A–C, E, Supplementary Table S1). The phenotype of crp-1D; 35S::FT plants was very similar to that of soc1-101D; 35S::FT plants (Fig. 1D), implying that crp-1D mutation causes up-regulation of genes acting downstream of FT, such as SOC1.

In contrast to the strong enhancement of the 35S::FT phenotype, *crp-1D* single mutation in the wild-type background had a moderate effect, leading to a slightly early-flowering phenotype.
in both inductive long-day and non-inductive short-day conditions (Fig. 1E, F, Supplementary Table S2). crp-1D also showed a dominant effect in the wild-type background (Fig. 1E, Supplementary Table S1).

**Identification of the CRP gene**

To obtain knowledge of the molecular nature of the CRYPTIC PRECOCIOUS (CRP) gene, map-based cloning was performed using crp-1D. The CRP locus was mapped to a 33.5 kb interval between F5L10 and F6N23 at the top of chromosome 4 where six genes are predicted (Supplementary Fig. S1). The only non-synonymous substitution found in the coding region of the crp-1D mutant was a C-to-T substitution in At4g00450. No obvious differences in expression levels of the other genes were observed, indicating that nucleotide substitutions in the intergenic regions causing elevated or ectopic

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**Fig. 1** Flowering phenotype of the crp-1D mutant and gene structure and expression patterns of CRP. (A–D) Effect of crp-1D and soc1-101D on the early-flowering phenotype of 35S::FT. 35S::FT #1-5C (A), 35S::FT #1-5C/--; crp-1D/+ (B), 35S::FT #1-5C; crp-1D (C) and 35S::FT #1-5C; soc1-101D plants (D). ‘Transgene symbol/–’ and ‘mutation symbol/+’ indicate the hemizygote and heterozygote, respectively. (E) Flowering time of 35S::FT #1-5C/+ plants under long-day conditions. There is a statistically significant difference (Student’s t-test, P < 0.005) with CRP+ (solid bars). (F) Flowering time of the crp-1D mutant under short-day conditions. There is a statistically significant difference (Student’s t-test, P < 0.005) between the two genotypes. (G) CRP gene structure and positions of mutations and T-DNA or Ds insertions in crp alleles. Boxes and lines indicate exons and introns of CRP, respectively; gray and green boxes represent untranslated regions and coding regions, respectively. (H) Semi-quantitative RT–PCR analysis of CRP expression in the wild type and crp mutants. Plants were harvested on day 10 (Col background; left panel) or 7 (Ler background; right panel). (I–L) Spatial expression patterns of CRP. Whole-mount preparation and longitudinal section through the SAM of 3-day-old (I, J) and 10-day-old (K, L) CRP::GUS seedlings. Plants were grown under long-day conditions and subjected to GUS staining for 12–48 h. (M–R) Subcellular localizaton of CRP and crp-1D proteins. Onion epidermal cells were bombarded with GFP fusion constructs, 35S::CRP-GFP (M–O) and 35S::crp-1D-GFP (P–R). Dark field images (M, P), bright field images (N, Q) and merged images (O, R). Scale bars: 5 mm in A and C, 2 mm in B and D, 1 mm in I and K, and 0.1 mm in J, L, O and R. In E and F, the numbers of rosette leaves are the average of at least 11 plants. Error bars indicate the SD. Additional data and statistics of the data are summarized in Supplementary Tables S1 and S2.
expression would be unlikely to be responsible for the crp-1D phenotype. Thus, we concluded that At4g00450 is the CRP gene. CRP was recently identified independently as the CENTER CITY (CCT) gene, which is required for proper embryogenesis (Gillmor et al. 2010). By cDNA cloning, we found that the actual coding region of CRP is comprised of 12 exons spanning 8.7 kb (Fig. 1G, DDBJ accession No. AB690343) and is different from the publicly available annotated unit adopted for CCT (encoding a protein of 2,144 amino acids; Gillmor et al. 2010). The CRP gene encodes a protein of 2,235 amino acids, which corresponds to the Arabidopsis counterpart of MED12 protein (Gillmor et al. 2010), a subunit of the transcriptional Mediator complex (Bäckström et al. 2007) (Supplementary Fig. S2). crp-1D mutation caused an amino acid substitution from serine (S) to phenylalanine (F) at position 99 (Fig. 1G, Supplementary Fig. S3). crp-1D mutation had no effect on the mRNA level (Fig. 1H).

Spatial expression patterns of CRP were examined using transgenic plants expressing the β-glucuronidase (GUS) reporter gene under the control of a 2.0 kb genomic sequence upstream of the initiation codon (CRP::GUS). Ubiquitous expression was observed in seedlings and young plants, with higher levels in vascular tissue and the shoot apex (Fig. 1I–L). In mature plants, strong expression was observed in young floral buds and developing floral organs as described above. To examine the subcellular localization of CRP protein, green fluorescent proteins (GFPs) fused to full-length wild-type CRP (CRP–GFP) and S99F mutant CRP (crp-1D–GFP) were expressed in onion epidermal cells. Both CRP–GFP and crp-1D–GFP fusion proteins were localized in the nuclear region in a similar manner (Fig. 1M–R).

Effects of crp loss-of-function mutations on flowering time

To investigate whether the CRP gene has any role in the flowering process, we obtained a Ds transposon insertion line in the Landsberg erecta (Ler) background (crp-2) and two T-DNA insertion lines in the Columbia (Col) background (crp-3 and crp-4) (Fig. 1G). Reverse transcription–PCR (RT–PCR) analysis revealed no accumulation of the CRP full-length transcript in crp-2, crp-3 or crp-4 plants (Fig. 1H). As homozygous crp loss-of-function plants were sterile, we analyzed the phenotypes in homozygous mutants segregating from self-fertilized heterozygous crp plants.

crp loss-of-function mutants flowered much later than the wild type in long-day conditions (Fig. 2A, Supplementary Table S2) (see also Gillmor et al. 2010), whereas no significant difference from the wild type was observed in short-day conditions (Fig. 2B, Supplementary Table S2). This phenotype is comparable with that of loss-of-function mutants of genes in the photoperiod pathway, such as ft, co and pft1/med25 (Koornneef et al. 1991, Cerdán and Chory 2003), suggesting that CRP is likely to be a promoter of flowering acting in the photoperiod pathway.

To explore further the molecular basis of the late-flowering phenotype in crp, we first examined expression levels of genes which are regulated by FT as well as genes with important roles in the FT-dependent flowering pathway, in crp loss-of-function mutants and in wild-type plants. While the mRNA level of CO, an upstream promoter of FT, remained unchanged, the mRNA level of FLC, a strong repressor of FT and TSF, was increased in crp-3 and crp-4 plants at every time point examined (Fig. 2C). Consistent with this, FT and TSF mRNA levels were much lower in crp loss-of-function mutants than in wild-type plants (Fig. 2C). The robust expression of the NRT1.7 gene in crp mutants, which has been reported as a marker gene of phloem companion cells of mature leaf minor veins (Fan et al. 2009), confirmed that the reduction of FT and TSF expression is likely to be due to the direct effect of crp mutation rather than the secondary effect of disturbed vascular patterning in crp (Supplementary Fig. S5A; details are described later). In the shoot apical region, expression of FD, encoding an FT partner bZIP transcription factor, and SOC1 and FUL, downstream targets of FT, were significantly reduced in crp mutants (Fig. 2D).

This is again consistent with the increased level of FLC which is a negative regulator of FD and SOC1 expression in the shoot apex (Searle et al. 2006). That the reduced expression of FD, SOC1 and FUL was not the consequence of possible abnormal shoot apical morphology in the crp mutant or unequal collections of shoot apical samples among genotypes was confirmed by monitoring the expression level of the SHOOTMERISTEMLESS (STM) gene (Long et al. 1996) (Supplementary Fig. S5B). In accordance with the reduced expression of flowering promoters, LFY and AP1 expression was not induced in crp mutants even at day 15 when these floral meristem identity genes are up-regulated in wild-type plants (Fig. 2D).

Because CRP seems to act both upstream and downstream of the FT-dependent flowering pathway, we next analyzed the genetic interaction between CRP and FT. Since the 3SS::FT transgene was silenced in F2 progeny from the cross between 3SS::FT and crp T-DNA insertion alleles (data not shown), we instead used a SULTR2;1::FT transgenic line in which the FT gene is specifically expressed in the phloem (Abe et al. 2005) to examine whether the artificial expression of FT can suppress the late-flowering phenotype of crp loss-of-function mutants. Introduction of SULTR2;1::FT partially, but not completely, suppressed the late-flowering phenotype of crp-3 and crp-4 (Fig. 2E, Supplementary Table S3). This result is consistent with CRP controlling not only the expression of FT, but also the expression of downstream genes of FT in an, in part, FT-dependent manner. This was further supported by the observation that crp; ft-2 double mutants flowered later than the ft-2 single mutant (Fig. 2A, Supplementary Table S4). A tsf loss-of-function mutant (tsf-1) had no effect on the flowering time of crp, whereas both ft and soc1 mutations showed an additive effect on the late-flowering phenotype of the crp single mutant (Fig. 2A, Supplementary Table S4).
function mutants, crp; flc-3 double mutants were compared with crp single mutants. The crp; flc-3 plants flowered earlier than crp single mutants under long-day conditions (Fig. 2A, Supplementary Table S4), suggesting that the increased expression of FLC does partially explain the flowering delay of crp mutants. This was further confirmed by the observation that the reduction of FT and TSF in the crp single mutant was partially restored in crp; flc-3 double mutant plants (Fig. 2F, Supplementary Fig. S5).

**Effect of overexpression of CRP on flowering time**

To understand further the function of CRP, we generated transgenic plants overexpressing CRP under the control of the 35S promoter (35S::CRP), and obtained 15 independent lines.
Interestingly, the majority of 35S::CRP transgenic plants showed a flowering time phenotype either indistinguishable from that of wild-type plants or similar to that of crp loss-of-function mutants in both long-day and short-day conditions. In the latter case, the extent of delay in long days was not as severe as that of crp loss-of-function mutants (Fig. S3A–C, Supplementary Table S5). A similar observation has been reported for Drosophila kohtalo (kto), which is the Drosophila counterpart of CRP encoding MED12 (Janody et al. 2003). It is likely that the excessive amount of MED12 protein (CRP in Arabidopsis and kto in Drosophila) interferes with the proper formation of protein complexes that contain MED12. Consistently, expression levels of FT and TSF were reduced in the 35S::CRP plants as in the case of crp loss-of-function mutants (Supplementary Fig. S6).

Based on the phenotypes of crp loss-of-function mutants and 35S::CRP transgenic plants, it is suggested that crp-1D acts as neither a dominant-negative mutation nor a gain-of-function mutation which increases protein stability as reported for other genes (El-Assal et al. 2001, Reed 2001, Dieterle et al. 2005). By taking advantage of 35S::CRP transgenic plants, we further addressed the question of whether the mutant crp-1D protein functions in the same molecular context as that of the wild-type CRP protein. If this is indeed the case, then increased amounts of wild-type CRP protein should attenuate the crp-1D phenotype. In contrast, there should be little effect if crp-1D protein functions in a different molecular context. As shown in Fig. 3D, the crp-1D phenotype was significantly attenuated by the introduction of the 35S::CRP transgene (see also Supplementary Table S6). Therefore, it is likely that crp-1D acts in the same molecular context as that which involves wild-type CRP, rather than acting as a ‘neomorphic’ allele whose activity is unrelated to normal CRP function. As described below, this was further supported by the observation that expression of the crp-1D phenotype required functional MED13/MAB2, another subunit functioning together with MED12/CRP (see Genetic interaction of MED12/CRP and MED13/MAB2 in the flowering process and auxin response).

### Genetic interaction between the dominant crp-1D mutation and flowering-related genes

Since crp-1D is likely to act in the same molecular context as the wild-type CRP, it will shed some light on the normal CRP function. With this in mind, genetic and regulatory interactions between crp-1D and flowering-related genes were investigated. We first examined the effect of crp-1D mutation on the precocious-flowering phenotype of 35S::TSF. As expected from redundancy between FT and TSF, F1 progeny of crosses between crp-1D and 35S::TSF showed a much earlier flowering phenotype than 35S::TSF (Fig. 4A, Supplementary Table S1). Previously, it was reported that FT, TSF, SOC1 and LFY have both overlapping and independent functions such that soc1-101D and 35S::LFY, respectively, had additive effects on early-flowering phenotypes of 35S::FT or 35S::TSF (Kardailsky et al. 1999, Kobayashi et al. 1999, Moon et al. 2005, Yamaguchi et al. 2005). This raises the possibility that crp-1D may enhance the precocious-flowering phenotype of FT- or TSF-overexpressing plants via up-regulation of SOC1 or LFY. To test the interaction between crp-1D and these genes, we generated plants which have a crp-1D mutation with soc1-101D or 35S::LFY. F1 progeny of a cross between crp-1D and 35S::LFY flowered slightly earlier than 35S::LFY (Fig. 4A, Supplementary Table S1). In contrast, crp-1D did not enhance the early-flowering phenotype of soc1-101D (Fig. 4A, Supplementary Table S1). In addition, no enhancement by crp-1D was observed for the early-flowering phenotype of 35S::AP1 (Fig. 4A, Supplementary Table S1). These results suggest that crp-1D may affect step(s) upstream of SOC1 and AP1.

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**Fig. 3** CRP expression and flowering phenotype of CRP-overexpressing plants. (A) CRP expression levels in wild-type Col and CRP-overexpressing plants. Aerial parts of seedlings grown under long-day conditions were harvested on day 12 for qRT–PCR analysis. (B and C) Flowering time of wild-type Col and CRP-overexpressing plants under long-day (B) and short-day (C) conditions. There is a statistically significant difference (Student’s t-test, P < 0.005) between the wild type and 35S::CRP in B. (D) Flowering time of crp-1D plants with the 35S::CRP transgene under long-day conditions. There is a statistically significant difference (Student’s t-test, P < 0.005) between CRP (solid bar) and crp-1D in the 35S::CRP background. Error bars in A indicate the SE. In B–D, the numbers of rosette leaves are the average of at least 10 plants. Error bars indicate the SD. Additional data and statistics of the data are summarized in Supplementary Tables S5 and S6.

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**Table**

- **Supplementary Table S5**: Description of the table.
- **Supplementary Table S6**: Description of the table.
Fig. 4 Effect of crp-1D mutation on the flowering phenotype. (A) Flowering time of plants overexpressing flowering regulator genes with or without the dominant crp-1D mutation. There is a statistically significant difference (Student’s t-test, P < 0.005) with CRP+ (solid bars). (B) CO, FLC, FT and TSF expression levels in wild-type Col and crp-1D plants. Aerial parts of seedlings grown under long-day conditions were harvested on days 5, 7 and 10 for qRT–PCR analysis. (C) Flowering time of double mutant plants with the crp-1D mutation and loss-of-function mutation of flowering regulator genes. (D) FD, SOC1, FUL, LFY and AP1 expression levels in wild-type Col and crp-1D plants. Shoot apical regions of seedlings grown under long-day conditions were harvested on days 5, 7 and 10 for qRT–PCR analysis. (E) Flowering time of SULTR2;1::FT−/−; crp-1D/++ plants with an fd-1 or soc1-2 mutation. (F) FD, SOC1, FUL, LFY and AP1 expression levels in wild-type Col, crp-1D, ft-2 and ft-2; crp-1D plants. Shoot apical region of seedlings grown under long-day conditions were harvested on days 7 and 10 for qRT–PCR analysis. In A, C and E, the numbers of rosette leaves are the average of at least eight plants. Error bars indicate the SD. Additional data and statistics of the data are summarized in Supplementary Tables S1, S7 and S8. Error bars in B, D and F indicate the SEM (n = 9).
We next compared the temporal regulation of FLC, CO, FT and TSF in crp-1D and wild-type plants. With the possible exception of FLC in young seedlings (5 d), no significant differences were observed (Fig. 4B). This suggests that crp-1D may have an effect on step(s) downstream of FT and/or TSF. To test for genetic interaction between crp-1D and FT and TSF, we generated crp-1D; ft-2 and crp-1D; tsf-1 double mutants. The early-flowering phenotype of crp-1D was suppressed by ft-2, but was not affected by tsf-1 in long-day conditions (Fig. 4C, Supplementary Table S7), suggesting that crp-1D affects flowering mainly in the FT-dependent pathway.

The results so far described prompted us to examine the relationship between crp-1D and developmental kinetics at the shoot apex in steps downstream of FT, such as FD, SOC1 and FUL. The expression level of FD was not significantly affected in crp-1D (Fig. 4D), and fd-1 had little effect on the early-flowering phenotype of crp-1D (Fig. 4C, Supplementary Table S7). In addition, fd-1 only very weakly suppressed the early-flowering phenotype of crp-1D/+; SULTR2;1::FT/+ (Fig. 4E, Supplementary Table S8). These results suggest that crp-1D is likely to regulate flowering independently of FD. In contrast, the expression level of SOC1 and FUL, both of which are regulatory targets of FT, was elevated in crp-1D at every time point tested (Fig. 4D). Consistently, the precocious-flowering phenotype of crp-1D plants was strongly suppressed by soc1-2 (Fig. 4C, Supplementary Table S7). In addition, soc1-2 suppressed the early-flowering phenotype of SULTR2;1::FT/–; crp-1D/+ plants (Fig. 4E, Supplementary Table S8). ful-2, which has little effect on flowering time in the wild-type background, did not delay flowering in crp-1D (Fig. 4C, Supplementary Table S7). These results suggest that crp-1D acts in the same pathway in which endogenous and overexpressed FT regulates SOC1 and FUL expression. This was further supported by the observation that up-regulation of SOC1 and FUL in crp-1D was strongly suppressed by introduction of ft-2, while the expression level of FD was little affected by genotypes (Fig. 4F). As expected from elevated SOC1 expression, levels of LFY and AP1 expression were increased in crp-1D compared with the wild type in an FT-dependent manner (Fig. 4D, F). Since Ify loss-of-function mutants (ify-1) had no effect on the precocious-flowering phenotype of crp-1D (Fig. 4C, Supplementary Table S7), crp-1D may affect LFY (and AP1, as well) expression indirectly.

Genetic interaction between MED12/CRP and MED13/MAB2 in the flowering process and auxin response

It was reported that MED12 and MED13 act as a pair in the CDK8 Mediator kinase module in yeast and Drosophila (Samuelsan et al. 2003, Kornberg 2005, Bäckström et al. 2007, Loncle et al. 2007). Moreover, mutations in Drosophila MED12 and MED13 cause identical phenotypes across multiple traits, again supporting the idea that these two components act closely together in the same module of the Mediator (Treisman 2001, Janody et al. 2003). To test whether this is also true for Arabidopsis MED12/CRP and MED13/MACCHI-BOU2 (MAB2), we compared the flowering phenotype of mab2 loss-of-function mutant (mab2-1) with that of crp-2 in the Ler background. Similar to the crp-2 mutant, mab2-1 flowered later than the wild type in long-day conditions and no difference in flowering time was observed in short-day conditions (Fig. 5A, B, Supplementary Table S2). We further examined the genetic interaction between CRP and MAB2. In Drosophila, the phenotype of med12/kohtalo; med13/skulid double mutants was indistinguishable from that of either single mutant (Janody et al. 2003, Loncle et al. 2007). Similarly, the crp-2; mab2-1 double mutant and each parental single mutant showed the same phenotype in seedling morphology and flowering time in long-day conditions (Fig. 5C, Supplementary Table S9) (Ito et al. 2011). These observations suggest that MED12/CRP and MED13/MAB2 act closely together in the same pathway in Arabidopsis development. We further examined genetic interaction between crp-1D and mab2, mab2-1 completely suppressed the early-flowering phenotype of crp-1D and the precocious-flowering phenotype of 35S::FT/+; crp-1D/+ (Fig. 5D, E, F–H, Supplementary Table S10), indicating that crp-1D requires functional MED13/MAB2 for its effect on flowering. These results confirmed that MED13/MAB2 is required for crp-1D-mediated floral induction as well.

In addition to a late-flowering phenotype, crp loss-of-function mutants showed pleiotropic phenotypes. These include occasional embryo lethality, such that a certain fraction of seeds on the selfed crp/+ heterozygote were aborted (Fig. 6A), abnormal shape of cotyledons (Fig. 6B), delay in early growth (Fig. 6C), dwarfism (Fig. 6D), abnormal leaf shape and vascular patterning (Fig. 6E), ectopic bract-like organs subtending the first few flowers as reported for mab2 (Ito et al. 2011) (Fig. 6F), abnormal development of floral organs including chimerism (Fig. 6G, H), and sterility due to poor production of pollen grains and anther dehiscence (Fig. 6I). In accordance with the floral phenotype, strong expression was observed in young floral buds and developing floral organs (Fig. 6K, L). Interestingly, crp-1D showed the opposite effect in some of the seedling phenotypes (Fig. 6A, B). These phenotypes were in agreement with a recent report that center city-1 (cct-1), another loss-of-function allele of MED12, affected the timing of a variety of morphogenetic events, including embryogenesis, specification of peripheral-abaxial identity and vascular patterning (Gillmor et al. 2010), supporting the generally accepted concept that MED12 is involved in diverse developmental aspects through gene regulation.

Interestingly, gynoecium morphology of crp loss-of-function mutants (Fig. 6J) resembles that of auxin-related mutants such as ettin (ett), namely shorter valves, longer style and elongated basal internode (Liu and Meyerowitz 1995, Nemhauser et al. 2000, Franks et al. 2002). A similar gynoecium phenotype was also observed in mab2 mutants. It was recently reported that double mutant combinations of mab2 and pinoid (pid), which
abolishes directional flow of auxin in developing embryo (Robert and Offringa 2008), caused severe defects in organogenesis such that cotyledons and bilateral symmetry are completely missing (Ito et al. 2011). These observations prompted us to generate pid; crp double mutants. Similar to the pid-2; mab2-1 double mutant, seedlings of pid-2; crp-2, pid-3; crp-3 and pid-3; crp-4 double mutants completely lacked cotyledons (Fig. 7). These results suggest that MED12/CRP has a similar role in auxin-dependent organogenesis to that of MED13/MAB2, supporting the notion that these two act closely together possibly within the same module of the Mediator complex.

Discussion

In this study, we identified a dominant mutation, crp-1D, as a genetic modifier of the precocious-flowering phenotype of 35S::FT. Map-based cloning revealed that crp-1D is a missense mutation of the Arabidopsis gene for the MED12 subunit of the Mediator. Genetic and molecular analysis of both dominant crp-1D and recessive crp loss-of-function alleles showed that MED12/CRP is a novel flowering regulator which suppresses FLC expression, promotes FT and TSF expression and up-regulates SOC1 and FUL mainly in an FT-dependent manner under long-day conditions (Supplementary Fig. S7).
Although protein interaction has not yet been successfully demonstrated for MED12/CRP and MED13/MAB2, our present work and previous work (Gillmor et al. 2010, Ito et al. 2011) strongly suggest that these two proteins act closely together in the same pathways to regulate various aspects of plant development from embryogenesis to floral morphogenesis, some of which are mediated through modulation of the auxin response (Ito et al. 2011). Among them, floral transition represents a developmental process largely independent of auxin signaling and a unique common target process shared by MED12/CRP and MED13/MAB2 and core subunits of the Mediator, such as MED8 and MED25/PFT1.

**CRP encodes the MED12 subunit of the Mediator**

We have shown that the CRP gene encodes a protein of 2,235 amino acids, which corresponds to the Arabidopsis counterpart of the MED12 subunit of the Mediator (Supplementary Fig. S2). The gene for MED12 was recently identified independently as the CCT gene required for proper embryogenesis (Gillmor et al. 2010). By cDNA analysis, we found that the actual coding region of MED12/CCT/CRP differs from the publicly available annotated unit (Fig. 1G). MED12/CCT/CRP is ubiquitously expressed in seedlings and young plants, with higher levels of expression in vascular tissues and the shoot apex.
A dominant \textit{crp-1D} mutation caused an S-to-F amino acid substitution at position 99. This serine residue is not located in either the conserved MED12 domain or signature sequence motifs (SSMs) of MED12 defined by Bourbon (2008), and is not conserved even among plant MED12 proteins (Supplementary Fig. S3). This makes interpretation of the \textit{crp-1D} mutation difficult. However, that \textit{crp-1D} is likely to act in the same molecular context as that involving wild-type MED12/CRP is suggested by its requirement for MED13/MAB2 to exert its phenotypic effect and attenuation of the \textit{crp-1D} phenotype by an increased level of wild-type CRP (Figs. 3, 5). One interesting feature of \textit{crp-1D} is its limited effect on development (mainly restricted to the floral transition) as opposed to pleiotropic effects throughout the entire life cycle observed in \textit{cct} and \textit{crp} loss-of-function mutants (Figs. 5–7) (Gillmor et al. 2010). Even during flowering, \textit{crp-1D} affects only a subset of genes whose expression is affected by \textit{crp} loss-of-function mutations (Figs. 2, 4). These findings are reminiscent of a recently reported dominant allele of \textit{CURLY LEAF} (\textit{CLF}), the Arabidopsis ortholog of \textit{Enhancer of Zeste} (Doyle and Amasino 2009). \textit{clf} loss-of-function mutations cause a severe pleiotropic phenotype and ectopic expression of various genes including \textit{AGAMOUS} (\textit{AG}). In contrast, the effect of a dominant \textit{clf-59} mutation (P704S) is limited to flowering, and ectopic \textit{AG} expression was not observed. \textit{crp-1D} and \textit{clf-59} may be useful tools to understand further the biochemical function of the respective proteins.

**MED12/CRP is a novel flowering regulator with multiple target steps**

Genetic and molecular analysis of both dominant \textit{crp-1D} and loss-of-function \textit{crp} alleles suggested that MED12/CRP acts as a flowering regulator with multiple target steps. That the Mediator plays multiple roles in the regulation of flowering is an emerging scheme, as clearly demonstrated by recent studies of MED25/PFT1 (Kidd et al. 2009, Elfving et al. 2011, In‘igo et al. 2011). Our present work with MED12/CRP gives further supports to this idea (Supplementary Fig. S7).

First, MED12/CRP is a negative regulator of \textit{FLC} expression (T-bar #1 in Supplementary Fig. S7). An increased \textit{FLC} mRNA level in \textit{crp} loss-of-function mutants (Fig. 2C) is in part responsible for the late-flowering phenotype (Fig. 2A). It also, at least in part, explains reduced expression levels of downstream target genes of \textit{FLC}, namely \textit{FT} and \textit{TSF} in the vasculature, and \textit{FD} and \textit{SOC1} in the shoot apex (Searle et al. 2006) (Fig. 2C, D). Genetic analysis suggests a similar role for MED13/MAB2 as well (Fig. 5). It is interesting to note that genome-wide association analysis of recombinant inbred lines revealed a promising association at the CRP locus with the \textit{FLC} mRNA level.
although its close linkage (<100 kb) with FRIGIDA, whose allelic variation is a major determinant of the FLC expression level among natural accessions, made it difficult to be conclusive (Atwell et al. 2010). Involvement of the Mediator in regulation of FLC expression has recently been shown for several core subunits, such as MED8 and MED25/PFT1 (Kidd et al. 2009, Eilving et al. 2011). Similar to crp loss-of-function mutants, med8 and med25/pft1 mutants have increased FLC and reduced FT expression levels and showed the late-flowering phenotype in long-day conditions. Taken together, these and our results suggest that the Mediator plays an important role in suppression of FLC. In zebrafish and humans, it was shown that the SOX9 transcription factor physically or functionally interacts with MED12 and MED25 (Zhou et al. 2002, Rau et al. 2006, Nakamura et al. 2011). Therefore, it is likely that MED12/CRP, and possibly MED13/MAB2 as well, act with MED25/PFT1 in the same Mediator complex in regulation of FLC.

Secondly, MED12/CRP also positively regulates FT and TSF independently of FLC (arrow #2 in Supplementary Fig. S7). Reduced expression levels of FT and TSF were observed in crp loss-of-function mutants. The increased FLC level mentioned above is not the sole cause, since only partial recovery of expression of these two genes was observed in crp; flc double mutants (Fig. 2F). There should be another pathway independent of FLC. This is not through the transcriptional regulation of CO, because neither dominant crp-1D nor loss-of-function crp mutants showed a significant change in CO expression (Figs. 2C, 4B). MED12/CRP, and possibly also MED13/MAB2, may act either downstream of CO expression or independently of the CO function. Interestingly, it was shown that MED25/PFT1 regulates FT (but not TSF) in both CO-dependent (through up-regulation of CO expression) and CO-independent pathways (Iníigo et al. 2011). MED12/CRP may participate in the latter pathway. Alternatively, since MED12/CRP regulates both FT and TSF, it may represent a unique pathway.

Thirdly, MED12/CRP acts downstream of FT in regulation of SOC1 and FUL (arrow #3 in Supplementary Fig. S7). SOC1 and FUL expression in the shoot apical region was severely reduced in crp-3 and crp-4 mutants and increased in the crp-1D mutant (Figs. 2D, 4D). Reduction of the SOC1 level in crp loss-of-function mutants is, again, partly explained by increased FLC, which is a negative regulator of SOC1 (Fig. 2F). In contrast, increased SOC1 and FUL levels in crp-1D clearly required functional FT (Fig. 4F), suggesting that MED12/CRP acts with or downstream of FT. That MED12/CRP acts in part downstream of FT is further supported by partial suppression of the precocious-flowering phenotype of FT-overexpressing (SULT2;1:FT) plants by crp loss-of-function mutations (Fig. 2E). It has been shown that FT is an upstream activator of SOC1 (Yamaguchi et al. 2005, Yoo et al. 2005, Searle et al. 2006) and FUL (Abe et al. 2005, Tepfer-Bammolker and Samach 2005, Wigge et al. 2005) expression. MED12/CRP, and possibly also MED13/MAB2, may act with FT and transcription factors such as FD in transcriptional regulation of FUL and SOC1. Again, it is interesting to note that it was recently suggested that MED25/PFT1 is also involved in regulation of SOC1 expression (Iníigo et al. 2011). MED12/CRP, and possibly also MED13/MAB2, and MED25/PFT1 may act in the same pathway for SOC1 activation.

Recent studies in animals showed that the CDK8 module (comprising CDK8, CycC, MED12 and MED13) is stable and biochemically active apart from the Mediator (Knuesel et al. 2009). CDK8 phosphorylates histone H3S10, and the kinase activity of the CDK8 module is dependent on MED12 in humans (Knuesel et al. 2009). MED12 can repress transcription through interaction with histone H3K9 methyltransferase in HeLa cells (Ding et al. 2008). MED12 acts as a chromatin regulator and a ‘hub’ protein that modifies expression of a large number of genes from multiple signaling pathways in C. elegans (Moghal and Sternberg 2003, Lehner et al. 2006). These observations may support the possibility that MED12/CCT/CRP could be involved in the epigenetic control of genes such as FLC or FT in the context of the CDK8 module outside of the Mediator. However, our observations discussed above are slightly in favor of the alternative view that it functions in the context of the Mediator with core subunits.

**MED12/CCT/CRP and MED13/GCT/MAB2 in plant development**

In addition to the control of flowering, MED12/CRP and MED13/MAB2 are involved in various developmental processes. It was shown that MED12/CCT and MED13/GCT are absolutely required for KANADI expression during early embryogenesis and are important for the proper temporal regulation of genes involved in radial pattern formation during embryogenesis (Gillmor et al. 2010). This is likely to be achieved in part through modulation of the auxin response, as clearly demonstrated for MED13/MAB2 (Ito et al. 2010). That MED12/CRP closely shares the role in the auxin response with MED13/MAB2 was supported by a similar genetic interaction of crp and mab2 mutations with pid (Fig. 7). The fact that the gynoecium phenotype of the crp loss-of-function mutants is reminiscent of that of auxin-related mutants such as ett (Fig. 6I) lends additional support for the role of MED12/CRP in the auxin response. Taken together, MED12/CCT/CRP and MED13/GCT/MAB2 function closely together to regulate the auxin response. Dwarfism (Fig. 6D), inflorescence defects including ectopic bract formation (Fig. 6F), and various floral defects (Fig. 6G–I) observed in crp loss-of-function mutants, some of which are shared by mab2 (Ito et al. 2010), imply that MED12/CCT/CRP, and possibly also MED13/GCT/MAB2, are involved in other aspects of development. Some are probably through modulation of the auxin response. In contrast to the role in regulation of flowering, which is shared by core subunits of the Mediator, such as MED8 and MED25/PFT1, the roles of MED12/CCT/CRP and MED13/GCT/MAB2 in other aspects of development, such as embryogenesis, seem to be unique to these two subunits of the Mediator.
MED12 and MED13 belong to the CDK8 module of Mediator which contains CDK8 and CycC subunits. There has been no report of a mutant phenotype and functional analysis of CycC orthologs in plants (Wang et al. 2004). In Arabidopsis, HUA ENHANCERS (HEN3) encodes the CDK8 subunit, and is expressed in proliferating tissues, such as the SAM, young leaves and floral buds (Wang and Chen 2004, Wang et al. 2004). However, hen3 mutants do not show pleiotropic developmental defects such as those observed in cct/crp and gct/mab2 mutants. HEN3 protein interacts with LEUNIG (LUG) and SEUSS (SEU), which repress AG and are involved in auxin signaling during floral organ development (Navarro et al. 2004, Pfleger and Zambrayski 2004, Gonzalez et al. 2007).

Interestingly, crp and gct/mab2 mutants showed floral defects reminiscent of lug and seu mutants (Liu and Meyerowitz 1995, Franks et al. 2002, Gillmor et al. 2010) (Fig. 6G–J). It was reported that gct: kan double and hen3; hua1; hua2 triple mutants showed enhanced loss of floral organ identity, although gct/mab2 or hen3 single mutants did not show major defects in floral organ specification (Wang and Chen 2004, Gillmor et al. 2010). These observations suggest that floral organ development may represent a shared developmental program regulated by the CDK8 module proteins of the Mediator possibly through modulation of the auxin response.

Does Mediator contribute to feed-forward loops in flowering regulation?

Flowering of monocarpic plants such as A. thaliana is an irreversible phase transition. It has been suggested that feed-forward loops constructed from key regulators are important to minimize external noises to achieve robustness of developmental systems (Mangan and Alon 2003, Dekel et al. 2005, Pastore et al. 2011). As summarized in a simplified model (Supplementary Fig. S7), MED12/CRP, and possibly also MED13/MAB2, are involved in multiple steps in the regulation of production of florigen (FT and TSF proteins) and in multiple downstream steps of florigen action. Similarly, it has been shown that a core subunit of the Mediator, MED25/PFT1, regulates multiple steps both upstream and downstream of FT florigen. These observations and the possible involvement of genetic variation at the CRP locus in natural variation of the FLC expression level among various accessions (Atwell et al. 2010) prompt us to propose a hypothesis that the Mediator may make an important contribution to flowering as a key factor to construct a cohesive feed-forward regulatory motif. Further studies to examine the role of other core subunits of the Mediator in flowering and identification of transcription factors involved will be important steps to test this hypothesis.

Materials and Methods

Plant materials and growth conditions

Col-0 and Ler were used as wild types. T-DNA insertion alleles of CRP in the Col background (SALK_108241, crp-3; and SALK_124276; crp-4) were obtained from the Arabidopsis Biological Resource Center (ABRC). crp-3 and crp-4 were backcrossed with wild-type Col-0 three times prior to analysis. crp-3 and crp-4 correspond to cct-2 and cct-3, respectively (Gillmor et al. 2010). A Ds-transposon insertion allele in the Ler background (SET_3.701; crp-2) was kindly provided by Dr. V. Sundaresan (Parinov et al. 1999), and was backcrossed with wild-type Ler three times prior to analysis. crp-1D was isolated as an enhancer mutation of the 35S::FT #1-5C in the Col background (Kobayashi et al. 1999). Mutagenesis was carried out by soaking seeds in 0.1% EMS for 16 h. The resulting M1 population (5,000 seeds) was sown and self-fertilized, and the M2 population was screened for enhancers of the early-flowering phenotype in constant light conditions (24°C). crp-1D L was obtained by five backcrosses with wild-type Ler. CRP::GUS and 35S::CRP (see below for the transgene construction) were generated in this work. The ft-2 allele from the Ler accession was introgressed into Col by five backcrosses to generate ft-2 (Col). Previously published plant materials used in this work are as follows: 35S::FT #11-1 (a strong line), 35S::FT #1-5C (a weak line), SULTR2;1::FT #1-a, 35S::TSF #4-1 (a weak line), 35S::LFY (DW151.2.5C), 35S::AP1, soc1-101D, ftc-3, tsf-1, ful-1 (Col). soc1-2, ful-2, lfy-1, pid-3 are in the Col background; mab2-1 and pid-2 are in the Ler background. Further information is given in Supplementary Table S11.

For analysis of the flowering time phenotype, plants were grown on soil or half-strength Murashige and Skoog (MS; Wako) medium supplemented with 0.5% sucrose and 0.4% Gellan Gum (Wako) at 22°C under long-day (16 h light/8 h dark) conditions with white fluorescent light (~80 μmol m⁻² s⁻¹) or short-day (8 h light/16 h dark) conditions with white fluorescent light (~100 μmol m⁻² s⁻¹). Flowering time was measured by counting rosette and cauline leaves. Bars in the figures show the number of rosette leaves. For expression analysis, plants were grown on half-strength MS medium supplemented with 0.5% sucrose and 0.4% Gellan Gum. Seeds were stratified by keeping them at 4°C for 2–4 d and then transferred to 22°C long-day conditions, which was defined as day 0. Plants were harvested at Zeitgeber time (ZT) 15 on each day.

RT–PCR analysis

RNA was extracted using TRizol reagent (Invitrogen) and was treated with RNase-free DNase I (Invitrogen) according to the manufacturer’s instructions. Total RNA (0.5 μg) was reverse-transcribed in a 20 μl reaction mixture using SuperScript III (Invitrogen). After the reaction, 10 μl of the mixture was diluted with 240 μl of water, and 5 μl aliquots were analyzed. Quantitative RT–PCRs (qRT–PCRs) were performed using SYBR Premix Ex Taq II (TAKARA). Primers used in this study are listed in Supplementary Table S12. qRT–PCR results normalized to ACT2, NRT1.7 or STM show the average of nine different reactions (biological×technical triplicate), except for the data shown in Fig. 3A (only technical triplicate).
Relative expression was obtained as the ratio to the level in wild-type Col harvested on the first day of the experiment. Semi-quantitative RT–PCR was performed using the primers listed in Supplementary Table S13. PCR products were electrophoresed on an agarose gel and visualized by ethidium bromide staining.

Plasmid construction and transgenic plants
To construct CRP::GUS, the GUS coding sequence from pB1101 was inserted downstream of the 2.0 kb CRP promoter fragment (2.0 kb sequence upstream of the presumptive initiation ATG codon) amplified by PCR from Col using the primers listed in Supplementary Table S14. The coding region of CRP from Col and crp-1D, respectively, was amplified by PCR using the primers listed in Supplementary Table S14 and recombined into the pENTR1A vector (Invitrogen). To construct CRP-GFP and crp-1D-GFP, the coding region of sGFP was fused, in-frame, to the C-terminus of CRP or crp-1D in the pENTR vector as described above and, after sequencing, this translational fusion construct was cloned into the binary vector pGWB2 (Nakagawa et al. 2007) using LR Recombination Reactions (Invitrogen). To construct 35S::CRP, the CRP coding sequence in the pENTR1A vector was transferred to the binary vector pGWB2 (Nakagawa et al. 2007) using LR Recombination Reactions (Invitrogen). To generate stable transgenic plants, the constructs were introduced into Agrobacterium tumefaciens strain pMP90, and Col plants were transformed by particle bombardment PDS-1000/He Biolistic Particle Delivery System (Bio-Rad). A 1 μg aliquot of plasmids was mixed with 8 μl of a pre-washed 1 μm diameter gold particle suspension (60 mg ml⁻¹), 3.3 μl of CaCl₂ (2.5 M) and 3.3 μl of spermidine (0.1 M). After incubation, the particles were washed with 80% ethanol and resuspended in 10 μl of 100% ethanol. The DNA-coated particles were fired into the onion cells using a 1,100 p.s.i. rupture disk. Onion cells were incubated in the dark at 23 °C for 24–40 h after particle delivery and the epidermal cells were observed with a confocal laser scanning microscope (FV1000, Olympus), excitation wavelength 488 nm by argon laser, emission wavelength 500–600 nm.

Supplementary data
Supplementary data are available at PCP online.

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