Mediator Subunit18 Controls Flowering Time and Floral Organ Identity in Arabidopsis

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Abstract

Mediator is a conserved multi-protein complex that plays an important role in regulating transcription by mediating interactions between transcriptional activator proteins and RNA polymerase II. Much evidence exists that Mediator plays a constitutive role in the transcription of all genes transcribed by RNA polymerase II. However, evidence is mounting that specific Mediator subunits may control the developmental regulation of specific subsets of RNA polymerase II-dependent genes. Although the Mediator complex has been extensively studied in yeast and mammals, only a few reports on Mediator function in flowering time control of plants, little is known about Mediator function in floral organ identity. Here we show that in Arabidopsis thaliana, MEDIATOR SUBUNIT 18 (MED18) affects flowering time and floral organ formation through FLOWERING LOCUS C (FLC) and AGAMOUS (AG). A MED18 loss-of-function mutant showed a remarkable syndrome of later flowering and altered floral organ number. We show that FLC and AG mRNA levels and AG expression patterns are altered in the mutant. Our results support parallels between the regulation of FLC and AG and demonstrate a developmental role for Mediator in plants.

Introduction

Mediator plays an important role in regulating RNA polymerase II (Pol II) transcription. The Mediator complex contains 22–28 subunits, and mediates interactions between transcriptional co-activators and Pol II [1,2]. Mediator is evolutionarily conserved and has an ancient eukaryotic origin [3]; it is found in organisms from fungi to mammals and plants, although the evolutionary conservation of individual subunits is moderate [4–6]. More than 30 different subunits have been described that are part of the Mediator complex in different organisms, but only about 20 subunits are found in all eukaryotes [6–8]. The others consist of either species-specific subunits or other ancillary subunits associated with activation of specific genes. Med18 is one subunit of the Mediator complex and a component of the head module that is involved in stimulating basal RNA Pol II transcription in yeast [4,9].

Arabidopsis: Mediator was first found to contain 27 subunits, and most of them are conserved in eukaryotes [5]. Until now, all the known yeast/metazoan Mediator components have been identified in plants [10]. PHYTOCHROME and FLOWERING TIME1 (PFT1), now known as MED25, was identified as a factor of a Phytochrome B (phyB) signaling pathway that promotes flowering and controls final organ size [5,11–14]. STRUWWELPETER (SWP)/MED14 was reported to be a nuclear protein that plays a role in defining the duration of cell proliferation [15]. Some Mediator subunits like MED25, MED8, MED16, and MED21 act as integrators in response to environmental cues in Arabidopsis [16–21]. MED18 is a subunit of the head submodule of the plant Mediator complex [10]. Recently, Kim et al. (2011) reported that several Mediator subunits including Mediator 18 (MED18) are required for microRNA biogenesis [22]. Little is known about Mediator function during floral organ formation or its role in the regulation of flowering time.

The transition from vegetative growth to reproductive development in Arabidopsis is regulated by multiple floral induction pathways. Genetic studies of the timing of flowering in Arabidopsis have revealed 5 major pathways [23]. The photoperiod and vernalization pathways integrate external signals into the floral decision, the autonomous and gibberellin (GA) pathways act independently of environmental cues, whereas the endogenous pathway adds plant age to the control of flowering time. The flowering pathways are interconnected and converge on a few floral integrators, such as FLOWERING LOCUS T (FT) and SUPPRESSOR OF OVEREXPRESSION OF CONSTANS1 (SOC1) or AG3 [24–26]. One important regulator of floral initiation is the MADS-box transcription factor FLOWERING LOCUS C (FLC), which acts as a negative regulator of flowering in response to both endogenous and environmental signals; it is also an integrator of the autonomous and vernalization pathways [23,27,28]. The autonomous and vernalization pathways both suppress the expression of FLC [29], resulting in a decreased expression of FLC and consequently increased expression of FT and/or SOC1 in a later developmental stage or after a prolonged exposure of plants
to low temperature [23,30]. The florigen, FT, directly regulates floral meristem identity genes such as APETALA1 (API) and initiates floral morphogenesis [31,32].

Following the vegetative to floral transition, Arabidopsis flowers develop four different organ types that are arranged in concentric whorls: the first whorl contains four sepals, the second whorl contains four petals, six stamens develop in the third whorl, and two fused carpels form in the fourth whorl. The control of floral organ identity has been intensively studied in the past 25 years. Analysis of floral homeotic mutants led to the proposal of a simple genetic model, explaining how three groups of regulatory genes (class A, B and C genes) alone or by interactions, control the organ identity of the four floral whorls [33,34]. The termination of stem cells in the floral meristem requires AGAMOUS (AG), a MADS-domain transcription factor [35]: As a class C floral homeotic gene, AG specifies stamen identity together with the B class and SEPALLATA (SEP) genes and carpel identity together with the SEP genes [34,36]. AG activates SPOROCYTELESS (SPL), which controls sporogenesis in both stamens and carpels [37]. Clearly, AG is one of the most important regulators for the floral transition, floral organ identity, and spore formation.

In this study, we describe the function of Arabidopsis Mediator subunit 18 (MED18), and show that it controls flowering time and floral organ identity by transcriptional regulation of FLC and AG.

Materials and Methods

Plant Materials and Growth Conditions

All the transgenic and mutant Arabidopsis lines used in this study were of ecotype Columbia (Col) except for the ag-1 and pi-1 mutants, which were in the Landsberg erecta (Lei) background. Plants were grown in a temperature controlled greenhouse at 22–24°C, with a relative humidity of 60%–70%, and 16 hours light and 8 hours dark photoperiod.

For short day experiments, Plants were grown in a growth chamber equipped with 40 W fluorescent light tubes with a 9 hours light and 8 hours dark photoperiod.

Analysis of Transcripts Levels

Semi-quantitative RT-PCR was used to measure the transcript levels of MED18 and the floral homeotic genes using ACTIN4 (At5g59370) and GAPC (At3G04120) as loading controls. Total RNA samples were treated extensively with RNase-free DNase I to remove any contaminating genomic DNA. First-strand cDNA was synthesized using 1 μg of total RNA in a 20 μl reaction volume using High Capacity cDNA Archive Kit from Applied Biosystems (Foster City, CA) according to the manufacture’s instructions, followed by phenol/chloroform purification, and ethanol precipitation. The cDNA were dissolved in 30 μl TE buffer and 1 μl was subjected to PCR in a 20 μl reaction volume. The RT-PCR runs were 20 to 30 cycles, depending on the linear range of PCR amplification for each gene, with cycle parameters of 94°C for 0.5 min, and 72°C for 1 min for each cycle, with a final incubation of 72°C for 10 min. API, AP2, AP3, PI, and AG, primers were designed according to published sequences [38]. All other primers designed in this study were in Table S3.

Quantitative RT-PCR was modified from a previously published method [39]. Mutant and wildtype seedling or flowers were dissected and pooled. Total RNA was extracted using RNeasy plus micro kit (Qiagen) and RNA quantity (>100 ng/μl) and purity (260/230>2.0, 260/280>1.63) were determined using a Nanodrop. RNA integrity (RIN>8.3) and 28S/18S ratio (>1.5) was assessed using a Bioanalyzer 2100 (Agilent Technologies). A quantity of 500 ng of high-quality RNA for each pooled sample was converted into cDNA using the iScript cDNA Synthesis Kit (Bio-Rad). Gene expression was determined using the CFX96TM Real Time system (Bio-Rad) with Act2/8 [40] as a control. FT, SOC1 and FLC Primers were designed according to Zhou and Ni [41]. The results were determined using ΔΔCt method [42]. 4 replicates of pooled samples were used for both wildtype and mutant seedlings and flowers.

In situ Hybridization

We used previously established methods for in situ hybridization [43] with the following modifications. To generate templates for in situ probe synthesis, a cDNA was PCR amplified using primers that contained the phage T7 RNA polymerase initiation sequence. The PCR product was used for in vitro transcription of digoxigenin-labeled probes using a DIG-RNA labeling kit (Roche Applied Science). DIG-labeled RNA probes were not hydrolyzed, and used at a final concentration of 400 ng/ml in the hybridization solution. Slides were photographed under bright field illumination.

Statistical Analysis

All group differences in our dependent variables were revealed using two-tailed Student’s T-tests, and α-Levels were set at 0.05.

Results

MED18 Controls Flowering Time and Floral Organ Identity

The MED18 (NP_565534; At2g22370) gene was originally identified through a phylogenomic comparison of single-copy genes conserved in angiosperms [44]. Additional database searches revealed that MED18 was a plant homolog of Mediator subunit 18 (Fig. S1, ref [3,5]). To gain further insight into the function of this gene in Arabidopsis, we examined the phenotypes of plants homozygous for T-DNA insertions in the MED18 coding sequence. We identified T-DNA insertion alleles (SAIL_889_C08, med18-1; SALK_027178, med18-2) from the SALK T-DNA insertion database [http://signal.salk.edu/cgi-bin/tdnaexpress] [45,46], and confirmed the location of the T-DNA inserts by PCR using MED18 and T-DNA specific primers (Fig. 1A). We also examined MED18 mRNA levels in plants homozygous for each of the alleles using RT-PCR. MED18 mRNA was undetectable in med18-1 plants compared to wild type. This result suggests that med18-1 is a null allele. Kim et al. [22] reported weak expression of MED18 mRNA in med18-2 plants, but we failed to detect any MED18 mRNA using primers located on either side of the T-DNA insertion (Fig. S2).

When either med18-1 or med18-2 was crossed with wildtype plants, the F1 showed a wildtype phenotype, which demonstrates that both med18-1 and med18-2 are recessive. A complementation test was also performed using the med18-1 and med18-2 alleles. The F1 plants from a cross of med18-1 and med18-2 plants showed a med18 phenotype (Table S1), which is described below. This result demonstrates that med18-1 and med18-2 are allelic.

In addition to the previously described phenotype [22], med18 mutations cause a syndrome of related phenotypes affecting flowering time, inflorescence structure, and flower morphology. Under long day conditions (16 hour light, 8 hour dark), both med18-1 and med18-2 mutants did not flower even after 35 days after germination (DAG) (Fig. 1C). In contrast, wildtype plants flowered approximately 14 DAG (12 leaves) (Fig. 1B). The med18-1 mutation also affects flower morphology. In wildtype flowers, the sepals fully enclose the developing flowers until shortly after the beginning of anthesis (Fig. 1D). The sepals of med18-1 flowers...
not fully enclose the developing flowers such that the buds appeared prematurely open (Fig. 1E).

Most striking of all was that med18-1 mutants showed dramatically altered floral organ numbers as compared to wildtype. All floral organs were affected (Table S1, Fig. 1F–H), more than 40 and 80 percent of the flowers on med18-1 plants had abnormal sepals and petals number respectively, while 80% of the flowers had fewer than 6 stamens. Approximately

Figure 1. Phenotypes of Mediator subunit 18 (med18) mutants. (A) Schematic diagram of the MED18 gene showing the locations of T-DNA insertions in the med18 mutants. Black rectangles represent exons, lines represent introns, and triangles represent T-DNA insertions; the med18-1 mutation corresponds to insertion line SAIL_889_C08, whereas the med18-2 mutation corresponds to insertion line, SALK_027178. (B, C) 35 day old wild type (B), med18-1 (C) plants. (D, E) Inflorescence of wild type (D), and med18-1 (E) plants. (F–H) Arabidopsis wild type (F), med18-1 (G), and dissected med18-1 (H) flower. (I, J) Transverse section of wild type (I), and med18-1 (J) carpels. (K, L) Anthers of wild type (K) and med18-1 (L) (stained with KI/I2) at time of flowering. Scale bars: 1 mm in D, E, F, G and H; 100 μm in I, J, K and L.

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25% of med18-1 mutant flowers had more than two carpels (Table S1, Fig. 1H, J). In addition to the altered floral organ number, med18-1 mutants also showed delayed stamen development and later maturation of pollen (Fig. 1K, L), which led to reduced seed set.

Overexpression of MED18 caused increased carpel and stamen numbers (Fig. S3A), and reduced petal numbers (Fig. S3B). Carpel-like sepals were also observed in MED18 overexpressing plants (Fig. S3C), and these MED18 overexpressing plants flowered earlier than wildtype (data not shown).

**MED18 Expression during Arabidopsis Development**

The flowering time and floral organ patterning defects in the med18 mutants suggests that MED18 plays a role in regulating genes important for flowering and floral organ development. To determine if MED18 expression coincides with the phenotype, we examined the RNA expression pattern of MED18 in wildtype plants using in situ hybridization. MED18 transcripts could be detected in developing seeds (Fig. 2A), the inflorescence meristem, the floral meristem, and floral organ primordia (Fig. 2B and C). Transcript abundance appeared highest in the developing stamens and pistils (Fig. 2D). In later stages, MED18 expression was abundant in developing ovules and pollen (Fig. 2E) and in addition, MED18 mRNA was weakly expressed in petals, sepals, and the walls of carpels up to stage 12 (Fig. 2E). The strong expression of MED18 in the inflorescence meristem and floral organs is consistent with the floral defects observed in med18-1 mutants.

**med18 Mutants Affect Flowering Time in Both Long and Short Days**

In long day condition (16 h light, 8 h dark), the mutant plants did not flower until more than 40 leaves; and in short day (9 h light, 15 h dark), after 95 days (more than 60 leaves), med18 mutants still did not flower, and many rosette and cauline leaves showed senescence (Fig. 3A). Compared to wildtype plants (12 leaves under long days and about 40 leaves under short days), med18 is late flowering under both long and short days (Fig. 3A).

**med18 Mutants Affect Flowering Time through up-regulation of FLC Expression**

To understand how MED18 regulates flowering time, we compared the expression levels of key genes of different pathways in mutant and wildtype seedlings using RT-PCR. FT and FLC show expression differences in the med18 mutants as compared to wildtype plants (Fig. S4). We performed QPCR and showed that FT mRNA was down regulated approximately 4.7 fold (Fig. 3B, p≤0.004), while the FLC transcript level was much higher (9.5 fold) in med18-1 than in the wildtype plants (Fig. 3B, p≤0.004).
p ≤ 0.001). For FT and SOCI, which are downstream of FLC, only FT mRNA was significantly suppressed by the loss of MED18 function, whereas SOCI mRNA levels show no significant difference (Fig. 3B, p = 0.106).

**med18** Mutants are Responsive to Vernalization but not GA Treatment

To examine the responsiveness of **med18** to vernalization, the **med18** plants were planted and kept at 4°C for 4 weeks in the dark and then transferred to normal growth temperature (23°C). The vernalization-treated **med18** plants flowered much earlier than the untreated plants, after producing about 14 leaves, which is comparable to that of wildtype plants (Fig. 3C). The untreated plants flowered after producing close to 40 leaves, which suggests that the flowering of the mutant plants was decreased by vernalization (Fig. 3C). To examine the effects of GA treatment, a GA solution of 20 mM was sprayed twice a week after germination until flowering. The results show that GA has no obvious effect on the flowering of **med18** mutants as compared to wildtype plants. The GA-treated **med18** plants initiated flowering after producing more than 35 leaves, which is not significantly different than untreated mutant plants (p = 0.115, Fig. 3D). Our results are consistent with previous results that show that vernalization promotes flowering by repressing FLC and releasing FT from repression [23,47]. The responsiveness of **med18** to vernalization suggests that MED18 regulates flowering time through the vernalization pathway.

**MED18** Affects Floral Organ Formation in all Four Whorls

Both mutation and overexpression of **MED18** altered the number of floral organs (Fig. 1G–I, Table S1, Fig. S3). The increased number of petals and fewer than normal stamens seen in the **med18** mutants was reminiscent of the floral phenotype of **agamous** (ag) mutants [48,49]. To further explore the floral developmental pathway in which **MED18** is involved, we constructed double mutants with the well-studied floral developmental regulators, **ag**, **pistillata** (**pi**), and **apetala2** (**ap2**). Flowers on the **med18** ag-1 double mutant showed the striking reiteration of sepals and petals characteristic of ag mutants (Fig. 4A–C, Table S2). The **med18** pi-1 double mutant flowers had abnormal carpels, but fewer sepals than the pi-1 single mutant (Fig. 4D, E, Table S2). The flowers on the **med18** ap2-5 double mutant displayed a much more complex phenotype. Carpels in both the first and fourth whorls were present as seen in the ap2-5 single mutant. In addition, double mutant flowers exhibited petaloid stamens and other petaloid structures in whorls 2 and 3 (Fig. 4F, G, Table S2).

**MED18** Affects Floral Organ Formation through Regulation of Floral Homeotic Gene Expression

The clear epistasis observed in the **med18** ag-1 double mutants suggested that **MED18** and **AG** function in the same pathway to control floral development. Therefore, we examined **AG** mRNA levels as well as the levels of mRNA for other key floral regulators in **med18** mutants using semi-quantitative RT-PCR. Our results showed that the mRNA expression levels of **AG**, **API**, and **PI** were
down regulated in medi8-1 mutants while AP2 and AP3 mRNA showed no obvious change (Fig. S5). QPCR results revealed AG mRNA was down regulated up to 4.8 fold (Fig. 5A, p≤0.007), AP1 and PI mRNA expression levels were also reduced in the medi8-1 mutant 1.9 (p≤0.014) and 2.4 fold (p≤0.011) respectively, while AP2 and AP3 transcript levels showed no significant differences between medi8-1 and the wildtype (Fig. 5A, p = 0.068 and 0.082). The decreased AG expression is likely to cause a stamen to petal transition as well as abnormal carpel development [49], as observed in medi8-1 and MED18 overexpression flowers (Fig. 1F–H, Fig. S3). These results support the hypothesis that MED18 controls floral organ identity through its regulation of floral homeotic gene expression.

Altered AG Expression Patterns are Observed in medi8 Mutants

To further examine the expression of AG in medi8-1 mutants, in situ hybridization using an AG antisense RNA probe was performed on tissue sections from wildtype (Fig. 5B, D, F, H) and medi8-1 plants (Fig. 5C, E, G, I). As described previously [50,51], AG is not expressed in the inflorescence meristem, nor in stage 1 or stage 2 floral meristems in wildtype plants. Strong AG expression is first found in the center of stage 3 and stage 4 wildtype flowers, but not in the emerging sepal primordia (Fig. 5B). During later stages of wildtype flower development, AG expression is present in stamens and carpels (Fig. 5D, F, H). In medi8-1 mutants, a pattern of AG expression similar to that seen in wildtype was observed in both the inflorescence meristem and in stage 1 and stage 2 floral meristems (Fig. 5C). In flowers from stage 4 to stage 7, weak AG expression was detected in the center of the carpel primordia, although no expression was observed in petal and sepal primordia (Fig. 5C, E, G). In later development stages (stage 11, Fig. 5H, I), AG expression was observed in developing pollen similar to that observed in wildtype flowers (Fig. 5H), but it was difficult to detect any signal in vascular bundles of stamens and carpels (Fig. 5I). This result, together with the results of the QPCR analysis, strongly suggests that MED18 is required to maintain the normal AG expression level and pattern during early stamen and gynoecium development.

Discussion

Under many conditions Mediator appears to function as a general transcription factor [52]. Nonetheless, expression profiling of yeast Mediator subunit mutants has revealed the direct regulation of specific sets of genes by Mediator [53], and analysis of Mediator in Arabidopsis has shown that the Mediator subunits are important in regulating specific developmental processes like early embryo patterning [54,55], cell number and organ size [12,15], flowering time control [5,14,56] environmental regulation and defense gene regulation [16,18–21,57].

MED18 was first characterized as a general transcription factor that promotes Pol II transcription through promotion of the transcription of mRNA, and knocking down MED18 expression caused abnormal cotyledon and silique development as well as a later flowering phenotype [22]. However, the mechanisms by which MED18 regulates flowering are poorly understood, and nothing is known about how MED18 regulates floral organ identity. In this study, we identified MED18 as a regulator of both flowering time and floral organ identity. Our findings show that MED18 controls flowering time by up-regulating FLC expression, which also affects the expression of the downstream gene, FT.
After the flowering transition, MED18 plays a role in floral organ identity by regulating the \( \text{AG} \) expression level and pattern as well as \( \text{AP1} \) expression levels (Fig. 5, 6). These results suggest that MED18 is important in the integration of key signaling pathways in plants by controlling target genes transcription (Fig. 6).

The \( \text{med18} \) mutants display various developmental defects, suggesting that MED18 regulates multiple pathways. This is in agreement with the function of animal and yeast Mediator proteins, which have been suggested to regulate both basic and specific transcription [8,58,59]. We have focused on a study of MED18 function during flowering and floral organ development. We found that MED18 mRNA is localized mainly to the precursor cells of inflorescence meristem, the four floral whorls and strongly in the pollen and ovule primordia. This suggests a role for MED18 in the control of floral and reproductive organ initiation and development. Floral primordia arise from the inflorescence meristem, and floral organs are then formed in the floral meristem. The ABC model of flower development explains how three classes of genes control sepal, petal, stamen, and carpel identity [33]. Furthermore, the model indicates that class A and C genes are mutually antagonistic [60]. The previously isolated floral homeotic genes all seem to code for potential transcription factors. AP1, AP3, PI, AG, and the SEP proteins contain the MADS domain known to bind to DNA [36]. AP2 contains another DNA binding domain, the AP2 domain [61]. General regulators like Mediator are likely to regulate these genes. Our studies have shown that mutations in \( \text{MED18} \) cause downregulation of several homeotic genes, such as \( \text{AP1} \), \( \text{PI} \), and \( \text{AG} \), but not all of them; this finding suggests that the effect of MED18 on homeotic gene expression is gene specific.

Because MED18 affects the expression of multiple genes, the flowers of \( \text{med18} \) mutants show variable phenotypes, such as 4–14 petals, 2–6 stamens, 0–6 sepals and 1–3 carpels. In one of the flowers that had 14 petals, four stamens are clearly visible (Fig. S6).

The \( \text{med18} \) mutation cannot be a simple homeotic transformation of the floral organs into petals. It is interesting to note that the \( \text{med18} \) mutation causes a down
regulation of AG expression, yet some flowers have three carpels. According to the ABC model, down regulation of an A class gene (such as AP1) will cause ectopic carpel formation.

The later flowering phenotype observed in med18 mutants is caused by up-regulation of FLC mRNA expression. FLC also contains a MADS domain, and interestingly, both FLC and AG expression levels are altered in med18 mutants, but in opposite directions. There are similarities between the DNA structure of FLC and AG. Most of the genes in the Arabidopsis thaliana genome have short (<1 kb) introns [62], but intron 1 in FLC and intron 2 in AG are both greater than 3.0 kb in length, and both of these introns have cis-elements that are important for transcriptional control [27,63,64]. HUA1, HUA2, HUA ENHANCER2 [HEN2] and HEN4 were shown to positively regulate AG expression, either by inhibiting premature polyadenylation within AG intron 2 or by promoting the splicing of this intron, and hua1 hua2 double mutants have reduced levels of FLC mRNA [65]. All these results and our data that MED18 regulates FLC and AG mRNA expression levels, strongly support the idea of parallel regulation of FLC and AG [66].

The result of up-regulation of FLC and down-regulation of AG suggest that MED18 is not a general transcription repressor, but rather it plays different roles depending on the identity of its target, and/or the developmental stage. The mechanism by which MED18 affects FLC and AG expression is currently unknown. Highly specific gene regulation is thought to be determined by activators and combinatorial use of cofactors. In yeast, Med18 acts downstream of CDK8, and may act as a direct processor of signaling pathways for determining specific gene expression [53]. Med18 was also reported to be required for proper termination of transcription of a subset of genes during yeast budding [67]. In Arabidopsis, the CDK8 homolog is HUA ENHANCER3 [HEN3], which also controls organ identity and show similar loss-of-C-function phenotypes (Wang and Chen, 2004), suggesting that HEN3 may regulate organ identity through MED18 in Arabidopsis (Fig. 6). Our results suggest a conserved mechanism may exist in yeast and plants.

Supporting Information

Figure S1 Phylogenetic tree of eukaryotic MEDIATOR SUBUNIT 18 (MED18). The Bayesian inference analysis was derived from 406 amino acid positions of MED18 in different species of Eukaryotes. The best model amino acid replacement for MED18 sequences was JTT and gamma model for substitution rate heterogeneity between sites. Bayesian phylogenetic inference was performed with MrBayes Version 3.0 using four chains and 2,000,000 generations. Numbers at node indicate posterior was performed with MrBayes Version 3.0 using four chains and rate heterogeneity between sites. Bayesian phylogenetic inference

Figure S2 RT-PCR result of MED18 showing that MED18 mRNA was not detected in med18-1, med18-2, and med18-1/med18-2 T1 plants using primers designed for the PCR product including both insertion sites. The GAPC gene was used as a control.

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Table S1 Number of different organ types in med18 flowers. a a 200 flowers were counted. b mean of 200 flowers. c Organ number is 6 or more. d Organ number is 4 or less. e Organ number is 1 or 1.5 (some carpels only have half or one third of normal size located at the tip of pistil). f Organ number is between 2 and 3 (same as e). Mutant plants were grown in the greenhouse (16 h light, 23±2°C), wildtype plants were grown under the same conditions. The floral organs on 200 wildtype plants were also counted, and all flowers showed 4 sepals, 4 petals, 6 stamens and 2 fused carpels, except 2 flowers showed 5 petals. The med18-1 allele is a strong allele and all four floral organs show significant differences from wildtype (**p<0.01), med18-2 is a weaker allele, and only petals and stamens show significant differences from wildtype. The F1 plants from a cross of med18-1 with med18-2 (med18-1 × med18-2) also show obvious floral organ number changes.

Table S3 Primers designed in this study.

Table S2 Number of different organ types in floral homeotic mutants and double mutants with med18-1 flowers. a, at least 15 flowers were counted for each mutant. b, the first whorl of each organ showed the normal organ number for that whorl. c, many flowers showed 1 connected carpel-like sepal.

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Author Contributions

Conceived and designed the experiments: ZZ DGO. Performed the experiments: ZZ HG PG. Analyzed the data: ZZ FL DGO. Wrote the paper: ZZ DGO.
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