Heritable endogenous gene regulation in plants with designed polydactyl zinc finger transcription factors

Xuen Guan*†‡, Justin Stege†§¶, Myoung Kim*, Zina Dahmani*, Nancy Fan*‖, Peter Heifetz*, Carlos F. Barbas III‡§, and Steven P. Briggs*

*Torrey Mesa Research Institute, San Diego, CA 92121; and §The Skaggs Institute for Chemical Biology and Department of Molecular Biology, The Scripps Research Institute, La Jolla, CA 92037

Zinc finger transcription factors (TFs$_{ZF}$) were designed and applied to transgene and endogenous gene regulation in stably transformed plants. The target of the TFs$_{ZF}$ is the *Arabidopsis* gene *APETALA3* (*AP3*), which encodes a transcription factor that determines floral organ identity. A zinc finger protein (ZFP) was designed to specifically bind to a region upstream of *AP3*. *AP3* transcription was induced by transformation of leaf protoplasts with a transformation vector that expressed a TF$_{ZF}$ consisting of the ZFP fused to the tetrameric repeat of herpes simplex VP16's minimal activation domain. Histochemical staining of β-glucuronidase (GUS) activity in transgenic AP3::GUS reporter plants expressing GUS under control of the *AP3* promoter was increased dramatically in petals when the *AP3*-specific TF$_{ZF}$ activator was cointroduced. TF$_{ZF}$-amplified GUS expression signals were also evident in sepal tissues of these double-transgenic plants. Floral phenotype changes indicative of endogenous *AP3* factor coactivation were also observed. The same *AP3*-specific ZFP^{AP3} was also fused to a human transcriptional repression domain, the mSIN3 interaction domain, and introduced into either AP3::GUS-expressing plants or wild-type *Arabidopsis* plants. Dramatic repression of endogenous *AP3* expression in floral tissue resulted when a constitutive promoter was used to drive the expression of this TF$_{ZF}$. These plants were also sterile. When a floral tissue-specific promoter from *APETALA1* (*AP1*) gene was used, floral phenotype changes were also observed, but in contrast the plants were fertile. Our results demonstrate that artificial transcriptional factors based on synthetic zinc finger proteins are capable of stable and specific regulation of endogenous genes through multiple generations in multicellular organisms.

floral development | APETALA3 | APETALA1

In nature, eukaryotic nuclear genes are tightly regulated at both the transcriptional and translational levels. Much of this control is achieved through DNA-binding transcription factors. The manipulation of plant traits in agricultural biotechnology would be greatly facilitated if preselected endogenous genes could be turned on or off in a controlled and selective manner. A conceptual approach to such manipulation is the engineered expression of specific native transcription factors that have evolved to control particular genes. Advances in whole-genome sequencing of *Arabidopsis* (1) and more recently rice (2), combined with informatics-based analysis have allowed the identification of numerous putative plant transcription factors (2, 3). However, the identification and characterization of the molecular targets of these transcription factors is still at a very early stage, and consequently it is not yet possible to use them broadly as gene-specific tools for controlled regulation of endogenous gene expression. Rational design of artificial transcription factors that target specific DNA sequences with non-native nucleotide binding domains fused to transcriptional activation or repression domains is therefore an attractive option. An especially promising approach of this kind utilizes synthetic DNA binding domains of the zinc finger protein (ZFP) class.

Numerous zinc finger DNA-binding domain motifs have been identified in genes originating from plants and other biological systems (4–7). Among these, the Cys-2–His-2 type of ZFP has been the subject of the most extensive structural, biochemical, and genetic studies (4–24). This highly modular zinc finger domain has been found to be particularly amenable to rational manipulation of target binding site specificity. Several design and selection strategies have been developed for construction of synthetic zinc finger-based DNA binding proteins that can be highly specific for given target sequences (8, 10–24). Recently, several studies have demonstrated targeting of endogenous genes in cultured mammalian cells using synthetic ZFP-based artificial transcription factors where the DNA binding domain of the ZFP has been fused to transcriptional activation or repression domains (18–24). Application of this technology to agriculture by means of designing plant-specific zinc finger transcription factors (TFs$_{ZF}$) would potentially enable a range of diverse applications. However, two critical issues remain to be addressed: the function and stability of TFs$_{ZF}$ in a multicellular organism that has been regenerated from a transformed cell, and the ability of these genes to be stably inherited in subsequent generations.

In this study, we describe artificial transcription factors based on a designed polydactyl ZFP that specifically targets the *AP3* floral development gene of *Arabidopsis thaliana*. Wild-type *Arabidopsis* flowers have four organ types (sepal, petal, stamen, carpel) arranged in concentric whorls (25). *AP3*, a member of the MADS box gene family (26, 27), is involved in specifying the organ identity of floral whorl 2 (petal) and whorl 3 (stamen) (28–30). Altered expression of *AP3* results in homeotic mutations where whorl-specific organ identity is perturbed. Severe *ap3* mutant alleles and mutations in the related gene *pistillata* cause organ identity changes and sterility (31, 32). Such readily observed phenotypes make the *Arabidopsis* flower a useful system in which to study manipulated gene regulation. A suitable target site for ZFP design using GNN repeat motifs (16–19) is located approximately 50 bp upstream of the *AP3* TATA box. A six-finger ZFP with predicted specificity for this 18-bp site was designed and synthesized (19). A TFs$_{ZF}$ for gene activation was prepared by fusion of the synthetic tetrameric repeat of herpes simplex VP16's minimal activation domain (VP64) to the designed ZFP. For gene-specific repression, a mammalian repression domain, mSin3 interaction domain (Sid), was fused to the ZFP (19, 21). Transformation of these TFs$_{ZF}$ into *Arabidopsis* yielded transgenic phenotypes similar to known *ap3* mutant alleles. Introduction of *AP3*-specific TFs$_{ZF}$ into *Arabidopsis*

---

plants expressing a β-glucuronidase (GUS) reporter gene under control of the *AP3* promoter resulted in GUS expression changes reflective of the various promoters used to drive TFs$_{ZF}$ expression. Our results demonstrate that artificial transcription factors based on engineered ZFPs can be used to manipulate endogenous transgene gene expression in multicellular organisms in both a transient and stable fashion.

Materials and Methods

Plant Materials and Promoters. A 1.9-kb *AP1* promoter sequence and transgenic plants expressing GUS under control of the *AP3* promoter originally generated by Tom Jack's laboratory (25) was obtained from Detlef Weigel (The Salk Institute, La Jolla, CA).

Construction and Characterization of AP3-Specific ZFP^AP3. A ZFP was synthesized to bind to the complementary strand of the18-bp sequence 5′-TACTTCTTCAACTCCATC-3′ found at −112 to −95 relative to the start of translation of the *APETALA3* genomic sequence (33). The gene was constructed and the protein expressed and purified as a fusion with maltose binding protein as described. ELISA specificity and electrophoretic mobility shift assays were performed as described.

Construction of Zinc Finger-Effector Domain Fusions and Transformation of Plants. TFs$_{ZF}$ bearing the VP64 activation domain and the Sid repression domain were prepared as described (19). For constitutive expression, the TFs$_{ZF}$ were cloned into a dicot expression vector pNOV102 (34) downstream from the UBQ3 promoter (35, 36) and upstream of the nos transcriptional terminator. The resulting constructs (UBQ3::ZFP^AP3-VP64//nos and UBQ3::Sid-ZFP^AP3//nos) were transformed into *A. thaliana* plants (Columbia) using the agrobacteria-mediated transformation method (37). Protoplast transient transformation and assay were conducted as described (34). Putative transgenic plants were selected for hygromycin resistance as described (34). A nontarget activation construct UBQ3::ZFP^m4-VP64//nos was generated and transformed into *Arabidopsis* at the same time. ZFP^m4 targets the sequence of maize *myo*inositol 1-phosphate synthase. ZFP^m4-VP64 activates transcriptions of *myo*inositol 1-phosphate synthase in maize cells (34). A transformation control vector UBQ3::GFP//nos was used for all transformations. For floral tissue-specific regulation, a 1.9-kb fragment containing the *AP1* promoter was substituted for the UBQ3 promoter fragment. The resulting constructs (AP1::ZFP^AP3-VP64//nos and AP1::Sid -ZFP^AP3//nos) were transformed into *Arabidopsis* as described above.

RT-PCR Analysis of AP3 Expression Level. Plant tissues (either protoplasts or floral tissue from stages 1 to approximately stage 15; ref. 38) were collected and frozen immediately in liquid nitrogen. Total RNA was prepared from these samples by using the Ambion RNAwiz kit (Ambion). The expression levels of the ZFP artificial transcription factors and endogenous *AP3* were monitored by RT-PCR. RT-PCRs were carried out in 25-μl volumes by using 200 ng of RNA and the Qiagen 1-step RT-PCR kit. *AP3*-specific primers were Ap3-F, 5′-GGCGAGAGGGAA-GATCCAG-3′ and Ap3–4R, 5′-CTCCTCTAATACGACT-CACTATAGGGACACTCACCTAGCCTCTG-3′. The thermocycler settings were 50°C for 30 min, 95°C for 15 min (94°C for 30 s, 60°C for 30 s, 72°C for 1 min) × 30 cycles, and 72°C for 10 min.

Quantitative PCR Analysis of *AP3* Expression Level. Total RNA used for RT-PCR was assayed quantitatively on an ABI Prism 7900 Sequence Detector (Taqman) according to the manufacturer's instructions (Beckman Coulter). All probes and primers were designed with the program PRIMER EXPRESS with the default setting (Perkin–Elmer). For *AP3* detection, the probe sequence

5′-CCATTTCATCCTCAAGACGACGCAGCT-3′ was used with primers 5′-TTTGGACGAGCTTGACATTCAG-3′ (forward) and 5′-CGCGAACGAGTTTGAAAGTG-3′ (reverse). Taqman PCRs were carried out by using 250 ng total RNA and Taqman one-step RT-PCR master mix reagent (Perkin–Elmer). Thermal cycling conditions were 48°C for 30 min, 95°C for 10 min for 1 cycle, then 40 cycles of 95°C for 15 s and 60°C for 1 min. Relative expression was quantified by using the comparative Ct method with the thioredoxin gene TRX3 as an internal expression standard. The probe sequence used for TRX3 was 5′-AGACTTCACTGCAACATGGTGCCCAC-3′ with primers 5′-GTGTGGAAATGACACAGATTGTGA-3′ (forward) and 5′-AGACGGGTGCAATGAAACG-3′ (reverse).

GUS Histochemical Staining and Analysis of Expression Pattern. GUS histochemical staining was conducted as described (39, 40). Freshly excised floral tissues were immersed immediately in GUS staining solution containing 0.25 mM 5-bromo-4-chloro-3-indolyl β-D-glucuronide cyclohexylammonium salt (X-glu, Rose Scientific, Edmonton, AB, Canada) in 50 mM sodium phosphate buffer (pH 7.3), and then incubated 20 h at 37°C. Stained tissues were dehydrated in an ethanol series and photographed before infiltration with Histo-Clear (International Diagnostics, Atlanta), and paraffin-cast specimen blocks (41) were sectioned on a microtome (Microm HM315, Mikron) and stained *in situ* with 0.1% aqueous Safranin-O for 5–60 s. Stained surfaces of specimen blocks were treated with immersion oil and visualized under a dissecting microscope (Olympus, New Hyde Park, NY).

Results

Synthesis and Characterization of ZFP. The target sequence of the designed *AP3*-specific ZFP^AP3 is an 18-bp sequence located in the *AP3* promoter region, at −112 to −95 relative to the ATG codon (Fig. 1*A*). This six-domain ZFP was assembled from zinc finger domains of predefined specificity as described (16, 17, 19, 23). ZFP^AP3 was expressed in *Escherichia coli* and purified. ZFP^AP3 demonstrated good specificity in ELISA assays using a panel of oligonucleotide target sequences (data not shown). The affinity of the ZFP^AP3 for its designed target was determined to be 2.3 nM by electrophoretic mobility assays. This affinity is in the range we have previously determined to be required for endogenous gene regulation (21, 23).
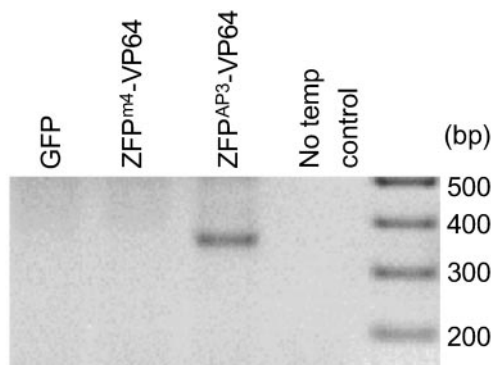
Transient Activation of Endogenous *AP3* in *Arabidopsis* Leaf Cells. *AP3* is normally expressed exclusively in developing flowers, and no expression of the gene has been reported in leaf mesophyll protoplasts. Such protoplasts were used to investigate transcriptional activation of *AP3*. The *AP3*-specific activation construct UBQ3::ZFP^AP3-VP64//nos and a nontarget activation construct UBQ3::ZFP^m4-VP64//nos (which targets the sequence of maize *myo*inositol 1-phosphate synthase) were transformed into *Arabidopsis* leaf protoplasts. UBQ3::GFP//nos was used as a transformation control. All samples were analyzed by RT-PCR to determine the baseline levels of endogenous *AP3* transcripts. The *AP3*-specific primers were designed to yield a 600-bp product if amplification is based on a genomic DNA template (indicating contamination) and 334 bp for transcribed *AP3* messages. The originally silent *AP3* gene was activated in cells that were transformed with the *AP3*-specific activation construct UBQ3::ZFP ^AP3-VP64//nos but not with any other constructs (Fig. 2). These results indicate that the ZFP^AP3 domain is able to bind *AP3* DNA and direct the activation domain (VP64) to the specific and otherwise silenced endogenous target *in vivo*. Furthermore, TFs$_{ZF}$ targeted to different genes (e.g., ZFP^m4) did not activate *AP3* in a nonspecific manner.
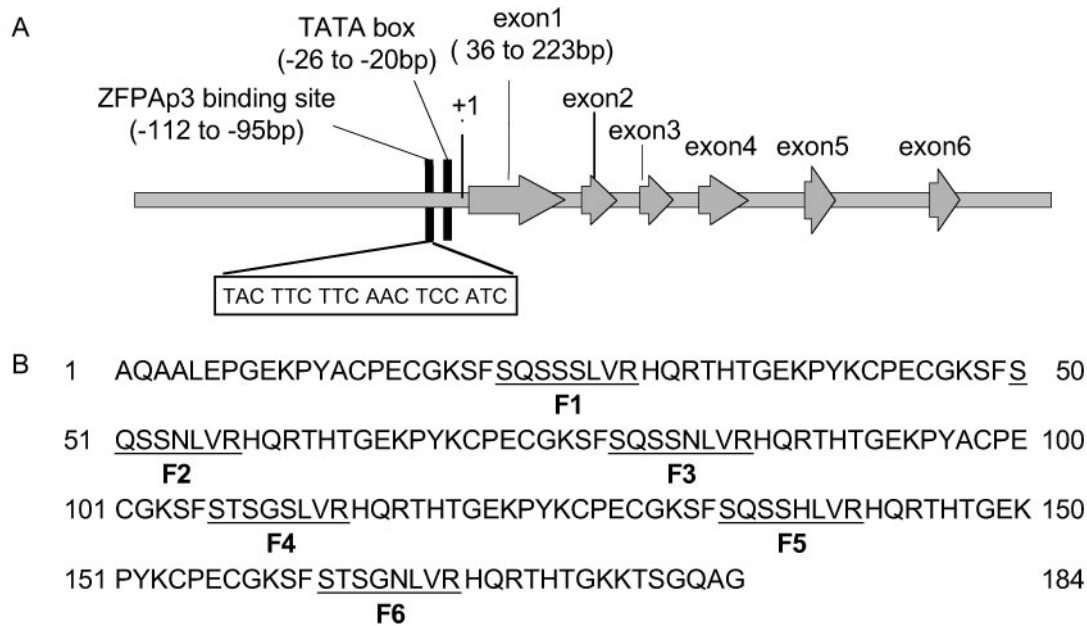
Fig. 1. (*A*) *ZFP*^AP3 target sequence (boxed) and its position in the *AP3* 5′ UTR. Numbers indicate the distance from the ATG translation initiation codon. The arrowed boxes indicate the exon of *AP3*. (*B*) DNA recognition helix sequences of the *ZFP*^AP3 protein. The underlined amino acids are the components of the new zinc fingers that provide specificity for the selected nucleotide sequences indicated in *A*. The recognition helices of fingers 1–6 (F1–F6) are underlined.

Phenotypic Consequences of *in Planta* Activation of *AP3*. Attempts to stably transform *Arabidopsis* with the construct UBQ3::ZFP^AP3-VP64//nos to constitutively activate *AP3* expression were inefficient. Few transformants could be obtained with this vector. Most had rearrangements that lacked the VP64 domain. None expressed ZFP^AP3-VP64. To circumvent this problem, we restricted *AP3* activation to floral tissue. For this, the *APETALA1* (*AP1*) promoter was used to direct expression of ZFP^AP3-VP64. To dissect *AP1*-specific regulation of *AP3* from the morphological consequence of altered *AP3* transcription factor activity, we examined the effects of AP1::ZFP^AP3-VP64//nos first in an *AP3*-driven GUS reporter background (AP3::GUS). This reporter has been characterized (25) and established a characteristic *AP3*-specific pattern of GUS staining. Floral tissue-specific activation construct AP1::ZFP^AP3-VP64//nos and control construct AP1::ZFP^AP3//nos were transformed into homozygous *Arabidopsis* plants harboring construct AP3::GUS. The expression of *AP3* starts from stage 3 of

flower development and is accumulated in petal and stamen primordia only. As a result of this late expression, AP3::GUS plants accumulate a moderate level of the stable reporter in the petals and stamens, as illustrated in Fig. 3*A*. In contrast, *AP1* is expressed at the very early stages (stages 1–3) of flower development (42, 43), when the floral organ primordia are just beginning to initiate. This results in an *AP1* signal that is uniformly distributed throughout the young floral primordia and later in development is restricted to the sepal and petal primordia (Fig. 3*A*). Consequently, we predict that a plant double-transformed with constructs AP1::ZFP^AP3-VP64//nos and AP3::GUS will initiate *AP3*-directed expression at the very early stage of floral primordia development (stages 1–3), coinciding with *AP1* expression, and will also show the petal and stamen pattern at later stages of the characteristics of normal *AP3* expression. To test this hypothesis, flowers from many double transgenic plants were stained for GUS and observed either with or without tissue mounting. The GUS staining patterns shown in Fig. 3 revealed strong activation of the *AP3* promoter in an *AP1*-dependent manner as predicted: (*i*) the GUS signals expanded throughout the entire flower primordia, starting at the very earliest stages of development (Fig. 3 *C* and *E*); (*ii*) the GUS signal was detected in the sepals of mature flowers (Fig. 3 *C* and *E*); and (*iii*) the intensity of the GUS signal increased dramatically in the petals of mature flowers (Fig. 3 *C* and *E*). These patterns were distinct from the petal and stamen staining of the AP3::GUS line. In our system, both the transgenic GUS gene and the native *AP3* transcription factor gene should each be subject to *AP1*-dependent activation by the *AP3*-targeted TFs_ZF. Reflective of this, we found that several independent transgenic plants showed distinct phenotypic changes in floral development, suggesting an altered pattern of *AP3* transcription factor activity. In these plants, some whorl 1 organs are replaced by whorl 2 organs, resulting in flowers having five or more petals (Fig. 4*B*) and reduced or absent sepals. The stamens appear normal and all plants are fertile. An interesting floral phenotype was observed in some of the floral-specific activation events obtained using construct AP1:ZFP^AP3-VP64. These plants exhibited a markedly higher percentage of young flowers in the

Fig. 2. Activation of the silenced endogenous *AP3* gene in *Arabidopsis* leaf cells. RT-PCR was used to detect *AP3* expression in *Arabidopsis* leaf protoplasts transformed with *GFP* (control), *ZFP*^m4-VP64 (nonspecific activation control), and *ZFP*^AP3-VP64 (*AP3*-specific activation). The last lane (−) is no template control amplification.
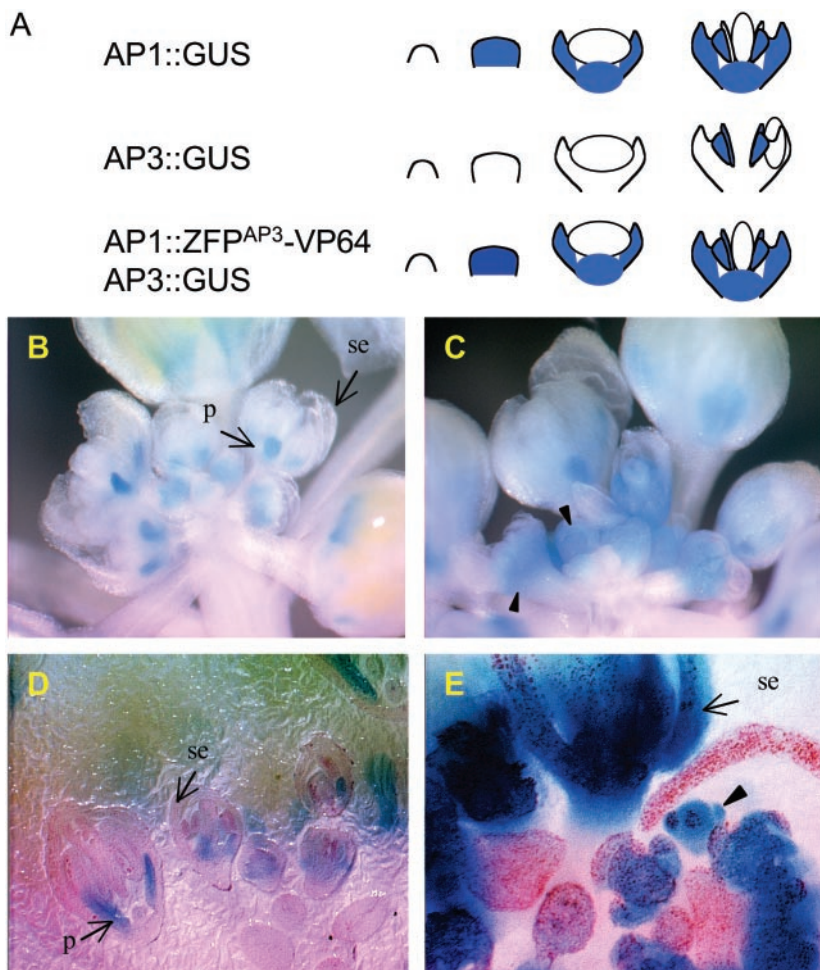
Fig. 3. GUS staining flowers of background plant with AP3::GUS only and double transgenic plant with both AP3::GUS and activation construct AP1::ZFP^{AP3}-VP64// nos. (*A*). Predicted GUS staining patterns of *AP3::GUS* and AP1::GUS (based on personal communication with Martin Yanofsky, University of California, San Diego). (*B*) Flowers from background plant with AP3::GUS only stained for GUS activity. GUS signal is detected only in the petal (p) and stamen (not visible here, see Fig. 6*A*), but not in the carpal (not visible) and sepal (se). Picture taken directly after staining procedure. (*C*) Flowers from double transgenic plant expressing AP3::GUS and AP1::ZFP^{AP3}-VP64//nos simultaneously. GUS signal is increased in petal and is detected throughout the young flower primordia. (*D*) Mounted flowers from background plant with AP3::GUS only stained for GUS activity. GUS signal is detected only in the petal (p) and stamen (not visible here, see Fig. 7*A*) and not in the carpal and sepal (se). (*E*) Mounted flowers from double transgenic plant expressing AP3::GUS and AP1::ZFP^{AP3}-VP64//nos simultaneously. GUS signal is increased in petal (p), extended to sepal (se), and detected throughout the young flower primordia (arrow).

inflorescence tissue as compared with controls (data not shown). Activation of the AP3::GUS transgene and the endogenous *AP3* gene were both found to be stable genetically and were transmitted faithfully to progeny over two subsequent generations (T3).

In Planta Repression of Endogenous AP3. For *in planta* repression, a constitutive UBQ3 promoter was used to drive the expression of an *AP3*-specific ZFP fused to a human transcriptional repression domain (19). This construct (UBQ3::Sid-ZFP^{AP3}//nos) was transformed into wild-type *Arabidopsis*. We found that both

TFs$_{ZF}$ and endogenous *AP3* expression levels varied over a wide range among independent transgenic events. However, endogenous *AP3* gene expression levels were repressed in most transgenic lines. Higher TFs$_{ZF}$ expression in line *ND0052–2e* (Fig. 5*A*) produced a marked down regulation of endogenous *AP3* expression (Fig. 5*B*). Quantitative RT-PCR analysis revealed a nearly 50-fold *AP3* repression in this line (data not shown). We also
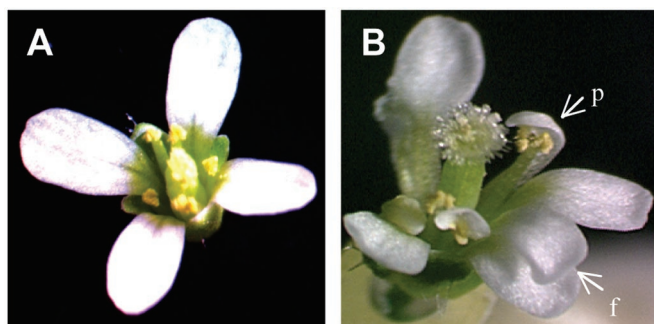
Fig. 4. Floral phenotypic changes in double transgenic plant expressing AP3::GUS and AP1::ZFP^{AP3}-VP64//nos simultaneously. A seven-petal flower is shown here. Two extra petals are fully converted (f), and the third one is partially converted (p).
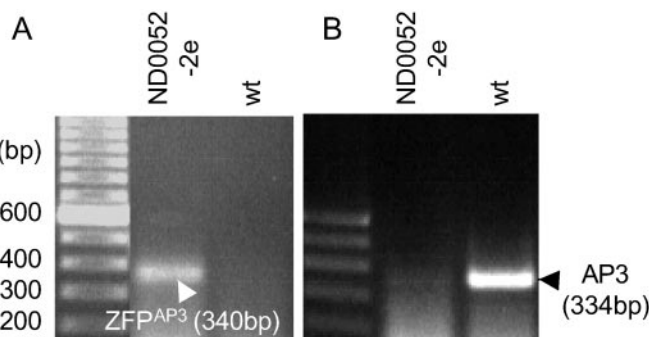
Fig. 5. Repression of endogenous *AP3* expression by the constitutive repression construct UBQ3::Sid-ZFP^{AP3}//nos in transgenic plant *ND0052–2e*. (*A*) RT-PCR identification of transgene *ZFP^{AP3}* in transgenic event *ND0052–2e* and wild-type control plant. (*B*) RT-PCR evaluation of endogenous gene *AP3* expression level in transgenic event *ND0052–2e* and wild-type plant. In plant *ND0052–2e*, the expression of *AP3* is significantly repressed by the expression of repressor *Sid-ZFP^{AP3}* fusion protein. Quantitative PCR indicated 46-fold repression.
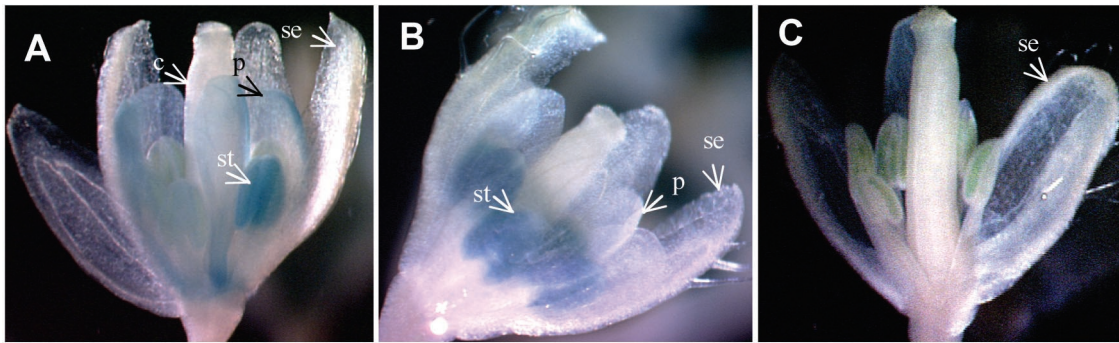
Fig. 6. GUS staining flowers of background plant with AP3::GUS only and double transgenic plant with both AP3::GUS and repression construct AP1::Sid-ZFP^{AP3}//nos. (*A*) Flowers from background plant with AP3::GUS only stained for GUS activity. GUS signal is detected only in the petal (p) and stamen (st) and not in the carpal and sepal (se). Picture taken directly after staining procedure. (*B*) Flowers from double transgenic plant expressing AP3::GUS and AP1::Sid-ZFP^{AP3}//nos simultaneously. GUS signal disappeared from petals but is detectable in stamens. (*C*) Flowers from a different double transgenic plant expressing AP3::GUS and AP1::Sid-ZFP^{AP3}//nos simultaneously. Low level GUS activity is detectable in stamens. In addition, the petals were absent in this flower.

observed that most of the transgenic plants having reduced *AP3* expression were sterile and had unopened flowers at maturity. Dissection of flowers from *ND0052–2e* plants revealed petals that were shorter and narrower than wild-type flowers of the same maturity. In addition, the lower portions of the petals were converted partially to sepal-like structures, and the stamens were greatly reduced in size compared with wild type. The floral phenotypes of the strongly *AP3*-repressed plants are similar to the flowers of the characterized *ap3* and *sap* mutants (44, 45).

Floral tissue-specific repression, the construct AP1::Sid-ZFP^{AP3}//nos and control construct AP1::ZFP^{AP3}//nos, lacking a repression domain, were transformed into *Arabidopsis* plants already carrying an AP3::GUS (25) transgene stably integrated into the genome. Because *AP1* is expressed in whorls 1 and 2 (sepals and petals), whereas *AP3* is expressed in whorls 2 and 3 (petals and stamens), we predicted that the GUS signal should be eliminated from whorl 2 but not whorl 3 in the double transgenic plant-expressing construct AP1::Sid-ZFP^{AP3}//nos in the presence of AP3::GUS. Fig. 6 shows this expected pattern of stamen-only GUS expression. Petals from some of these flowers are absent, as expected if the endogenous *AP3* gene is repressed (Fig. 6*C*). Two types of petal morphology were observed in these double transformants: missing petals (Figs. 6*C* and 7*C*) and partial conversion of petals to sepals (sepaloid petals) (Fig. 7*B*). These plants were fully fertile despite the floral alterations. Only the constitutive expressed ZFP^{AP3} plants were sterile.

Discussion

We have shown that ZFP-based artificial transcription factors can be designed and synthesized to manipulate transgene and endogenous gene expression levels in transgenic plants. Although regulation of only one transgene (GUS) and one endogenous gene (*AP3*) are presented here, we believe this approach will be generally applicable to all genes. This study supports and extends previous studies of TFs$_{ZF}$ that have been designed to activate and repress endogenous genes in mammalian cells (8, 21–24). Therefore, we believe that this approach should be viable in other transgenic organisms as well.

Our studies indicate that transcriptional activation and repression with our TFs$_{ZF}$ are specific for the targeted *AP3* gene. Preliminary GeneChip (47) analysis of transgenic plants with or without ZFP^{AP3}-effector fusion revealed no significant changes in gene expression in the 8,000 nontargeted floral or nonfloral genes examined (data not shown). Despite this apparent specificity, recovery of plant lines that constitutively expressed TFs$_{ZF}$ activators (UBQ3::ZFP^{AP3}-VP64//nos) was inefficient. In addition, we observed that the VP64 tetrameric repeat was subject to somatic rearrangement in plant cells. This result parallels difficulties encountered in the generation of transgenic animals and cell lines expressing the VP16 activation domain or designed versions of this domain like VP64, suggesting that this activation domain itself carries with it an intrinsic toxicity (48).

We believe that our TFs$_{ZF}$ approach to gene regulation can be further enhanced by combination with other gene regulation
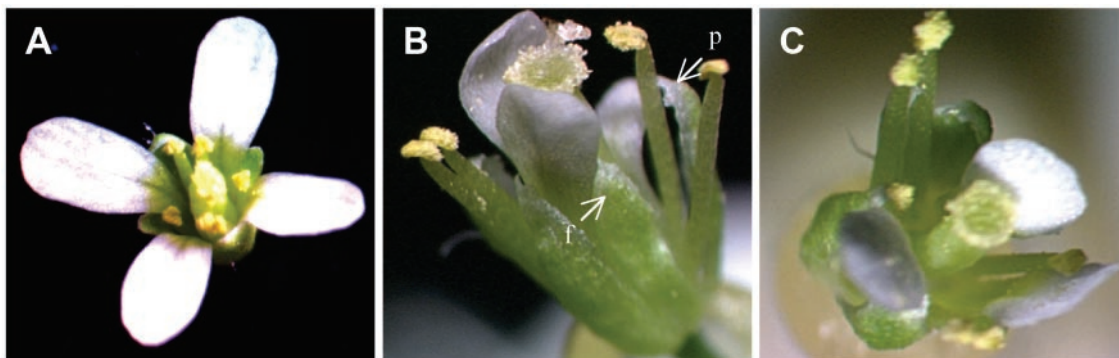
Fig. 7. Floral phenotypic changes in double transgenic plant expressing AP3::GUS and AP1::Sid-ZFP^{AP3}//nos simultaneously. (*A*) Flowers from background plant with AP3::GUS only. (*B*) Flowers from double transgenic plant expressing AP3::GUS and AP1::Sid-ZFP^{AP3}//nos simultaneously. A three-petal flower is shown here. In this flower, an extra sepal is fully replaced by one petal (f), and another petal is partially replaced (p). (*C*) Flowers from a different double transgenic plant expressing AP3::GUS and AP1::Sid-ZFP^{AP3}//nos simultaneously with one missing petal.

technologies, such as inducible gene expression, which has been recently adapted to create chemically regulated TFs$_{ZF}$ (49). Tissue-specific expression of the TFs$_{ZF}$ provides for another level of control as demonstrated here. Jack and colleagues have shown that overexpression of *AP3* causes conversion of sepals to petals, carpels to stamens, as well as loss of fertility (32, 44). Presumably, tissue-specific activation of *AP3* in our *AP1*-driven activation plants allows them to maintain their fertility. Likewise, transcriptional repression with the human Sid domain in plants

was potent and yielded phenotypes analogous to those observed in plants harboring a temperature-sensitive allele of *AP3* (46), suggesting that both gain of function and loss-of-function phenotypes are accessible by using zinc finger technology in whole organisms.

Note Added in Proof. In a companion article, we have demonstrated the efficacy of our TF$_{ZF}$ approach in transgenic tobacco plants maintained over several generations (50).

1. The *Arabidopsis* Genome Initiative (2000) *Nature (London)* **408,** 796–815.
2. Goff, S., Ricke, D., Lan, T., Presting, G., Wang, R., Dunn, M., Glazebrook, J., Sessions, A., Oeller, P., Varma, H., *et al.* (2002) *Science* **296,** 92–100.
3. Riechmann, J., Heard, J., Martin, G., Reuber, L., Jiang, C., Keddie, J., Adam, L., Pineda, O., Ratcliffe, O. J., Samaha, R. R., *et al.* (2000) *Science* **290,** 2105–2110.
4. Berg, J. M. (1990) *Annu. Rev. Biophys. Biophys. Chem.* **19,** 405–421.
5. Ashworth, A. & Denny, P. (1991) *Mamm. Genome* **1,** 196–200.
6. Pieler, T. & Bellefroid, E. (1994) *Mol. Biol. Rep.* **20,** 1–8.
7. Takatsuji, H. (1998) *Cell. Mol. Life Sci.* **54,** 582–596.
8. Beerli, R. & Barbas, C. F., III (2002) *Nat. Biotechnol.* **20,** 135–141.
9. Elrod-Erickson, M., Rould, M. A., Nekludova, L. & Pabo, C. O. (1996) *Structure (London)* **4,** 1171–1180.
10. Desjarlais, J. R. & Berg, J. M. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 7345–7349.
11. Wu, H., Yang, W. & Barbas, C. F., III (1995) *Proc. Natl. Acad. Sci. USA* **92,** 344–348.
12. Rebar, E. J. & Pabo, C. O. (1994) *Science* **263,** 671–673.
13. Choo, Y. & Klug, A. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 11163–11167.
14. Wolfe, S. A., Grant, R. A., Elrod-Erickson, M. & Pabo, C. O. (2001) *Structure (London)* **9,** 717–723.
15. Greisman, H. A. & Pabo, C. O. (1997) *Science* **275,** 657–661.
16. Segal, D. J., Dreier, B., Beerli, R. R. & Barbas, C. F., III (1999) *Proc. Natl. Acad. Sci. USA* **96,** 2758–2763.
17. Dreier, B., Segal, D. J. & Barbas, C. F., III (2000) *J. Mol. Biol.* **303,** 489–502.
18. Liu, Q., Segal, D. J., Ghiara, J. B. & Barbas, C. F., III (1997) *Proc. Natl. Acad. Sci. USA* **94,** 5525–5530.
19. Beerli, R. R., Segal, D. J., Dreier, B. & Barbas, C. F., III (1998) *Proc. Natl. Acad. Sci. USA* **95,** 14628–14633.
20. Kang, J. S. & Kim, J. (2000) *J. Biol. Chem.* **275,** 8742–8748.
21. Beerli, R. R., Dreier, B. & Barbas, C. F., III (2000) *Proc. Natl. Acad. Sci. USA* **97,** 1495–1500.
22. Zhang, L., Spratt, S. K., Liu, Q., Johnstone, B., Qi, H., Raschke, E. E., Jamieson, A. C., Rebar, E. J., Wolffe, A. P. & Case, C. C. (2000) *J. Biol. Chem.* **275,** 33850–33860.
23. Dreier, B., Beerli, R., Segal, D., Flippin, J. & Barbas, C. F., III (2001) *J. Biol. Chem.* **276,** 29466–29478.
24. Liu, P., Rebar, E. J., Zhang, L., Liu, Q., Jamieson, A. C., Liang, Y., Qi, H., Li, P. X., Chen, B., Mendel, M. C., *et al.* (2001) *J. Biol. Chem.* **276,** 11323–11334.
25. Weigel, D. (1995) *Annu. Rev. Genet.* **29,** 19–39.
26. Theissen, G. & Saedler, H. (1995) *Curr. Opin. Genet. Dev.* **5,** 628–639.
27. Bowman, J. L., Baum, S. F., Eshed, Y., Putterill, J. & Alvarez, J. (1999) *Curr. Top. Dev. Biol.* **45,** 155–205.
28. Yanofsky, M. (1995) *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **46,** 167–188.
29. Irish, V. F. (1999) *Dev. Biol.* **209,** 211–220.
30. Jenik, P. D. & Irish, V. F. (2001) *Development (Cambridge, U.K.)* **128,** 13–23.
31. Jack, T., Brockman, L. L. & Meyerowitz, E. M. (1992) *Cell* **68,** 683–697.
32. Jack, T., Fox, G. L. & Meyerowitz, E. M. (1994) *Cell* **76,** 703–716.
33. Irish, V. F. & Yamamoto, Y. T. (1995) *Plant Cell* **7,** 1635–1644.
34. Barbas, C. F., III, Stege, J., Guan, X. & Dalmia, B. (2001) PCT WO 01/52620.
35. Norris, S. R., Meyer, S. E. & Callis, J. (1993) *Plant Mol. Biol.* **21,** 895–906.
36. Callis, J., Raasch, J. A. & Vierstra, R. D. (1990) *J. Biol. Chem.* **265,** 12486–12493.
37. Bechtold, N. & Pelletier, G. (1998) in *Arabidopsis Protocols*, eds. Martinez-Zapater, J. & Salinas, J. (Humana, Clifton, NJ), pp. 259–266.
38. Bowman, J., ed. (1993) in *Arabidopsis, An Atlas of Morphology and Development* (Springer, New York), pp. 135–145.
39. Jefferson, R. A., Kavanagh, T. A. & Bevan, M. W. (1987) *EMBO J.* **6,** 3901–3907.
40. Sessions, A., Weigel, D. & Yanofsky, M. (1999) *Plant J.* **20,** 259–263.
41. Ruzin, S. E., eds. (1999) in *Plant Microtechnique and Microscopy* (Oxford Univ. Press, New York), pp. 57–86.
42. Mandel, A. M., Gustafson-Brown, C., Savidge, B. & Yanofsky, M. F. (1992) *Nature (London)* **360,** 273–277.
43. Gustafson-Brown, C., Savidge, B. & Yanofsky, M. F. (1994) *Cell* **76,** 131–143.
44. Byzova, M. V., Franken, J., Aarts, M. G., de Almeida-Engler, J., Engler, G., Mariani, C., Van Lookeren Campagne, M. M. & Angenent, G. C. (1999) *Genes Dev.* **13,** 1002–1014.
45. Yi, Y. & Jack, T. (1998) *Plant Cell* **10,** 1465–1477.
46. Ren, D., Collingwood, T. N., Rebar, E. J., Wolffe, A. P. & Camp, H. S. (2002) *Genes Dev.* **16,** 27–32.
47. Zhu, T. & Wang, X. (2000.) *Plant Physiol.* **124,** 1472–1476.
48. Akagi, K., Kanai, M., Saya, H., Kozu, T. & Berns, A. (2001) *Nucleic Acids Res.* **29,** 1–5.
49. Beerli, R. R., Schopfer, U., Dreier, B. & Barbas, C. F., III (2000) *J. Biol. Chem.* **275,** 32617–32627.
50. Ordiz, M. I., Barbas, C. F., III, & Beachy, R. N. (2002) *Proc. Natl. Acad. Sci. USA* **99,** 13290–13295.