Targeted gene silencing by diverse epigenetic pathways

Plant gene silencing is usually achieved through chromatin modifications and repressive transcription factors. We used a gain-of-function approach in *Arabidopsis* that identified 14 proteins that can repress gene expression via diverse epigenetic pathways, including DNA methylation, histone modifications and interference with RNA polymerase II transcription.

This is a summary of:
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The problem

Cytosine DNA methylation has been used in plants to induce gene repression, and targeted DNA methylation can be heritable in subsequent plant generations even after the methylation-inducing transgenes have been segregated away[1–3]. However, only some genes are amenable to this type of modification — for instance, those with high cytosine density in their promoters — and for some applications, such as the silencing of developmental genes, it may be desirable for the silencing to be easily reversible. Furthermore, for dosage-sensitive genes it would be desirable to be able to fine-tune the levels of gene repression. Thus, having a broad range of gene silencing tools would enable the targeting of a wide range of genes and applications. An extensive array of gene silencing tools would facilitate basic research and could be used in crop improvement.

The discovery

Previous research had shown that when components of the RNA-directed DNA methylation system (a pathway unique to plants in which non-coding RNAs guide the methylation of selected DNA sequences) were fused to an artificial zinc finger (ZF) designed to bind to the promoter of the *FWA* gene, these fusion proteins could cause the establishment of *FWA* DNA methylation and silencing when they were transformed into *Arabidopsis thaliana* plants containing a hypomethylated *fwa* epigenetic mutant allele[1,3]. To discover other proteins that could also target *FWA* gene silencing, we fused a collection of 270 putative *Arabidopsis* chromatin-related proteins with the ZF, created transgenic *Arabidopsis* plants for each fusion, and screened the plants for fusions that caused *FWA* silencing, which leads to an early-flowering phenotype. Successful ZF fusions were analysed to determine the mechanism by which they caused *FWA* silencing. The ZF that we used to generate the fusions binds not only to *FWA*, but also to thousands of 'off target' sites, which enabled an analysis of the effects of targeting many additional genes. In addition, some of the effector proteins that were successful as ZF fusions were also developed into CRISPR-based gene targeting systems[2,4].

The screen uncovered 14 effector proteins that were able to silence *FWA*, some by inducing DNA methylation and others via other mechanisms, including inducing H3K27me3 deposition (that is, trimethylation of lysine 27 of the histone H3 protein), H3K4me3 demethylation, histone deacetylation, inhibition of RNA polymerase II (Pol II) transcription, or Pol II dephosphorylation (Fig. 1a). The ZF fusions could also silence hundreds of additional genes in the genome, with each gene silencing tool providing different levels of gene silencing. Some genes were more amenable to silencing by certain effector proteins. A machine learning model using pre-existing chromatin features of the target genes as inputs was able to accurately predict which genes in a test dataset would be effectively silenced by each gene silencing tool (Fig. 1b). Furthermore, genes that were silenced by DNA-methylation-independent fusions showed an immediate reversal of silencing once the silencer was removed.

The implications

This work provides a list of plant genes that are capable of being silenced. These genes participate in biological processes such as development, hormone signalling and disease resistance, suggesting that the silencing tools described here could be useful in modulating varied plant traits. The machine learning algorithm was also used to predict all *Arabidopsis* genes in the genome that should be susceptible to target silencing by each mechanism, which should make the tools more useful for future gene expression engineering research and for crop improvement projects.

Additional experimental evidence will be needed to confirm the accuracy of the machine learning predictions. It will also be important to test whether these tools are equally effective in plants other than *Arabidopsis*.

The screen for effector proteins was done by targeting the *FWA* gene, which has a particular set of pre-existing chromatin marks, meaning that it is possible that additional effectors could be discovered using different reporter genes. Future work in which various effectors are combined may also result in more powerful and universally effective gene silencing tools.

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EXPERT OPINION

"The authors utilized a hypomethylated *FWA* gene as a reporter to screen for regulators of gene silencing in *Arabidopsis thaliana*, identifying 14 proteins that restored the early-flowering phenotype of the *fwa* epiallele. Their results provide important information for understanding how different silencing proteins specifically target different sets of genes, and they provide possible tools for precisely silencing target genes with different chromatin features." **An anonymous reviewer.**

FIGURE

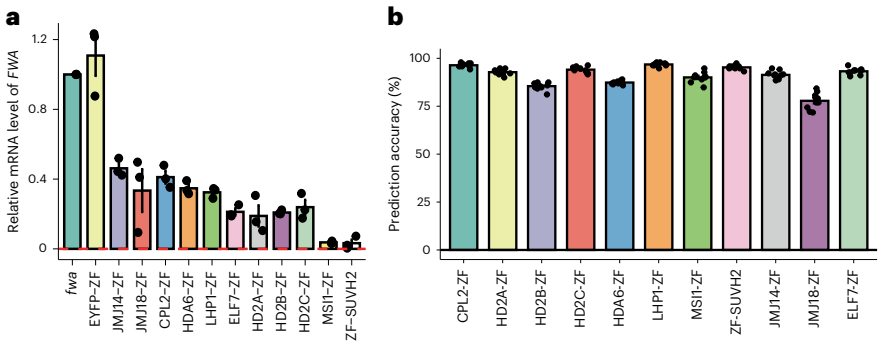

Fig. 1 | Target gene silencing by various plant proteins. a, Relative mRNA level of *FWA* in non-transgenic control plants (*fwa*) and three representative second-generation ZF fusion lines using normalized reads of RNA-sequencing data (reads per kilobase of transcript, per million mapped reads). Error bars, s.e.m. of the three replicates (dots) for each sample. **b**, Accuracy of the machine learning model using a tenfold cross validation for each ZF line. The input dataset was divided into ten groups, of which nine were used as training data and one was used as test data, and this process was iterated ten times.

BEHIND THE PAPER

The project was inspired by earlier work in the laboratory showing that ZF fusions with components of the DNA methylation pathway could efficiently induce *FWA* silencing, and the project was motivated by the desire to find additional gene silencing effectors that could eventually be combined with DNA methylation effectors to create more potent gene silencing systems. The project took more than six years to complete, in part because of the massive amount of cloning and plant transformation required and in part because our first transgene designs produced very few positive hits in the screen, meaning that the entire screen was performed twice. We also learned in hindsight that many of the gene silencing fusion constructs were, ironically, undergoing natural gene silencing themselves, meaning that we may have underestimated the number of effector proteins that can cause gene silencing in this system. **M.W., Z.Z. & S.E.J.**

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FROM THE EDITOR

"Previously, RNA-directed DNA methylation (RdDM) effectors have been fused with zinc finger proteins for target gene silencing. This study stands out because it not only identified many new non-RdDM silencers by a comprehensive screen on chromatin proteins, but also enhanced our understanding of the silencing pathways in plants." **Jun Lyu, Senior editor, *Nature Plants*.**