induce epigenetic modifications at a target locus is desirable as this may allow for controlled expression of the locus (*e.g.* control over gene expression). Moreover, there is currently no robust method for selectively demethylating and activating the expression of plant genes.

[0006] Accordingly, a need exists for epigenetic regulators that are capable of being targeted to specific loci to induce epigenetic modifications at those loci in plants.

BRIEF SUMMARY

[0007] In one example, the present disclosure relates to a method for reducing methylation of a target nucleic acid in a plant, including: (a) providing a plant including a recombinant polypeptide including a DNA-binding domain and a TET1 polypeptide or fragment thereof; and (b) growing the plant under conditions whereby the recombinant polypeptide is targeted to the target nucleic acid, thereby reducing methylation of the target nucleic acid. In some embodiments, the DNA-binding domain includes a zinc finger domain. In some examples, the zinc finger domain includes two, three, four, five, six, seven, eight, or nine zinc fingers. In some examples, the zinc finger domain is a zinc finger array. In some examples, the zinc finger domain is selected from the group of a Cys2His2 (C2H2) zinc finger domain, a CCCH zinc finger domain, a multi-cysteine zinc finger domain, and a zinc binuclear cluster domain. In some examples, the DNA-binding domain is selected from the group of a TAL effector targeting domain, a helix-turn-helix family DNA-binding domain, a basic domain, a ribbon-helix-helix domain, a TBP domain, a barrel dimer domain, a real homology domain, a BAH domain, a SANT domain, a Chromodomain, a Tudor domain, a Bromodomain, a PHD domain, a WD40 domain, and a MBD domain. In some examples, the DNA-binding domain includes a TAL effector targeting domain. In some examples, the DNA-binding domain includes three C2H2 zinc finger domains. In some examples that may be combined with any of the preceding examples, the TET1 polypeptide includes an amino acid sequence that is at least 80% identical to SEQ ID NO: 8. In some examples that may be combined with any of the preceding examples, the target nucleic acid is an endogenous nucleic acid. In some examples that may be combined with any of the preceding examples, the target nucleic acid is a heterologous nucleic acid. In some examples that may be combined with any of the preceding examples, expression of the target nucleic acid is activated as compared to a corresponding control nucleic acid.

[0008] In another example, the present disclosure provides a recombinant nucleic acid including a plant promoter and which encodes a recombinant polypeptide including a DNA-binding domain and a TET1 polypeptide or fragment thereof. The present disclosure further relates to expression vectors including the recombinant nucleic acid of the preceding example, and a host cell including the expression vector of the preceding example. The present disclosure also relates to a recombinant plant including the recombinant nucleic acid and/or polypeptide of the preceding examples.

[0009] In another example, the present disclosure provides a plant having reduced methylation of a target nucleic acid as a consequence of the method of any one of the preceding examples, as well as a progeny plant of the plant of the preceding embodiment. In some embodiments, the progeny plant has reduced methylation of the target nucleic acid and does not include the recombinant nucleic acid and/or polypeptide.

[0010] In another example, the present disclosure provides a method for reducing methylation of a target nucleic acid in a plant, including: (a) providing a plant including a recombinant polypeptide including a nuclease-deficient CAS9 polypeptide (dCAS9) or fragment thereof and a TET1 polypeptide or fragment thereof; and a crRNA and a tracrRNA, or fusions thereof; and (b) growing the plant under conditions whereby the recombinant polypeptide is targeted to the target nucleic acid, thereby reducing methylation of the target nucleic acid. In some examples, the TET1 polypeptide includes an amino acid sequence that is at least 80% identical to SEQ ID NO: 8. In some examples that may be combined with any of the preceding examples, the target nucleic acid is an endogenous nucleic acid. In some examples that may be combined with any of the preceding examples, the target nucleic acid is a heterologous nucleic acid. In some examples that may be combined with any of the preceding examples, expression of the target nucleic acid is activated as compared to a corresponding control nucleic acid.

[0011] In another example, the present disclosure provides a recombinant nucleic acid including a plant promoter and which encodes a recombinant polypeptide including a nuclease-deficient CAS9 polypeptide (dCAS9) or fragment thereof and a TET1 polypeptide or fragment thereof. The present disclosure further relates to expression vectors including the recombinant nucleic acid of the preceding example, and a host cell including the expression vector of the preceding example. The present disclosure also relates to a

recombinant plant including the recombinant nucleic acid and/or polypeptide of the preceding examples.

[0012] In another example, the present disclosure provides a plant having reduced methylation of a target nucleic acid as a consequence of the method of any one of the preceding examples, as well as a progeny plant of the plant of the preceding example. In some examples, the progeny plant has reduced methylation of the target nucleic acid and does not include the recombinant nucleic acid and/or polypeptide.

[0013] In another example, the present disclosure provides a method for reducing methylation of a target nucleic acid in a plant, the method including: (a) providing a plant including a recombinant TET1-like polypeptide or fragment thereof; and (b) growing the plant under conditions whereby the recombinant polypeptide is targeted to the target nucleic acid, thereby reducing methylation of the target nucleic acid.

[0014] In another example, the present disclosure provides a method for reducing methylation of a target nucleic acid in a plant, the method including: (a) providing a plant including a recombinant nucleic acid encoding a TET1-like protein or fragment thereof; and (b) growing the plant under conditions where the recombinant nucleic acid is expressed and where the recombinant polypeptide is targeted to the one or more target nucleic acids, thereby reducing methylation of the target nucleic acid.

[0015] In another example, the present disclosure provides a method for reducing methylation of a target nucleic acid in a plant, the method including: (a) providing a plant including a recombinant nucleic acid encoding a TET1-like protein or fragment thereof; and a crRNA and tracrRNA, or fusions thereof, and where the plant expresses a dCAS9 protein; and (b) growing the plant under conditions where the recombinant nucleic acid is expressed and where the recombinant polypeptide is targeted to the one or more target nucleic acids, thereby reducing methylation of the target nucleic acid. In some examples, the recombinant polypeptide includes a dCAS9 protein or fragment thereof. In some examples, the recombinant polypeptide includes an MS2 protein or fragment thereof. In some examples, the recombinant polypeptide includes an scFV antibody or fragment thereof.

[0016] In another example, the present disclosure provides a method for reducing methylation of a target nucleic acid in a plant, including: (a) providing a plant including: a first recombinant polypeptide including a nuclease-deficient CAS9 polypeptide (dCAS9) or

fragment thereof and a multimerized epitope; a second recombinant polypeptide including a TET1 polypeptide or fragment thereof and an affinity polypeptide that specifically binds to the epitope; a crRNA and a tracrRNA, or fusions thereof; and (b) growing the plant under conditions whereby the first and second recombinant polypeptides are targeted to the one or more target nucleic acids, thereby reducing methylation of the target nucleic acid. In some examples, the dCAS9 polypeptide has an amino acid sequence that is at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical, or 100% identical to SEQ ID NO: 125. In some examples that may be combined with any of the preceding examples, the multimerized epitope includes a GCN4 epitope. In some examples, the multimerized epitope includes about 2 to about 10 copies of a GCN4 epitope. In some examples that may be combined with any of the preceding examples, the first polypeptide includes one or more linkers that link polypeptide units in the recombinant polypeptide. In some examples that may be combined with any of the preceding examples, the first polypeptide includes a nuclear localization signal (NLS). In some examples that may be combined with any of the preceding examples, the TET1 polypeptide includes an amino acid sequence that is at least 80% identical to SEQ ID NO: 8. In some examples that may be combined with any of the preceding examples, the affinity polypeptide is an antibody. In some examples, the antibody is an scFv antibody. In some examples, the antibody includes an amino acid sequence that is at least 80% identical to SEQ ID NO: 132. In some examples that may be combined with any of the preceding examples, the second polypeptide includes one or more linkers that link polypeptide units in the recombinant polypeptide. In some examples that may be combined with any of the preceding examples, the crRNA and the tracrRNA are fused together, thereby forming a guide RNA (gRNA). In some examples that may be combined with any of the preceding examples, expression of the nucleic acid is increased in the range of about 2-fold to about 100-fold as compared to a corresponding control. In some examples that may be combined with any of the preceding examples, expression of the nucleic acid is decreased in the range of about 2-fold to about 100-fold as compared to a corresponding control.

[0017] In another example, the present disclosure provides a recombinant vector including: a first nucleic acid sequence including a plant promoter and that encodes a recombinant polypeptide including a nuclease-deficient CAS9 polypeptide (dCAS9) or fragment thereof and a multimerized epitope; a second nucleic acid sequence including a plant promoter and

that encodes a recombinant polypeptide including a TET1 polypeptide or fragment thereof and an affinity polypeptide that specifically binds to the epitope; and a third nucleic acid sequence including a promoter and that encodes a crRNA and a tracrRNA, or fusions thereof.

[0018] Also provided are host cells including the vector or one or more of the recombinant polypeptides or nucleic acids of any of the preceding examples, and a recombinant plant including the vector or one or more of the recombinant polypeptides or nucleic acids of any of the preceding examples.

[0019] In another example, the present disclosure provides a plant having reduced methylation of a target nucleic acid as a consequence of the method of any of the preceding examples. Also provided is a progeny plant of the plant of the preceding example. In some examples, the progeny plant has reduced methylation of the target nucleic acid and does not include the recombinant polypeptides.

[0019a] In one aspect, there is provided a method for reducing methylation of a target nucleic acid in a plant, comprising:

> (a) providing a plant comprising a recombinant methylcytosine dioxygenase polypeptide that comprises the amino acid sequence of SEQ ID NO: 189 and that is capable of being targeted to a target nucleic acid; and
>
> (b) growing the plant under conditions whereby the recombinant polypeptide is targeted to the target nucleic acid, thereby reducing methylation of the target nucleic acid.

[0020] In another example, the present disclosure provides a method for reducing methylation of a target nucleic acid in a plant, including: (a) providing a plant including a recombinant polypeptide including a DNA-binding domain and a methylcytosine dioxygenase polypeptide that includes the amino acid sequence of SEQ ID NO: 189; and (b) growing the plant under conditions whereby the recombinant polypeptide is targeted to the target nucleic acid, thereby reducing methylation of the target nucleic acid. In some examples, the DNA-binding domain comprises a zinc finger domain. In some examples, the zinc finger domain includes two, three, four, five, six, seven, eight, or nine zinc fingers. In some examples, the zinc finger domain is a zinc finger array. In some examples, the zinc finger domain is selected from the group consisting of a Cys2His2 (C2H2) zinc finger domain, a CCCH zinc finger domain, a multi-cysteine zinc finger domain, and a zinc binuclear cluster domain. In some examples, the DNA-binding domain is selected from the group consisting of a TAL effector targeting domain, a helix-turn-helix family DNA-binding domain, a basic domain, a ribbon-helix-helix domain, a TBP domain, a barrel dimer domain, a real homology domain, a BAH domain, a SANT domain, a Chromodomain, a Tudor domain, a Bromodomain, a PHD domain, a WD40 domain, and a MBD domain. In some examples, the DNA-binding domain includes a TAL effector targeting domain. In some examples, the DNA-binding domain includes three C2H2 zinc finger domains. In some examples that may be combined with any of the preceding examples, the methylcytosine dioxygenase polypeptide is a TET polypeptide. In some examples, the TET polypeptide is a TET1

polypeptide. In some examples, the TET1 polypeptide includes the catalytic domain of TET1. In some examples, the TET1 polypeptide includes an amino acid sequence that is at least 80% identical to SEQ ID NO: 8. In some examples that may be combined with any of the preceding examples, the target nucleic acid is an endogenous nucleic acid. In some examples that may be combined with any of the preceding examples, the target nucleic acid is a heterologous nucleic acid. In some examples that may be combined with any of the preceding examples, expression of the target nucleic acid is activated as compared to a corresponding control nucleic acid.

[0020a] In another aspect, there is provided a recombinant nucleic acid comprising a plant promoter and which encodes a recombinant polypeptide comprising a DNA-binding domain and a methylcytosine dioxygenase polypeptide that comprises the amino acid sequence of SEQ ID NO: 189.

[0021] In another example, the present disclosure provides a recombinant nucleic acid including a plant promoter and which encodes a recombinant polypeptide including a DNA-binding domain and a methylcytosine dioxygenase polypeptide that includes the amino acid sequence of SEQ ID NO: 189.

[0022] In another example, the present disclosure provides a method for reducing methylation of a target nucleic acid in a plant, including: (a) providing a plant including: a first recombinant polypeptide including a nuclease-deficient CAS9 polypeptide (dCAS9) or fragment thereof and a multimerized epitope; a second recombinant polypeptide including a methylcytosine dioxygenase polypeptide that includes the amino acid sequence of SEQ ID NO: 189, and an affinity polypeptide that specifically binds to the epitope; and a crRNA and a tracrRNA, or fusions thereof; and (b) growing the plant under conditions whereby the first and second recombinant polypeptides are targeted to the one or more target nucleic acids, thereby reducing methylation of the target nucleic acid. In some examples, the dCAS9 polypeptide has an amino acid sequence that is at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical, or 100% identical to SEQ ID NO: 125. In some examples that may be combined with any of the preceding examples, the multimerized epitope includes a GCN4 epitope. In some examples, the multimerized epitope includes about 2 to about 10 copies of a GCN4 epitope. In some examples that may be combined with any of the preceding examples, the first polypeptide includes one or more linkers that link polypeptide units in the recombinant polypeptide. In some examples that may be combined with any of the preceding examples, the first polypeptide includes a nuclear localization signal (NLS). In some examples that may be combined with any of the preceding examples, the methylcytosine dioxygenase polypeptide is a TET polypeptide. In some examples, the TET polypeptide is a TET1

polypeptide. In some examples, the TET1 polypeptide includes the catalytic domain of TET1. In some examples, the TET1 polypeptide includes an amino acid sequence that is at least 80% identical to SEQ ID NO: 8. In some examples that may be combined with any of the preceding examples, the affinity polypeptide is an antibody. In some examples, the antibody is an scFv antibody. In some examples, the antibody includes an amino acid sequence that is at least 80% identical to SEQ ID NO: 132. In some examples that may be combined with any of the preceding examples, the second polypeptide includes one or more linkers that link polypeptide units in the recombinant polypeptide. In some examples that may be combined with any of the preceding examples, the crRNA and the tracrRNA are fused together, thereby forming a guide RNA (gRNA). In some examples that may be combined with any of the preceding examples, expression of the nucleic acid is increased in the range of about 2-fold to about 100-fold as compared to a corresponding control. In some examples that may be combined with any of the preceding examples, expression of the nucleic acid is decreased in the range of about 2-fold to about 100-fold as compared to a corresponding control.

[0022a] In another aspect, there is provided an expression vector comprising the recombinant nucleic acid disclosed herein.

[0022b] In another aspect, there is provided a recombinant vector comprising:

-a first nucleic acid sequence comprising a plant promoter and that encodes a recombinant polypeptide comprising a nuclease-deficient CAS9 polypeptide (dCAS9) or fragment thereof and a multimerized epitope;

-a second nucleic acid sequence comprising a plant promoter and that encodes a recombinant polypeptide comprising a methylcytosine dioxygenase polypeptide that comprises the amino acid sequence of SEQ ID NO: 189, and an affinity polypeptide that specifically binds to the epitope; and

-a third nucleic acid sequence comprising a promoter and that encodes a crRNA and a tracrRNA, or fusions thereof.

[0023] In another example, the present disclosure provides a recombinant vector including: a first nucleic acid sequence including a plant promoter and that encodes a recombinant polypeptide including a nuclease-deficient CAS9 polypeptide (dCAS9) or fragment thereof and a multimerized epitope; a second nucleic acid sequence including a plant promoter and that encodes a recombinant polypeptide including a methylcytosine dioxygenase polypeptide that includes the amino acid sequence of SEQ ID NO: 189, and an affinity polypeptide that specifically binds to the epitope; and a third nucleic acid sequence including a promoter and that encodes a crRNA and a tracrRNA, or fusions thereof.

[0023a] In another aspect, there is provided a host cell comprising the expression vector disclosed herein. In another aspect, there is provided a recombinant plant comprising the recombinant nucleic acid disclosed herein.

[0024] Also provided are host cells including the vector or one or more of the recombinant polypeptides or nucleic acids of any of the preceding examples, and a recombinant plant including the vector or one or more of the recombinant polypeptides or nucleic acids of any of the preceding examples.

[0024a] In another aspect, there is provided a plant having reduced methylation of a target nucleic acid as a consequence of the method disclosed herein. In another aspect, there is provided a progeny plant of the plant disclosed herein. In another aspect, there is provided a host cell comprising the vector disclosed herein. In another aspect, there is provided a recombinant plant comprising the vector disclosed herein.

[0025] In another example, the present disclosure provides a plant having reduced methylation of a target nucleic acid as a consequence of the method of any of the preceding examples. Also provided is a progeny plant of the plant of the preceding example. In some examples, the progeny plant has reduced methylation of the target nucleic acid and does not include the recombinant polypeptides.

CLAIMS

What is claimed is:

1. A method for reducing methylation of a target nucleic acid in a plant, comprising:

(a) providing a plant comprising a recombinant methylcytosine dioxygenase polypeptide that comprises the amino acid sequence of SEQ ID NO: 189 and that is capable of being targeted to a target nucleic acid; and

(b) growing the plant under conditions whereby the recombinant polypeptide is targeted to the target nucleic acid, thereby reducing methylation of the target nucleic acid.

2. The method of claim 1, wherein the recombinant methylcytosine dioxygenase polypeptide is targeted to the target nucleic acid via a SunTag targeting system.

3. The method of claim 1, wherein the recombinant methylcytosine dioxygenase polypeptide is targeted to the target nucleic acid via a DNA-binding domain.

4. The method of claim 1, wherein the methylcytosine dioxygenase polypeptide is a TET polypeptide.

5. The method of claim 4, wherein the TET polypeptide is a TET1 polypeptide

6. The method of claim 5, wherein the TET1 polypeptide comprises the catalytic domain of TET1.

7. The method of claim 6, wherein the TET1 polypeptide comprises an amino acid sequence that is at least 80% identical to SEQ ID NO: 8.

8. The method of claim 1, wherein expression of the target nucleic acid is activated as compared to a corresponding control nucleic acid.

9. A recombinant nucleic acid comprising a plant promoter and which encodes a recombinant polypeptide comprising a DNA-binding domain and a methylcytosine dioxygenase polypeptide that comprises the amino acid sequence of SEQ ID NO: 189.

10. An expression vector comprising the recombinant nucleic acid of claim 9.

11. A host cell comprising the expression vector of claim 10.

12. A recombinant plant comprising the recombinant nucleic acid of claim 9.

13. A plant having reduced methylation of a target nucleic acid as a consequence of the method of claim 1.

14. A progeny plant of the plant of claim 13.

15. The progeny plant of claim 14, wherein the progeny plant has reduced methylation of the target nucleic acid and does not comprise the recombinant methylcytosine dioxygenase polypeptide.

16. A recombinant vector comprising:

-a first nucleic acid sequence comprising a plant promoter and that encodes a recombinant polypeptide comprising a nuclease-deficient CAS9 polypeptide (dCAS9) or fragment thereof and a multimerized epitope;

-a second nucleic acid sequence comprising a plant promoter and that encodes a recombinant polypeptide comprising a methylcytosine dioxygenase polypeptide that comprises the amino acid sequence of SEQ ID NO: 189, and an affinity polypeptide that specifically binds to the epitope; and

-a third nucleic acid sequence comprising a promoter and that encodes a crRNA and a tracrRNA, or fusions thereof.

17. A host cell comprising the vector of claim 16.

18. A recombinant plant comprising the vector of claim 17.