Targeted manipulation of DNA methylation in plants
Outline

1. DNA methylation and tools available for targeted manipulation

2. Targeted methylation with ZF fused to RdDM in plants
   - Use of targeted methylation to explore the hierarchy of action of RdDM components
   - Co-targeting RdDM components to enhance methylation

4. Targeted methylation with Suntag fused to DRM in plants

5. Targeted demethylation with ZF and Suntag fused to TET1 in plants
DNA methylation

✧ Heritable covalent epigenetic mark

✧ Gene silencing, imprinting, genome stability, chromatin architecture

Law and Jacobsen. 2010
DNA methylation levels and distribution differs between organisms

<table>
<thead>
<tr>
<th>Species</th>
<th>CG</th>
<th>CHG</th>
<th>CHH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apis mellifera</td>
<td>0.93%</td>
<td>0.26%</td>
<td>0.17%</td>
</tr>
<tr>
<td>Ciona intestinalis</td>
<td>31.1%</td>
<td>0.17%</td>
<td>0.12%</td>
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<tr>
<td>Mus musculus</td>
<td><strong>74.2%</strong></td>
<td>0.30%</td>
<td><strong>0.29%</strong></td>
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<tr>
<td>Danio rerio</td>
<td>80.3%</td>
<td>1.22%</td>
<td>0.91%</td>
</tr>
<tr>
<td>Chlamydomonas reinhardtii</td>
<td>5.38%</td>
<td>2.59%</td>
<td>2.49%</td>
</tr>
<tr>
<td>Oryza sativa</td>
<td>59.4%</td>
<td>20.7%</td>
<td>2.18%</td>
</tr>
<tr>
<td>Arabidopsis thaliana</td>
<td><strong>22.3%</strong></td>
<td><strong>5.92%</strong></td>
<td><strong>1.51%</strong></td>
</tr>
<tr>
<td>Populus trichocarpa</td>
<td>41.9%</td>
<td>20.9%</td>
<td>3.25%</td>
</tr>
</tbody>
</table>

Gene body

Transposable elements

Feng et al, PNAS, 2010
DNA methylation enzymes in mammals and plants

**Mammals**

**Methylation**
- DNMT1: CG methylation maintenance
- DNMT3 (A and B): *de novo* CG methylation

**Demethylation**
- TET proteins

**Plants**

**Methylation**
- MET1 (DNMT1 homolog): CG maintenance
- CMT2, CMT3: CHH and CHG maintenance (H is C, T, A)
- DRM2 (DNMT3 homolog): *de novo* methylation (all context)

**Demethylation**
- ROS1/DMT proteins
DNA methylation affects gene expression

- Methylated promoter
- Gene body methylation

**Gene silencing**
- Unmethylated promoter
- Methylated promoter
- Chromatin remodeller Histone modifier
- Methyl reader

**Gene activation**
- Transcriptional machinery
- DNAJ1,2
- SUVH1,3

Arrows indicate the direction of gene expression regulation.
Loss of promoter methylation causes gene up-regulation

Manipulation of DNA methylation is desirable to:

✧ study its effect on gene expression and chromatin features

✧ modulate gene expression and generate novel epialleles (biotechnological tool)

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**Fruit development**

- **EgDEF1**
  - Good Karma
  - Transcription
  - Spliced transcripts
  - Bad Karma
  - Transcription

**Fruit ripening**

- Mantled oil palms: Dramatic reduction in oil yields
- Tomato fruit ripening
  - Epimutation
  - CNR dynamic demethylation

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*fwa* epiallele

**Col-0**

- Early flowering
- 10 leaves (10-17)

**fwa**

- Late flowering
- 30 leaves (25-50)

Methylated *FWA* promoter

Unmethylated *FWA* promoter
Manipulation of DNA methylation is desirable to:

✧ study its effect on gene expression and chromatin features

✧ modulate gene expression and generate novel epialleles (biotechnological tool)

How to manipulate DNA methylation?

Use of mutants
\(met1, ddm1, drm2, ros1, ibm2\ldots\)

Pharmacological treatments
(5-azacytidine, zebularine)

Genome-wide effects

Mathieu et al, Cell, 2007

Mock

Zebularine (uM)

20 40 80

Baubec et al, TPJ, 2008
Targeted DNA de/methylation using programmable DNA-binding domains

- Artificial Zinc Finger
- TAL effectors
- CRISPR/dCas9

Thakore, Nature Methods, 2016
Multiple tools to manipulate DNA methylation

Targeted methylation and demethylation using ZF and Suntag in plants

Gallego-Bartolome et al. PNAS 2018
Gallego-Bartolome et al. Cell 2019
Papikian et al. Nat Comm 2019
Preparation of programmable DNA-binding domain fusions

- Which effector? DNMT3cd and TET1cd

- Which DNA-binding protein?
  - ZF, TALE
  - dCas9: easier reprogram and multiplex
    - Direct fusion
    - RNA aptamers
    - Suntag
    - Other Cas proteins: SaCas9, Cas12

- N- or C- fusions. Linkers: flexible, rigid. Promoter: Constitutive, cell-type specific
- gRNAs: one, multiple
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RNA-directed DNA methylation
Targeted methylation using artificial Zinc Fingers

fwaf

Late flowering

Wild type

Early flowering

Unmethylated FWA promoter

Methylated FWA promoter

RdDM

ZF

SINE
Multiple RdDM components can be used for targeted methylation

- SHH1
- NRPD1
- RDR2
- CLSY
- DRM2
- DNMT
- AGO4/6/9
- SUVH9
- MORC6
- DCL1/2/3/4
- Pol V recruitment

siRNA biogenesis

k9me
Study the hierarchy of action of RdDM components
DMS3-ZF acts upstream of DRD1 and Pol V

Pol V recruitment is sufficient to target methylation in FWA

Gallego-Bartolomé et al. Cell. 2019
Small number of off-targets become methylated

DMS3-ZF + NRPD1-ZF

Gallego-Bartolomé et al. Cell. 2019
Combination of NRPD1-ZF and DMS3-ZF enhances the efficiency of targeted methylation
Off-target methylation near the TSS represses gene expression.

Gallego-Bartolomé et al. Cell. 2019
Highly heritable methylation occurs at CG context
ZF, Pol V and siRNA all contribute to efficient targeted methylation
Summary

- ZF-RdDM can be used to target methylation in plants
- Targeted Pol IV (NRPD1) or Pol V (DMS3) is sufficient to trigger methylation
- Co-targeting of Pol IV and Pol V enhances targeted methylation
- Targeted methylation near the TSS silences gene expression
- CG targeted methylation is more heritable
- ZF binding, siRNA and Pol V accumulation contribute to efficiency
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Suntag-based targeted methylation

UBQ10 promoter

UBQ10 promoter

U6 promoter

gRNA1

dCas9 + HA + NLS

10xGCN4

scFv

sfGFP

NtDRMcd

SV40 NLS

GB1+ REX NLS

Methylated promoter (early flowering)

Unmethylated promoter (late flowering)

fwa

Papikian et al. Nature Comm. 2019
Suntag target recognition is very specific
Targeted methylation with one gRNA
Longer GCN4 linkers allow more efficient methylation.
Expression of multiple gRNAs enhances methylation.

Papikian et al. Nature Comm. 2019
SunTag-DRM triggers heritable methylation and *FWA* silencing

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**Figure 1:**

- **AT4G25530.1**
- **Chr4:13,018,700–13,063,605**
- **fwa**
- **Col-0**
- **T2 22aa Sun DRMcd g4+g10+g18-2 (+)**
- **T2 22aa Sun DRMcd g4+g10+g18-2 (−)**

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Papikian et al. Nature Comm. 2019
Suntag-DRM induces high CHH background
High CHH background is independent of gRNAs

dCas9-DNMT3 expression in mammalian cells also causes genome-wide ectopic methylation
Removing NLS in scFv-DRM reduces methylation background
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Removing NLS in scFv-DRM reduces methylation background
Summary

• Suntag-DRM is a powerful tool for targeted methylation in plants

• Suntag shows very reduced number of off-targets

• Higher targeted methylation with 22aa linker than 5aa

• Higher targeted methylation with 3 gRNAs than 1 gRNA

• Presence of NLS in scFV-DRM triggers ectopic CHH methylation

• Construct optimization is important to achieve high and specific methylation
Targeted DNA demethylation using the human TET1 catalytic domain.

**ZF-based tool**

- ZF6, ZF6, ZF6, ZF6, ZF6, ZF6
- CGG, AAA, GAT, GTA, TGG, GCT
- *FWA* target sequence

**Suntag-based tool**

- dCas9
- 14aa and 22aa linkers

**Methylated promoter** (early flowering)  $\Rightarrow$ **Unmethylated promoter** (late flowering)

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**FWA** promoter

TET1CD

Target

**dCas9**
Targeted DNA demethylation and up-regulation of FWA using ZF-TET1cd

Gallego-Bartolomé et al. PNAS. 2019
FWA ZF-TET1cd is highly specific.
Targeted DNA demethylation using SunTag-TET1cd
Conclusions

• Different tools can be exploited to manipulate DNA methylation

• Mammalian systems are based on the use of DNMT3 and TET1

• Targeted methylation in Arabidopsis can be achieved with ZF-RdDM fusions

• Simultaneous targeting of two RdDM components dramatically enhances methylation

• Suntag can be used to target methylation with high specificity in Arabidopsis

• ZF and Suntag can be exploited for targeted demethylation in Arabidopsis

• Proper experiments needed to investigate off-target activity. Construct optimization
Thanks for your attention!!