Patterns of Histone Methylation and Chromatin Organization in Grapevine Leaf

Rachel Schwope

EPIGEN
May 24-27, 2016
Plant of interest: *Vitis vinifera*

**Culturally important**

An economically vital organism for Italy and beyond

**Scientifically intriguing**

A clonal organism whose genome has not undergone meiosis – or its developmental program – in centuries.
Chromatin – the structural and functional complex of DNA and binding proteins

ase.org.uk

http://www.structuralbiology.uzh.ch/
Chromatin is specialized to maintain genomic states

- A recent ChIP-Seq meta-study identified 9 states of chromatin in *Arabidopsis* (Sequeira-Mendes, Plant Cell 2014)

  - 4 types = high transcription
  - 5 types = low transcription

![Gene expression (RPKM) vs Chromatin state](Sequeira-Mendes, Plant Cell 2014)

- Promoter and CDS-rich
- Intergenic and TE-rich
What defines a Chromatin State?

1) DNA methylation (CG, CHG, CHH)
2) Post-translational modification of histones (H3K9ac, etc.)
3) Histone variants (H3.1, H2AZ, etc.)

(Sequeira-Mendes, Plant Cell 2014)

(www.promega.com)
### Functions of Specific Histone Modifications at H3

<table>
<thead>
<tr>
<th>H3K4me3</th>
<th>H3K9me2</th>
<th>H3K27me3</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Recruits <strong>transcription initiation</strong> factors</td>
<td>- <strong>In Arabidopsis</strong>, bound by CMT3 DNA methylase</td>
<td>- Associated with <strong>Polycomb</strong></td>
</tr>
<tr>
<td>- Promotes <strong>open</strong> conformation</td>
<td>- <strong>In maize</strong>, increased at <strong>transposons</strong> and some genes</td>
<td>- <strong>In maize</strong>, found in gene-dense chromatin arms, not at pericentric chromatin</td>
</tr>
</tbody>
</table>

---

Chromatin Immunoprecipitation (ChIP) Methodology

Chromatin from fixed leaf tissue or nuclei → ChIP with Abcam Plant Kit → Library production (Nugen Ultralow)

Library production (Nugen Ultralow) → 125 bp PE Illumina Sequencing → Quantification and Quality Control

Bowtie Alignment MACS2 Peak Call
Where is H3K4me3 found in grapevine leaves?

- Peaks are generally at the 5’ ends of genes
- Have a broader “shoulder” at the 3’ side of the peak

**Chromosome 2: 3,198,150..3,225,396**
H3K4me3 coverage over gene body

- As gene size increases, H3K4me3 covers a proportionally smaller amount of the gene.
How Enriched is H3K4me3 in Grapevine leaf?

Enrichment of H3K4me3 peaks in grapevine leaf

Fold Enrichment vs Total Peaks

Enrichment level Distribution

Fold Enrichment
What does H3K4 methylation do?

H3K4me3 Enrichment generally increases with Peak Length

H3K4me3 Peak Enrichment vs. peak length
What does H3K4 methylation do? …But only correlates weakly with Transcript Accumulation.

H3K4me3 enrichment versus FPKM for Pinot Noir

FPKM data from M. Miculan
Peak length also correlates weakly with Transcript Accumulation

H3K4me3 Peak length versus FPKM for Pinot Noir

FPKM data from M. Miculan
Where else do we find H3K4me3 enrichment?

**Long non-coding RNAs**: at least 200 bp long

- 2044 intergenic long-ncRNAs in *Vitis* genome (*Genoscope*)
- In leaf, 219 of these (11%) correlate with H3K4me3 signal
What does H3K4 methylation do?

What are the lncRNA species co-occur with H3K4me3?

Total: 219 lncRNAs

• 15 of these encode miRNAs (miRbase.org)

• 204 have no discernable miRNA structure
How do Heterochromatinic modifications compare?

Distribution of Enrichment in grapevine leaf

Enrichment level Distribution

- H3K4me3
- H3K9me2
- H3K27me3

Total Peaks

Fold Enrichment

- 1
- 730
- 1459
- 2188
- 2917
- 3646
- 4375
- 5104
- 5833
- 6562
- 7291
- 8020
- 8749
- 9478
- 10207
- 10936
- 11665
- 12394
- 13123
- 13852
- 14581
- 15310
- 16039
- 16768
- 17497
- 18226
- 18955
- 19684
- 20413

Fold Enrichment
Where do we find H3K9me2 and H3K27me3?

<table>
<thead>
<tr>
<th>Transposon Types Associated with H3K9me2 and H3K27me3</th>
</tr>
</thead>
<tbody>
<tr>
<td>RLG</td>
</tr>
<tr>
<td>RLR</td>
</tr>
</tbody>
</table>

- K9me2: Total peaks = 11,487
  - 51.8% LTR-Gypsy
  - 26.0% LTR-Copia
  - 3.3% RIL

- K27me3: Total peaks = 12,270
  - 50.0% LTR-Gypsy
  - 26.5% LTR-Copia
  - 2.4% RIL
Many, but Not All Highly Repetitive TEs are Enriched

What does H3K4 methylation do?
What happens when I ask MACS2 to find peaks from an *unenriched* alignment?
What does H3K4 methylation do?

Separating Signal from Noise

kmers

‘Input mock’

H3K27me3

H3K9me2

TEs

‘True Positives’

‘False Positives’
What does H3K4 methylation do?

What is the DNA methylation state at loci with modified histones?

Genome-wide average methylation

H3K4me3

H3K9me2

M. Celii
*Vitis* results support *Arabidopsis* data that DNA and histone methylation form feedback loop.

Adapted from Stroud et al., 2013
Hi-C: a method of inferring the global three-dimensional relationships from paired-end sequence data
Hi-C Methodology

In the lab:
- Crosslink DNA
- Cut with restriction enzyme
- Fill ends and mark with biotin
- Ligate
- Purify and shear DNA; pull down biotin
- Sequence using paired-ends

Adapted from Lieberman et al, 2009

In silico:

**HOMER** (v4.8, 1-13-2016)
Software for motif discovery and next generation sequencing analysis

[Integrative Genomics and Bioinformatics Core](#) at the [Salk Institute](#)
What does H3K4 methylation do?

- Each chromosome shows a unique pattern of organization.

A. Tocci
What does H3K4 methylation do?

ChIP data corresponds with 3D structure

LSD

CSD

Chr 8

H3K4me3

H3K9me2

H3K27me3
What does H3K4 methylation do?

- Each chromosome shows a unique pattern of organization.
What does H3K4 methylation do?

ChIP data corresponds with 3D structure

LSD

CSD

Chr 17

H3K4me3

H3K9me2

H3K27me3
What does H3K4 methylation do?

• Each chromosome shows a unique pattern of organization

PCA vs Heatmap vitis chr4

“Loose Structural Domain”

“Compact Structural Domain”
What does H3K4 methylation do?

ChIP data corresponds with 3D structure
What does H3K4 methylation do?

Chromatin Organization is Highly Stable

Biological Replicate 1

Biological Replicate 2
Pinot Noir Genomic Contact Map

Easily visualize the actual locations of unassigned regions of the genome assembly

A. Tocci
Conclusions and Future Directions

1. **Much to uncover with ChIP-Seq**
   - H3K4me3 results = high confidence
   - Allele specific analysis in progress
   - Other histone modifications will require finer analyses

2. **Hi-C shows chromatin structure very stable**
   - Try varieties other than Pinot Noir – do they have the same organization?
   - Use Hi-C to improve genome assembly (in progress - Aldo Tocci)
Acknowledgements

IGA
Prof. Michele Morgante
Emanuele de Paoli
Fabio Marroni
Aldo Tocci
Mirko Celii
Mara Miculan
Gabriele Magris

IGA Technology Services
Emanuela Aleo
Federica Magni
Giacomo Prete

Lab and Administration
Giusi Zaina
Nicoletta Felice

Stefano Grasso

Supported by the ERC project NOVABREED - Novel variation in plant breeding and the plant pan-genomes (Grant agreement no.: 294780)
What does H3K4 methylation do?

- CHH
- CHG
- CG
- H3K9me2
- H3K27me3
- H3K4me3
- Transposon Elements
- Genes
Hi-C shows H3K4me3 is located preferentially in Loose Structural Domains

H3K4me3 Peaks

- Average # of peaks per 50 kb window
  - CSD: 0.709, 3524
  - LSD: 2.65, 11988

Genes

- Average # of genes per 50 kb window
  - CSD: 2.54, 12636
  - LSD: 4.89, 22098

A. Tocci
What does H3K4 methylation do?

H3K9me2 distribution mimics that of total TEs

Average # of peaks per 50 kb window

H3K9me2 Peaks

Transposon Elements

Average # of TEs per 50 kb window

CSD

LSD

CSD

LSD

A. Tocci
Transposons with K9me2 or K27me3 have higher k-mer count

K-mer count of TEs from Chromosome 2

- TEs with H3K9me2
- TEs with H3K27me2
- All TEs in Chr2
Enriching for Antibody-associated DNA with Chromatin Immunoprecipitation

1. Isolate and shear chromatin mechanically
2. Add antibody specific for acetylated N-terminal histone tail
3. Immunoprecipitate
4. Release immunoprecipitated DNA and assay by PCR

Cross-linked chromatin
What does H3K4 methylation do?

H3K9me2 Enrichment generally increases with Peak Size.
Transposons with H3K9me2 or H3K27me3 have higher k-mer count

K-mer count of TEs in Chromosome 2
How do heterochromatin modifications at histones relate to DNA methylation?
How do heterochromatic modifications at histones relate to DNA methylation?

- H3K4me3
- H3K27me3
- H3K9me2

position (percentiles)