Proteome Analysis of Chromatin Associated Proteins During Endosperm Development in Rice (*Oryza sativa*)

Mississippi State University

Zhaohua Peng, Dwight Kanter, Jiaxu Li, Carolyn Boyle, John Boyle

The Ohio State University

Guo-Liang Wang

Endosperm is economically the most important organ in plants

Endosperm represents up to 70% of the world’s food supply

It is estimated that rice alone feeds half of the world population
Like all other flower plants, rice uses double fertilization for reproduction—an unique feature of higher plants.

**3n endosperm**
- Two sets of the genomes from mother side
- One set from father side

**2n embryo**
- One from mother side
- The other one from father side
Chromatin Plays a Critical Role in Regulating Endosperm Development

1. Ploidy barrier of hybridization
2. Parental gene dosage effect (maternal:parental ratio of 2:1 is critical for endosperm development)
3. Endoreduplication-up to 690C in maize
4. Chromatin remodeling has been shown to be critical for high level expression of genes encoding storage proteins
5. Effect of Parental-of-Origin-Imprinting

**IMPRINTING**

**Imprinting** is a form of epigenetic gene regulation by which the expression of a gene depends on the parent from which it is derived

**Gene-specific imprinting**
Anthocyanin production in maize aleurone cells is controlled by the *rl* locus. Kermicle (1970) discovered that fully colored R allele is transmitted only maternally.

**Genome or chromatin-wide imprinting in plants**
While studying the chromosome regions regulating endosperm development in maize, Lin *et al* (1982,1984) found that maternal chromosome B^{10}'s were unable to compensate for the absence of paternal B^{10}, indicating genome imprinting.
Imprinting in Endosperm Development in Arabidopsis

1. After having studied 19 transposon-tagged and GUS fused genes that encode a broad range of cellular functions, Vielle-Calzada et al (2000) concluded that a genome-wide imprinting mechanism occurs during early embryogenesis. Reporter expression was delayed up to the mid-globular stage when inherited from male.

2. med/fis1, fix2, and fie mutants generate diploid endosperm autonomously without fertilization. Wildtype allele from pollen did not restore the mutant phenotype-indicating imprinting. The products of these genes are chromatin associated proteins that regulate histone modifications, change higher order chromatin structure, and result in gene repression.

Imprinting at Molecular Level

Mammals: Histone and DNA methylation-Allele specific, de novo

Plants: Probably use a different approach

Kinoshita et al, 2004

1. Arabidopsis FWA gene imprint depends on the maintenance of DNA methyltransferase MET1, suggesting the role of DNA methylation in plant imprinting

2. Maternal specific expression of FWA is established by maternal specific activation-suggesting that silent methylated state is the default state
Histones are basic proteins that associated tightly with DNA in the chromosomes.

The NH2-terminal and COOH-terminal “tail” of histones are the primary sites for post-translational modifications. The histones of active genes are preferentially acetylated. Meanwhile the histones of inactive genes are hypoacetylated.

Histone code—more complicated
Although chromosome and chromatin have been extensively studied, the high level structure and the molecular composition of chromatin and chromosome are still unknown.

Chromatin is not simply a way of DNA packaging in nucleus. It represents a highly conserved regulatory entity that provides a means of integrating multiple endogenous and exogenous signals for the establishment and maintenance of gene expression profile.

**SUMMARY**

1) Chromatin plays multi-facet role in regulating endosperm development.

1) Many genetically identified genes that regulating endosperm development encode chromatin associated proteins and subjected to imprinting regulation.
**INTERESTING QUESTIONS**

1) What is the unique feature of endosperm chromatin subproteome?

2) What is the dynamic change of endosperm chromatin during development?

3) What is the difference of the imprinting mechanism between plants and mammals?

4) Any particular posttranslational modification in endosperm contributes to imprinting regulation?

---

**Objectives of the Project**

1) Identifying the rice chromatin sub-proteome in both root and endosperm

2) Identifying chromatin proteins specific to endosperm development

3) Identifying posttranslational modifications correlated to endosperm development

4) Generating at least 45 mutants of chromatin genes that regulate endosperm development
Workflow of the Project

Purification of Chromatin from Rice Tissues

Identification of Endo-Sperm Specific Proteins by Chromatin Sub-proteome Comparison of Different Tissues

Extraction and Fractionation of Chromatin Proteins

Separation of the Chromatin Proteins by 2-D Gels

Identification of the Proteins by Mass Analyses

Study Temporal Regulation of Endosperm Chromatin Subproteome by Comparison of Different Time Points

Study Endosperm Specific Post-translational Modifications of the Chromatin Associated proteins

Purification of Chromatin

Chromatin is a DNA and protein Supercomplex. It is among the most rapidly pelletable components of tissue homogenate. It can be purified when multiple Differential and Sucrose Density Gradient Centrifugations are combined

Electron microscopy photo of purified *Arabidopsis* chromatin
Preliminary Results of Mass Analyses of the Purified Chromatin Proteins

1. Typical chromatin associated proteins have been identified

   Histones, DNA repair proteins, DNA helicase, DNA polymerase, retrotransposon proteins, DNA binding proteins, elongation factors, RNA polymerase, histone acetyl transferase, polycomb proteins, and so on have been identified

2. Many low abundance transcription factors have been identified
Table 1: Identified chromatin associated proteins from Arabidopsis thaliana using 2-Dgel followed by MALDI-TOF.

<table>
<thead>
<tr>
<th>No</th>
<th>Acc #</th>
<th>Protein name</th>
<th>Exp. molar mass (Da)</th>
<th>Theoritical Mol.mass (Da)</th>
<th>Coverage %</th>
<th>Number of matched peptides</th>
<th>Exposition significant score</th>
<th>E-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Q93WU9</td>
<td>Probable WRKY transcription factor</td>
<td>51000</td>
<td>51000</td>
<td>50</td>
<td>53</td>
<td>1.15E-03</td>
<td>0.0077</td>
</tr>
<tr>
<td>2</td>
<td>O82256</td>
<td>Zinc finger protein</td>
<td>35000</td>
<td>35000</td>
<td>35</td>
<td>32</td>
<td>0.0000</td>
<td>0.0077</td>
</tr>
<tr>
<td>3</td>
<td>Q9STX0</td>
<td>Probable WRKY transcription factor</td>
<td>36000</td>
<td>36000</td>
<td>36</td>
<td>30</td>
<td>0.0000</td>
<td>0.0086</td>
</tr>
<tr>
<td>4</td>
<td>P28689</td>
<td>Histone H1.2</td>
<td>25000</td>
<td>24870</td>
<td>25</td>
<td>13</td>
<td>0.0000</td>
<td>0.0051</td>
</tr>
<tr>
<td>5</td>
<td>Q82776</td>
<td>Histone lysine N-methyltransferase</td>
<td>75000</td>
<td>75000</td>
<td>75</td>
<td>59</td>
<td>0.0000</td>
<td>0.0007</td>
</tr>
<tr>
<td>6</td>
<td>Q9SGM2</td>
<td>Double-strand break repair protein MRE11</td>
<td>55000</td>
<td>25524</td>
<td>55</td>
<td>48</td>
<td>0.0000</td>
<td>0.0049</td>
</tr>
<tr>
<td>7</td>
<td>Q47C8X</td>
<td>Zinc finger protein</td>
<td>44000</td>
<td>44000</td>
<td>44</td>
<td>33</td>
<td>0.0000</td>
<td>0.0008</td>
</tr>
<tr>
<td>8</td>
<td>P45553</td>
<td>Zinc finger protein</td>
<td>7500</td>
<td>7500</td>
<td>75</td>
<td>59</td>
<td>0.0000</td>
<td>0.0004</td>
</tr>
<tr>
<td>9</td>
<td>Q9M223</td>
<td>Double-strand break repair protein MRE11</td>
<td>70000</td>
<td>27132</td>
<td>70</td>
<td>54</td>
<td>0.0000</td>
<td>0.0056</td>
</tr>
<tr>
<td>10</td>
<td>Q48520</td>
<td>Zinc finger protein</td>
<td>48000</td>
<td>51367</td>
<td>48</td>
<td>37</td>
<td>0.0000</td>
<td>0.0006</td>
</tr>
<tr>
<td>11</td>
<td>P95226</td>
<td>Zinc finger protein</td>
<td>10000</td>
<td>10000</td>
<td>10</td>
<td>8</td>
<td>0.0000</td>
<td>0.0032</td>
</tr>
<tr>
<td>12</td>
<td>P95226</td>
<td>Zinc finger protein</td>
<td>10000</td>
<td>10000</td>
<td>10</td>
<td>8</td>
<td>0.0000</td>
<td>0.0029</td>
</tr>
<tr>
<td>13</td>
<td>P94883</td>
<td>Zinc finger protein</td>
<td>25000</td>
<td>25448</td>
<td>25</td>
<td>17</td>
<td>0.0000</td>
<td>0.0039</td>
</tr>
</tbody>
</table>

Methods for Quantitative Chromatin Proteome Comparison

**Primary Method**

2-D Fluorescence Difference Gel Electrophoresis (2-D DIGE) System

**Alternative Methods**

1) Cleavable Isotope-Coded Affinity Tag (cICAT) method

2) Applied Biosystems iTRAQ™ method

A new type of isobaric tags specifically label all primary amines for quantitative proteomics. Up to four samples, absolute quantitation maintaining PTMs, and so on.
2-D DIGE System for Quantitative Proteomics

The internal standard is a mixture of all samples in equal amount. The fluorescent Cydyes are very sensitive (0.12-0.6 ng/band). For quantitative analysis, it does not take too much sample (about 50 µg for each labeling reaction). For mass analysis, a preparative gel with more sample loading has to be run simultaneously.

Examples of DIGE Gels

The colorful spots reveal proteins with differential expression. Most post-translational modified proteins will appear as new spots.
Comparison of rice root and endosperm chromatin proteome

Red represents endosperm sample and green represents root sample

Chromatin Mutant Isolation

Once endosperm specific chromatin proteins have been identified, rice mutants of these genes will be isolated. Meanwhile, chromatin genes known to regulate endosperm development in other organisms will also be investigated.

Guo-Liang’s lab has searched insertion mutants for 55 candidate genes, 17 putative insertion lines have been identified from TOS17 in Japan and French group collections.

In case the desired mutants are not available in public mutant collections, RNAi mutant lines or over-expression lines will be generated. Overall, minimal 45 mutant lines will be generated.
**Deliverables**

1) A chromatin subproteome database of rice endosperm and root

2) A quantitative analysis database revealing the dynamic change of chromatin proteome in development

3) A collection of insertion, RNAi or over-expression mutants of endosperm specific chromatin genes

**Broader Impact of the Project**

1) The rice chromatin proteome database will have reference value in general biological science and biomedical community

2) The identified endosperm specific chromatin proteins and post-translational modifications might be very useful in the studies of other cereal crops

3) The mutants identified in this project can be used as a tool to study other biological questions
Long-Term Goals of the Project

1) Identifying the unique features of rice endosperm chromatin proteome

2) Identifying the key chromatin components that regulate endosperm development

3) Manipulating these key regulators to create high quality and high yield rice varieties

4) Understanding the molecular mechanisms of chromatin mediated regulation of endosperm development

ACKNOWLEDGMENTS

Mississippi State University
Dwight Kanter, Jiaxu Li
Carolyn Boyle, John Boyle

The Ohio State University
Guo-Liang Wang
Tel: (614) 292 9280 (O)
Email: wang.620@osu.edu

My Lab
Zhaohua Peng
Tel: 662-325-0685
Email: zp7@ra.msstate.edu

Li-Feng Zhang
Brahma Chitteti

USDA CSREES
Ed Kaleikau and the Panel
1. Ploidy Barrier of Hybridization

If parents with different ploidy are crossed, the seeds develop abnormally and often abort in most flowering plants. Even if diploid are crossed with their autotetraploids, Lin, et al (1984) has shown that a maternal to parental ratio of 2:1 is critical for normal endosperm development. Any other ratio produced abnormal endosperm in maize.

2. Parental Gene Dosage Effect

Lin, et al (1984) has shown that a maternal to parental ratio of 2:1 is critical for normal endosperm development. Any other ratio produced abnormal endosperm in maize.