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

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Endosperm is economically the most important organ in plants

Cereal crop 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—a unique feature of higher plants.

**Double Fertilization**

Generate endosperm

3n endosperm

two sets of the genomes from mother side
one set from father side

2n embryo

One from mother side
and the other one from father side
Chromatin Plays a Critical Role in Endosperm Development

1. Ploidy barrier of hybridization

2. Parental gene dosage effect (maternal:paternal ratio of 2:1 is critical for endosperm development)

3. Effect of Parental-of-Origin-Imprinting*

4. Endoreduplication-up to 690C in maize

5. Chromatin remodeling has been shown to be critical for high level expression of genes encoding storage proteins and starch synthesis enzymes

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

BACKGROUND SUMMARY

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

2) Many genetically identified genes that regulating endosperm development encode chromatin associated proteins and subjected to imprinting regulation

3) The molecular composition and high level structure of chromatin are still obscure.
**Objectives of the Project**

1) Identifying the rice chromatin sub-proteome in both suspension cells 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 in rice

**Methods**

Proteomics-Comparison of Chromatin Associated Proteins between Suspension Cells and Endosperm Cells

To avoid organelle contamination, we use purified nuclei to extract chromatin and then chromatin associated proteins
Chromatin Purification from Rice Suspension Cells in Large Scale

- Suspension Cells
  - Protoplasts
    - Nuclei
      - Chromatin
  - Nuclei
    - Chromatin

DAPI Stain of Nuclei

- Suspension Cell Crude Extract
- Purified Suspension Cell Nuclei
Purification of Endosperm Nuclei

Extract sample from milky stage endosperm

Ground Tissue and Sucrose Gradient Centrifugation

Sample
1.7 M Sucrose
Nuclei
2.0 M Sucrose
Starch Grain

DAPI Stain of Nucleus

Endosperm Nucleus  Purified Endosperm Nucleus
DAPI Stain of Chromatin Mass

Purified Chromatin under Electromicroscopy
Chromatin Protein Extraction

Phenol Extraction Method was used to separate DNA from proteins

MudPIT method for basic protein identification

MudPIT: Multidimensional Protein Identification Technology
SCX: Strong Cation Exchanger Chromatography
RP: Reverse Phase Chromatography
SEQUEST Search Parameters

Xcorr: +1/1.9; +2/2.5; +3/3.75

Delta Corr: > 0.1

P factor: < 0.001

Over one hundred proteins have been identified

Examples of Identified Proteins

1. gi|50934746|emb|XP_476900.1| putative Natone H1 [Oryza sativa (japonica cultivar-group)]
2. gi|37534399|emb|XP_501497.1| putative histone H2A [Oryza sativa (japonica cultivar-group)]
3. gi|3488989|emb|XP_503922.1| putative histone H2B [Oryza sativa (japonica cultivar-group)]
4. gi|50947133|emb|XP_483094.1| putative Histone H2B.2 [Oryza sativa (japonica cultivar-group)]
5. gi|34898309|emb|XP_501498.1| histone H3 [Oryza sativa (japonica cultivar-group)]
6. gi|206241|emb|AAA39155.1| H4.1 [Emericella nidulans]
7. gi|206241|emb|AAA39155.1| histone H4.2 [Emericella nidulans]
8. gi|50904844|emb|XP_470448.1| putative centromere/microtubule binding protein [Oryza sativa (japonic
9. gi|5700677|gb|AKW57682.1| putative histone deacetylase HD2 [Oryza sativa (japonica cultivar-group)]
10. gi|50934369|emb|XP_476712.1| putative Natone histone deacetylase [Oryza sativa (japonica cultivar-group)]
11. gi|3288883|gb|AAA31261.1| SAD DNA binding protein [Oryza sativa]
12. gi|50934783|emb|XP_479419.1| putative nuclear protein [Oryza sativa (japonica cultivar-group)]
13. gi|50915665|emb|XP_466771.1| nuclear RNA-binding Nop1p-like protein [Oryza sativa (japonica cult
14. gi|50901616|emb|XP_483094.1| putative U3 small nuclear ribonucleoprotein complex-associated prote
15. gi|50947133|emb|XP_483094.1| GTP-binding nuclear protein RAN-81 [Oryza sativa (japonica cultivar-gr
16. gi|50934369|emb|XP_476712.1| putative nuclear protein [Oryza sativa (japonica cultivar-group)]
17. gi|75533102|gb|ABA55856.1| trp protein [Oryza sativa (japonica cultivar-group)]
18. gi|5230272|gb|AAA44286.1| putative ribokinase [Oryza sativa (japonica cultivar-group)]
19. gi|50932757|emb|XP_475809.1| unknown protein [Oryza sativa (japonica cultivar-group)]
20. gi|51789582|emb|XP_500852.1| PREDICTED OJ1781_B03.34 gene product [Oryza sativa (japonica cultivar-
21. gi|51789582|emb|XP_500852.1| PREDICTED OJ1014_E09.28 gene product [Oryza sativa (japonica culti
22. gi|50921265|emb|XP_470448.1| putative histone [Oryza sativa (japonica cultivar-group)]
23. gi|77551701|gb|ABA94498.1| histone [Oryza sativa (japonica cultivar-group)]
24. gi|51789582|emb|XP_473872.1| OJ1014_E09.28 [Oryza sativa (japonica cultivar-group)]
25. gi|52291607|gb|BAD62530.1| histone [Oryza sativa (japonica cultivar-group)]
Conclusions from the MudPIT results

1. Chromatin proteins has been successfully purified

2. The Basic proteins can be successfully identified using MudPIT approach

3. Low abundance proteins are difficult to be identified due to the interference of high abundance proteins

Research in Progress

We are making progress in building the chromatin subproteome map using 2-D gel based methods
Identification of Rice Mutants of Chromatin Genes

Priority of Gene Selection

1. Chromatin genes with known function in endosperm development
2. Chromatin Genes with known function in parental imprinting
3. Function unknown genes specific to endosperm tissues

Mutant Identification

Guo-Liang’s group used 57 genes we selected to blast rice insertion mutant collections worldwide

30 putative mutant lines were found in Korea collection

15 putative mutant lines were found in Tos17 collection of Japan

10 putative mutant lines were found in French collection

Seeds from the Tos17 collection have been received
Characterization of these lines is in progress
RNAi Approach

For genes without insertion mutants being found, RNAi approach have been used to generate mutants

12 constitutive RNAi constructs have been made, transgenic rice (Nipponbare) of some constructs have been obtained. A few constructs might have lethal effect to the cells.

2 inducible RNAi constructs (XVE system) have been made. Transformation is in progress.

ACKNOWLEDGMENTS

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Feng Tan
Brahma Chitteti
**GO slims:**

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<th>GO Slim type</th>
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**Metabolic view of ‘few’ differentially expressed proteins:**

- Differentially expressed proteins during cell dedifferentiation were painted on Arabidopsis metabolic view using ‘AraCyc’ tool.
- AraCyc was computationally predicted for the sequenced Arabidopsis genome.
- Reaction lines (and protein icons, where present) are color-coded according to the concentration of the enzyme that catalyzes that reaction step.
Gene Ontology:

- **gene ontology** - a controlled vocabulary used to describe the biology of a gene product in any organism. There are 3 independent sets of vocabularies, or ontologies, that describe the molecular function of a gene product, the biological process in which the gene product participates, and the cellular component where the gene product can be found.
  - Molecular function describes activities, such as catalytic or binding activities, at the molecular level.
  - A biological process is a series of events accomplished by one or more ordered assembles of molecular functions.
  - A cellular component is just that, a component of a cell.

Imprinting at Molecular Level

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

**Mammals:** Histone and DNA methylation-allele specific, de novo

**Plants:** use a different approach with unique feature

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

2. Maternal specific expression of *FWA* and *MEDEA* is established by maternal specific demethylation of these two genes using the *DEMETER* gene product, suggesting that imprinting is default state.

3. Paternal silencing of *MEDEA* is controlled by maternal expression of *MEDEA* instead of DNA methylation, indicating that imprinting could occur without DNA methylation.

Note: *MEDEA* and its homologous are chromatin associated proteins regulating endosperm development.
Modification of DNA and Histones are correlated with chromatin structure and gene expression

Although chromosome and chromatin have been extensively studied, the high level structure and the molecular composition of chromatin and chromosome are still not well defined.