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

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

It is estimated that rice alone feeds half of the world population

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are needed to see this picture.

Double Fertilization in reproduction

Like all other flower plants, rice uses double fertilization for reproduction-an unique feature of higher plants

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3n endosperm
two sets of the genomes from mother side one set from father side

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are needed to see this picture.

2n embryo
One from mother side and 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, fis2, 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

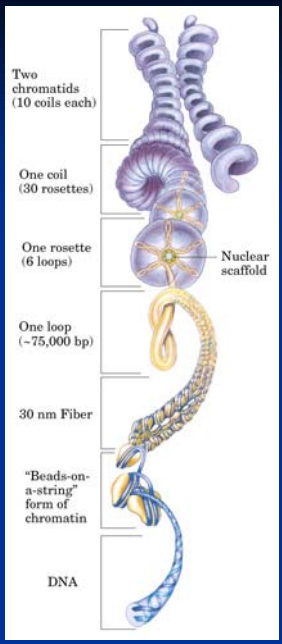
Imprinting at Molecular Level

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



Structure of Chromosome

Histones are basic proteins that associated tightly with DNA in the chromosomes

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Histone Modification Regulates Gene Expression

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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

Chromatin Regulates Gene Expression

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

Although chromosome and chromatin have been extensively studied, the high level structure and the molecular composition of chromatin and chromosome are still unknown

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

INTERSTING 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

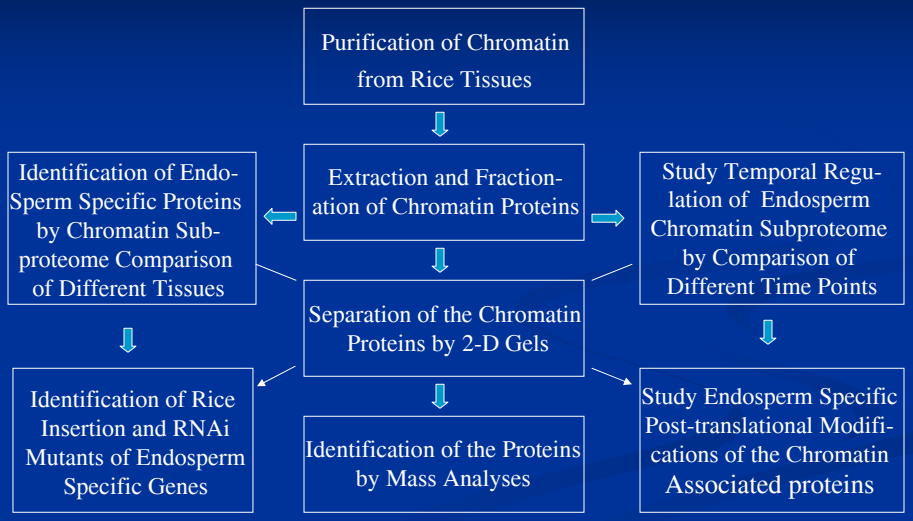
1) Identifying the rice chromatin sub-proteome in both root and endosperm

2) Identifying chromatin proteins specific to endosperm development

3) Identifying posttranslational modifications correlated to endosperm development

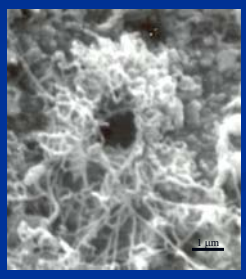
4) Generating at least 45 mutants of chromatin genes that regulate endosperm development

Workflow of the Project



Purification of Chromatin

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

Table1: Identified chromatin associated proteins from *Arabidopsis thaliana* using 2-Dgel followed by MALDI-TOF

No	Acc #	Protein name	Exp.mol .mass (Da)	Theoritical Mol.mass (Da)	Coverage	Number of Matched peptides	Exp.score/ Significant score	E-value
1	Q93WU9	Probable WRKY transcription factor51	20000	22030	20	11	60/48	0.0034
2	O82256	Zinc finger protein constans-like 10	35000	37486	22	18	82/48	0.0027
3	Q9STX0	Probable WRKY transcription factor 7	36000	38325	32	10	46/48	0.086
4	P26569	Histone H1. 2	25000	28470	54	13	49/48	0.051
5	O82175	Histone-lysine N-methyltransferase	75000	88097	16	18	56/48	0.0097
6	Q9XGM2	Double-strand break repair protein MRE11	77000	80242	18	19	49/48	0.049
7	Q9C7E8	Zinc finger protein constans-like 3	44000	47576	23	13	56/48	0.0099
8	P42551	DNA binding protein S1FA	7500	8208	43	8	56/48	0.0084
9	Q9M223	Probable U3 small nucleolar RNA-associated protein 11	29000	27132	33	19	48/48	0.056
10	O48520	DNA polymerase delta small subunit	48000	51307	19	14	58/48	0.0066
11	P59226	Histone H3	18000	15127	42	12	61/48	0.0032
12	P59169	Histone H3.3	16000	15266	34	11	51/48	0.029
13	P46665	Homeobox-leucine zipper protein HAT14	23000	25448	17	7	50/48	0.039
14	Q93WY4	Probable WRKY transcriptionfactor 12	26000	24533	33	11	88/48	0.056
15	O04336	Probable WRKY transcriptionfactor 21	39000	42979	41	14	50/48	0.039
16	Q9M9B3	Hypothetical zinc finger protein	33000	36783	35	15	55/48	0.011
17	Q9LJ44	Zinc finger protein	36000	37549	20	14	61/48	0.0032
18	P13905	Elongation factor 1-alpha	53000	49471	42	21	58/48	0.0057
19	P56762	DNA-directed RNA polymerase alpha chain	37000	38113	26	17	52/48	0.024
20	Q38859	DNA-directed RNA polymerase II 13.6 kDa polypeptide	15000	13555	39	3	55/48	0.011

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

QuickTime™ and a
TIFF (Uncompressed) decompressor
are needed to see this picture.

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

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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

2. Parental Gene Dosage Effect

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