Transcriptomes and Epigenomes in Specific Plant Developmental Processes

Jigang Li
Prof. Xing-Wang Deng Lab
Yale University
Two plant developmental processes have been studied at Xing-Wang’s Lab.

**Light signaling**
- Dark
- Light

**Heterosis**
- B73
- B/M
- M/B
- Mo17
Outline

• Genome-wide binding sites of transcription factors
  ELONGATED HYPOCOTYL 5 (HY5)
  FAR-RED ELONGATED HYPOCOTYL 3 (FHY3)

• Transcriptomes and epigenomes in hybrid plants
  two rice subspecies and their reciprocal hybrids
  two Arabidopsis ecotypes and their reciprocal hybrids
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Li et al., 2011, The Arabidopsis Book
Light signals and photoreceptors

Jiao et al., 2007, Nat Rev Genet
Red light

phyB

Far-red light

cop/DET/FUS

Blue/UV-A light

CRY1

CRY2

HY5, etc

Photomorphogenesis
Analysis of transcription factor HY5 genomic binding sites revealed its hierarchical role in light regulation of development

Jungeun Lee
Kun He

Plant Cell, 2007
Experimental Design

• ChIP-chip
  Materials: 35S:HA-HY5/hy5 transgenic lines
  Conditions: grown in white light conditions for 4 d, then ChIP assay was performed with anti-HA antibodies
  60-nucleotide oligomer microarray (contains one probe for every 500 nucleotides over the entire Arabidopsis genome)

• Expression analysis
  Materials: wild-type and hy5 plants
  Conditions: grown in white light conditions for 4 d
  70mer oligonucleotide microarray (covers 25,676 unique Arabidopsis genes)
3894 putative HY5 binding target genes were identified
HY5 preferentially binds to gene promoters

HY5 binding sites

Whole genome

- 61.3% promoter
- 15.6% 0.5kb
- 18% non-promoter

- 31.11% promoter
- 14.14% 0.5kb
- 6.33% non-promoter

ATG
Functional classification of the HY5 binding target genes
Comparison of ChIP-chip data with genome-wide expression analysis

White: the number of differentially expressed genes that also have HY5 binding sites

A

Differentially Expressed Genes (1144)

B

Downregulated Genes (594)

Upregulated Genes (550)
HY5 preferentially mediates an early event of phyA- and phyB-regulated gene expression
Main Conclusions

• This analysis showed that HY5 binds preferentially to promoter regions *in vivo* and revealed 3894 genes as putative HY5 binding target genes.

• HY5 binding targets tend to be enriched in the early light-responsive genes and transcription factor genes.

• Our data indicate that HY5 is a high hierarchical regulator of the transcriptional cascades for photomorphogenesis.
Genome-wide mapping of the HY5-mediated gene networks in *Arabidopsis* that involve both transcriptional and post-transcriptional regulation.

Huiyong Zhang

Hang He

Plant J., 2011
Experimental Design

• ChIP-chip

**Materials:** wild-type *Arabidopsis* plants

**Conditions:** 4-d-old seedlings under white light or light-to-dark transition, then ChIP assay was performed with anti-HY5 antibodies

*Affymetrix* *Arabidopsis* genome tiling array (covers 97% of the *Arabidopsis* genome)

• Expression analysis

**Materials:** wild-type and *hy5* plants

**Conditions:** grown in white light conditions for 4 d

mRNA sequencing
HY5 binding loci using an *Arabidopsis* genome tiling array
11,797 genes as putative HY5 target genes
Distribution of HY5 binding loci

Number of HY5-binding sites in corresponding regions

WL
Dark transition

% of transcribed region
Comparison of ChIP-chip data with mRNA-seq results

Blue: the number of differentially expressed genes that also have HY5 binding sites
HY5 directly binds to 8 miRNA genes
HY5 regulates the expression of miRNA genes
HY5 regulates the expression of miRNA target genes
Over-expression of miR408 leads to increased chlorophyll and anthocyanin contents
Main Conclusions

- HY5 binds to over 9000 genes, detectably affecting the expression of over 1100 genes, either positively or negatively.
- HY5 regulates eight miRNA genes that in turn control the transcript abundance of specific target genes.
- Over-expressing HY5-targeted miR408 resulted in phenotypes that are opposite to the hy5 mutants.
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Phenotypes of phyA-pathway mutants

(a) wild-type

(b) FR light

Control of phyA nuclear accumulation

Li et al., 2010, Plant Cell
Genome-wide Binding Site Analysis of FAR-RED ELONGATED HYPOCOTYL 3 Reveals Its Novel Function in *Arabidopsis* Development

Xinhao Ouyang  Jigang Li  Gang Li  Bosheng Li

Plant Cell, 2011
Experimental Design

• ChIP-seq
  **Materials:** 35S:3FLAG-FHY3-3HA/fhy3-4 transgenic lines
  **Conditions:** grown in darkness (D) or far-red (FR) light conditions for 4 d, then ChIP assay was performed with anti-FLAG antibodies
  **Illumina high-throughput sequencing**

• Expression analysis
  **Materials:** FHY3p:FHY3-GR/fhy3-4 transgenic lines
  **Conditions:** grown in darkness (D) or far-red (FR) light conditions for 4 d, then treated with DEX or MOCK for 2h
  **Affymetrix Arabidopsis ATH1 genome arrays**
FHY3 Binding Sites in the *Arabidopsis* Genome
FH3 binds to the FBS motifs in the gene promoters in vivo
Identification of FHY3 directly regulated genes

A) Venn diagrams showing the overlap between genes regulated by FHY3 in different conditions:
- Dark (643): 555 genes, 88 overlapping with Far-red (143).
- Far-red (143): 55 genes.

B) Venn diagrams showing the overlap between FHY3 target genes by ChIP-seq and microarray:
- Dark: 1362 genes, 197 overlapping with 446 FHY3-regulated genes by microarray.
- FR: 923 genes, 86 overlapping with 57 FHY3-regulated genes by ChIP-seq.

C) Pie charts showing the distribution of promoter regions:
- Promoter 68%: Exon 24%, 3'UTR 2%, 5'UTR 3%, Intron 3%.
- Promoter 81%: Exon 13%, 5'UTR 5%, Intron 1%.

Table showing the overlap between activated and repressed genes:
- Activated (154) 78% in 555 genes.
- Repressed (43) 22% in 55 genes.
- Activated (70) in 88 overlapping genes.
- Repressed (1) in 143 Far-red genes.
- Activated (85) 99% in 923 FR genes.
FHY3 coregulates a large number of common target genes with HY5
Functional classification analysis of FHY3 direct target genes
FHY3 and FAR1 are involved in the control of chloroplast development.

ARC5: a structural component of the latter stages of chloroplast division in Arabidopsis.
FHY3 directly activates *ARC5* transcription.
Restoration of ARC5 rescued the chloroplast defects of *fhy3* mutants
Main Conclusions

- FHY3 binds to 1559 and 1009 target genes in darkness (D) and far-red (FR) light conditions, respectively, in the *Arabidopsis* genome.
- FHY3 preferentially binds to promoters through the FHY3/FAR1 binding motif (CACGCGC).
- Comparison between the ChIP-seq and microarray data indicates that FHY3 quickly regulates the expression of 197 and 86 genes in D and FR, respectively.
- FHY3 co-regulates a number of common target genes with HY5.
- FHY3 controls chloroplast development by directly activating *ARC5* transcription.
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Heterosis in hybrid rice and maize

Longping Yuan
Heterosis

• **Utilization of heterosis in crops**
  - Heterosis has been utilized in agricultural production since the 1930s
  - Hybrid shows 20-30% in grain yield increase over inbred lines

• **Genetic explanations for heterosis**
  - Dominance hypothesis, overdominance hypothesis, and epistasis hypothesis
  - Although each hypothesis is supported by many lines of evidence, little consensus has yet been reached.
  - These hypotheses are largely conceptual and not connected to molecular principles, and are therefore far from explaining the molecular basis of heterosis.
Project background

• Molecular mechanism of heterosis suggested by genomic studies
  • Differential gene expression between hybrids and their parental inbred lines may be responsible for heterosis
• Advance of genomic and epigenomic researches in plants
  • Publicly available genome sequences and their annotation in rice and maize
  • Development of high-throughput genomic and epigenomic approaches: microarray-based technologies and deep sequencing-based technologies
Global epigenetic and transcriptional trends among two rice subspecies and their reciprocal hybrids

Guangming He  Xiaopeng Zhu  Axel Elling

Plant Cell, 2010
Experimental Design

- **Materials**: Rice seedling shoots at four-leaf stage
- **4 genotypes**: Nipponbare, 93-11, and their reciprocal hybrids Nipponbare × 93-11 and 93-11 × Nipponbare
- **Illumina high-throughput sequencing**
  - mRNA and smRNA transcriptomes
  - DNA methylomes
  - Genome-wide distribution of histone modifications (H3K4me3, H3K9ac, and H3K27me3)
- **56,275** (including 15,232 TE genes) rice genes were included in the analysis
EXPERIMENTAL STRATEGIES

Gene expression

- mRNA-Seq
- (Total RNA)
- DNase I digestion
- Oligo(dT) purification
- (mRNA)
- ds-cDNA synthesis
- (ds-cDNA)
- Sonication

DNA methylation

- McrBC-Seq
- (Genomic DNA)
- McrBC digestion
- (≤ 500 bp)
- (> 500 bp)
- Gel extraction
- (≤ 500 bp)

Histone modification

- ChIP-Seq
- Sonication and ChIP
- Dissociation
- Reverse cross-link

Small RNA

- SmRNA-Seq
- (Total RNA)
- Gel extraction
- (Small RNA)
- Adaptors ligation
- Reverse transcription
- PCR

Illumina sequencing library construction

High-throughput sequencing
Distribution of DNA methyl, smRNAs, histone modifications, and genes on rice chromosomes
Distribution patterns of DNA methylation and histone modification in genes and their roles in transcription
Number and percentage of non-TE genes and TE-related genes identified with epigenetic modifications
Relationships between Gene Expression and Epigenetic Modifications

H3K4me3: activating mark
H3K9ac: activating mark
H3K27me3: repressive mark
Patterns of gene expression and epigenetic modifications in hybrids
Non-additive patterns of gene expression and epigenetic modifications in hybrids
Main Conclusions

• We generated highly integrated maps of the epigenome, mRNA, and small RNA transcriptomes of two rice (*Oryza sativa*) subspecies and their reciprocal hybrids.

• We found that gene activity was correlated with DNA methylation and both active and repressive histone modifications in transcribed regions.

• We observed distinct patterns in gene expression and epigenetic modifications in reciprocal hybrids.
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Prof. Xing Wang Deng

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