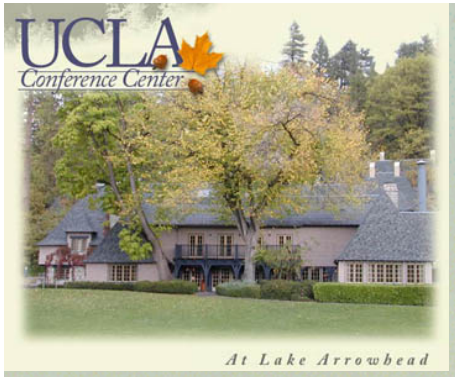


Using Genomics to Dissect Seed Development
Goldberg Lab

MCDB Research Conference
December 2 – 4, 2011

Why Seeds? Our Food is Derived From 14 Major Food Crops & Over Half Produce Seeds For Human and Animal Consumption

Seed Crops

- Wheat
- Rice
- Corn
- Barley
- Sorghum
- Soybean
- Common Bean
- Coconut

Non-Seed Crops

- Potato
- Sweet Potato
- Cassava
- Sugar Beet
- Sugar Cane
- Banana

22,300 Seed-Bearing Plant Species (90% of all known plants)

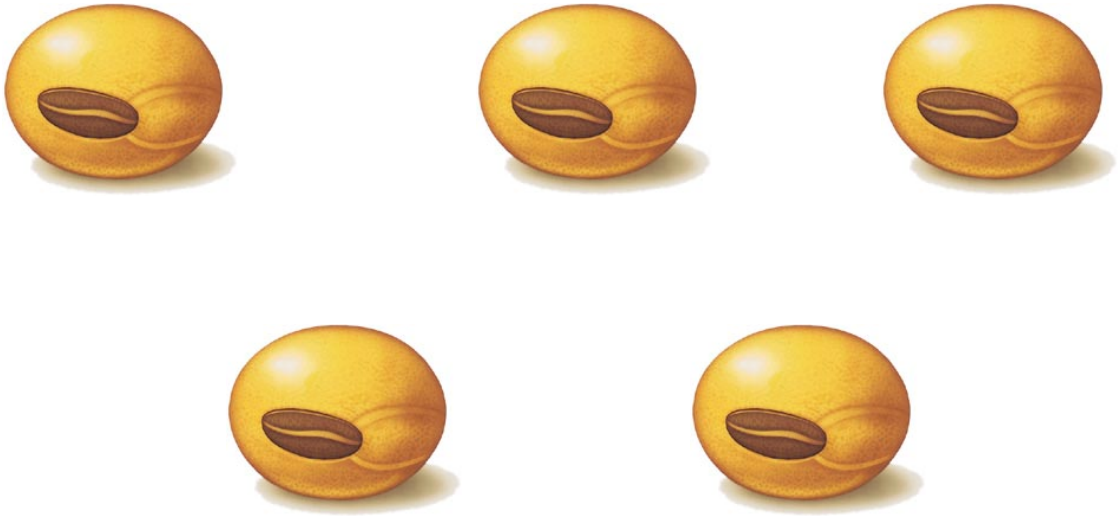
$36.5 Billion Dollars is the Value of the World Seed Market (2010)

OVER THE NEXT 50 YEARS WE WILL NEED TO DOUBLE THE WORLD'S FOOD SUPPLY IN ORDER TO PRODUCE MORE FOOD THAN IN ALL OF HUMAN HISTORY – Yield, Yield Yield!!!

Why Soybean?-A Reminder

- Second Major US Crop
- Total Crop Value $32Billion (50% Value Exported)
- Major Food Source
- Important Biofuel Source (Biodiesel ~20% of US Soybean Oil Production)
- Excellent Model Plant (Transformation, Knockdowns, Genetics)
- Genome Sequenced
- Seed Gene Expression Data

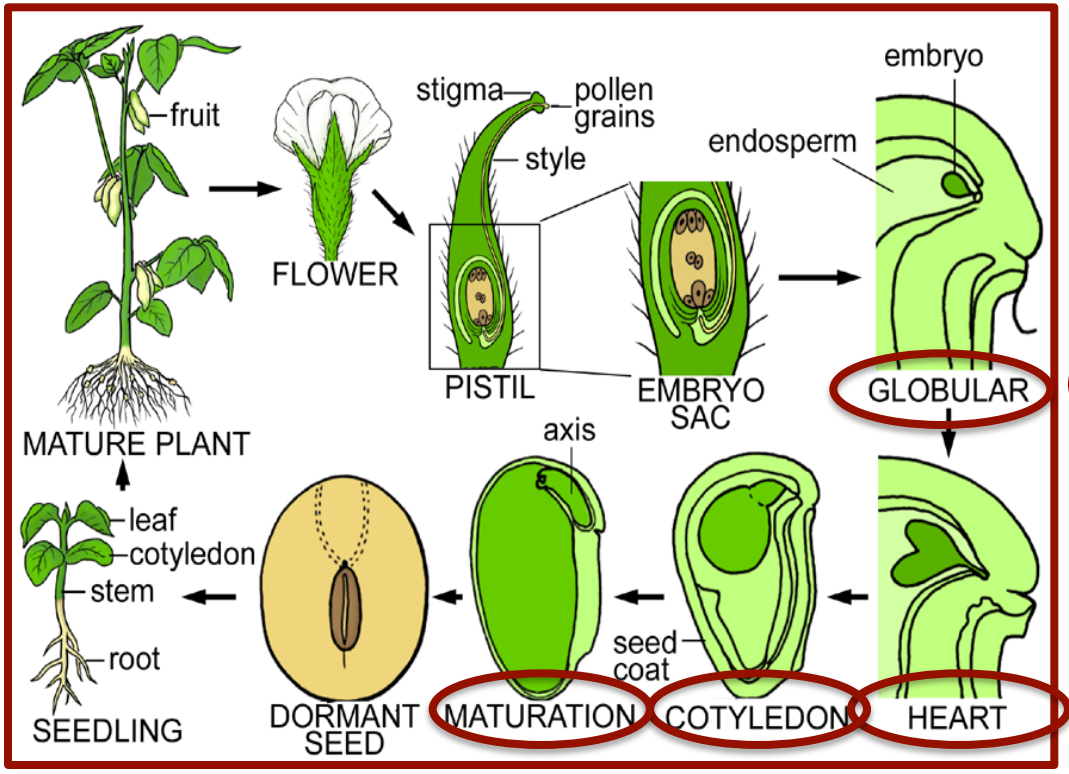
How Is a Seed Formed?



In the Beginning....

What Are the Major Questions?

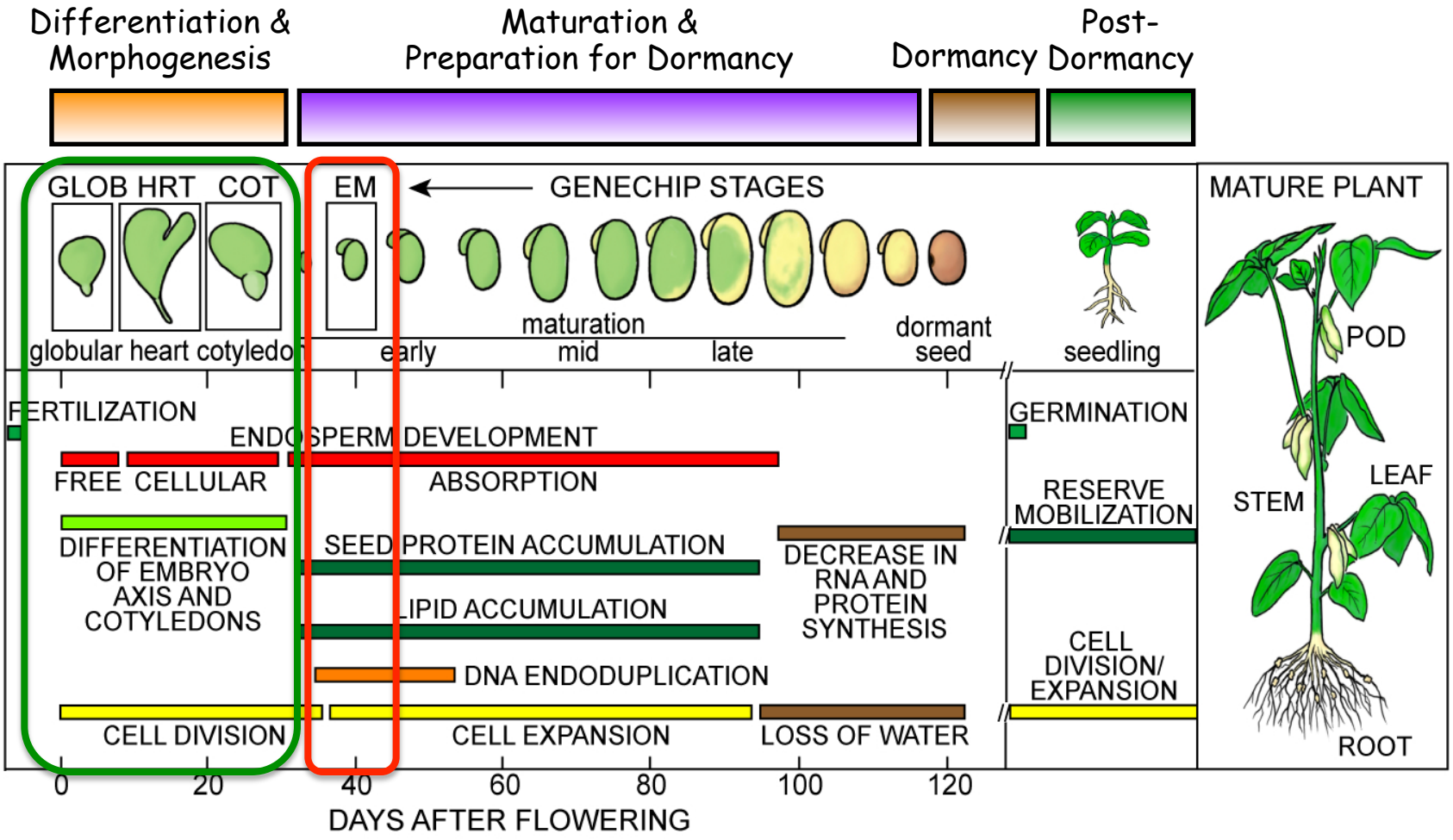
Seed Development



Seed Stages Studied

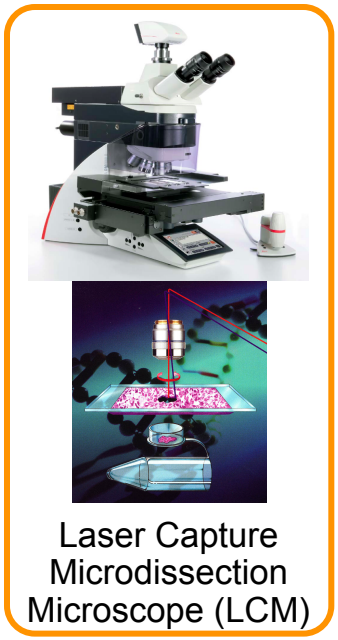
- What are the genes and regulators that are active in specific seed compartments throughout soybean seed development?
- How does gene activity change during seed development?
- What biological processes are unique or prevalent in different seed compartments throughout development?
- What are the genes required to make a seed?

What Soybean Developmental Stages Were Studied?

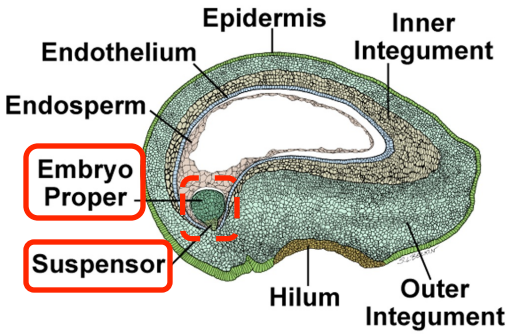


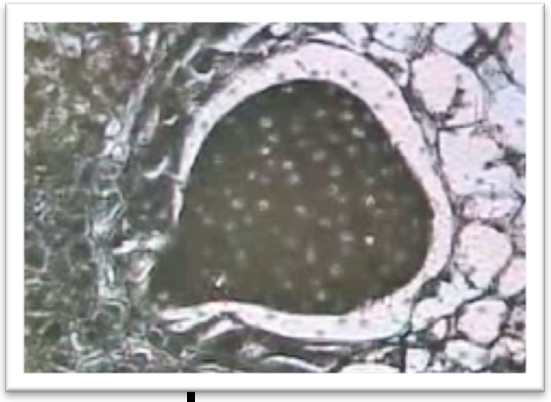
- The **differentiation** of the embryo, endosperm, and seed coat happens at *Globular, Heart, and Cotyledon stage*
- The developmental program is switched to **storage protein deposition and preparation for dormancy** at *Early-Maturation stage*

What is Our Approach?



Laser Capture Microdissection Microscope (LCM)





A Paraffin Cross Section

LCM Captured Tissues

RNA Isolation

DNA Isolation

mRNA Selection

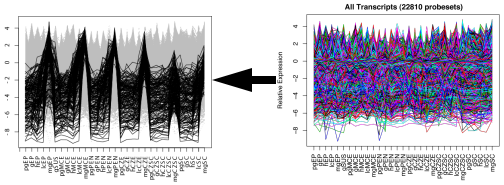
Small RNA Enrichment

Bisulfite Conversion

cDNA Sequencing Library

Small RNA Sequencing Library

Bisulfite Converted Sequencing Library



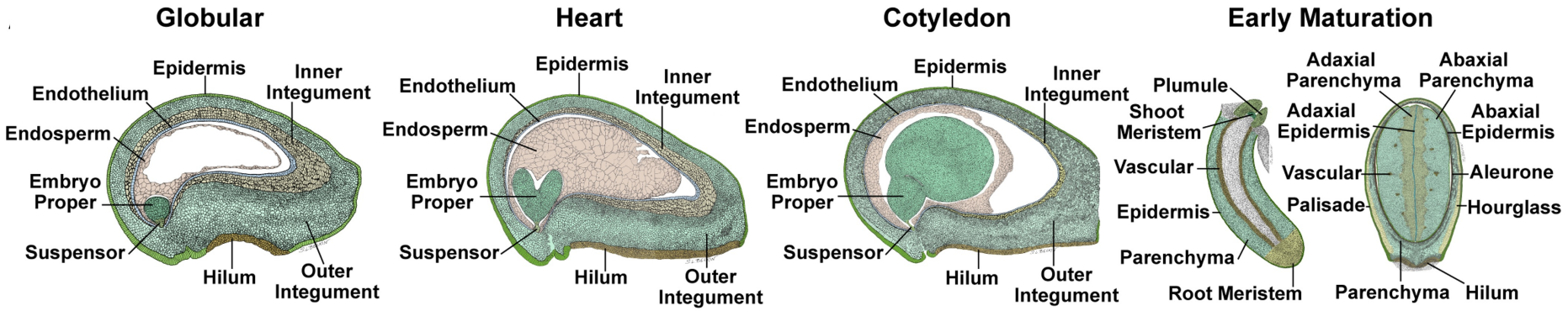
Data Analysis

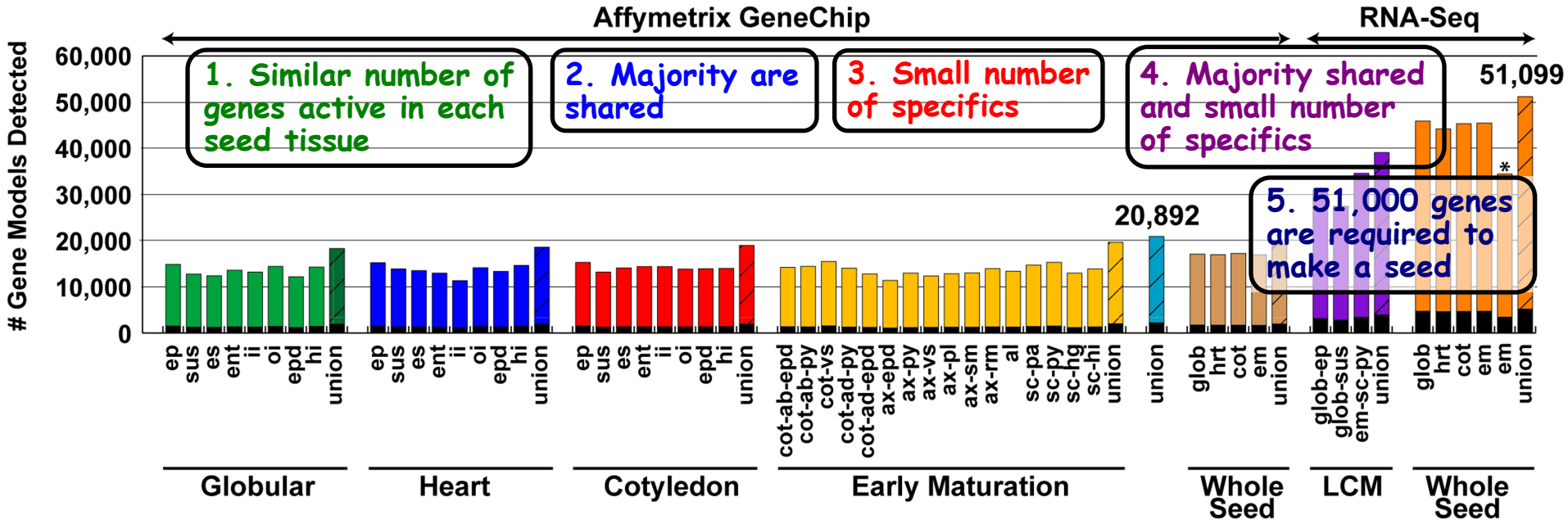
TGTCATTACCTCCACCTCAAACAC
CTCAATGTACTGGGCGTAGAAAGG
ATCAACTGCGTGGTTGTATCAATG
ACGGTTACAGTTCAGATGACATTG
TTCCCACTAGGGCTAATATGATTA
ATGATTCTTTGTTCCTTCATTATT
ACGAAGAAGATGGGATGGATGATG
TTATCGACGATGAAATGCACGATA
CAGCATTGTTTGACTCTTGTCATT
CTAAGGGTATTATTAAGGAGCCCA
CAGCTATTTCATATGCCACAGGAA

GeneChip

Next Generation Sequencing

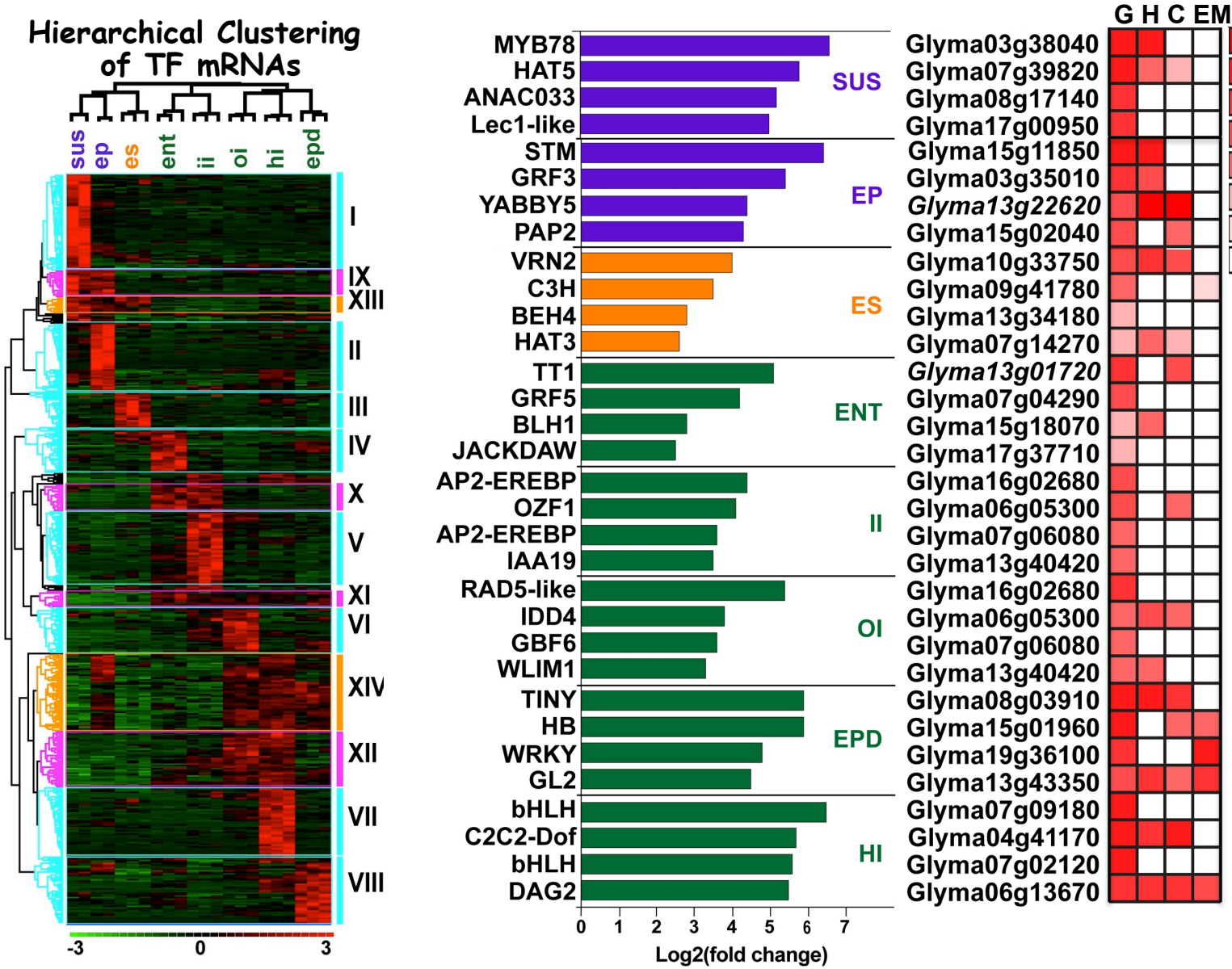
What Are the Genes Active in Soybean Compartments, Regions, and Tissues throughout Development?





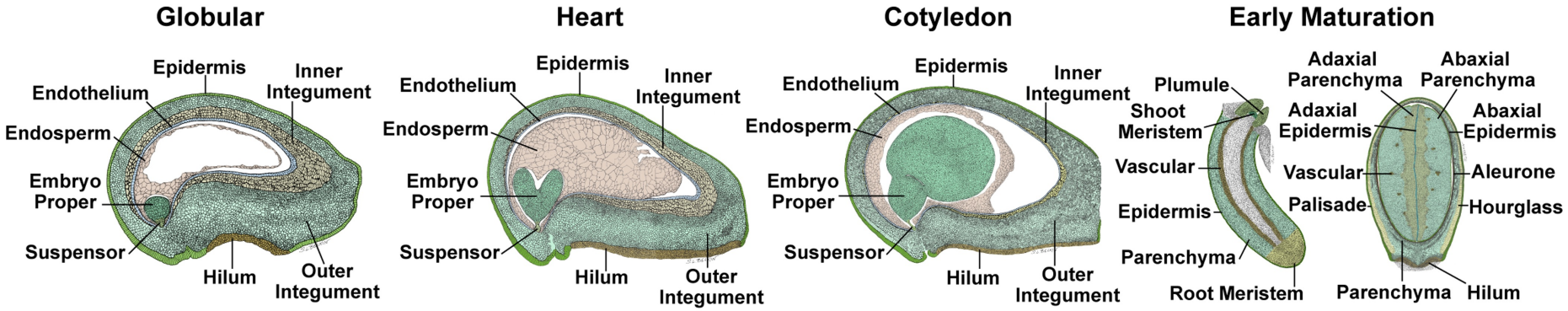
*Total RNA was amplified using NuGEN Ovation RNA-Seq System

What Transcription Factor mRNAs Are Prevalent or Unique in Different Seed Compartments at Globular Stage?

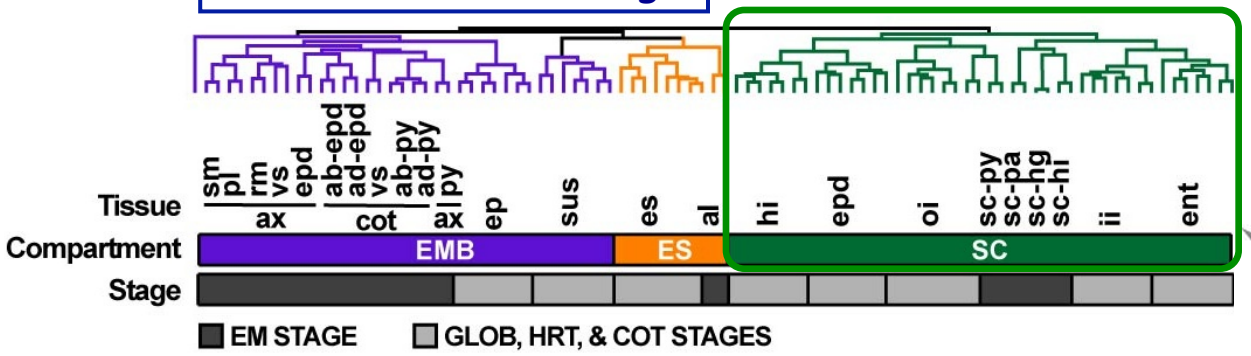


Distinct Set of TF mRNAs Are Up-Regulated in Different Seed Compartments

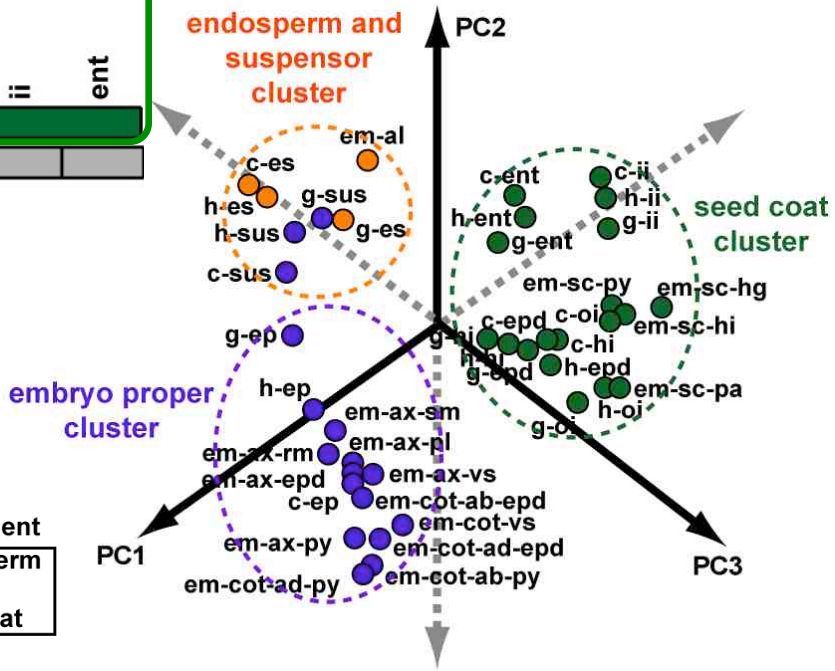
What Biological Relationships Are Observed among 40 Different Seed Compartments throughout Development?



Hierarchical Clustering



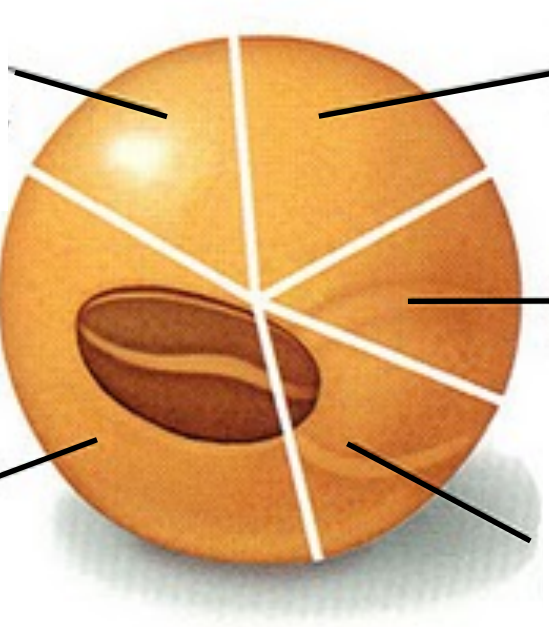
Principal Component Analysis



Samples cluster with respect to seed compartment (Seed Coat Tissues)

Are Different Seed Compartments Specialized For Specific Metabolic Processes?

15% Soluble Carbohydrates
(Sucrose, Raffinose, Stachyose)



20% Oil (Oleate, Linoleate etc)

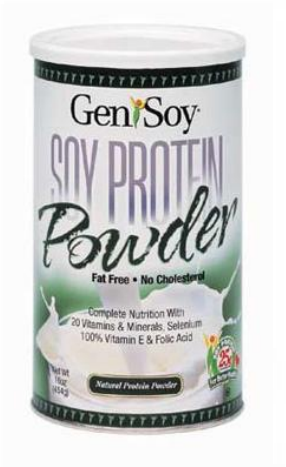
10% "Other"
(**Isoflavones**, Vitamins, etc)

15% Insoluble Carbohydrates
(Dietary fiber)

40% Protein
(Glycinin, Beta-conglycinin, etc)

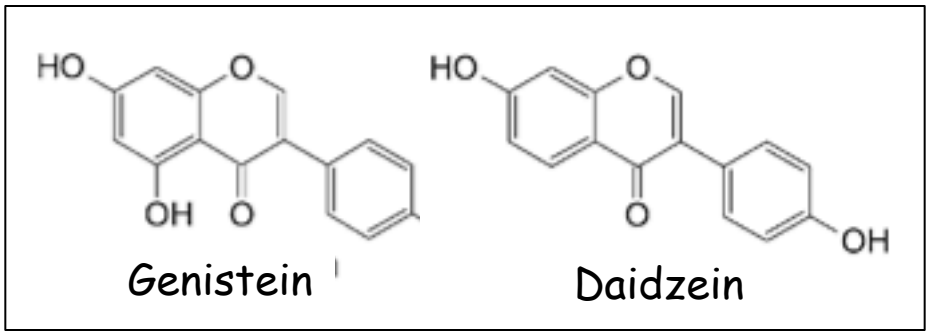
Composition of Soybean Seed

Where Are the Pathways for Soybean Health-Related Products Localized within the Seed?



What are the Health-Related Benefits of Isoflavone?

Isoflavones

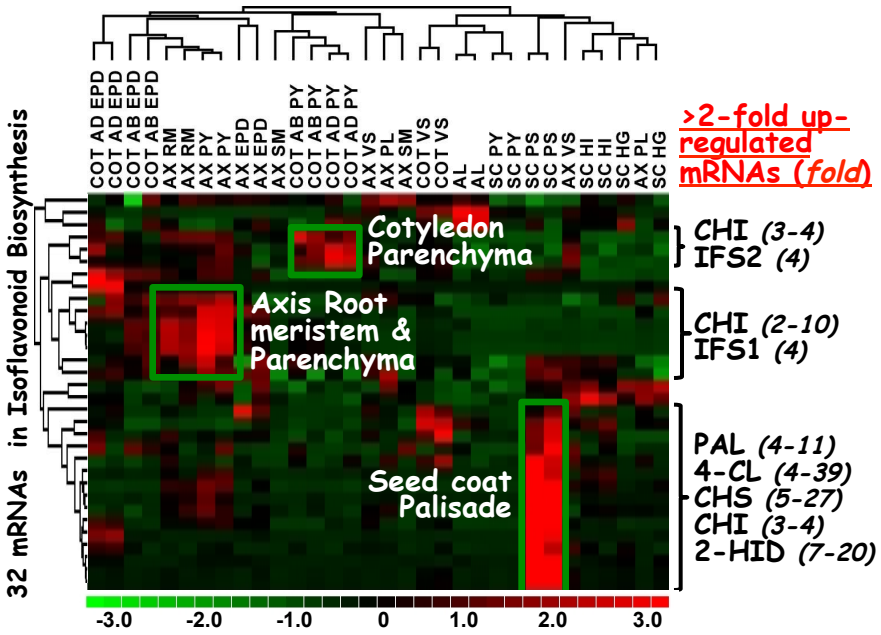


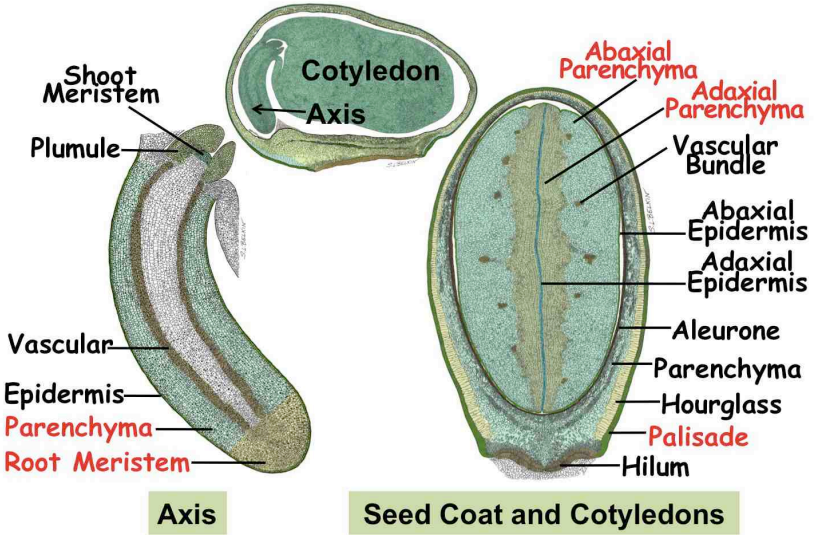
- Soybean is best known source of Isoflavones
- The chemical structure of Isoflavones is very similar to Estrogen

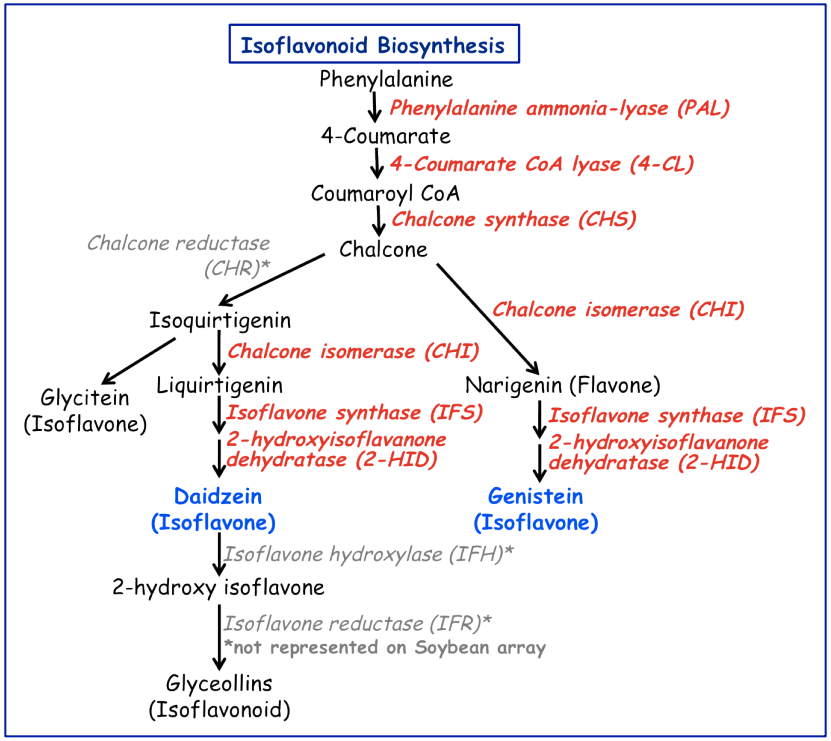
Health Benefits of Isoflavones

- Ease menopause symptoms
- Improve bone health by increasing bone density
- Reduce heart disease risk
- Reduce cancer risk

Where Are the Pathways for Isoflavone Biosynthesis Localized Within the Seed?

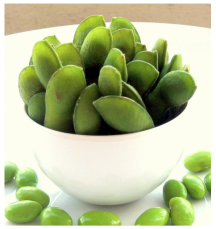






mRNAs Encoding Enzymes in *Isoflavone biosynthesis* are up-regulated in Early maturation-stage seed *Cotyledon Parenchyma*, *Axis Parenchyma* and Root *meristem* and *Seed coat Palisade*.

Where Are the Pathways For Soybean Health-Related Products Localized Within the Seed?



AXIS

SHOOT MERISTEM
Storage Protein (β-conglycinin)
Amino acids (Ser, Trp)

PARENCHYMA
Isoflavones (Genistein, Daidzein)
Storage Protein (β-conglycinin)

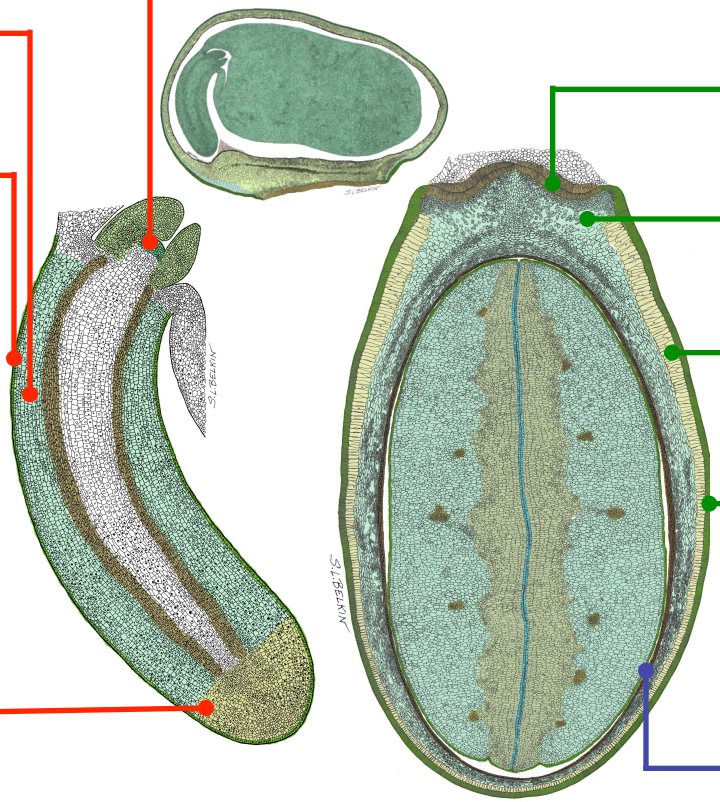
EPIDERMIS
Vitamin B1

ROOT MERISTEM
Isoflavones (Genistein, Daidzein)

COTYLEDON

PARENCHYMA
Isoflavonoes (Genistein,Daidzein)
Vitamin B1, B2, B5, B9, C, E
Fatty acids (Linoleate, Oleate)
Amino acids (Asn, Asp, Cys, His, Ile, Leu, Lys, Met, Thr, Val)
Storage Proteins (β-conglycinin, Glycinin)
Sucrose, Stachyose

ADAXIAL EPIDERMIS
Storage Protein (Glycinin)



SEED COAT

HILUM
Amino acids (Asn, Phe)

PARENCHYMA
Sucrose, Stachyose
Amino acid (Gln)

HOUR GLASS
Vitamin B9,C, E
Sucrose
Amino acids (Cys, Gln, Gly, Lys)

PALISADE
Isoflavones (Genistein, Daidzein)
Fatty acids (Linoleate, Oleate)
Sucrose
Amino acids (Gln, Phe, Ser)

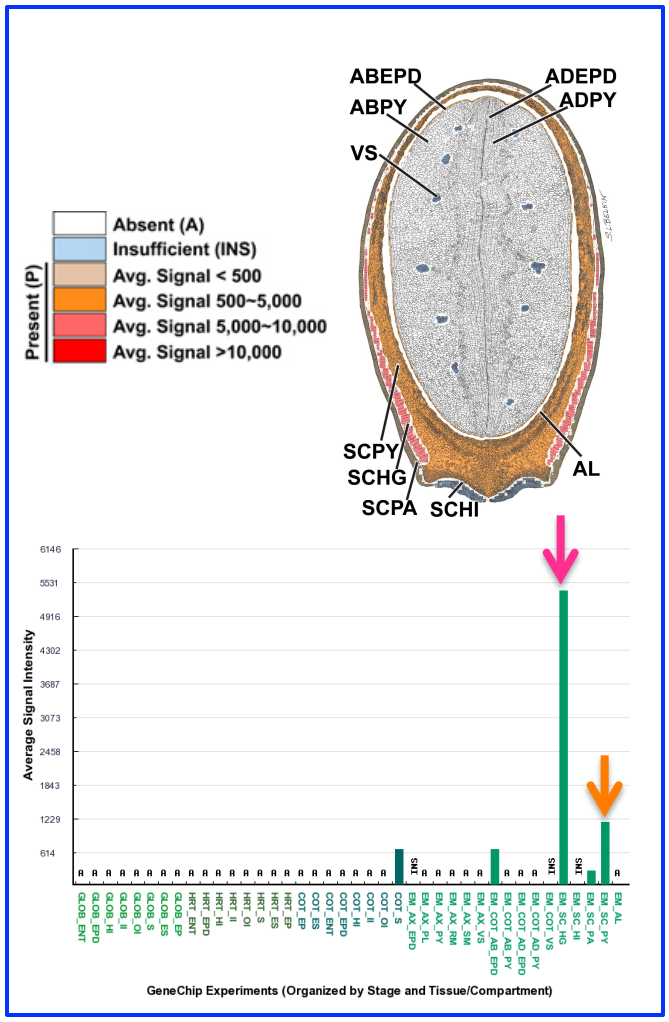
Aleurone

Vitamin B2, B9
Stachyose
Amino acid (Gln)

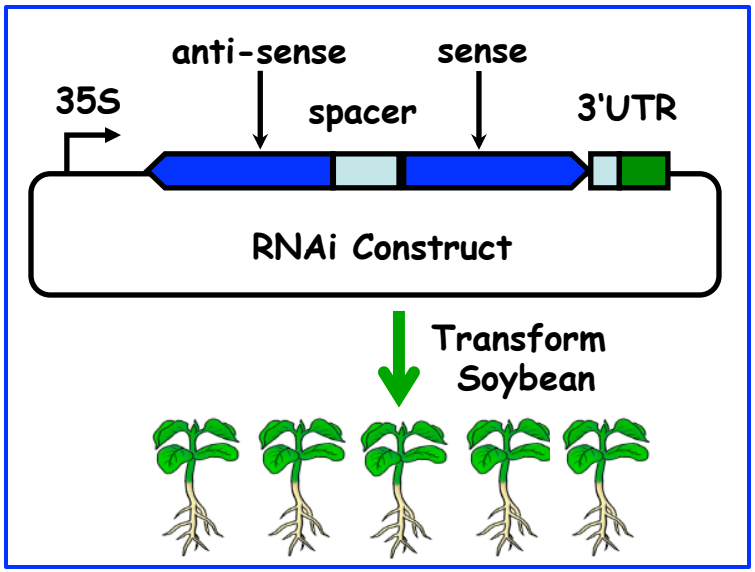
mRNAs Encoding Enzymes in Health-Related Metabolic Pathways are Up-Regulated in Specific Seed Tissues

Using RNAi to Knock-Down Seed Compartment-Specific Transcription Factor mRNAs
-- An Example – GRAS Transcription Factor --

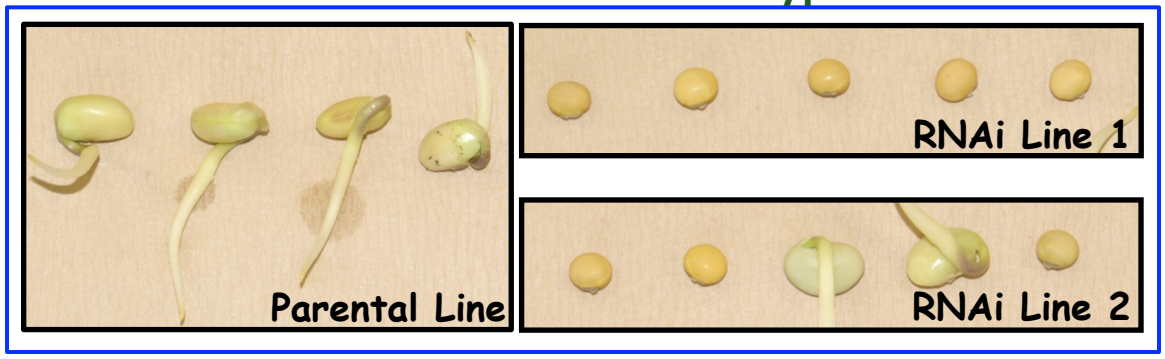
1. Transcript Profiling Data



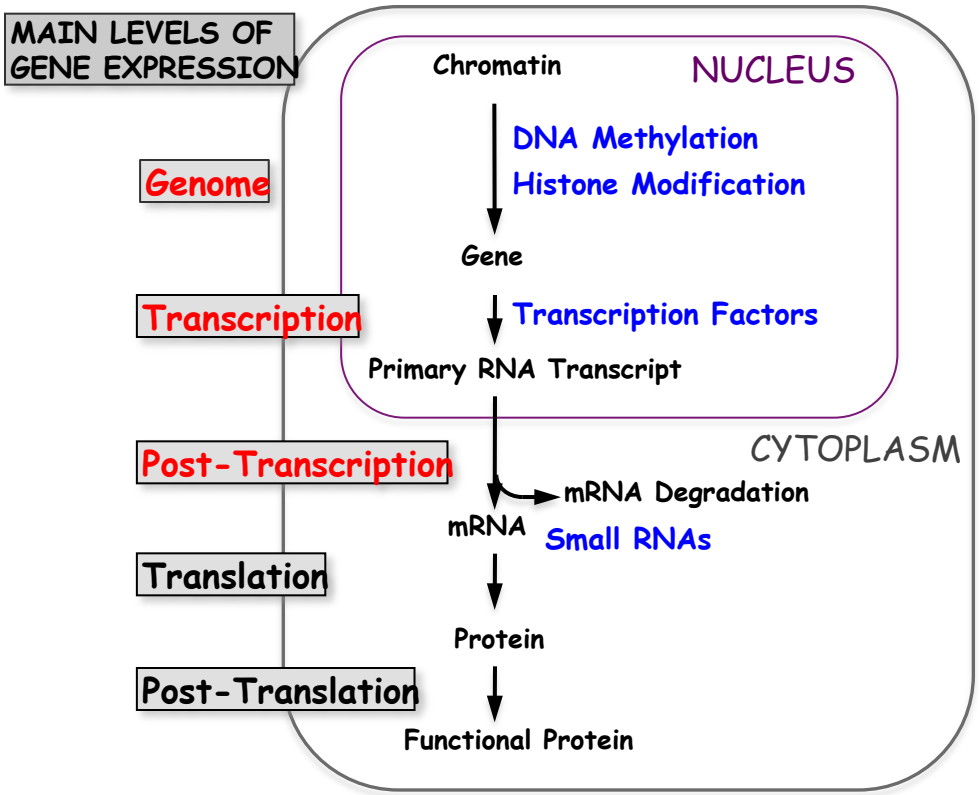
2. RNAi Knockdown



3. Observe Phenotypes



What Are the Major Questions?



1. What is the spectrum of genes that are active in different seed compartments on a whole-genome basis?

2. What are the regulatory processes required to make a soybean seed?

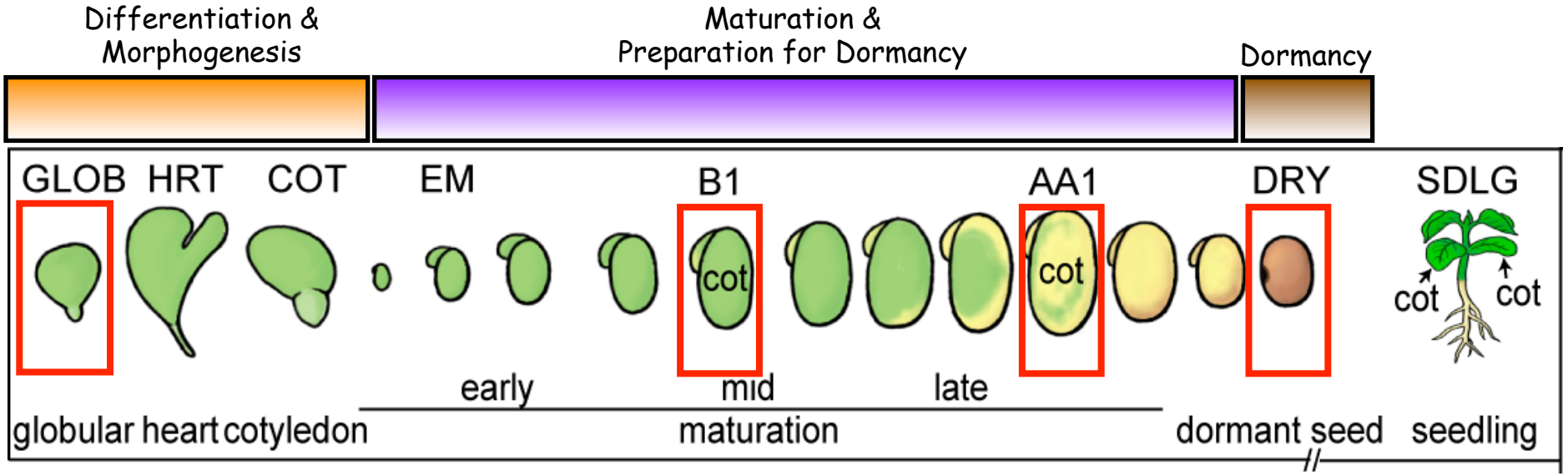
   - What microRNAs are present in specific tissues and what are their targets?

   - What are epigenetic changes that occur in the genome in specific tissues?

3. What are the correlations between methylome, microRNAs, and compartment specific gene expression?

4. What are the gene networks required to program seed differentiation and maturation?

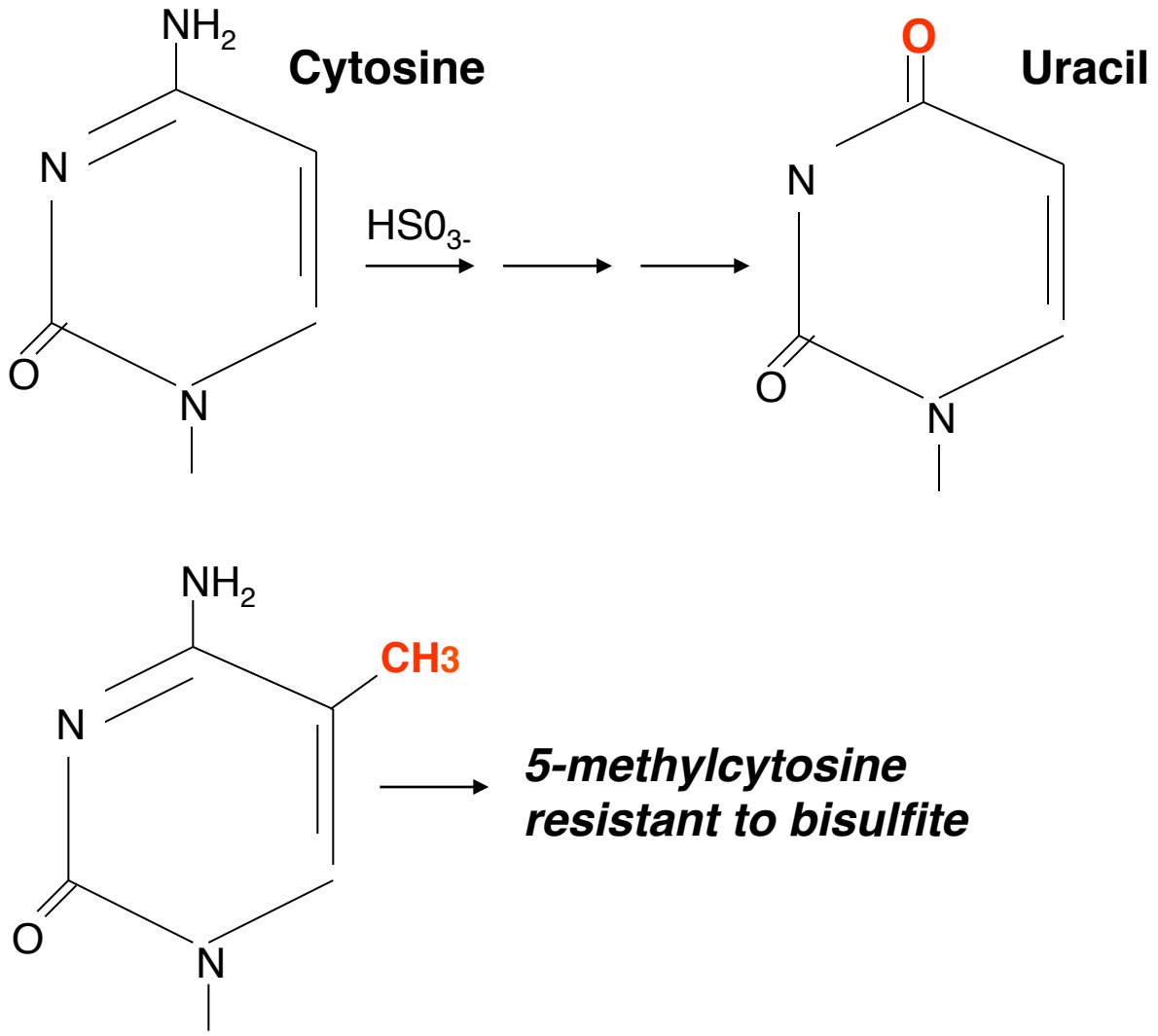
What Are the Epigenetic Changes Occurring in Specific Tissues Throughout Seed Development?



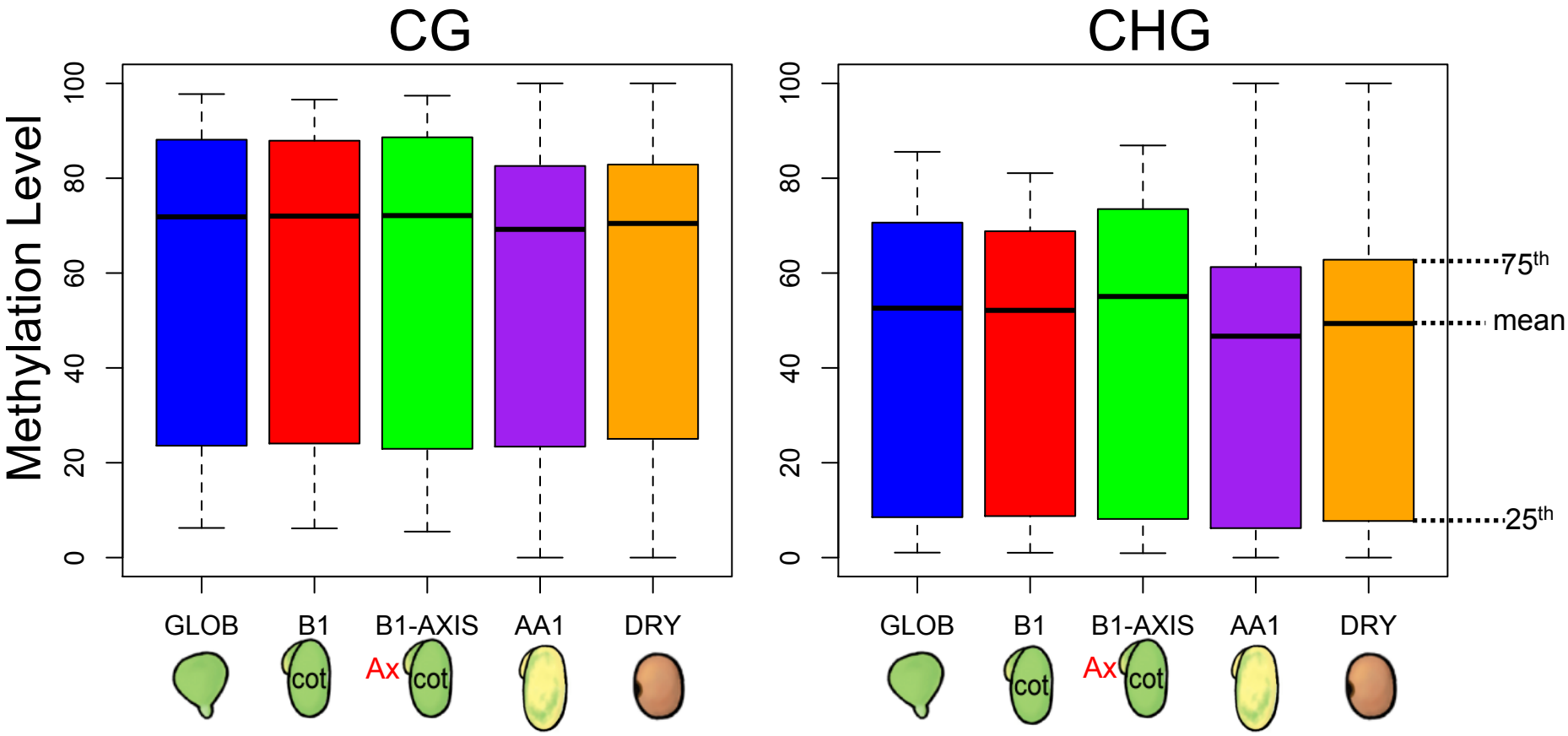
LIBRARY	GLOB	MM (B1)	MM-AXIS (B1)	LM (AA1)	DRY
# Reads (Bases)	120M (12Gb)	336M (33.6Gb)	191M (19.1Gb)	144M (14.4Gb)	449M (44.7Gb)
# Mapped Unique Reads	70.5M (7.1Gb)	160M (16.0Gb)	107M (10.7Gb)	82M (8.2Gb)	85M (8.4Gb)
Genome Coverage Per Strand	3.5 fold	8 fold	5.3 fold	4.1 fold	4.2 fold

LM, Late-maturation; MM, Mid-maturation

Bisulfite deaminates Cytosine to Uracil



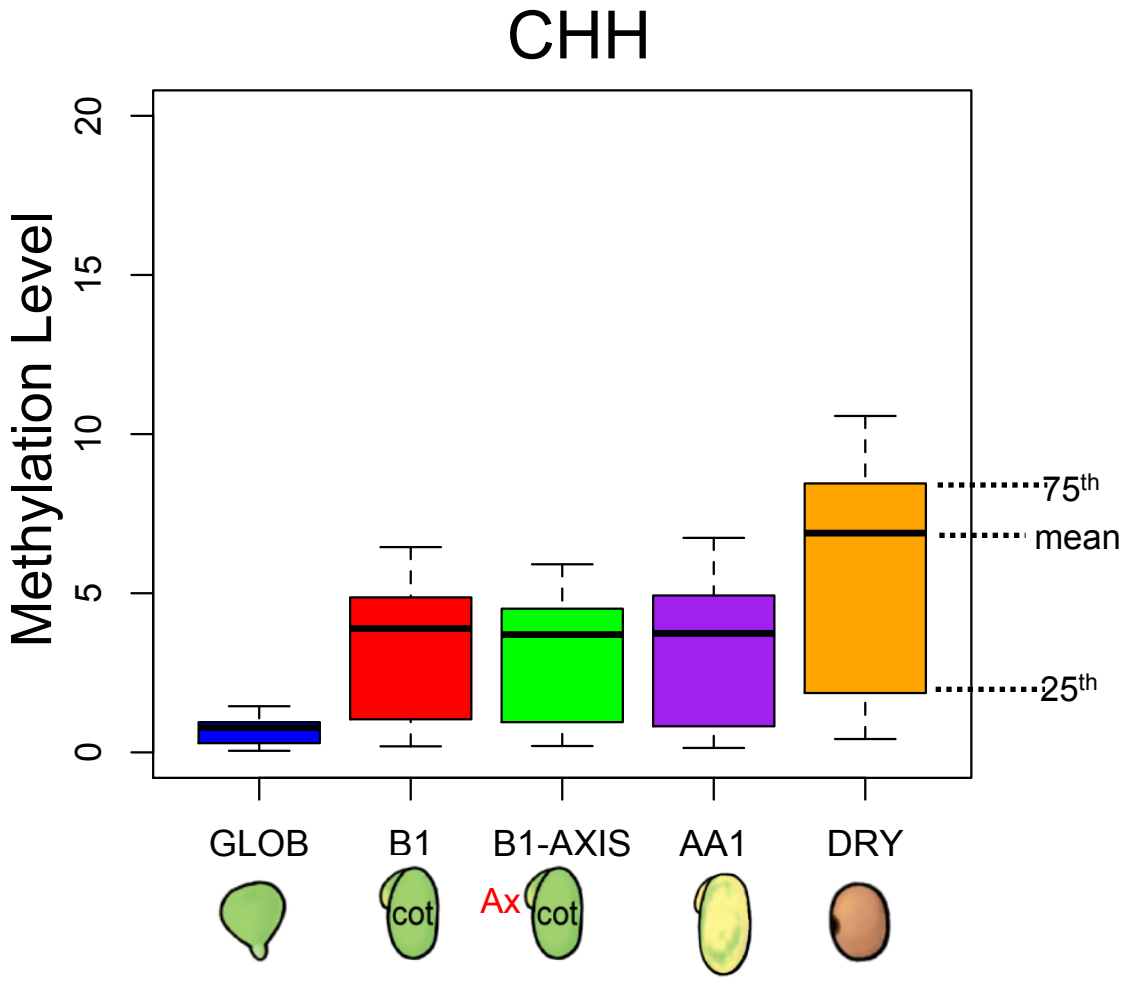
What is the Methylation Landscape During Soybean Seed Development?



Similar methylation levels in whole seeds in both the *CG* and *CHG* DNA Context

H = A, C, or T

What is the Methylation Landscape During Soybean Seed Development?



Gradual increase in methylation level relative to stage of development

H = A, C, or T

Use Genomics to Identify Genes To Improve Seeds

The 21st Century Crop!!!!

Future Seed Biology

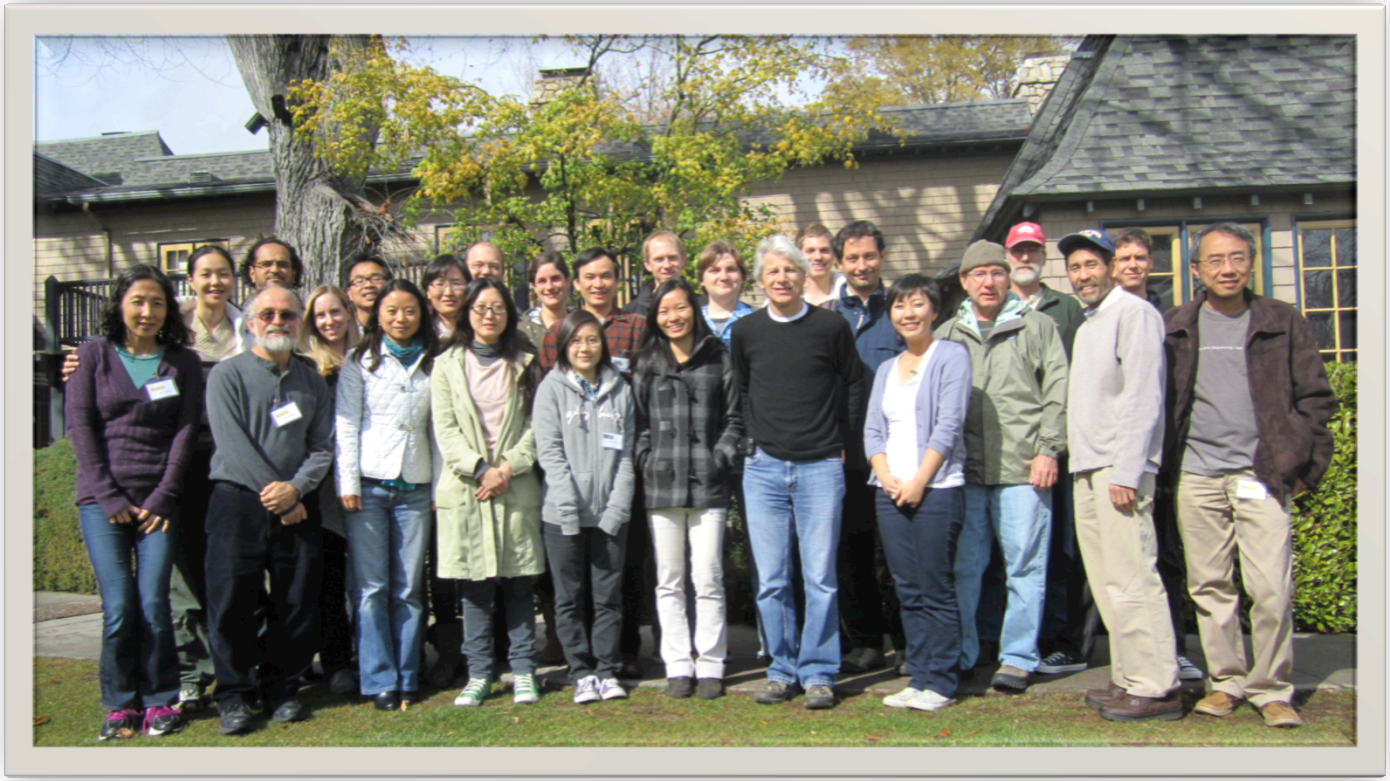
- Seeds Without Fertilization
- Hybrids
- Reduced Pod Shattering
- Architecture Designed For Specific Growth Conditions

Future Seed Biology

- More Seeds
- Bigger Seeds
- Seeds Optimal For Human/Animal Health & Nutrition

.....and → **Re-Engineering Existing Crops** *increase yields*

Acknowledgement



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UC Berkeley
Bob Fischer
Tzung-Fu Hsieh

UC Davis
John Harada
Ryan Kirkbride
Julie Pelletier
Meryl Hashimoto
Tina Wang

Funding
NSF Genome Grant