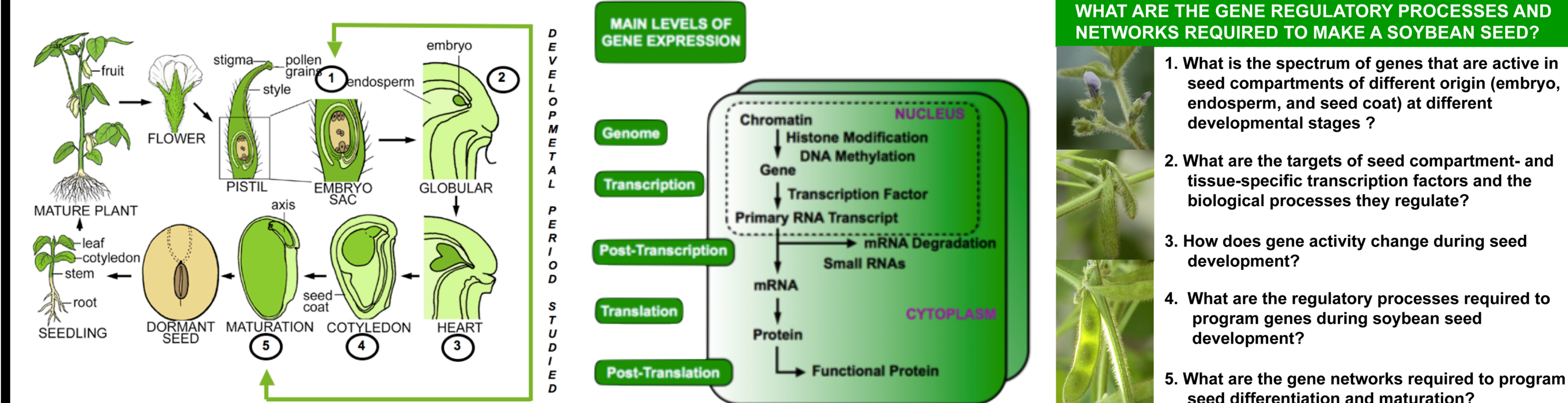


WHAT ARE THE GENES REQUIRED TO MAKE A SOYBEAN SEED?

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INTRODUCTION



WHAT ARE THE GENE REGULATORY PROCESSES AND NETWORKS REQUIRED TO MAKE A SOYBEAN SEED?

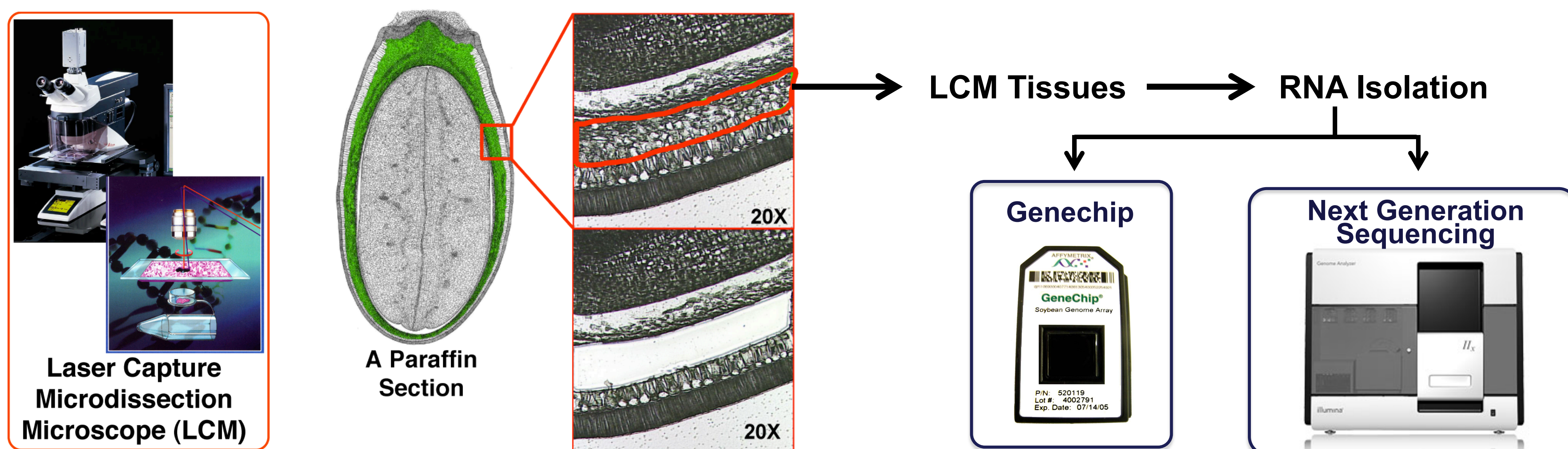
1. What is the spectrum of genes that are active in seed compartments of different origin (embryo, endosperm, and seed coat) at different developmental stages?
2. What are the targets of seed compartment- and tissue-specific transcription factors and the biological processes they regulate?
3. How does gene activity change during seed development?
4. What are the regulatory processes required to program genes during soybean seed development?
5. What are the gene networks required to program seed differentiation and maturation?

Soybean Seed Development - Developmental Period Studied -

Unraveling the Gene Regulatory Processes That are Required to "Make a Seed"

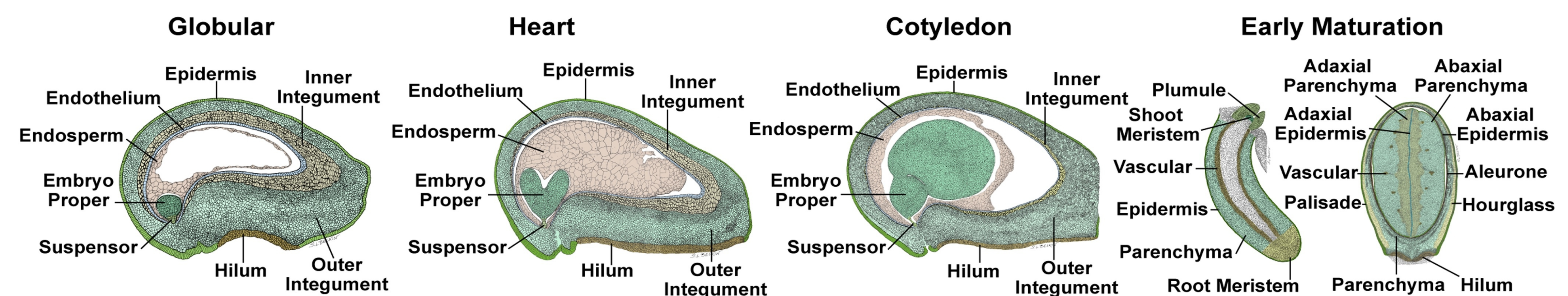
Major Questions Addressed in the Research

RESEARCH PLAN & STRATEGY



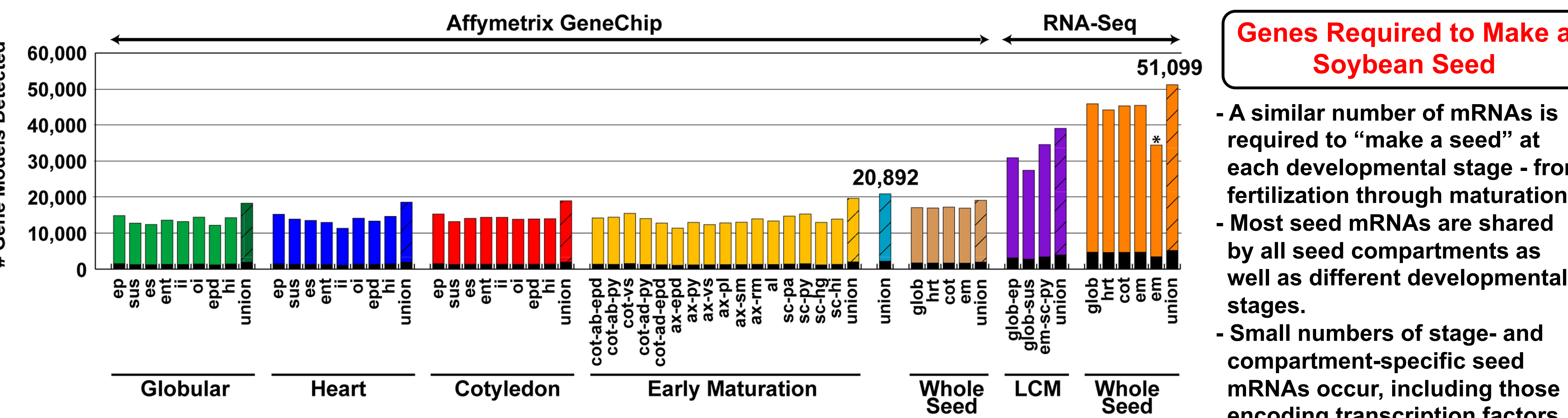
RESULTS

Overview of Soybean Seed Transcriptional Profiling and Genomics Experiments



SEED STAGE	GLOB	HRT	COT	EM	TOTAL
# Compartments Studied	8	8	8	16	40
# GeneChip Experiments	26	21	18	34	99
# Genes Validated by qRT-PCR (TF)	42 (28TFs)	36 (20TFs)	55 (55TFs)	21 (21TFs)	154
# Seed Sections	97,318				
Next-Gen Sequencing	718 million reads				

	GLOB	HRT	COT	EM
EMBRYO REGIONS				
EMBRYO PROPER (EP)				
SUSPENSOR (S)				
ENDOSPERM (ES)				
SEED COAT (SC)				
ENDOTHELIUM (ENT)				
INNER INTEGUMENT (II)				
OUTER INTEGUMENT (OI)				
EPIDERMIS (EPD)				
HILUM (HI)				



Genes Required to Make a Soybean Seed

- A similar number of mRNAs is required to "make a seed" at each developmental stage - from fertilization through maturation.
- Most seed mRNAs are shared by all seed compartments as well as different developmental stages.
- Small numbers of stage- and compartment-specific seed mRNAs occur, including those encoding transcription factors.

- At least 51,000 diverse mRNAs are required to program the differentiation of all compartments, regions, and tissue types of soybean seeds throughout development.

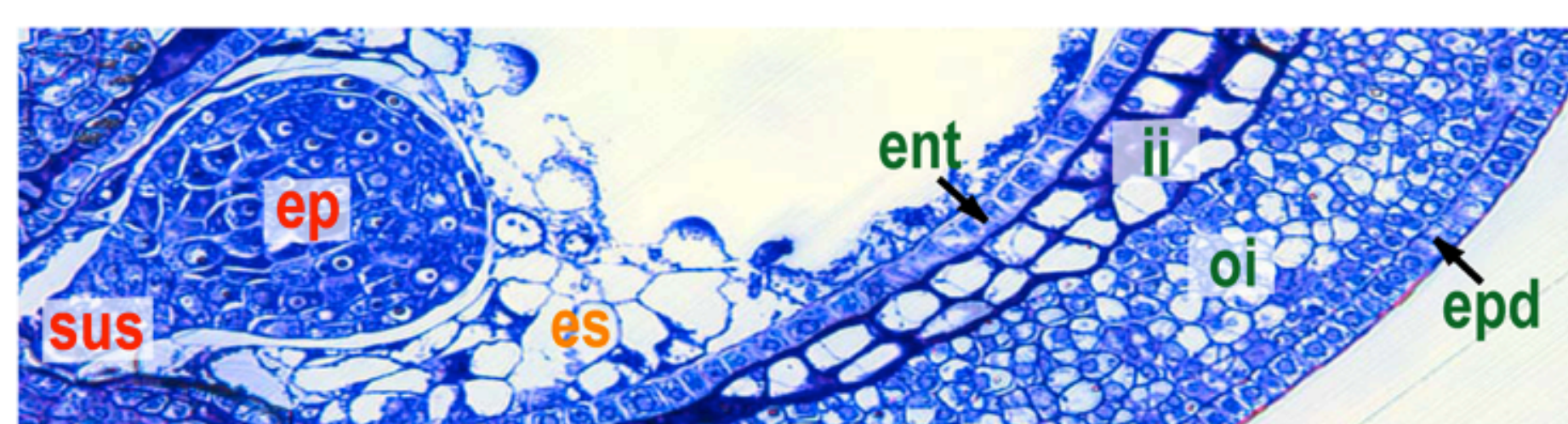
QUESTIONS REMAINED TO ANSWER

1. What is the spectrum of genes that are active in 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?

* Under Investigation

RESULTS

Gene Activity & Regulation in a Soybean Globular-Stage Seed Compartments, Regions, and Tissues



	WS	sus	ep	es	ent	ii	oi	epd	hi
No. mRNAs Detected									
At least 2 BRs	19,938	14,177	16,998	13,880	15,274	14,767	16,402	13,451	16,153
No. TF mRNAs	1,373	936	1,129	872	976	952	1,102	865	1,086
Unique mRNAs	62	70	115	47	44	79	29	66	
Unique TF mRNAs	2	14	13	4	5	7	2	8	
Shared mRNAs	7,664 (400)								

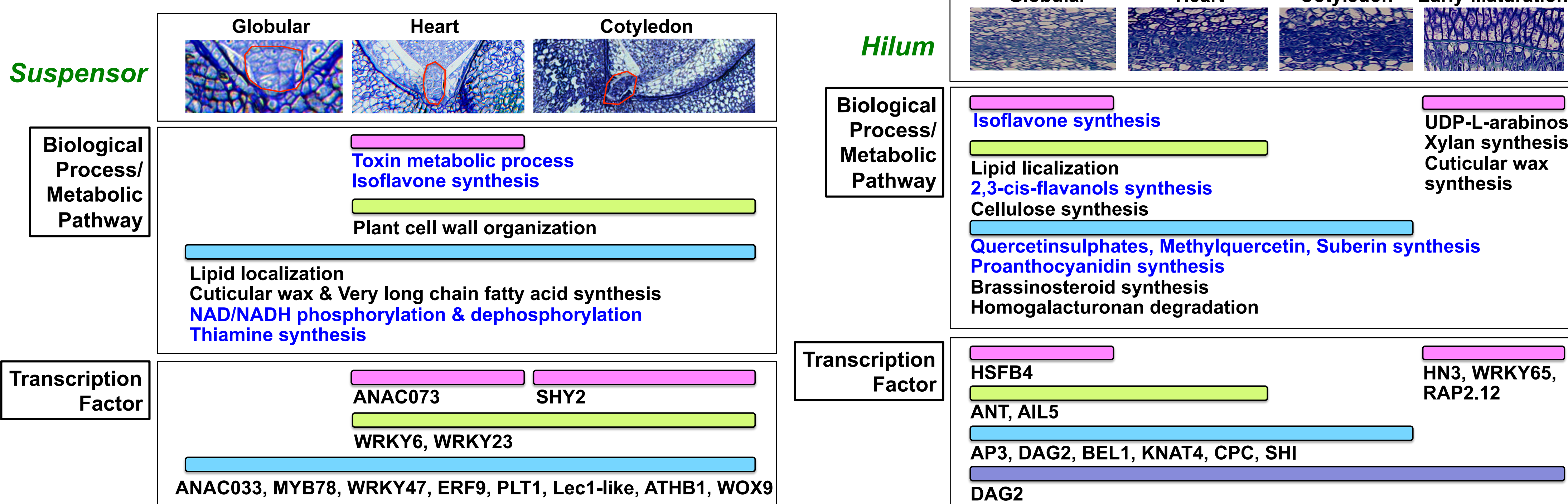
- A similar number of diverse mRNAs is required for the differentiation and/or function of each seed compartments, regions, and tissues.
- Each seed compartment has a small set of unique mRNAs and most mRNAs are shared by all soybean seed compartments, regions, and tissues.
- Most of active soybean seed mRNAs, including TF mRNAs, are quantitatively regulated.
- Distinct GO Terms, Metabolic Pathways, and different set of TF mRNAs are prevalent in soybean seed compartments, regions, and tissues.

Several Spatial and Temporal mRNA Accumulation Patterns Occur During Soybean Seed Development

1. **Spatial pattern of mRNA accumulation during early development (Glob-HRT-COT):** mRNAs cluster spatially with respect to seed compartments, regions, and tissues irrespective of developmental stage. e.g. Embryo Proper (EP)
2. **Spatial pattern of mRNA accumulation during early maturation (EM):** clusters of mRNAs appear spatially in compartments, regions and tissues. e.g. Seed coat Palisade
3. **mRNA sets up-regulated throughout development (Glob to EM):** clusters of up-regulated mRNAs are present in seed tissues throughout development. e.g. GHC-Endosperm/EM-Aleurone

Hierarchical clustering of most varying mRNAs, enzyme mRNAs, and transcription factor mRNAs in all compartment, region, and tissues throughout development indicated that **Enriched GO terms, metabolic processes and distinct set of transcription factor mRNAs are compartmentalized temporally and spatially.**

The Summary - Functions of Soybean Seed Compartments during Development



Compartmentalization of Soybean Health-Related Products Within the Seed

