Genetic and Epigenetic Responses to Environmental Stress (drought) in Common Bean (*Phaseolus vulgaris L.)*

Combining Genetics-Molecular Genetics-Transcriptomics-Epigenomics

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Project Overview

- Grow the same genotype in two different locations (DE and NE)
- Have fully-irrigated plot and terminal-drought plot in both locations
- Compare differential expression of drought-responsive genes between the two locations

The Common Bean

- A major crop cultivated around the world:
  - -High in protein, fiber, and micronutrients
  - -Long storage life
  - -Ability to fix atmospheric nitrogen
  - -Has been adapted to different environments

Different Growth Areas:

Average Temperatures (°F)

Location	June	July	August	September	Average
Smyrna, DE	73 F	80 F	80 F	73 F	77 F
Scottsbluff, NE	74 F	76 F	71 F	64 F	71 F

Average Rainfall (Inches)

Location	June	July	August	September	Total
Smyrna, DE	4.1	7.5	2.8	10.3	24.8
Scottsbluff, NE	1.1	1.3	1.9	1.4	5.6

Genotype used: NE-28-15-16

- A red bean cultivar developed by Dr. Carlos Urrea- A bean breeder from University of Nebraska Lincoln
- Developed using shuttle breeding in Puerto Rico
- A determinate bush type (Andean)

Experimental Design

- Terminal drought- irrigation was applied weekly during vegetation, but ceased once flowers appeared (R1) ≈ 2 month stress
- Leaf, root, and developing pod samples were harvested from 4 different plants per row
- Samples were taken at R8 stage of development

Sample Examples (2016)

Non-drought sample(NE)

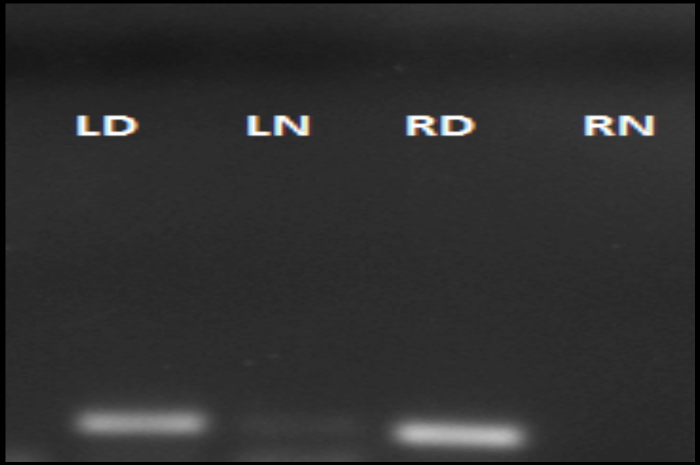
Drought sample(NE)

Molecular Analysis Overview

- RNA isolated from samples
- RNA converted into cDNA
- cDNA used in PCR for drought-responsive genes
- RNA sent for RNA-Seq for full transcriptome (future)

BURP Gene Expression

Delaware Samples



Nebraska samples

LD= Leaf Drought
LN= Leaf Non-drought
RD= Root Drought
RN= Root Non-drought

- BURP-Domain is a RD22 (responds to dehydration) homolog
- Known to be induced by salt and drought stress, but not temperature stress

CA1 Expression


Delaware Samples


Nebraska Samples

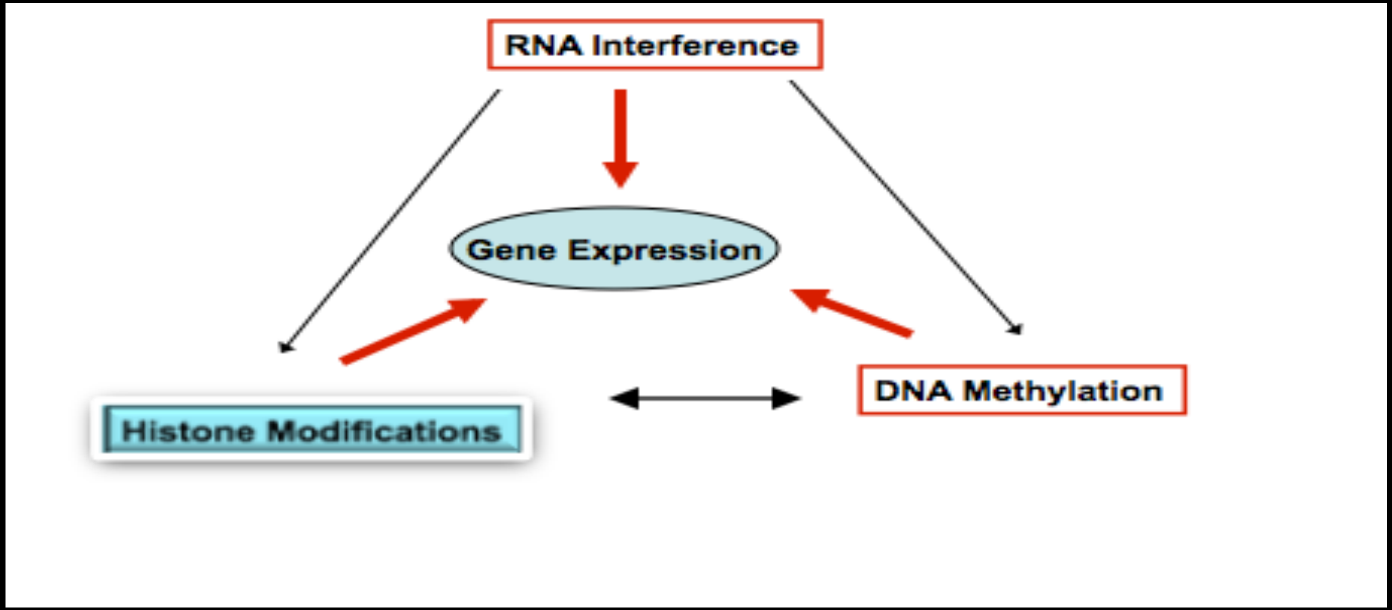
- Ethylene-responsive transcription factor-
- Notice difference in root expression compared with BURP gene

Big Picture

- Genotypes will express genes differently depending on locations
- Breeders can benefit from knowing which genes are more active in their certain region
- Breeders can breed for more specific region based genotypes

Epigenetic mechanisms

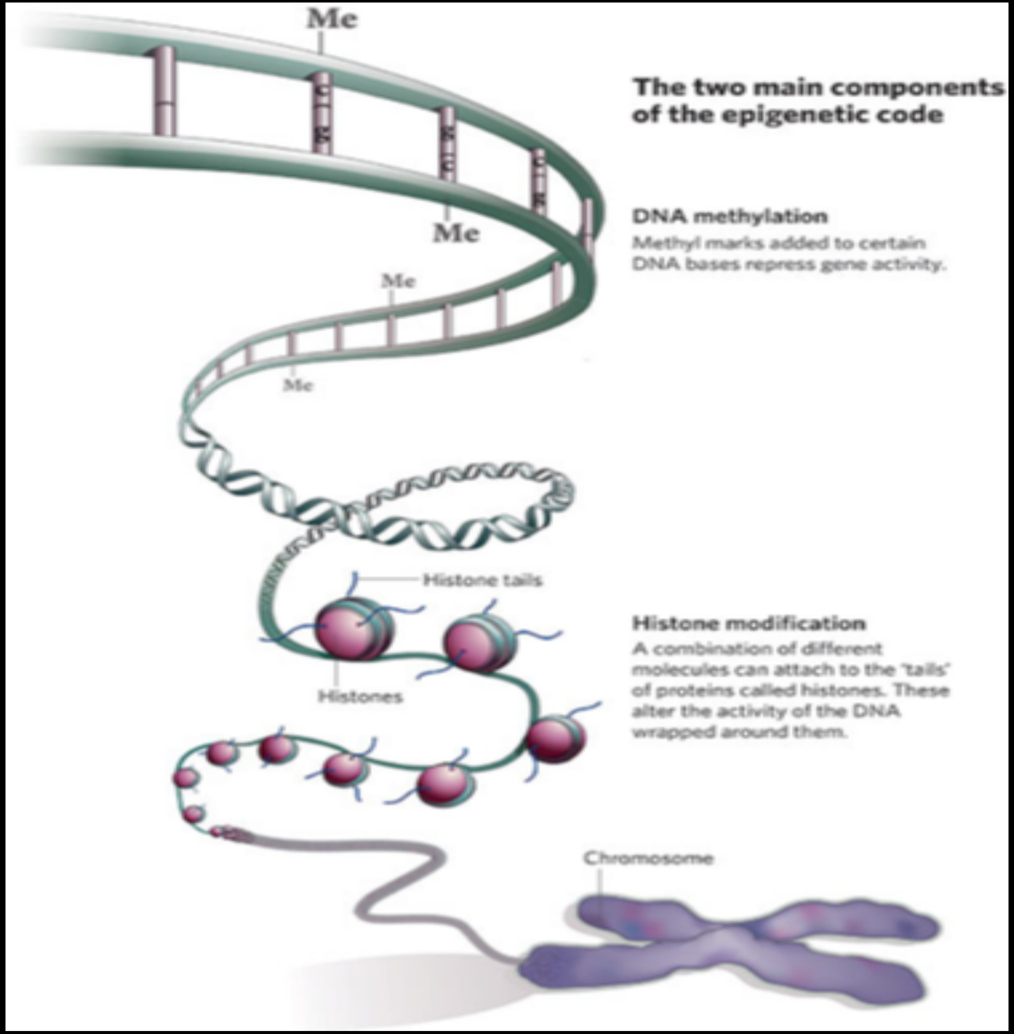
- Epigenetics- “upon genetics”: “A change in molecular or morphological phenotype without a change in the underlying nucleotide sequence”


RNA Interference

Gene Expression

Histone Modifications

DNA Methylation

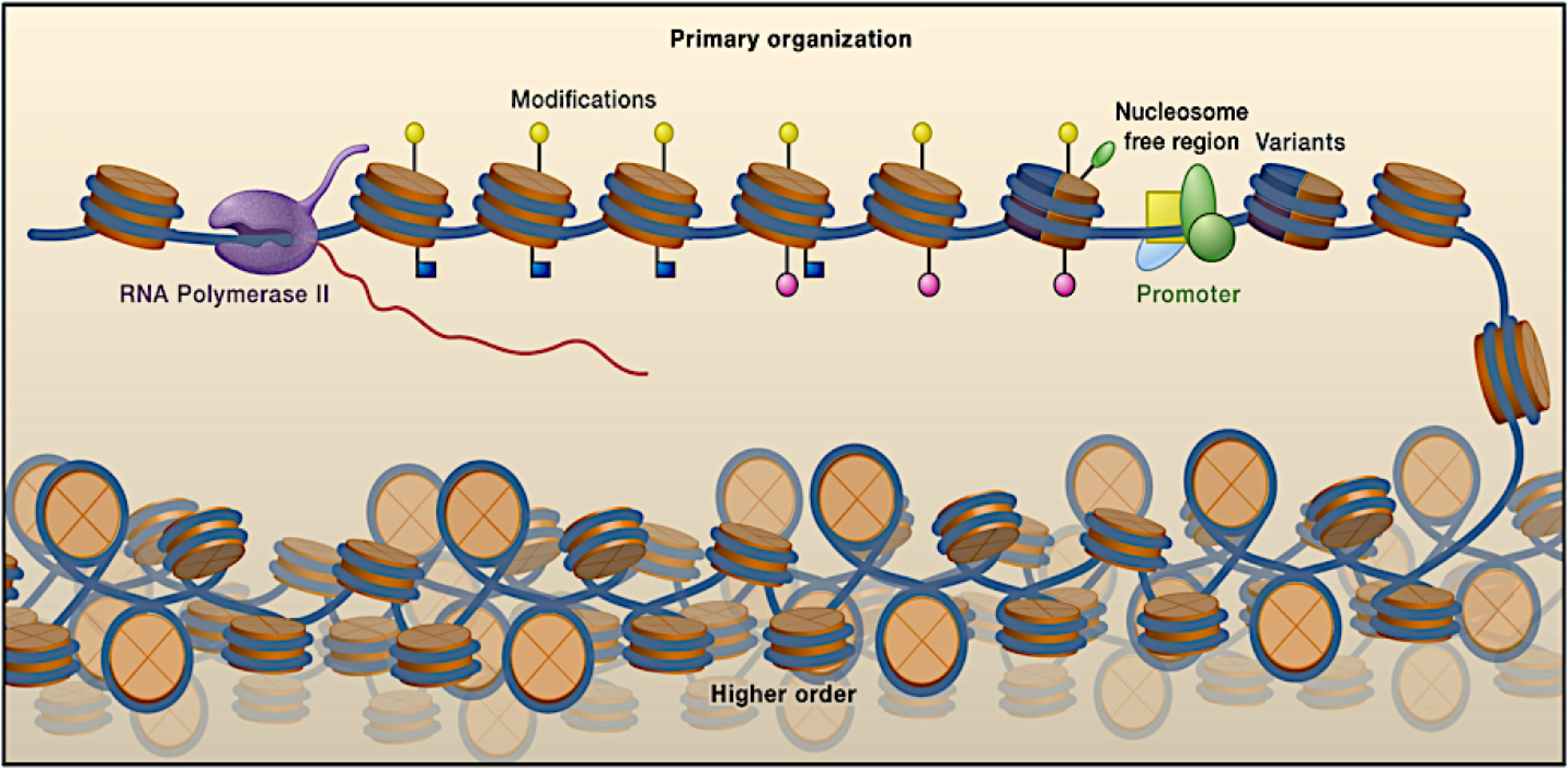

The two main components of the epigenetic code

DNA methylation
Methyl marks added to certain DNA bases repress gene activity.

Histone modification
A combination of different molecules can attach to the 'tails' of proteins called histones. These alter the activity of the DNA wrapped around them.

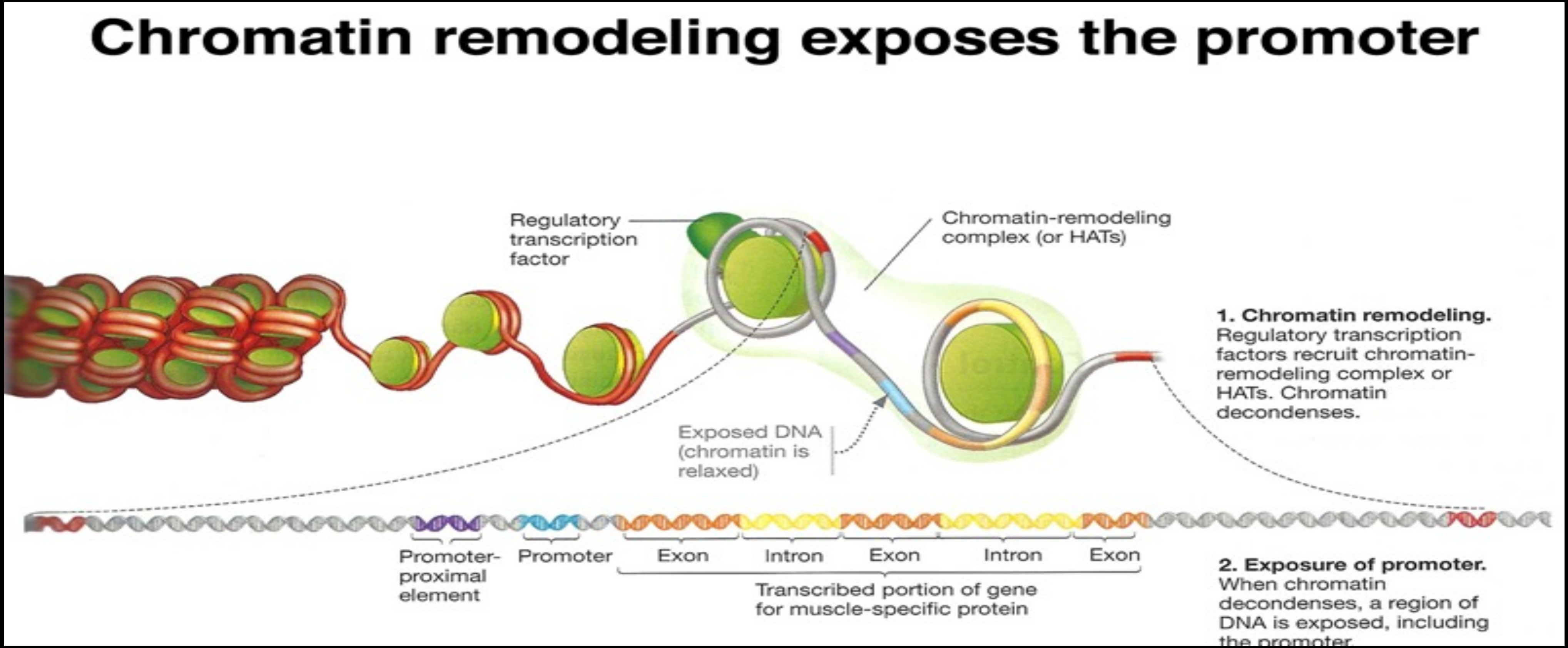
Nucleosome occupancy in drought response

- Structure of chromatin regulates accessibility of genes-position & spacing
- ↑ nucleosome occupancy = ↓ transcription
- ↓ nucleosome occupancy = ↑ transcription



Source: Zhang and Pugh , 2011

Chromatin reorganization-Stress Memory

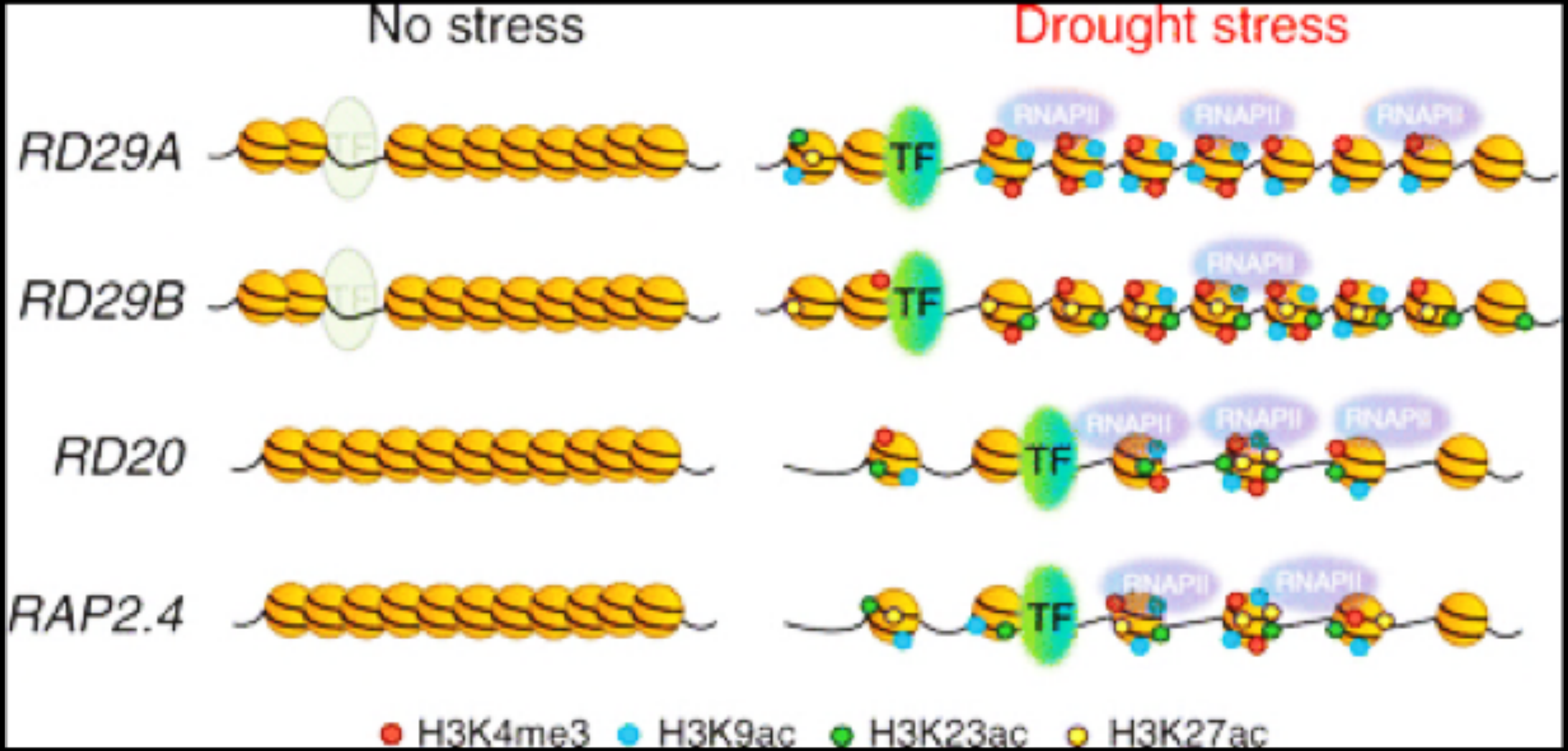

Chromatin remodeling exposes the promoter

1. Chromatin remodeling. Regulatory transcription factors recruit chromatin-remodeling complex or HATs. Chromatin decondenses.

2. Exposure of promoter. When chromatin decondenses, a region of DNA is exposed, including the promoter.

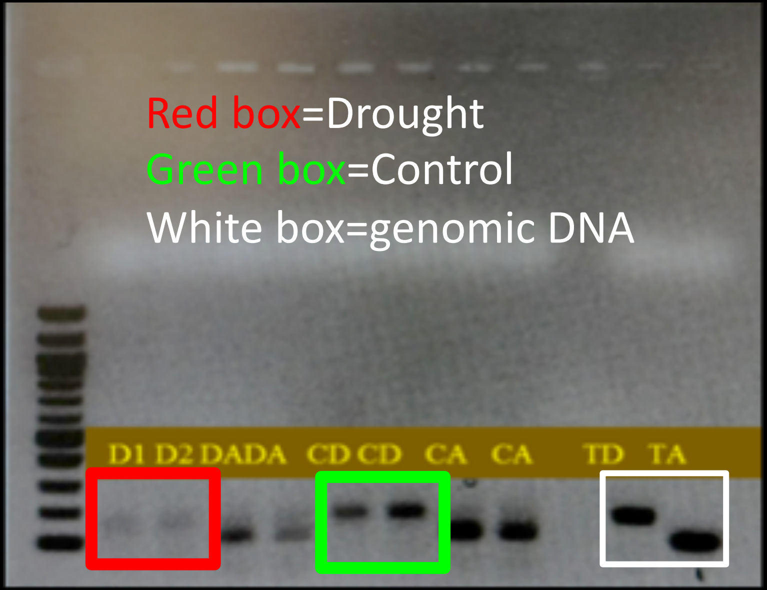
- In our study, drought potentially caused a shift in the nucelosomes in the DREB6B region
- Incomplete loss of nucleosomes suggests stress memory

Nucleosome occupancy and the enrichment of histone modification



Kim et al. 2008 Arabidopsis

Real time-PCR amplification of leaf mononucleosomal DNA with DREB6B primers



D1, D2: Drought-with DREB

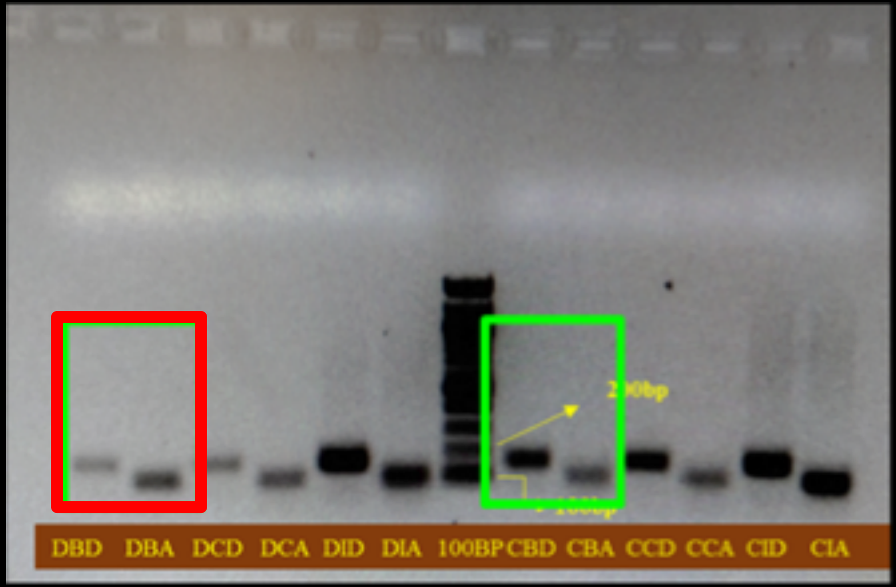
DA: Actin Primers

CD: Non Drought DREB

CA: Control with Actin primers

TD: Genomic DNA with DREB and Actin

PCR amplified H3 pulled out DNA with DREB and actin primers



DBD: Drought H3 pulled DNA with DREB6B,
DBA: H3 and Actin
CBD: Control and H3 and DREB6B
CBA: H3 and Actin from control

- Drought caused a shift in the nucelosomes in the DREB6B region,
- Low amplification yield in PCR of mononucleosomal DNA
- Also confirmed by Histone H3 ChIP PCR as well
- Not complete loss of nucleosome-hints of “Drought Memory”

Conclusions/Future Directions

- Understanding why the same genotype/cultivar reacts differently to different environments
- Can we discover epigenetic markers that go beyond traditional genetic markers
- Need for large-scale, collaborative studies that integrate genetics, molecular genetics, and genomics
- Working on beans, wheat, and other crops with breeders and other geneticists

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Common Bean Genome Projects