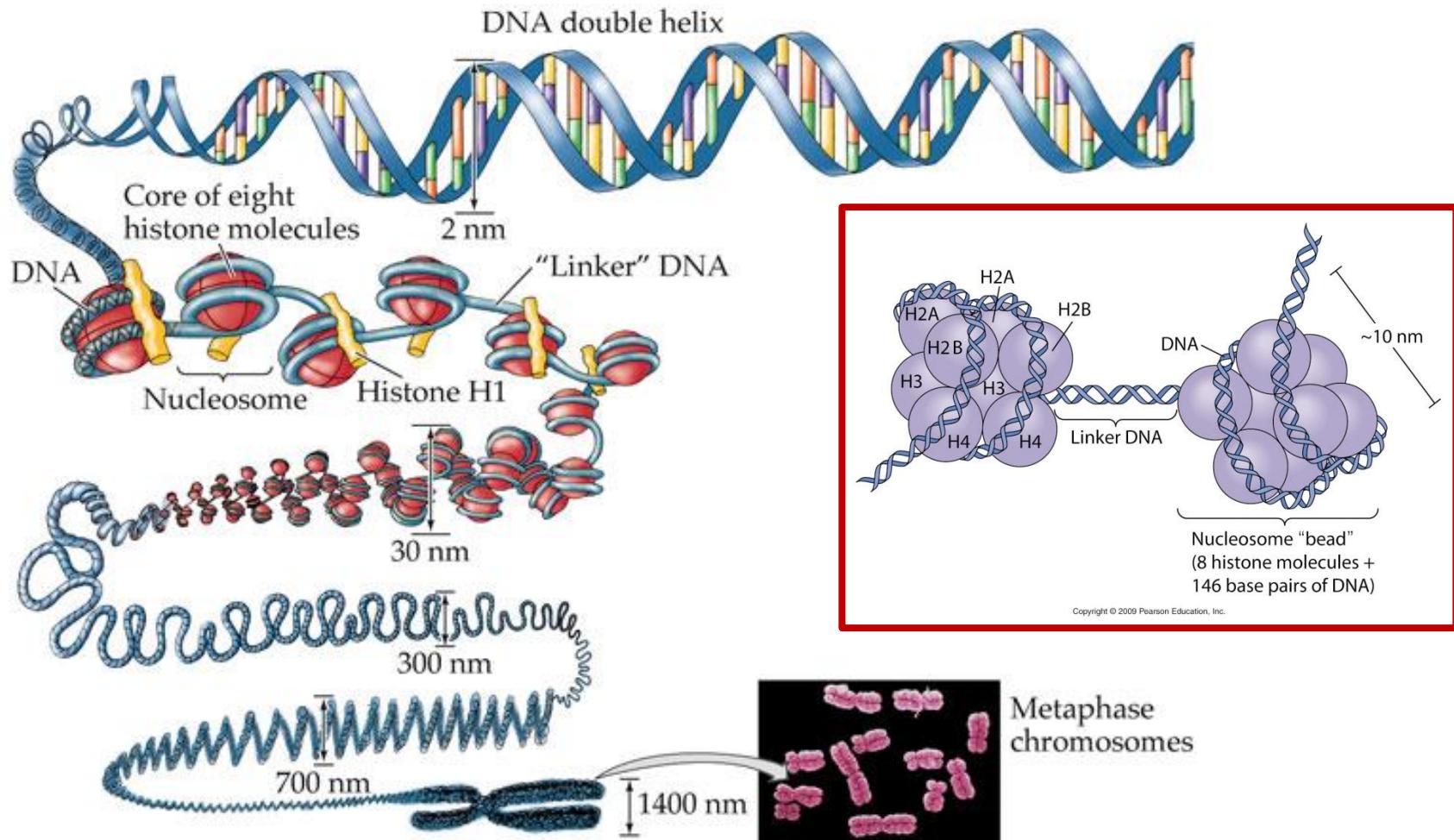


Histone modifications and their regulatory roles in plants

TS. Nguyễn Hoài Nguyên
Khoa Công nghệ Sinh học, Trường Đại học Mở TP.HCM

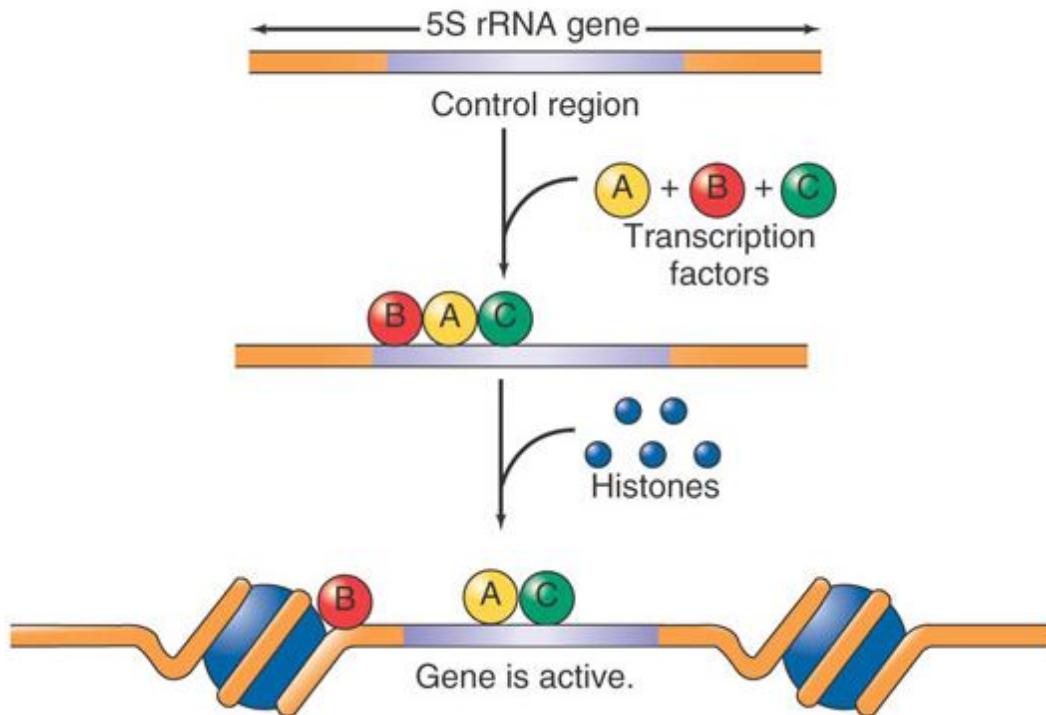
Chromosome and histone



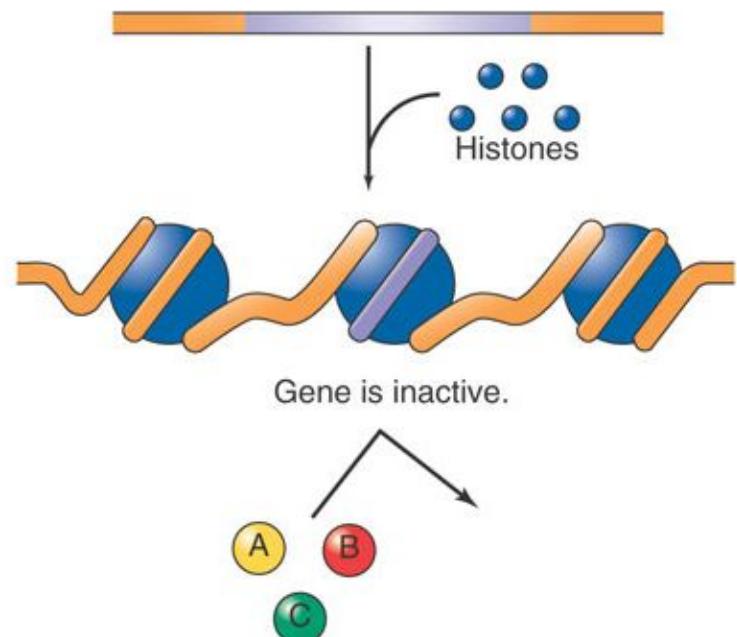
LIFE: THE SCIENCE OF BIOLOGY, Seventh Edition, Figure 9.6 DNA Packs into a Mitotic Chromosome
© 2004 Sinauer Associates, Inc. and W. H. Freeman & Co.

Chromatin structure and gene activity

(a) Transcription factors win:



(b) Histones win:



Chromosome and histone

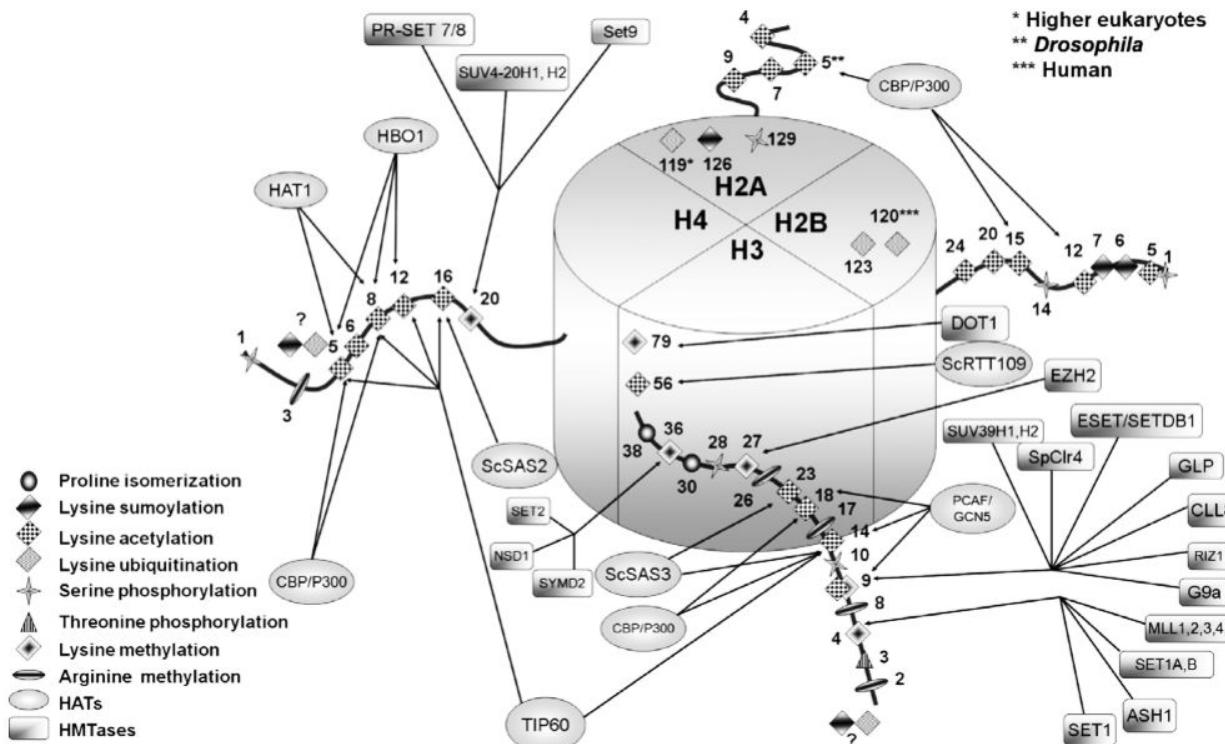
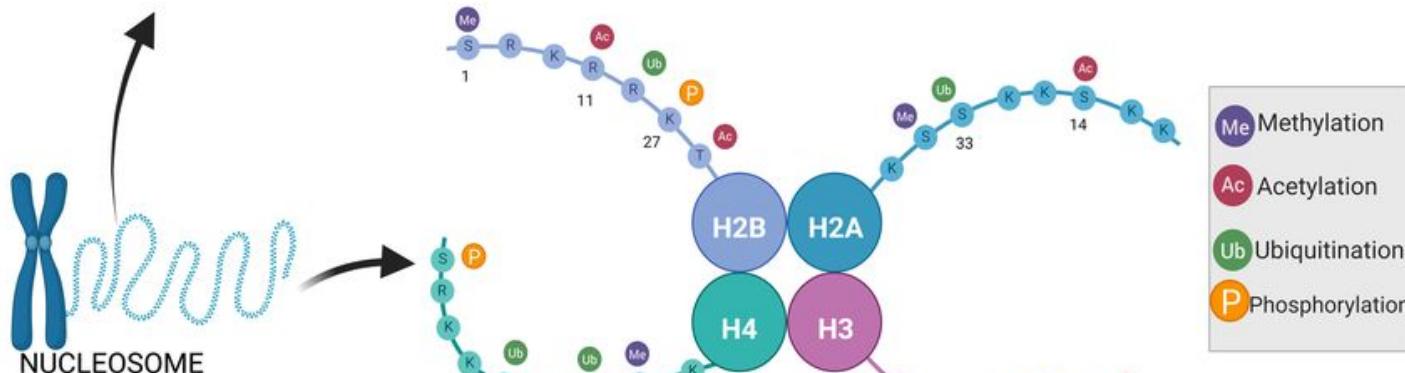
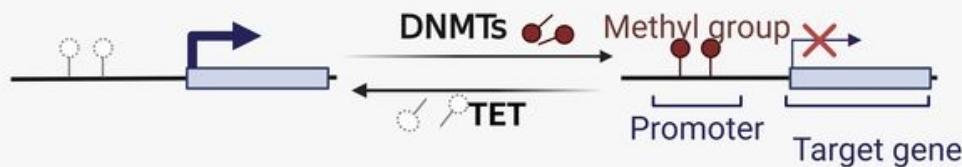


FIGURE 3.1

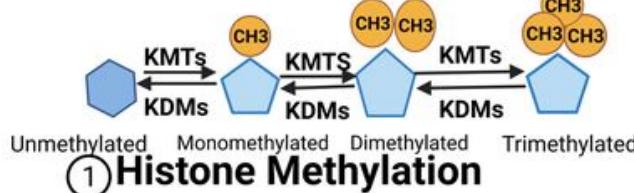
Schematic representation of a nucleosome and major histone modifications. Post-translational modifications of histones occur primarily on N-terminal tails of core histones (H2A, H2B, H3, and H4) and include acetylation, methylation, phosphorylation, and ubiquitination. Note that several lysines (e.g. Lys 9) can be either acetylated or methylated.

Histone modifications

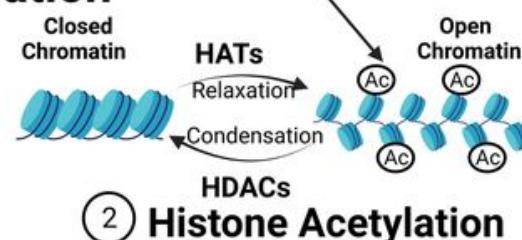
(A) DNA Methylation



(B) Histone Modification

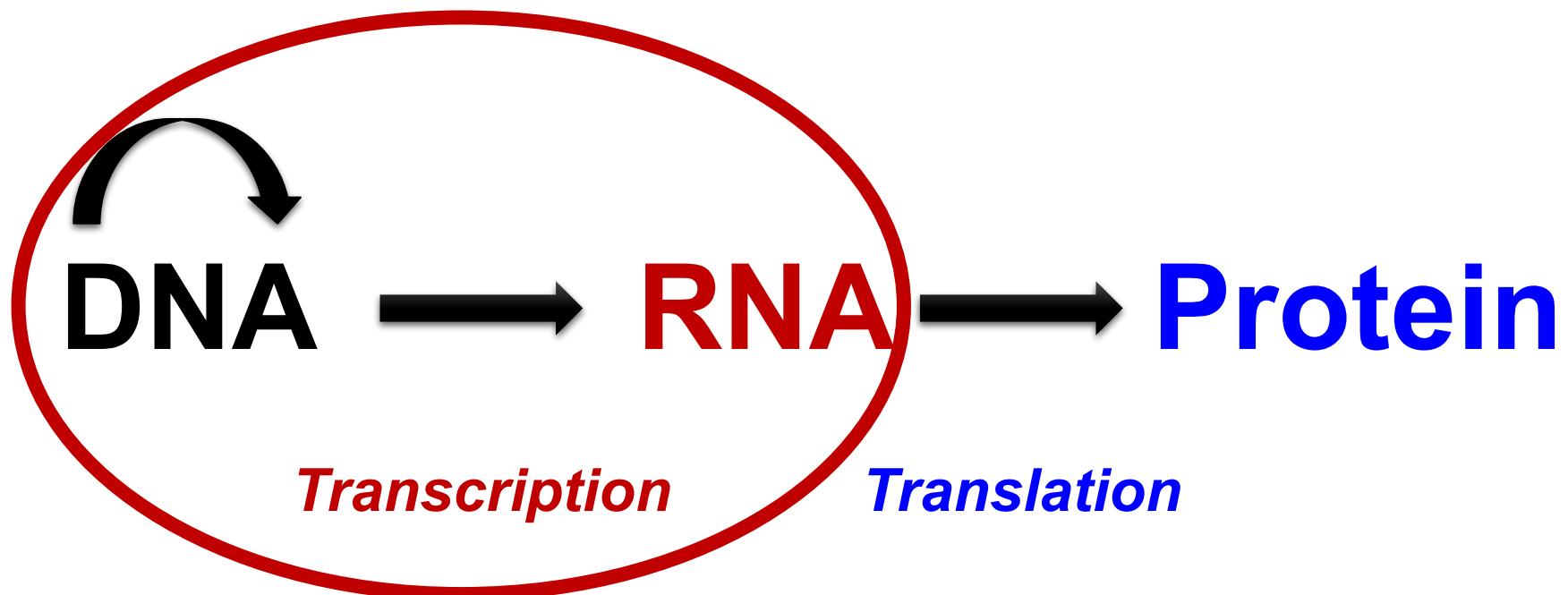


① Histone Methylation



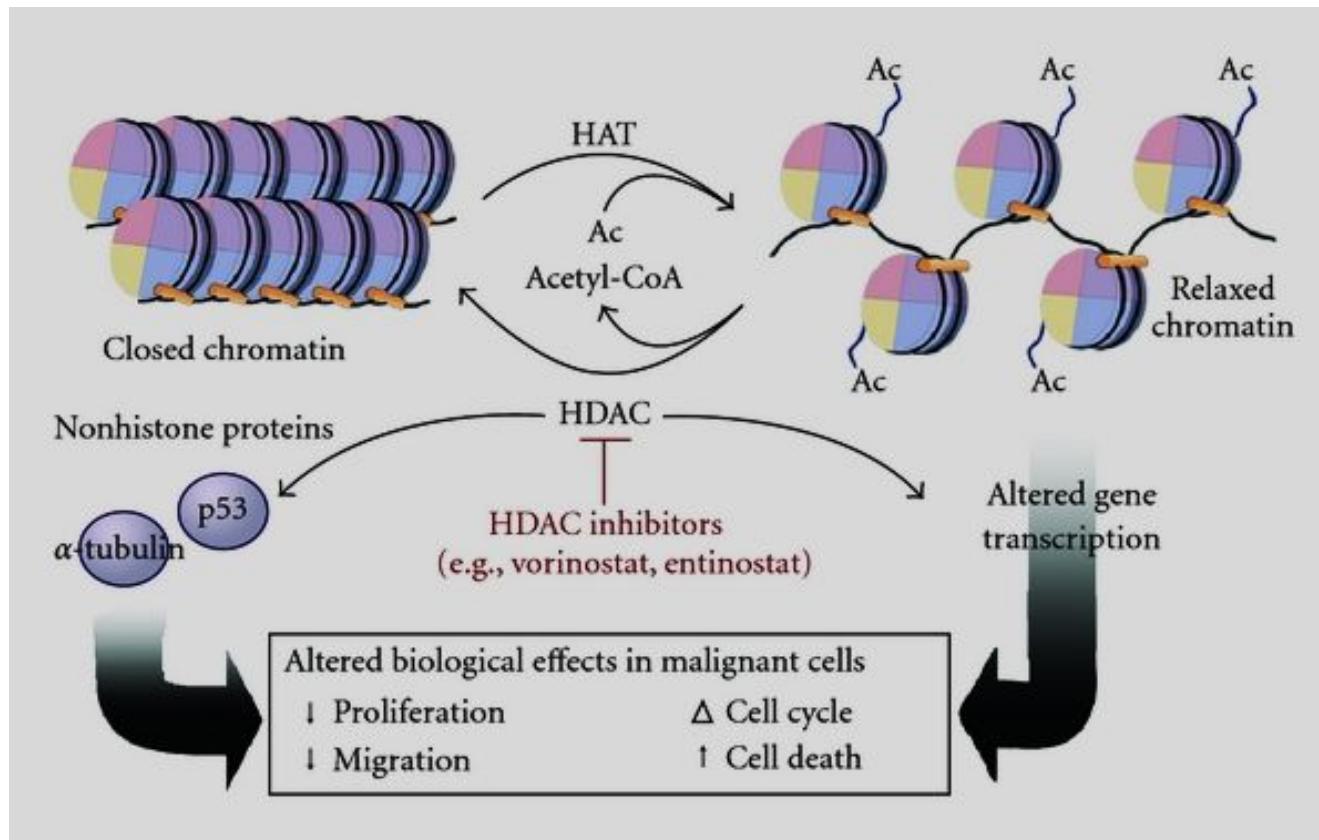
② Histone Acetylation

Central dogma of molecular biology



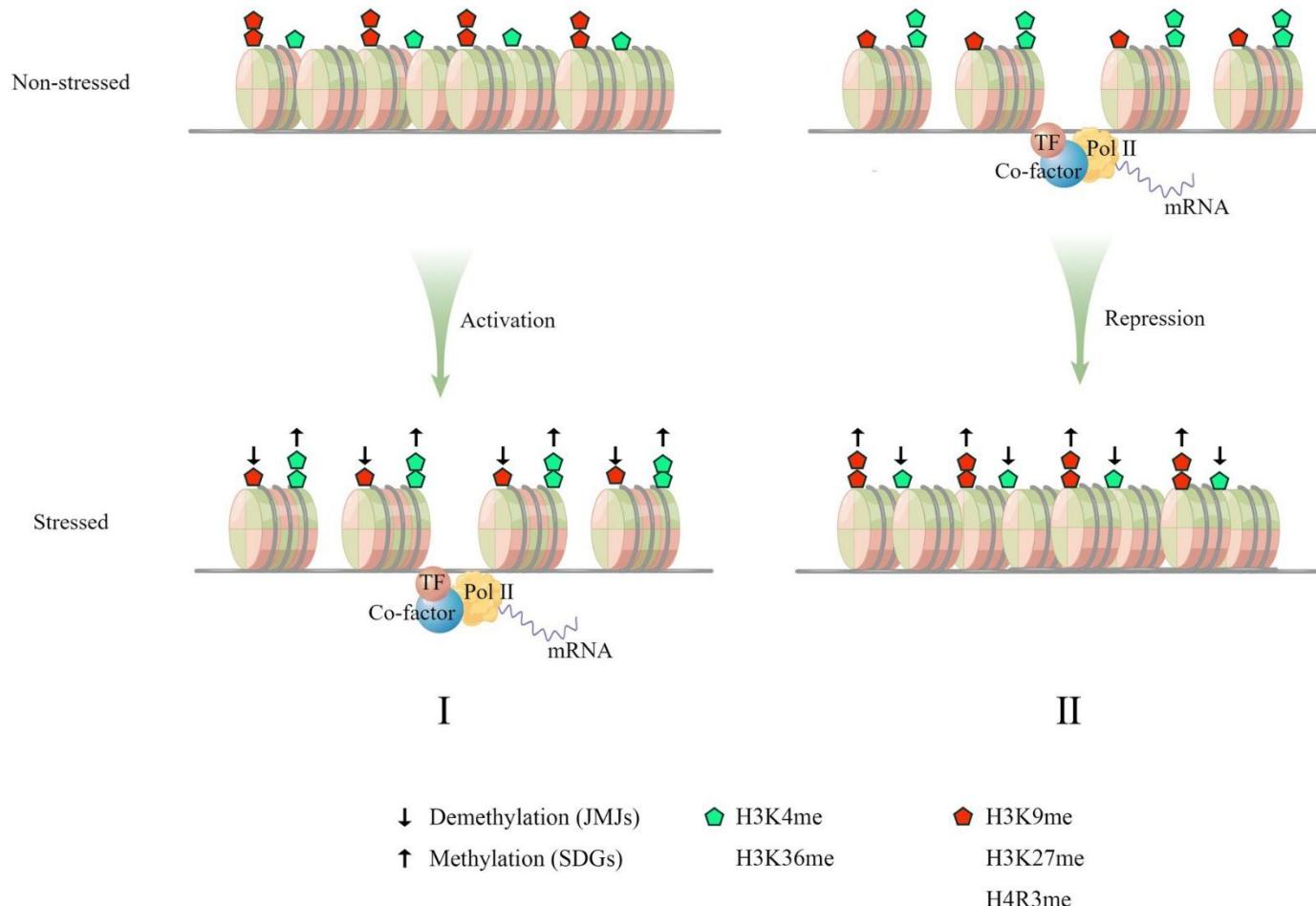
Transcription and histone modifications

Histone acetylation

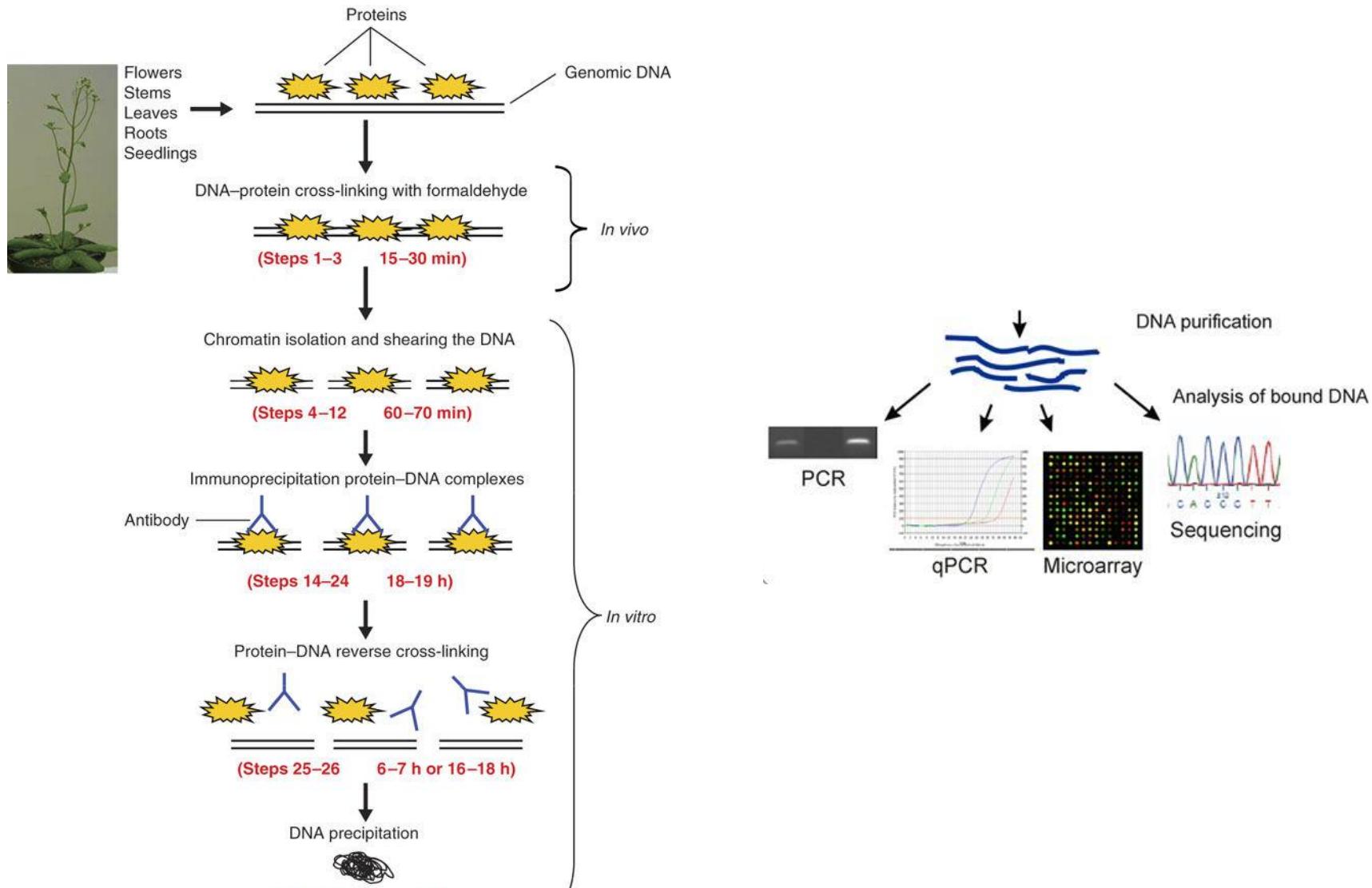


Transcription and histone modifications

Histone methylation



Chromatin immunoprecipitation (ChIP)



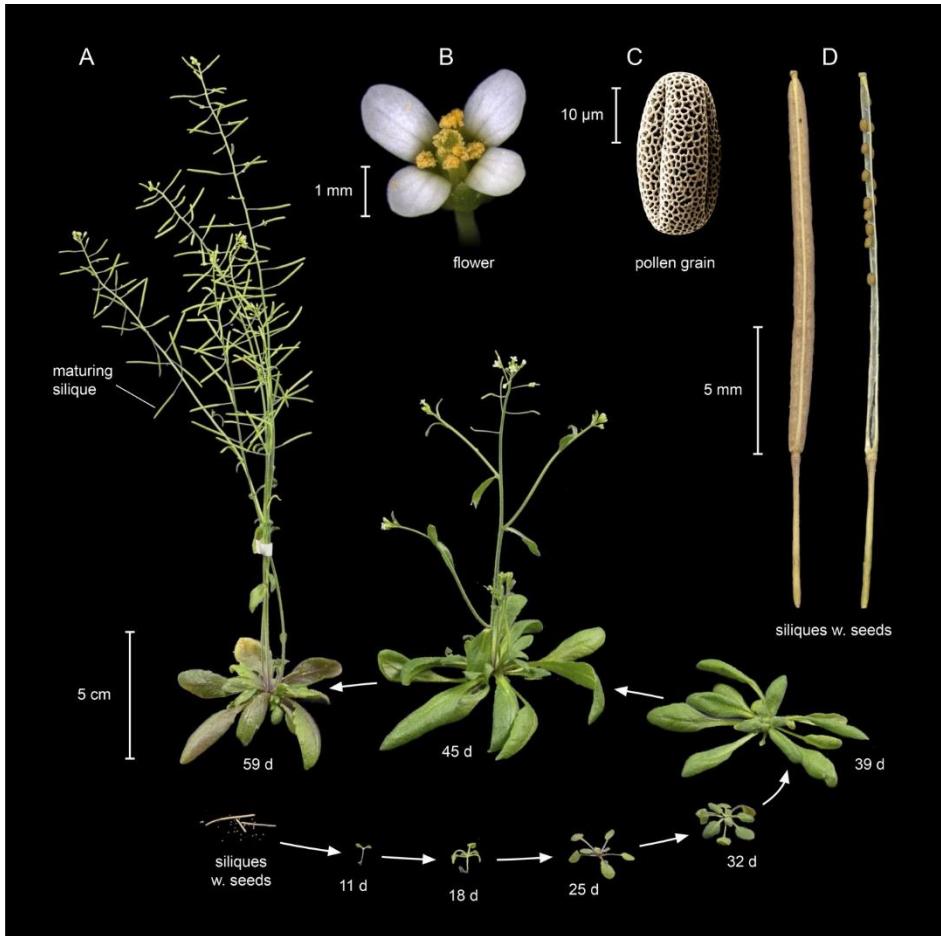
Chromatin modifications associated with AtMYB44 modulating environmental stress tolerance in *Arabidopsis thaliana*

Nguyen Hoai Nguyen and Jong-Joo Cheong

Center for Food and Bioconvergence, Seoul National University



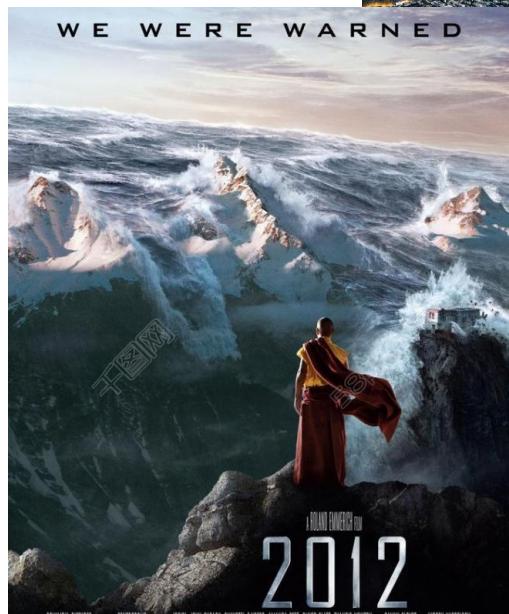
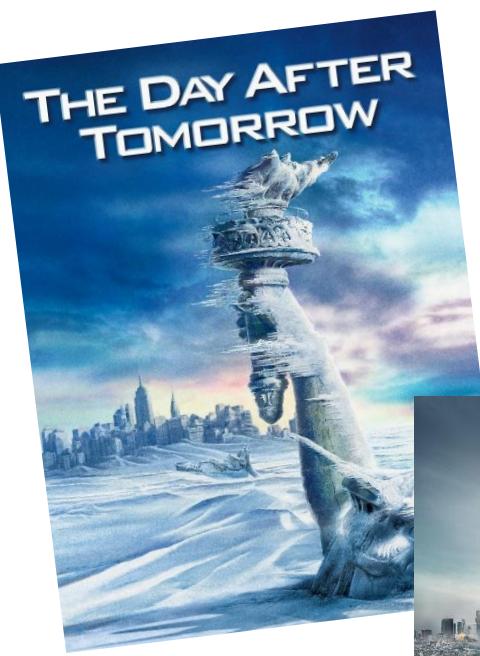
Why *Arabidopsis thaliana*?



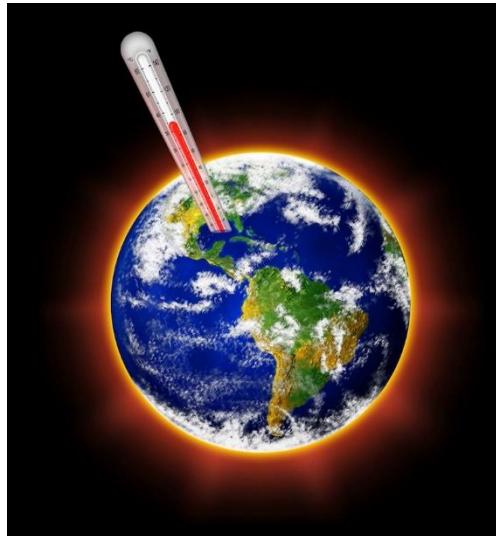
Ute Krämer (2015) eLife, 4, e06100 DOI: 10.7554/ELIFE.06100

Why? (www.arabidopsis.org)

- A rapid life cycle.
- Easy cultivation.
- Small genome (114.5 Mb/125 Mb total) has been sequenced in the year 2000.
- Extensive genetic and physical maps of all 5 chromosomes.
- Efficient transformation methods utilizing *Agrobacterium tumefaciens*.
- A large number of mutant lines and genomic resources many of which are available from Stock Centers.
- Multinational research community of academic, government, and industry laboratories.



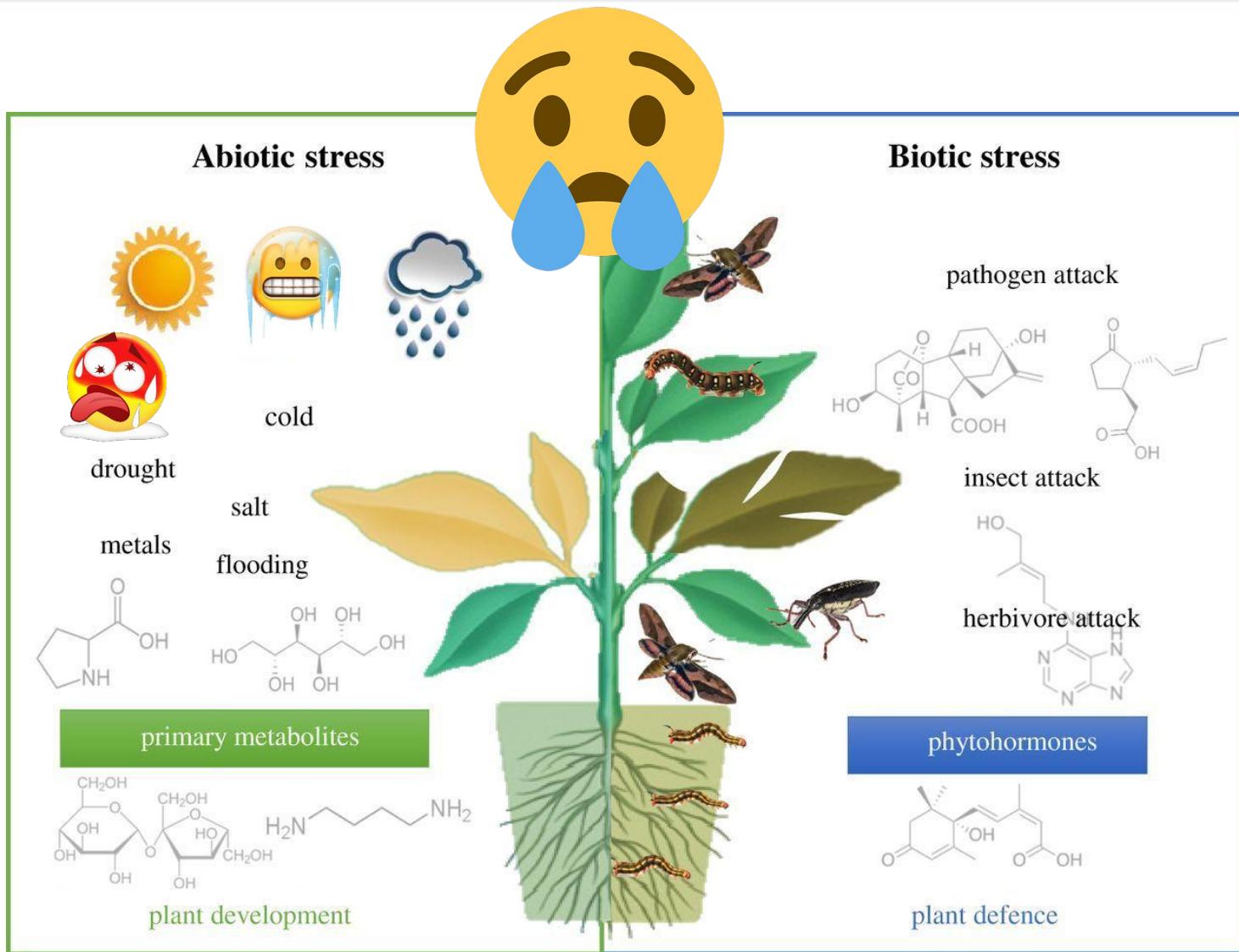
Abiotic stress



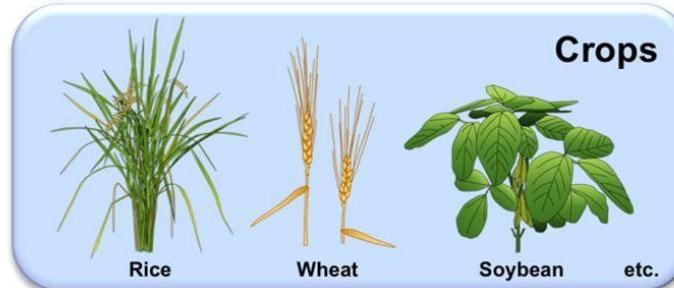
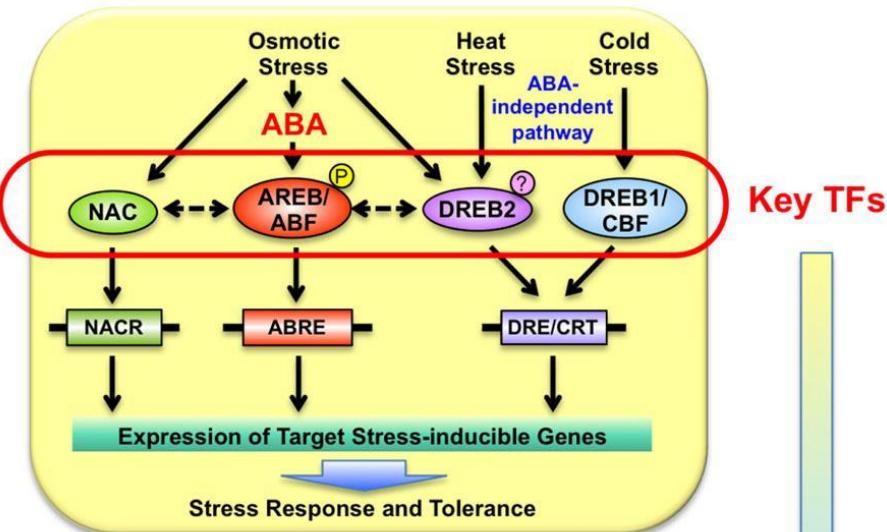
Salt sensitive (left) and tolerant (right) soybean varieties, shown after 8 days of treatment with 120 mM NaCl.



Plants also feel stressful!!!

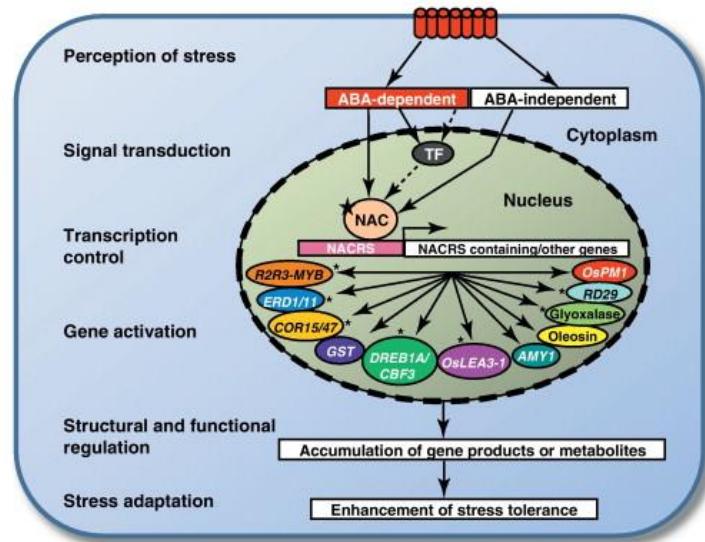


Abiotic stress

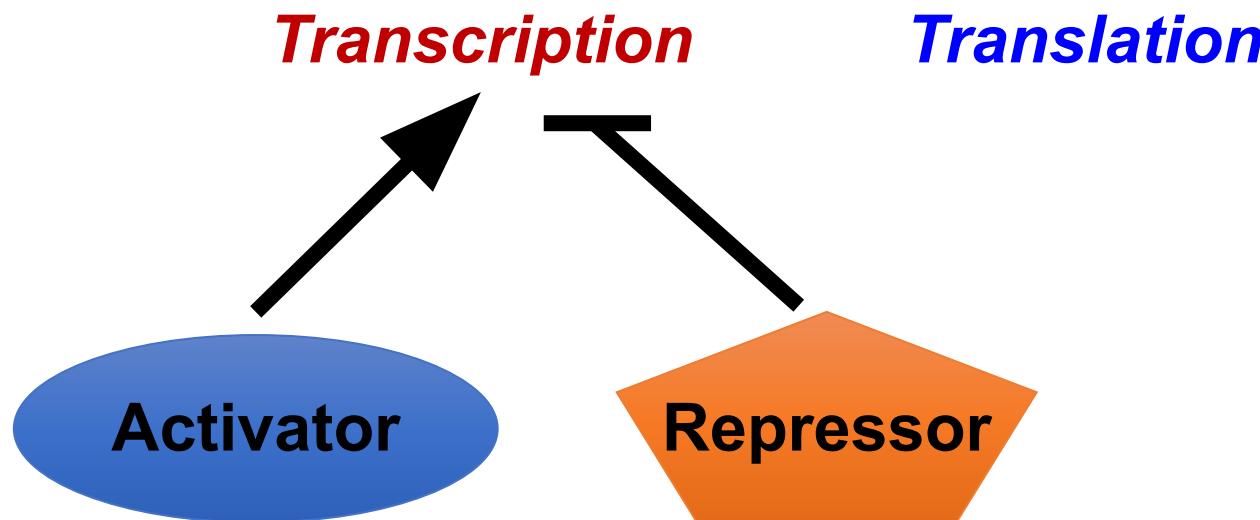


Over-expression
using
suitable
promoters

Improvement of Drought Tolerance

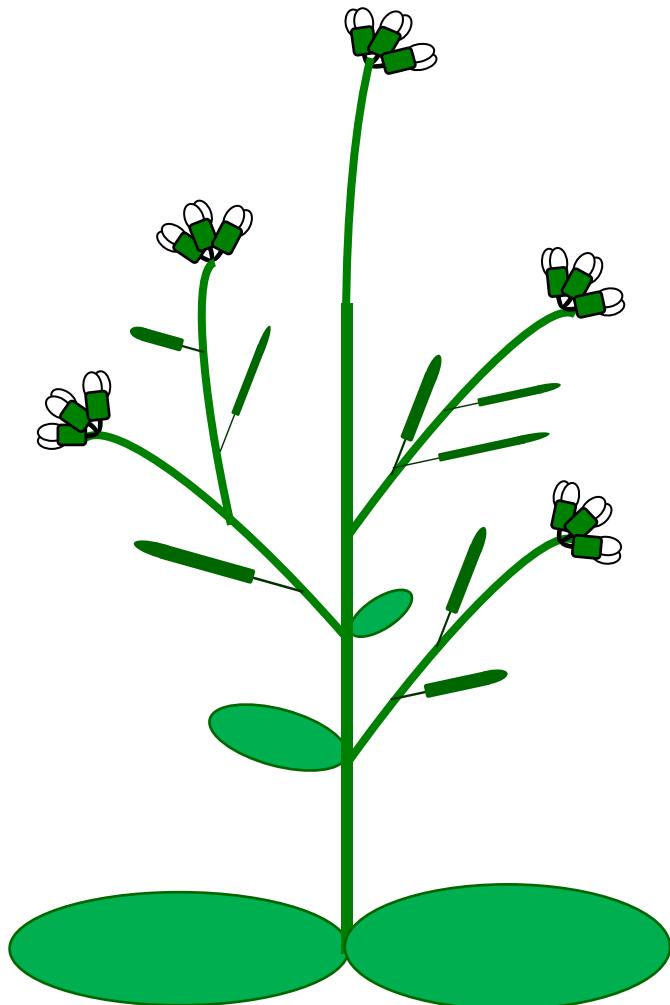


Central dogma of molecular biology



Transcription factors

Plant transcription factors



bHLH
bZIP
MYB
NAC
WRKY
...

Arabidopsis thaliana AtMYB44

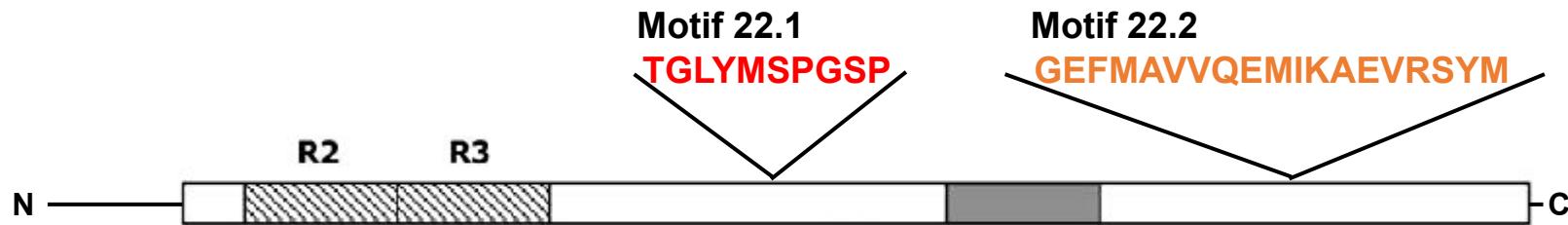
| | |
|-----------------------|---------------------|
| Transcription Factors | 1,600 (6% of genes) |
| MYB TF | 163 |
| R2R3 MYB TF | 126 |

Subgroup 22

AtMYB44
AtMYB70
AtMYB73
AtMYB77

AtMYB44

At5g67300
918 bp
305 a.a.
33.3 kDa



Arabidopsis thaliana AtMYB44

Overexpression of *AtMYB44* Enhances Stomatal Closure to Confer Abiotic Stress Tolerance in Transgenic *Arabidopsis*^{1[C][W][OA]}

Choonkyun Jung, Jun Sung Seo, Sang Won Han, Yeon Jong Koo, Chung H Baek Hie Nahm, Yang Do Choi, and Jong-Joo Cheong*

Department of Agricultural Biotechnology and Center for Agricultural Biomass University, Seoul Nutrition, Seoow Bioinformatics, M

Biochemical and Biophysical Research Communications 499 (2018) 1039–1043



Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc



Contents lists available at ScienceDirect

Other Sections ▾

AtMYB44 belongs to a group of transcription factors that are induced by abscisic acid (ABA) and other phytohormones in response to various abiotic stresses, such as salt stress, cold, heat, and drought. AtMYB44 is a member of the MYB protein family and is highly conserved across different plant species. It has been shown to play a role in the regulation of gene expression in response to various stress conditions. In this study, we report that overexpression of AtMYB44 in *Arabidopsis thaliana* enhances stomatal closure under salt stress conditions. This results in increased salt tolerance and reduced water loss. The mechanism involves the induction of H2A.Z-containing nucleosomes at the AtMYB44 promoter region, which leads to the eviction of H2A.Z-containing nucleosomes and subsequent transcriptional activation. Our results suggest that AtMYB44 may act as a key regulator of gene expression in response to salt stress.

H2A.Z-containing nucleosomes are evicted to activate AtMYB44 transcription in response to salt stress



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ARTICLE INFO

Article history:

Received 6 April 2018

Accepted 7 April 2018

Available online 10 April 2018

Keywords:

Arabidopsis

AtMYB44

H2A.Z

Nucleosome

Salt stress

Transcription

ABSTRACT

Transcripts of the *Arabidopsis* transcription factor gene, *AtMYB44*, accumulate rapidly to mediate a salt stress tolerance mechanism in response to salt stress. The *AtMYB44* promoter is activated by salt stress, as illustrated in *AtMYB44pro::GUS* transgenic plants. Chromatin immunoprecipitation (ChIP) assays revealed that RNA polymerases were enriched on the *AtMYB44* gene, especially on TSS-proximal regions, and nucleosome density was markedly reduced in the *AtMYB44* gene-body region in response to salt stress. In addition, H2A.Z occupation was significantly decreased at the *AtMYB44* promoter, transcription start site (TSS), and gene-body regions. Histone modifications including histone H3 lysine 4 trimethylation (H3K4me3) and histone H3 and H4 acetylation (H3ac and H4ac) were not affected under the same stress conditions. We found a decrease in the number of *AtMYB44* proteins bound to their own gene promoters in response to salt stress. These results suggest that salt stress induces the eviction of H2A.Z-containing nucleosomes from the *AtMYB44* promoter region, which may weaken its affinity for binding *AtMYB44* protein that acts as a repressor for *AtMYB44* gene transcription under salt stress-free conditions.

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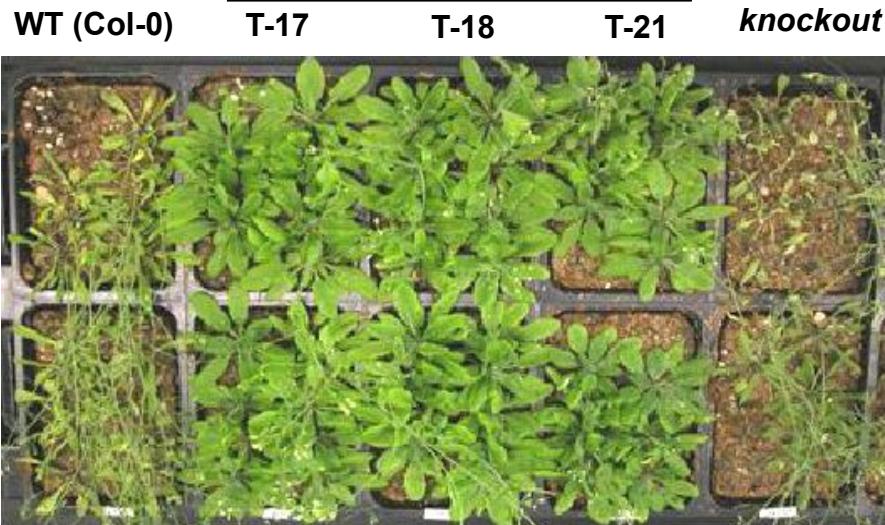
Mol. Cells 2010; 29(1): 71~76 DOI: 10.1007/s10059-010-0009-z
Non-Specific Phytohormonal Induction of AtMYB44 and Suppression of Jasmonate-Responsive Gene Activation in *Arabidopsis thaliana*
Choonkyun Jung^{1,3}, Jae Sung Shim¹, Jun Sung Seo¹, Han Yong Lee¹, Chung Ho Kim², Yang Do Choi¹, and Jong-Joo Cheong^{1,*}
1, 2, Korea, ³Present address: Laboratory of Plant
10065, USA
als, Seoul National University, Seoul 151-921,
2, Korea, ³Present address: Laboratory of Plant
10065, USA

; Published online December 10, 2009.

n 10 min by treatment with me-thyl jasmonate but not in distal leaves, illustrating jasmonate insensitivity in *Arabidopsis* mutants insensitive to jasmonate signaling hormones. Moreover, various growth hormones gene activation appears to not be induced by ABA as JR2, VSP, LOXII, and AOS was attenuated in nificantly enhanced in atmyb44 knockout mutants. ed primary root growth inhibition, indicating that the alterations in the jasmonate signaling pathway. jasmonate-induced anthocyanin accumulation, of ABA-mediated responses. These observations abscisic acid-mediated signaling pathways. plant

Arabidopsis thaliana AtMYB44

AtMYB44-overexpression



Jung et al. (2008) *Plant Physiol.* 146: 623-635

대한민국 특허등록 10-0781059 (2007)

대한민국 특허등록 10-0792169 (2007)

Drought (7 days)

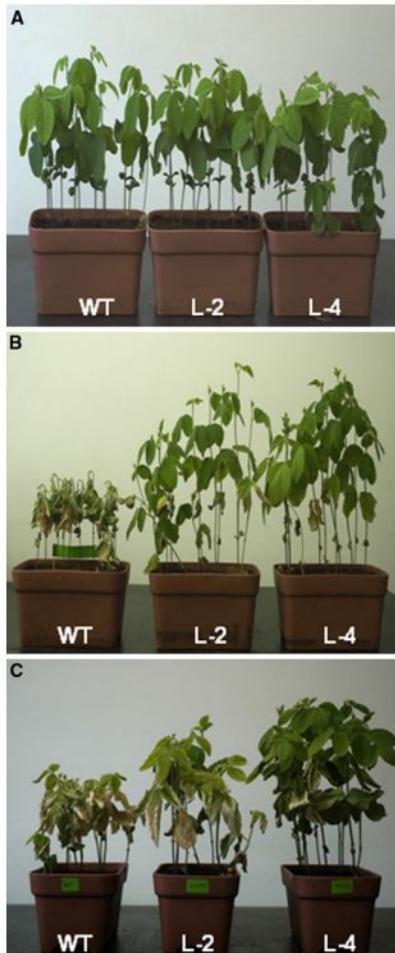
WT (Col-0) T-17 T-18 T-21 knockout



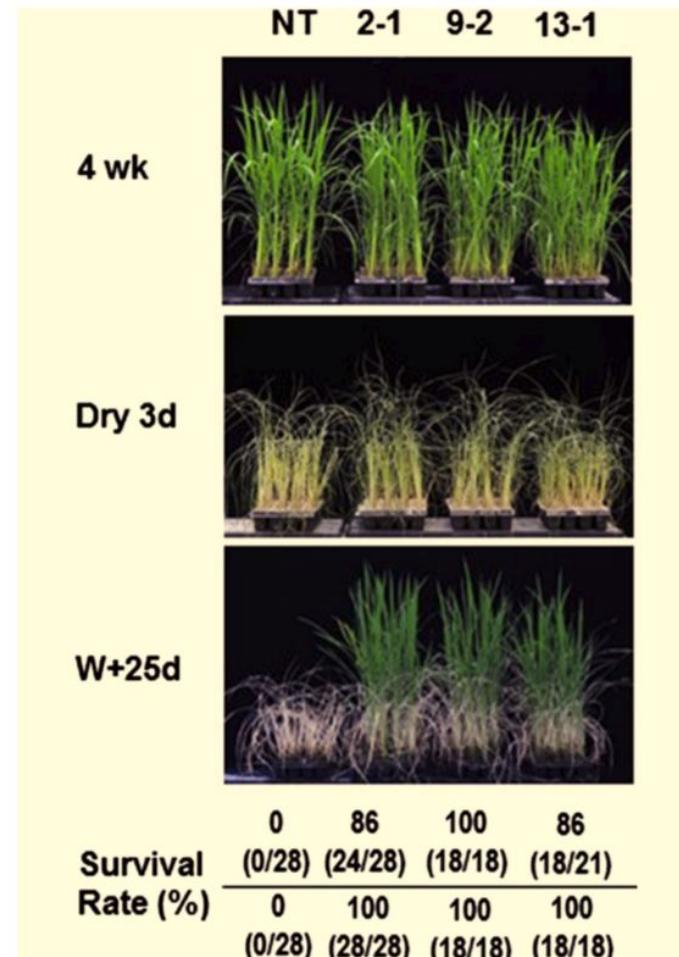
Salt (200 mM NaCl)

Overexpression of *AtMYB44*

Overexpression of *AtMYB44* confers abiotic stress tolerance in soybean and rice



Seo et al. (2012)



Joo et al. (2017)

Expression Microarray

PP2Cs

35S::AtMYB44 (T-21) vs. NT (Fold^a)
250 mM NaCl.
Affymetrix GeneChip® ATH1

| AGI No. ^b | Description | No treatment | | Salt treatment | |
|----------------------|-----------------------------------------|--------------|--------|----------------|--------|
| | | Expt 1 | Expt 2 | Expt 1 | Expt 2 |
| At4g26080 | <i>Protein phosphatase 2C (ABI1)</i> | NC | -2.0 | -1.4 | -3.2 |
| At5g57050 | <i>Protein phosphatase 2C (ABI2)</i> | NC | -1.6 | -2.0 | -6.9 |
| At3g11410 | <i>Protein phosphatase 2C (AtPP2CA)</i> | -2.0 | -1.6 | -1.2 | -6.9 |
| At1g72770 | <i>Protein phosphatase 2C (HAB1)</i> | NC | -1.6 | -2.2 | -7.4 |
| At1g17550 | <i>Protein phosphatase 2C (HAB2)</i> | NC | -1.4 | -1.5 | -1.8 |
| At5g59220 | <i>Protein phosphatase 2C</i> | -2.1 | -4.0 | -3.7 | -19.6 |
| At1g07430 | <i>Protein phosphatase 2C</i> | -1.8 | -4.5 | -3.0 | -29.8 |

^a Relative gene transcript level compared with the same gene in wild-type plants.

^b Arabidopsis Genome Initiative number.

^c NC : No change.

Main goal

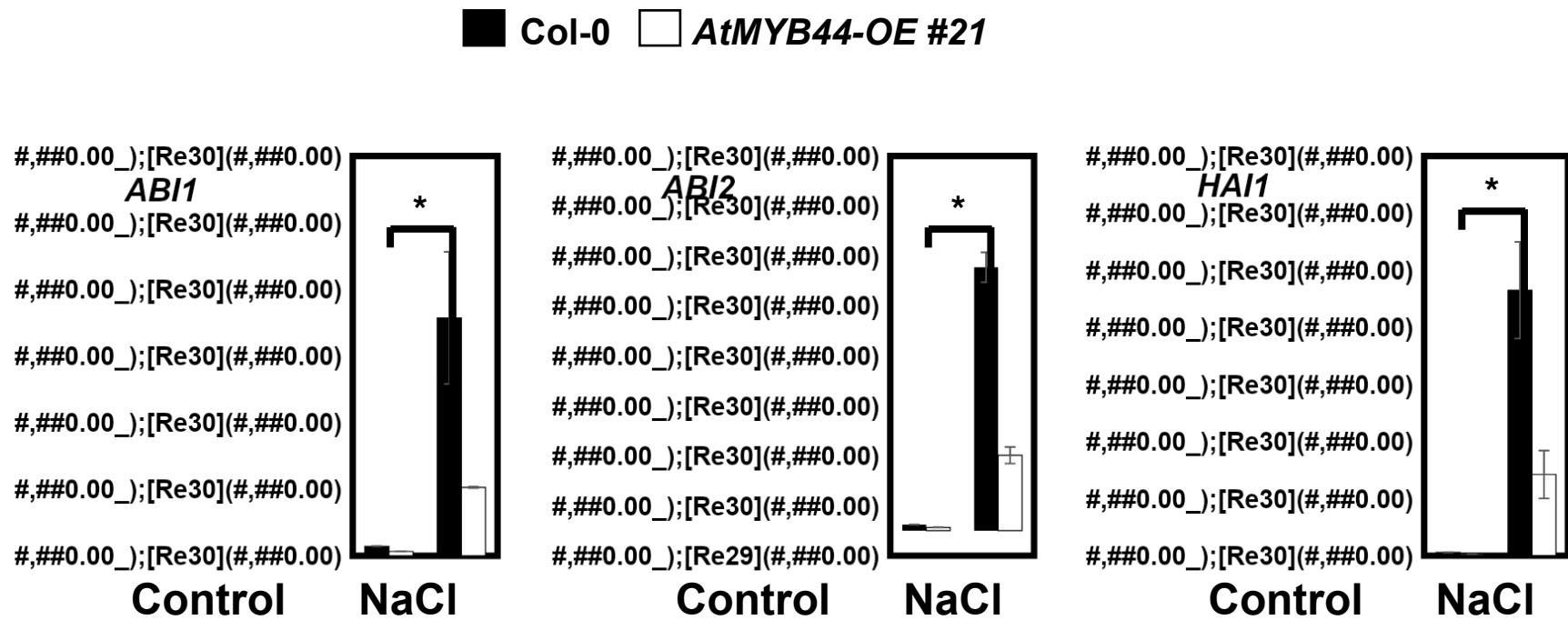


How does *AtMYB44* repress its downstream genes (*PP2Cs*)?

AtMYB44 and *PP2Cs* transcription

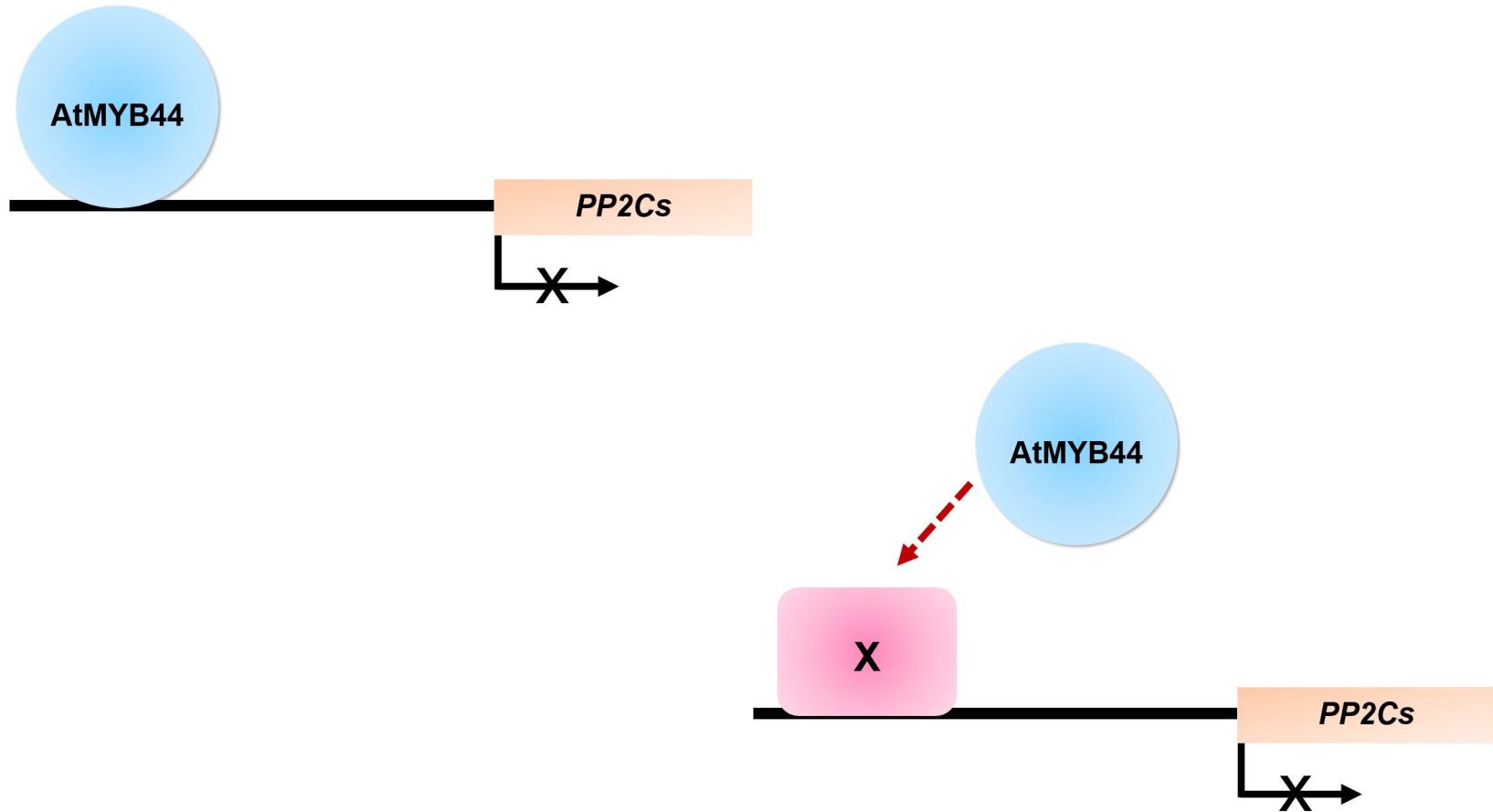
qRT-PCR

Relative transcript level



□ AtMYB44 represses *PP2Cs* transcript levels

AtMYB44 and *PP2Cs* transcription



35S::AtMYB44:GFP

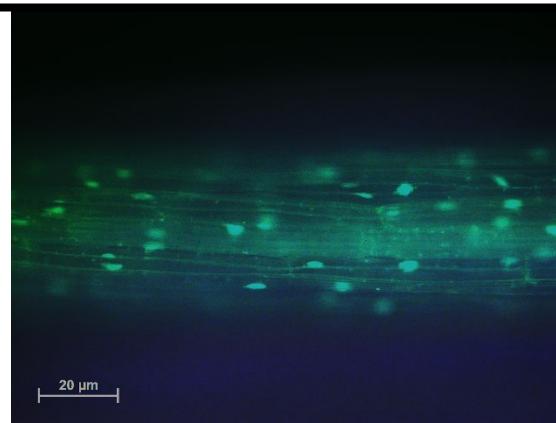
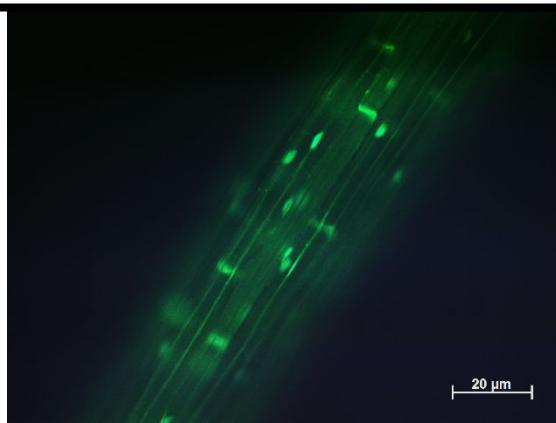
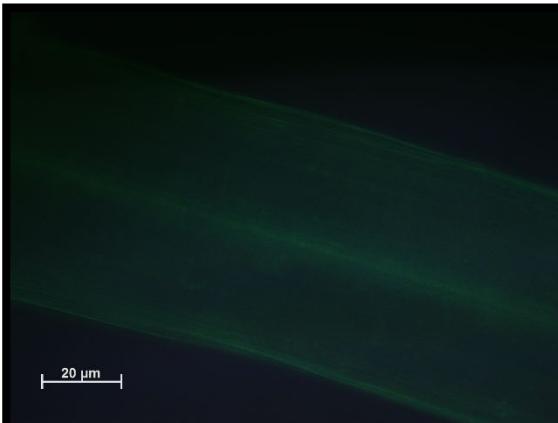
Col-0

AtMYB44:GFP

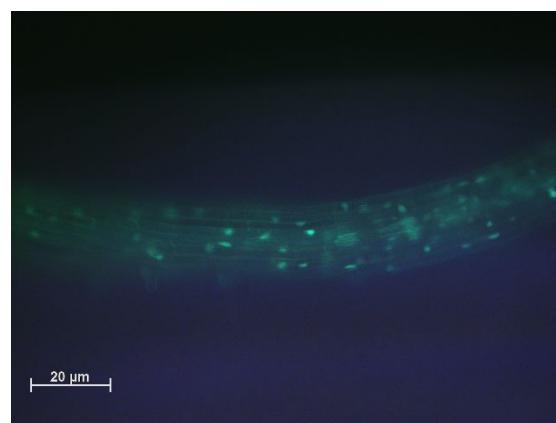
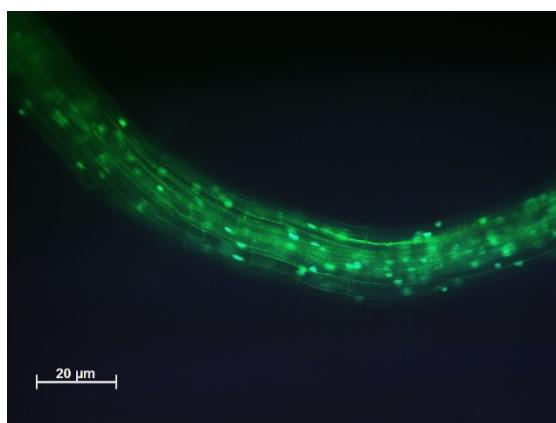
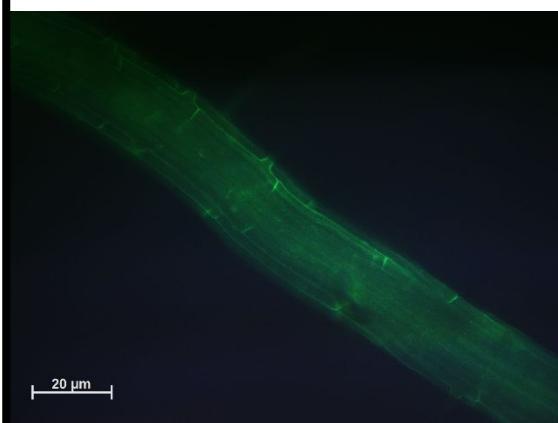
#2-5

#28-3

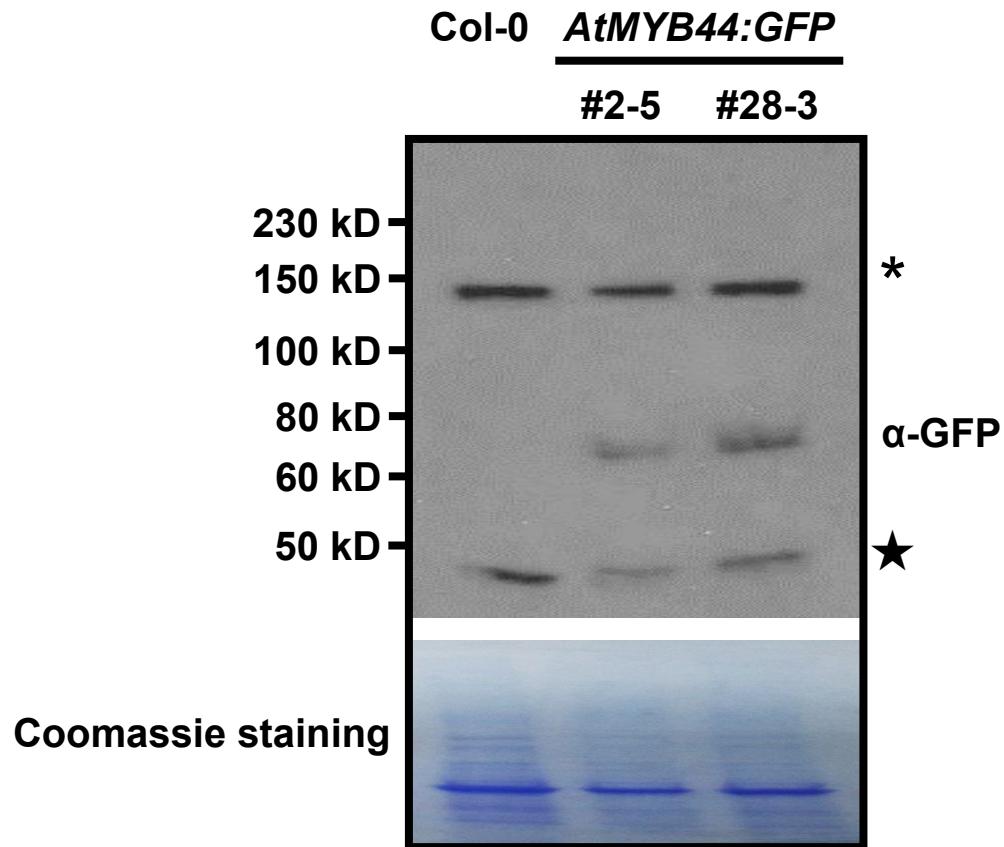
Hypocotyl



Root

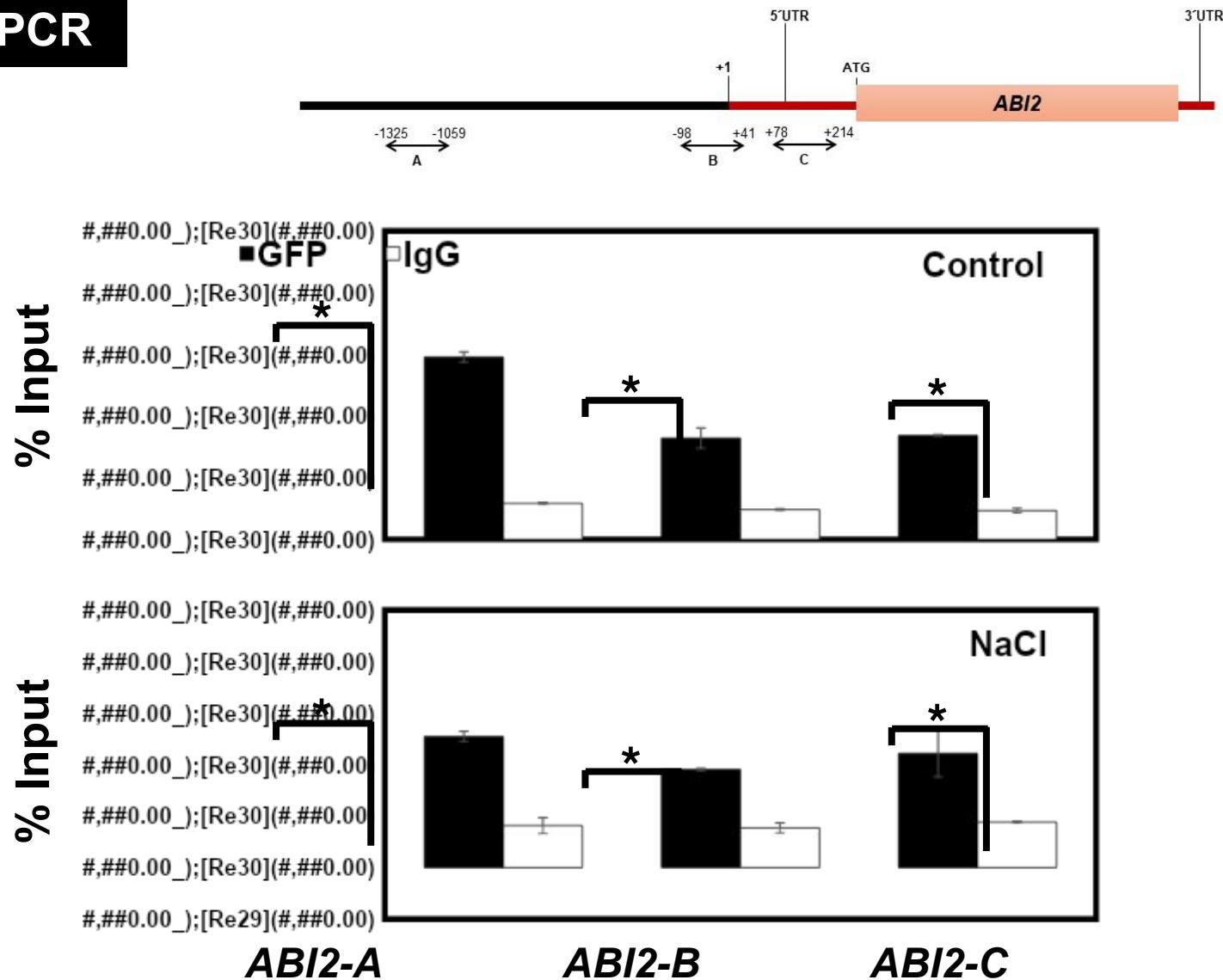


35S::ATMYB44:GFP

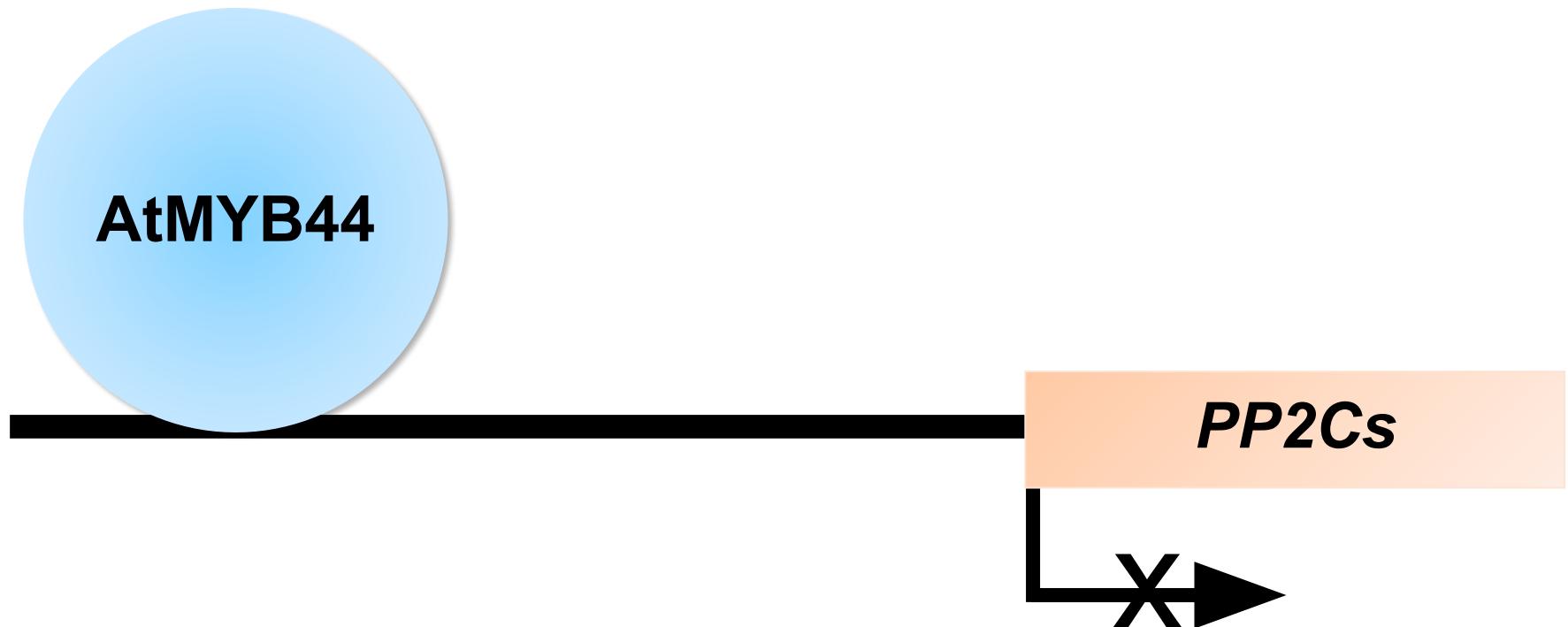


AtMYB44 and *PP2Cs* transcription

ChIP-qPCR



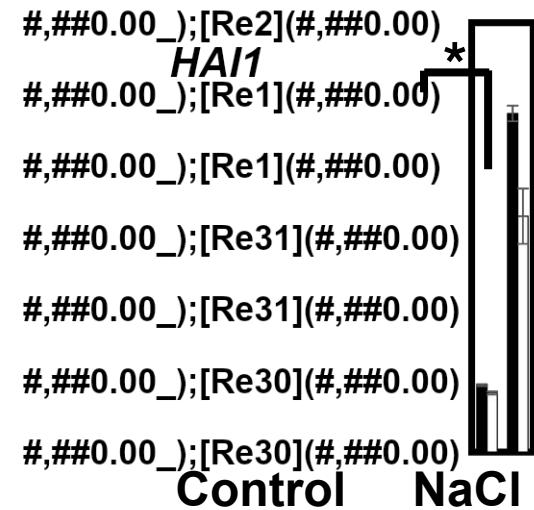
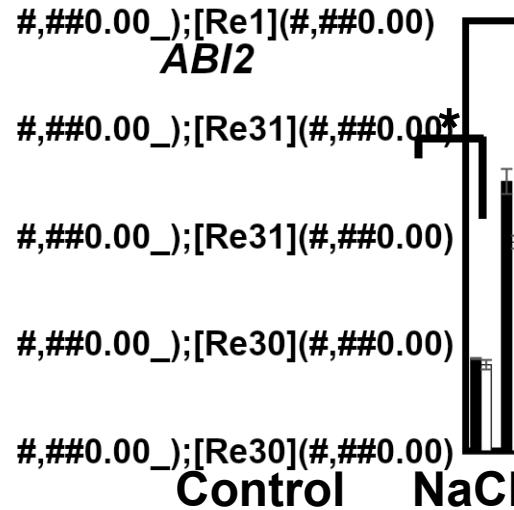
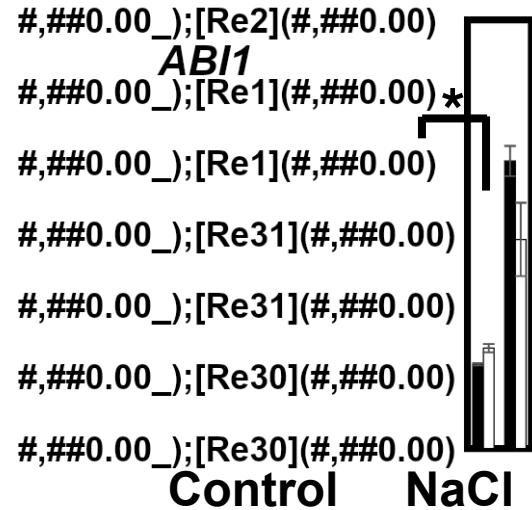
AtMYB44 and *PP2Cs* transcription



AtMYB44 and *PP2Cs* transcription

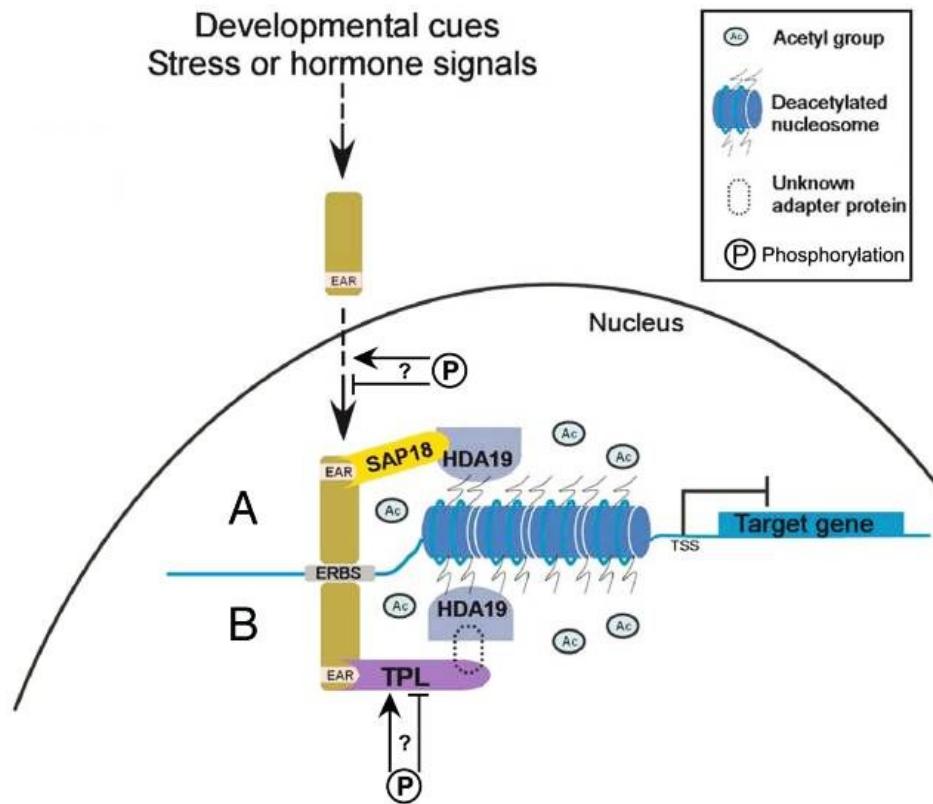
ChIP-qPCR with anti-RNA polymerase II (RNAPII) antibody

Relative RNAPII level

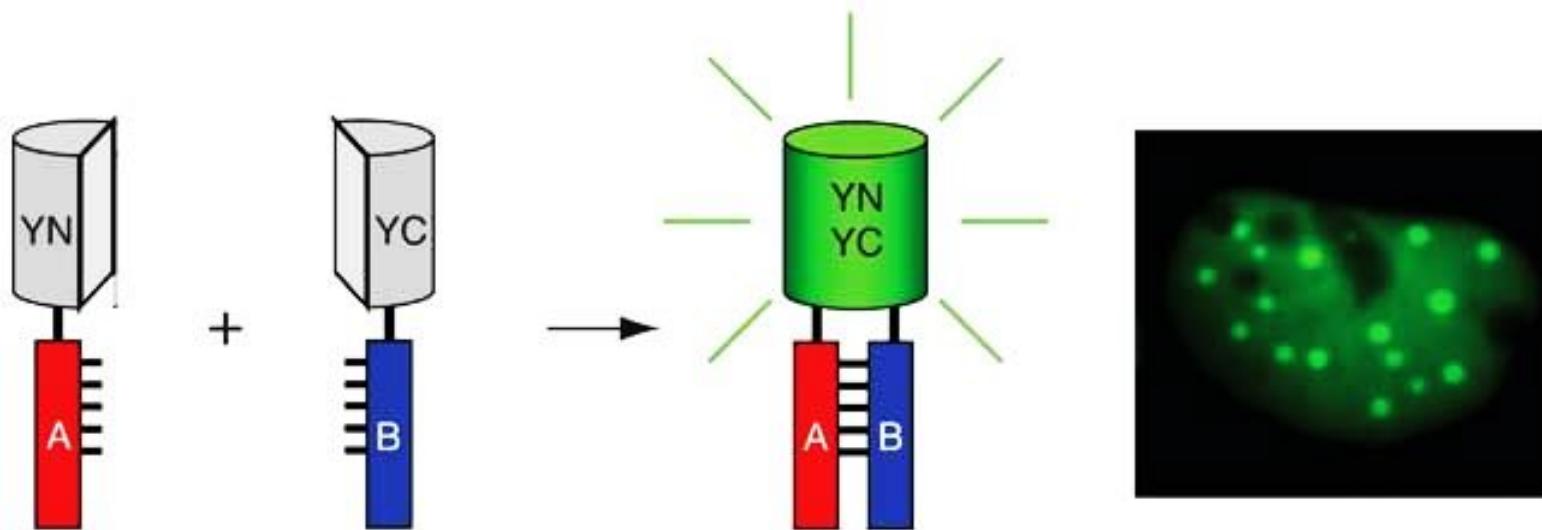


□ AtMYB44 directly represses the *PP2Cs* transcription

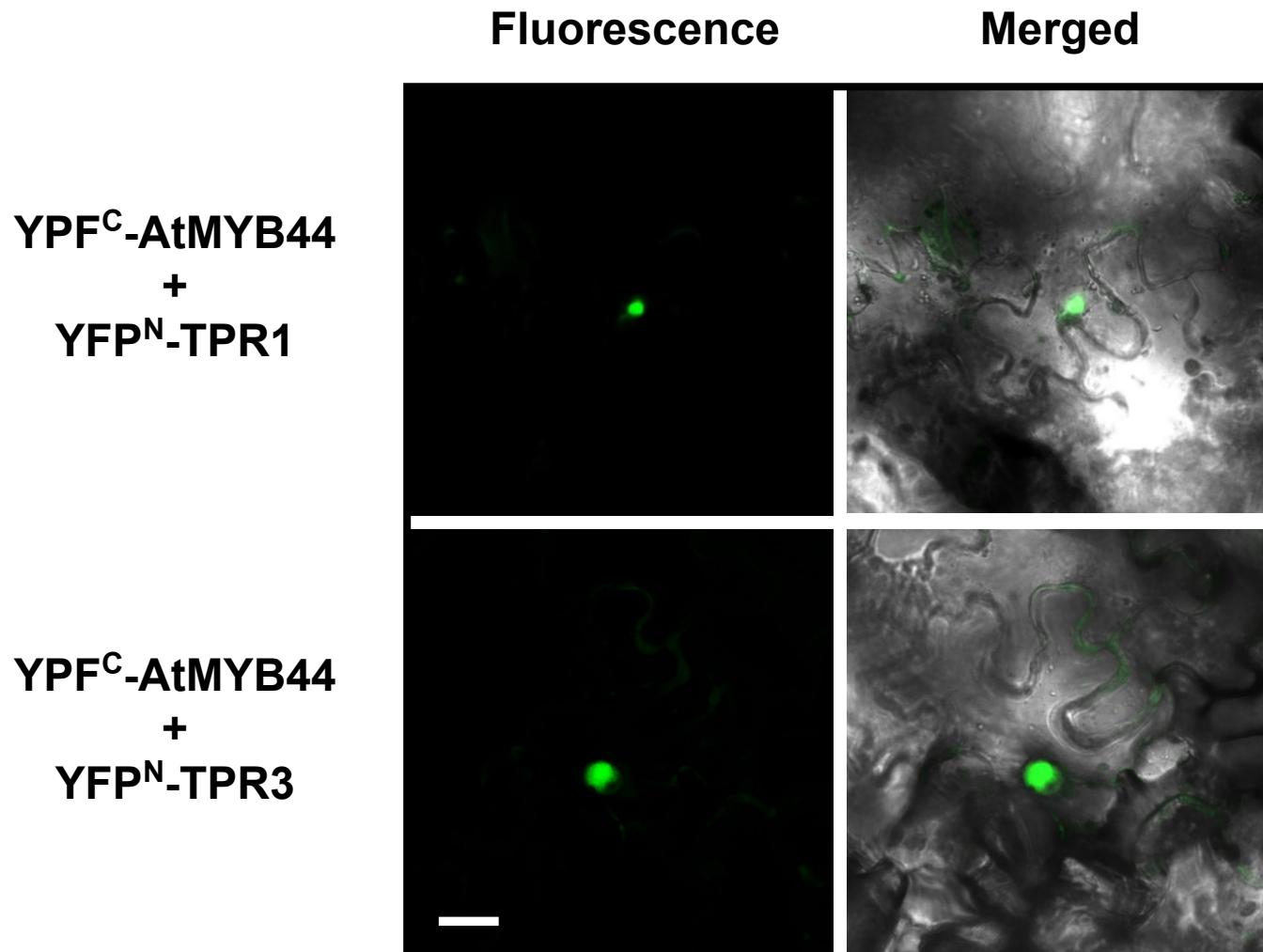
A repressor suppresses its downstream genes in association with histone modifications



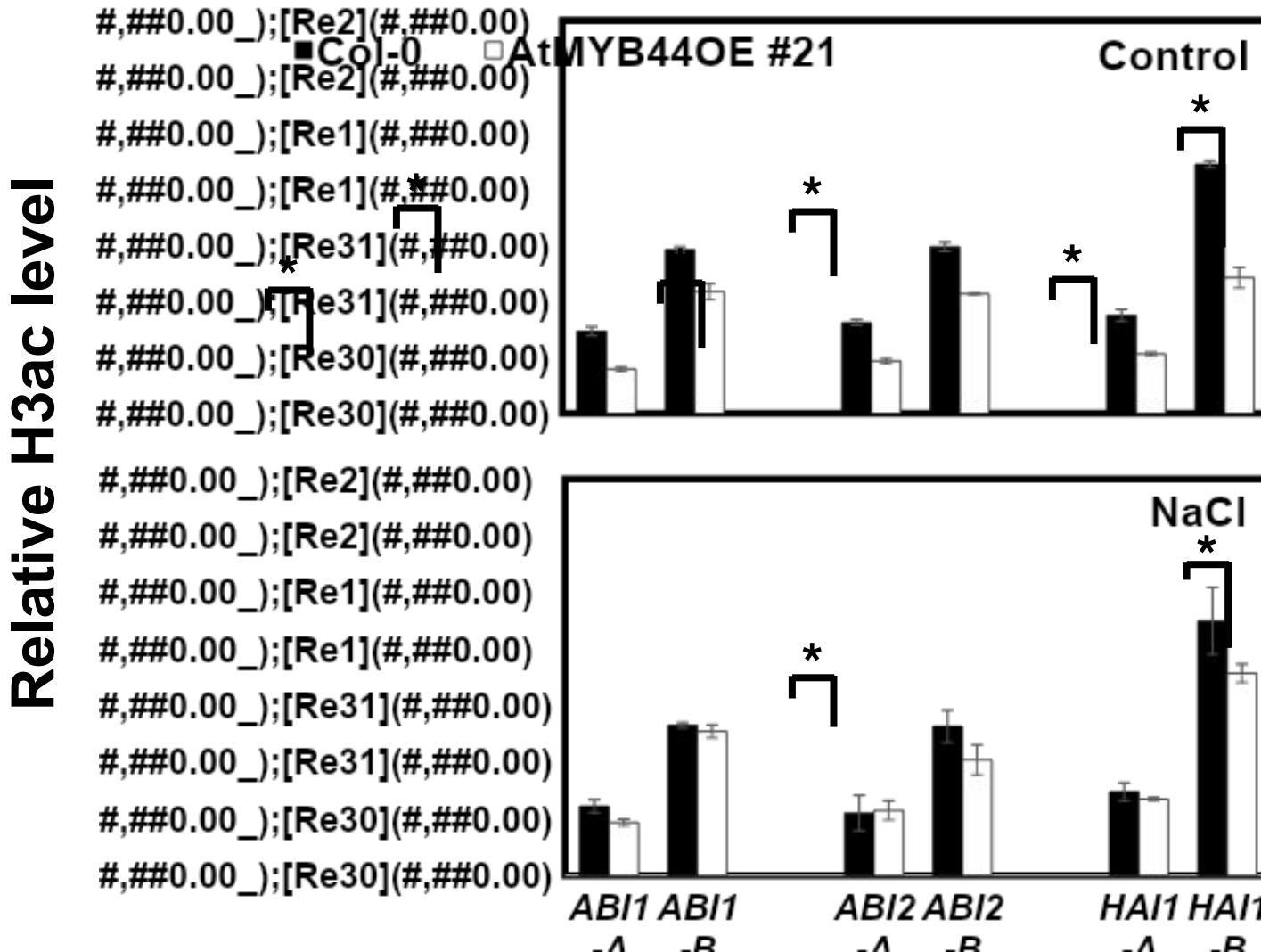
Bimolecular fluorescence complementation (BiFC)



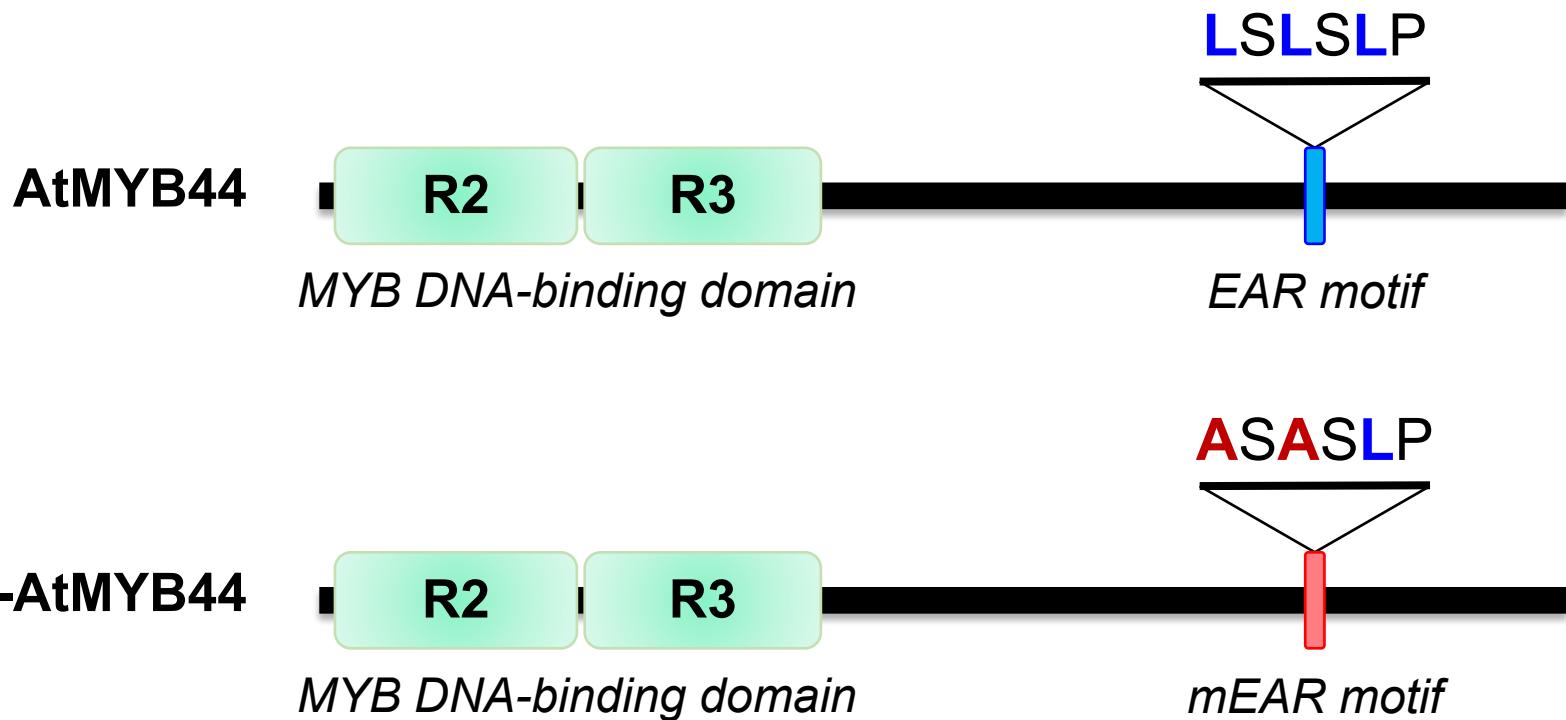
AtMYB44 interacts with co-repressors (TPR1/3)



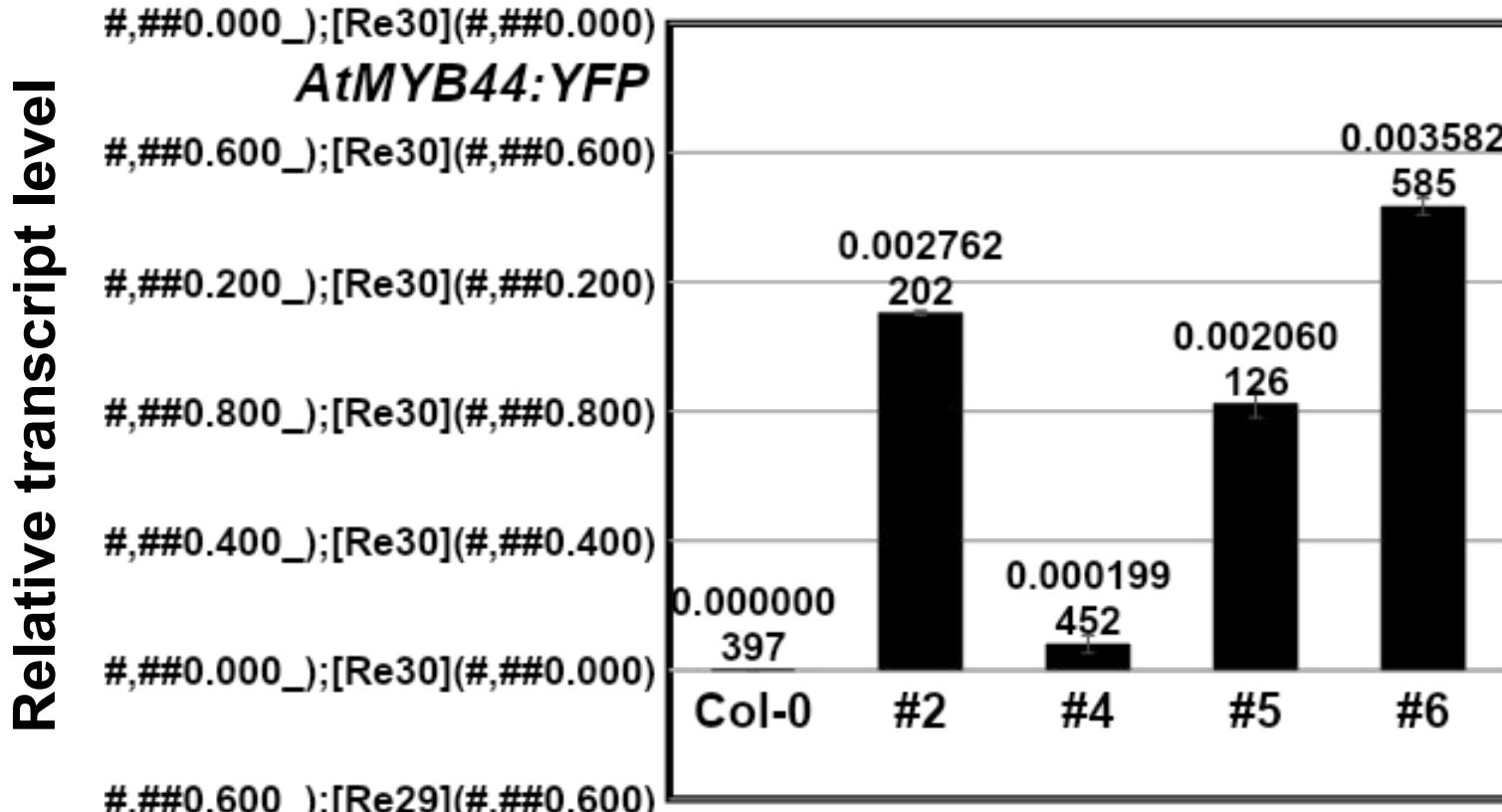
AtMYB44 and *PP2Cs* transcription



EAR motif mutation of AtMYB44



EAR motif mutation of AtMYB44



EAR motif mutation of AtMYB44

Col-0

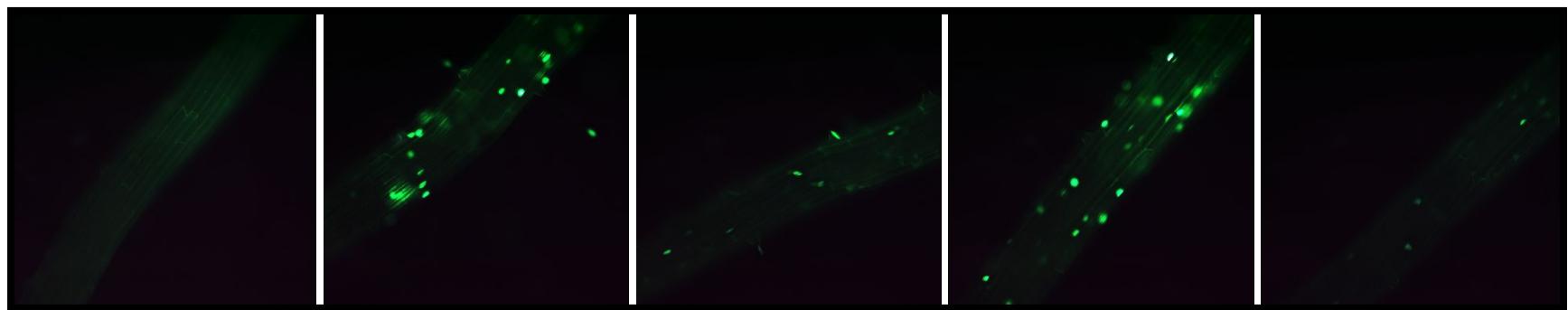
AtMYB44-mEAR:YFP

#2

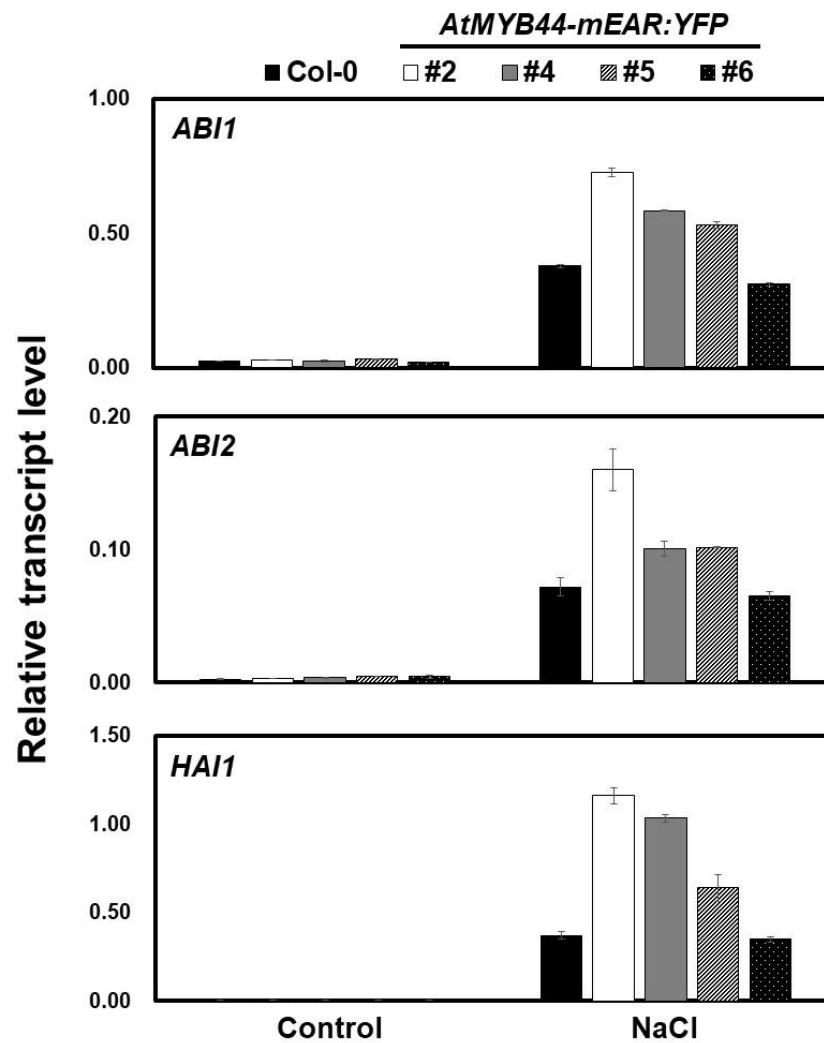
#4

#5

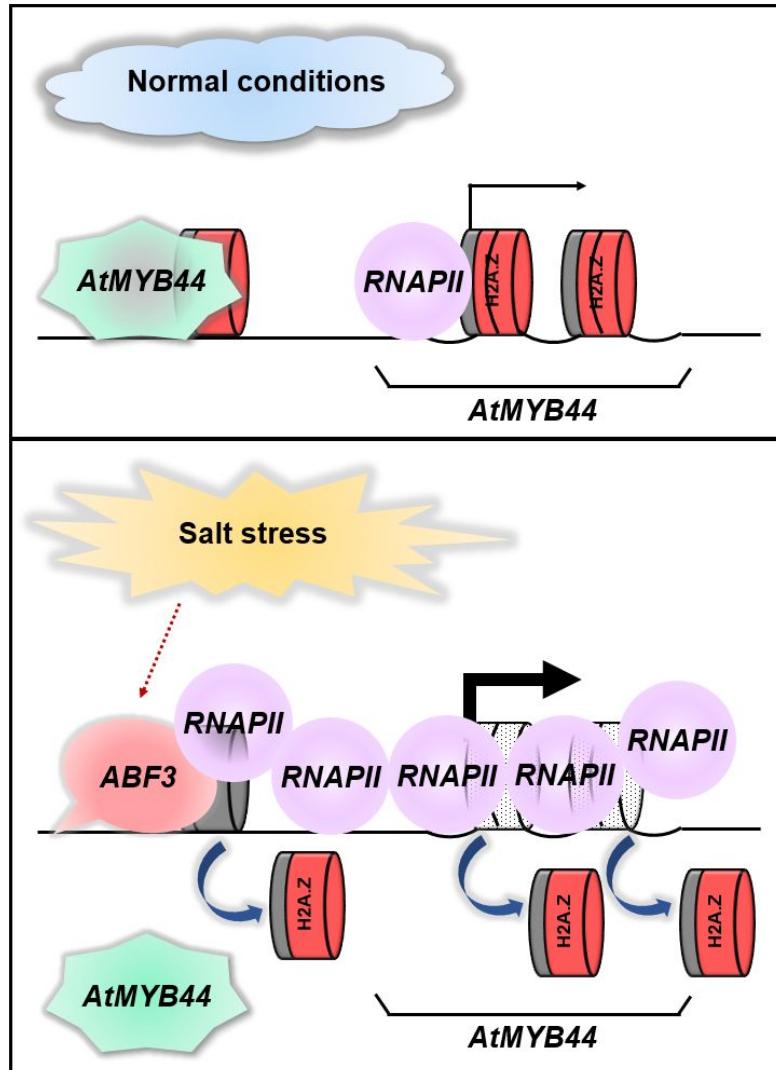
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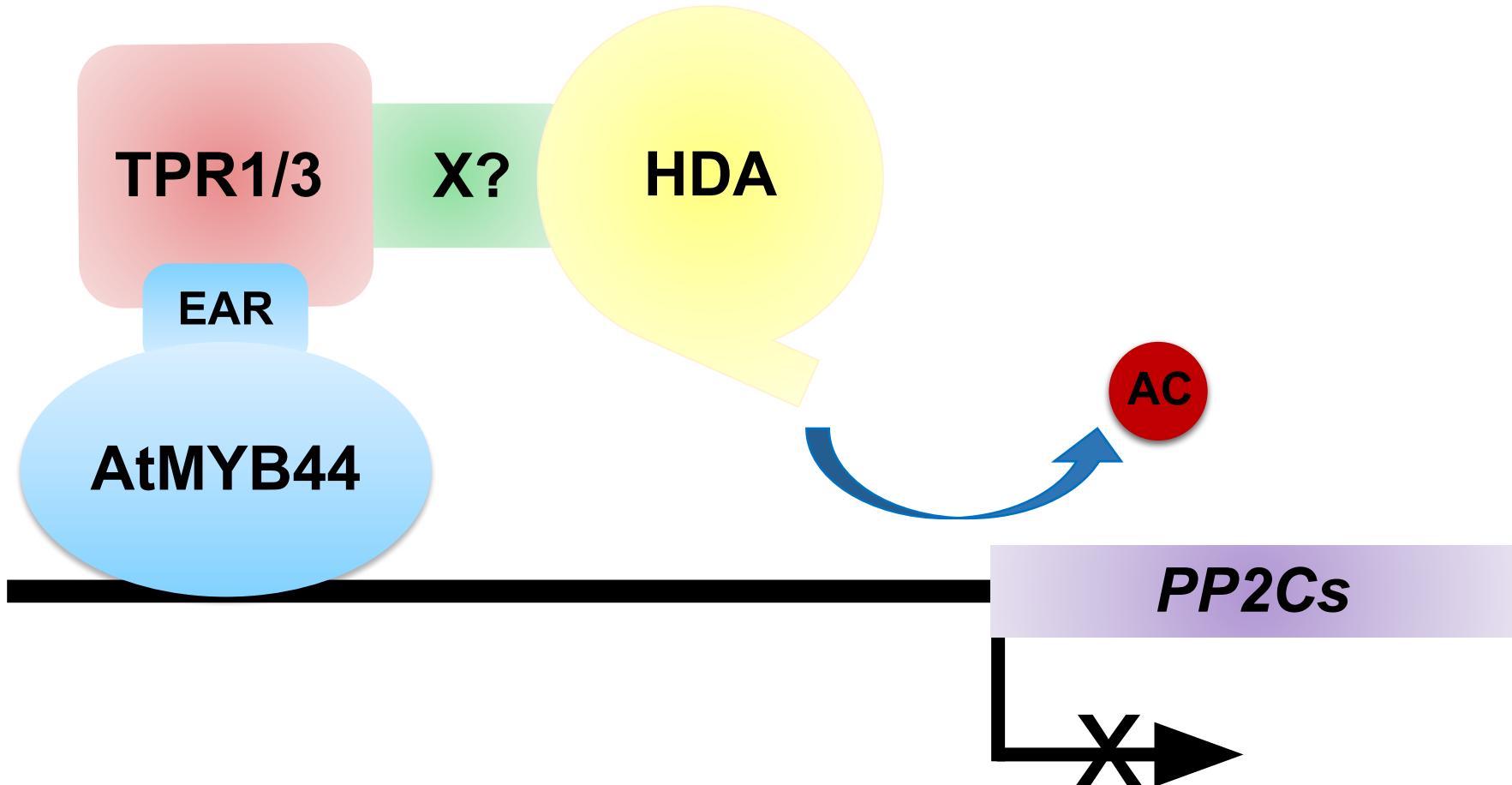
EAR motif mutation of AtMYB44



Conclusions



Conclusions



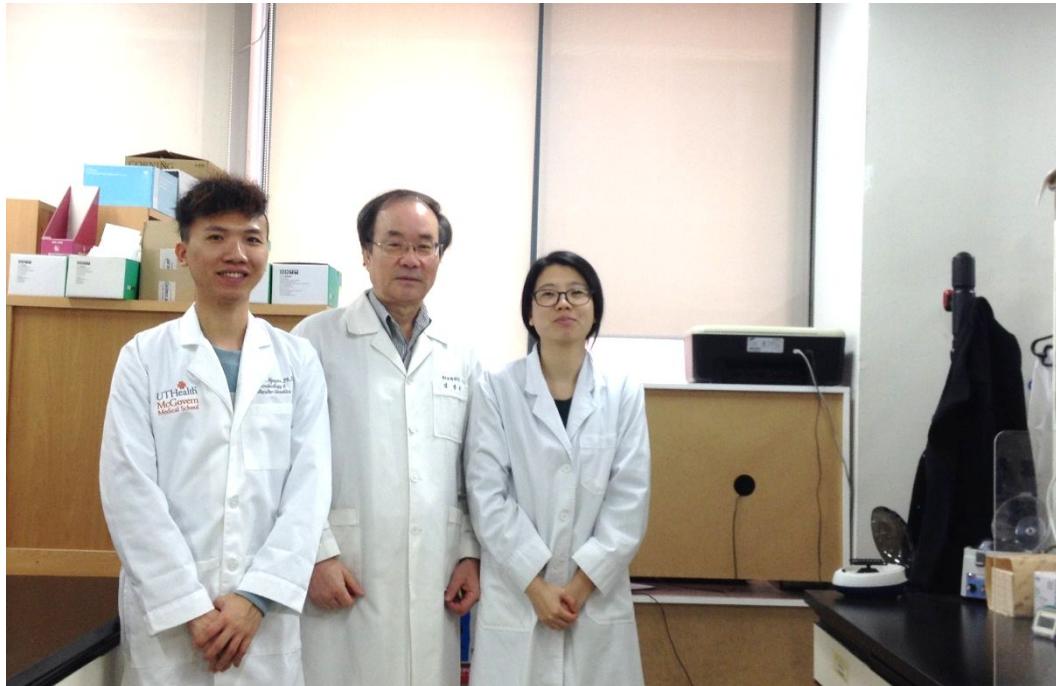
Acknowledgements



Center for Food and Bioconvergence
Seoul National University



National Research
Foundation of Korea



A close-up photograph of a chocolate cake. The cake has a dense, dark brown crumb. It is covered in a thick layer of white frosting, which is decorated with intricate, wavy patterns made from melted chocolate. Several fresh red roses are tucked into the top left corner of the cake, and a single large green leaf is visible in the foreground, partially obscuring the cake's base.

Thank You

Very Much!