

DNA methylation – does it matter?

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Feb. 15, 2024



goldy the gopher and thundar the bison from north dakota
state hanging out

Introduction

Background

epigenetics/epigenomics, 3 contexts matter

A *Setaria* methylation mutant

What are we missing?

How dynamic is DNA methylation?



USDA-Agricultural Research Service Wheat and Oat Geneticist



Started with USDA-ARS in September 2023

My office and lab are on the UMN St. Paul campus
(Borlaug Hall)

Currently just a technician and myself - but looking to grow

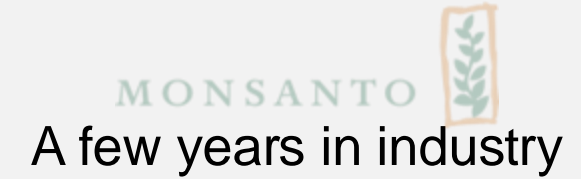
Something to think about:

USDA funded postdoc minimum \$64k/year

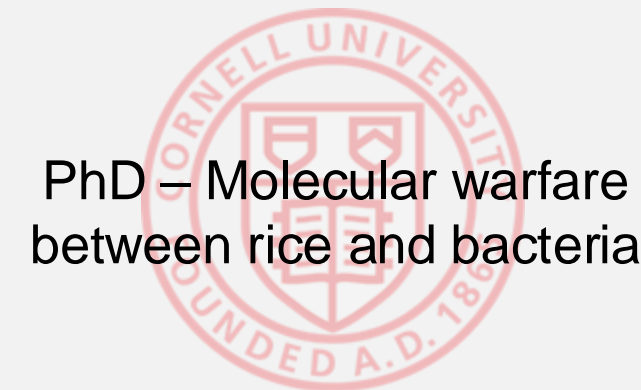
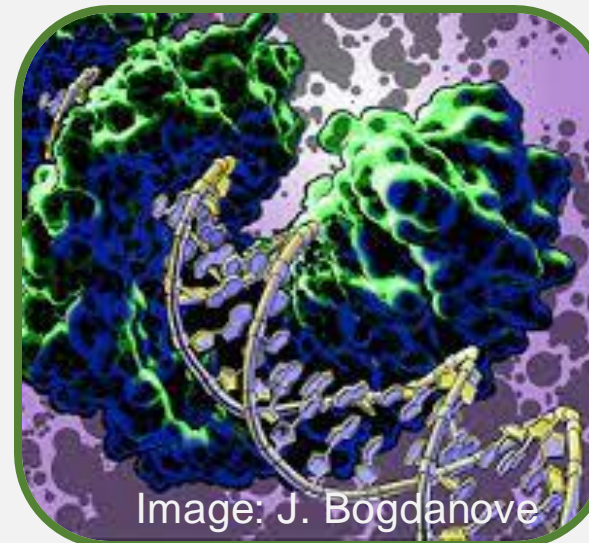
Not all postdocs in USDA labs are USDA funded



My scientific journey

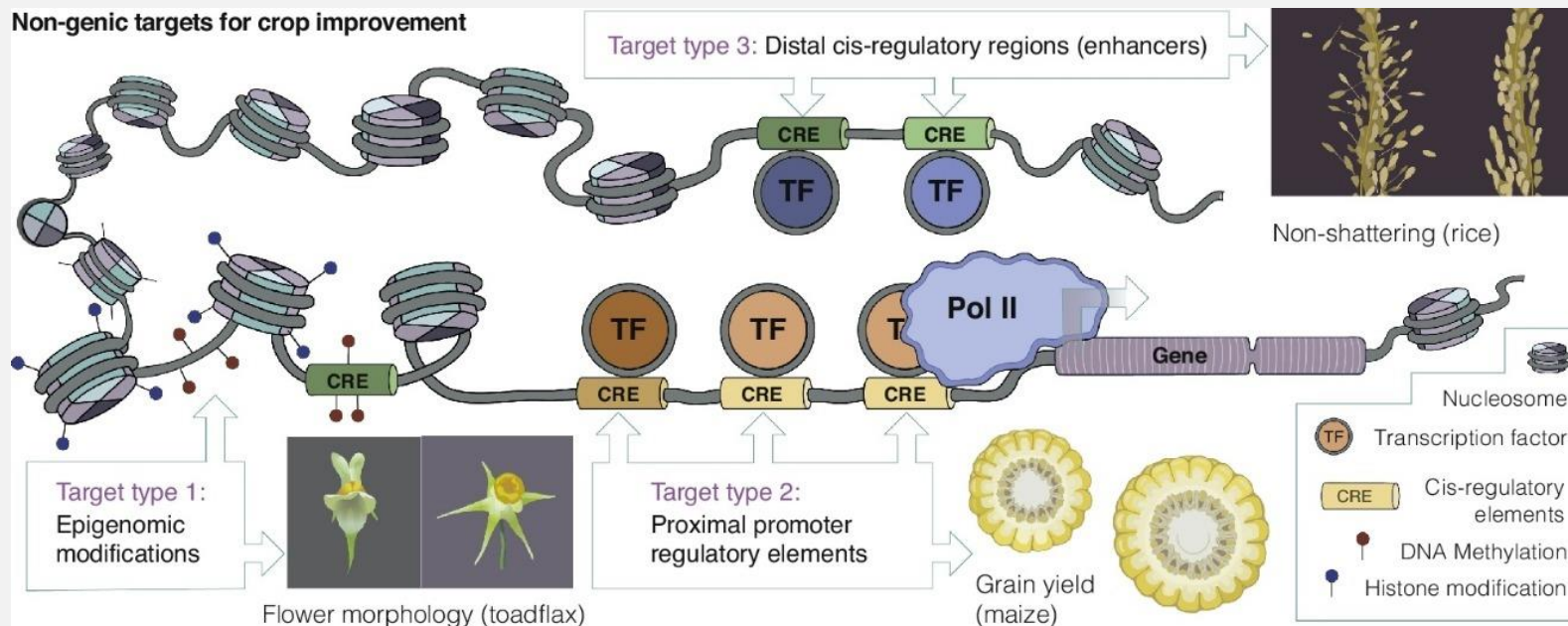


Maize and Setaria
(epi)genomes and
transposon biology



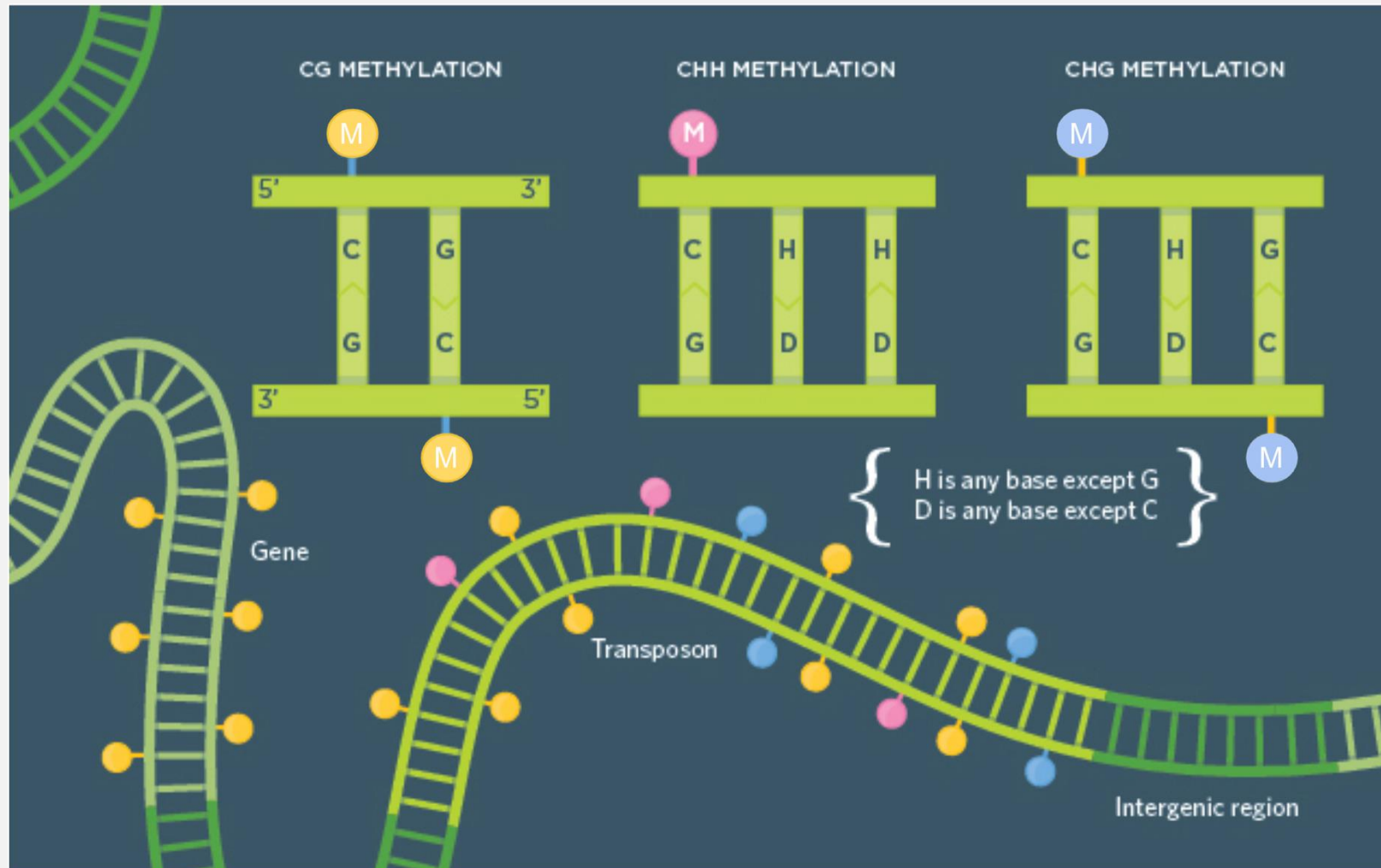
What is epigenetics?

Epigenetics: Genomic modification that alter gene expression that cannot be attributed to modification of the primary DNA sequence and that are **heritable** mitotically and meiotically (**DNA methylation and histone modifications**)



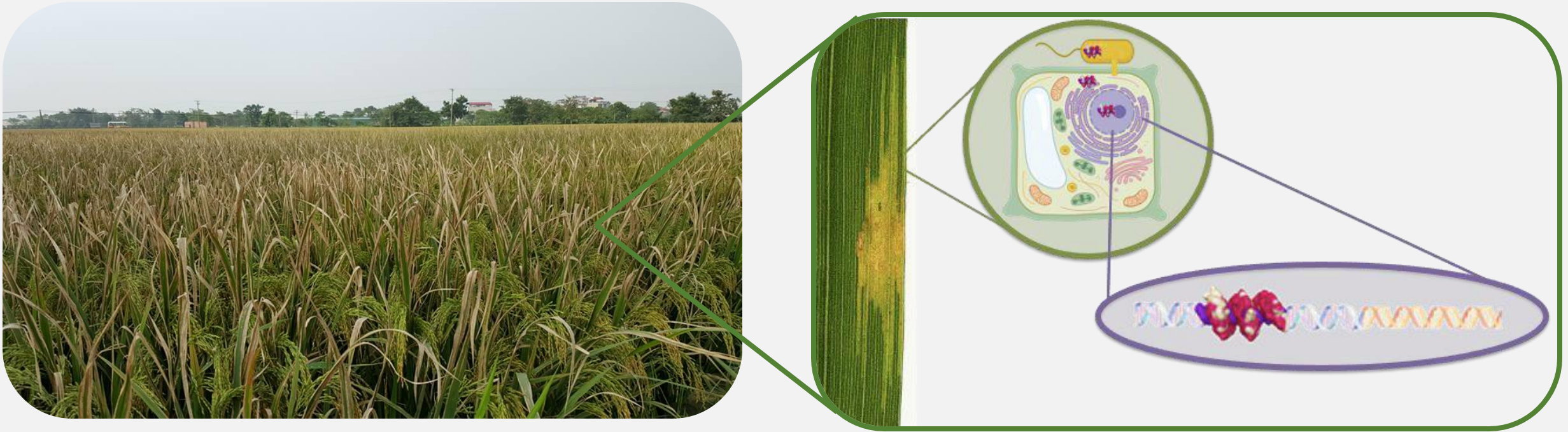
Crisp et al. (2021) Current Opinion in Biotech.

DNA methylation – context matters



Infographic: Plant Methylation Basics. Akst, Jef. The Scientist (2017)

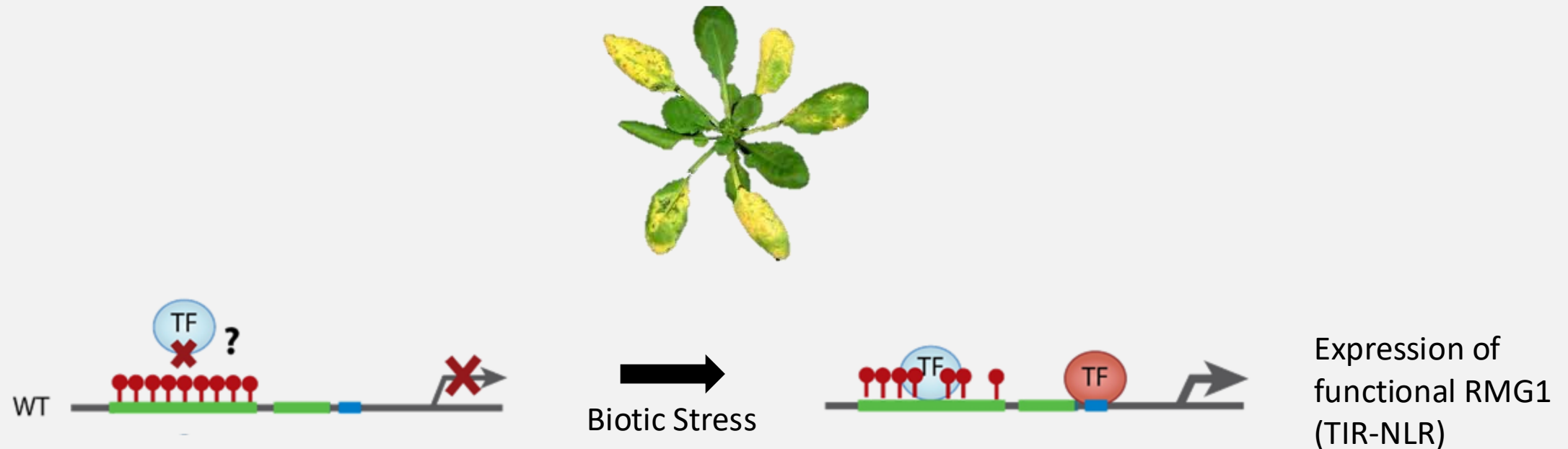
Why do I care?



Bacterial leaf streak of rice – major problem in Asia and emerging problem in West Africa

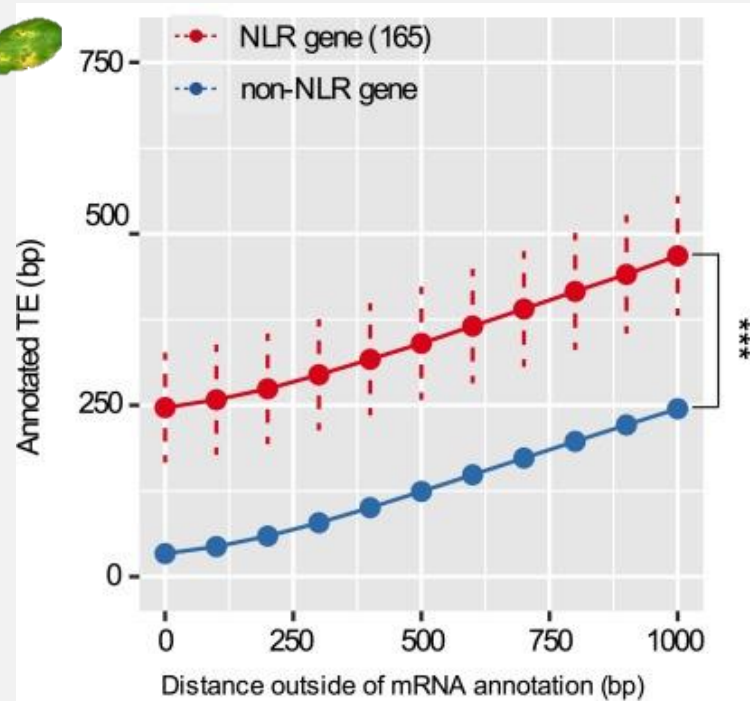
The plant immune system is diverse and complex – must be **highly regulated** to balance robust response to pathogens and avoid auto-immunity

How is DNA methylation related to gene expression?



Deleris et al. (2016) Annual Rev.

Transposable elements and plant immunity genes (NLRs)



Lai et al. (2020) PLoS Genet.

PLANT IMMUNITY (NLRS)



DNA METHYLATION

TRANSPOSONS

PLANT IMMUNITY (NLRS)



Perturb

DNA METHYLATION

Observe

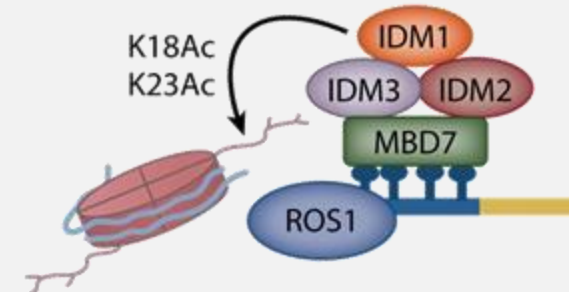
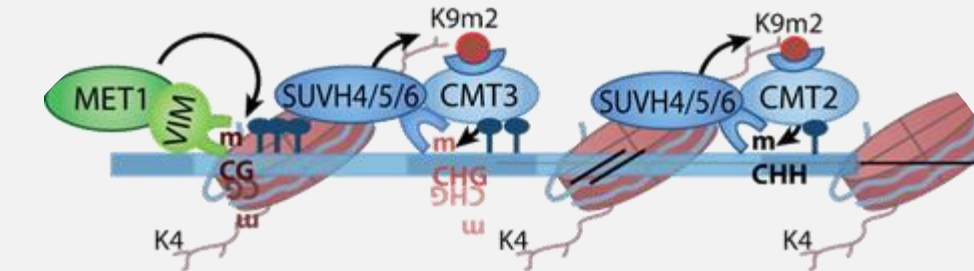
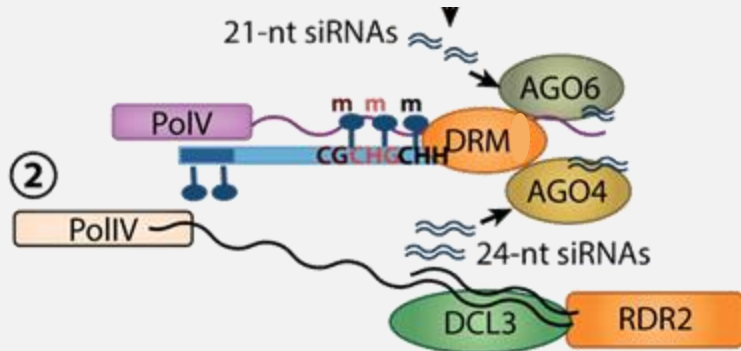
TRANSPOSONS

Setaria viridis – a model monocot



- Small C4 grass
- Short life-cycle (8-10 weeks)
- Small genome ~400 Mb
- Transformable
A DNA methylation pathway mutant library is being built

A *Setaria viridis* methylation pathway mutant library



DRM

De novo methylation
mCHH maintenance

MET1

mCG maintenance

CMT3

mCHG maintenance

CMT2

mCHH maintenance
deep in transposons

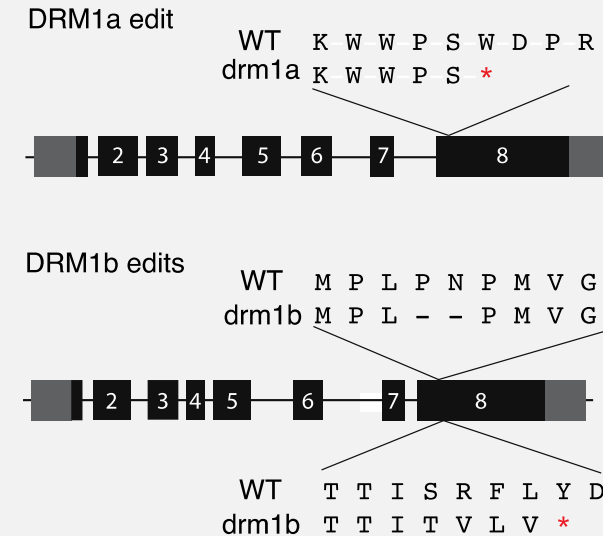
ROS1

Active Demethylation

The first methylation pathway mutant - Δdrm1ab



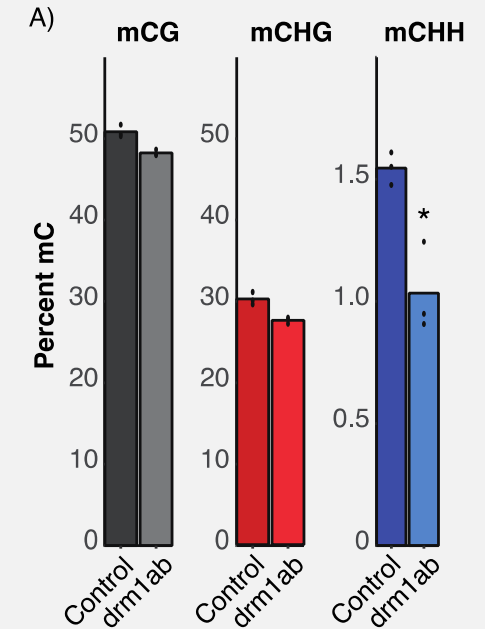
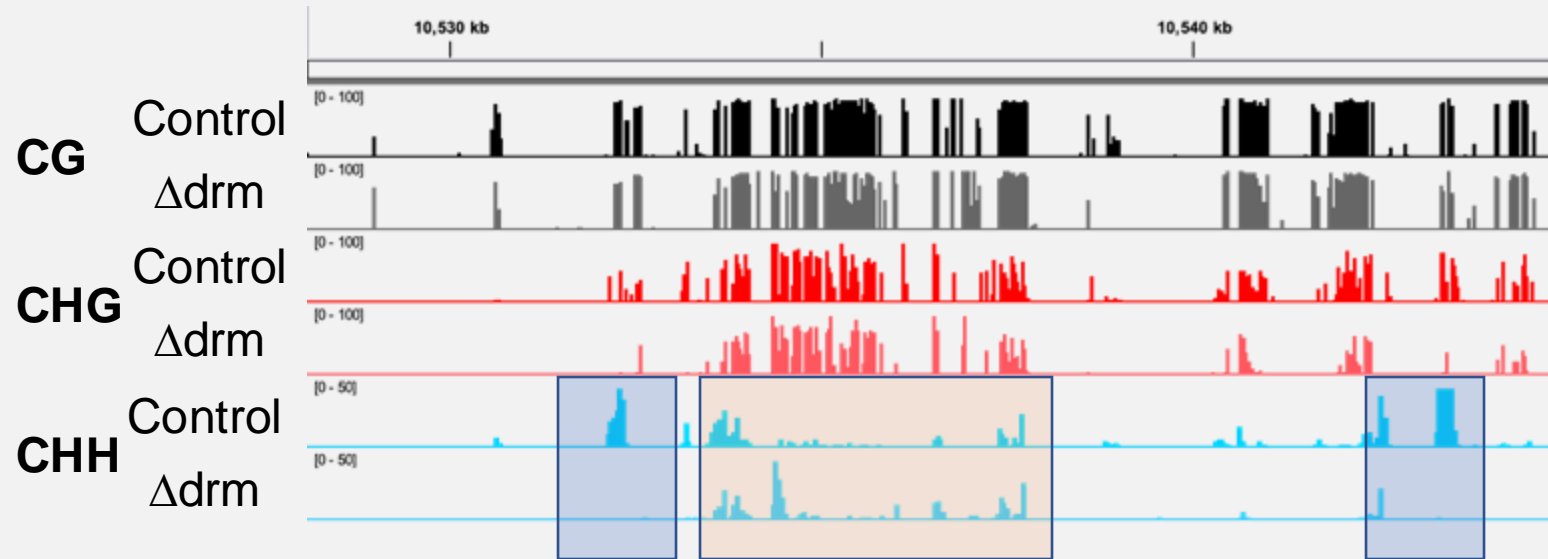
De novo methylation
mCHH maintenance



CRISPR/Cas generated pre-mature stop codons – prior to motif required for activity

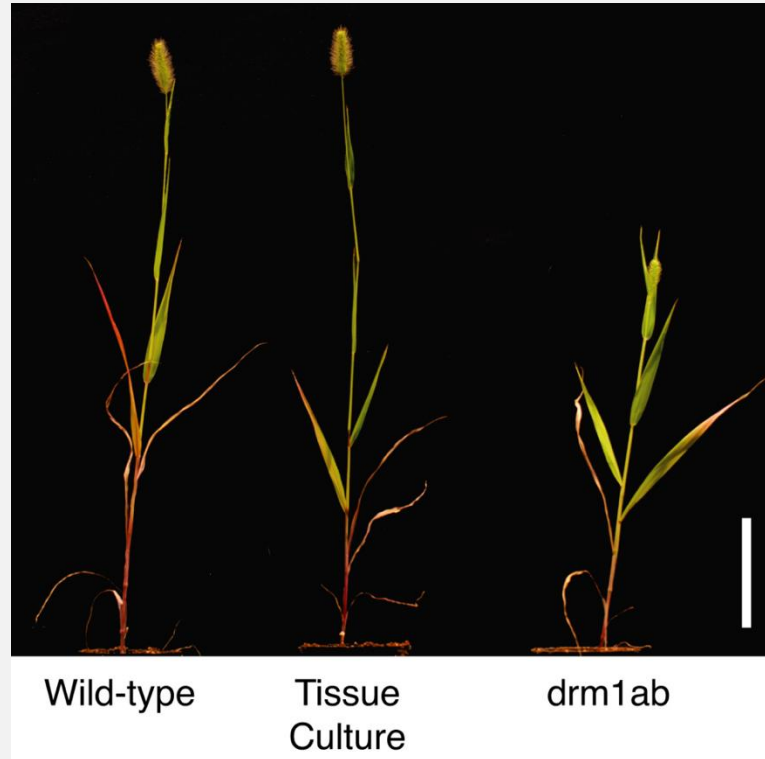
What do we expect to observe when we look at DNA
methylation and Transposons?

Methylation changes in the Δdrm mutant



- Globally, most CHH methylation was lost
- Some sites also lost CG and CHG methylation
- Gene expression changes were limited and not predicted by mCHH changes
- Expression of some transposons was up-regulated in the mutant

No major growth defects in Δdrm in standard growth conditions

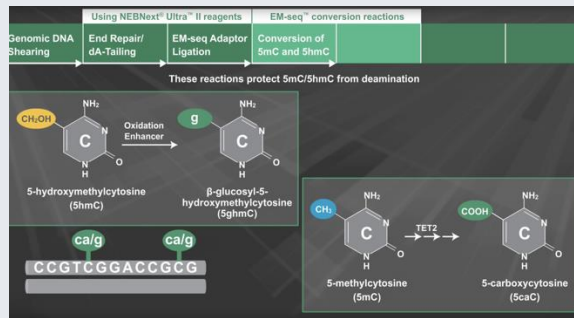


What will happen under stress?

Measuring DNA methylation – What are we missing?

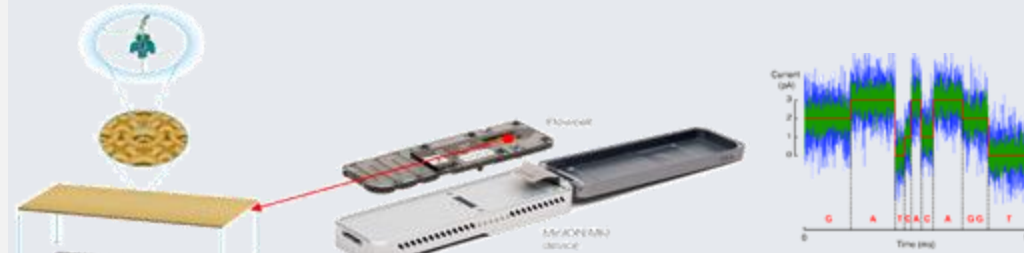


Existing methods:



- Whole tissue homogenate
 - Signal is an average of cell types
- chemical/enzymatic conversion
- Short read sequencing
 - Challenging over repetitive areas (TEs)

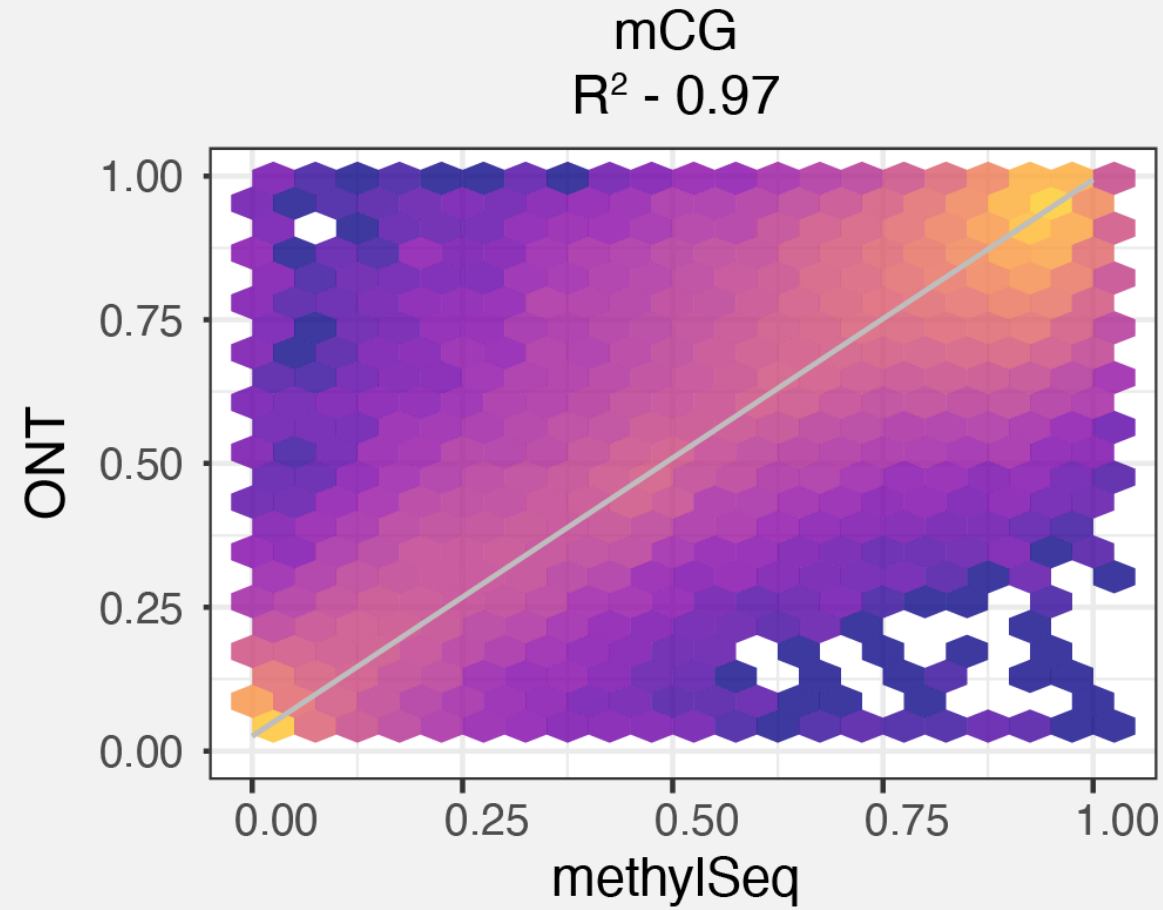
Applying new technologies:



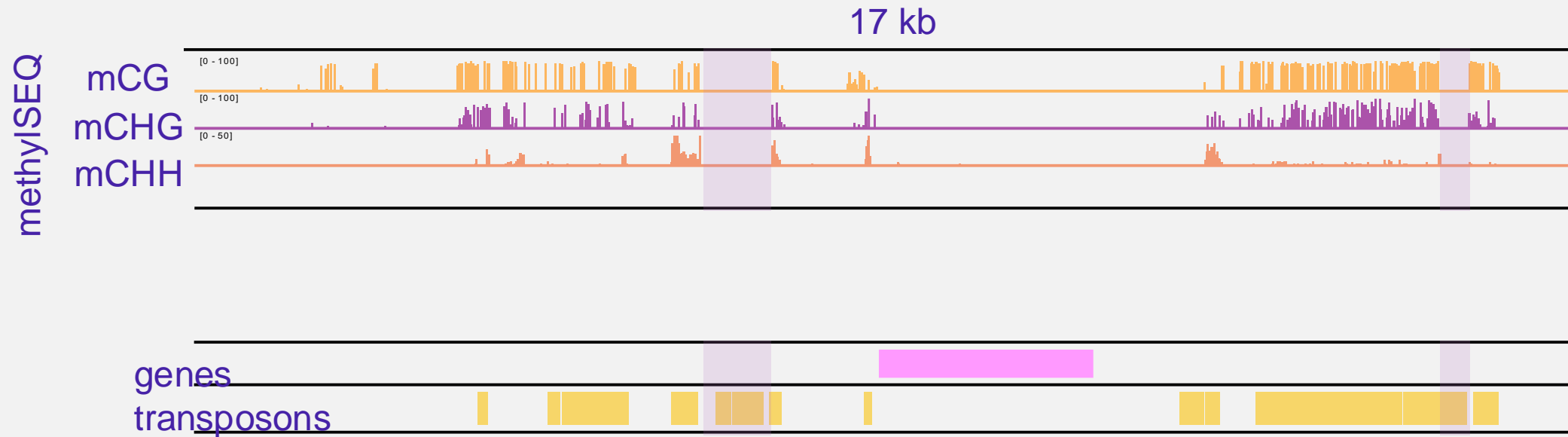
- Nanopore sequencing
 - No conversion required
 - Long reads map to complex regions of the genome

I ♥
ONT

Calling DNA methylation with Nanopore

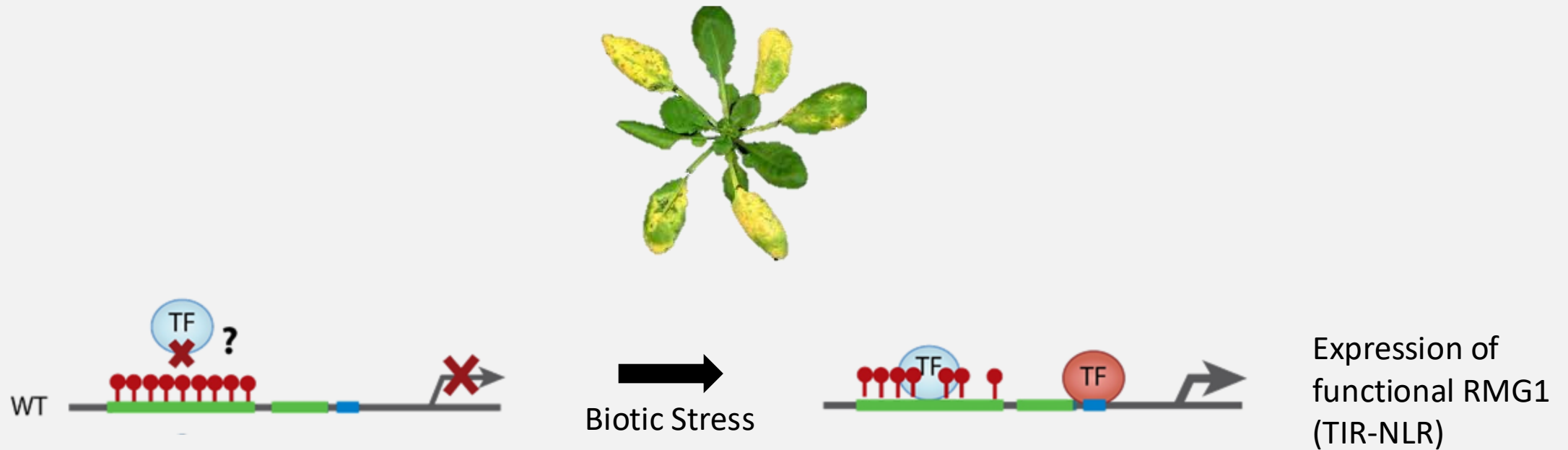


Calling DNA methylation with Nanopore



Nanopore seems like a promising tool to characterize DNA methylation in future experiments

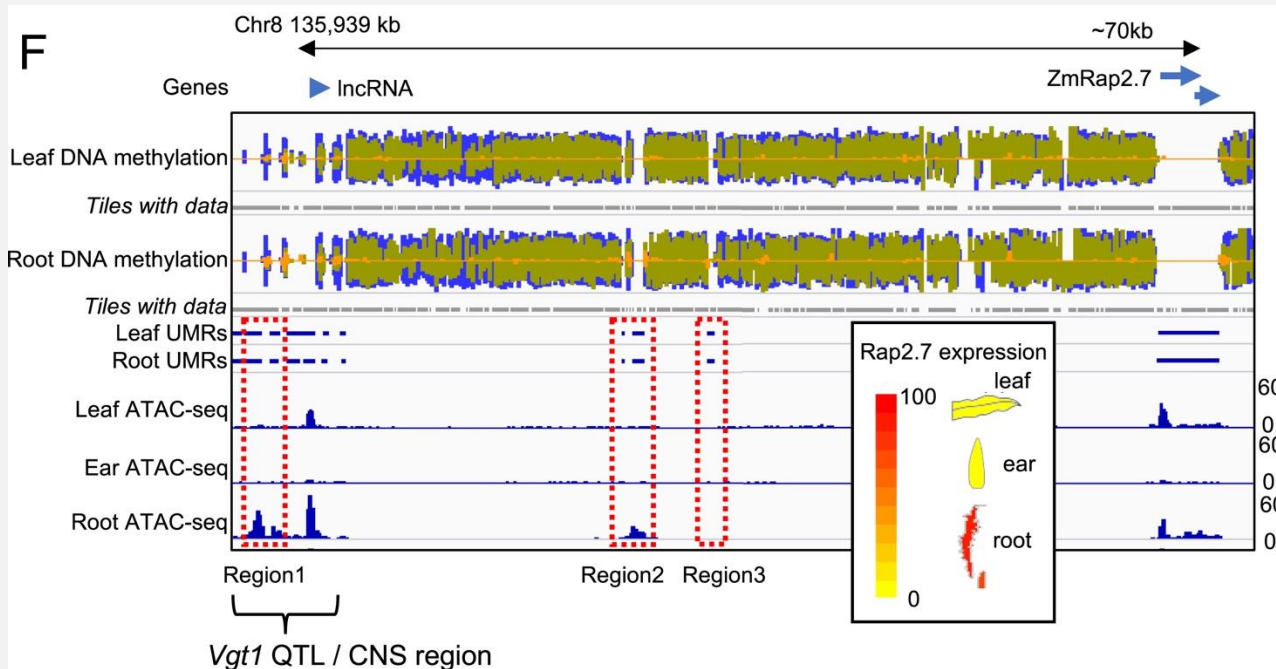
Is DNA methylation static or dynamic?



Deleris et al. (2016) Annual Rev.

Is DNA methylation static or dynamic?

Mostly static



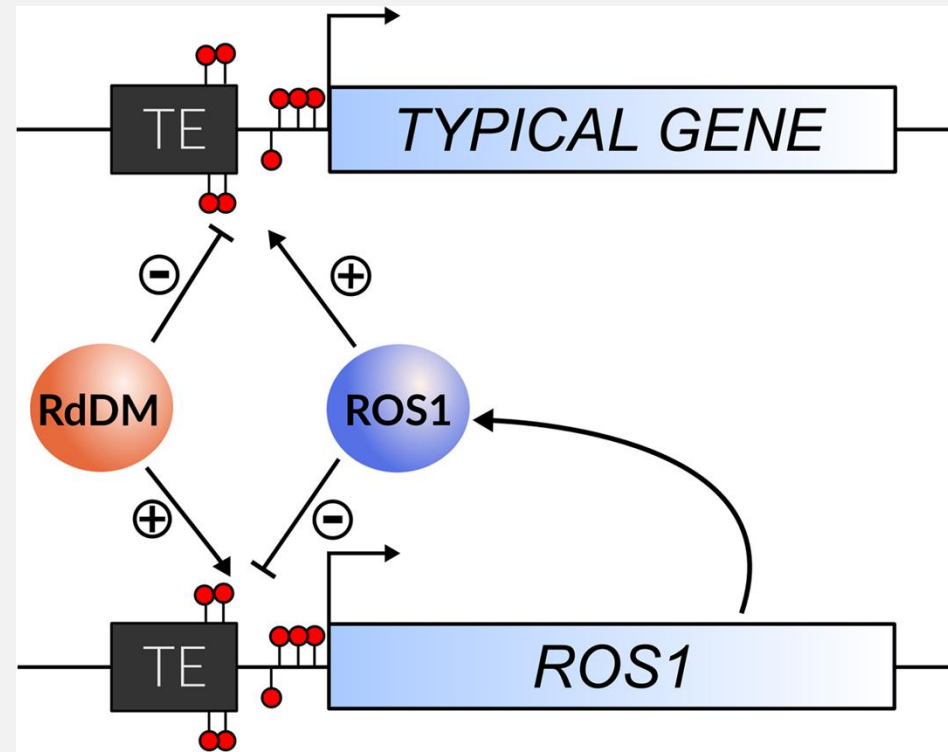
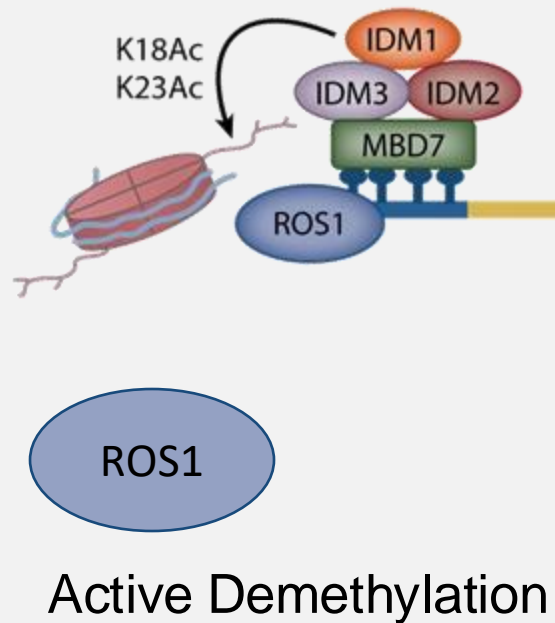
Crisp et al. (2020) PNAS

Recent work has demonstrated the utility of using DNA methylation profiles, rather than chromatin accessibility or histone modifications, to identify candidate CREs [57,58*]. Unmethylated regions profiled in a single tissue appear to mark both the regions accessible in that tissue and the regions that will become accessible in other tissues, enabling the rapid identification of entire portfolios of candidate CREs. The development of a pan-cis-regulatory atlas [59,60] will assist the selection of targets for gene editing and the prioritisation of intergenic QTL. For unmethylated regions identified in this way

Crisp et al. (2021) Current Opinion in Biotech.

Is DNA methylation static or dynamic?

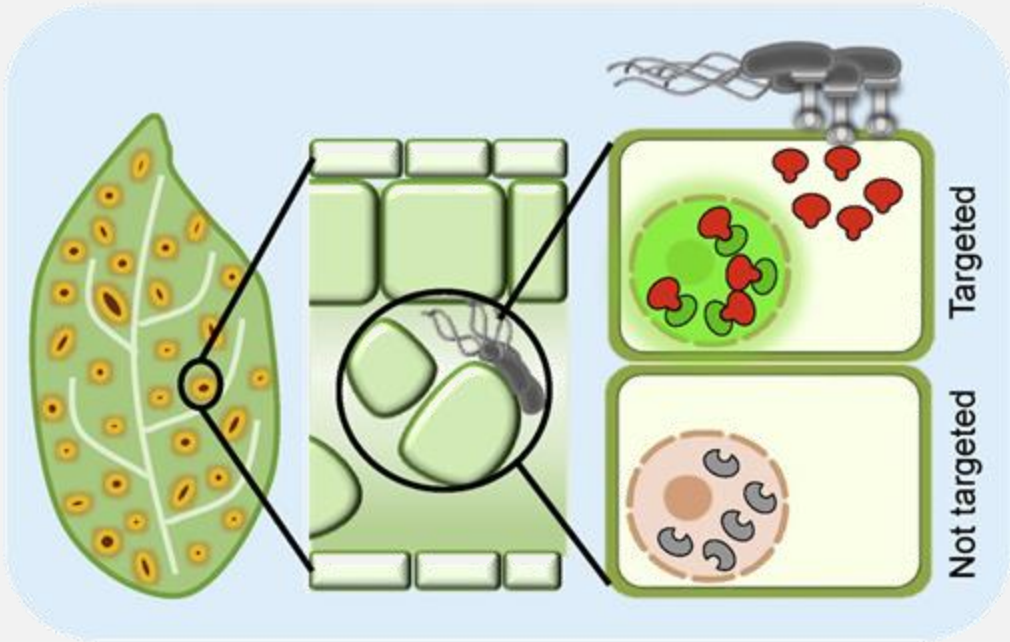
...but sometimes dynamic



Williams et al. (2015) PLOS Genetics

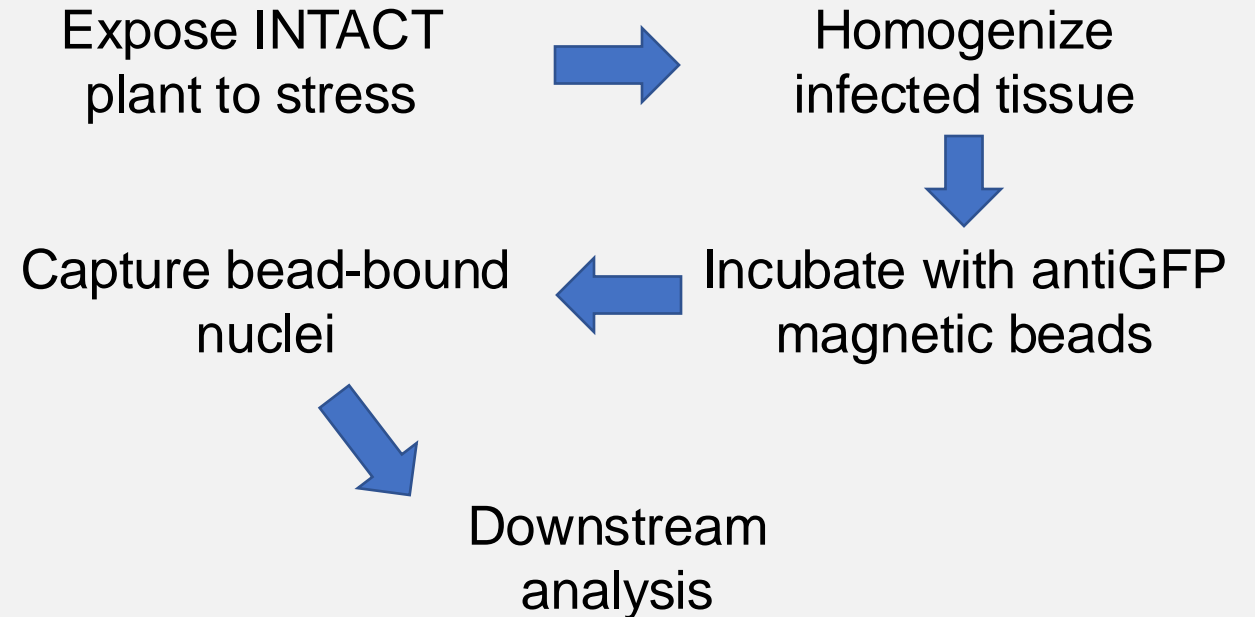
Can we improve signal by enriching for cells experiencing stress?

Heterogeneity of immune responses

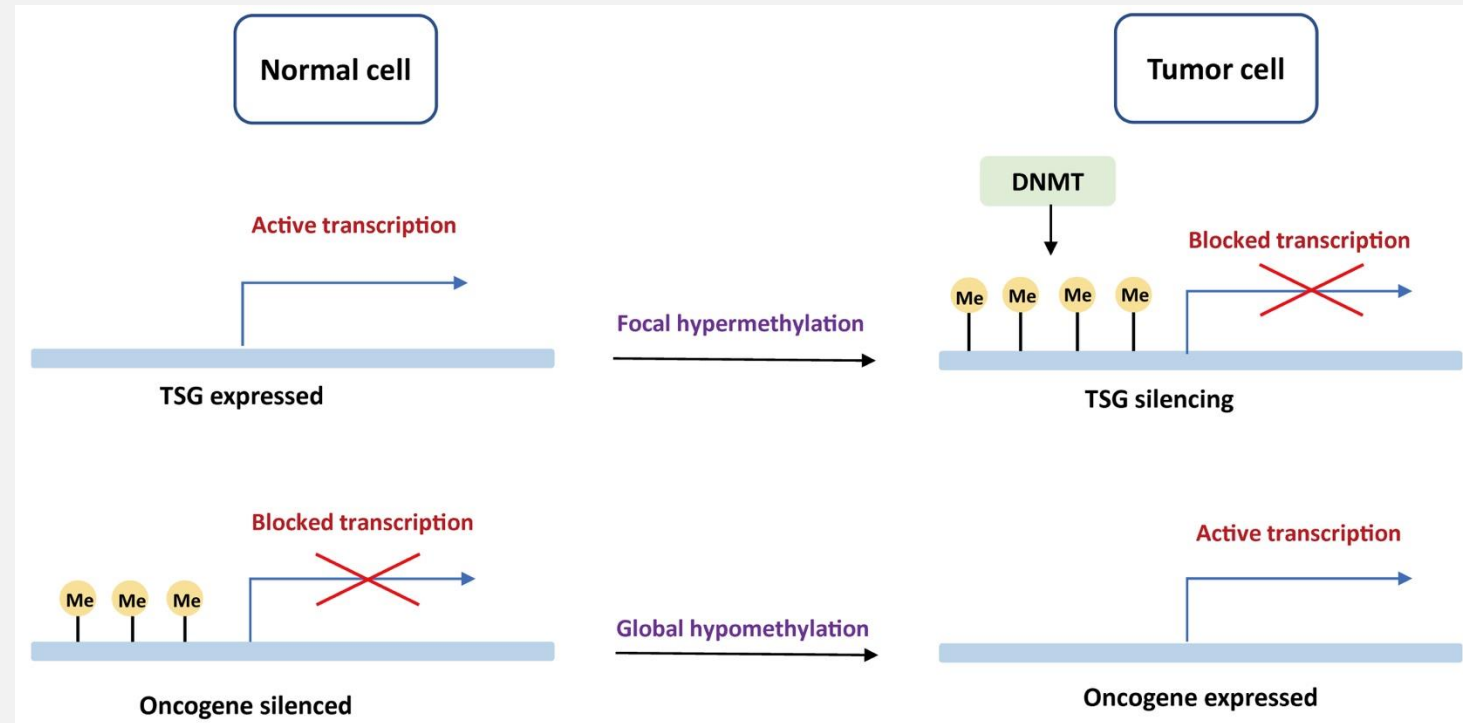


Zhang et al. (2020) Mol. Plant

INTACT allows antibody-based nuclei sorting



In humans, changes in DNA methylation are associated with cancers



Chen et al. (2022) Front Bioscience-Landmark

Take homes:

DNA methylation occurs in three contexts: CG, CHG, CHH – these have different impacts on DNA accessibility and gene expression

DNA methylation is *mostly* static – Unmethylated regions identified in a single tissue are associated with open chromatin and expression in at least one tissue type.

Some DNA methylation is dynamic, impacted by stress or developmental stage – how important is this?

DNA methylation data can highlight genomic loci that can guide breeding or engineering efforts