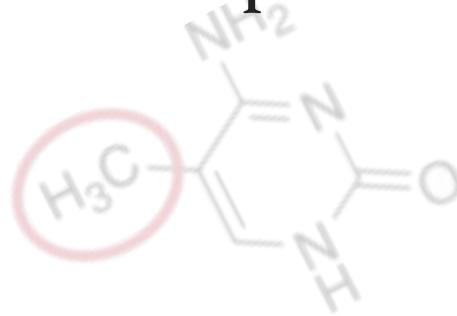


What does DNA methylation have to do with plant immunity?

Andy Read
Springer Lab
UMN - PMB



methylated Cytosine

Introduction

B.A. in Ecology, Evolution, Conservation Bio from

University of Hawai'i at Hilo

-Research in a plant virology lab

Research Associate with Monsanto in Cambridge, MA

-Insect-control proteins from bacteria

PhD in Plant-Microbe Biology from Cornell U.

-Molecular warfare during bacterial infection of rice

NSF PRFB Postdoc Fellow with Nathan Springer

-Epigenetics, what even is it?



@hashtag_read

read0094@umn.edu

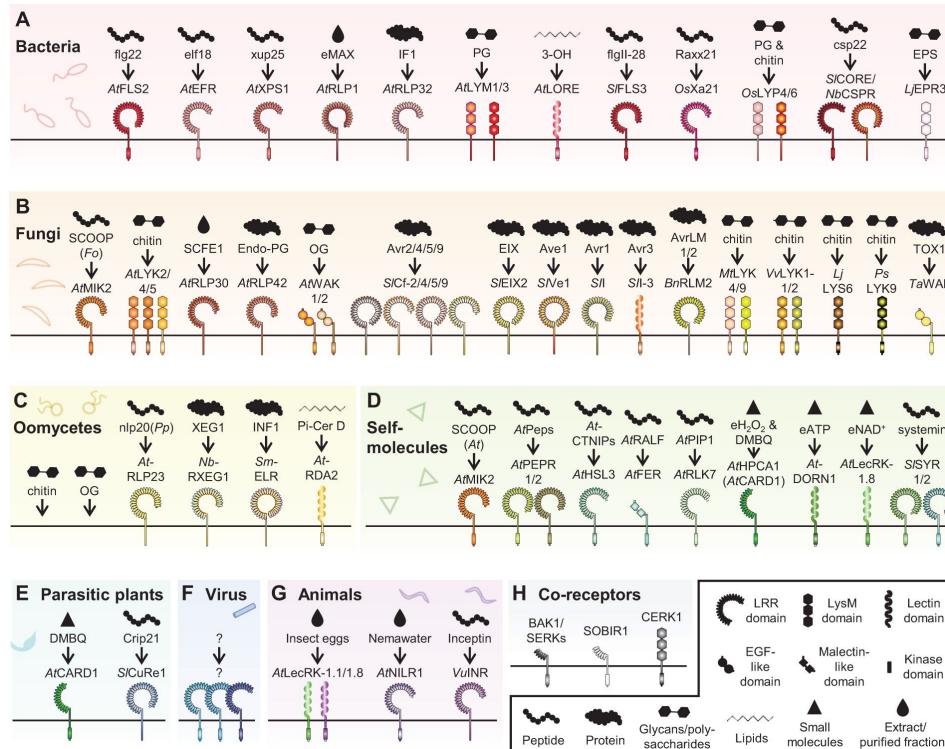
Introduction



Introduction



How do plants defend themselves?



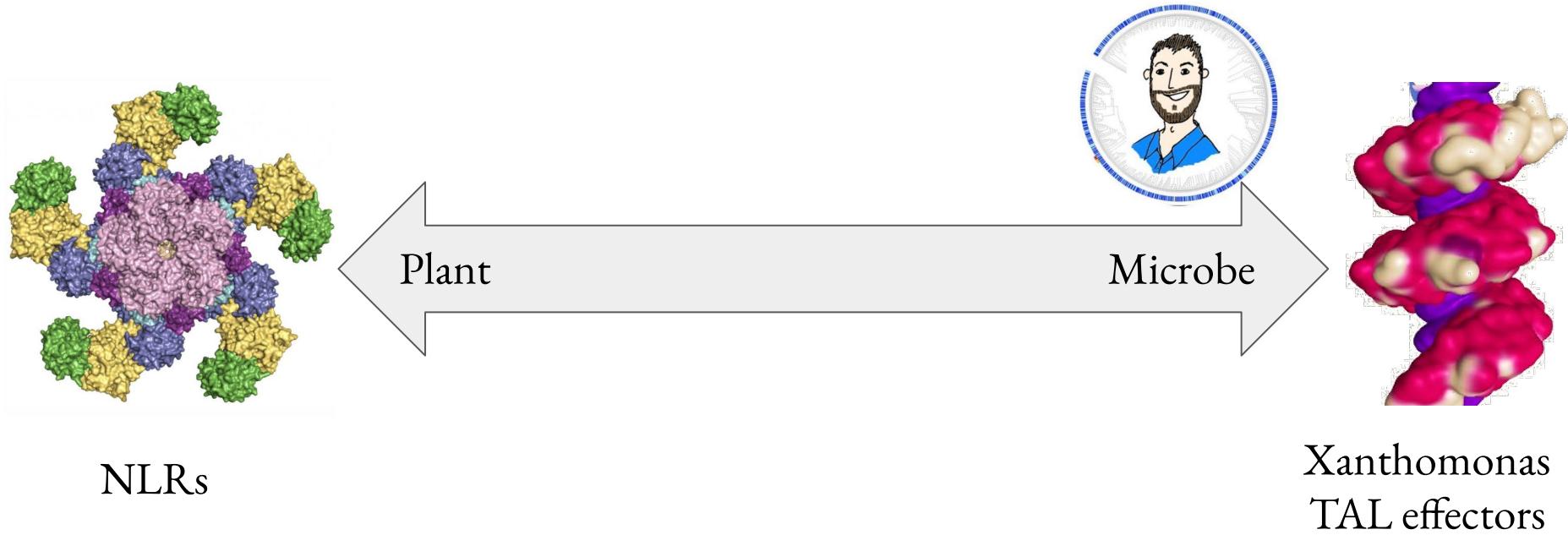
Outline:

- How did I become interested in epigenetics, disease, and resistance?
Work from my graduate research on
Xanthomonas-rice interactions
- What do I mean by epigenetics, how do we measure it, and
can we improve this with Nanopore sequencing?
- Using nuclei sorting to separate stressed from unstressed
plant cells during disease

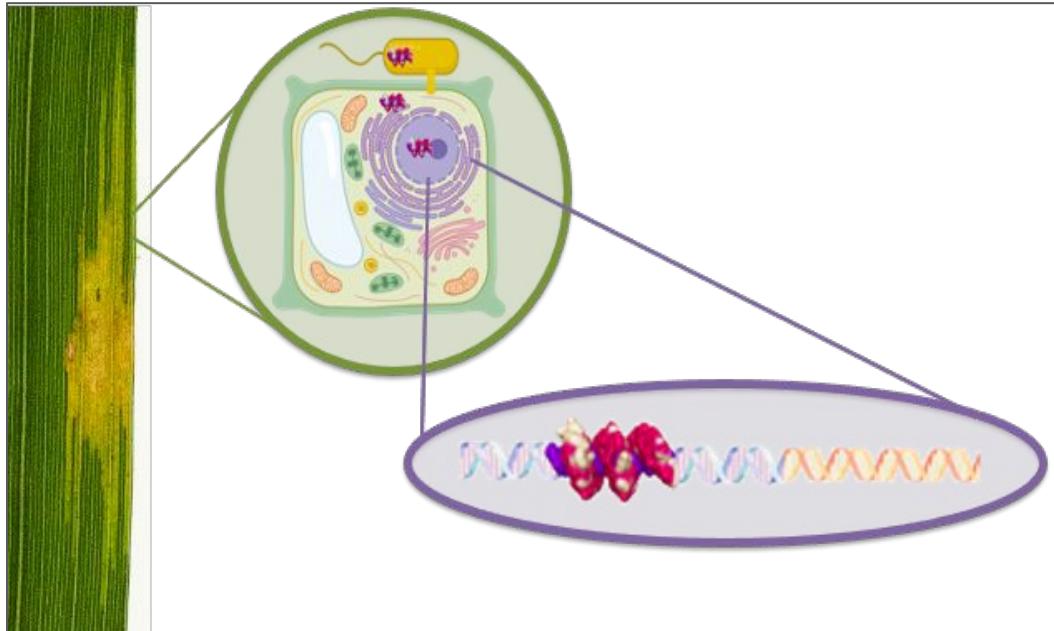
Outline:

- How did I become interested in epigenetics, disease, and resistance?
Work from my graduate research on
Xanthomonas-rice interactions
- What do I mean by epigenetics, how do we measure it, and
can we improve this with Nanopore sequencing?
- Using nuclei sorting to separate stressed from unstressed
plant cells during disease

The Andy plant-microbe interest spectrum



TAL effectors are amazing



Transcription Activator-Like effectors

- Nuclear localization signal helps it get to the plant cell nucleus
- Central repeat region coils around specific DNA regions
- Activation domain up-regulates nearby genes
- Can result in increased Susceptibility or Resistance

Xanthomonas infection in Vietnamese rice field



Yield loss of 10-20% and up to 40%

Dramatic impacts on food security

No good chemical control – must rely on **genetic plant defense**

Nearly identical bacterial strains can have very different disease outcomes

Resistance:

Plant immunity gene
recognizes a bacterial
avirulence gene - triggers HR



Susceptibility:

The addition of an additional
avirulence gene leads to
susceptibility - a suppressor
of Resistance?



Resistance is **triggered** by NLR Xo1 recognition of TAL effectors (different from canonical TAL-induced resistance)

- Cloning of the Rice Xo1 Resistance Gene and Interaction of the Xo1 Protein with the Defense-Suppressing Xanthomonas Effector Tal2hAC Read, M Hutin, MJ Moscou, FC Rinaldi, AJ Bogdanove Molecular Plant-Microbe Interactions 33 (2020)
- Genome assembly and characterization of a complex zfBED-NLR gene-containing disease resistance locus in Carolina Gold Select rice with Nanopore sequencing AC Read, MJ Moscou, AV Zimin, G Pertea, RS Meyer, MD Purugganan, PLOS Genetics 16 (2020)

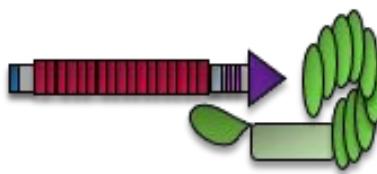
Resistance is **blocked** by an unusual truncated TAL effector previously thought to be a pseudogene

- Suppression of Xo1-mediated disease resistance in rice by a truncated, non-DNA-binding TAL effector of *Xanthomonas oryzae* AC Read, FC Rinaldi, M Hutin, YQ He, LR Triplett, AJ Bogdanove Frontiers in plant science 7 (2016)
- Interfering TAL effectors of *Xanthomonas oryzae* neutralize *R*-gene-mediated plant disease resistance. Ji, Z., Ji, C., Liu, B. et al. *Nat Commun* 7, 13435 (2016)

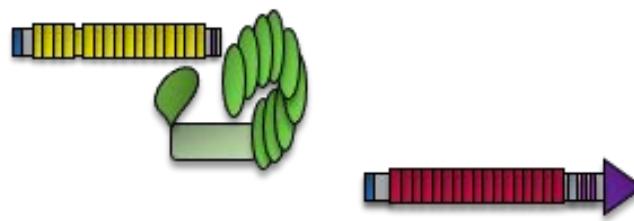
An Xo1 model



Xo1 in 'off' state



TALE recognition
Xo1 triggered
Defense

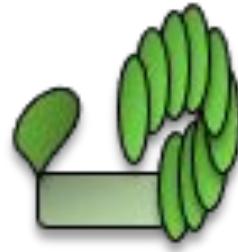


truncTALE disrupts
Xo1 resistance
Counter-Defense

Down the NLR rabbit hole

Presence/Absence Variation

Difficult to sequence and
annotate

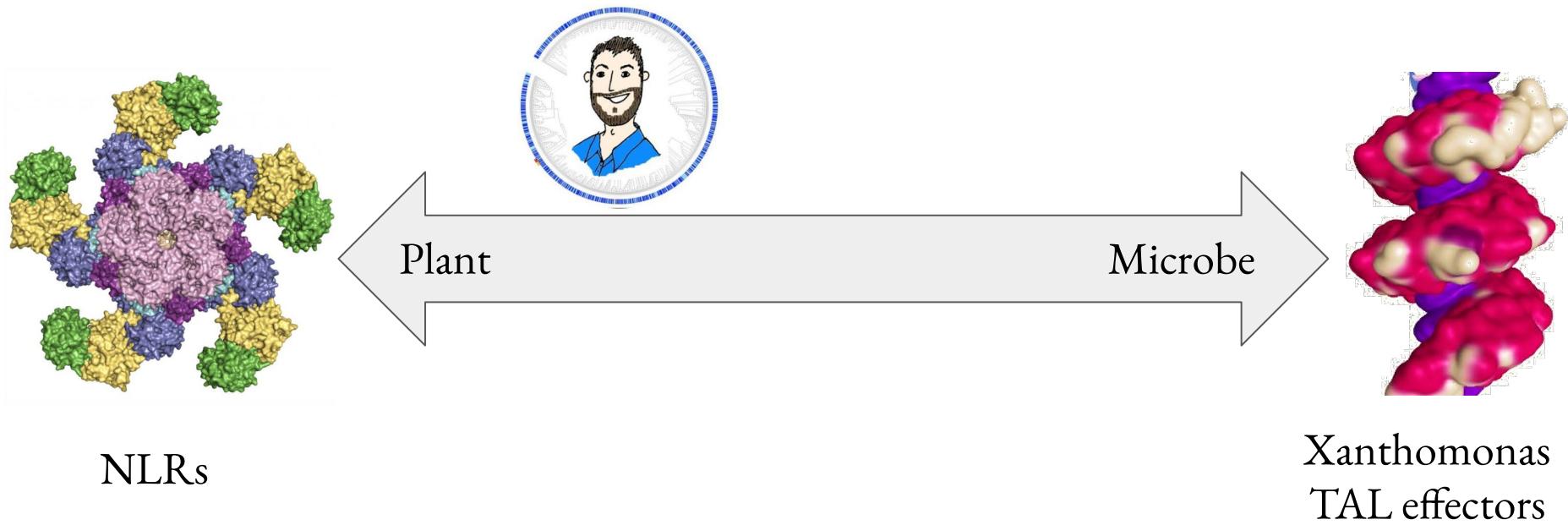


Evolve in interesting ways

Regulated at every level

Autoimmunity

The Andy plant-microbe interest spectrum

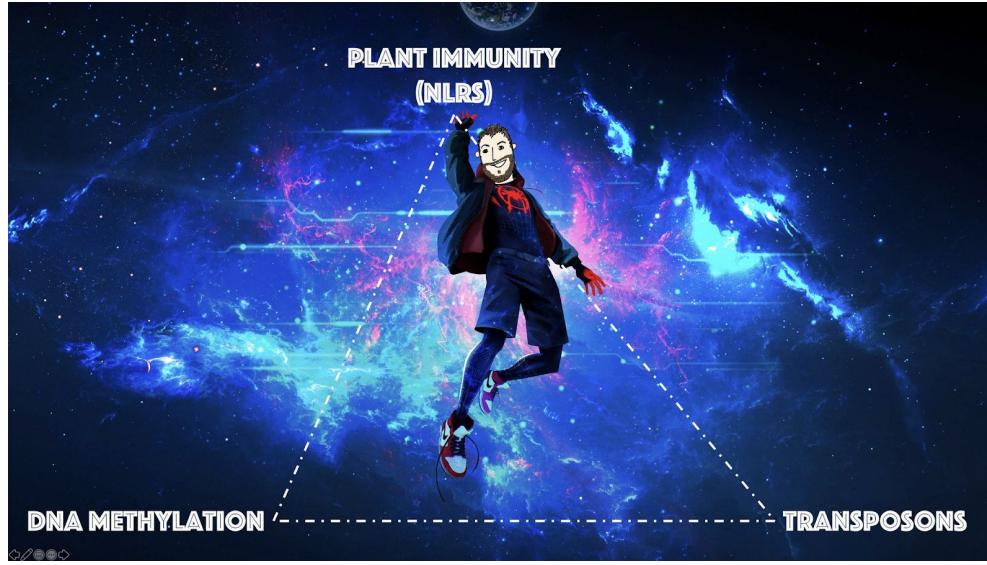


PLANT IMMUNITY (NLRS)



DNA METHYLATION

TRANSPOSONS



Within generations: Dynamic methylation, especially at transposons, can impact immunity gene expression

Across generations: Transposon movement may aid in rapid diversification of the plant immune system

Meet our model: *Setaria viridis*



Benefits

- Small C4 grass
- Short life-cycle (8-10 weeks)
- Genome ~ 400 Mb
- 2 high quality genomes available
 - A10 - platinum
 - ME034V - high quality genome, transformable genotype

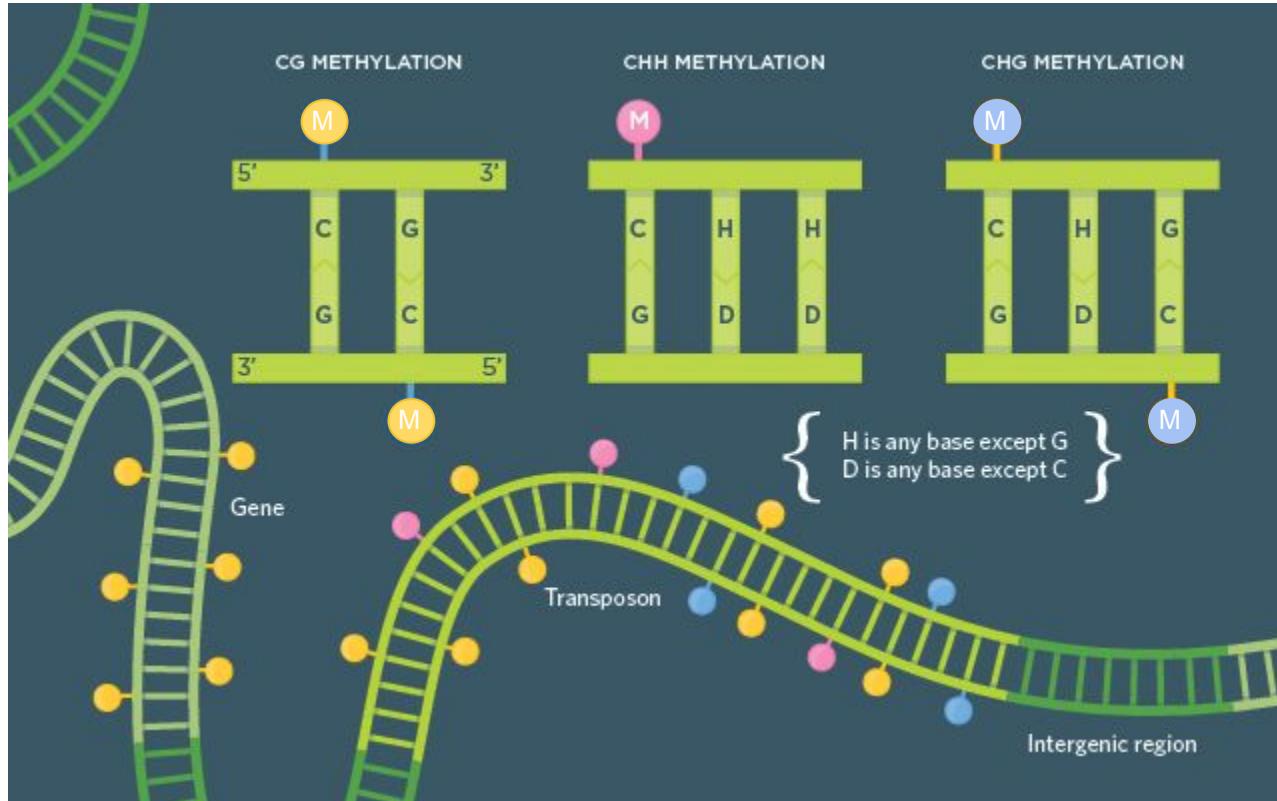
Challenges

- Not much disease knowledge

Outline:

- How did I become interested in epigenetics, disease, and resistance?
Work from my graduate research on
Xanthomonas-rice interactions
- What do I mean by epigenetics, how do we measure it, and
can we improve this with Nanopore sequencing?
- Using nuclei sorting to separate stressed from unstressed
plant cells during disease

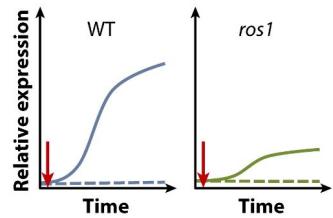
When I say ‘epigenetics’ I mean DNA methylation



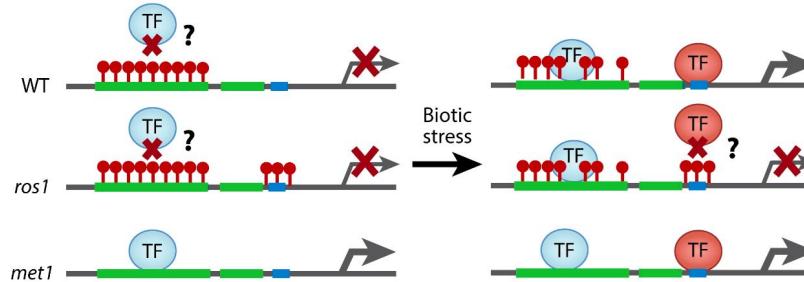
Infographic: Plant Methylation Basics. Akst, Jef. The Scientist (2017)

DNA methylation is dynamic during disease

a RMG1 expression

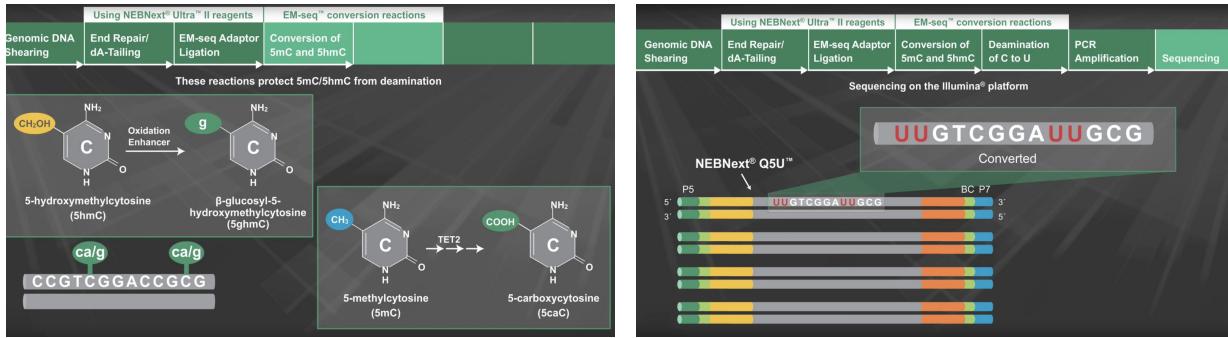


C RMG1 expression changes upon treatment



Deleris A, et al. 2016.
Annu. Rev. Phytopathol. 54:579–603

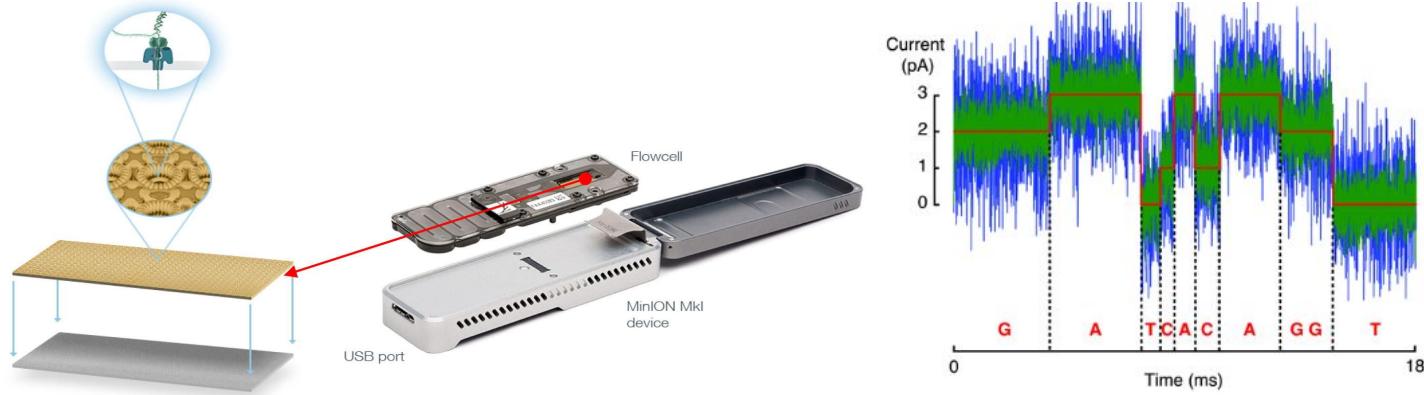
How do we measure DNA methylation?



Traditionally:

- Bisulfite or enzymatic conversion of un-methylated cytosines to uracil
- Short-read Illumina sequencing
- Align to reference

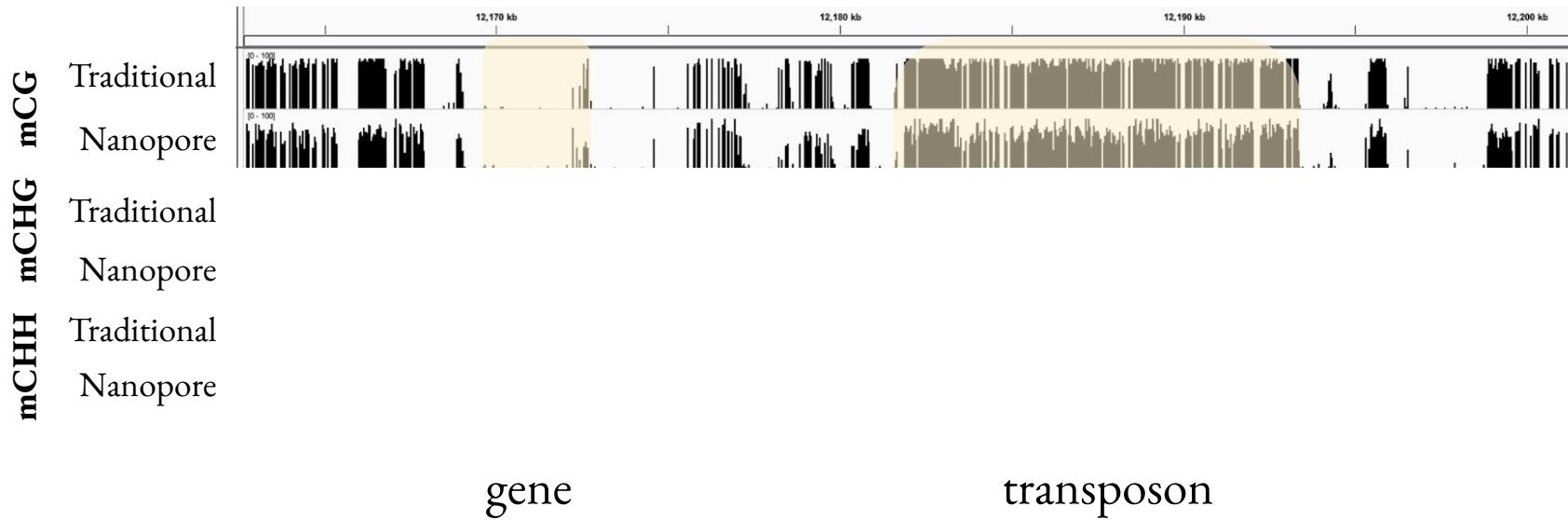
How do we measure DNA methylation?



Alternatively:

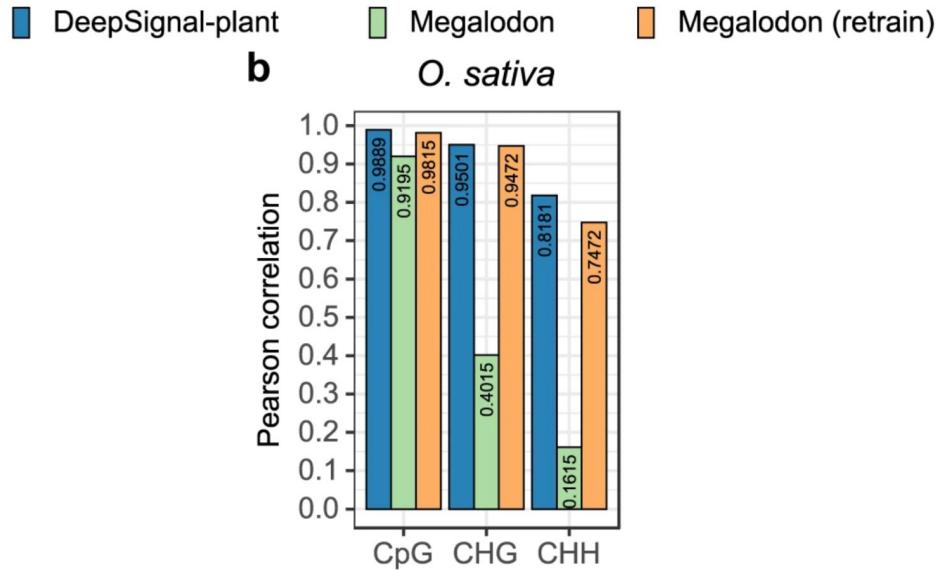
- No conversion
- Long-read Nanopore sequencing
- Methylation status determined from raw sequence signal
- Align to reference

Comparing traditional methylSeq and Nanopore



Currently Nanopore does a good job of identifying methylated regions, but the detection of mCHG and mCHH have a lot of room for improvement

....but there's hope

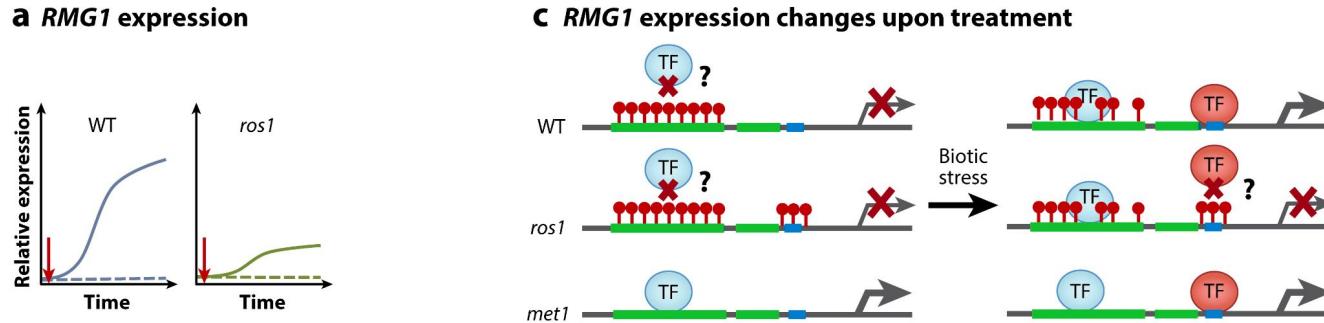


A deep learning algorithm for plant DNA modification calling improves mCHG and mCHH - I am working on validating with our Setaria Nanopore data

Outline:

- How did I become interested in epigenetics, disease, and resistance?
Work from my graduate research on
Xanthomonas-rice interactions
- What do I mean by epigenetics, how do we measure it, and
can we improve this with Nanopore sequencing?
- Using nuclei sorting to separate stressed from unstressed
plant cells during disease

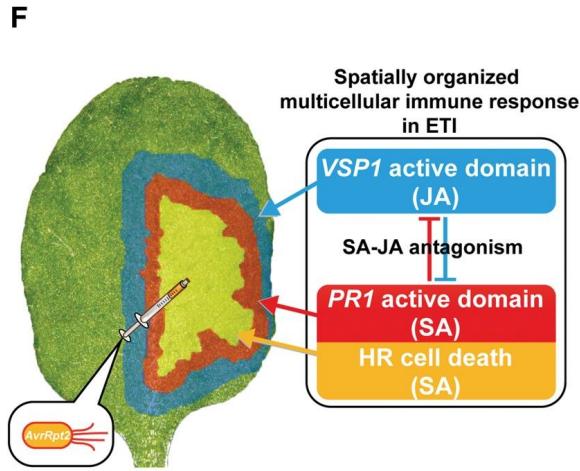
DNA methylation is dynamic during disease



Deleris A, et al. 2016.
Annu. Rev. Phytopathol. 54:579–603

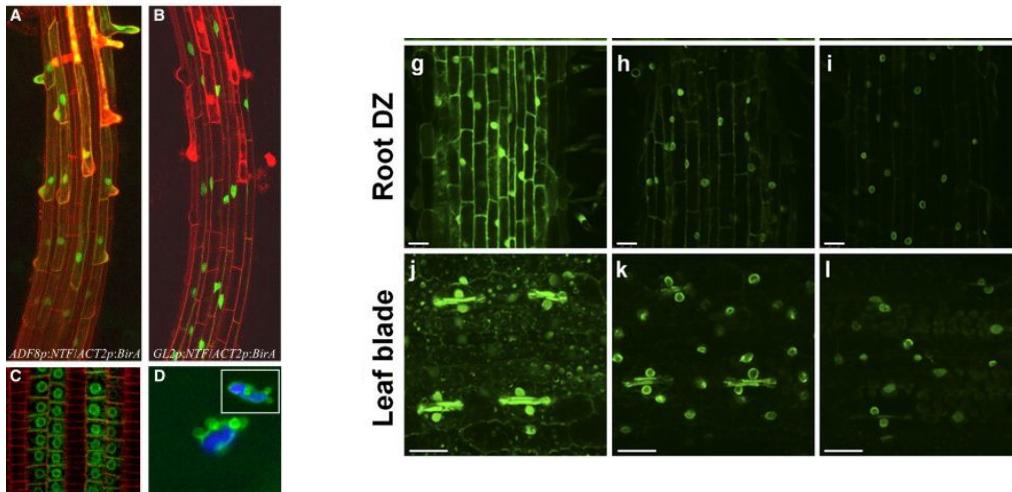
But.... reported methylation changes are relatively small and it has been challenging to demonstrate causation

Not all cells experience the same stress during infection



If we can separate the cells from each region, we may learn more about the dynamics and impacts of DNA methylation changes on plant immunity

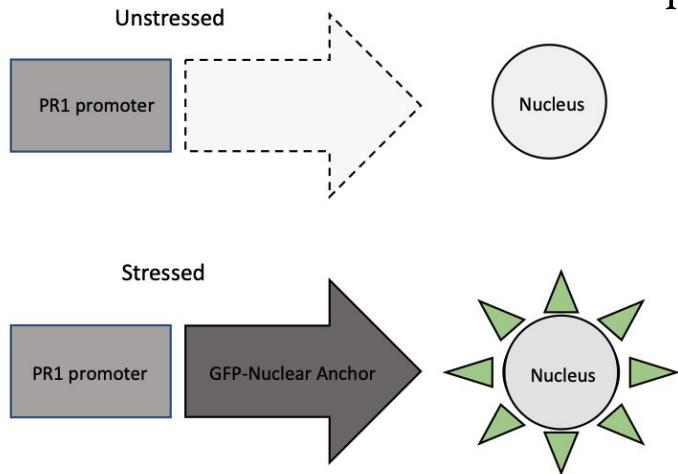
Isolation of nuclei from tagged specific cell types -INTACT



First applied in *Arabidopsis* to mark and sort cell types
Later implemented in rice and tomato

INTACT - how it works

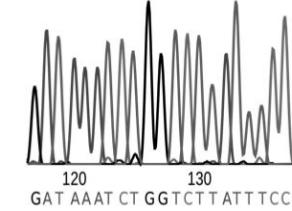
Marker activation



Tissue homogenization



Sequencing (mC and RNA)

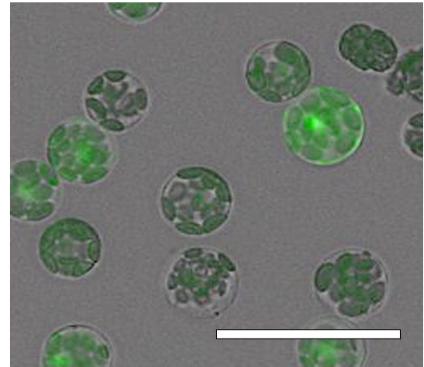


GFP studded
nuclei

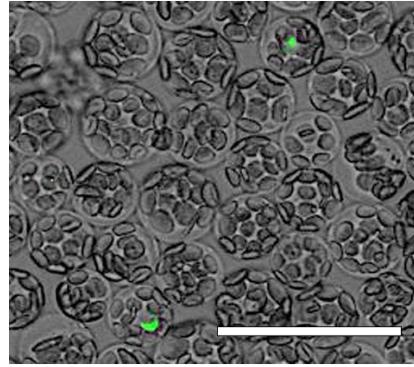
Magnetic
purification

Building an INTACT system in Setaria

35s::GFP



35s::GFP_WIP



Adding a nuclear membrane anchor (WIP - WPP interacting protein)
to a Voytas Lab plasmid

Expression in Setaria protoplasts shows distinct foci in GFP-WIP

Parallels with Jacobs Lab ‘effector detector’ plants - but objective is nuclei sorting, not imaging/tracking

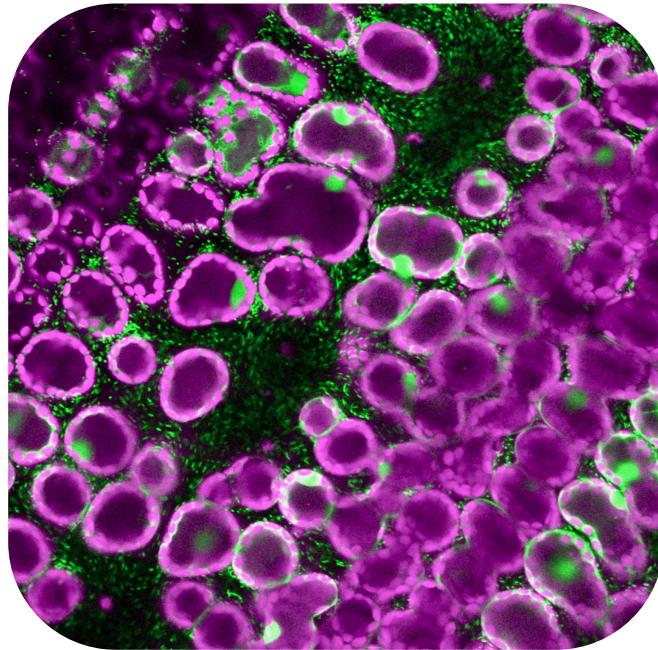


Image from Jacobs Lab website - GFP expressing *Xanthomonas* and TAL effector dependent GFP expression in plant cells. Magenta = chlorophyll autofluorescence

Developing a model Setaria-Xanthomonas pathosystem

Host of origin	Number of fields sampled ^a	Number of leaves sampled	Number of <i>Xanthomonas translucens</i> ^b
<i>Avena fatua</i>	8	17	11
<i>Bromus inermis</i>	6	8	4
<i>Elymus repens</i>	14	24	31
<i>Hordeum jubatum</i>	9	22	4
<i>Hordeum vulgare</i>	3	7	6
<i>Lolium perenne</i>	1	4	7
<i>Setaria faberi</i>	6	12	0
<i>Setaria pumila</i>	7	11	0
<i>Setaria viridis</i>	5	11	5
<i>Triticum aestivum</i>	8	9	9
Total	16	128	77

Xanthomonas translucens pv. undulosa Identified on Common Weedy Grasses in Naturally Infected Wheat Fields in Minnesota
Kristi E. Ledman, Rebecca D. Curland, Carol A. Ishimaru, and Ruth Dill-Macky Phytopathology (2021)

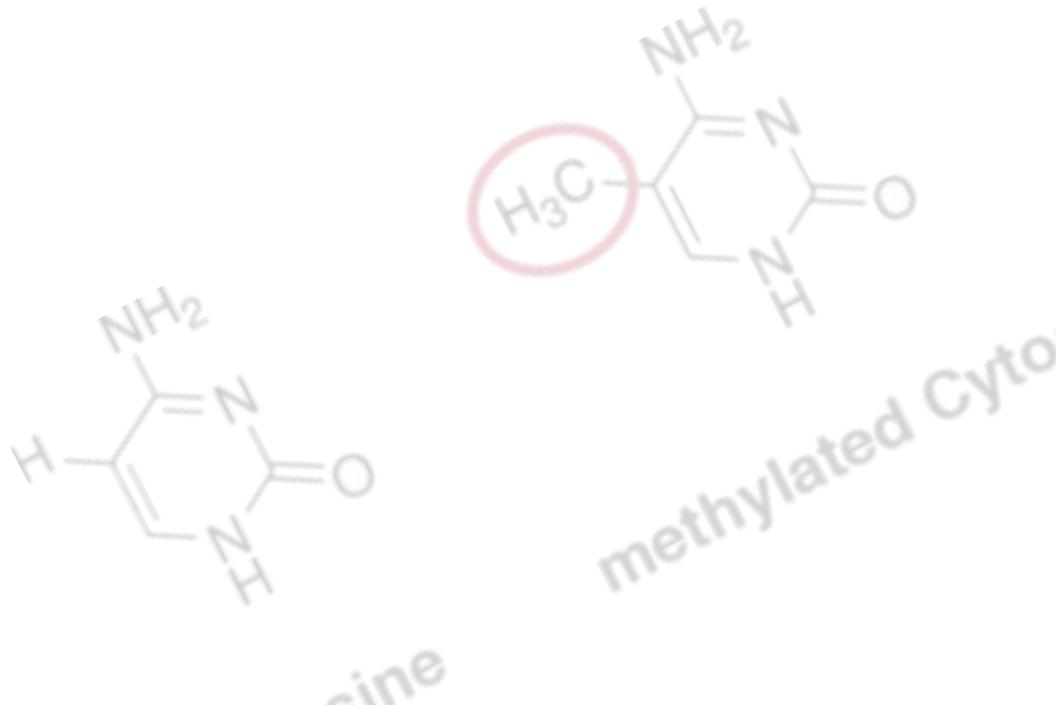
1. Check for symptoms on our Setaria cultivar
2. Select 2-3 candidate disease responsive promoters for INTACT constructs (RNAseq informed?)
3. Generate stable Setaria transformants
4. Start sorting nuclei and sequencing

Still a lot of work left



Plate I

GREEN FOXTAIL
(Setaria viridis (L.) Beauvois)



Acknowledgements

The Springer Lab



Help with protoplasts:

Jitesh Kumar - post-doc Zhang Lab

Xanthomonas strains for upcoming assays:

Professor Ruth Dill-Macky

Rebecca Curland

Kristi Ledman

Funding

NSF PRFB

Questions, concerns, and suggestions

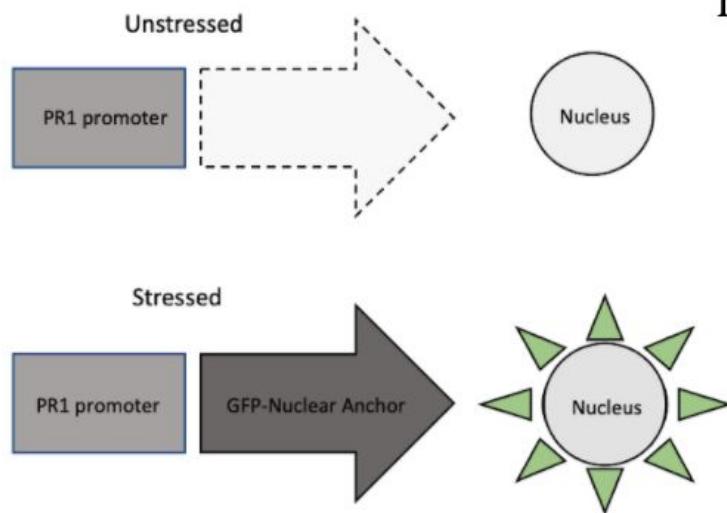


Stay in touch! I'd like to be more connected (professionally and socially) to the plant pathology dept!

read0094@umn.edu

INTACT - how it works

Marker activation



GFP studded
nuclei

Tissue homogenization



Magnetic
purification

Sequencing (mC and RNA)

