

Arabidopsis BORDER proteins put the breaks on RNA polymerase II elongation



Scott Michaels



Xuhong Yu

 INDIANA UNIVERSITY



Pascal GP MARTIN

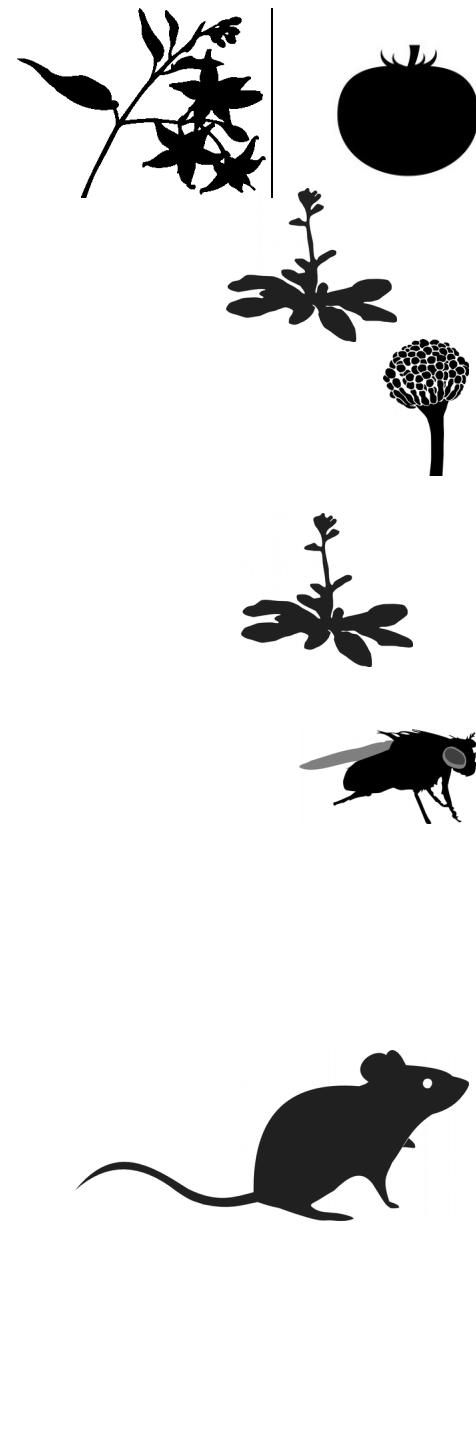


pascal.martin@inrae.fr



[@PgpMartin](https://twitter.com/PgpMartin)

My career path... in logos...



since 2021 INRAE BFP

2019-20 IU Pikaard lab

2018-19 INRA - E5 / BTM

2015-17 IU Michaels lab

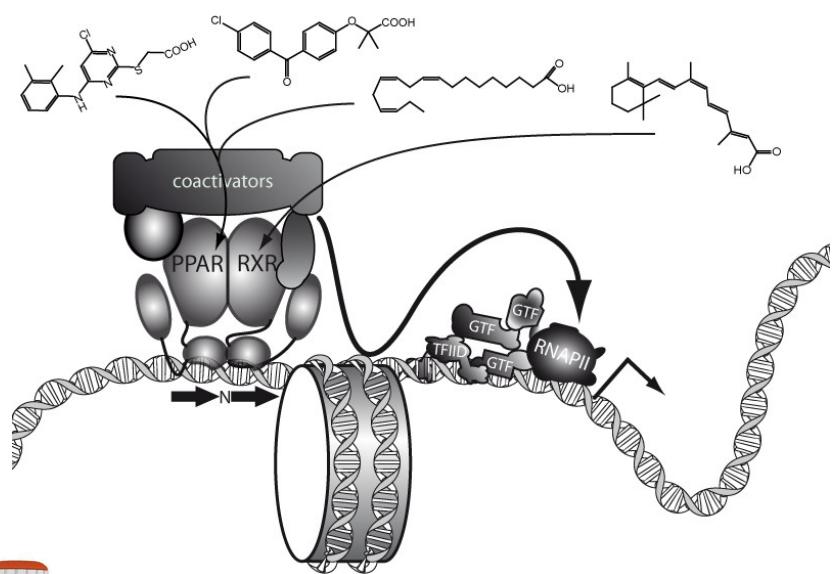
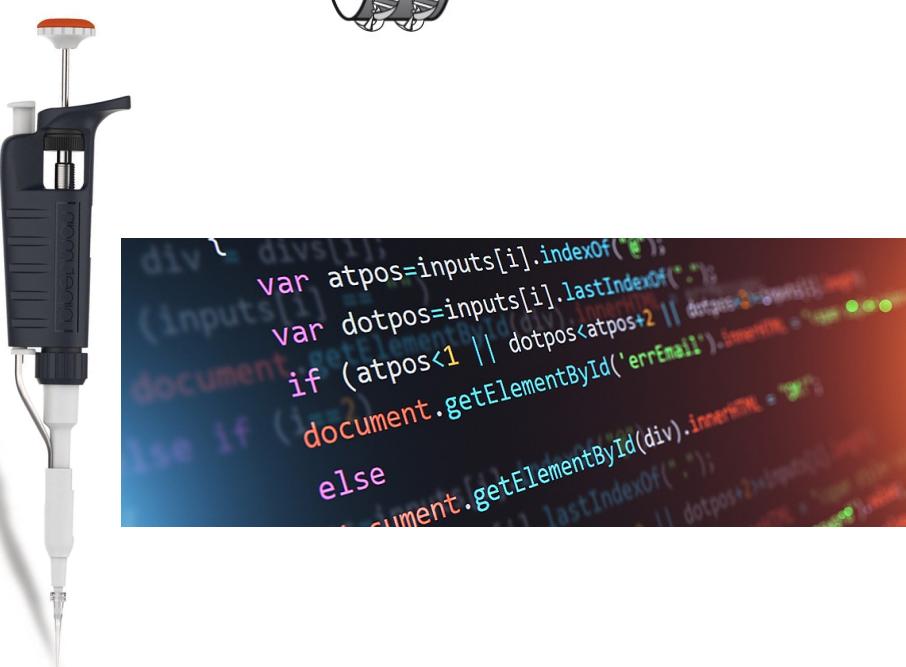
2013-15 LBME / Cuvier lab

2011-13 TRiX

2007-11 INRA - E1 / TIM

2004 INRA Research Engineer (IR)

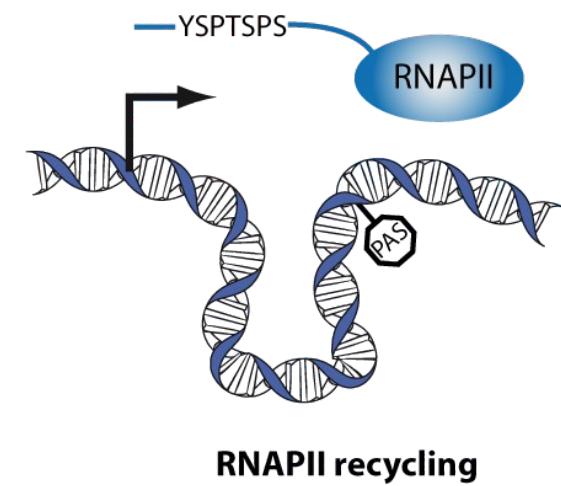
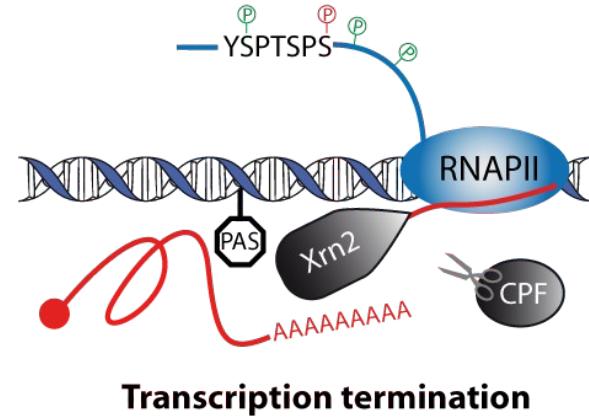
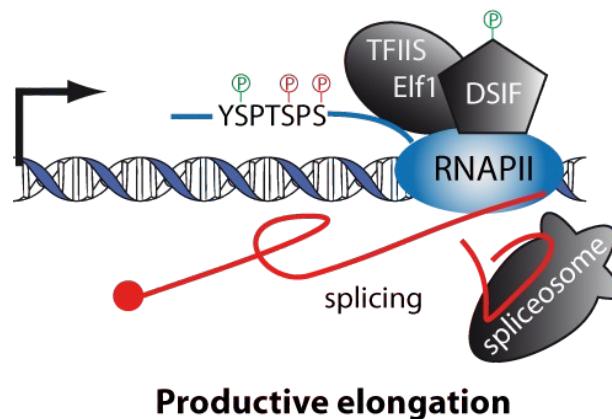
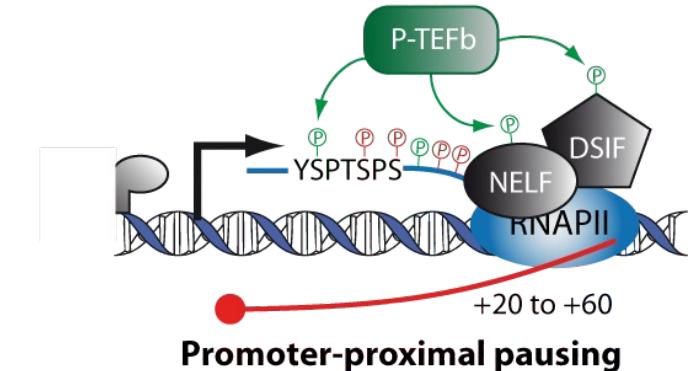
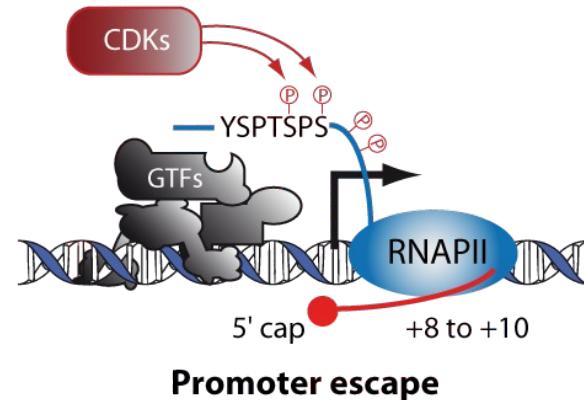
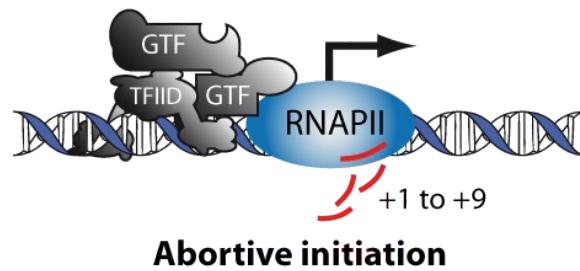
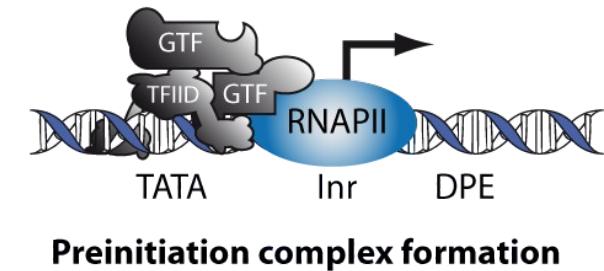
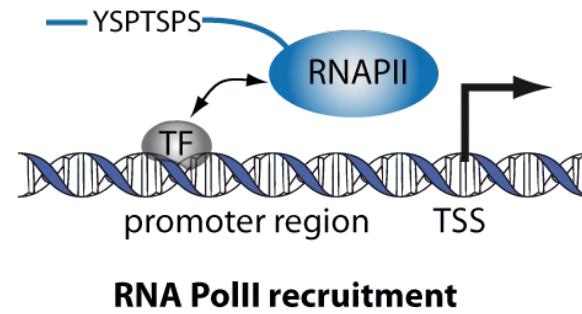
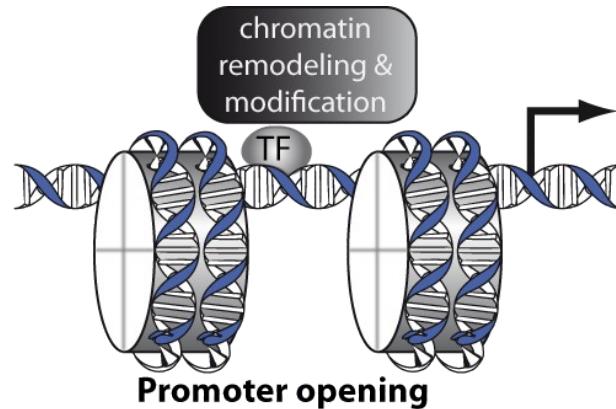
2000 MsC - PhD Pharmaco-Toxicology

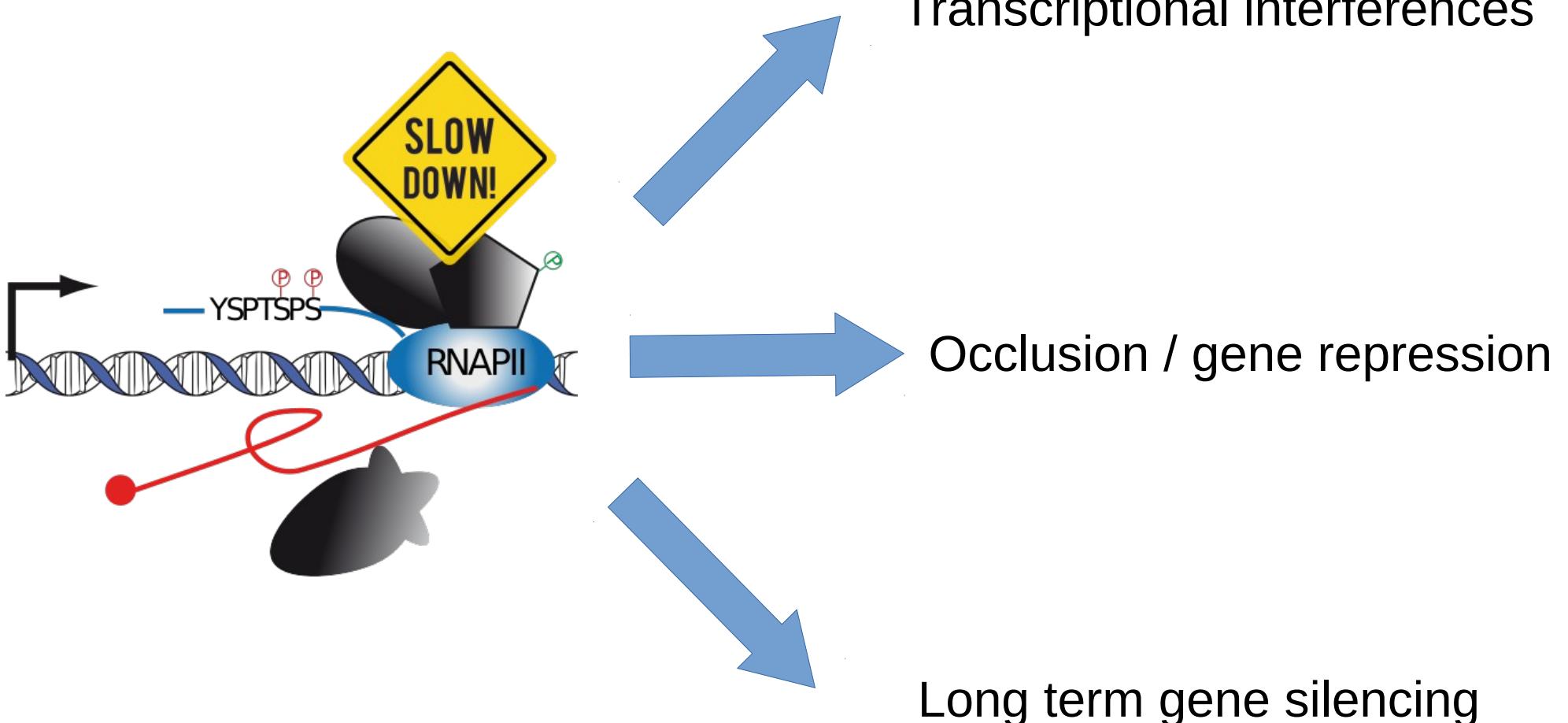


gene expression transcription
transcription factor RNA
biostatistics stimulus plant
nutrition nucleosome
microarray qPCR ChIP animal DNA drug
bioinformatics metabolism elongation
transcriptomics
genomics mouse protein sequence genome chromatin
R code NGS fly
data analysis pharmacology physiology
nuclear receptor cell
lipids histone insulator liver annotations
epigenomics motif toxicology
chromatin modification

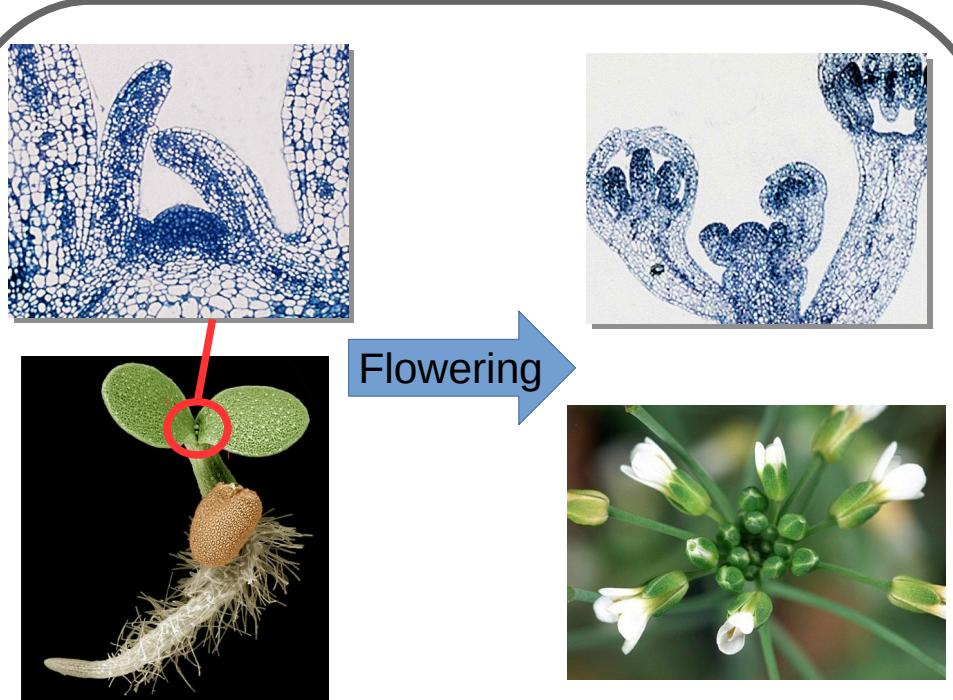
RNA polymerase II transcription

Roeder, TIBS 1996
 Margaritis & Holstege, Cell 2008
 Zaborowska et al., NSMB 2016
 Ngoc et al., Genes Dev 2017





New actors in the regulation of flowering time



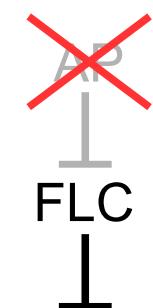
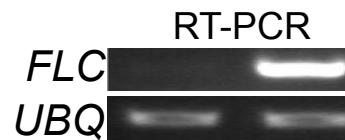
"Autonomous pathway" genes repress FLC



Wild-type

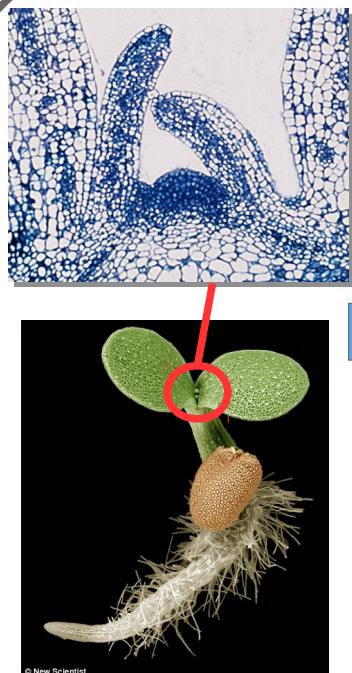


AP mutant



Michaels & Amasino, Plant Cell 1999
Amasino & Michaels, Plant Physiol 2010
Wu et al., Plant Physiol 2020

New actors in the regulation of flowering time



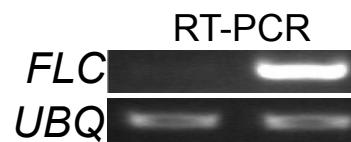
"Autonomous pathway" genes repress FLC



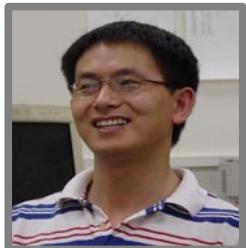
Wild-type



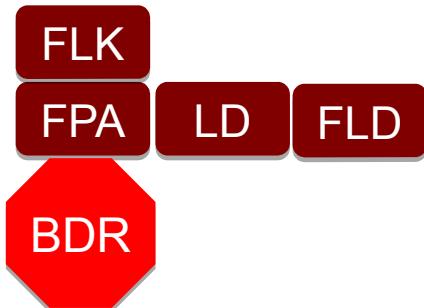
AP mutant



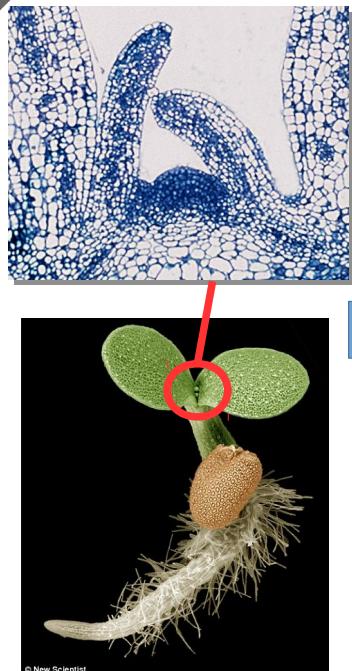
Michaels & Amasino, Plant Cell 1999
Amasino & Michaels, Plant Physiol 2010
Wu et al., Plant Physiol 2020



Xuhong Yu



New actors in the regulation of flowering time



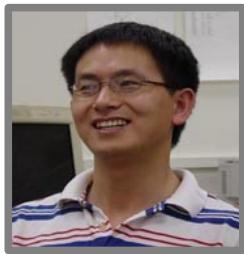
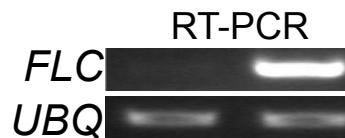
"Autonomous pathway" genes repress FLC



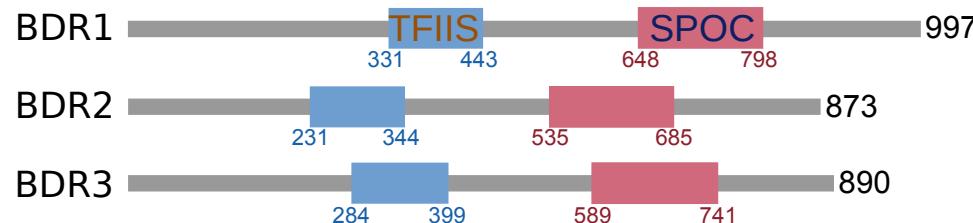
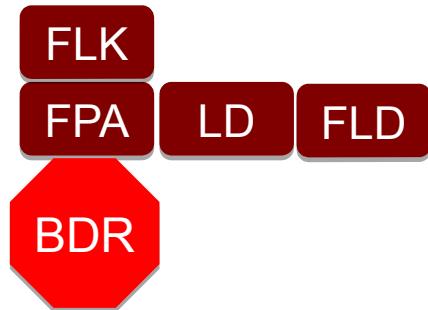
Wild-type



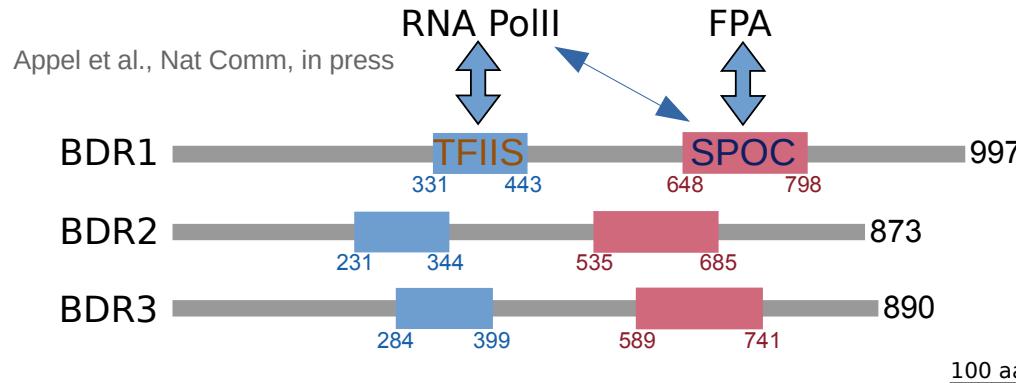
AP mutant



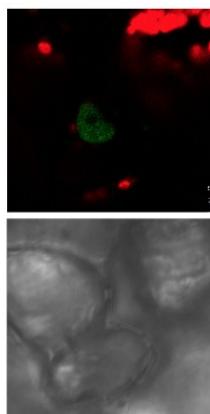
Xuhong Yu



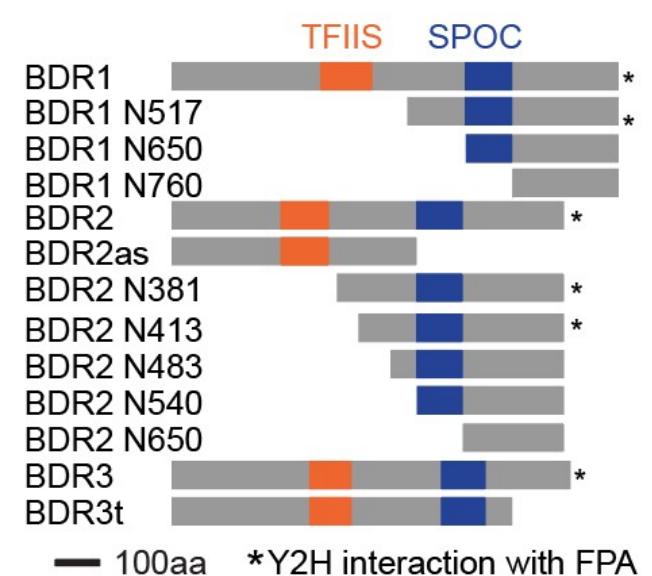
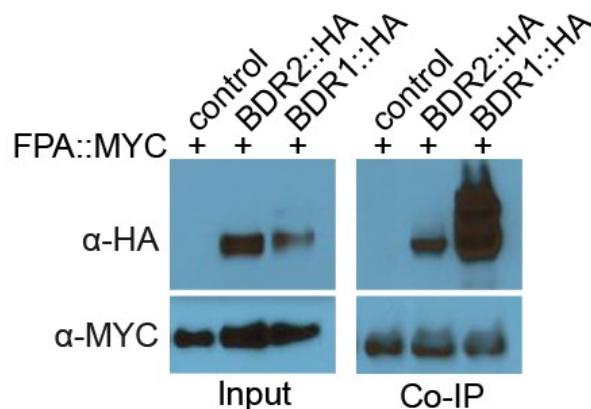
BORDER proteins interact with FPA



BiFC



pNYFP::FPA+
pCCFP::BDR1



+ IP-MS
+ ChIP-seq

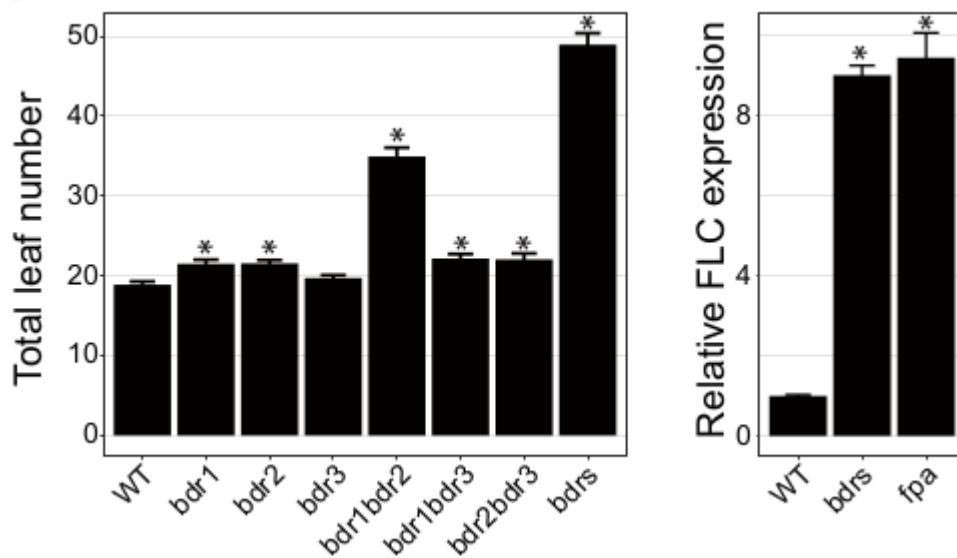
BDRs redundantly regulate flowering in *Arabidopsis thaliana*

A.

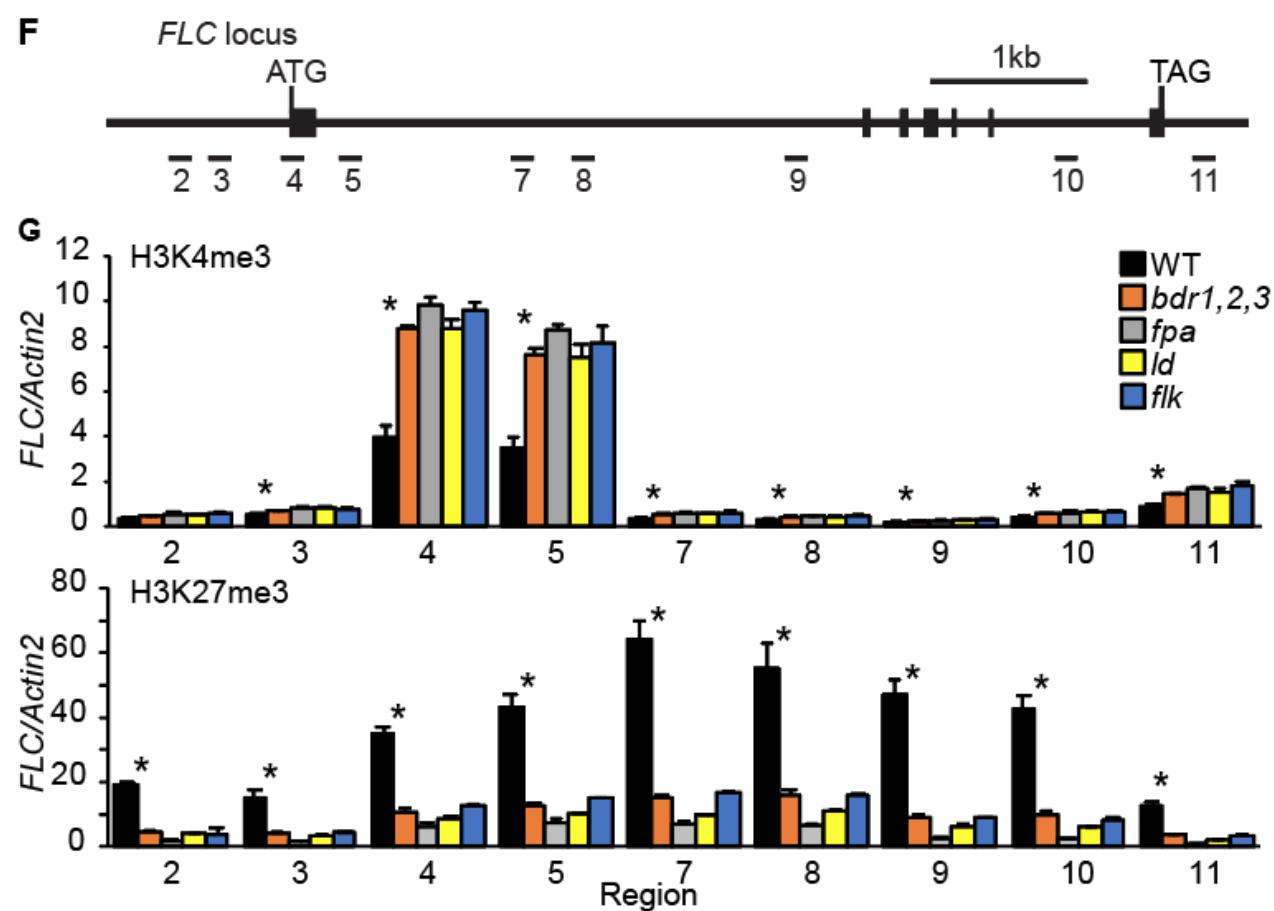
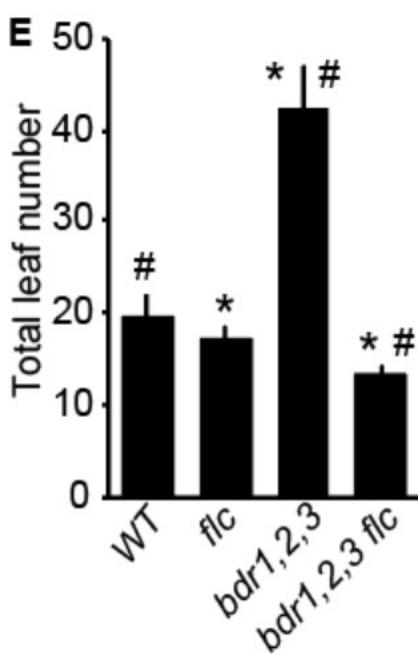
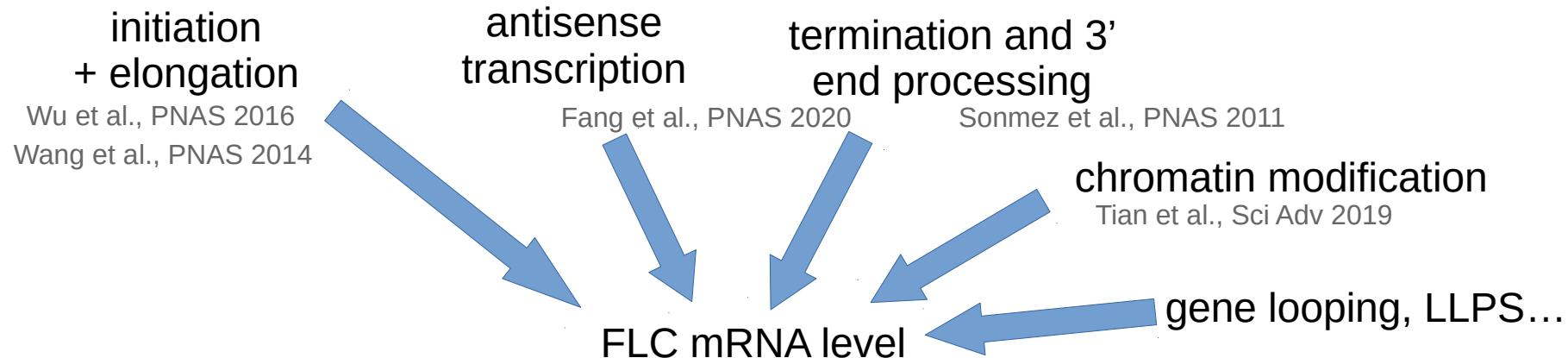


- Triple mutant is very late flowering
- High level of FLC expression

B.

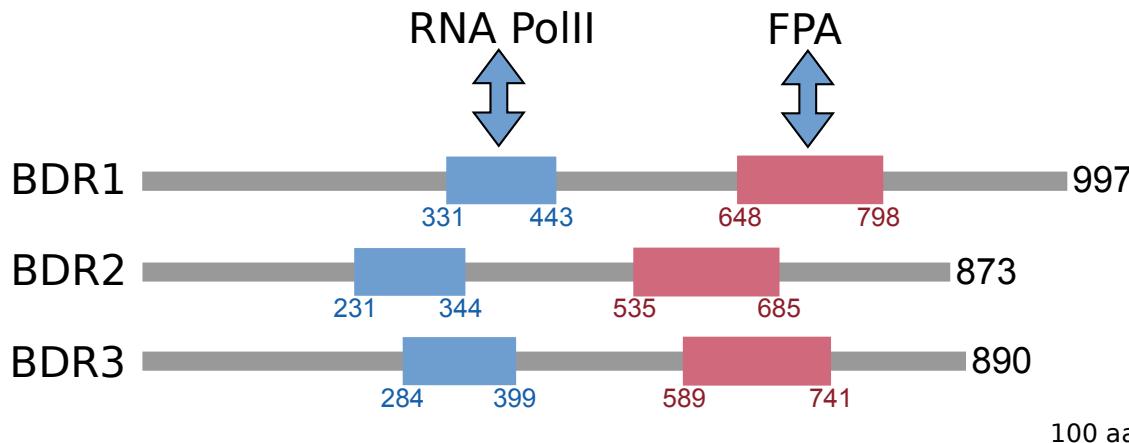


BDRs redundantly regulate flowering in *Arabidopsis thaliana*



A family of protein with common structural domains

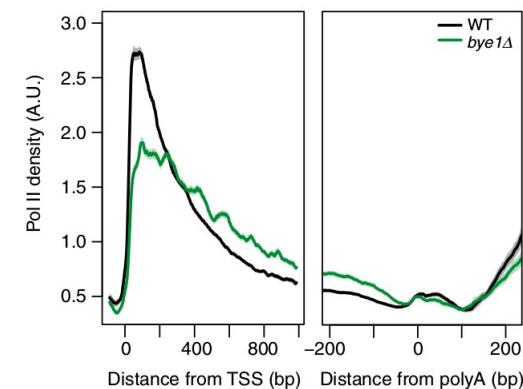
PFAM
HMMER



Wu et al., Genetics 2003
Pinskaya et al., PloS One 2014
Harlen & Churchman, Mol Syst Biol 2017

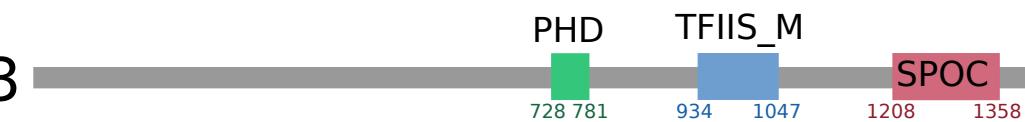


M Das Murtey & P Ramasamy



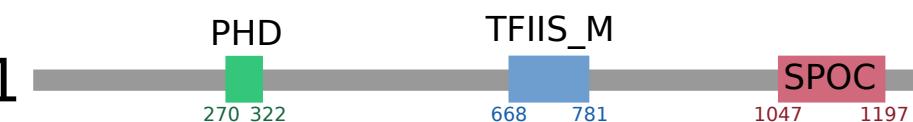
Zoch et al., Nature, 2020

PHF3

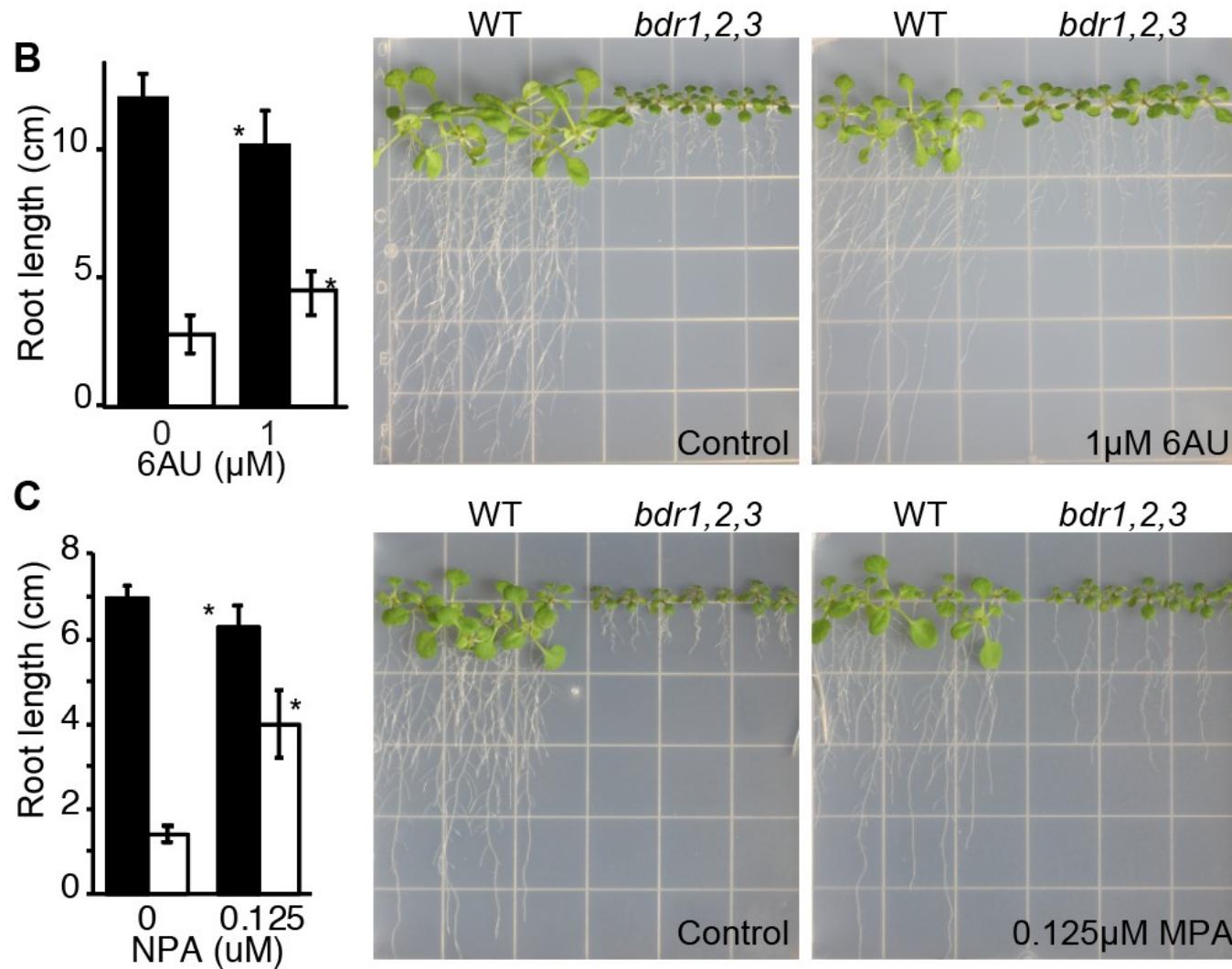


Appel et al., Nat Comm, in press

DIDO1

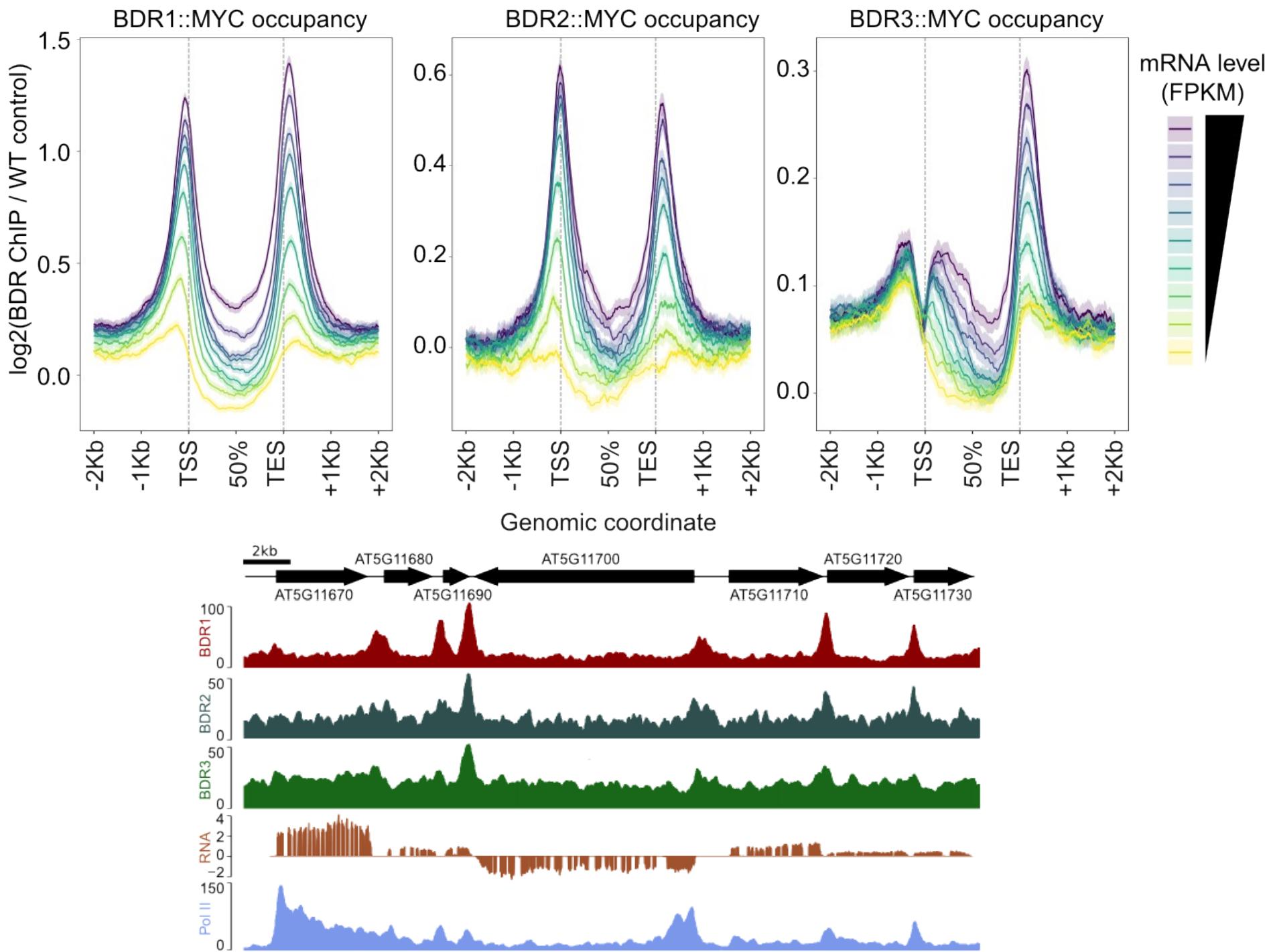


BDRs are putative negative transcription elongation factors

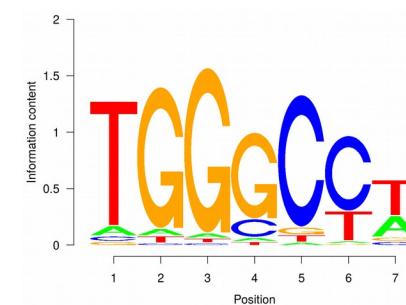
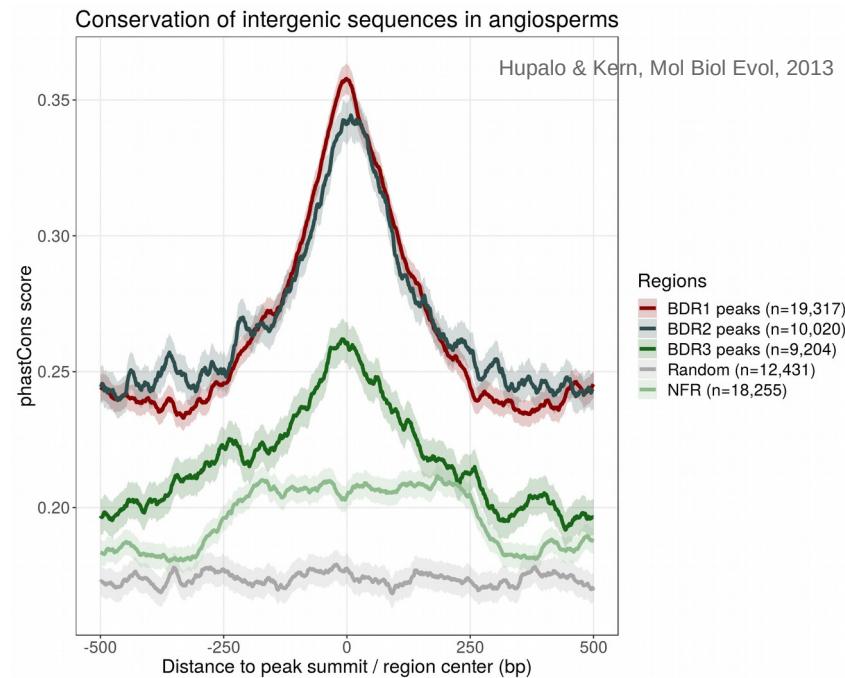
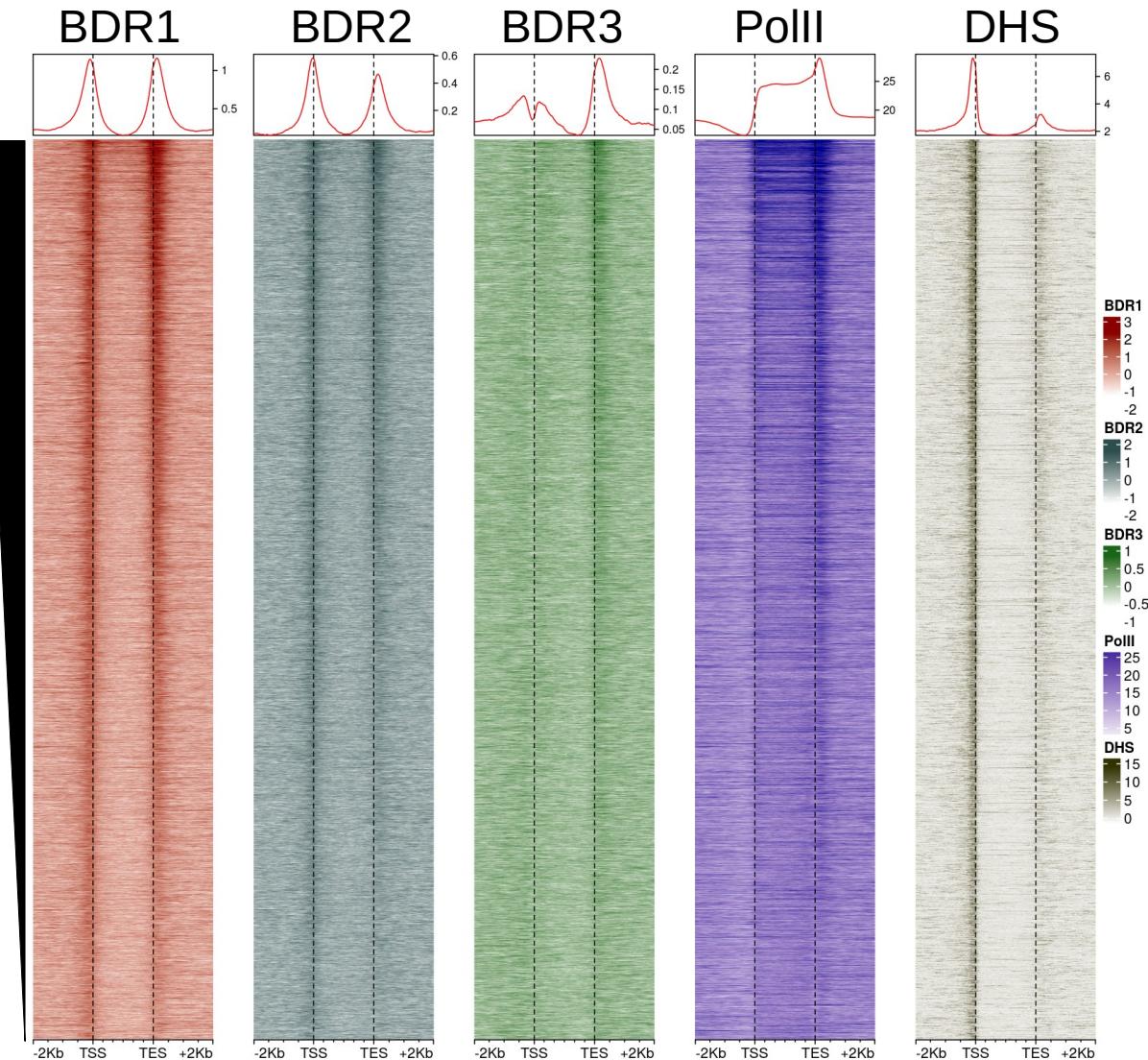


→ Short root phenotype in *bdr1,2,3* is partially reversed by inhibitors of transcription elongation

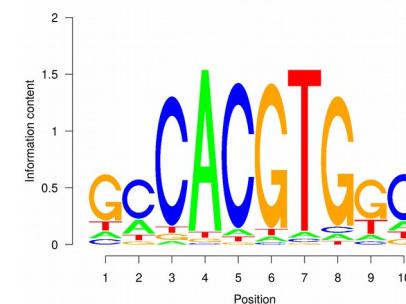
BDRs are enriched at gene borders



BDR peaks contain evolutionarily conserved sequences

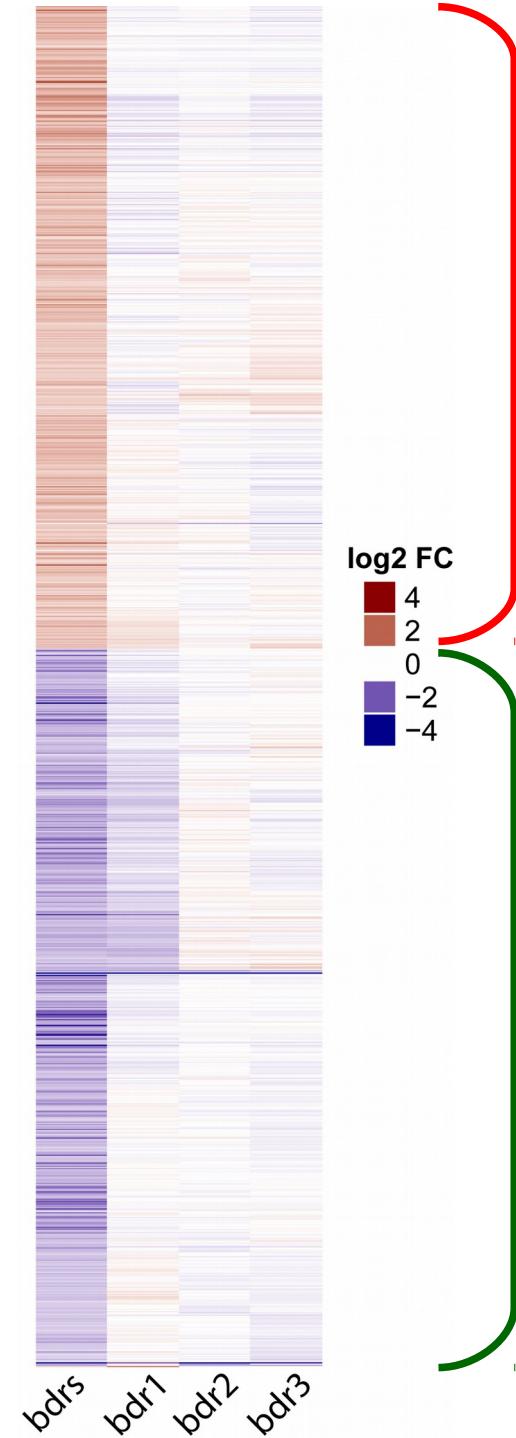


TCP-like
(~45 % of peaks)



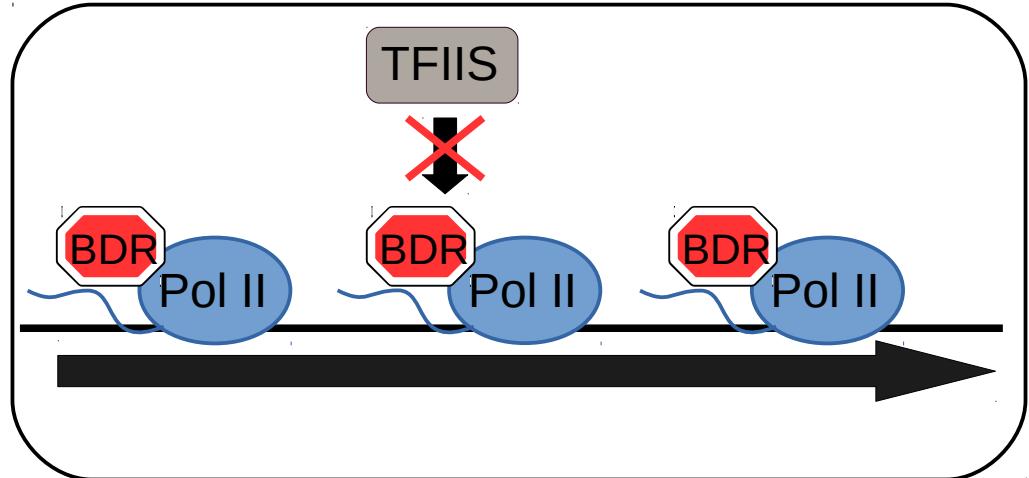
E-box
(7 % of peaks)

BDR proteins regulate the expression of hundreds of genes



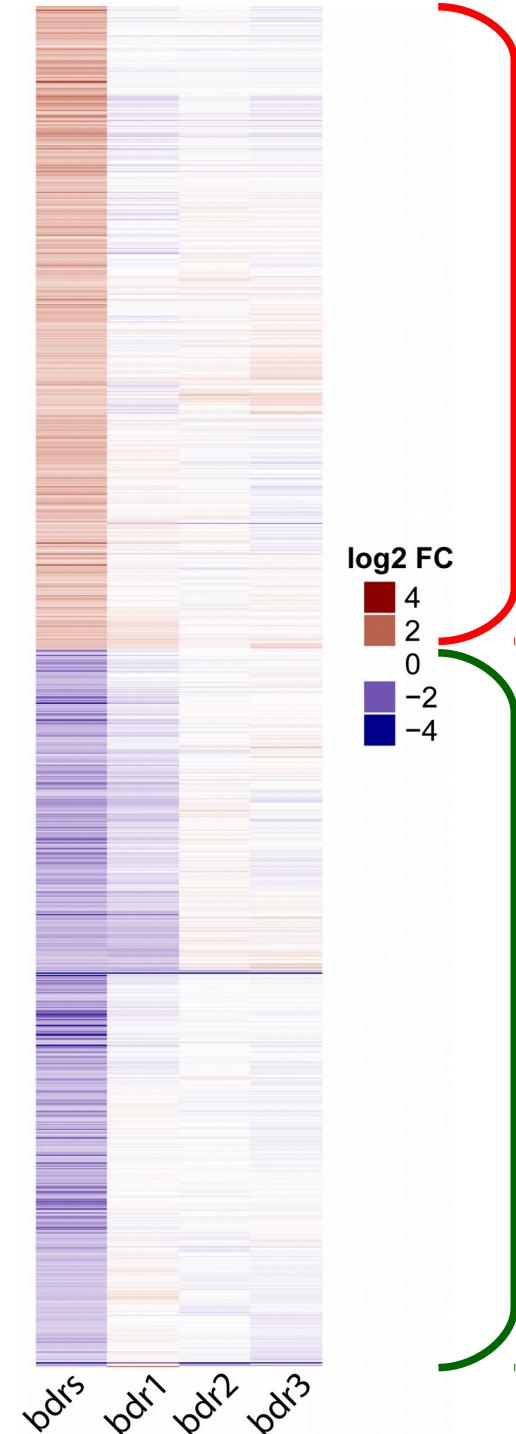
529 genes
upregulated

Negative transcription elongation factor

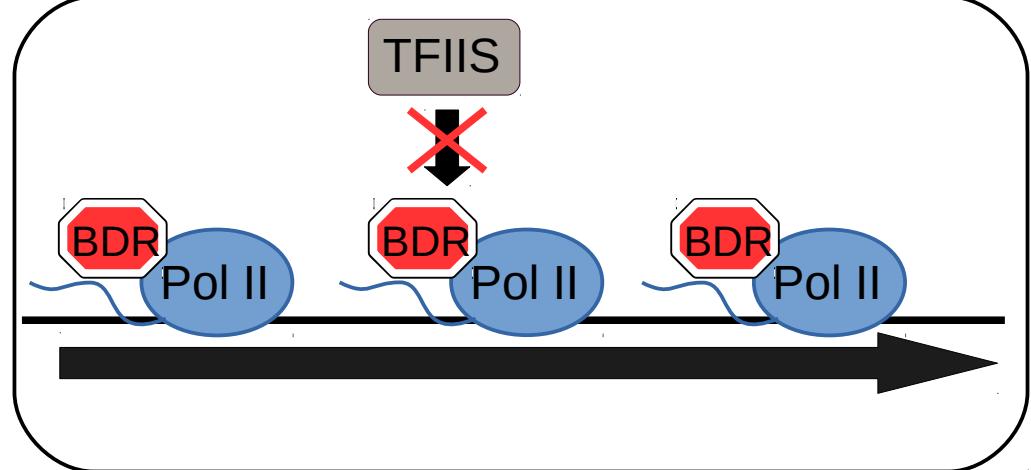


592 genes
downregulated

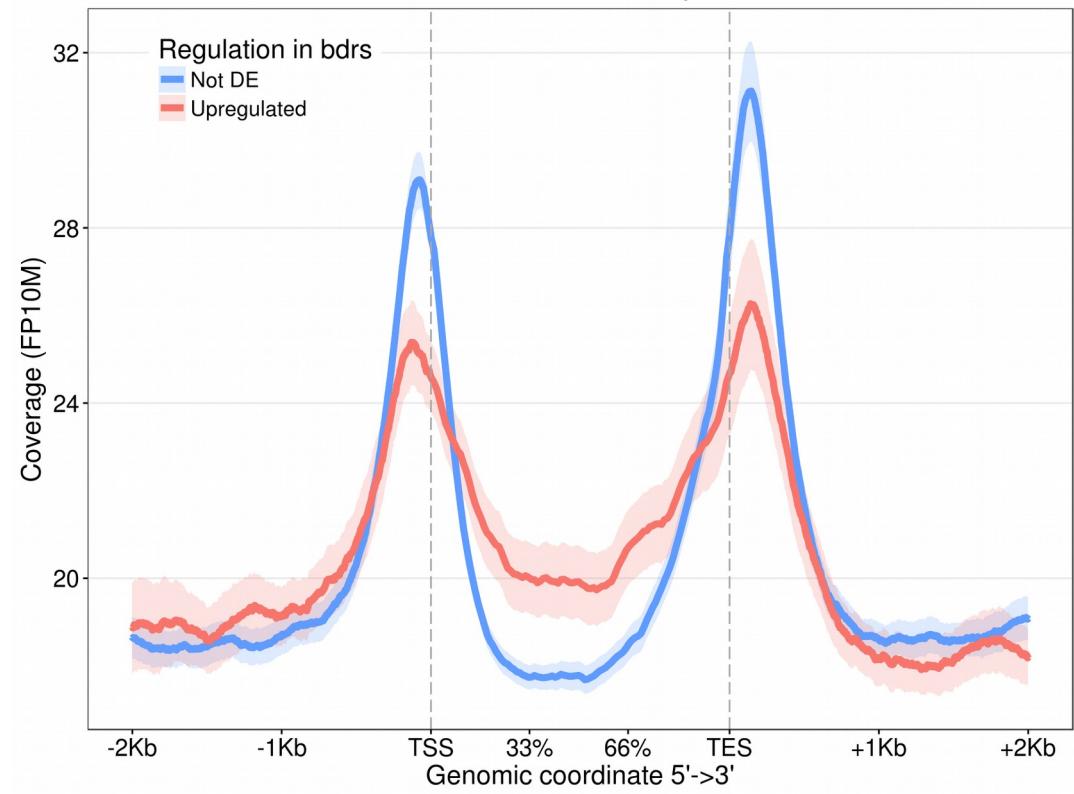
BDR proteins regulate the expression of hundreds of genes



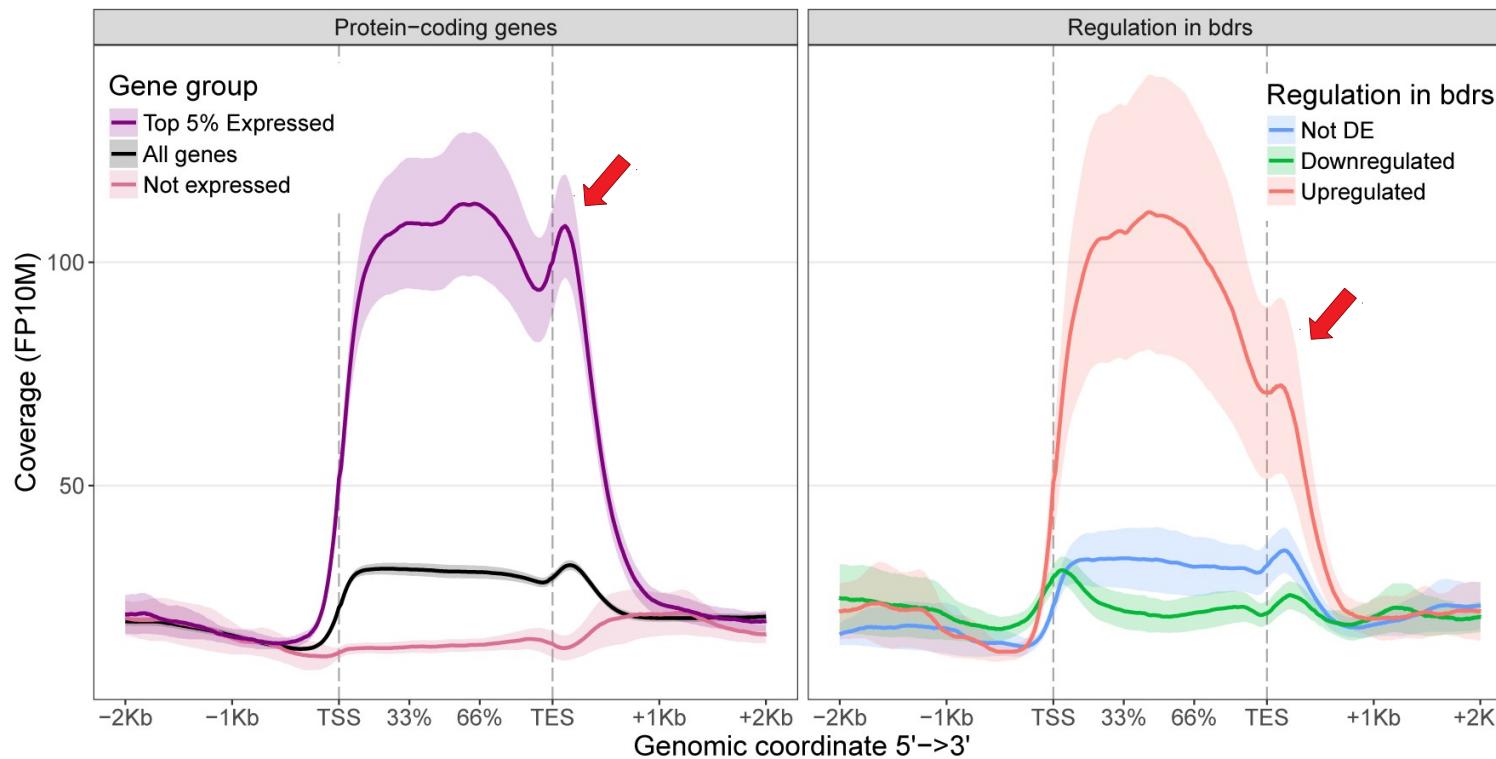
Negative transcription elongation factor



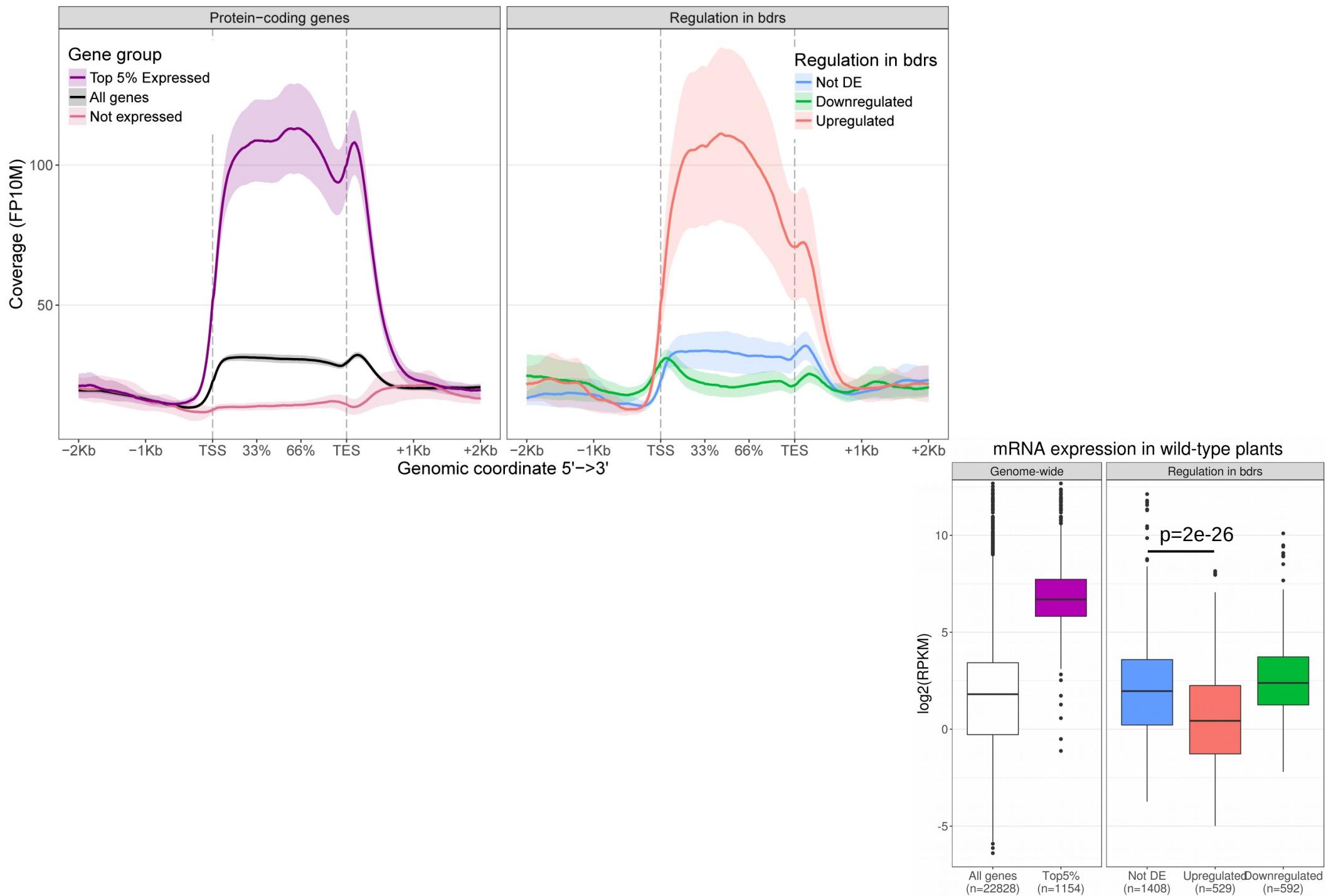
BDR1 ChIPseq



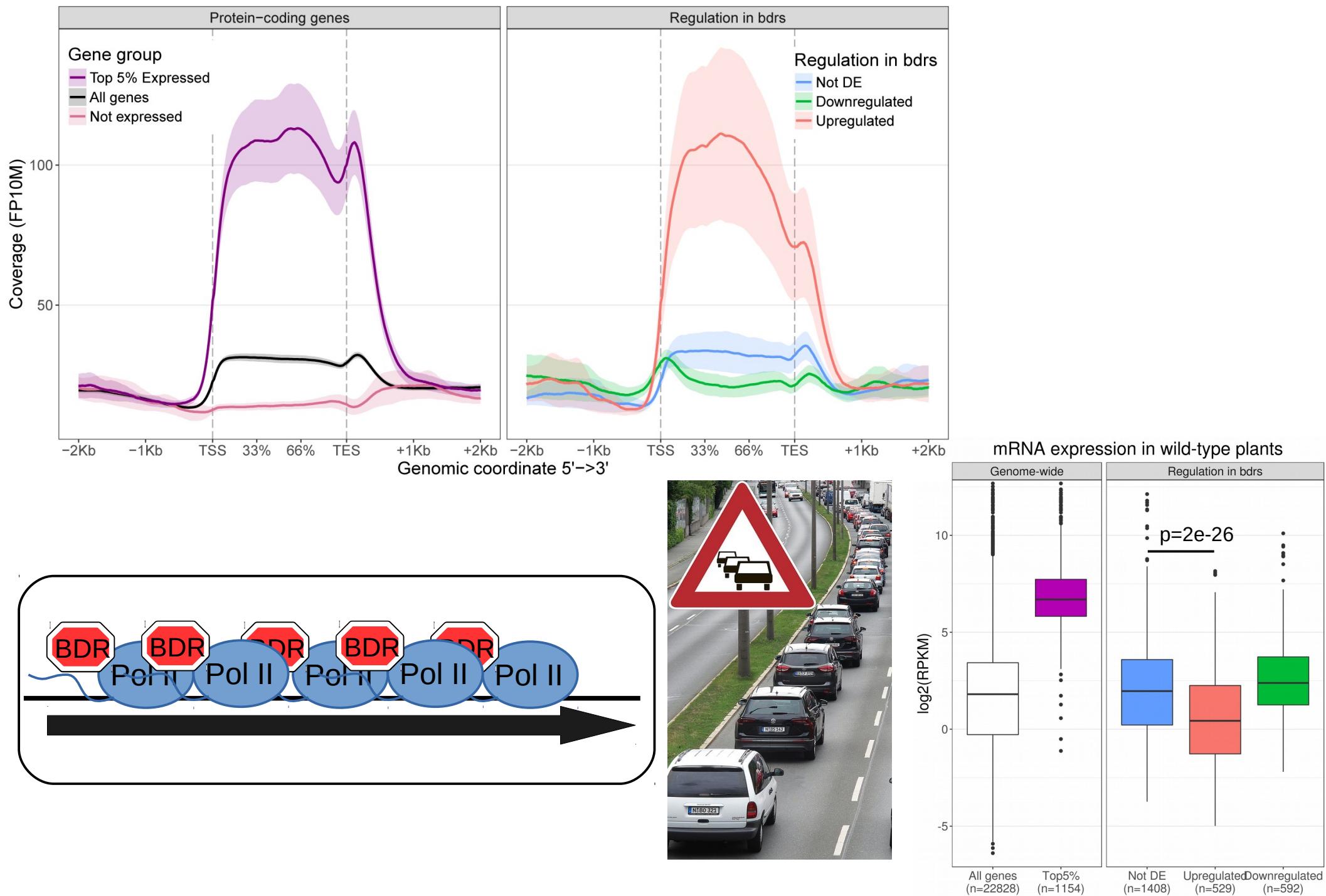
Upregulated genes have high RNAPII but low RNA levels



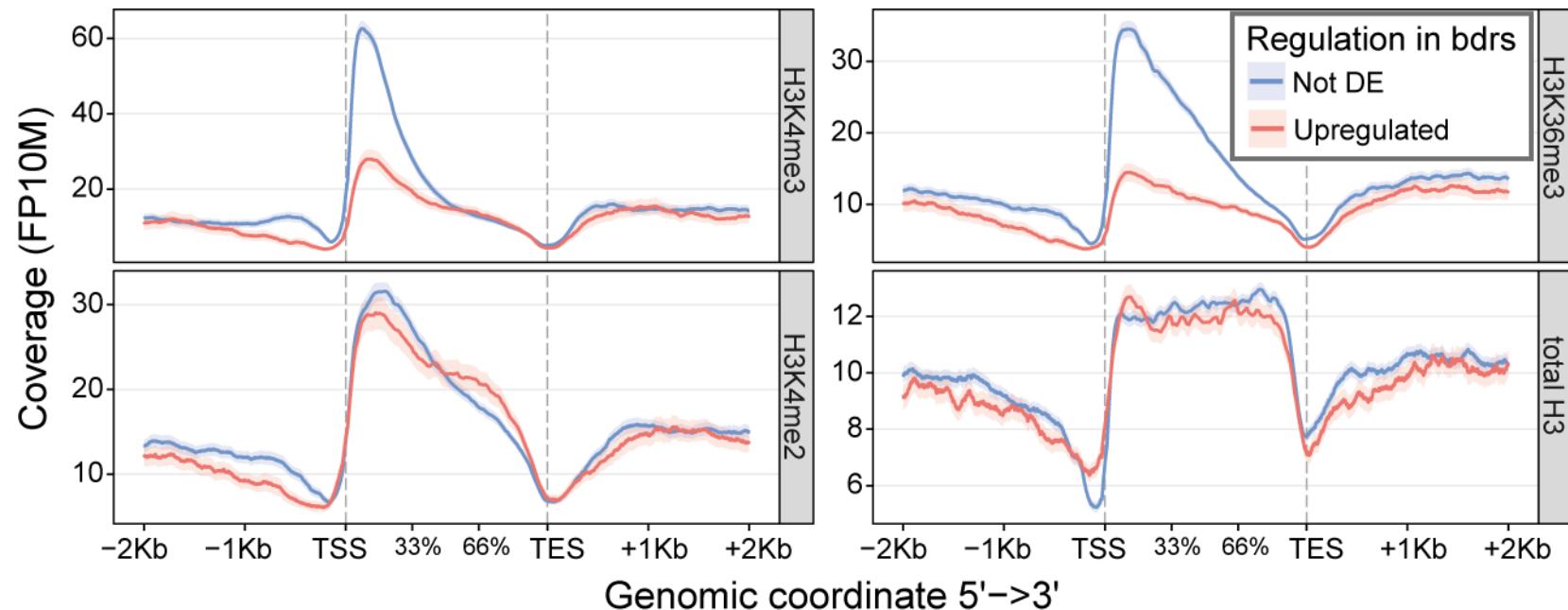
Upregulated genes have high RNAPII but low RNA levels



Upregulated genes have high RNAPII but low RNA levels



Evidences of increased elongation in bdr1,2,3 mutant



Genes

← AT3G44100
protein_coding_gene

← AT3G44110
protein_coding_gene

→ AT3G44115
protein_coding_gene

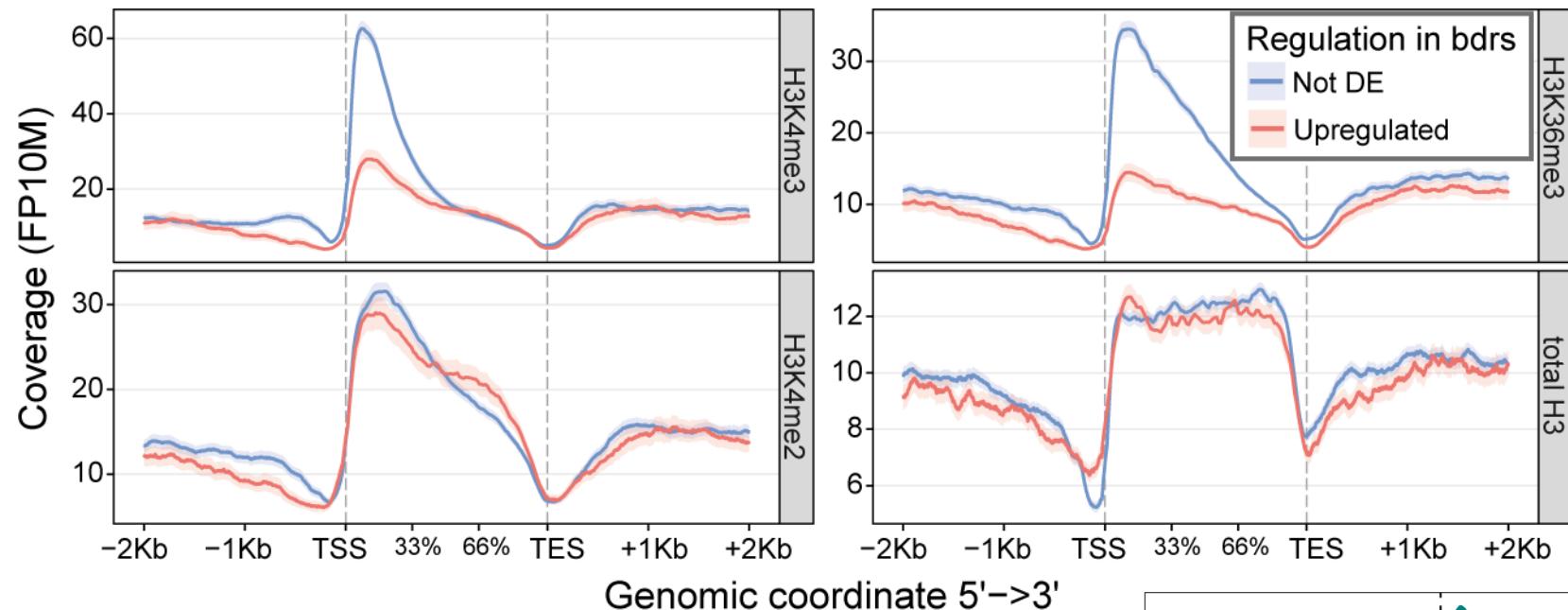
RNA

H3K36me3

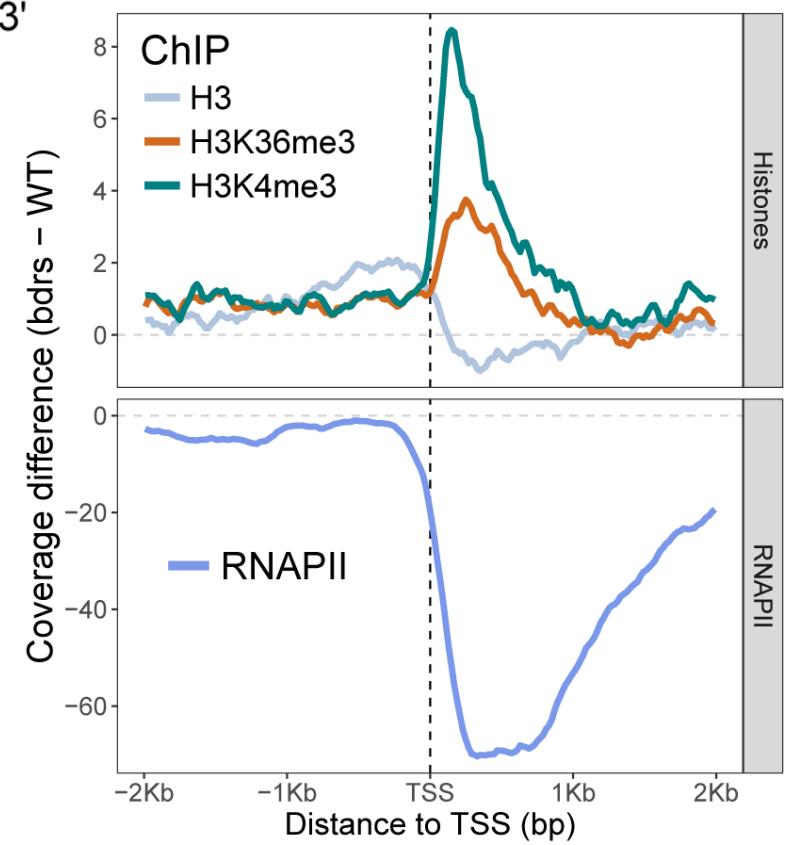
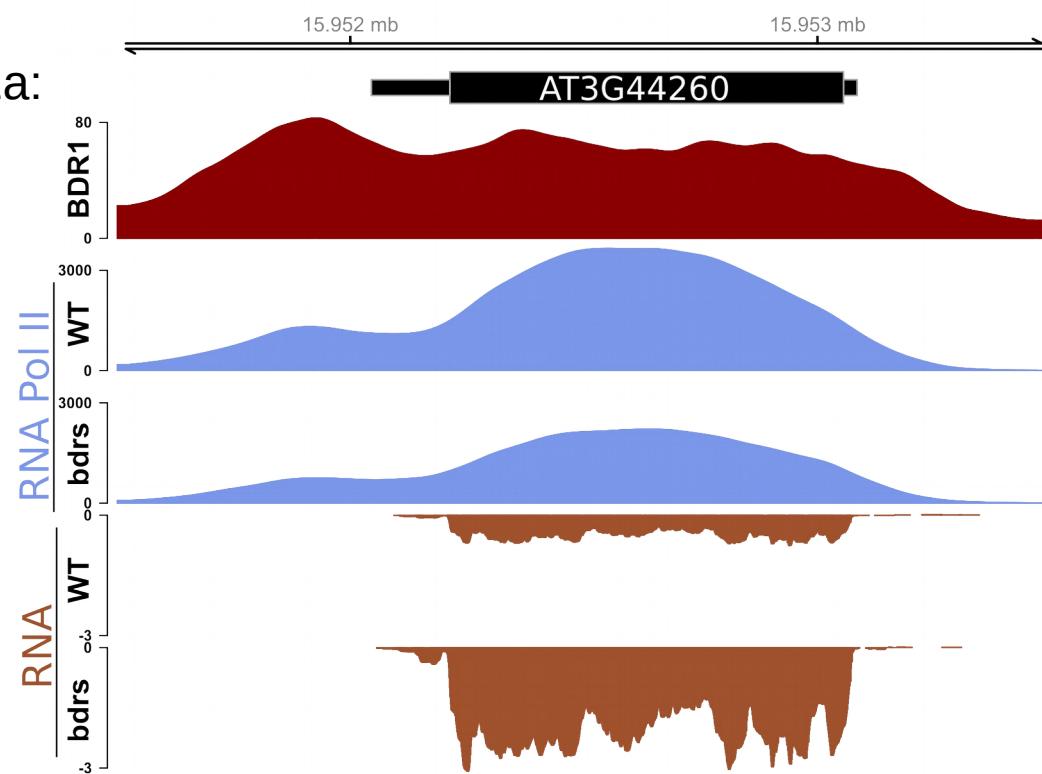
H3K4me3

RNAPII

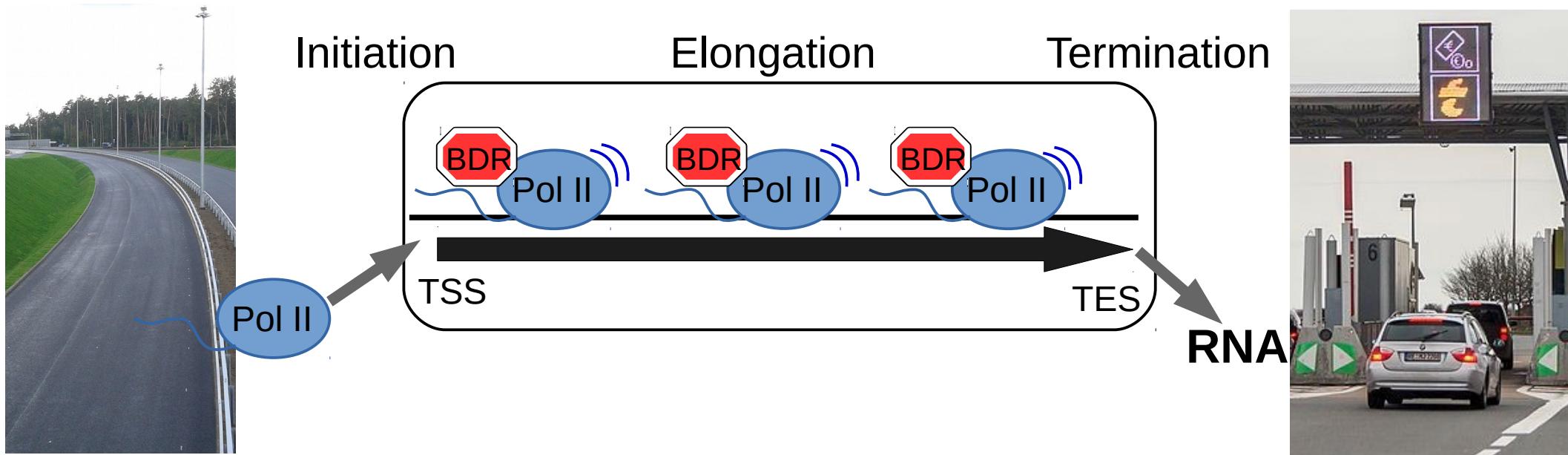
Evidences of increased elongation in bdr1,2,3 mutant



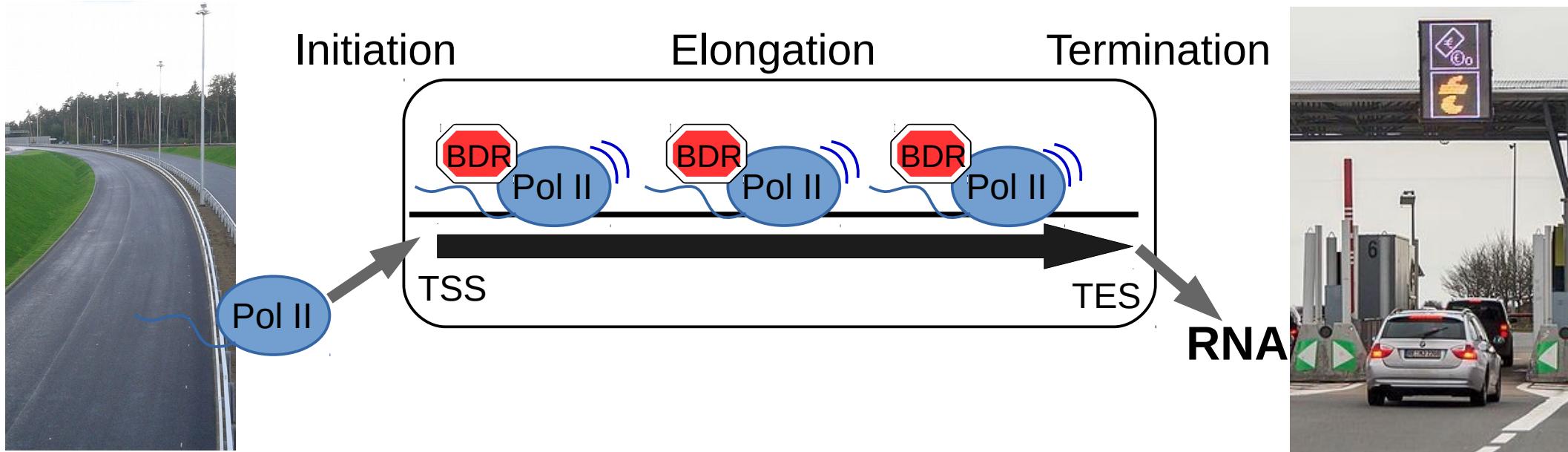
CAF1a:



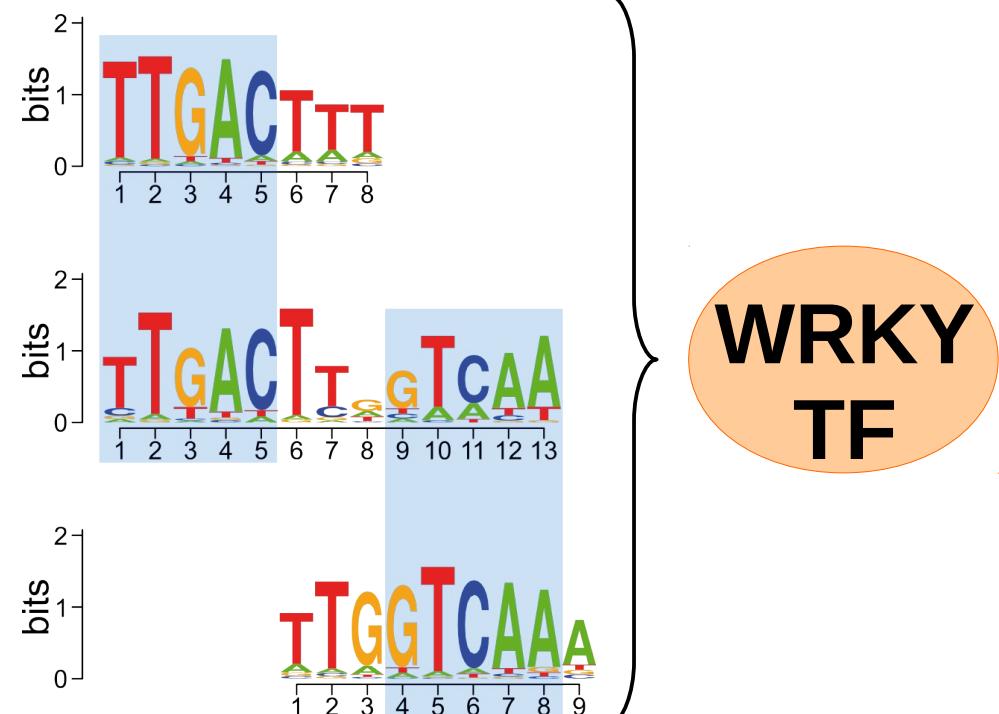
The environment/TFs define the repertoires of upregulated genes



The environment/TFs define the repertoires of upregulated genes



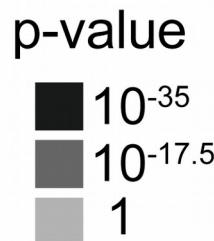
W-box motifs



GO functions enriched in genes upregulated in bdrs mutant

salicylic acid mediated signaling pathway
systemic acquired resistance
salicylic acid biosynthetic process
response to wounding
jasmonic acid mediated signaling pathway
protein targeting to membrane
response to ethylene
defense response to bacterium
respiratory burst involved in defense response
response to chitin
defense response to fungus
amino acid transport
negative regulation of programmed cell death
cellular response to hypoxia
regulation of plant-type hypersensitive response
oligopeptide transport
MAPK cascade
negative regulation of defense response

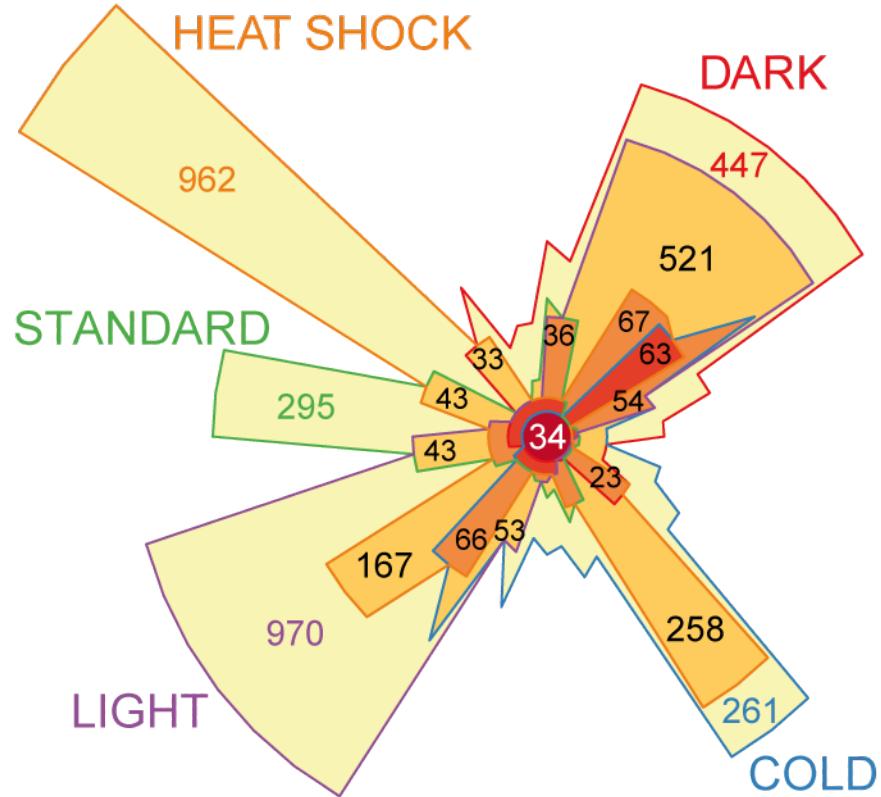
regulation of defense response
response to molecule of bacterial origin
regulation of hydrogen peroxide metab...
defense response to fungus, incompat...
hypersensitive salinity response
intracellular signal transduction
regulation of multi-organism process
regulation of immune response
response to wounding
response to bacterium
ethylene-activated signaling pathway
endoplasmic reticulum unfolded prot...
response to virus
defense response by callose deposition
detection of biotic stimulus
detection of bacterium
ethylene biosynthetic process
response to jasmonic acid
response to nitrate
jasmonic acid biosynthetic process
hypothalamic catabolic pathway
response to other organism
abscisic acid-activated signaling pathway
systemic acquired resistance, salicyl...
response to herbicide
regulation of defense response
pathogen-associated molecular pattern...
indoleacetic acid biosynthetic process



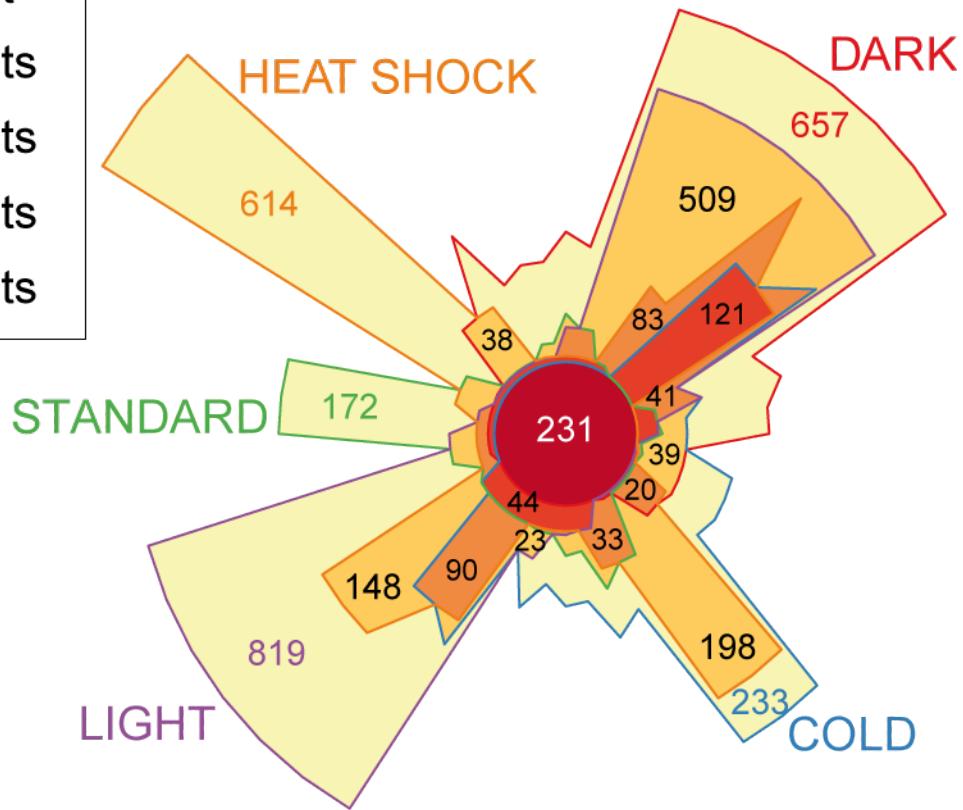
Increased mRNA expressions depend on growth conditions

Regulation in bdrs mutant vs wild-type

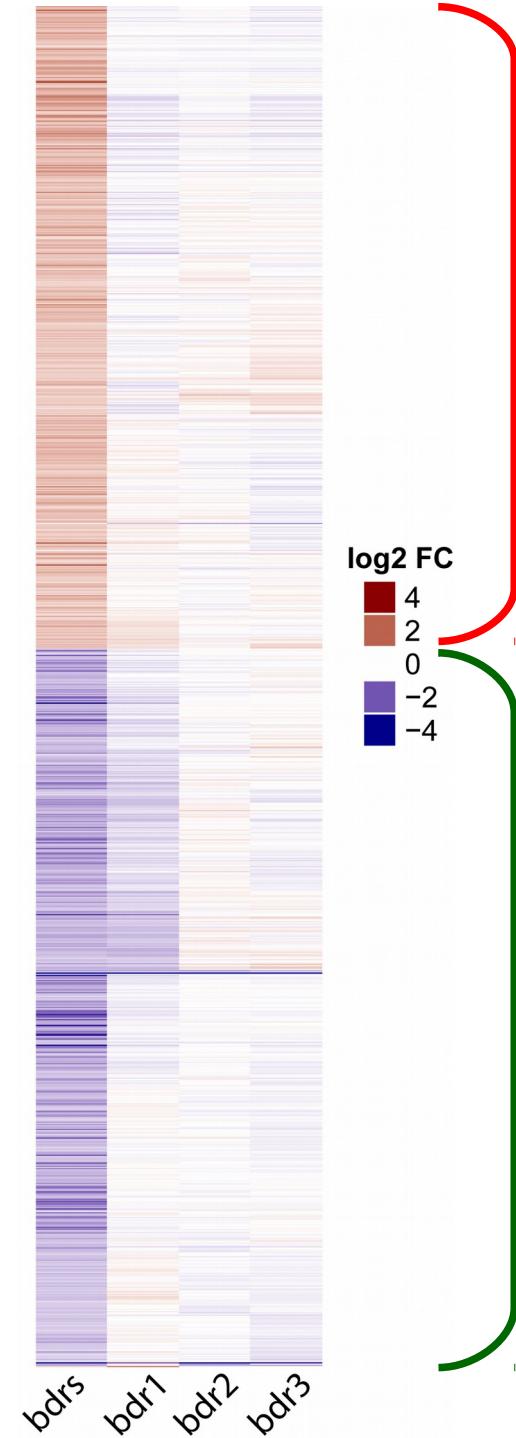
Genes upregulated



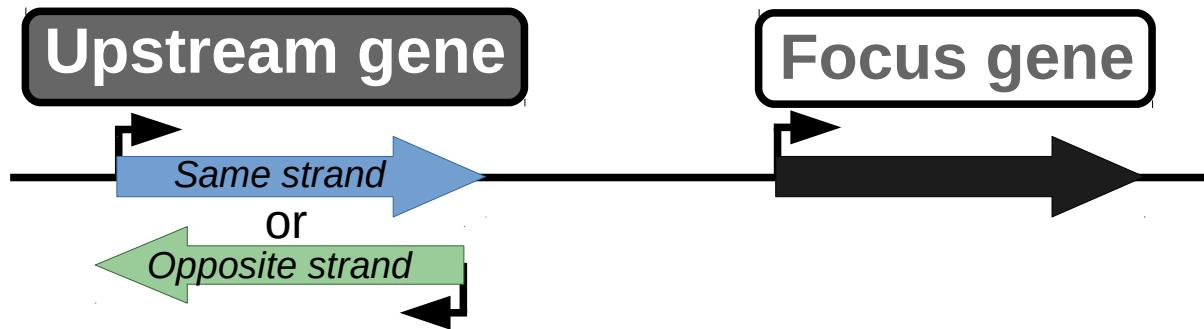
Genes downregulated



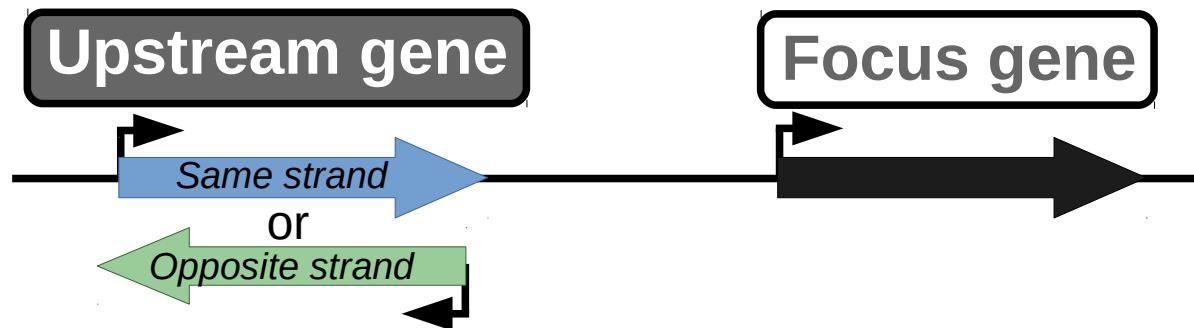
BDR proteins regulate the expression of hundreds of genes



Downregulated genes have a specific upstream gene neighborhood

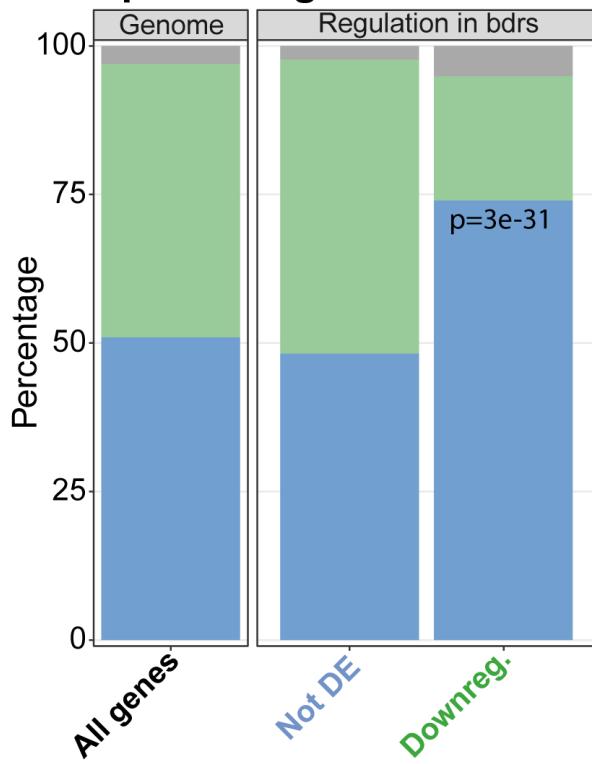


Downregulated genes have a specific upstream gene neighborhood



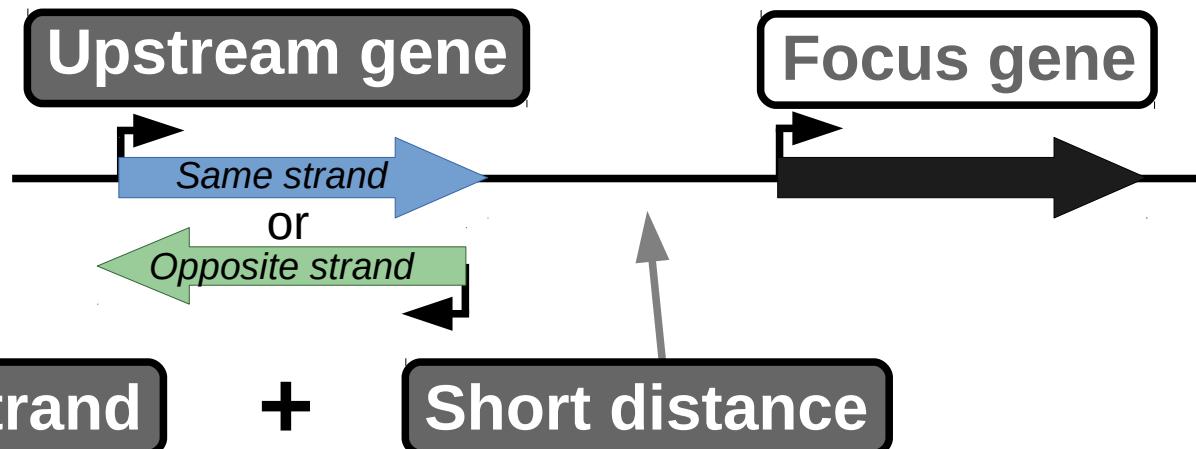
Same Strand

Upstream gene orientation



- Overlap
- Opposite Strand
- Same Strand

Downregulated genes have a specific upstream gene neighborhood

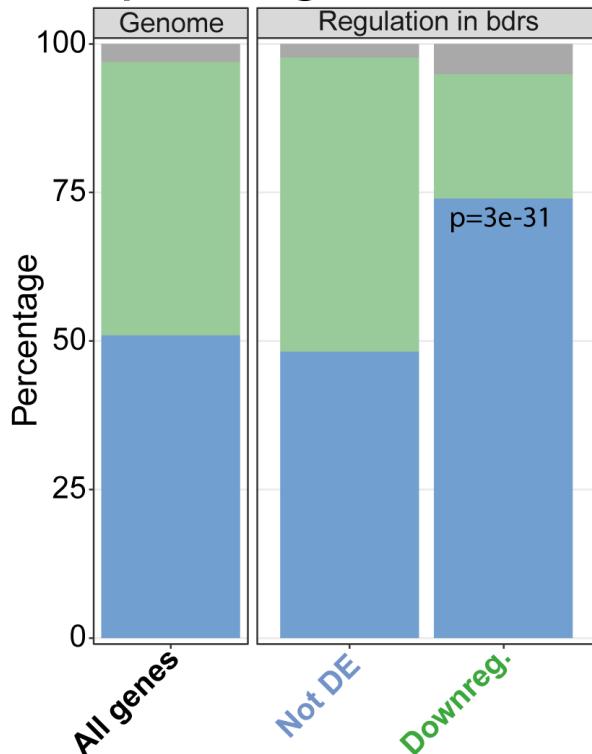


Same Strand

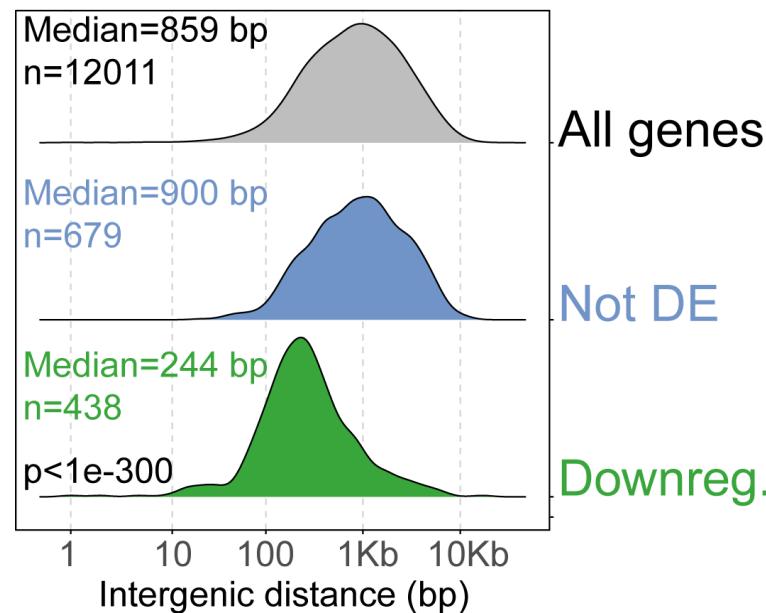
+

Short distance

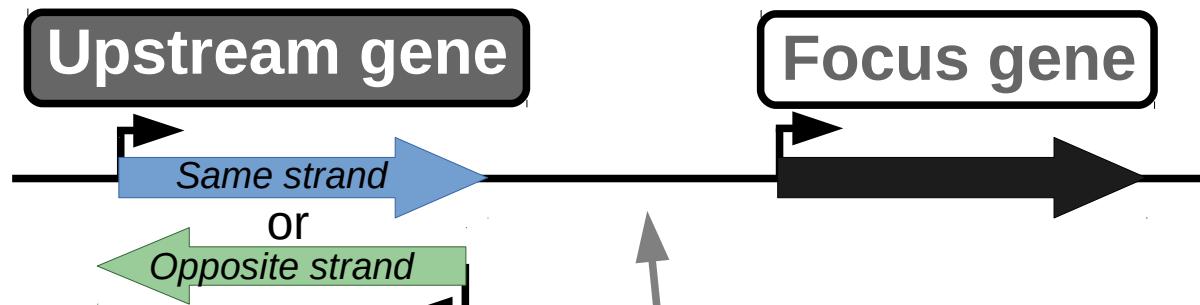
Upstream gene orientation



Upstream intergenic distance



Downregulated genes have a specific upstream gene neighborhood

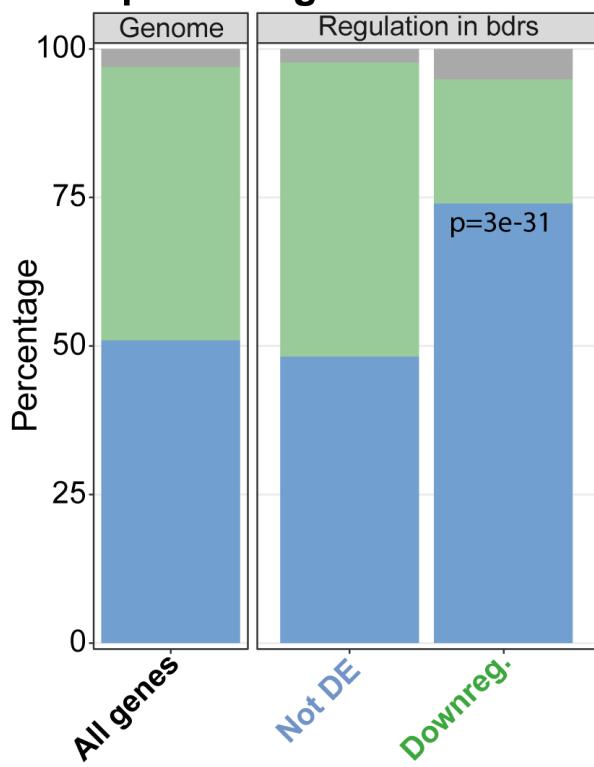


Same Strand

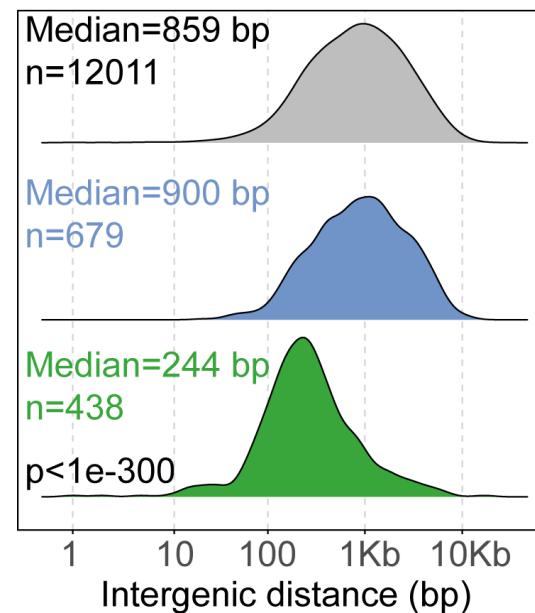
Short distance

Highly expressed

Upstream gene orientation



Upstream intergenic distance

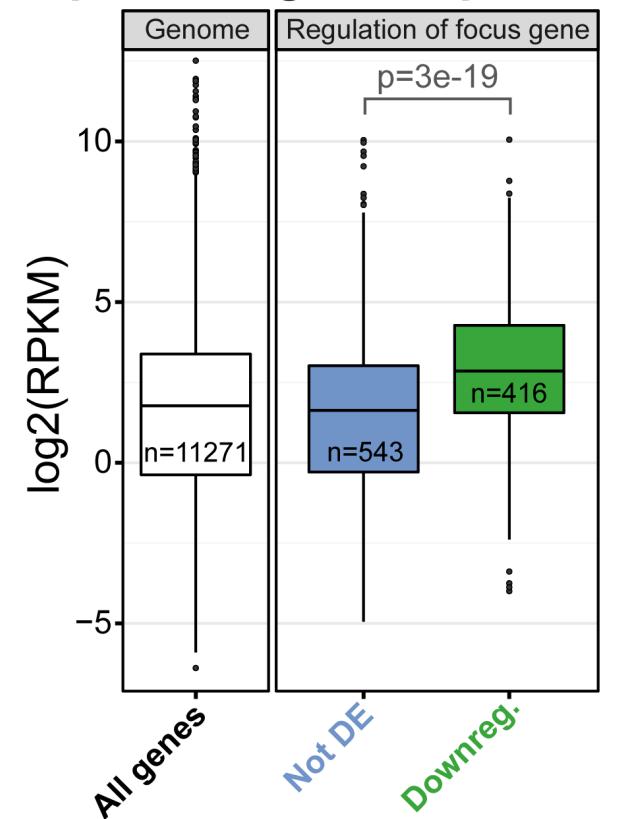


All genes

Not DE

Downreg.

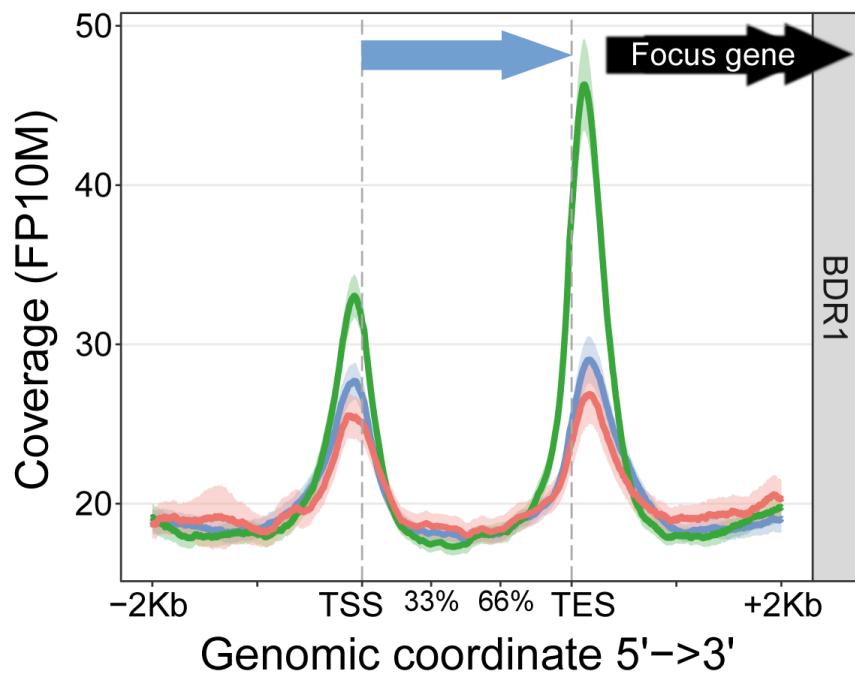
Upstream gene expression



Downregulated genes have a specific upstream gene neighborhood

+ High BDR at 3' end

BDR occupancy at upstream tandem gene



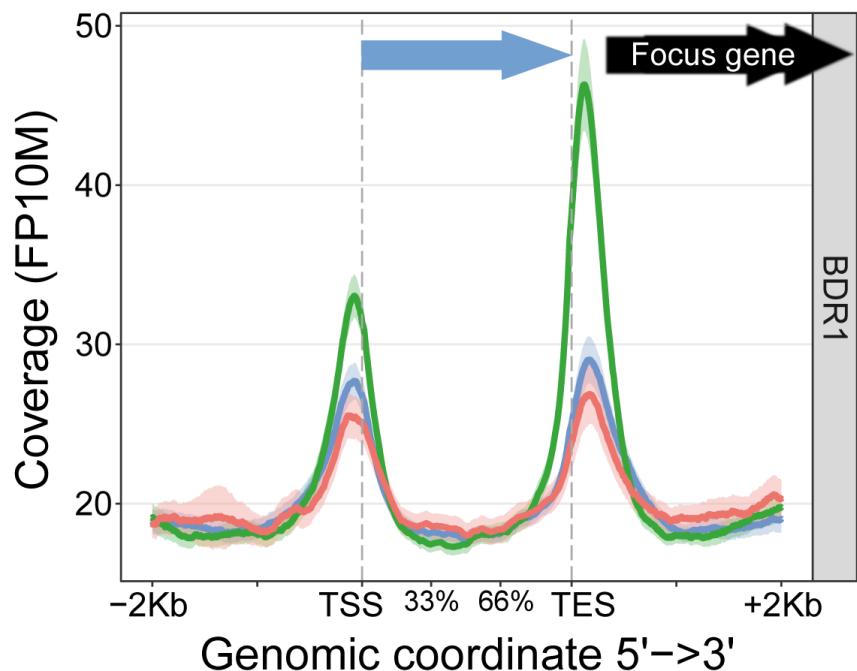
Focus gene in bdrs:

- Not DE
- Downregulated
- Upregulated

Downregulated genes have a specific upstream gene neighborhood

+ **High BDR at 3' end**

BDR occupancy at upstream tandem gene

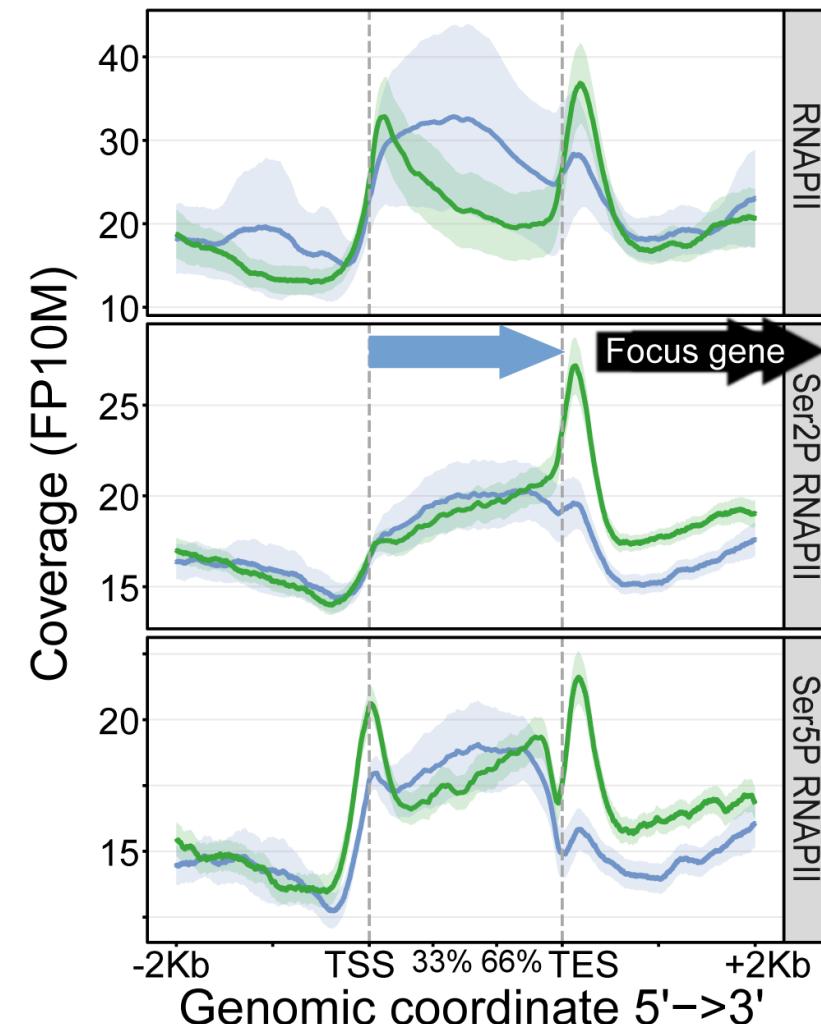


Focus gene in bdrs:

- Not DE
- Downregulated
- Upregulated

+ **3' RNAPII pausing**

RNAPII at upstream tandem genes

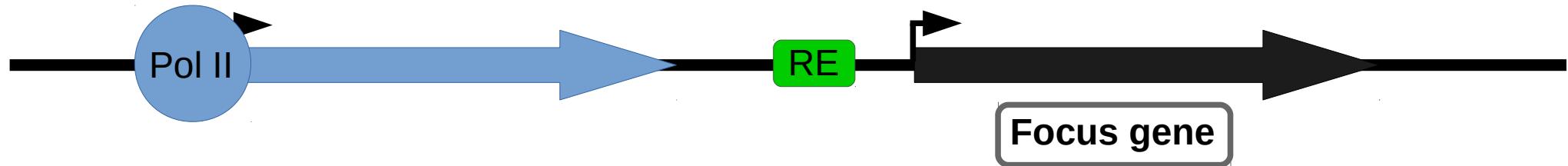


Focus gene in bdrs:

- Not DE
- Downregulated

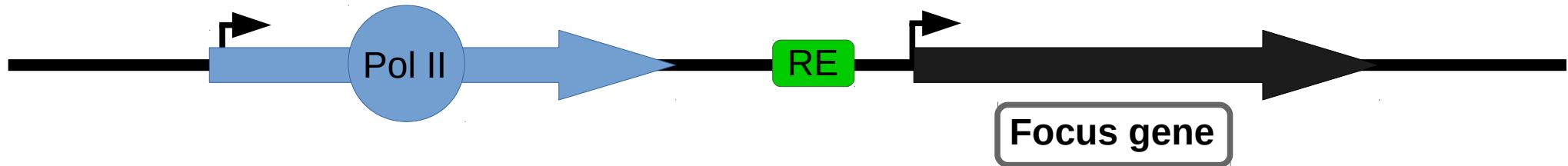
Transcriptional interference ?

Shearwin et al., Trends Genet 2005



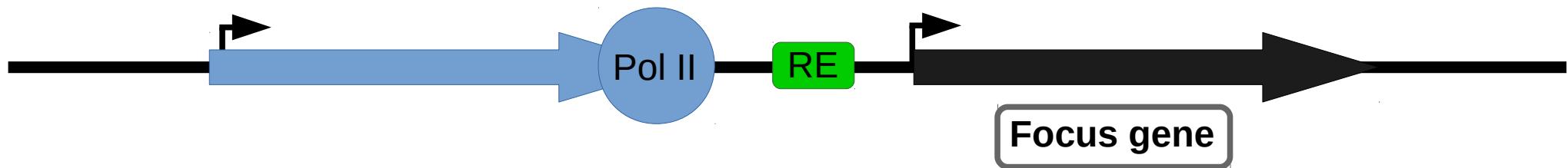
Transcriptional interference ?

Shearwin et al., Trends Genet 2005



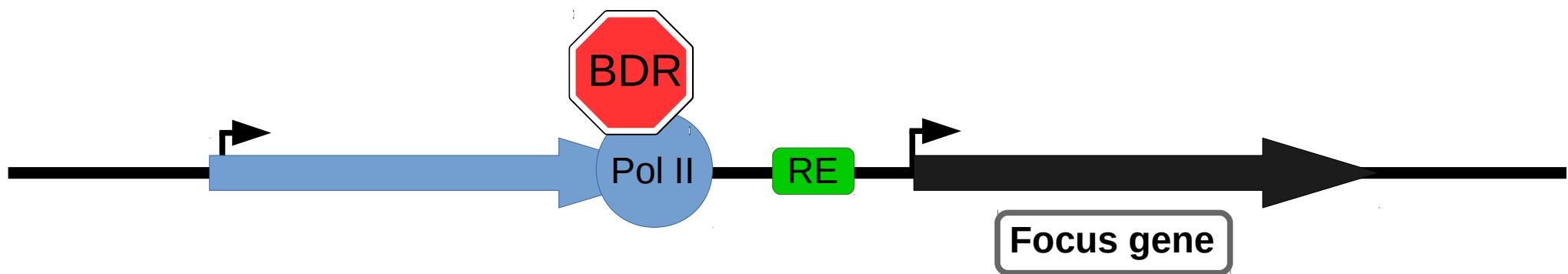
Transcriptional interference ?

Shearwin et al., Trends Genet 2005



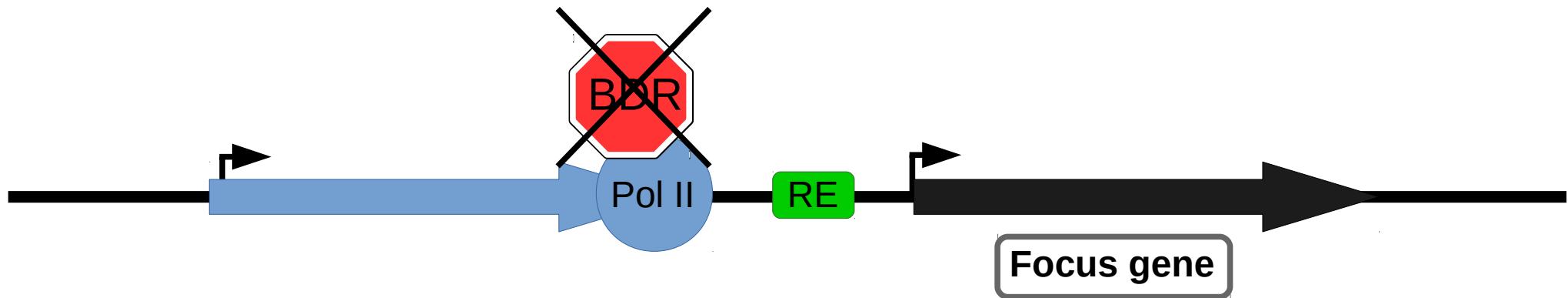
Transcriptional interference ?

Shearwin et al., Trends Genet 2005



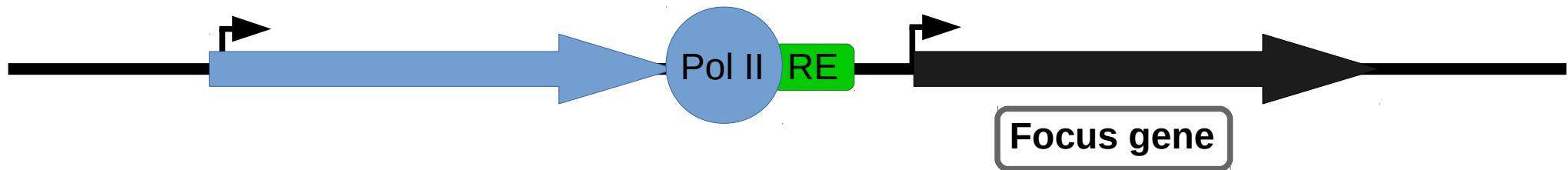
Transcriptional interference ?

Shearwin et al., Trends Genet 2005



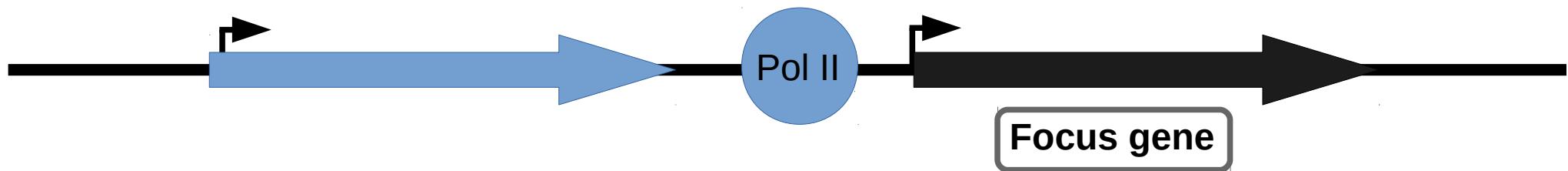
Transcriptional interference ?

Shearwin et al., Trends Genet 2005



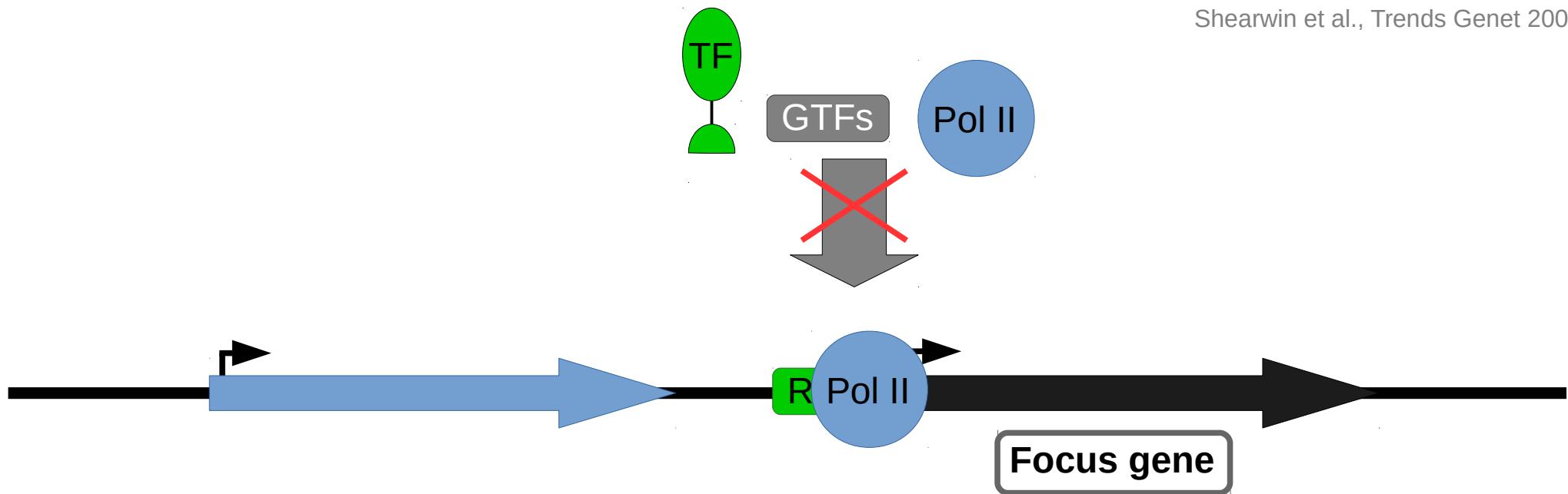
Transcriptional interference ?

Shearwin et al., Trends Genet 2005



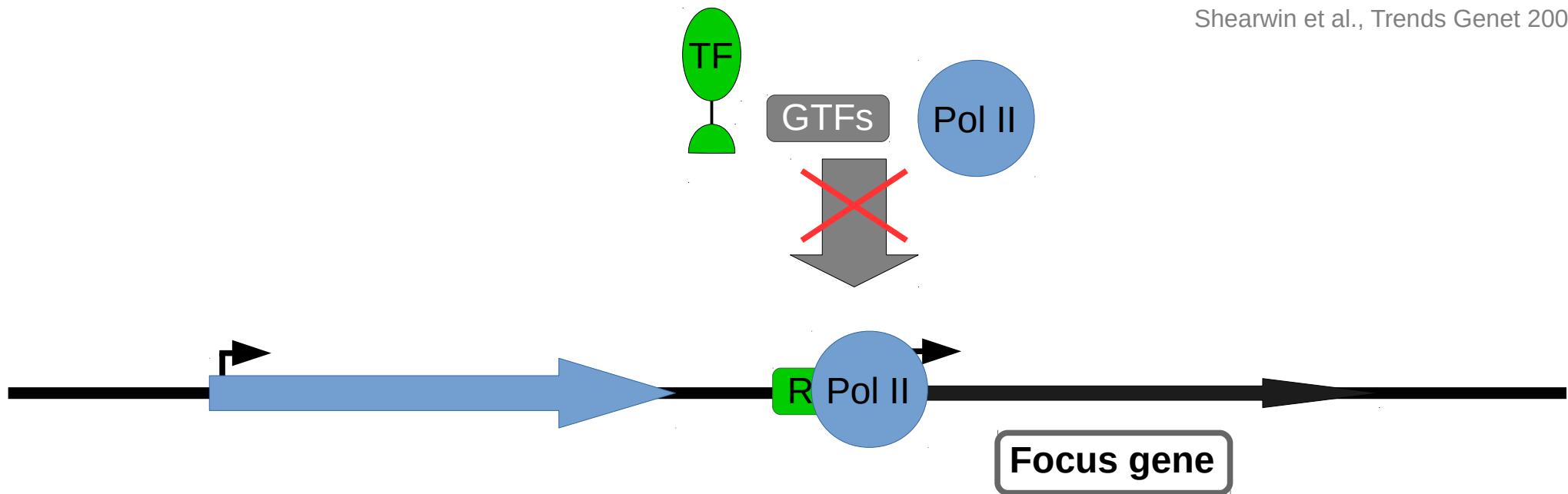
Transcriptional interference ?

Shearwin et al., Trends Genet 2005



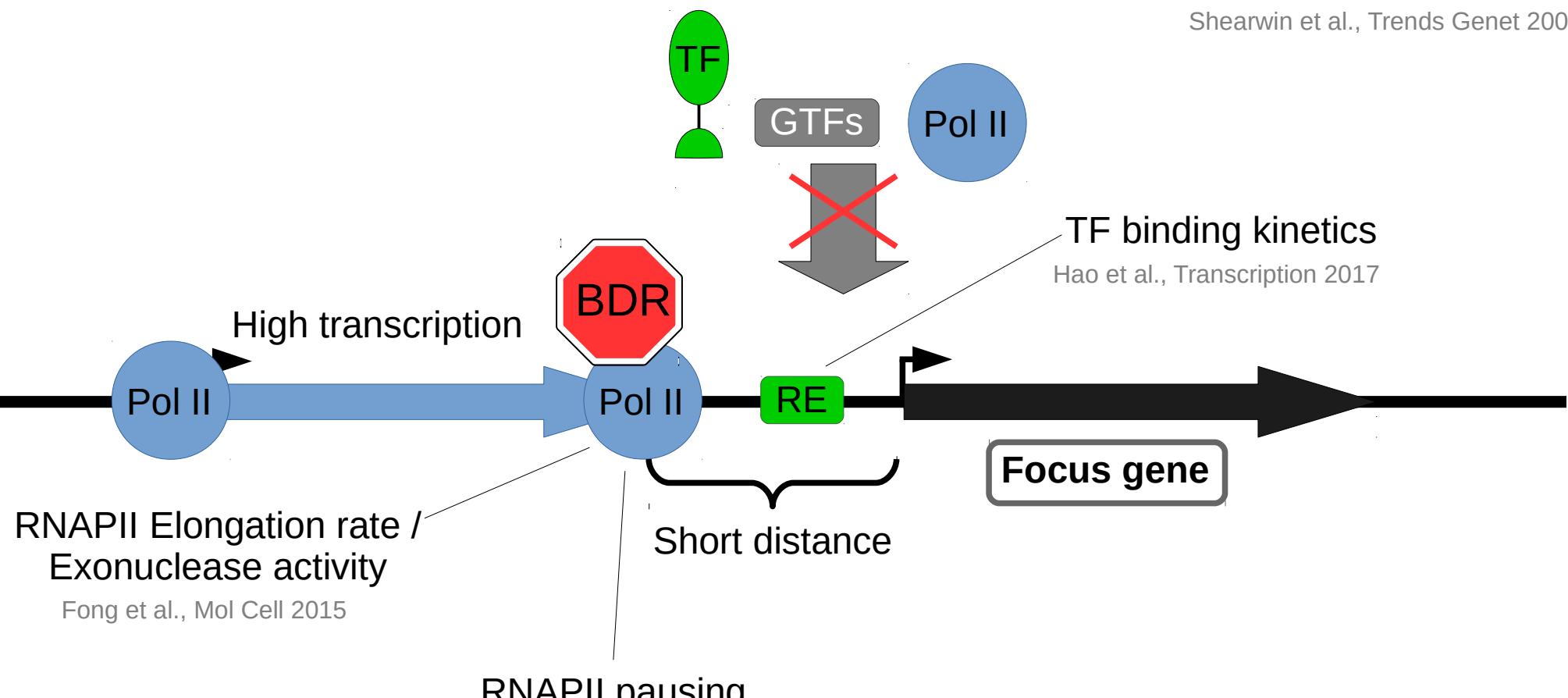
Transcriptional interference ?

Shearwin et al., Trends Genet 2005

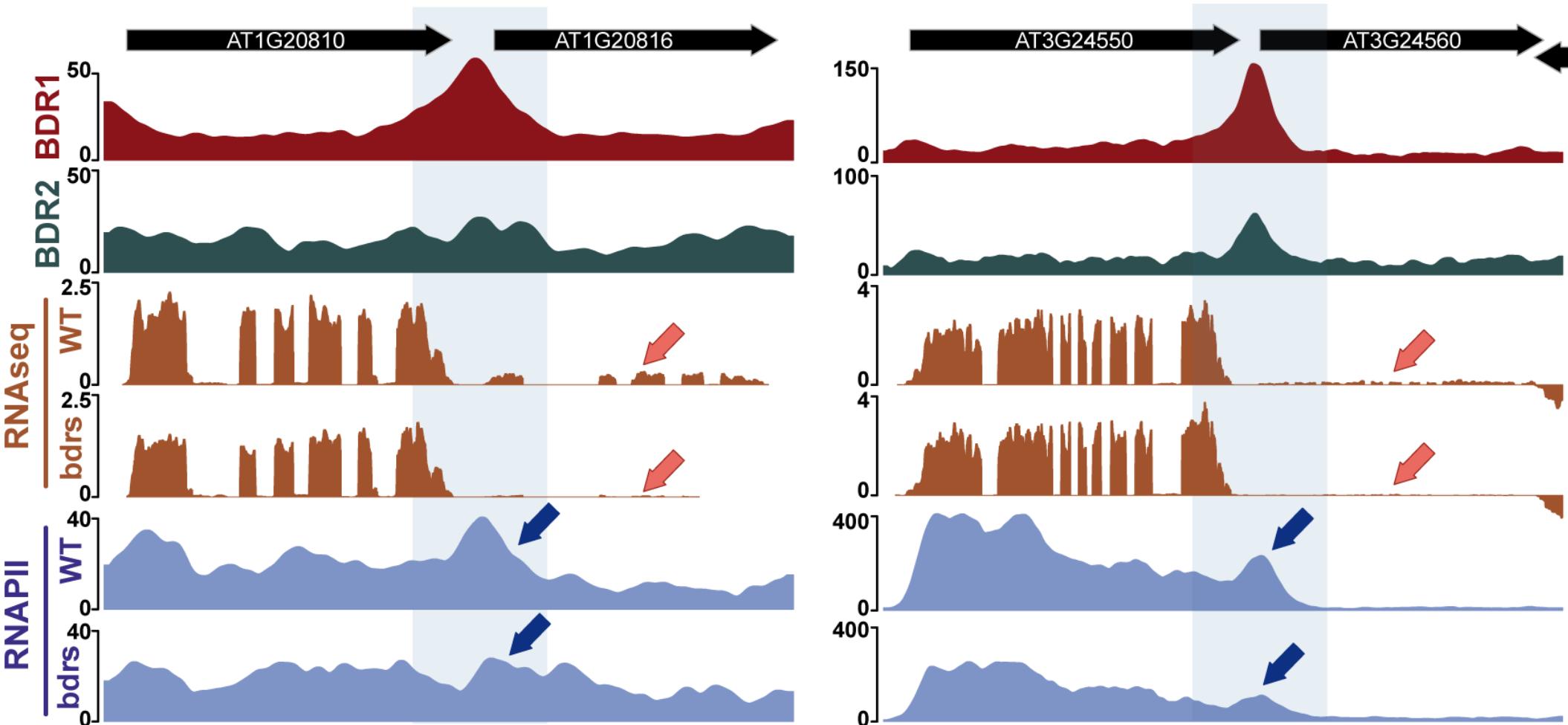


Transcriptional interference ?

Shearwin et al., Trends Genet 2005

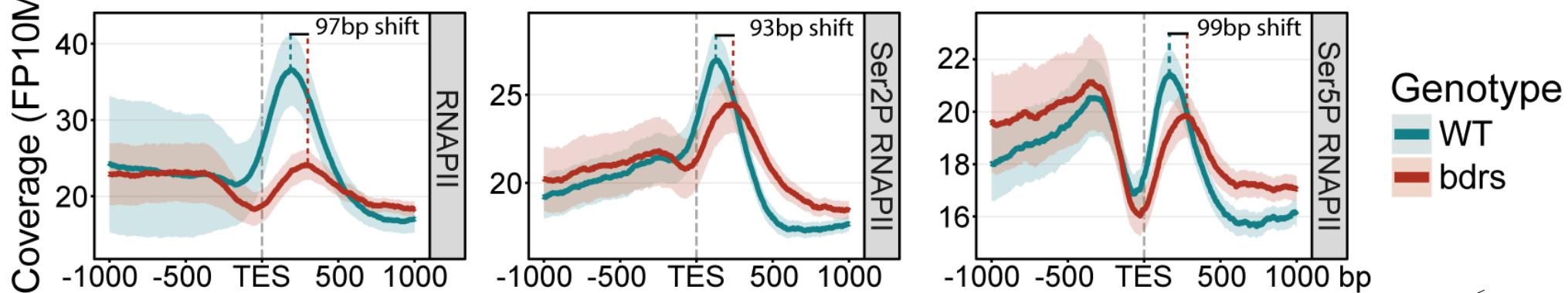


Shift of RNAPII at the 3' end in bdr1,2,3 triple mutant



Shift of RNAPII at the 3' end in bdr1,2,3 triple mutant

3' shift of RNAPII at the TES of genes upstream of **downregulated** genes



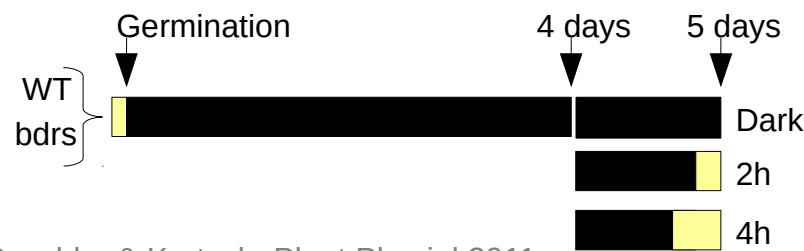
Pol II

TES

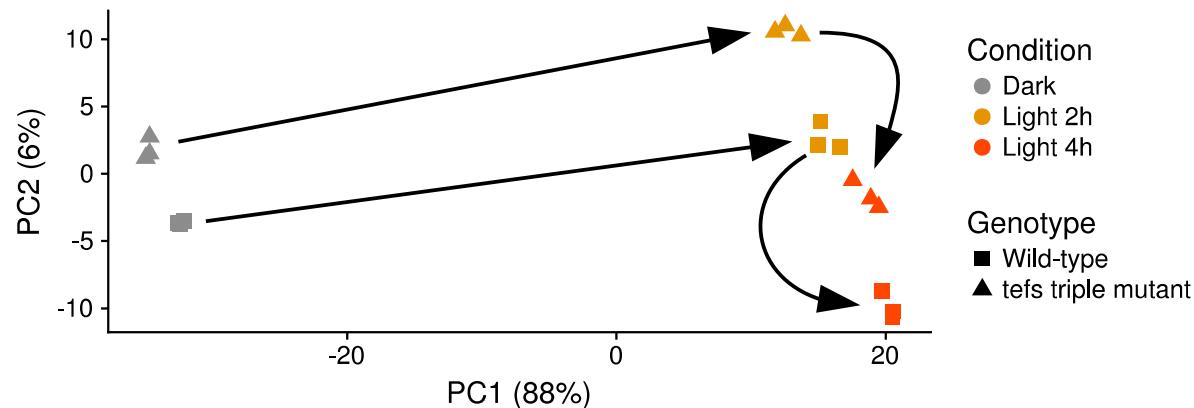
Downregulated
in bdrs mutant

Modulation of transcriptional interferences in bdr1,2,3 mutant

PHOTOMORPHOGENESIS

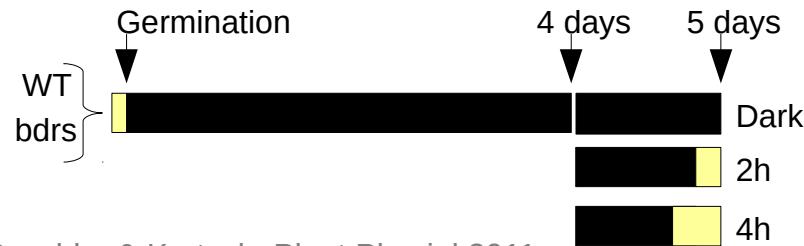


Peschke & Kretsch, Plant Physiol 2011
Bourbousse et al., PloS Genet 2012
Bourbousse et al., PNAS 2015

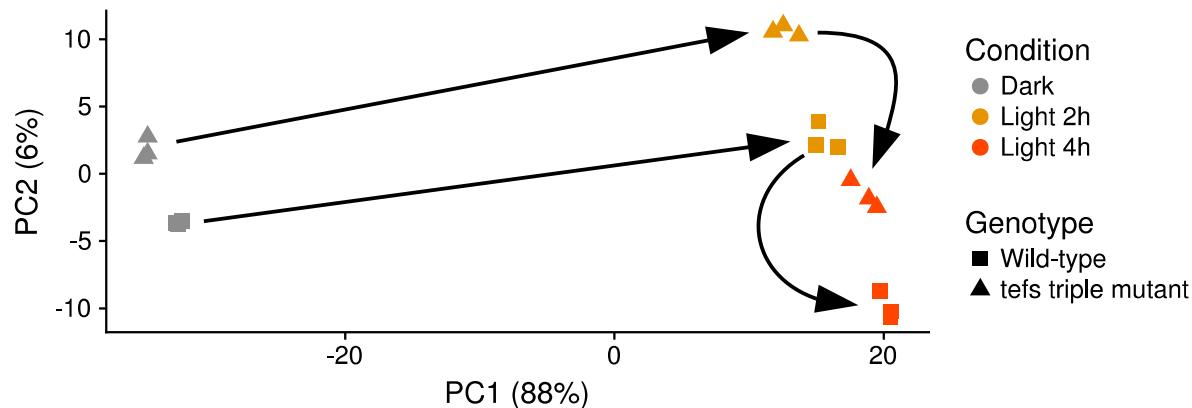


Modulation of transcriptional interferences in bdrs mutant

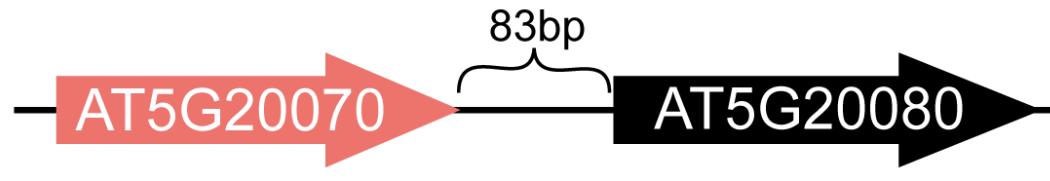
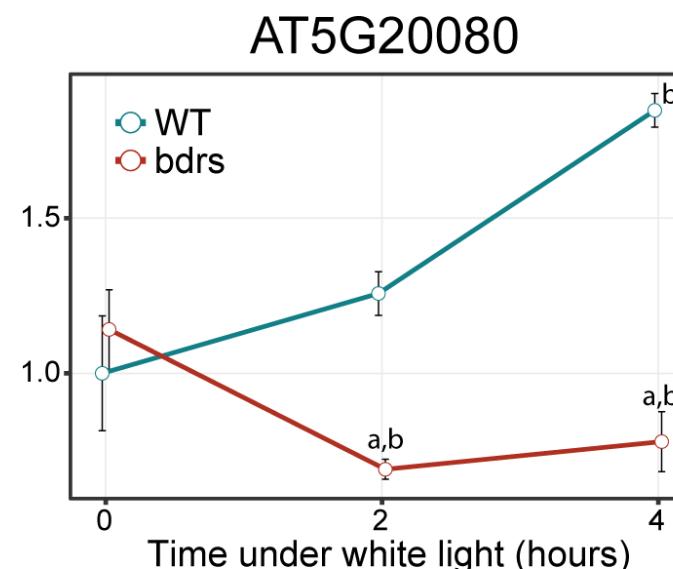
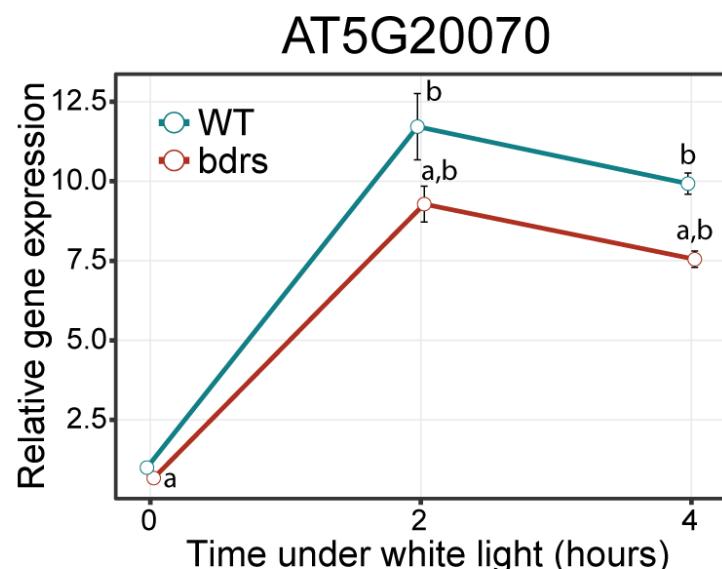
PHOTOMORPHOGENESIS



Peschke & Kretsch, Plant Physiol 2011
Bourbousse et al., PloS Genet 2012
Bourbousse et al., PNAS 2015



New transcriptional interferences in bdrs mutant



induced by light in both genotypes

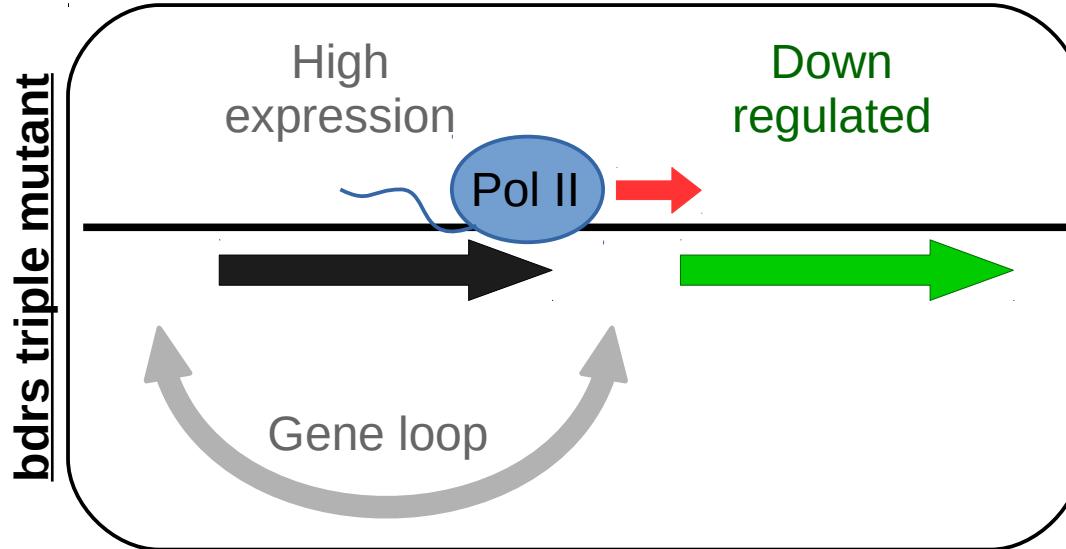
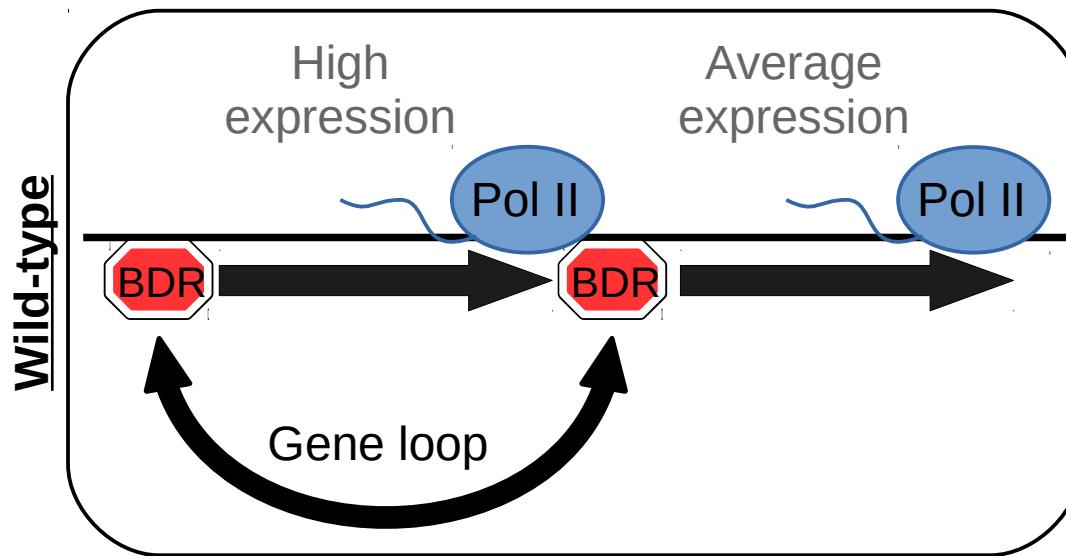
transcriptional interference in bdrs only

Conclusions

BDRs apparent functions:

- slow down PolII (negative elongation factor / 3' pausing factor)
- contribute to gene looping

Downregulated in bdrs triple mutant



Conclusions

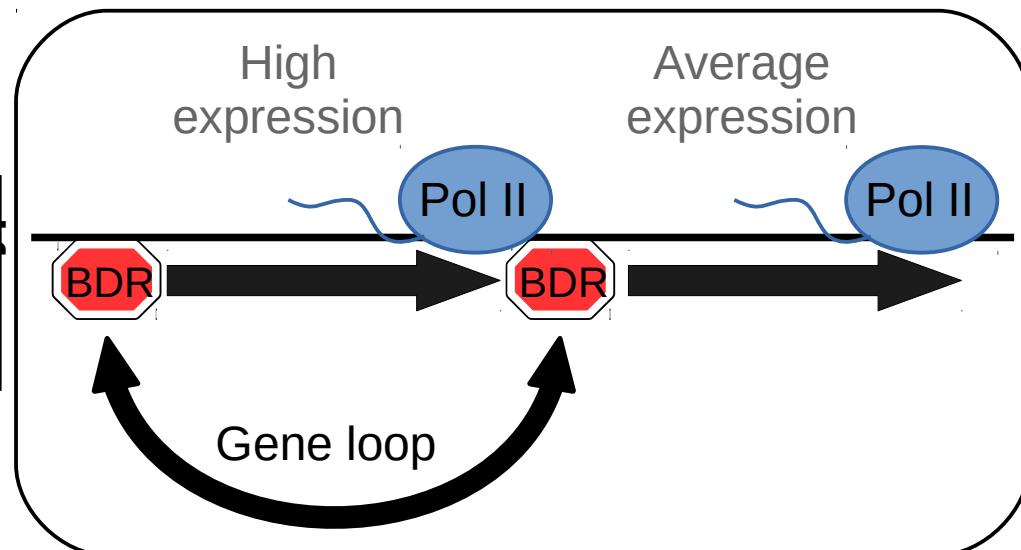
BDRs apparent functions:

- slow down PolII (negative elongation factor / 3' pausing factor)
- contribute to gene looping

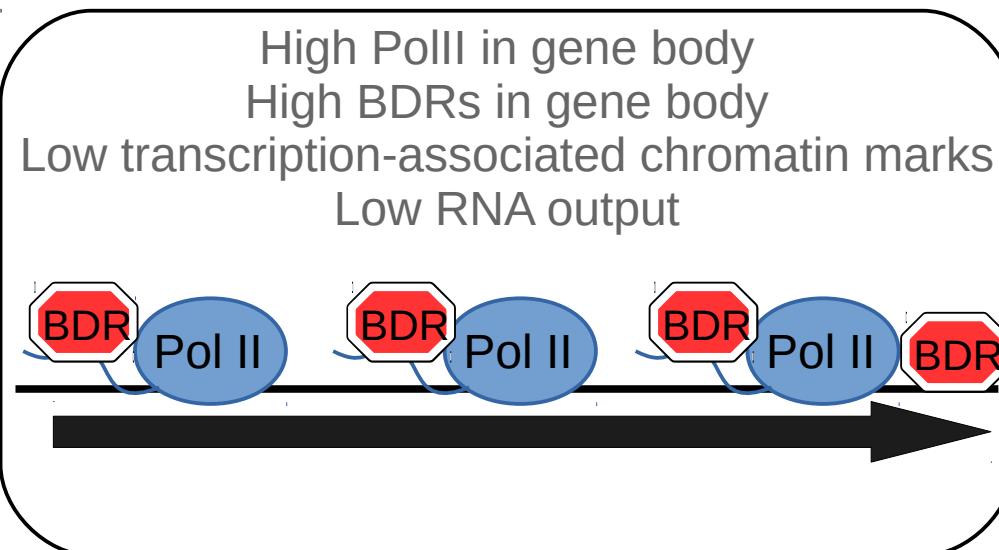
Yu*, Martin*, et al., Michaels, *Curr Biol*, 2021

Downregulated in bdrs triple mutant

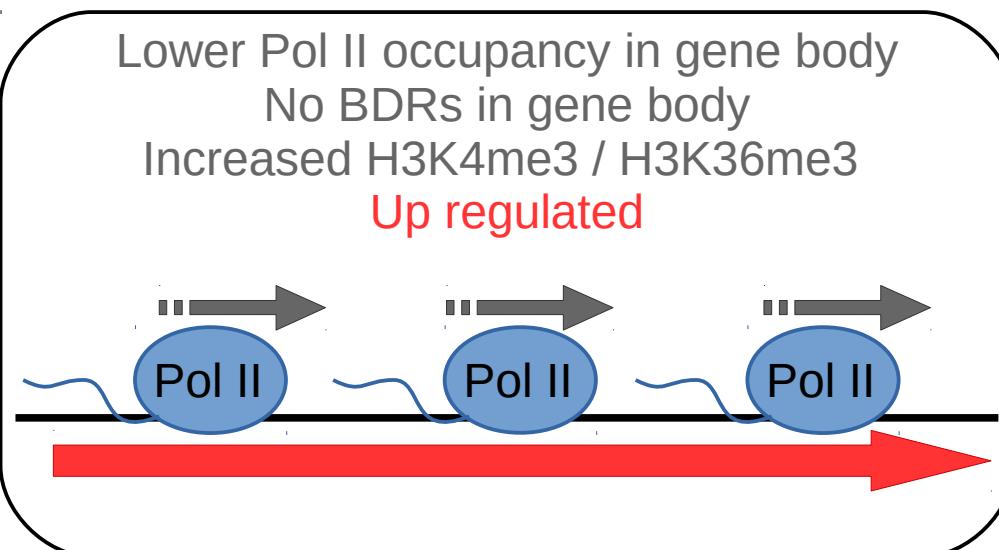
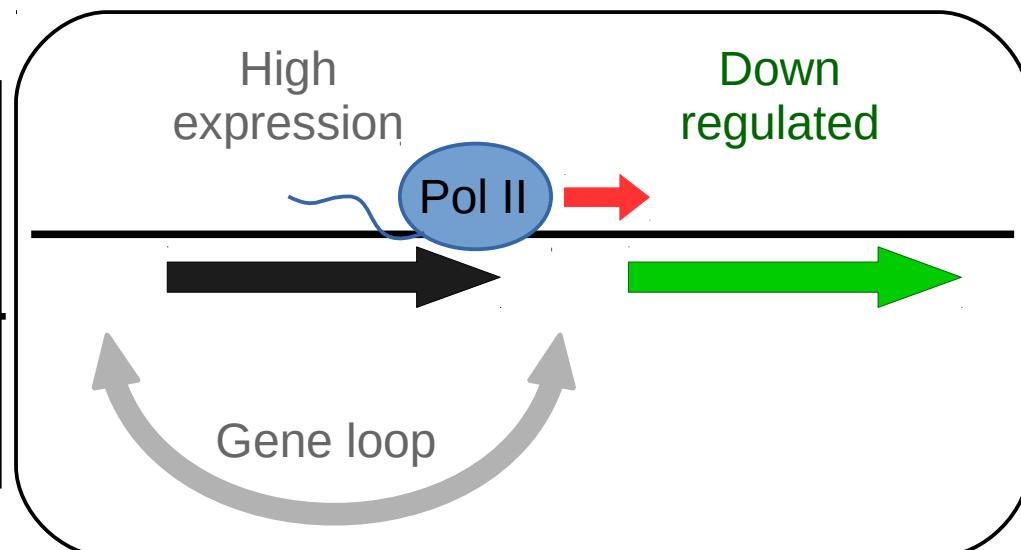
Wild-type



Upregulated in bdrs triple mutant



bdrs triple mutant



Thank you for your attention !



<https://github.com/pgpmartin/GeneNeighborhood>

https://github.com/pgpmartin/ChIPseq_functions



pascal.martin@inrae.fr



[@PgpMartin](https://twitter.com/PgpMartin)



<http://perso.pgpmartin.fr>

