FlowPlast

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Plasticity of flowering time in response to environmental signals in *Arabidopsis thaliana*

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FlowPlast Partners

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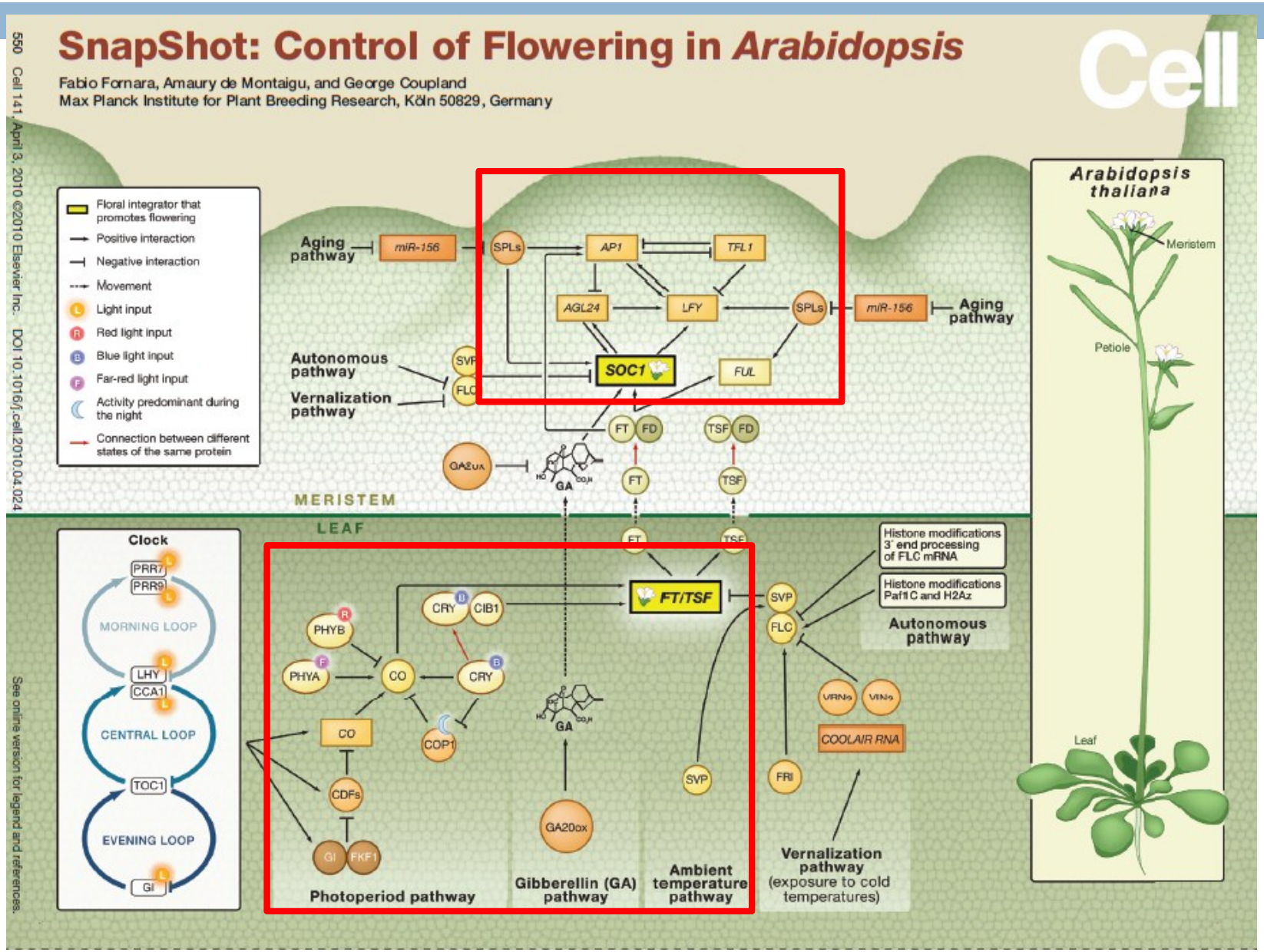
- Brendan Davies, Leeds University, Uk
- Pawel Krajewski, Polish Academy of Sciences, Poland

FlowPlast Timeline

- April – September 2014
  - Activation of individual grants
- May 2014
  - Consortium Agreement signed
- September 26, 2014
  - Kick-off Meeting, Heidelberg, Germany

Regulation of Flowering in *Arabidopsis thaliana*



Main Objectives

- Role of changes in chromatin landscape (in relation with transcriptome) on flowering time
  - As part of the photoperiod pathway
  - As part of the ambient temperature pathway
  - Focusing on the shoot apical meristem (SAM)
- Understand cross-talk between pathways
  - between photoperiod and GA pathways (converge in SAM)
- Role of alternative splicing in flowering time control

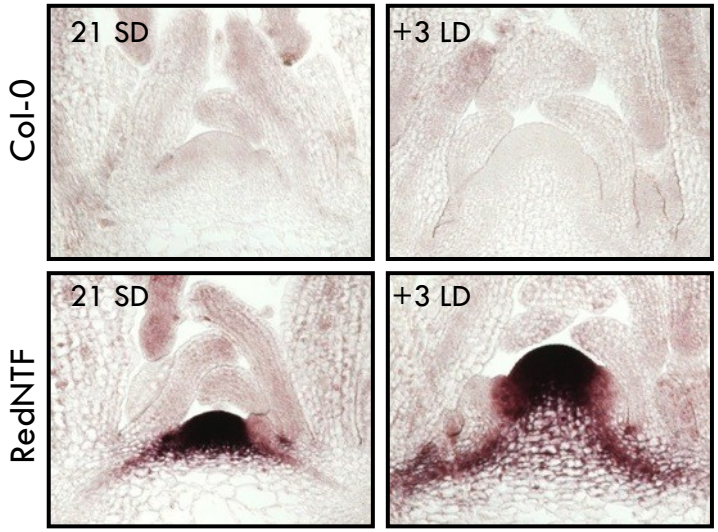
Technical Challenges

- Isolation of nuclei from the SAM
  - INTACT

- Analysis of mRNA expression and chromatin/DNA modifications from very limited material
  - nano-ChIP-seq
  - RNA-seq
  - DNAseI-seq accessibility profiling
  - DNA methylation profiling

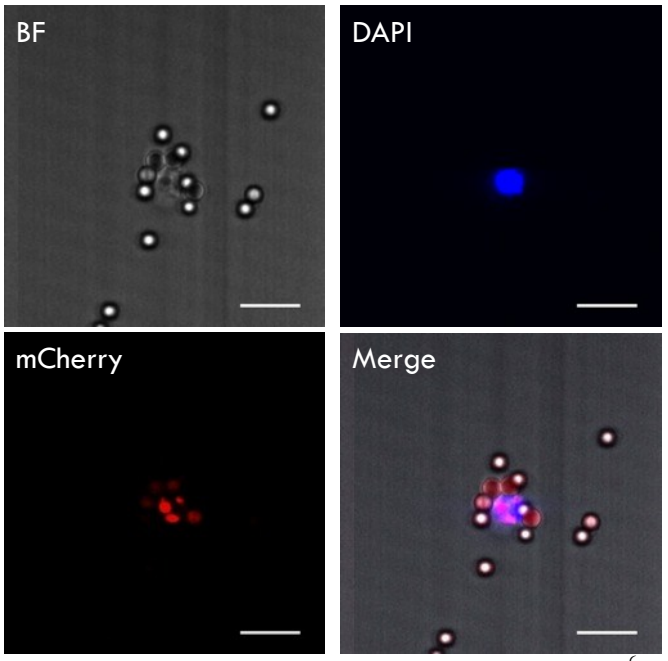
A. thaliana SAM-specific INTACT (WP1)

- RedNTF reporter line



Immunohistochemistry with anti-Streptavidin antibody

- Isolation of SAM nuclei
  - optimized protocol



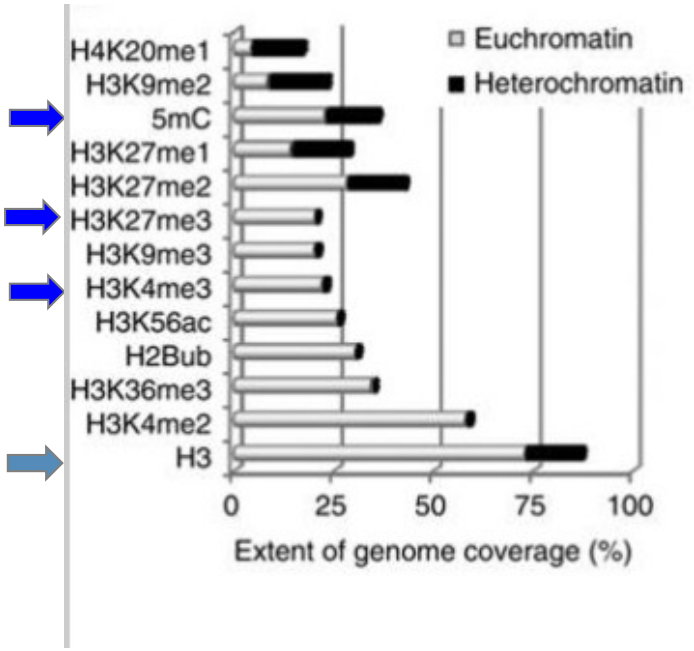
Bar = 10 ⌈m

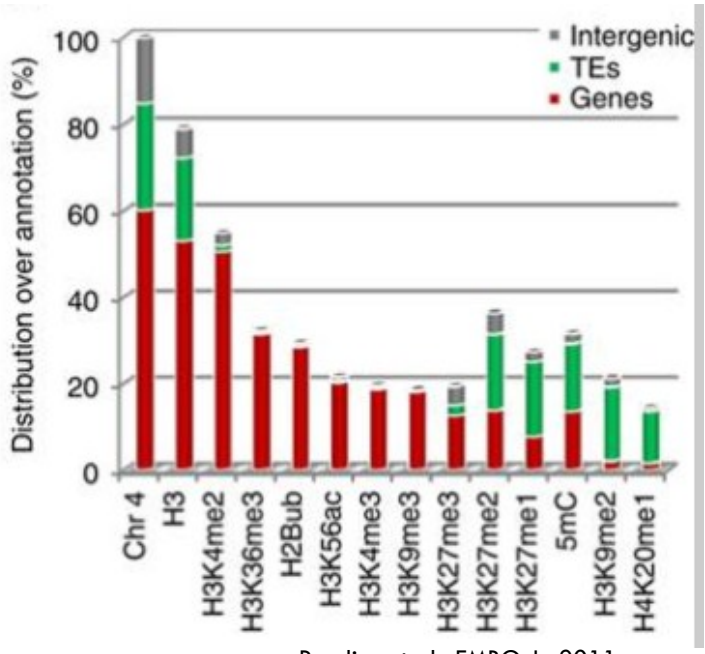
  - >95% pure SAM nuclei

- Reporter lines sub-domains of the SAM on the way

Establishing nanoChIP-seq (WP1)

- Low amount of material from INTACT will (severely) limit the number of possible downstream analyses
  - 3 informative chromatin marks selected for profiling
  - H3 serves as control

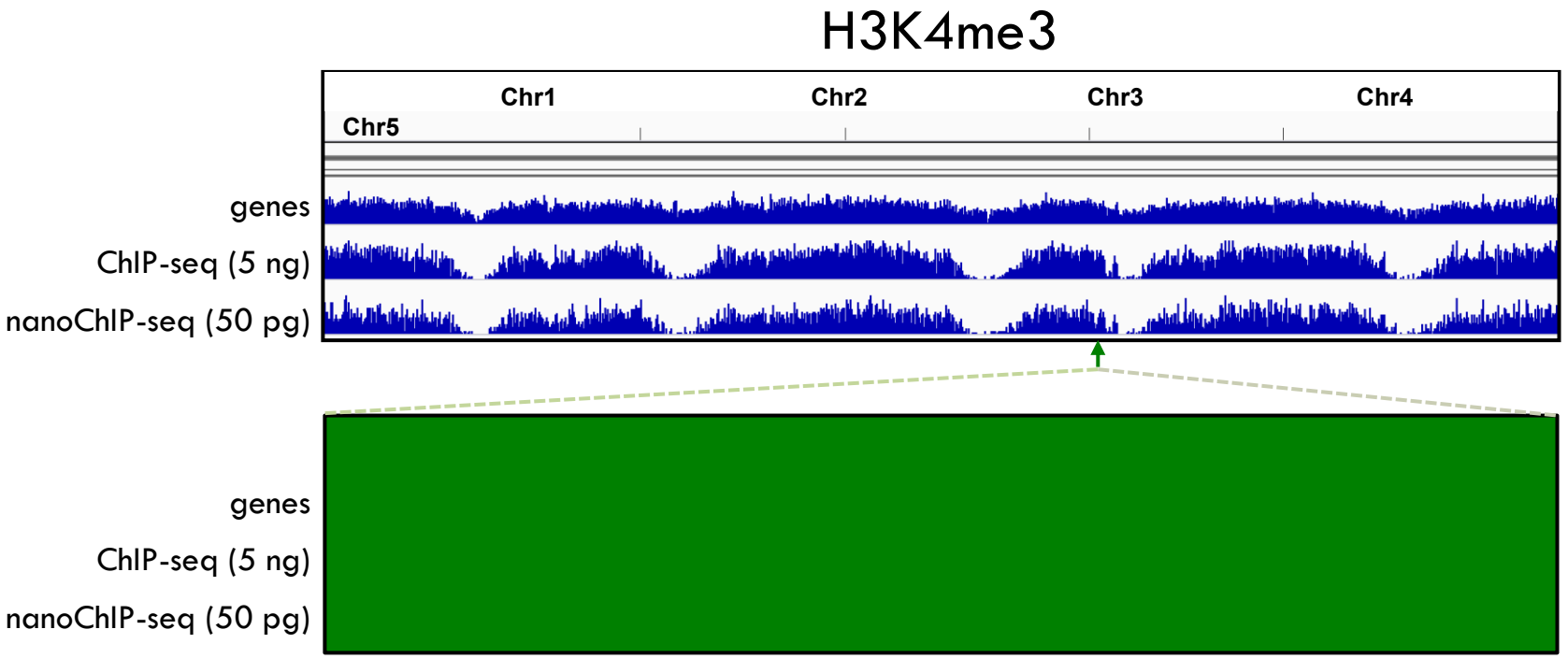




Roudier et al., EMBO J., 2011

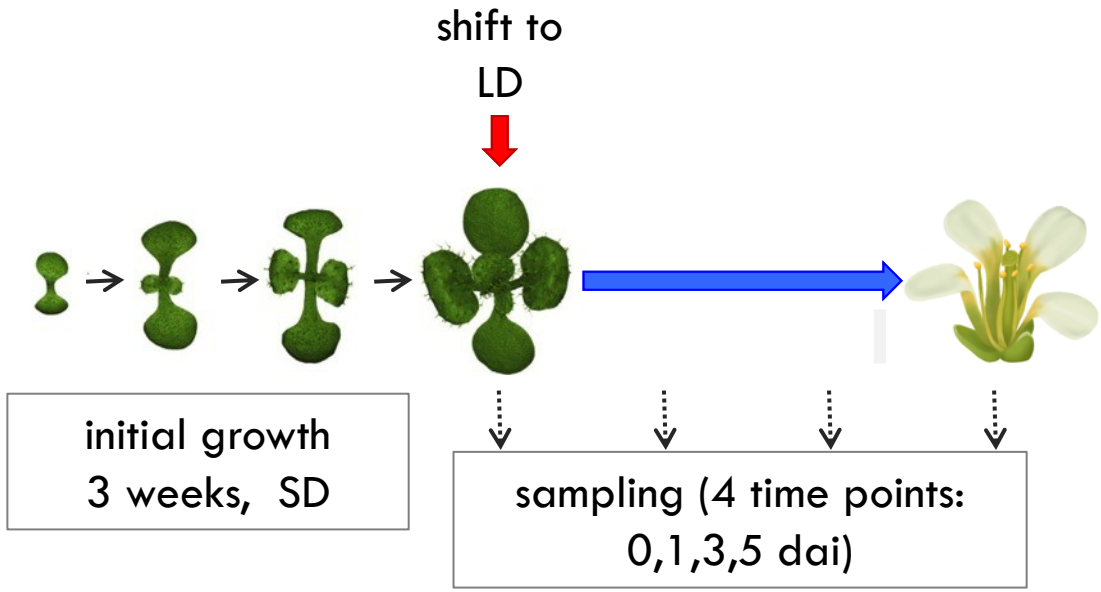
Establishing nanoChIP-seq (WP1)

- Testing nanoChip-seq using 50pg input DNA



Induction of Flowering by Photoperiod (WP2)

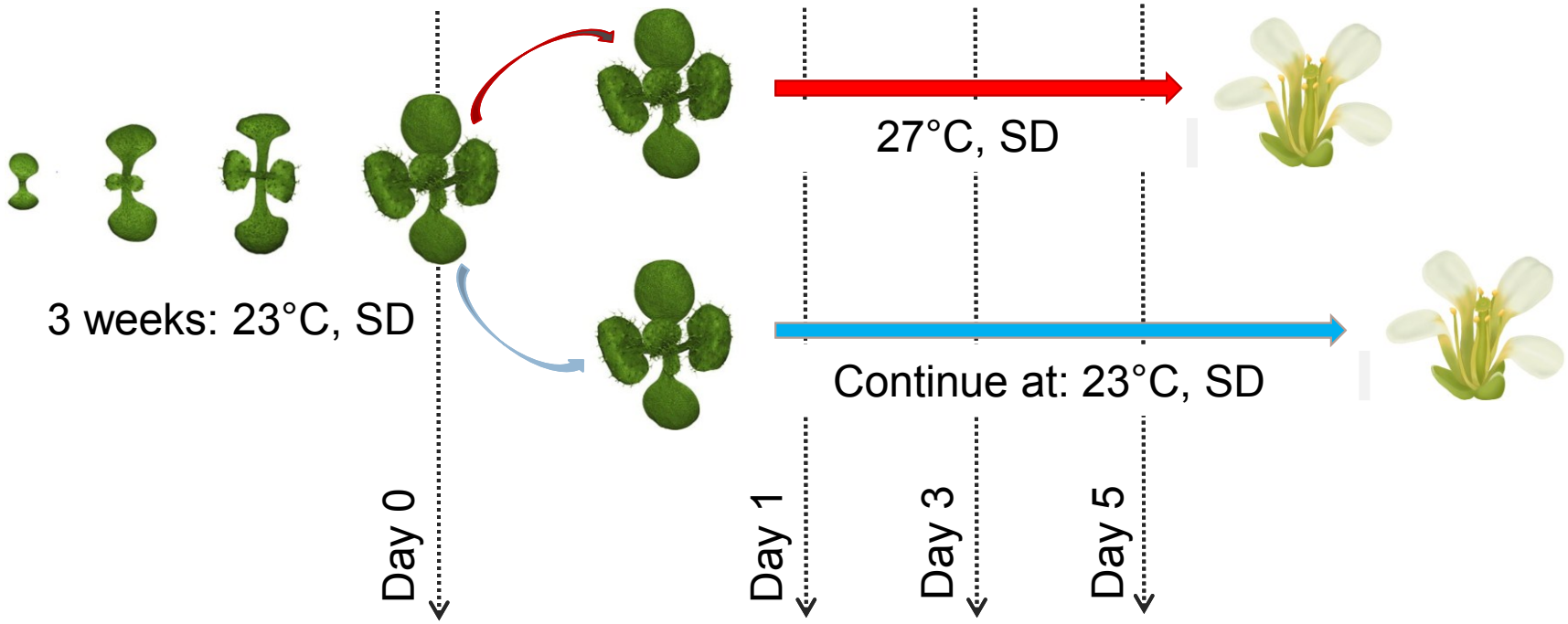
- INTACT reporter lines will be shifted from SD to LD to synchronously induce flowering
- Effects of photoperiod induction on the SAM (and sub-domains)



Genome wide analyses of temperature-dependent molecular processes at the SAM (WP3 and WP4)

ERA-CAPS

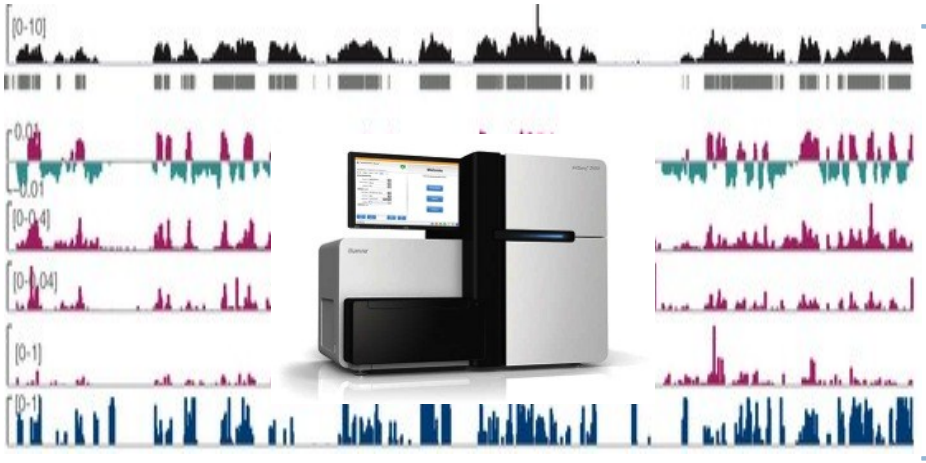
Induction of flowering will be triggered by shifting SD-grown plants from 23°C to 27°C



Genome wide analyses of molecular processes at the SAM

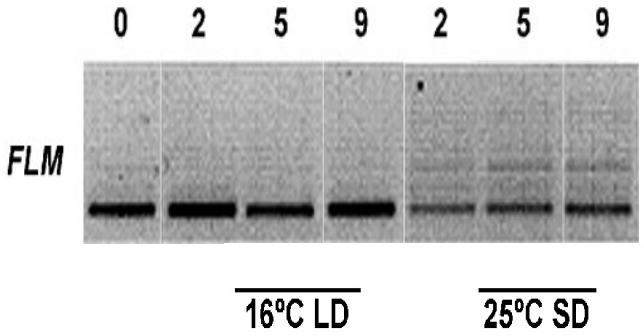
- day-length-dependent (WP2)
- Ambient temperature dependent (WP3)

- *Diff expression:*
- *Diff AS:*
- *Chromatin (DNaseI):*
- *Histon marks:*

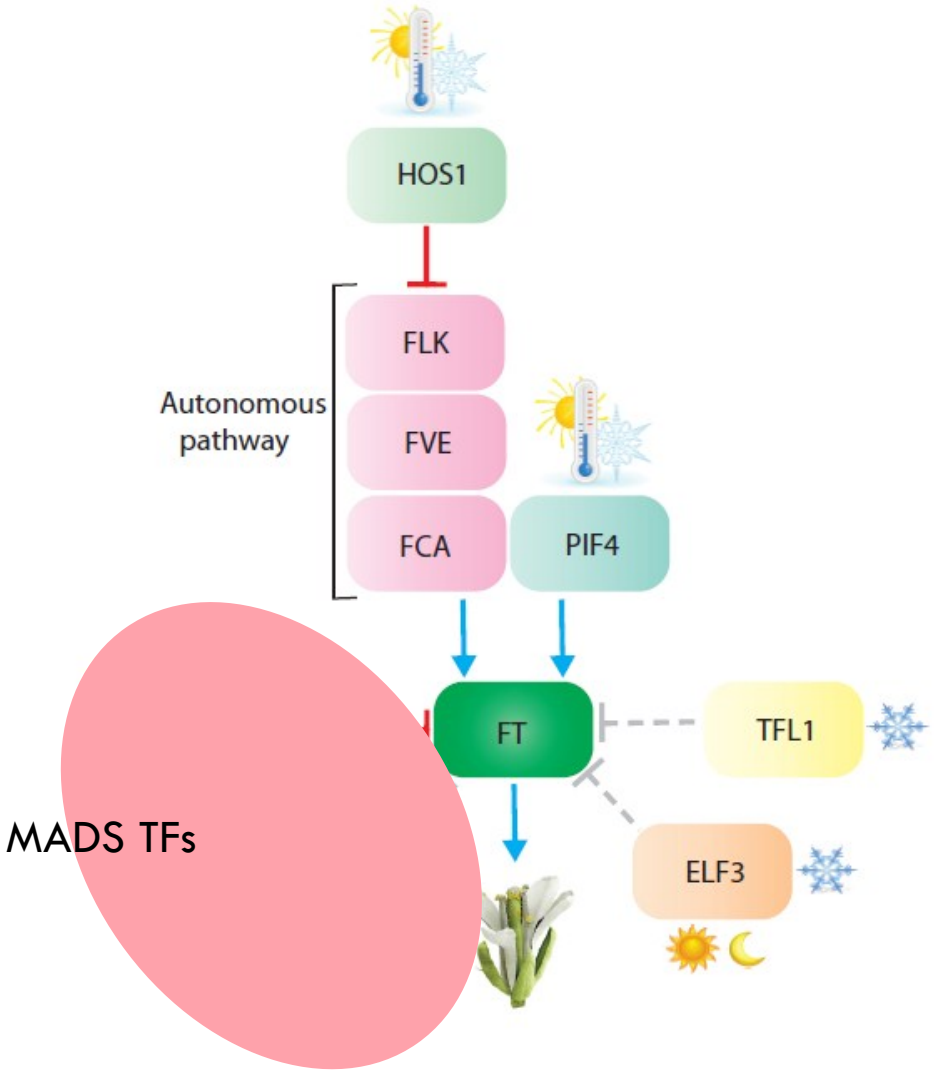


Conservation (ecotypes)

The ambient temperature pathway

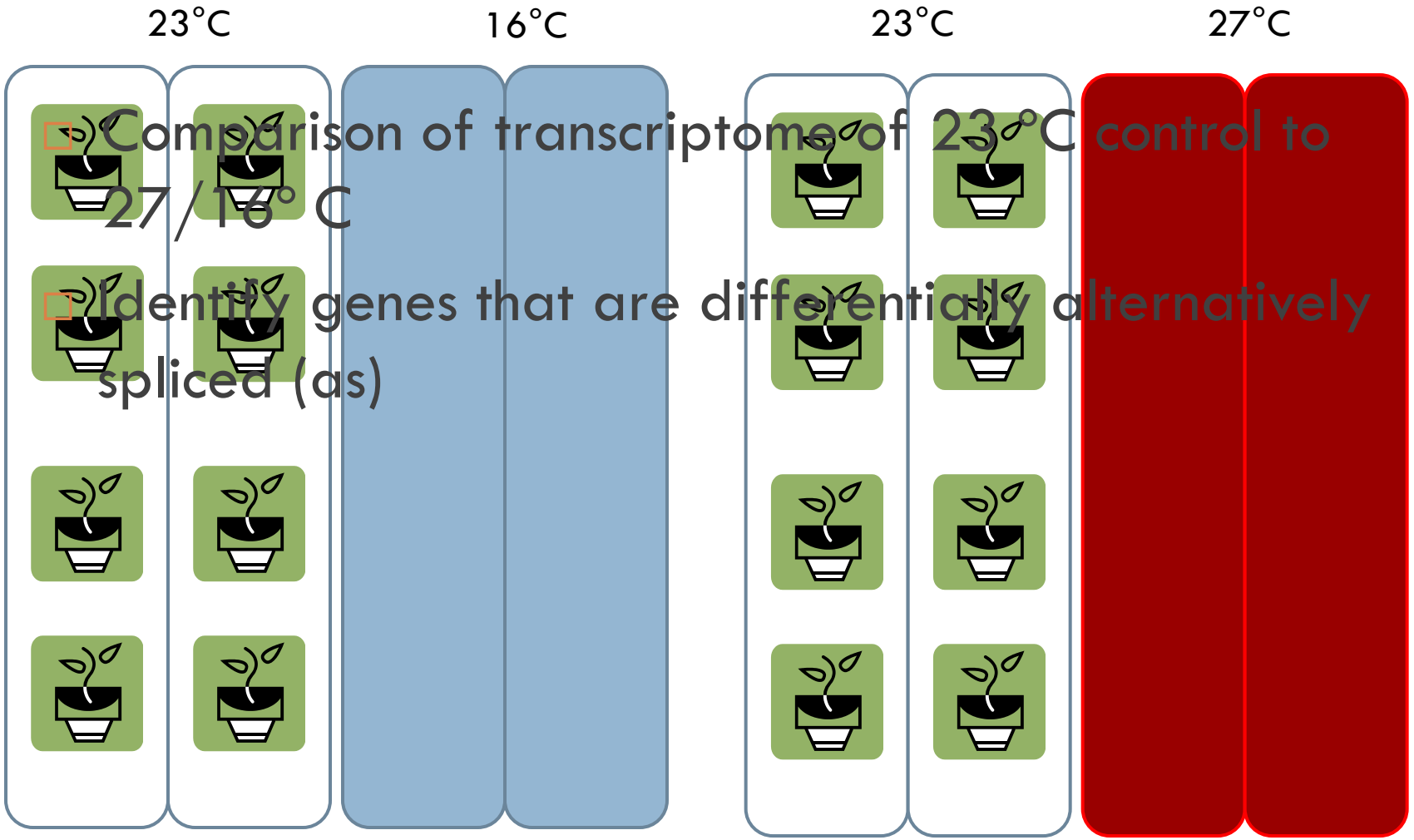


Balasubramanian et al, PLoS Genetics, 2006



Verhage, Angenent, Immink (2014) TiPS, Sept.

RNAseq to screen for temperature affected alternative splicing (AS)



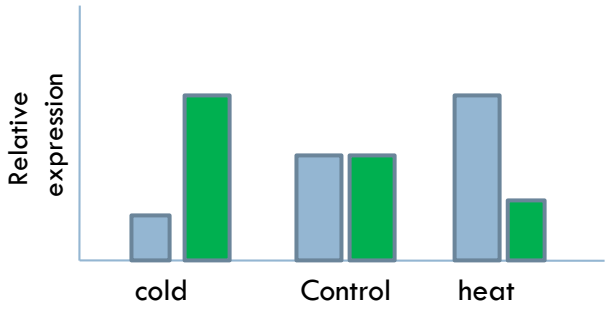
- Comparison of transcriptome of 23°C control to 27/16° C
- Identify genes that are differentially alternatively spliced (as)

Results

- Total number of differentially alternatively spliced genes
  - 23°C → 27°C: 1107 genes
  - 23°C → 16°C: 897 genes
  - Reciprocal: 135 genes (15%)

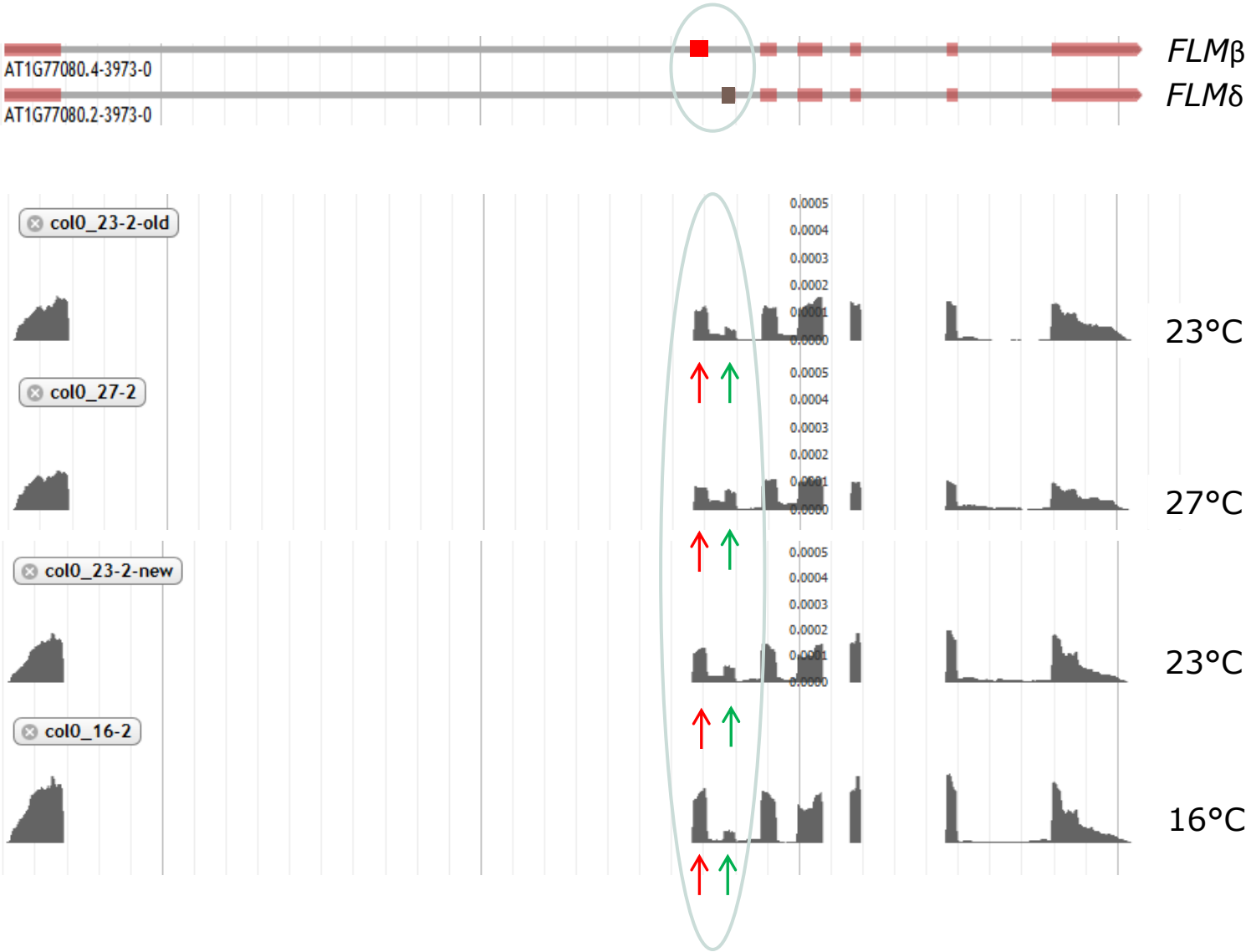
- Differentially alternatively spliced flowering genes
  - 23°C → 27°C: 17 genes
  - 23°C → 16°C: 15 genes
  - Overlap: 7 genes
  - Reciprocal: 4 genes

    (including ***FLM***)

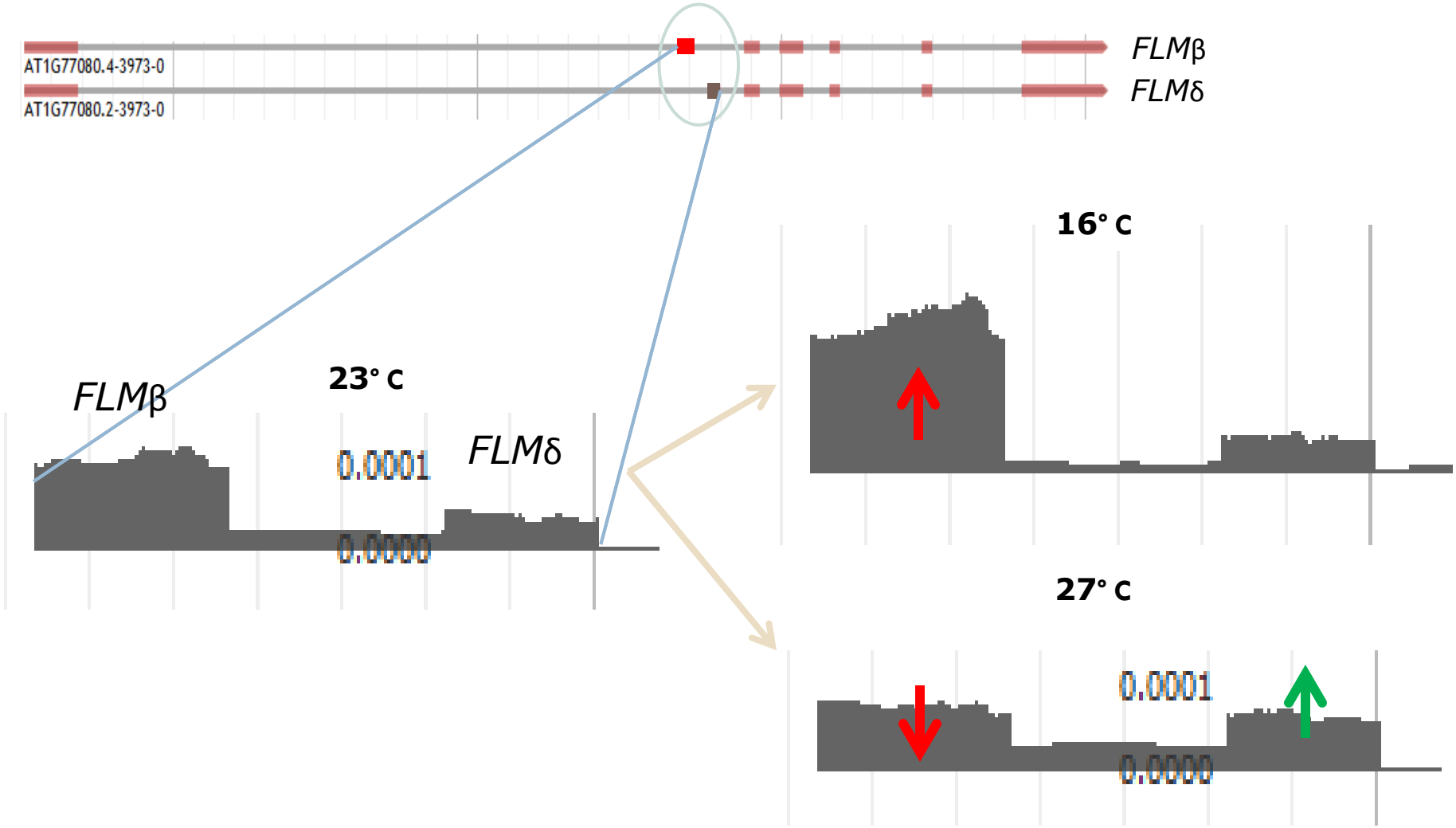


Reciprocal

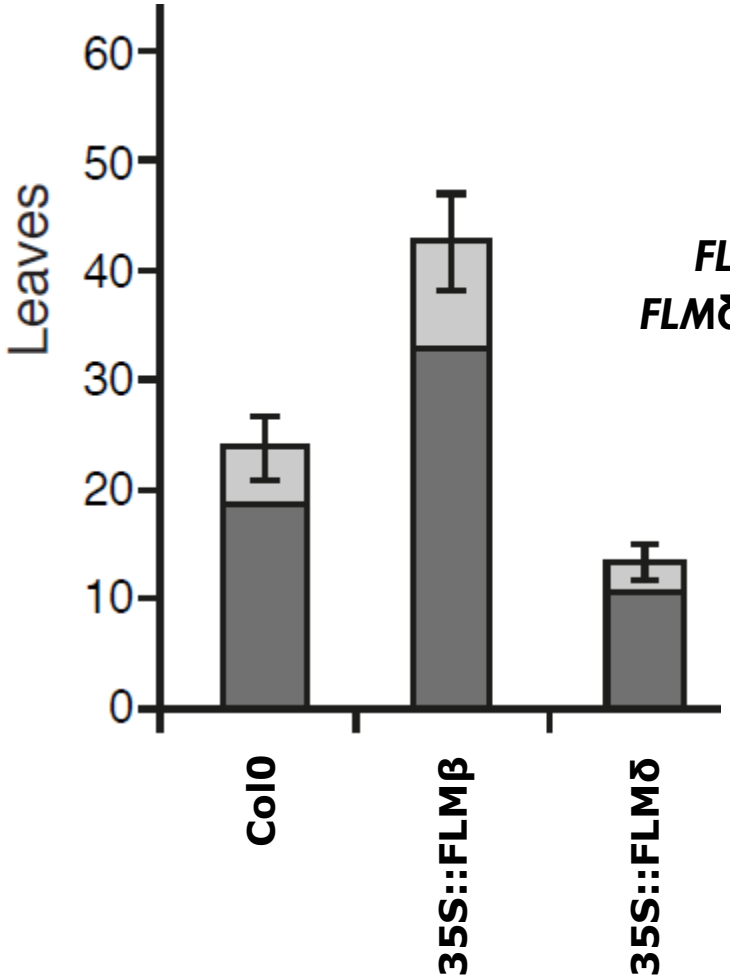
RNAseq results: *FLM* alternative splicing



RNAseq results: *FLM* alternative splicing



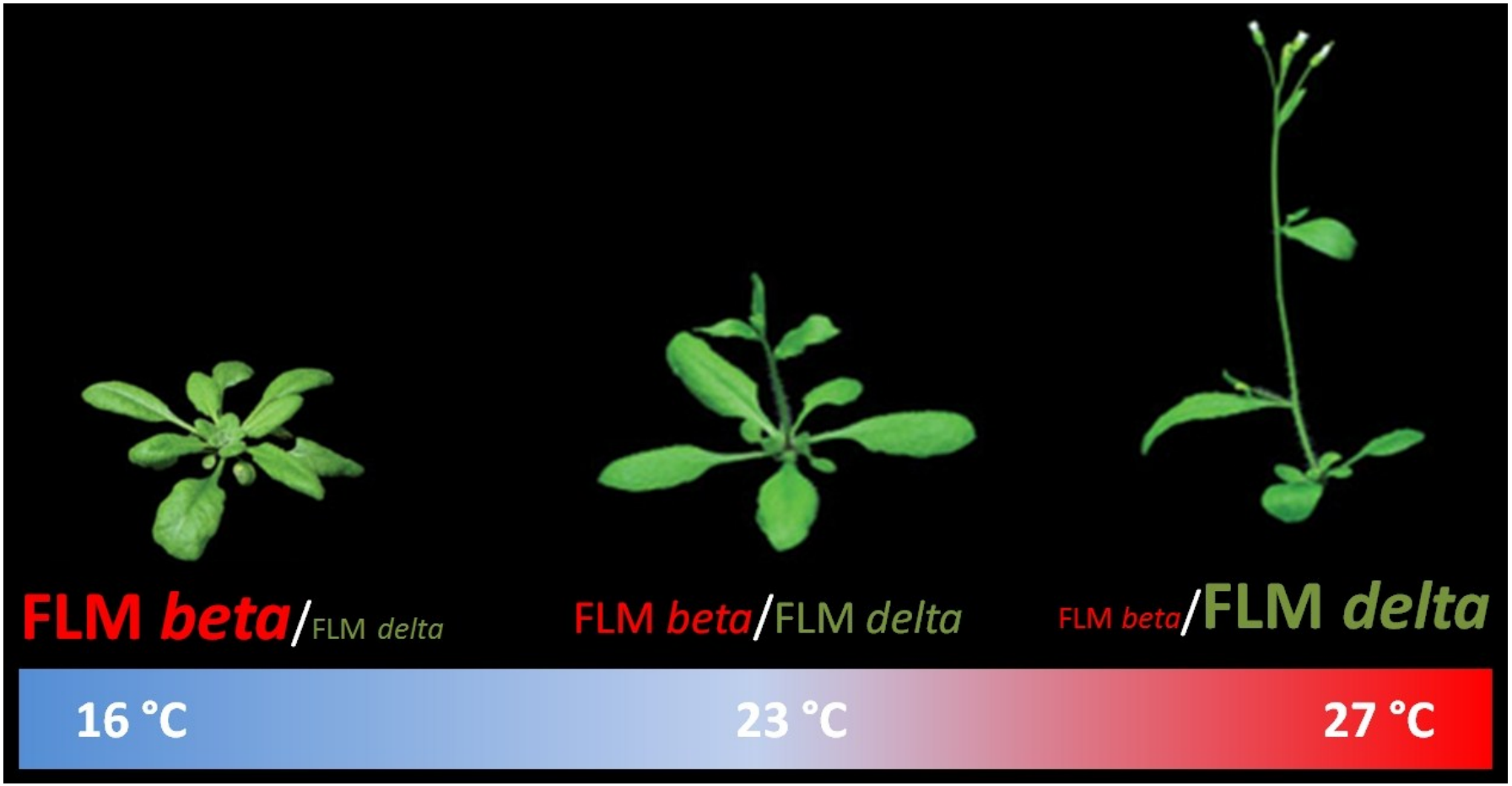
Functional analysis *FLM* isoforms



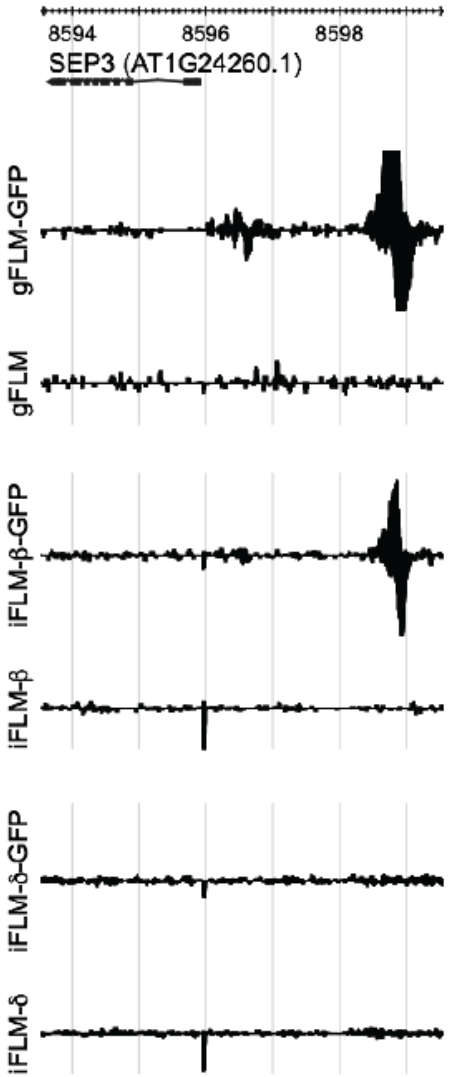
***FLM*β isoform acts as a repressor and *FLM*δ isoform acts as activator of flowering**

Posé, et al (2013) Nature, 503(7476), 414-

FLMβ and FLMδ biological functions



*FLM*β and *FLM*δ isoforms differ in DNA binding capacity



ChIP-seq gFLM

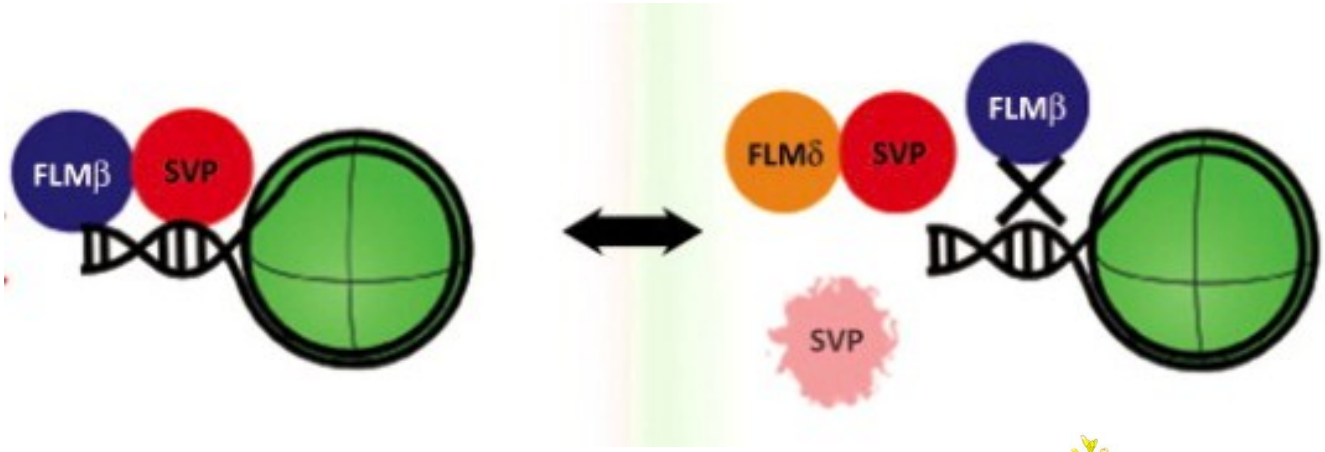
ChIP-seq FLMβ

ChIP-seq FLMδ

Posé, et al (2013) Nature, 503(7476), 414-

FLMβ/ FLMδ competition is the underlying mechanism

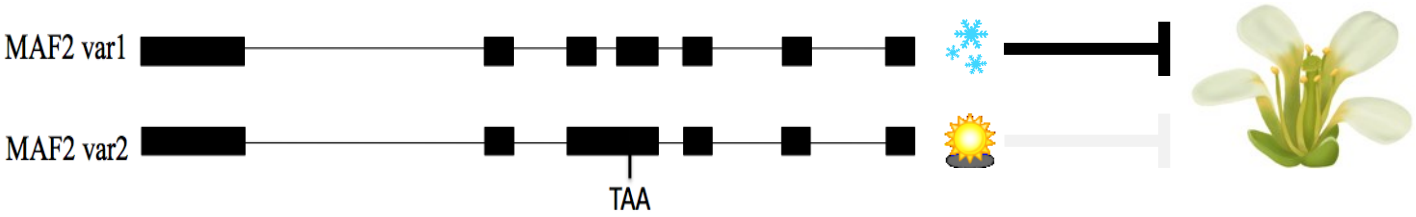
suppressor SVP can not bind to DNA anymore



Posé, et al (2013) Nature, 503(7476), 414-
Verhage, Angenent, Immink (2014),TiPS, Sept

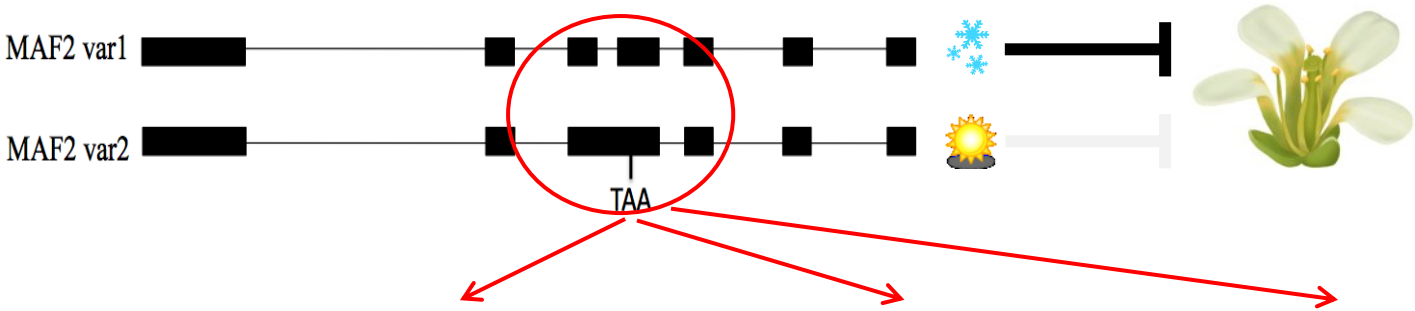
Temperature-dependent alternative splicing (WP4)

- Temperature-dependent alternative RNA splicing (TD-AS) has been shown to affect flowering
  - MAF2 represses flowering at low temperatures (16°C)
  - no effect at 27°C
- This is achieved through TD-AS of *MAF2*



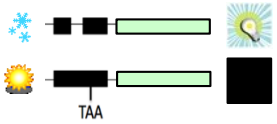
Temperature-dependent alternative splicing (WP4)

Q: What is the molecular basis for TD-AS



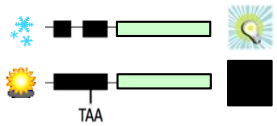
Candidate approach

Construct LUC reporter.



Test mutants in SRs/PTBs & splicing factors identified in other WPs.

Forward genetics



Screen for mutants that are LUC+ at high temperature and/or LUC- at low temperature .

RNA pulldown/MS

Tag MAF2 with MS2 aptamers

↓

INTACT

↓

Pulldown RNAs using MS2 tag

↓

Mass Spec. analysis of RNA-associated proteins at high and low temp.

Compare to global TD-AS analysis, ecotype analysis and TD chromatin data

Data Analysis & Integration (WP5) Pawel Krajewski

- Challenges:
  - Variety of protocols used (ChIP, RNA, DNAseI)
  - Multifactorial experiment design (time points, conditions, genotypes)
- Tasks:

Project question	Required analysis
Are changes in chromatin structure and changes in gene expression correlated?	Integration of ChIP-Seq, DNAse-Seq and RNA-Seq results
Are the same genes affected by thermal and photoperiod induction?	Differential analysis of RNA-Seq data (incl AS mRNAs)
How the transcriptome of the rib meristem changes during floral transition in *ft tsf* or GA biosynthetic mutants?	Factorial analysis of RNA-Seq data

Expected Outcome

- Genome-wide information on the control of flowering within (sub-) domains of the SAM in response to different environmental stimuli
  - How much does epigenetic regulation contribute to transcriptional changes during floral transition
  - To what extent is flowering modulated by alternative splicing
  - How variable are the responses to environmental stimuli between accessions of *A. thaliana*

Thank you for your attention.