



IIT ROORKEE



NPTEL ONLINE
CERTIFICATION COURSE

Molecular Genetics of Plant Development-cont..III

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Approaches to the study of plant development

- **Reverse Genetics:** An approach to study function(s) of gene(s)
(Genotype to Phenotype)
 - *Identifying & isolation of gene for functional study (gene of interest)*
 - *Analyzing expression pattern of the gene (differential, spatial & temporal)*
 - *Functional characterization of the gene (gain or loss-of-function)*
 - *Gene interaction study (genetic, physical or regulatory interaction)*



Gene interaction study during plant development

(When multiple genes regulate a developmental process)



Genetic interaction:

- Same or different pathways
- Combinatorial mutants: suppressors or enhancers



Physical interaction:

- Protein-protein interaction
- Protein-DNA/RNA interaction



Regulatory interaction:

- Co-expression
- Regulation of gene expression



Genetic interaction during plant development

- *Modifiers*: suppressors or enhancers (extra-genic)
- *Same or different pathways*: upstream or downstream
- *Genetic redundancy*
- *Combinatorial mutant analysis*



CONSTANS activates suppressor of overexpression of CONSTANS T through flowering locus T to promote flowering in Arabidopsis

FT and *SOC1* in parallel pathways downstream of *CO*

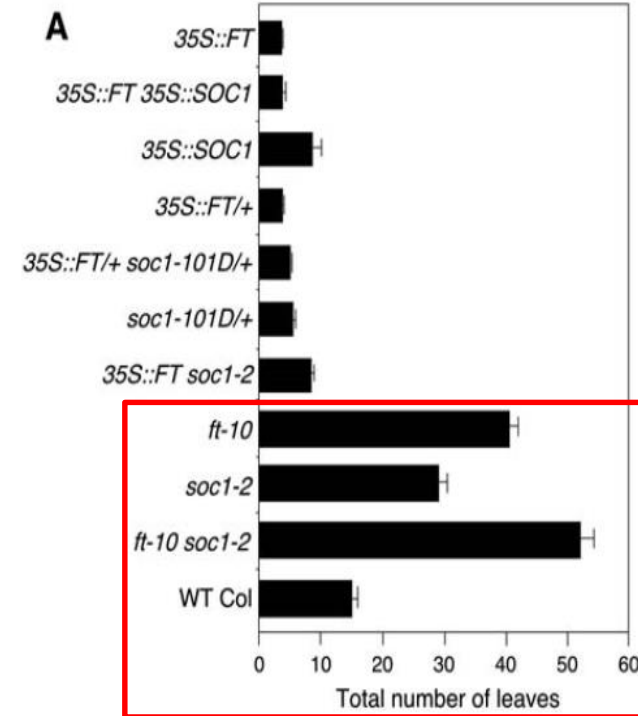
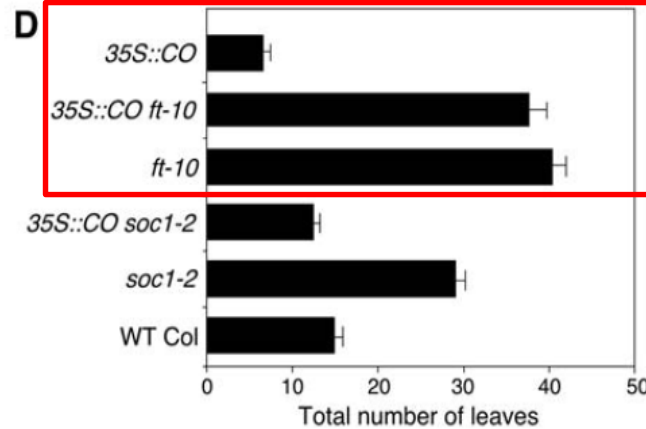
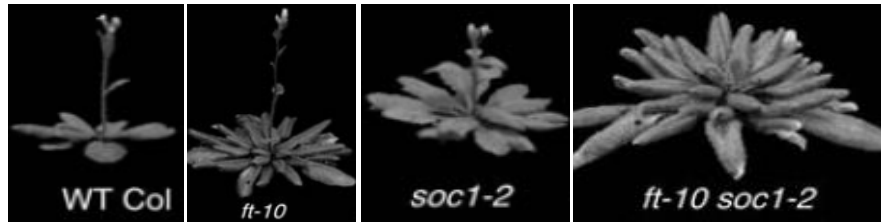
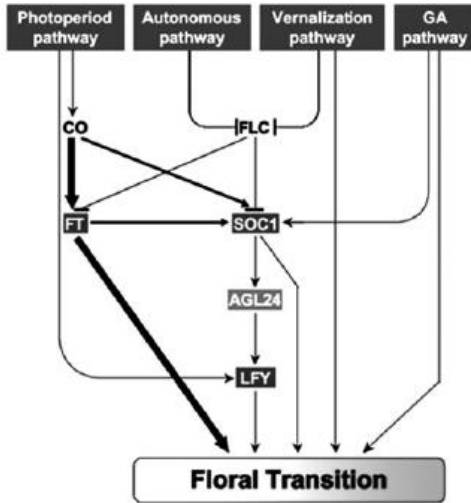


Image source: Yu et al. (2002) PNAS, 99, 16336-41.
Yoo et al. (2005) Plant Physiology, 139, 770-78.

AGAMOUS-LIKE 17, a novel flowering promoter, acts in a *FT*-independent photoperiod pathway

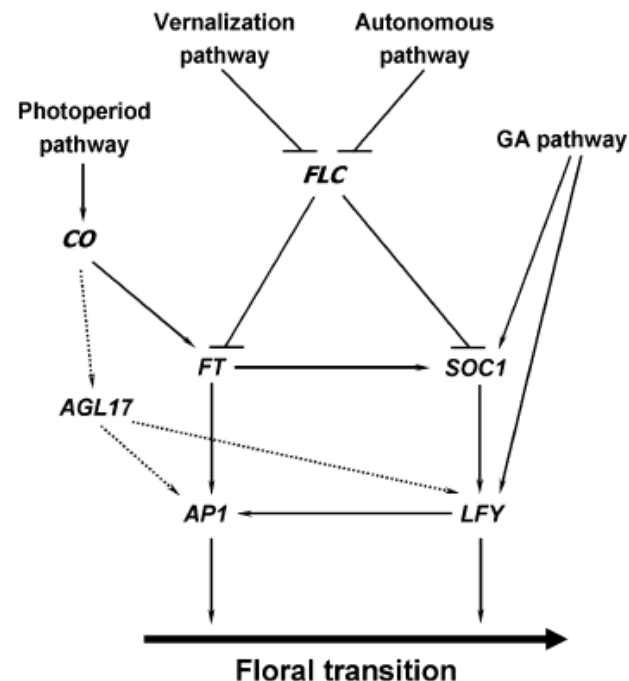
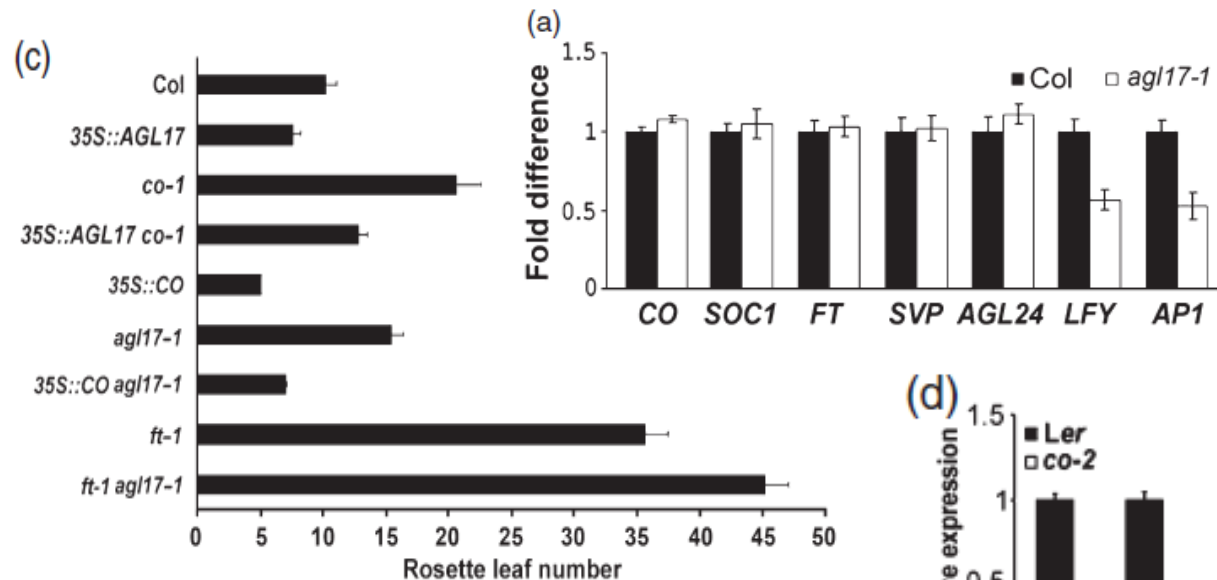


Image source: Han et al.
(2008) Plant J., 55, 253-65.

Genetic Redundancy

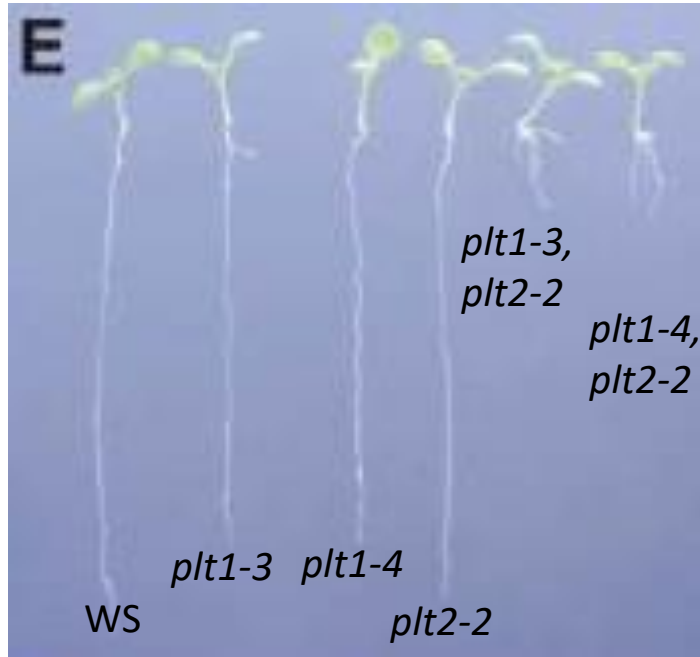


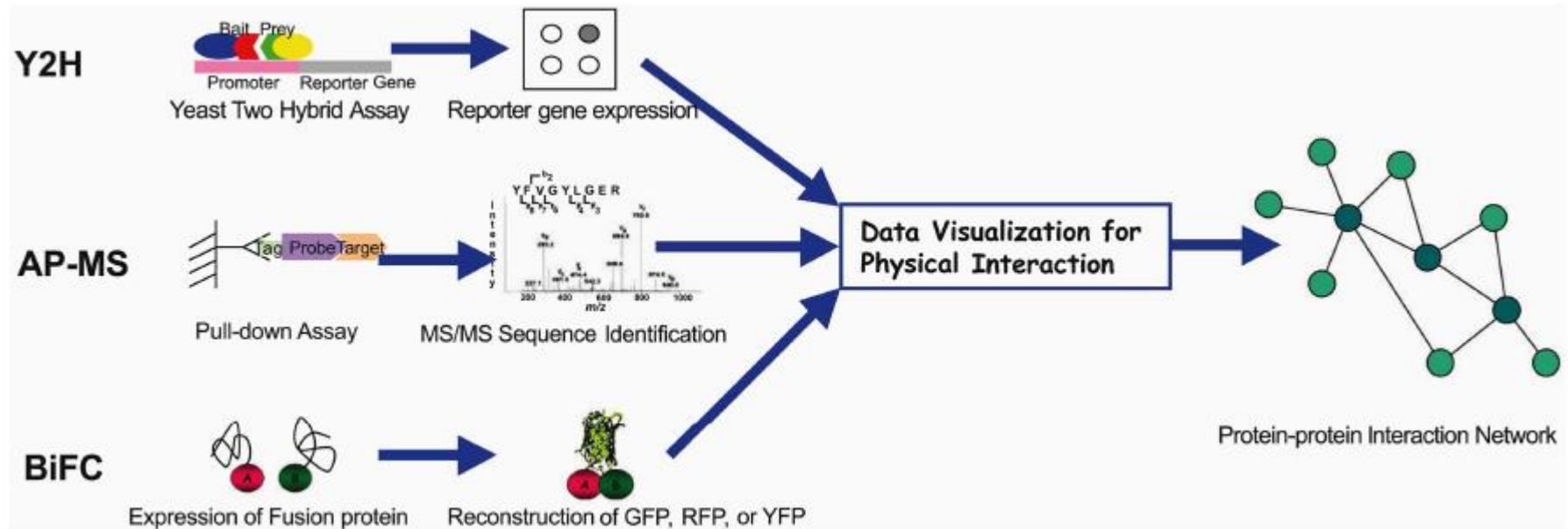
Image source: Aida et al. (2004)
Cell, 119, 109-20.



Image source: Okushima et al. (2005)
Plant Cell, 17, 444-63.

Physical interaction study during plant development

➤ Protein-protein interaction



bimolecular fluorescence complementation

Image source: Zhang et al. (2011) *Current Genomics*, 11, 40-46.

Physical interaction study during plant development

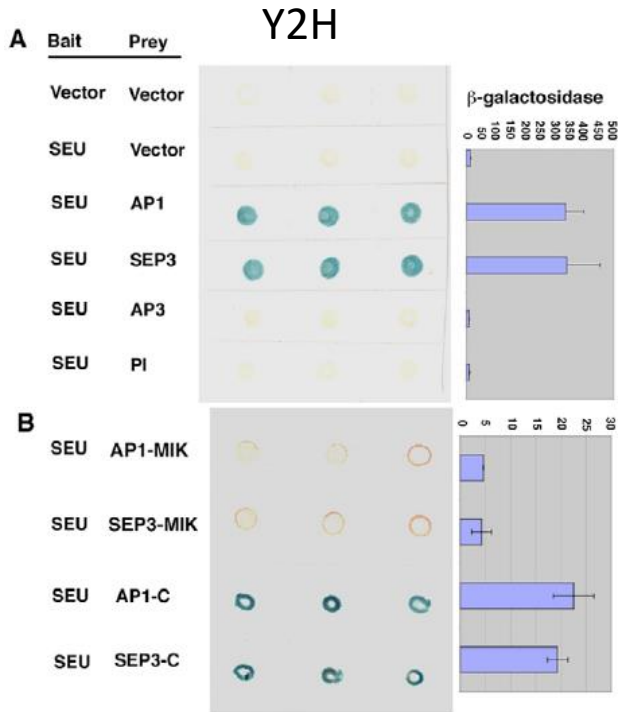
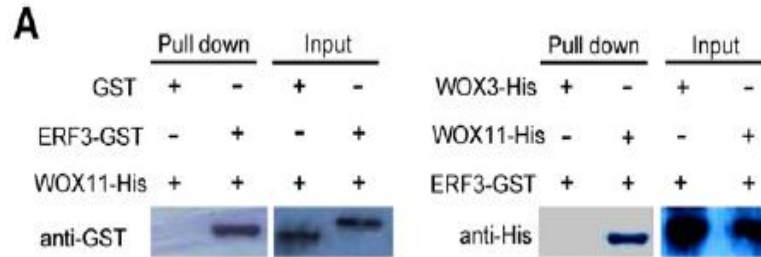
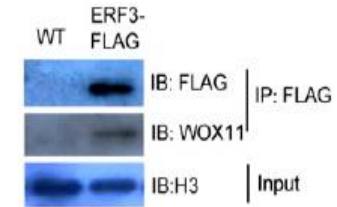


Image source: Sridhar et al. (2006)
Development 133, 3159-66.

Pull-down assay



Co-IP assay



Bimolecular fluorescence complementation (BiFC)

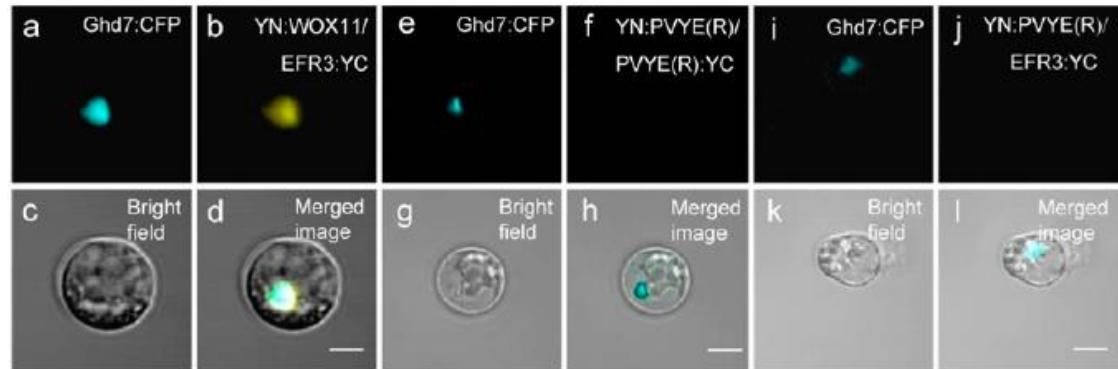


Image source: Zhao et al. (2015) Plant Cell, 27: 2469–83.

Nano-LC-MS/MS analysis

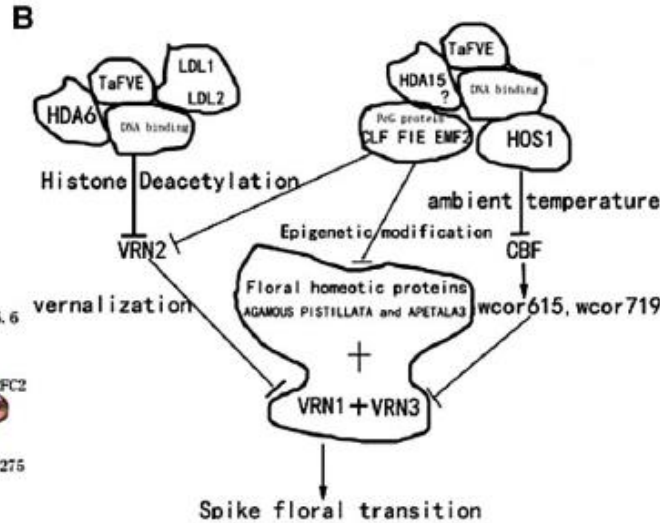
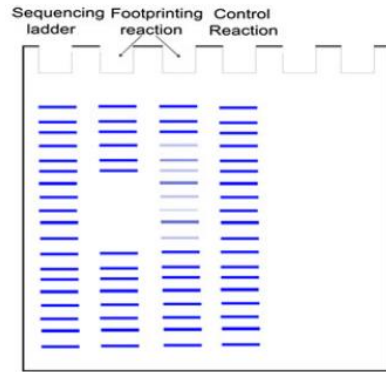


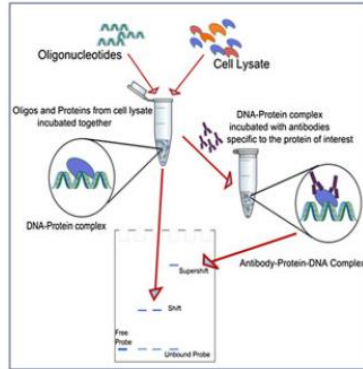
Image source: Zheng et al. (2017) Proteomics 17, 9, 1600331.

DNA-protein interaction

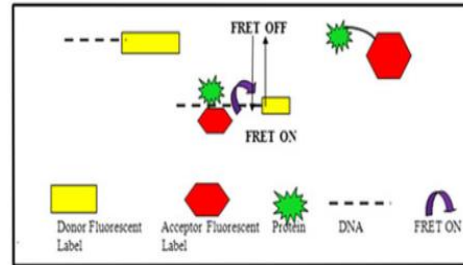
Footprinting



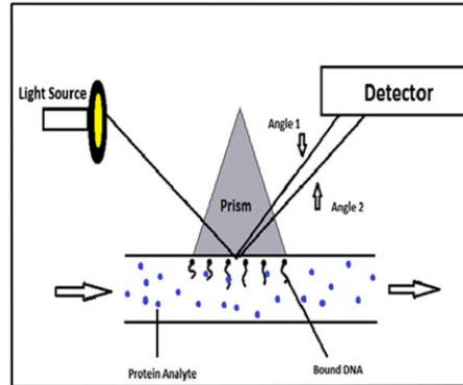
EMSA



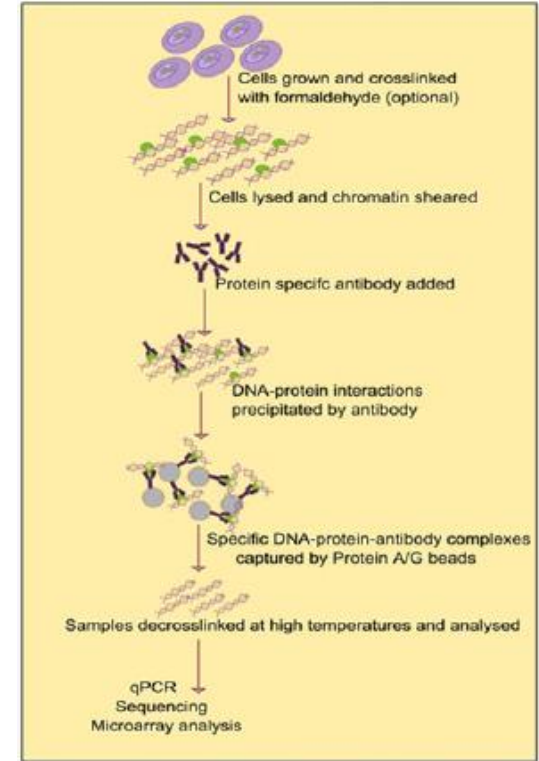
FRET



SPR



ChIP assay



Yeast-one-hybrid assay

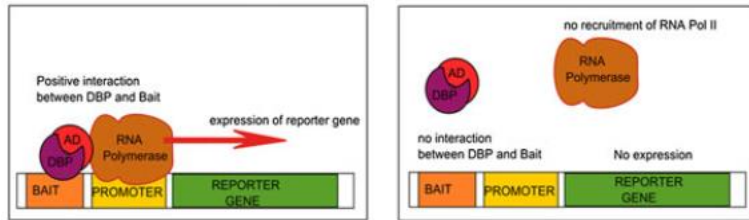


Image source: Dey et al. (2012) Mol Cell Biochem, 365:279–99.

DNA-protein interaction: Gel-shift assay

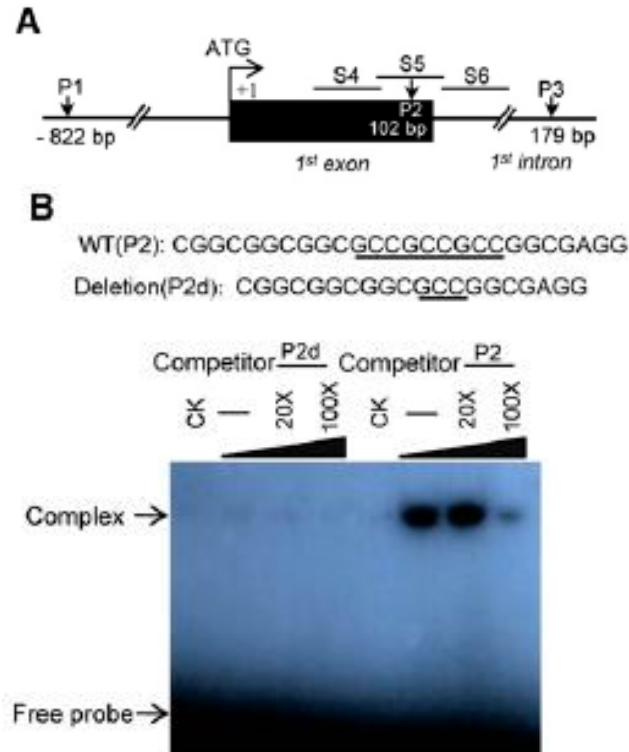


Image source: Zhao et al. (2015)
Plant Cell, 27: 2469–83.

DNA-protein interaction: ChIP assay

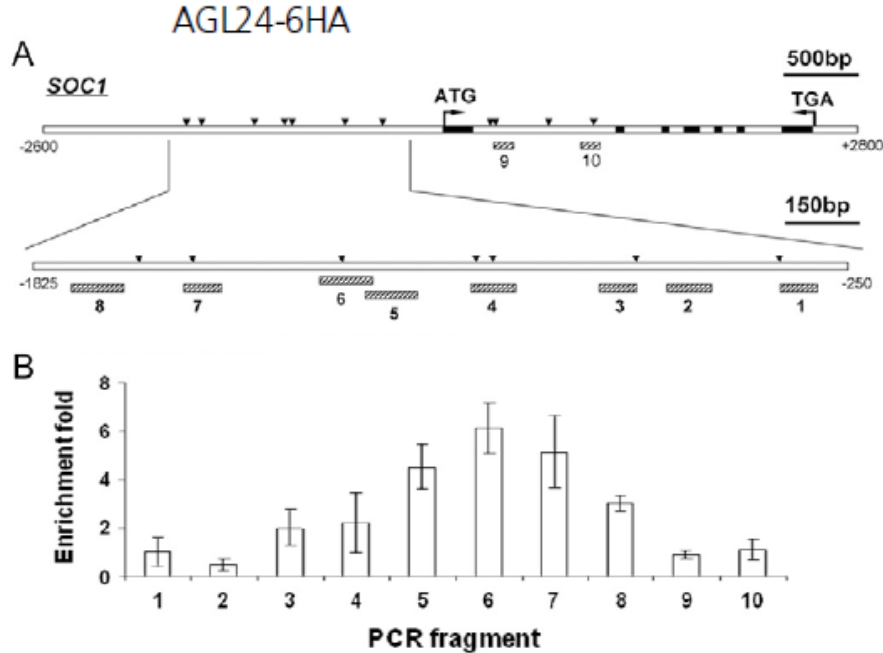


Image source: Liu et al. (2008)
Development 135, 1481-91.

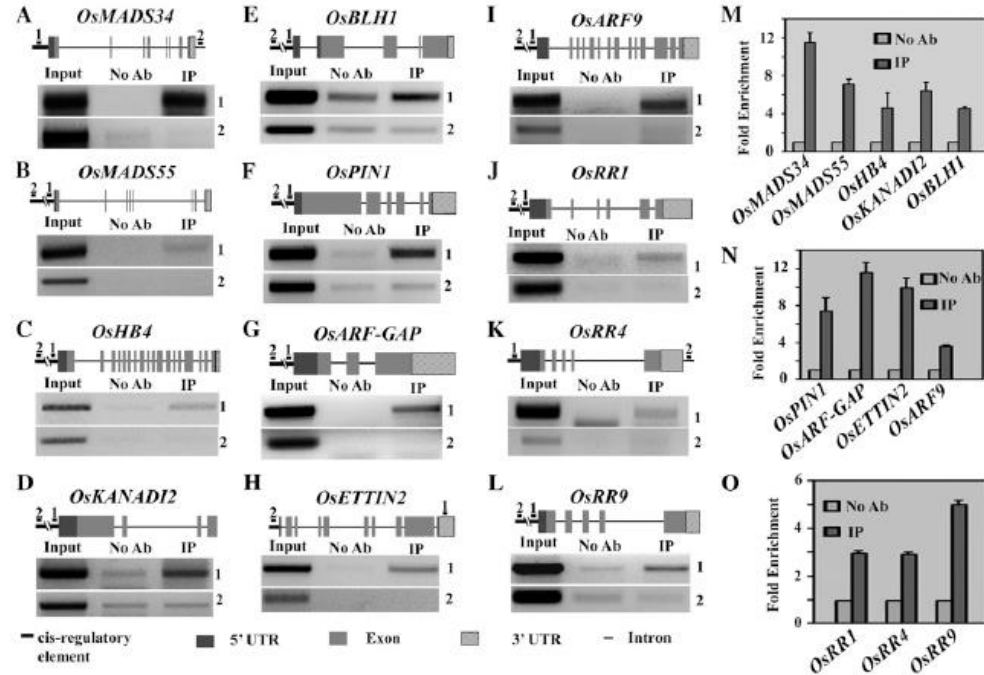
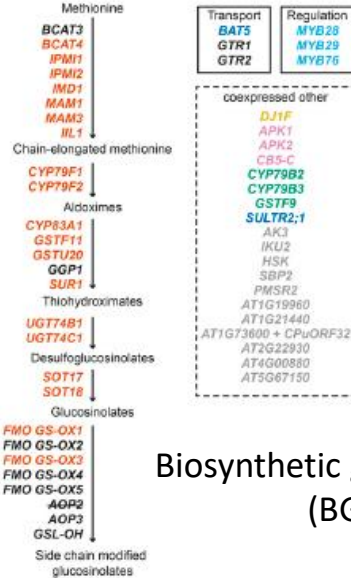
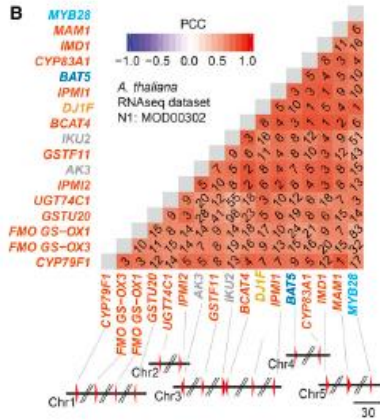
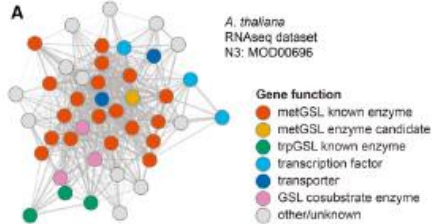


Image source: Khanday, Yadav et al. (2013)
Plant Physiology, 161:1970-83.

-Co-expression: Meta-analysis

A Global Coexpression Network Approach for Connecting Genes to Specialized Metabolic Pathways in Plants

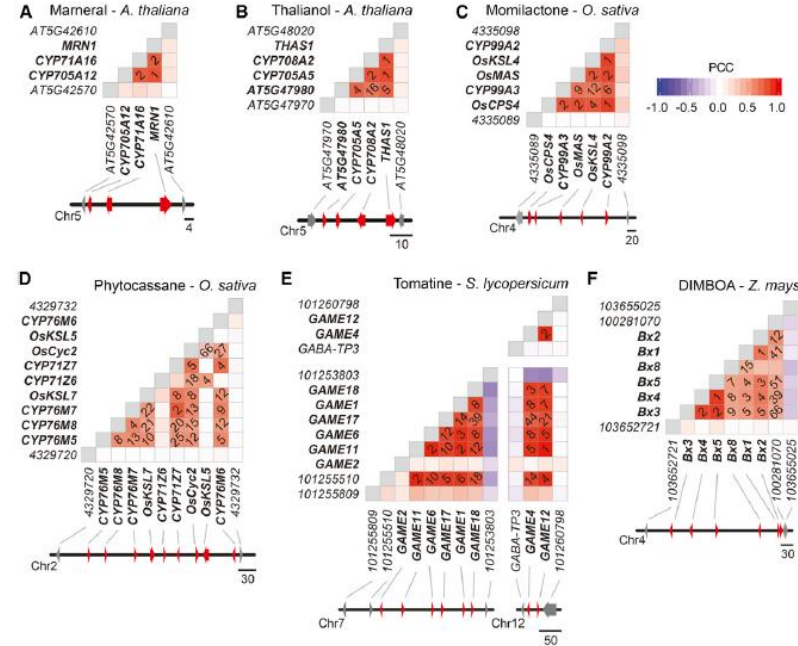
Coexpression Modules Efficiently Recover the Majority of Genes for metGSL Biosynthesis in Arabidopsis



Biosynthetic gene clusters
(BGCs)

Image source: Wisecaver et al.
(2017) Plant Cell, 29:944–59.

Coexpression Patterns of Six Functionally Characterized BGCs in Plants



Regulatory interaction: Mode of action

The PLETHORA Gene Regulatory Network Guides Growth and Cell Differentiation in Arabidopsis Roots [OPEN](#)

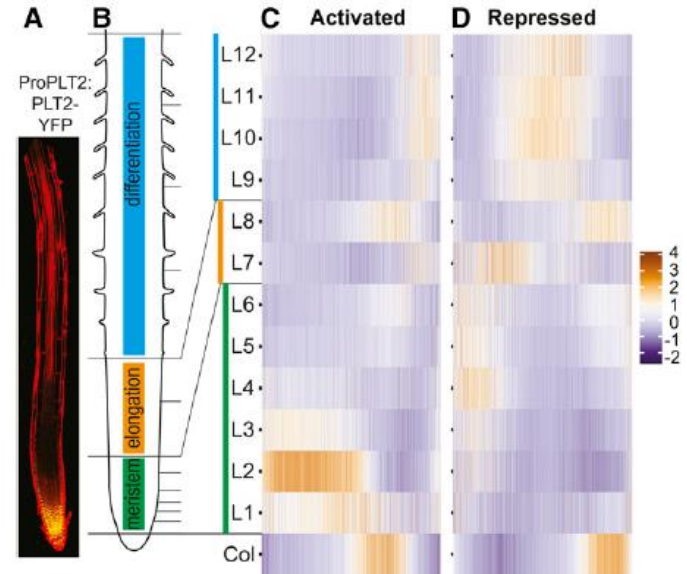
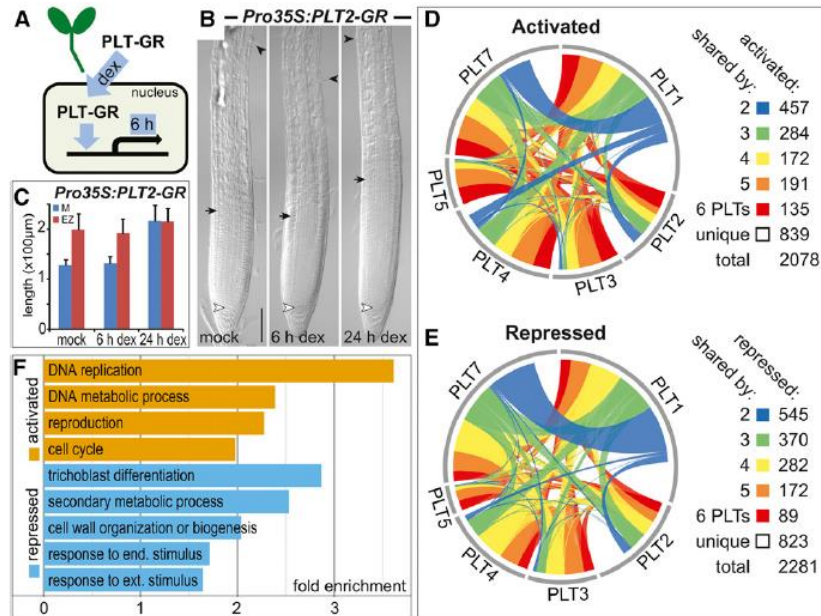


Image source: Santuari et al. (2016) Plant Cell, 28, 2937-51.

Architecture of gene regulatory networks controlling flower development in *Arabidopsis thaliana*

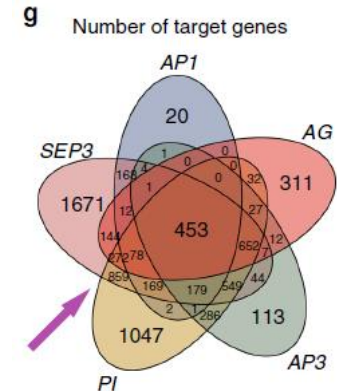
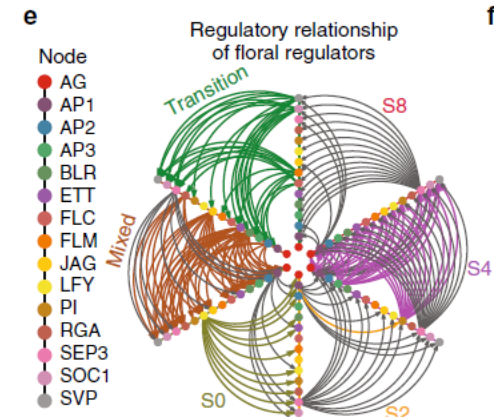
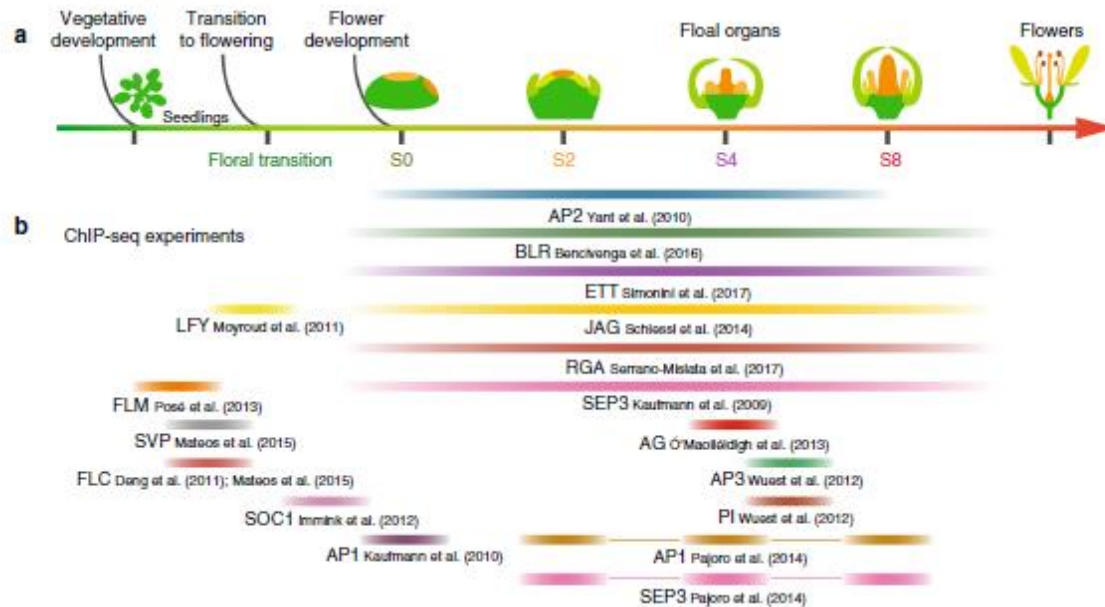


Image source: Chen et al. (2018) NAT. COMM., 9:4534.

Direct downstream targets: Mode of action

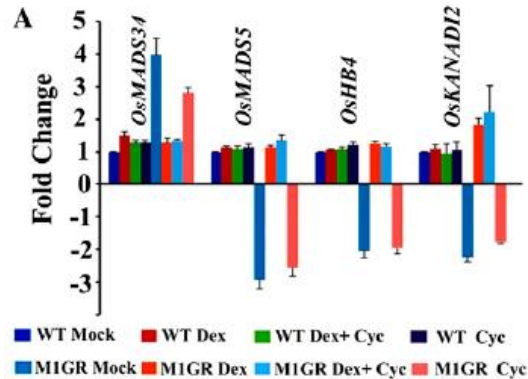
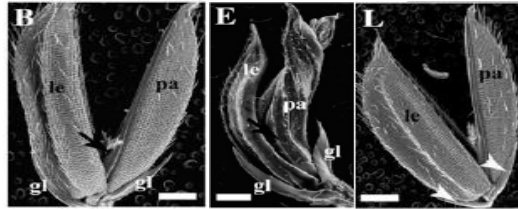


Image source: Khanday, Yadav et al. (2013)
Plant Physiology , 161:1970-83.

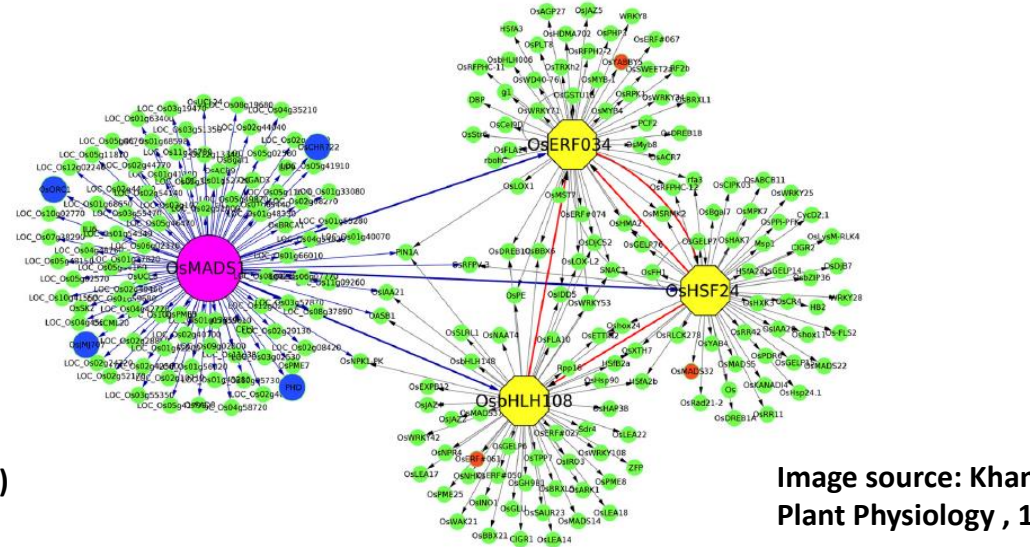
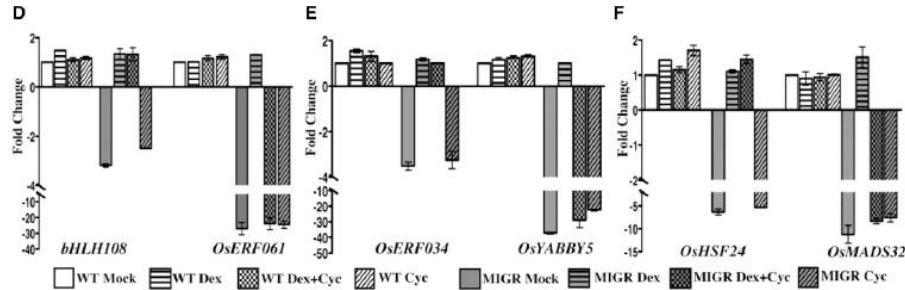


Image source: Khanday et al. (2016)
Plant Physiology , 172:372-88.