Dormancy and Chilling research notes

Justin Ngo
Wolkovich Lab 2024

Epigenetic silencing of callose synthase by VIL1 promotes bud-growth transition in lily bulbs
Authors

Methylation / Callose / Myosin / FT / FLC / VIL1 / PRC2 / H3K27me3 / H3K9ac / CALS3 / NFYA7 / HDA13 / HDA14 /

Study system: *Lilium* 'Siberia'

CHILLING (LTCT): 4 degC
WARMING: 12 degC
DAYLENGTH: not mentioned

- **The overall model**
  - NFYA7 → VIL1-PRC2 –(H3K27me3)–| CALS3 –| growth transition
  - VIL1-PRC2 –| FLC –| FT
  - VIL1-PRC2 → H3K27me3
  - HDA13/14 –| H3K9ac
  - HDA13/14 |—| NFYA7 → H3K27me3/H3K9ac
- **Bud development**
  - Long term cold treatment (LTCT) for at least 8 weeks led to central bud development and emergence; treatment of 12 degC afterward led to floral meristem differentiation
    - Plasmodesmata closed in dormant buds but open in buds of bulbs exposed to LTCT for 8 weeks
    - Callose accumulation significantly higher in dormant buds
  - Transcriptome revealed upregulation of genes involved in histone lysine methylation and glucan metabolism
  - Treatment with myosin inhibitor BDM and callose biosynthesis inhibitor DDG led to acceleration of bud growth
    - Symplastic transport in the SAM was enhanced
  - Treatment with myosin ATPase blocker NEM led to delayed bud growth
    - Symplastic transport in the SAM was decreased
  - FT1 expression increased with extending LTCT
- **LoVIL1**
  - VERNALIZATION INSENSITIVE-LIKE 1 (VIL1): in Arabidopsis, involved in photoperiod detection and vernalization through master regulation of the FT repressors FLC and FLM, flowering loci
  - Dormant buds hardly express VIL1, but LTCT induces expression specifically within SAM and leaf-scale primordia
  - VIL1 silencing leads to delayed central bud growth and shorter buds in general
    - Also increased callose deposition at plasmodesmata
    - Decreased FT1 mRNA in the SAM
    - Sprouting and flowering time significantly delayed
  - WT VIL1 therefore induces floral meristem development and increases FT1 expression
  - Overexpression of VIL1 led to evergreen phenotypes that did not enter dormancy with very low plasmodesmata closure

- **LoVIL1 and LoCALS3**
    - CALS3: Callose synthase 3
    - High expression in the dormant SAM, decreasing expression when SAM entered a stage of active growth
    - Expression pattern is opposite of VIL1
        - VIL1 putatively represses CALS3 activity
        - VIL1 silencing led to upregulation of CALS3 expression
    - CALS3 silenced plants displayed significantly enhanced central bud elongation even after just 6 weeks of LTCT
        - Also led to a decrease in callose biosynthesis by 50% compared to WT
        - Accompanied by an upregulation in FT1 accumulation
- **LoNFYA7**
    - NFYA7: Nuclear Factor Y family subunit A7
    - MULTICOPY SUPPRESSOR OF IRA1 (MSI1): needed for transition into flowering in Arabidopsis; the LOF mutant leads to proliferation of unfertilized ovules and endosperm tissue
    - POLYCOMB REPRESSIVE COMPLEX 2 (PRC2): epigenetic repressor of gene expression; heavily involved in the accumulation of H3K27me3
        - VIL1-PRC2 also suppresses FLC expression, indirectly upregulating FT expression
    - Overexpression of VIL1 led to upregulation of trimethylation at H3K27 (H3K27me3)
        - VIL1 is a subunit of PRC2
        - Yeast-2-hybrid assay revealed NFYA7 as a candidate interactor with the VIL1-PRC2 complex
    - NFYA7 recruits PRC2 via recognizing and binding VIL1
    - High expression in dormant central buds, particularly SAM, and decreased after LTCT, the same as CALS3
    - NFYA7 silencing led to faster central bud, along with 33% decrease in callose accumulation at the plasmodesmata and also an increase in FT1 expression
        - NFYA7 therefore negatively regulates growth transition by increasing callose deposition
    - NFYA7 overexpression led to upregulation in CALS3 mRNA accumulation, and vice versa
        - NFYA7 directly binds to the promoter of CALS3 via CCAAT *cis*-elements (Cces)
    - If both NFYA7 and VIL1 are present, activity of the CALS3 promoter is significantly decreased
- **H3K27me3**
    - H3K27me3 was significantly enriched at the CALS3 locus under the presence of an NFYA binding site
        - VIL1 promoted H3K27me3 enrichment on the CALS3 locus in VIL1 overexpressed plants
        - NFYA7 alone can enrich H3K27me3, but co-overexpression of NFYA7 and VIL1 leads to a much larger enrichment
        - NFYA7 silencing, even in VIL1 overexpressing plants, significantly reduced H3K27me3 enrichment at the CALS3 locus, indicating the importance of VIL-PRC2 recruitment via NFYA7
- **H3K9ac**
    - NFYAs are often associated with acetylation of H3K9 (H3K9ac) and compete with HISTONE DEACETYLASE (HDA13) in soybean (GmHDA13)

- NFYA7 silencing led to significant decrease in H3K9ac enrichment, while overexpression led to an increase in enrichment
    - Lily orthologue HDA14 competes against NFYA7 to regulate CALS3 expression through histone modification (methylation H3K27me3 or acetylation H3K9ac)

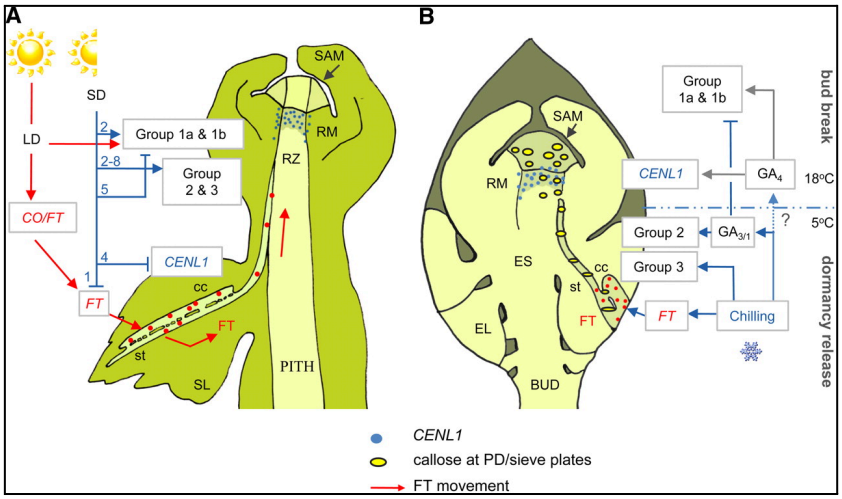
[Chilling of Dormant Buds Hyperinduces FLOWERING LOCUS T and Recruits GA-Inducible 1,3-β-Glucanases to Reopen Signal Conduits and Release Dormancy in Populus]
Authors

FT / Glucanase / GA / GA3ox / GA20ox / CO / CENL1 / DELLA / GID / GIP / Lipid bodies / GH17

Study system: *Populus tremula* × *tremuloides*

CHILLING: 5 degC
WARMING: 18 degC
DAYLENGTH: 10 hour SD / 18 hour LD

- **The overall model**



- **Chilling on primordia**
    - Six weeks of chilling optimal for bud burst, elongation, and leaf growth
- **GA_3 and GA_4**
    - GA_4 induced canonical bud burst while GA_3 failed and instead led to callus-like growth at the leaf bases
    - GA_4 induced shoots had smaller and paler leaves than those that were chilled, suggesting only partial substitution
- **Transport in dormant buds**
    - Dormancy is enforced by blockages in the symplastic pathways in the phloem and shoot apex
    - Chilled plants had symplasm blockages released
    - GA_4 improved this transport capacity
    - GA_3 led to no change in transport capacity compared to dormant plants
- **GH17 genes**
    - GH17: 1,3-β-glucanases of the glucan hydrolase family 17

        - Glycosylphosphatidylinositol (GPI) anchor: a lipid anchor for cell-surface proteins that allows attachment of a protein to the lumen or extracellular surface
        - CBM43/X8 motif: a protein motif that enables callose binding
        - LB: lipid bodies
    - Callose is characterized by glucan bonds of this 1,3-β class
    - GH17 groups:
        - Group 1a: CBM43, sometimes GPI anchor, localized at the cell wall or plasmodesmata
        - Group 1b: GPI anchor, localized to plasmodesmata
        - Group 2: lipid-body associated proteins
        - Group 3: GPI anchor, localized at plasma membrane
    - GA induction
        - GA_4 upregulated expression of 1a and 1b GH17s
        - GA_3 upregulated expression of Group 2 GH17s
        - Group 3 was not significantly affected by either GA application
    - Chilling induction
        - Group 1a peaked at 0 weeks of chilling and decreased with chilling
        - Group 1b peaked at 2 weeks of chilling and decreased after
        - Group 2 peaked at 6 weeks of chilling, but declined right after, aligning with the finding that 6 weeks was optimal for inducing all 3 elements of dormancy release (budburst, elongation, leafing)
        - Group 3 peaked at 2 weeks of chilling
    - Short days induction
        - Groups 1a and 1b peaked early on during short day treatment but declined with increasing short day condition length
        - Group 2 peaked later in SD (5 weeks) except for 1 (GH17_101)
        - Group 3 increased with longer short day conditions
- **GA biosynthesis and signalling**
    - GA3ox1 and GA20ox8 are upregulated during chilling
    - GA biosynthesis gene families have non-redundant functions in this dormancy mechanism
    - GA2ox1, involved in GA deactivation, were upregulated near the end of chilling
    - GID1 (GIBBERELLIN INSENSITIVE DWARF 1), a nuclear GA receptor, was upregulated during short day conditions
    - GIP-like1 (GIBBERELLIN INDUCIBLE PROTEIN-like1) was downregulated during short days, but expression increased following warming if chilling was sufficient
    - DELLA-like1 was downregulated during short days
        - DELLAs are GA signalling repressors
        - Longer than 6 weeks of chilling led to the upregulation of DELLA-like1
- **Flowering locus T (FT), CONSTANS, and CENL1**
    - CONSTANS (CO): a protein that induces floral meristem differentiation and photoperiod sensing, involved in short-day and long-day detection mechanisms
    - CENTRORADIALIS-LIKE 1 (CENL1): an orthologue of Arabidopsis TFL1, a regulator or floral development both temporally and physiologically; involved in transition into dormancy in *Populus*
    - FT protein is expressed in leaf vasculature and localizes to the shoot apex to facilitate elongation

        - Short days cause FT downregulation
        - Chilling induces rapid FT upregulation
        - Budburst leads to rapid FT downregulation
    - Dormant buds have elevated CONSTANS (CO) expression after 2 weeks of chilling, after which it plateaus
        - CO expression further increased when plants are exposed to warm LD conditions after sufficient chilling (8 weeks here, not 6)
    - CENL1: a *P. trichocarpa* orthologue of AtTFL1, encoding a signalling peptide that is FT-like
        - Localized at the rib meristem (RM) underneath the shoot apical meristem (SAM)
        - Chilling alone did not induce CENL1 expression; needed a period of warmth after sufficient chilling
        - Similar expression pattern to long day operating genes like GA biosynthetic GA20ox and GA3ox, GA inactivity GA2ox1, CO, DELLA-like1, and GIP-like1
        - CENL1 expression peaks just before bud burst, which occurs first in the lower lateral buds which also burst first
            - CENL1 therefore positively correlates with the event of budburst itself
    - GA_4 also induced CENL1 expression before budburst
- **GA_4 and lipid bodies**
    - After 5 days of GA_4 feeding, lipid bodies no longer aligned with the plasma membrane and plasmodesmata
    - Lipid bodies congregate at the SAM and RM during dormancy
        - All disappear after GA_4 exposure
    - Coincided with bud swelling
    - Typically, GH17-decorated lipid bodies localize to the cell wall/plasmodesmata; these GH17-LB aggregates are formed during short day conditions and chilling causes them to move to their targets
    - Consequently, reopening of the plasmodesmata via GA_4 application is likely not due to lipid bodies

---

Temperature-dependent growth contributes to long-term cold sensing
Authors

VIN3 / NTL8 / NTL14

Study system: *Arabidopsis thaliana*

CHILLING: 4 degC
WARMING: 20 degC
DAYLENGTH: 16/8 photoperiod for flowering

- **The overall model**
    - NTL8/14 → VIN3
    - VIN3
- **NTL8/14**
    - VERNALIZATION INSENSITIVE 3 (VIN3): PHD protein involved in the PRC2 complex to silence FLC expression epigenetically

    - NAC TRANSMEMBRANE MOTIF1-LIKE 8 (NTL8): a NAC TF involved in various biological processes like salt signalling, trichome development, etc.
    - VIN3 overexpression mutants (VIN3 expression even during warmth) displayed premature termination of translation of NTL8 and dwarfism and leaf deformation
        - NTL8 lacking in the C-terminus putatively led to their constitutive activation
    - In the LOF *ntl8* mutant, VIN3 expression was not affected
    - The closest genetic relative NTL14 can induce VIN3 expression and the NTL8 overexpressing mutant also displays VIN3 constitutive expression
        - The *ntl8 ntl14* double mutant displayed significantly reduced VIN3 expression, even in the cold
    - *ntl8* LOF also displayed reduced silencing of FLC
    - NTL8 and NTL14 therefore represent two redundant members of the NAC TF family that are involved in cold induction of VIN3 and floral development
    - NTL8 overexpressing mutants did not display uniform upregulation of cold-stress response genes, indicating that specificity of NTL8-induced VIN3 upregulation for vernalization
- **Slow degradation of NTL8**
    - NTL8 isoforms have relatively short half-lives (2 days)
    - In warm conditions with rapid growth; dilution of NTL8
    - In cold conditions of slow growth or dormancy; accumulation of NTL8
- **Mathematical model**
    - NTL8 is stable over many weeks during the cold
    - Visualization of localization of NTL8 with GFP revealed accumulation in reduced growth and vice versa
    - Warm-induced new growth had low GFP signal; GFP was only strongly observed in the root tip and cells that were no longer actively growing

---

EARLY BUD-BREAK 1 and EARLY BUD-BREAK 3 control resumption of poplar growth after winter dormancy

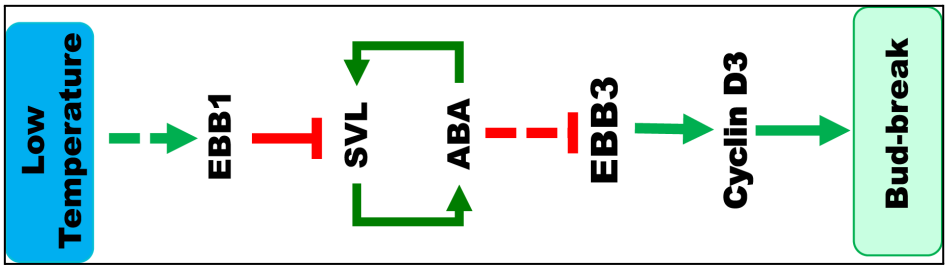
Authors

EBB1 / EBB3 / SVL / FT / CYCD3.1 / AP2/ERF / H3K27me3 / ABA / GA / GA2ox1 / GA3ox2

Study system: *Populus tremula* × *P. alba*

CHILLING: 4 degC
WARMING: 20 degC light, 18 degC dark
DAYLENGTH: 8 hour SD / 16 hour LD

- **The overall model**
    - EBB1 –| SVL --| EBB3 → CYCLIND3.1 → bud cell proliferation
        - EBB1 binds to GCC-box on SVL and represses
        - SVL putatively downregulates EBB3 through ??? mechanism
        - EBB3 binds to GCC-box on CYCD3.1



- **The *ebb3D* mutant displays early bud break phenotype**
  - In this mutant, L34e-like and AP2/ERF-containing TF like PtERF113 are upregulated
    - L34e is a ribosomal protein
    - AP2 refers to APETALA2 and ERF is ethylene-response element (ERE) binding factor; the AP2/ERF domain is a common component in proteins that bind to the GCC-box
      - The GCC-box plays a role in ethylene and jasmonate response
- **EBB3 is PtERF113**
  - Overexpression → early budbreak; Suppression → delay
    - WT function is therefore to initiate budbreak
  - Strong expression in active apical meristem
    - Expression is downregulated during entry into dormancy
    - Highly upregulated during the winter and spring, suggesting positive signalling through low temperatures
    - Expression peaks just before budbreak in spring but downregulated shortly after
  - Trimethylations are sensitive to low temps in peach and pear
    - Particularly at H3K27 (H3K27me3)
    - Low temperature induced demethylation at H3K27
      - Temporal correlation between release from trimethylation and EBB3 upregulation indicates that methylation is responsible for suppression of its expression in warmth
- **EBB1 and SVL**
  - EBB1: AP2/ERF TF
  - SVL: SHORT VEGETATIVE PHASE-LIKE protein; a repressor of FLOWERING LOCUS T (FT)
    - SVL is upregulated during short day conditions and binds to FT2 promoter as a transcription repressor
      - FT2 induces shoot elongation during long days
        - FT2 downregulation induces growth cessation and bud set
        - FT2 also upregulates 13-hydroxylation pathway in GA biosynthesis and also regulates the expression of some GA biosynthesis genes like GA2ox1 and GA3ox2
    - SVL Decreases bioactive GA concentration
  - Consequently SVL accelerates growth cessation and bud set
  - EBB1 upregulated during low temperatures but downregulated during short days
    - After low temp, warmth and long days further upregulate EBB1 expression, i.e. budbreak conditions
    - SVL expression is the exact opposite of EBB1
  - EBB1 likely acts upstream of SVL as a negative regulator
  - SVL contains a GCC-box that the AP2/ERF domain in EBB1 can bind to

        - EBB1 directly binds to the SVL promoter at the GCC-box
- **EBB3 is downregulated in the SVL overexpression mutant**
    - Suggesting its placement downstream of SVL
    - EBB1 is therefore upstream of SVL and EBB3
- **Role of ABA**
    - ABA upregulates SVL and SVL reciprocally upregulates ABA biosynthesis genes
    - ABA downregulates EBB3 expression
        - EBB3 is not responsive to increasing GA; that is, the upregulation of EBB3 in the ABA biosynthetic LOF mutant is not through increased GA but specifically reduced ABA
- **CYCLIND3.1**
    - the only statistically significantly downregulated gene in EBB3 knockdown plants
    - CYCD3.1 is the key driver of progression past the G1/S checkpoint in mitosis and its expression correlates strongly with bud reactivation during budbreak
    - CYCD3.1 is upregulated in EBB3 overexpression mutants; CYCD3.1 is downstream of EBB3
    - CYCD3.1 bears a GCC-box with which EBB3 can bind to as a direct target

---

Cold-induced Arabidopsis FRIGIDA nuclear condensates for FLC repression
Authors

FLC / FRIGIDA / COOLAIR / H3K27me3 / H3K36me3

Study system: *Arabidopsis thaliana*

CHILLING: 5 degC, 8/16 light/dark photoperiod
WARMING: 20 degC, 16/8 light/dark photoperiod
DAYLENGTH: 8 hour SD / 16 hour LD

- **The overall model**
    - Cold → COOLAIR → FRIGIDA → FLC
    - Cold –| FRIGIDA activity → FLC
- **FRIGIDA**
    - A protein needed for flowering in Arabidopsis, associated with vernalization
    - Forms nuclear condensates under cold conditions
    - Nuclear condensates accumulate both in size and number, but are delocalized away from the FLC locus
    - Warmth leads to rapid dissociation of FRIGIDA nuclear condensates which then localize around FLC locus
    - FLC transcription is thus enabled under warm conditions, initiating the reproductive phase
- **COOLAIR**
    - Heterochromatin region at near the terminus of the FLC locus
    - Produces long non-coding RNA in the antisense form of the FLC open reading frame
    - Inducible by small RNA

- Increasing trimethylation at H3K27 (H3K27me3) leads to further repression of FLC expression, opposite occurs when H3K36me3 is decreased (i.e. stronger trimethylation at H3K36 leads to higher FLC expression)
- H3K36me3 is deposited during cold-induced, FRIGIDA-mediated transcription of FLC