Regulation of the seed to seedling developmental phase transition by the LAFL and VAL transcription factor networks

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In the seed, a fundamental transition between embryo and vegetative phases of plant development is coordinated by the interaction between the AFL and VAL sub-clades of the plant specific B3 domain transcription factor family. The AFL B3 factors together with LEC1-type HAP3 transcription factors promote embryo maturation; whereas the VAL B3 factors repress the LEC1/AFL (LAFL) network during seed germination. Recent advances reveal that genes in key developmental programs and hormone signaling pathways are downstream targets of the LAFL network highlighting the central role of the LAFL network in integration of intrinsic developmental and hormonal signals during plant development. The VAL B3 proteins are proposed to mediate repression by recruiting a histone deacetylase complex (HDAC) to LAFL genes that contain the Sph/RY cis-element recognized by AFL and VAL B3-DNA-binding domains. In addition to VAL B3 factors, epigenetic mechanisms are implicated in maintaining repression of LAFL network during vegetative development. © 2013 Wiley Periodicals, Inc.

INTRODUCTION

The evolution of the seed was a key adaption that contributed to the success and diversification of the land plants. Regulation of seed formation and the critical transition between seed and seedling phases of plant development is controlled in part through concerted alterations in the biosynthetic and signaling pathways for major plant hormones including auxin, abscisic acid (ABA), and gibberellins (GA). The plant-specific B3 domain transcription factors were first discovered as mutants of maize [viviparous1 (vp1)] and Arabidopsis [abscisic acid insensitive 3 (abi3)] that alter ABA signaling in the developing seed. In Arabidopsis, seed development is regulated by a network of transcription factors that includes the AFL clade of B3 domain proteins [ABI3, FUSCA3 (FUS3), and LEAFY COTYLEDON 2 (LEC2)] (Figure 1) and two LEC1-type HAP3 family CCAAT-box binding factors, LEC1 and LEC1-LIKE (L1L). Together these genes comprise the LAFL transcription factor network. The program for seed development is refined by mutual interactions of LAFL genes combined with inputs from various hormone, sugar, and light signaling pathways. Key downstream targets of the LAFL network include genes that control major hormone metabolism and signaling pathways, as well as other transcription factor networks that program the transcriptome of the developing seed. Genetic analyses show that this elaborate program must be repressed during germination of the seed in order for the embryo to complete a transition.
to the vegetative phase of the plant life cycle. The VAL/HSI B3 factors [VAL1 (HSI2), VAL2 (HSL1), and VAL3 (HSL2)] which form a sister-clade to the AFL subfamily (Figure 1),10,11 play a central role in coordinating repression of the LAFL network during seed germination through recruitment of chromatin remodeling complexes.

THE LAFL TRANSCRIPTION FACTOR NETWORK

Genetic analyses show that the LAFL network is organized by complex mutual interactions among the LAFL genes (Figure 2). In this respect, the network is neither strictly hierarchical nor linear. While LEC1 can activate ABI3, FUS3, and LEC2 expression14,16; ectopic expression of LEC2 is sufficient to up-regulate LEC1 and FUS3 in vegetative tissue.13 ABI3 and FUS3 in turn are regulated by mutual positive interactions.14 Moreover, LIL was shown to be regulated by FUS3 in a transcriptome analysis.15 While the molecular basis for the genetic interactions among LAFL factors is not yet fully understood, recent insights have been gained through ChIP (chromatin immunoprecipitation)-on-chip analyses. For example, LIL was identified as a potential direct target of LEC1;28 whereas, FUS3 physically interacts with regulatory regions of the LEC1, FUS3, and ABI3 genes22; and, FUS3 was identified as a putative ABI3 target.21

The LAFL transcription factor network regulates diverse seed-specific processes including deposition of storage reserves (starch, storage proteins, and lipids), acquisition of desiccation tolerance, developmental arrest of the embryo, and dormancy.12,14,16,49–51 Important direct targets of LAFL factors include (1) seed storage protein (SSP) and late-embryogenesis-abundant (LEA) genes, (2) genes encoding transcription factors that control lipid biosynthesis and other seed specific processes (Figure 2 and Table 1), and (3) genes that function in hormone metabolism and signaling pathways (Table 2).

LAFL ACTIVATION OF SSP AND LEA GENES

Gene activation by AFL B3 factors is mediated by the Sph/RY cis-element (CATGCA) that is specifically recognized by the B3-DNA-binding domain.26,32–35 Ectopic expression of ABI3 or FUS3 in vegetative tissues causes activation of SSP genes, such as 2S albumin storage protein 3 (At2S3) and Cruciferin C (CRC).16,17 The LEC1 HAP3 factor activates CRC expression indirectly through regulation of AFL B3 factors,12 as well as via a direct interaction with the ABA-response element (ABRE) binding factor basic-leucine-zipper protein 67 (bZIP67).18 An important subset of LAFL regulated genes, including LEA genes, which have both Sph/RY and ABRE motifs in their promoters, are regulated by a combinatorial interaction between ABI3 and ABI5-related bZIP transcription factors.19,20 Hence, coupling of the LAFL network to ABA signaling is mediated by physical interaction of the N-terminal COAR (co-activator/co-repressor) domain of ABI3 with ABI5 and related bZIP factors.19,20 ABREs are also found in the promoters of other target genes of LAFL factors (Table 1), suggesting that other components of the LAFL network are potentially co-regulated by ABA.21,22,28 In addition, elegant studies in Phaseolus vulgaris have delineated the role of histone modifications in transcriptional activation of the phaseolin gene by ABI3 ortholog PVALF and ABA.56

LAFL ACTIVATION OF DOWNSTREAM TRANSCRIPTION FACTOR NETWORKS

Recent studies reveal that combinatorial interactions of LAFL factors up-regulate a diverse array of downstream transcription factor networks (Table 1). These include Zinc finger (Zf) factor PE11, NAC factor CUP-SHAPED COTYLEDON 1 (CUC1), APETALA2 (AP2) family factor BABY BOOM (BBM), and
WRINKLED (WRI1). PEI1 is a potential direct target of ABI3 that is also up-regulated in response to FUS3 over-expression. CUC1, BBM, and WRI1 are identified as targets of FUS3. WRI1 is up-regulated by LEC1 and LEC2. As summarized in Table 1, the downstream transcription factors in turn regulate critical pathways in seed development. Additional targets of FUS3 include FLOWERING LOCUS C (FLC), a key regulator of flowering and vegetative phase transition, as well as diverse NAC, MYB, bHLH, WRKY, bZIP, and Homebox family genes.

LAFL REGULATION OF MULTIPLE HORMONE SIGNALING PATHWAYS

A key function of the LAFL network is re-programming of the major plant hormone signaling pathways in the seed. A set of target genes of LEC1, LEC2, and FUS3 that are implicated in ABA, GA, auxin, brassinosteroid (BR), cytokinin (CK), ethylene, and jasmonic acid (JA) metabolism and signaling pathways is summarized in Table 2. FUS3 establishes the critical balance between dormancy and seed germination inducing signals by simultaneously regulating biosynthesis and turnover of ABA and GA in the seed. While LEC2 also contributes to regulation of ABA, GA, and ethylene biosynthesis pathways; LEC1 and LEC2 principally regulate auxin signaling through activation of YUCCA and IAA genes. By contrast, as noted above, ABI3 has a unique role in integrating ABA signaling with the
### TABLE 1 | Key Developmental Genes Regulated by the LAFL Network

| AGI Code | Gene Name | Protein Family | Cis-Element | Up-Regulated in val1 val2 | LEC1 | LEC2 | FUS3 | ABI3 | References | Potential Function (TAIR) |
|----------|-----------|----------------|-------------|---------------------------|------|------|------|------|------------|--------------------------|
| AT5G47670 | L1L       | HAP3           | Sph/RY; CCAAT box | ✓             | ✓   | ✓    | ✓    |     | 10,15,18,24,28 | Regulator of embryo development |
| AT1G28300 | LEC2      | B3             | Sph/RY       | ✓             | ✓   | ✓    |     |     | 10,12 | Plays critical roles during early and late embryo development |
| At3g26790 | FUS3      | B3             | Sph/RY       | ✓             | ✓   | ✓    | ✓    | ✓    | 10,12–14,21,22,24 | Regulator of gene expression during late embryogenesis |
| AT3G24650 | ABI3      | B3             | Sph/RY; ABRE  | ✓             | ✓   | ✓    | ✓    | ✓    | 10,12,14,22,24 | Regulator of the transition between embryo maturation and early seedling development |
| AT2G30470 | VAL1/HSI2 | B3             | Sph/RY       |               | ✓   |      |      |     | 15,22 | Repression of seed maturation program during germination |
| AT5G07500 | PEI1      | Zf             | Sph/RY       | ✓             | ✓   | ✓    |     |     | 10,15,21 | Required for heart-stage embryo formation |
| AT3G15170 | CUC1      | NAC            | Sph/RY       | ✓             | ✓   | ✓    |     |     | 10,22 | Shoot apical meristem formation and auxin-mediated lateral root formation |
| AT5G17430 | BBM       | AP2            | —            | ✓             | ✓   | ✓    |     |     | 10,22 | Promotes cell proliferation, differentiation and morphogenesis, especially during embryogenesis |
| AT3G54320 | WRI1      | AP2            | Sph/RY; ABRE  | ✓             | ✓   | ✓    | ✓    | ✓    | 10,15,22–24 | Control of lipid biosynthetic and metabolic processes |
| AT5G10140 | FLC       | MADS box       | Sph/RY       | ✓             | ✓   | ✓    |     |     | 10,15 | A repressor of floral transition |
| AT5G13790 | AGL15     | MADS box       | Sph/RY       | ✓             | ✓   | ✓    |     |     | 10,15,22,26 | Embryonic and post embryonic development |
| AT3G27785 | MYB118    | MYB            | —            | ✓             | ✓   | ✓    |     |     | 21    | Regulates the embryonic pathway by up-regulating LEC1 |
| AT1G03770 | RING1b    | PRC1           | Sph/RY       | ✓             | ✓   | ✓    |     |     | 10,22 | Core component of Polycomb Repressive Complex1 (PRC1). Interacts physically with CLF and LHP1 and function together to repress target gene expression. |
| AT2G46685 | MIR166    | —              | Sph/RY       | ✓             | ✓   | ✓    |     |     | 22    | Encodes a microRNA that targets several HD-ZIPIII family members including PHV and PHB |
### TABLE 2 | Overview of Hormone Pathway Genes Regulated by the LAFL Network

| Hormone | Pathway | AGI      | Gene      | LEC1 | LEC2 | FUS3 | Method | References |
|---------|---------|----------|-----------|------|------|------|--------|------------|
| ABA     | Biosynthesis | AT1G30100 | Nced5 | ✓   |      |      | Cc     | 22         |
|         |         | AT3G24220 | Nced6 | ✓   |      |      | M      | 15         |
|         |         | AT1G78390 | Nced9 | ✓   |      |      | M      | 15         |
|         |         | AT1G52340 | ABA2  | ✓   |      |      | M      | 15         |
|         | Signaling | AT3G44460 | bZIP67 | ✓   |      |      | M      | 15         |
|         |         | AT2G41070 | bZIP12/EEL | ✓ | ✓ | | M, Cc, Q, E | 15,22,26 |
|         |         | AT1G42990 | bZIP60 | ✓ | ✓ | | Cc | 28         |
|         |         | AT3G58120 | bZIP61 | ✓ | ✓ | | Cc | 22         |
| GA      | Biosynthesis | AT1G05160 | CYP88A3 | ✓ |     |     | M     | 15         |
|         |         | AT1G80340 | GA3OX2 | ✓ | ✓ |     | Q,E   | 27         |
|         |         | AT4G21690 | GA3OX3 | ✓ | ✓ |     | Cc     | 22         |
|         |         | AT1G80330 | GA3OX4 | ✓ | ✓ |     | M      | 15         |
|         |         | AT5G51810 | GA200X2 | ✓ | ✓ | | M | 15         |
|         |         | AT5G07200 | GA200X3 | ✓ | ✓ | | M | 15         |
|         | Catabolism | AT1G47990 | GA20X4 | ✓ | ✓ |     | Cc | 22         |
|         |         | AT5G56300 | GAMT2 | ✓ | ✓ | | Cc | 22         |
| Auxin   | Biosynthesis | AT4G13260 | YUC2 | ✓ | ✓ |     | M, C, Q | 13,15      |
|         |         | AT5G11320 | YUC4 | ✓ | ✓ |     | M, C, Q | 13,15      |
|         |         | AT1G04180 | YUC9  | ✓ | ✓ |     | Cc | 22         |
|         |         | AT1G48910 | YUC10 | ✓ | ✓ |     | M, C, Q | 15,28      |
|         |         | AT5G20960 | AA01  | ✓ | ✓ | | M | 15         |
|         |         | AT3G44300 | NIT2  | ✓ | ✓ | | M | 15         |
|         | Catabolism | AT5G555250 | IAMT1 | ✓ | ✓ | | Cc | 22         |
|         |         | AT1G44350 | ILL6  | ✓ | ✓ | | Cc | 22         |
|         | Signaling | AT3G62980 | TIR1  | ✓ | | | Cc | 28         |
|         |         | AT5G62000 | ARF2  | ✓ | ✓ | | Cc | 22         |
|         |         | AT1G30330 | ARF6  | ✓ | ✓ | | Cc | 22         |
|         |         | AT1G19220 | ARF19 | ✓ | ✓ | | Cc | 22         |
|         |         | AT4G14560 | IAA1  | ✓ | ✓ | | Q | 13         |
|         |         | AT1G04550 | IAA12 | ✓ | ✓ | | Cc | 22         |
|         |         | AT1G04250 | IAA17 | ✓ | ✓ | | Cc, Q | 13,22      |
|         |         | AT3G04730 | IAA16 | ✓ | | | Cc | 28         |
|         |         | AT3G15540 | IAA19 | ✓ | ✓ | | Cc, Q | 28         |
|         |         | AT3G62100 | IAA30 | ✓ | ✓ | | M, Q | 26         |
|         |         | AT3G17600 | IAA31 | ✓ | ✓ | | M | 15,26      |
| BR      | Biosynthesis | AT3G50660 | DWF4 | ✓ |     | | Cc, Q | 28         |
|         |         | AT4G36380 | ROT3  | ✓ |     | | M | 15         |
|         |         | AT3G30180 | BR6OX2 | ✓ | | | M | 15         |
|         | Catabolism | AT2G36800 | DOGT1 | ✓ | | | Cc, Q | 28         |
|         | Signaling | AT1G19350 | BES1  | ✓ | | | Cc, Q | 28         |
|         |         | AT3G61460 | BRH1  | ✓ | | | Cc, Q | 28         |
TABLE 2 | Continued

| Hormone | Pathway  | AGI | Gene   | LEC1 | LEC2 | FUS3 | Method | References |
|---------|----------|-----|--------|------|------|------|--------|------------|
| CK      | Biosynthesis | AT1G68460 | IPT1 | ✓    |      |      | M      | 15         |
|         |          | AT1G25410 | IPT6 | ✓    |      |      | M      | 15         |
| Catabolism |          | AT1G75450 | CKX5 | ✓    |      |      | Cc     | 22         |
| Ethylene | Biosynthesis | AT1G01480 | ACS2 | ✓    |      |      | M      | 15         |
|         |          | AT2G22810 | ACS4 | ✓    | ✓    |      | Q      | 13         |
|         |          | AT4G11280 | ACS6 | ✓    | ✓    | ✓    | M, Q   | 29         |
|         | Signaling | AT4G17500 | ERF1 | ✓    | ✓    | ✓    | M, Q   | 29         |
|         |          | AT5G47220 | ERF2 | ✓    | ✓    | ✓    | M      | 29         |
|         |          | AT1G28360 | ERF12 | ✓    | ✓    | ✓    | M      | 29         |
|         |          | AT5G61600 | ERF104 | ✓    | ✓    | ✓    | M      | 29         |
|         |          | AT1G25560 | ERF12 | ✓    | ✓    | ✓    | M, Q   | 29         |
|         |          | AT1G13260 | ERF12 | ✓    | ✓    | ✓    | M, Q   | 29         |
|         |          | AT5G25190 | ESE3 | ✓    | ✓    | ✓    | M      | 29         |
| JA      | Catabolism | AT1G19180 | JAZ1 | ✓    | ✓    | ✓    | Cc     | 22         |
|         | Signaling | AT1G19640 | JMT  | ✓    | ✓    | ✓    | M      | 15         |

Method: C, ChIP; Cc, ChIP-on-chip; M, Microarray; Q, Quantitative PCR; E, Electrophoretic mobility shift assay.

LAFL network through interactions mediated by its N-terminal COAR domain with bZIP factors.19,20 Interestingly, LAFL factors also play a role in postembryonic plant development by coordinating hormone signaling networks. For instance, FUS3 was shown to regulate vegetative phase transitions (juvenile to adult phase) by controlling the ethylene-responsive gene expression.29 In addition, LEC1 was found to be involved in regulation of hypocotyl elongation-related functions by targeting genes in auxin, BR, and light signaling networks.28 Therefore, the LAFL network participates in integration of hormonal and intrinsic developmental signals during seed development and other developmental stages. The implications of LAFL regulation of CK and JA signaling pathways remain to be determined.

REGULATION OF THE LAFL NETWORK

Genes implicated in activation of the LAFL network early in seed development (Figure 2 and Table 1) include the MADS-box factor AGAMOUS-LIKE15 (AGL15),30,31 HD-ZIP III family factors PHABULOSA (PHB) and PHAVOLUTA (PHV),32 and MYB115/118.33 LEC1 and LEC2 are up-regulated in transgenic plants over-expressing of AGL15.30 Moreover, AFL B3 genes were identified as direct targets of AGL15.31 While these lines of evidence indicate that AGL15 acts upstream of the AFL B3 network, AGL15 is also regulated by LAFL factors. For example, AGL15 was identified as a direct target of FUS322 and its expression is induced by LEC2.26 LAFL factors are activated in vegetative tissues by over-expression of adaxial/abaxial polarity genes PHB and PHV, and PHB has been shown to physically associate with the LEC2 promoter.32 In addition, LEC1 is up-regulated by over-expression of MYB115 or MYB118.33 Interestingly, ectopic expression of ABI3 in transgenic seedlings also up-regulates MYB118 transcription in presence of ABA.21 These findings indicate that upstream regulators and LAFL factors mutually regulate each other. To varying degrees, ectopic expression of individual LAFL genes and upstream regulators can induce expression of embryonic traits in vegetative tissues.4–6,17,30,32,33,49

REPRESSION OF THE LAFL NETWORK DURING GERMINATION

Genetic studies show that repression of the LAFL embryonic pathway during germination is necessary to enable the transition to seedling development. Key pathways that maintain repression of the LAFL network in the embryo prior to its transition to seedling development are summarized in Figure 2. The corresponding mutants commonly display embryonic traits during vegetative development though with
variable penetrance (Table 3). In addition, the subset of genes in the LAFL network that are derepressed differ among mutants (Table 3). Genes implicated in direct repression of the LAFL network include the VAL B3 factors, chromatin modifiers, and trihelix factors (Table 3), whereas, other mechanisms such as the miRNA (miR166) pathway most likely act indirectly via silencing of upstream regulator PHB and PHV.32

REPRESSION OF THE LAFL NETWORK BY VAL B3 DOMAIN FACTORS

Repression of the LAFL network is mediated by a family of VAL B3 domain factors that are closely related to the AFL B3 factors (Figures 1 and 2, Table 3).10,11 No other mutants implicated in repression display full activation of LAFL network and the extent of embryonic seedling phenotypes observed in the val1 val2 mutant (Table 3). GA signaling can enhance the repression of LAFL network by VAL factors.10 Although the DNA binding specificity of VAL B3 domain has not been directly determined, transcriptomics analyses of val mutants are consistent with the hypothesis that the VAL B3 domain binds the same Sph/RY motif recognized by the AFL B3 domain.10 In addition, VAL proteins contain conserved PHD-L (plant homeodomain-like) Zf, CW-Zf, and EAR [ethylene response factor (ERF)-associated repression] domains (Figure 1). The CW-Zf domain of VAL1 was shown to interact with the histone 3 lysine 4 trimethylation (H3K4me3) marks.58 Although the PHD domain has been shown to be H3K4me3 reader,59 the specificity of the divergent VAL PHD-L domain is not yet known. A mutation in VAL1 PHD-L domain leads to derepression of seed-specific genes, including FUS3 and AGL15 confirming that the PHD-L domain has a critical role in VAL mediated transcriptional repression.60 EAR motifs mediate transcriptional repression through interacting with co-repressors, such as SIN3 (SWI-independent 3) and TOPLESS (TPL), to recruit a histone deacetylase complex (HDAC) to target genes.61 VAL1 was identified as a SIN3-LIKE 1 (SNL1) interacting protein in a yeast two-hybrid (Y2H) assay36; however, it is not yet confirmed that the EAR motif is necessary for this interaction. Many genes up-regulated in the val1 val2 double mutant are also identified as direct targets of LAFL factors (Table 1), suggesting that VAL may directly target LAFL factors to shut off the network upon germination. This hypothesis is supported by a recent study showing that HDA19 interacts directly with the CW-Zf domain of VAL2 to repress expression of LEC1, LEC2, and other seed maturation genes.35 HDA6 and HDA19 were also shown to act redundantly to repress of ABI3, FUS3, and LEC1 expression in the leaf tissues (Table 3).34 Hence, one possible mechanism underlying VAL B3-mediated transcriptional repression is that VAL proteins recruit an HDAC to target genes that contain Sph/RY-motifs.

### Table 3

| Mutant or RNAi | Protein Family | Embryonic Trait | EC Penetrance | Up-Regulation of LAFL Factors | References |
|---------------|----------------|-----------------|---------------|-------------------------------|------------|
| val1 val2     | B3             | EC and arrested growth | High          | LAFL                          | 10,11      |
| HDA6/HDA19 RNAi| HDAC           | ELS and arrested growth | n.a.          | LEC1, FUS3, and ABI3          | 34         |
| clf swn       | PcG            | EC and arrested growth | No data       | LEC1, LEC2 and FUS3           | 41,62      |
| Atring1a Atring1b | PcG            | EC and arrested growth | Intermediate | LAFL                          | 39         |
| Atrbmi1a Atrbmi1b | PcG            | EC and arrested growth | Intermediate | LAFL                          | 39         |
| pkl pkr2      | CHD3           | EC               | Low           | LEC1, LEC2, and FUS3          | 41,57      |
| RBR RNAi or RBR overexpression | RB | ECP and arrested growth | n.a.          | ABI3 and LEC2 (induced by sucrose) | 43         |
| Brm           | SWI/SNF        | Arrested growth   | n.a.          | FUS3                          | 46         |
| asil1         | Trihelix       | Arrested growth   | n.a.          | LAFL                          | 47         |

ELS, embryo-like structure; ECP, embryonic cell proliferation; Penetrance: low, 10–30%; intermediate, 30–70%; high, 70–100%. n.a., not applicable. Embryonic traits: EC, embryonic callus. All mutants accumulate SSPs and lipids.1 Shoot and root.2 Primary root tip.3 Shoot.4 Cotyledon and root.
recognized by the B3 domain and specific chromatin marks recognized by the PHD-L and CW-Zf domains.

**REPRESSION OF THE LAFL NETWORK BY CHROMATIN MODIFICATIONS**

Chromatin modifications are emerging as a key mechanism for maintaining repression of the LAFL network during vegetative development. At least three distinct interacting chromatin modification systems are implicated in repression of the LAFL network (Table 3): (1) polycomb repressive complex 2 (PRC2), (2) polycomb repressive complex 1 (PRC1), and (3) CHD3 (chromodomain, helicase/ATPase, and DNA binding domain) and SWI/SNF (SWITCH/SUCROSE NON-FERMENTING) families of chromatin remodeling factors.

PRC2 proteins [CURLY LEAF (CLF), SWINGER (SWN), and MEDEA (MEA)] add H3K27me3 marks at repressed loci. Consistent with PRC2 involvement in repression of LAFL network, LEC1, LEC2, FUS3, and ABI3 genes have H3K27me3 marks in vegetative tissues, and FUS3 has been identified as a direct target of MEA. However, the mechanisms for PRC2 recruitment to target loci remain unclear in plants. Recent work identifying a cis-element, repressive LEC2 element (RLE), that is required for H3K27me3 modification and transcriptional repression of LEC2 during vegetative growth, sheds new light on the mechanism of PRC2 recruitment. Other proteins that partner with PRC2 include RETINOBLASTOMA-RELATED PROTEIN (RBR) which interacts with the MULTICOPY SUPPRESSOR OF IRA1 (MSI1) component of PRC2. RBR interacts with the promoter of LAFL member ABI3, and is required for establishing H3K27me3 modification.

Acting in concert with PRC2, PRC1 proteins including LIKE HETEROCHROMATIN PROTEIN1 (LHP1), EMBRYONIC FLOWER 1 (EMF1), RING1a-b and BMI1a-b recognize the H3K27me3 marks and induce histone 2A lysine 119 mono-ubiquitination (H2AK119ub1) to maintain a stable repressed state of target loci. Consistent with the up-regulation of LAFL genes observed in PRC1 mutants, LEC2, FUS3, and ABI3 were identified as direct targets of EMF1 in ChIP analyses. In addition to the PRC complexes, CHD3 and SWI/SNF families of chromatin remodeling ATPases encoded by the PICKLE (PKL), PICKLE-RELATED 2 (PKR2), and BRAHAM (BRM) genes, respectively, are implicated in repression of the LAFL network.

Recent studies suggest that PKL regulation of LAFL genes is mediated by interaction with PRC2. For instance, during seed germination, PKL is bound to the promoter regions of LEC1, LEC2, and FUS3 genes that are enriched for H3K27me3 modification. In addition, PKL and PKR2 may indirectly promote H3K27me3 modification at target loci by controlling the expression of PRC2 genes including EMF2, CLF, and SWN. BRM in turn contributes to repression of FUS3 and ABA-response factor ABI5 in leaf tissues where it physically interacts with target promoters.

Other potential players include a plant specific trihelix factor, ARABIDOPSIS 6b-INTERACTING PROTEIN LIKE1 (ASIL1), which binds to a GT cis-element (CGTGATT) found in promoters of LAFL genes where it frequently overlaps ABRE and Sph/Ry elements recognized by bZIP and B3 proteins, respectively.

While the VAL B3 proteins evidently play a central role in mediating repression of the LAFL network during germination through recruitment of an HDAC; it is still elusive how VALs physically and functionally interact with other chromatin modification pathways. Interestingly, VAL1, RING1b, and miR166 were shown to be direct targets of FUS3, and PKL expression is enhanced when LEC1 is over-expressed, which suggest that LAFL factors (mainly FUS3) have a role in controlling the feedback regulation of the network (Table 1 and Figure 2). Consistent with this hypothesis, PKR2 and RING1b are up-regulated in val1 val2 seedlings.

**CONCLUSION**

Recent findings advance our understanding of the role of LAFL network in integrating the complex hormonal and intrinsic developmental signals that control seed development. While the resulting seed is superbly adapted for enabling propagation of the seed plants in diverse environments, a massive reprogramming of the transcriptome and attendant hormone signaling pathways is evidently required before the plant can resume vegetative development. We propose that repression is initiated by recruitment of an HDAC to genes that contain a combination of active chromatin marks recognized by PHD-L and CW-Zf domains and the Sph/Ry motif recognized by the B3-DNA-binding domain. However, key predictions of this model including the binding specificities of the VAL B3 and PHD-L domains remain to be tested.
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