Focus Article

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.

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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*)][1] and *Arabidopsis [abscisic acid insensitive 3* (*abi3*)][2] 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,[2] FUSCA3 (FUS3),[3] and LEAFY COTYLEDON 2 (LEC2) [4]] (Figure 1) and two LEC1-type HAP3 family CCAAT-box binding factors, LEC1[5] and LEC1-LIKE (L1L).[6] 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.[7–9] 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

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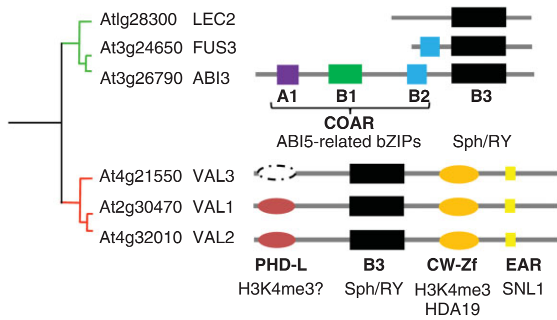

FIGURE 1 | Domain architectures of AFL and VAL B3 transcription factors. The AFL and VAL groups are sister clades in the ABI3/VP1 family of B3 proteins in *Arabidopsis*. AFL and VAL proteins have distinct domain architectures: B3 domain (dark), B1 (green), B2 (blue), A1 (purple), PHD-L domain (red), CW domain (orange), and EAR motif (yellow). ABI3 has an N-terminal co-activator/co-repressor (COAR) domain that physically interacts with ABI5-related-bZIP factors. VAL3 has an incomplete PHD-L domain (dashed circle). AFL B3 domains specifically bind to the Sph/RY motif (CATGCA), and VAL B3 domains are proposed to bind the same motif. PHD and CW-Zf domains are identified as the histone modification readers that recognize the H3K4me3 mark. VAL2 CW-Zf interacts with HDA19 to repress target gene transcription (see text). EAR motif may mediate the interaction of VAL1 with co-repressor SNL1 (see text).

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* expression[14,16]; 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, *L1L* 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, *L1L* was identified as a potential direct target of LEC1[28]; whereas, FUS3 physically interacts with regulatory regions of the *LEC1*, *FUS3*, and *ABI3* genes[22]; 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.[26,52–55] 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 PEI1, NAC factor CUP-SHAPED COTYLEDON 1 (CUC1), APETALA2 (AP2) family factor BABY BOOM (BBM), and

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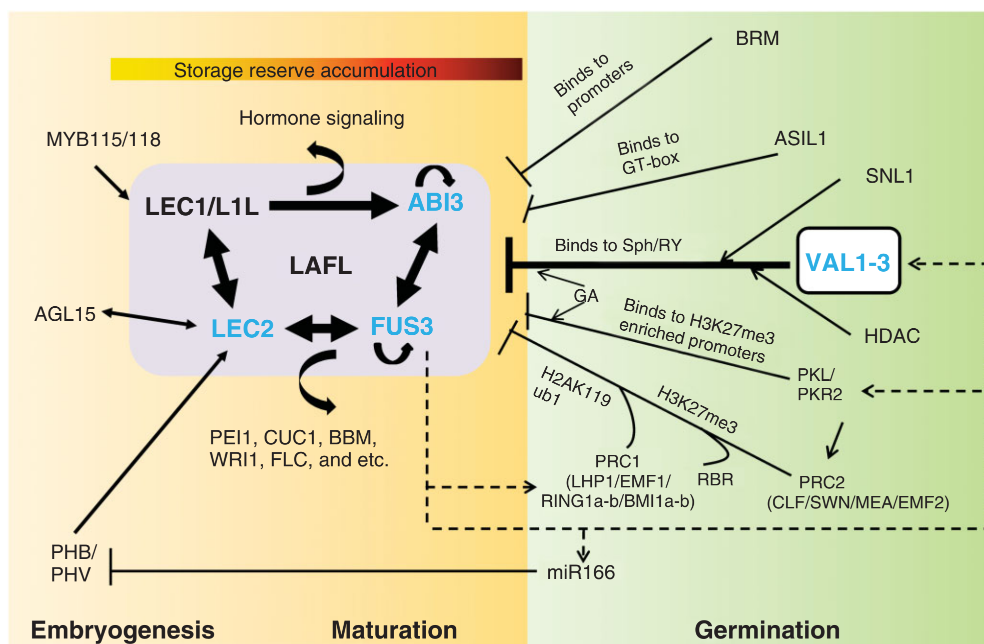

FIGURE 2 | LAFL and VAL networks regulate the seed to seedling phase transition. Spatial and temporal patterns of LAFL gene expression are refined by mutual interactions.[12–15] Important direct targets of LAFL factors include (1) SSP [12,16–18] and LEA genes,[19,20] (2) transcription factor genes that control seed specific processes including *PEI1*,[15,21] *CUC1*,[22] *BBM*,[22] *WRI1*[22–24] and *FLC*,[15,25] and (3) genes that function in major hormone metabolism and signaling pathways.[13,15,22,26–29] AGL15,[30,31] PHB/PHV,[32] and MYB115/118 [33]are proposed to act upstream of LAFL network. The LAFL network is repressed by VAL B3 factors and other repressors during seed germination to enable the transition to seedling development. VAL factors play a central role in repression of LAFL network in part by binding to the Sph/RY motif and recruiting an HDAC.[10,11,34,35] The interaction of VAL B3 factors with HDAC may be mediated by the co-repressor SNL1.[36] PRC2 proteins (CLF/SWN/MEA/EMF2) add H3K27me3 marks to LAFL genes.[37,38] Acting in concert with PRC2, PRC1 proteins (LHP1/ EMF1/RING1a-b/BMI1a-b) bind to H3K27me3 marks and deposit H2AK119ub1 to maintain a stable repressed state of *LAFL* genes.[37,39,40] The CHD3 chromatin remodeling factors, PKL and PKR2, can indirectly promote H3K27me3 modification by up-regulating genes encoding PRC2 proteins.[41] PKL is also present in the promoter region of *LEC1*, *LEC2* and *FUS3* genes that are enriched for H3K27me3.[42] RBR can interact with the promoter of *ABI*3, and is required for establishing H3K27me3 modification by cooperating with PRC2.[43,44] The SNF2 chromatin remodeling ATPase, BRM, can repress seed maturation genes by physically interacting with their promoters.[45,46] The plant-specific trihelix factor, ASIL1, contributes to repression of *LAFL* genes by binding to the GT element (CGTGATT).[47] miR166 indirectly represses *LEC2* transcription by targeting upstream *PHB* and *PHV*.[32] GA signaling enhances VAL and PKL repression of the LAFL network.[10,48] Negative feedback loops: LAFL factors (FUS3 and LEC1) up-regulate *VAL1*, *RING1b*, *miR166*, and *PKL*.[22,24] Black lines with arrows indicate activation, and black lines ending with bars indicate repression. Inferred functions with less experimental evidence are indicated by dashed lines.

WRINKLED (WRI1). *PEI1* is a potential direct target of ABI3[21] that is also up-regulated in response to *FUS3* over-expression.[15] *CUC1*, *BBM*, and *WRI1* are identified as targets of FUS3.[22] *WRI1* is up-regulated by LEC1[24] and LEC2.[23] 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)*,[15] a key regulator of flowering and vegetative phase transition,[25] as well as diverse NAC, MYB, bHLH, WRKY, bZIP, and Homebox family genes.[22]

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.[13,15,22,26–29] Table 2 highlights the central role of FUS3 in coordinating developmental regulation of hormone signaling. For example, 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.[27,49] While LEC2 also contributes to regulation of ABA, GA, and ethylene biosynthesis pathways[13,26]; LEC1[28] and LEC2[13,26] 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

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TABLE 1 | Key Developmental Genes Regulated by the LAFL Network

AGI Code	Gene Name	Protein Family	Cis-Element Enriched in LAFL Bound Promoter	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

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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	*GA20OX2*			√	M	15
		AT5G07200	*GA20OX3*			√	M	15
	Catabolism	AT1G47990	*GA2OX4*			√	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	*AAO1*			√	M	15
		AT3G44300	*NIT2*			√	M	15
	Catabolism	AT5G55250	*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

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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*			√	Cc	22
		AT5G61600	*ERF104*			√	M	29
		AT1G25560	*EDF1*			√	M, Q	29
		AT1G68840	*EDF2*			√	M, Q	29
		AT1G13260	*EDF4*			√	M, Q	29
		AT5G25190	*ESE3*			√	M	29
JA	Catabolism	AT1G19640	*JMT*			√	M	15
	Signaling	AT1G19180	*JAZ1*			√	Cc	22
		AT1G72450	*JAZ6*	√			Cc	28

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-ZIPIII 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 FUS3[22] 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

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TABLE 3 | Mutants Causing Ectopic Embryonic Traits and Up-Regulation of LAFL Network during Vegetative Development

Mutant or RNAi	Protein Family	Embryonic Trait	EC Penetrance	Up-Regulation of LAFL Factors	References
val1 val2	B3	EC[1] 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[3] and arrested growth	No data	LEC1, LEC2 and FUS3	41,62
Atring1a Atring1b	PcG	EC[4] and arrested growth	intermediate	LAFL	39
Atbmi1a Atbmi1b	PcG	EC[4] and arrested growth	Intermediate	LAFL	39
pkl	CHD3	EC[2]	Low	LEC1, LEC2, and FUS3	48,57
pkl pkr2	CHD3	EC[2]	Intermediate	LEC1, FUS3, ABI3	41
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.

variable penetrance (Table 3). In addition, the subset of genes in the LAFL network that are de-repressed 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 de-repression 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) assay[36]; 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

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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.[40] Consistent with PRC2 involvement in repression of LAFL network, *LEC1*, *LEC2*, *FUS3*, and *ABI3* genes have H3K27me3 marks in vegetative tissues,[37,38] and *FUS3* has been identified as a direct target of MEA.[62] 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,[63] sheds new light on the mechanism of PRC2 recruitment. Other proteins that partner with PRC2 include RETINOBLASTOMA-RELATED PROTEIN (RBR) which interacts with the MULTICOPYSUPPRESSOR OF IRA1 (MSI1) component of PRC2.[44] RBR interacts with the promoter of LAFL member *ABI3*, and is required for establishing H3K27me3 modification.[43]

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 monoubiquitination (H2AK119ub1) to maintain a stable repressed state of target loci.[37,39,40] 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.[37]

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.[41,42] For instance, during seed germination, PKL is bound to the promoter regions of *LEC1*, *LEC2*, and *FUS3* genes that are enriched for H3K27me3 modification.[42] 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*.[41] BRM in turn contributes to repression of *FUS3*[46] and ABA-response factor *ABI5*[45] 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)[47], 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,[22] and *PKL* expression is enhanced when *LEC1* is over-expressed,[24] 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.[10]

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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