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Review Article

Angiosperm–Specific PcG Protein EMF1 Mainly Functions in Vegetative Development

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ABSTRACT

Polycomb group (PcG) proteins are relatively conserved in animal and plant, associating with the epigenetic modification and transcriptional silencing of target genes. Imaginably, PcG proteins in animal and plant should display different properties in some aspects as well, such as protein sequence, function, expression, and composition, based on both almost opposite behaviors. Here, we review a plant–specific PcG protein Embryonic Flower 1 (EMF1) which is necessary for maintenance of vegetative development. Sequence blast and phylogenic analysis indicated that EMF1 homologs are only found in angiosperm, divided into dicot and monocot groups. Domain searching showed that no obviously established domains are discovered in EMF1–like proteins. Then we introduce its expression pattern, subcellular localization and detailed functions both in diverse developmental stages and as a PcG role based on published results.

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INTRODUCTION

Polycomb group (PcG) proteins were initially identified in *Drosophila melanogaster*, to repress the expression of the homeotic Hox genes related to embryo segmentation (Lewis, 1978; Jurgens, 1985). PcG proteins mainly form polycomb repressive complex 1 (PRC1) and PRC2, together acting as an epigenetic memory system that is conserved in plants and animals. In animal, the initial complex PRC2 catalyzes and introduces H3K27me3 into the target genes, and then the maintenance complex PRC1 recognizes and binds this epigenetic mark, and subsequently catalyzes histone H2A lysine119 monoubiquitylation (H2AK119ub) (Lund and van Lohuizen, 2004; Cernilogar and Orlando, 2005).

Drosophila core PRC2 components are comprised of Enhancer of Zeste [E(z), histone methyltransferase], Suppressor of Zeste12 [Su(z)12], and Extra Sex Combs (ESC), and p55. Correspondingly, Arabidopsis PRC2–like proteins include three E(z) homologs CLF, MEAEA (MEA), and SWINGER (SWN); three Su(z)12 homologs EMBRYONIC FLOWER2 (EMF2), FERTILIZATION–INDEPENDENT SEED2 (FIS2), and VERNALIZATION2 (VRN2); one Esc homolog FERTILIZATION–INDEPENDENT ENDOSPERM (FIE); five p55 homologs MULTICOPY SUPPRESSOR OF IRA1(MSI1)-5 (see review in Pien and Grossniklaus, 2007). These proteins can form different PRC2 complexes, which function at different development stages and events, but also share some target genes. The EMF2 complex (CLF/SWN, EMF2, FIE and MSI1) suppresses precocious flowering and cell dedifferentiation; the VRN2 complex (CLF/SWN, VRN2, FIE and MSI1) is responsible for FLC silencing after vernalization; and the FIS2 complex (MEA, SWN, FIS2, FIE and MSI1) prevents endosperm development in the absence of fertilization (see review by Hennig and Derkacheva., 2009).

Human core PRC1 components consist of BMI1, HPH1/EDR1, HPC2/CBX4, and RING1s (RING1A/RING1 and RING1B/RING2/RNF2), homologs of Drosophila Posterior sex combs (Psc), Polyhomeotic (Ph), Polycomb (Pc) and dRingl/Sce, respectively (Shao et al., 1999; Francis et al., 2001). Human BMI1, RING1A and RING1B all belong to Ring finger proteins, together forming an E3 ubiquitin ligase complex that monoubiquitinates histone H2A at lysine 119 (H2A–K119ub), where RING1B performs most of catalytic activity, RINGA only plays a minor role, but BMI1 alone does not show any E3–ligase activity and only enhances RING1B's activity (Wang et al., 2004; Buchwald et al., 2006). PRC1 complex is highly conserved in plant as well (Molitor and Shen, 2013). In Arabidopsis, there are a Pc–functional equivalent, LIKE HETEROCHROMATIN PROTEIN1/TERMINAL FLOWER2 (LHP1/TFL2), two RING1 homologs, AtRING1a and AtRING1b; and three BMI1 homologs, AtBMI1a, AtBMI1b, and AtBMI1c (Zhang et al., 2007; Xu and Shen, 2008; Chen et al., 2010). It has been confirmed that AtRING1 proteins can interact with AtBMI1 proteins and LHP1 (Chen et al., 2010). Sequence homology showing Arabidopsis LHP1 encodes a HETEROCHOMATIN (HP1) homolog, but LHP1 performs the function similar to Pc. LHP1 associates with gene loci marked by H3K27me3 in vivo (Turck et al., 2007), which chromodomain binds H3K27me3 created by PRC2 in vitro (Zhang et al., 2007). Disruption of the chromodomain abolishes H3K27me3 recognition and mimics *lhp1* null mutants (Exner et al., 2009). AtRING1 proteins are required for suppressing ectopic meristem production by repressing the misexpression of KNOX I genes (Xu and Shen, 2008). RING–finger proteins including AtRING1a/b and AtBMI1a/b are involved in repressing the vegetative–to–embryo conversion by suppressing the misexpression of stem cell–related and embryogenesis–related regulators (Chen et al., 2010). Additionally, AtBMI1c displays materially imprinting

expression in endosperm (Bratzel et al., 2012). However, different from the key catalytic role of RING1B in human, all these RING–finger proteins in Arabidopsis can monoubiquitinate H2A in vitro or in vivo (Bratzel et al., 2010; 2012), indicating possibly functional divergence of PRC1 between animal and plant. Notably, DRIP1/AtBMI1a and DRIP2/AtBMI1b have been confirmed to mediate DREB2A ubiquitination in vitro, consequently act as negative regulators in drought–induced gene response by targeting DREB2A to 26S proteasomes (Qin et al., 2008).

Embryonic Flower 1 (EMF1) is thought to be a plant–specific PcG protein, no significant homolog found in other organisms (Calonje et al., 2008). Defection in EMF1 results in a broad spectrum of phenotype. Weak allele *emf1–1* displays sessile cotyledons with oval shape, short hypocotyl, and lacks rosette leaves. In fact, a small inflorescence develops directly from the germinated embryo or callus; the inflorescence shoot contains sessile leaves and few sterile flowers with incomplete floral organ development, usually devoid of petals. Strong allele *emf1–2* additionally displays delayed germination, and carpelloid structures formed from all lateral organs, including the cotyledons (Chen et al., 1997).

EMF1 Homologs are Only Found in Angiosperm

Previous reports show that EMF1 homologs are only found in plant. Here, we screened EMF1–like proteins by protein BLAST using Arabidopsis EMF1 as query against phytozome database, finding that EMF1 homologs are only exist in angiosperm, including dicot and monocot plants, not in clubmoss, brytophyte, or lower plants. Phylogenic analysis suggested that EMF1 homologs are grouped into two classes: dicot lineage and monocot lineage (Fig.1).

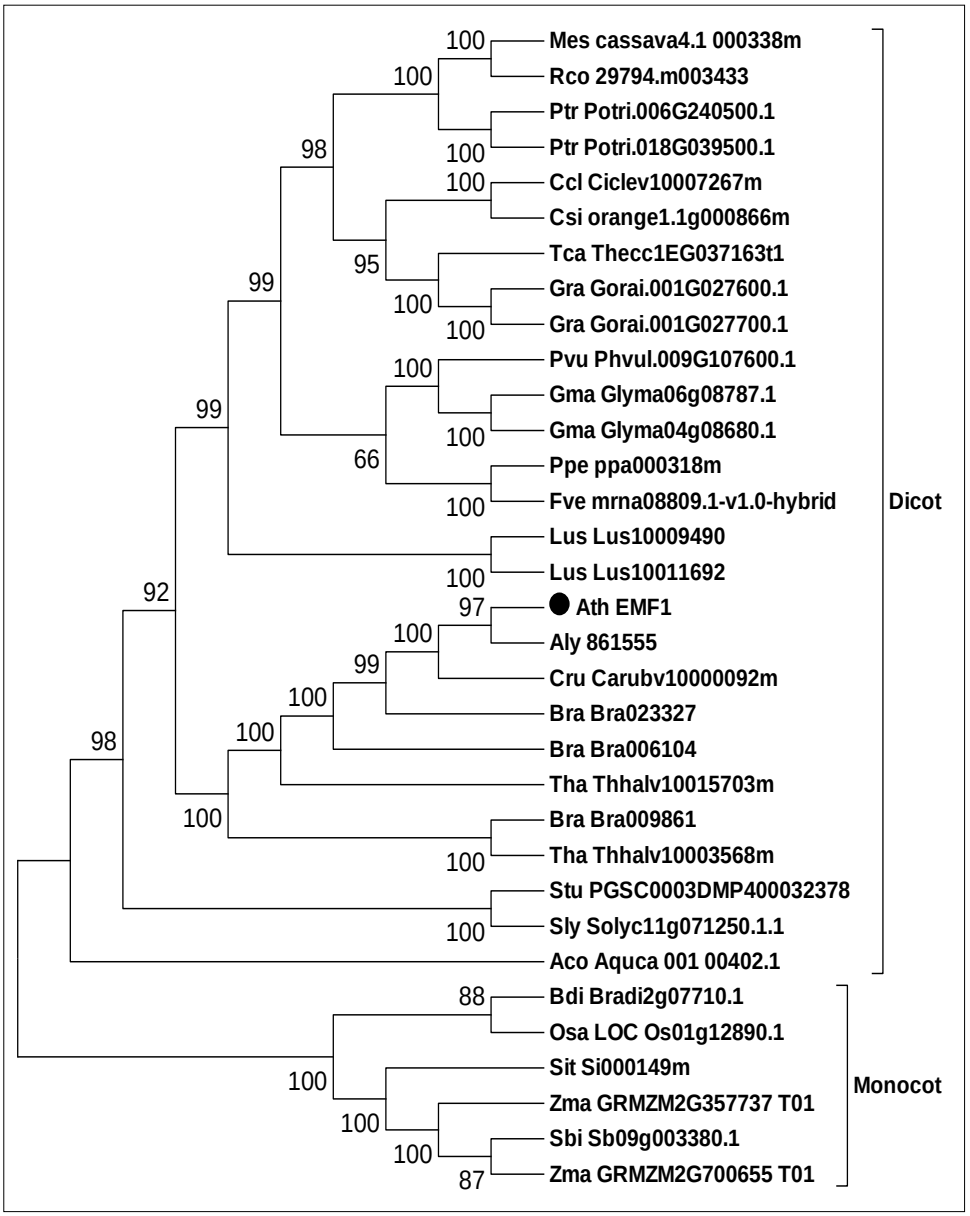

Figure 1: The phylogenic analysis of EMF1–like proteins in plant.
Some typical EMF1 homologs were identified by protein BLAST using Arabidopsis EMF1 as query against phytozome database (www.phytozome.net). Multiple sequence alignments of ~30 EMF1–like proteins were conducted via the ClustalW program (Thompson et al., 1994). The resulting file was subjected to phylogenic analysis using the MEGA5.1 program (Tamura et al., 2007). The trees were constructed using Minimum Evolution (ME) with the following settings: Bootstrap test of 500 replications for internal branch reliability; Substitution Model: p–distance. Rates among Sites: uniform rates; Gaps/missing data treatment: partial deletion with 95% site coverage cutoff. The first three letters before each EMF homologs represent the corresponding species. Ata, *Arabidopsis thaliana*; Ccl, *Citrus clementina*; Csi, *Citrus sinensis*; Mes, *Manihot esculenta*; Rco, *Ricinus communis*; Ptr, *Populus trichocarpa*; Gma, *Glycine max*; Ppe, *Prunus persica*; Fve, *Fragaria vesca*; Lus, *Linum usitatissimum*; Aly, *Arabidopsis lyrata*; Cru, *Capsella rubella*; Bra, *Brassica rapa*; Tha, *Thellungiella halophila*; Tca, *Theobroma cacao*; Stu, *Solanum tuberosum*; Sly, *Solanum lycopersicum*; Aco, *Aquilegia coerulea*; Pvu, *Phaseolus vulgaris*; Gra, *Gossypium raimondii*; Bdi, *Brachypodium distachyon*; Osa, *Oryza sativa*; Sit, *Setaria italica*; Zma, *Zea mays*; Sbi, *Sorghum bicolor*. Arabidopsis EMF1 was marked by solid circle.

Before, it has been reported that Arabidopsis EMF1 contains nuclear localization signals (NLSs), ATP/GTP binding motif (P–loop), and LXXLL motif (Aubert et al., 2001). In fact, based on our multiple BLAST analyses, these putatively specific motifs are not conserved in the other most of EMF1 homologs. So, detailed domain architecture prediction in SMART database (http://smart.emblheidelberg.de/) was performed, showing there are no well–established domains existing in EMF1–like proteins. Additionally, multiple sequence alignment showed there are three highly conserved regions (CRs) with unknown function found in both dicot and monocot lineages (Fig.2).

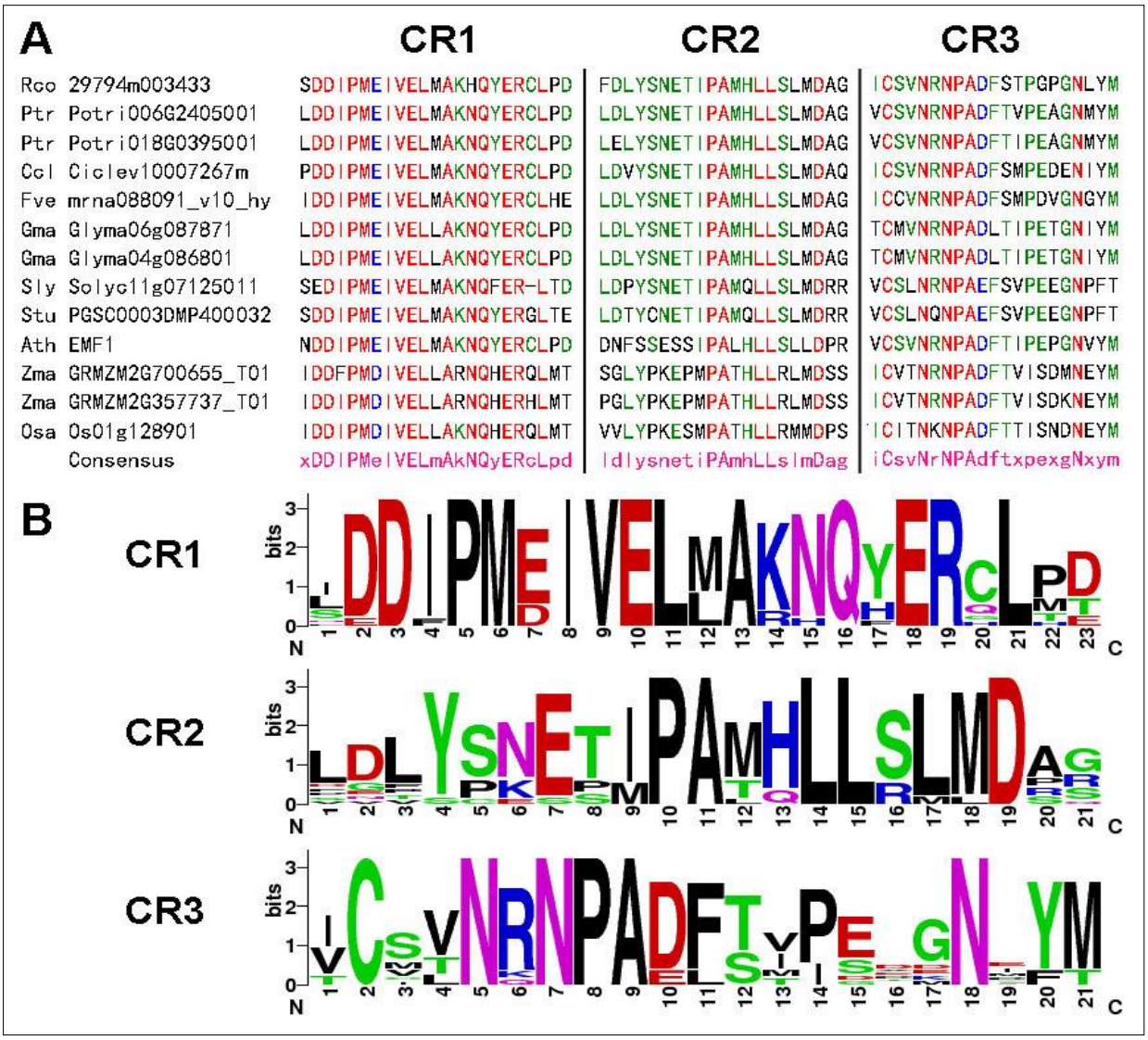

Figure2: Three Conserved regions (CRs) in EMF1–like proteins.
(A) Multiple alignments of these three CRs of EMF1 homologs in some representative species; (B) Sequence logos analysis of these three CRs.

Expression Pattern and Subcellular Localization of EMF1

RNA blot showed that EMF1 have ubiquitous and constitutive expression in all examined organs, such as root, leave, stem, and different stages of flowers (Aubert et al., 2001). Western blot using *emf2*–2/EMF1::EMF1–FLAG recovery transgenic line also confirmed constitutive expression of EMF1 in protein level (Calonje et al., 2008). But detailed temporal and spatial expression analysis by EMF1::GUS construct showed EMF1 promoter still has some tissue specificity, through is active in most developmental stages. The GUS activity was detected in mature embryo, in cotyledon and shoot apex in 7–day–old seedlings; in rosette leaf blade; in the 15–day–old root tips; in the stigma and anthers in opening flower, but absent in 4–day–old seedlings and floral buds excluding developing stigma papilla (Sanchez et al., 2009; Park et al., 2011). Indeed, analysis on Gene investigator also shows EMF1 have ubiquitous expression on the whole but inconsistent levels in different organs (supplemental Fig.1).

Different types of EMF1–GFP fusion constructs including full–length or truncated EMF1 versions transiently expressed in tobacco leaves showed EMF1 localized exclusively to the nucleus in a speckle–like pattern and the region between residues 337and 866 might be responsible for EMF1 subnuclear pattern (Calonje et al., 2008).

The Role of EMF1 in Different Development Phases

Maintenance of shoot apical meristem (SAM) is coordinately controlled by both KNOX pathway and WUS pathway, critical for the development of aerial parts in plant. EMF1 can directly bind the chromatin of some genes belonging to KNOX I pathway (STM, KNAT1, and KNAT2) and WUS pathway (WUS and CLV3) (Kim et al., 2012). But removal of EMF1 activity only leads to the upregulated expression of KNOX I genes, indicating that EMF1 influences the plant development mainly through directly repressing the misexpression of KNOX genes rather than WUS pathway. Overexpression of either *STM* or *KNAT2* is able to induce ectopic carpel formation (Scofield et al., 2007), consistent with the ectopic carpelloid structure produced in *emf1* mutant.

Microarray analysis showed floral time genes and floral pathway integrators, such as LFY, FT, CO and SOC1 have similar expression levels in *emf1* mutant compared with WT; whereas flower organ identity genes AGAMOUS (AG), PISTILLATA (PI), APETALA1 (AP1), AP3, SEPALLATA1 (SEP1), SEP2, and SEP3 are upregulated in *emf* (Moon et al., 2003), consistent with the phenotype of inflorescence–to–flower transition in *emf1*. Indeed, LFY::GUS activity in *emf* mutants is similar as WT, and is confined strictly to shoot apical region, whereas AP1::GUS construct has ectopic expression in cotyledon and hypocotyl at 7DAG (Moon et al., 2003). Further ChIP analysis suggests EMF1 directly bind to the promoter and 2nd intron of AG, not the downstream region. However, EMF1 fail to bind to AG sites in the *emf2–2* background, indicating EMF1 requires EMF2 to bind to AG gene and EMF1 acts downstream of EMF2. EMF1 could also be associated with the AP3 and PI promoter. But EMF1 binding to AP3 and PI loci is less influenced in *emf2–2* (Calonje et al., 2008). FLC is upregulated in *emf*, and is also the direct target of EMF1 (Kim et al., 2009), but *emf1* is extremely early flowering, indicating FLC is not the key regulator for flowering time in *emf* mutant. On the other hand, plenty of seed maturation genes are upregulated, moreover, embryonic regulator ABI3 and some seed storage genes are also the direct targets of EMF1 (Kim et al., 2010; 2012). So, expression level and pattern analysis suggested that EMF1 maintains vegetative development mainly by repressing the misexpression of the genes involved in flowering and embryo development.

The *emf1* mutants skip vegetative growth, flowering upon germination (Sung et al., 1992), but EMF1 overexpression cannot delay flowering or induce other obvious phenotype in wild–type plants. As we known, KNAT1 promoter is active in the SAM, stronger in peripheral and rib zone than in the central zone (Lincoln et al., 1994). LFY promoter is initially active in leaf primordia at 4 day after germination (DAG) (Blazquez et al., 1997), is gradually up–regulated in leaf primordia during vegetative development, peaking in flower meristem (FM) (Nilsson et al., 1998), and also has activity in embryo, but no expression in the SAM. Seed storage protein At2S3 is expressed in embryo (Guerche et al., 1990), and in cotyledon of germinating seedling, but absent in shoot apex and rosette leaves (Sanchez et al., 2009). Tissue–specific EMF1 constructs, such as KNAT1::EMF1, LFY::EMF1, and At2S3::EMF1, failed to completely or partially recovery the *emf1* phenotype. However, tissue–specific reduction of EMF1 expression in WT leads to different degrees of developmental defects. For example, SAM–specific KNAT1::asEMF1 (antisense EMF1) transgenic plants displayed obvious *emf* phenotype, lacking vegetative development and directly proceeding to reproductive stage upon germination, combined with no obvious phenotype in KNAT1::EMF1 line, together indicating that EMF1 activity in the SAM is essential but not sufficient to maintain vegetative development (Sanchez et al., 2009). Unexpectedly, FM–specific AP1::asEMF1 transgenic lines exhibit WT–like phenotype with normal flowering time and floral organs, indicating EMF1 is not important for flower development. So it is predicted that the defective floral organ development in *emf1* might be the secondary or comprehensive effects caused by widespread release of multiple flower homeotic genes (Sanchez et al., 2009). Leaf primordial/FM–specific LFY::asEMF1 transgenic line is similar to PRC2 mutant *clf*, producing curly leaves, early flowering and terminal flower. LFY::asEMF1 also displays abnormal flower absent of petal. Moreover, due to EMF1 reduction in FM having no obvious effect on plant development testified by AP1::asEMF1 line, together indicating that EMF1 expression in leaf primordial has no effect on true leaf development, but has a secondary effect on flower development (Sanchez et al., 2009). Seed–specific At2S3::asEMF1 transgenic lines initially germinate as *emf*–like phenotype, but develop rosette leaves after 2 weeks, subsequently producing early flowering, abnormal and terminal flowers (Sanchez et al., 2009). So, reduction of EMF1 activity only in embryogenesis can greatly influence the development of next generation possibly through disturbing stable epigenetic memory deposited by EMF1. In summary, based on the analysis on expression and function aspects, EMF1 mainly maintain vegetative shoot apical meristem via directly repressing the ectopic expression of the genes related to flower development and embryogenesis

In addition, identification of EMF1 interacting proteins (EIPs) further suggested the role of EMF1 in regulating flowering time and repressing vegetative–to–embryo conversion (Table 1). Damage of each EIP1/WNK8, EIP6/BBX32, or EIP9 results in early flowering, whereas overexpression of each leads to later flowering. EIP7/ASIL1 participates in repressing seed maturation genes during vegetative development (Gao et al., 2009). Besides, EMF1 interacting factors are implicated in other pathways, such as regulation of circadian clock period (EIP10/ZTL), pathogen resistance (EIP2/RD21, EIP8/BRG3), repression of root development in shoot (TPL, TPR3), and PcG silencing (AtRING1, AtBMI1, MSI1).

EMF1 Serves as a Novel PcG-like Protein

EMF1 has putative PRC1 function. Firstly, phenotype analysis of *emf1;emf2* double mutant showed that *emf1* is epistatic to or downstream of PRC2 component *emf2* in the same pathway (Calonje et al., 2008). **Secondly**, EMF1 can interact with multiple PRC1 core components, such as RING–finger proteins RING1a/b, BMI1a/b. Indeed, the amount of H2Aub was reduced in *emf1–2* mutants compared to WT, suggesting EMF1 is required for H2AK119 monoubiquitination activity on specific target genes (Bratzel et al., 2010). **Thirdly**, EMF1 can bind DNA and RNA in a non–sequence–specific fashion via its M1 (amino acids 337–617) and Ct (amino acids 866–1096) regions, and interfere with in vitro transcription fulfilled by RNA polymerase II and T7 RNA polymerase via its M1, M2 (amino acids 622–866) and Ct regions, consistent with the Drosophila PRC1 core component PSC inhibitory effect on transcription in vitro (King et al., 2002). **Additionally**, RING1–like proteins usually contain a conserved RING domain in N terminus and RAWUL domain in C terminus. PSC is RING1–like protein, but much larger than its homologs in human and plant. Besides both RING domain and RAWUL domain (amino acids 371–465) localized in N terminus, PSC also contains another highly charged and structurally disordered C–terminal region (PSC–CTR) (amino acids 456–1603), which binds DNA tightly and inhibit chromatin remodeling efficiently (Beh et al., 2012). Repressive PSC–CTR is absent in plant RING1 homolog,

instead a putatively functional analog EMF1 which displays similarly physical properties rather than sequence similarity with PSC–CTR performs relevant function to inhibit chromatin remodeling efficiency. For instance, EMF1 homologs in both *Arabidopsis thaliana* and *Aquilegia vulgaris* exhibit largely disordered sequence with low contiguous negative charge, consistent with the criteria for repressive PSC–CTRs, showing a disordered domain with dispersed negative charges. So plant EMF1 has PSC–CTR–like properties (Beh et al., 2012). .

Table 1: Arabidopsis EMF1 interacting partners.

Partner	Locus	Function annotation in TAIR	Method	Reference
EIP1/WNK8	At5g41990	WNK family protein kinase, nucleus localization.	Y2H, BiFC, pull–down	Park et al, 2011
EIP6/BBX32	At3g21150	B–box zinc–finger protein, cytoplasm and nucleus localization.	Y2H, BiFC, pull–down	Park et al, 2011
EIP9	At5g64360	DnaJ–domain protein, cytoplasm and nucleus localization.	Y2H, BiFC	Park et al, 2011
EIP2/RD21	At1g47128	Responsive to dehydration 21. Cysteine proteinase precursor–like protein, having peptide ligase activity and protease activity in vitro. Involved in immunity to the necrotrophic fungal pathogen *Botrytis cinerea*.	Y2H	Park et al, 2011
EIP4/ARP5	At3g12380	Actin–related protein. Member of nuclear ARP gene family.	Y2H	Park et al, 2011
EIP5	At5g25754	RNA polymerase I–associated factor PAF67.	Y2H	Park et al, 2011
EIP7/ASIL1	At1g54060	Member of the trihelix DNA binding protein family. Nuclear localized. Involved in repressing seed maturation genes during seed germination and seedling development	Y2H	Park et al, 2011
EIP8/BRG3	At3g12920	Encodes one of the BRGs (BOI–related gene) involved in resistance to *Botrytis cinerea*	Y2H	Park et al, 2011
EIP10/ZTL	At5g57360	ZEITLUPE/FKF1–like protein 2 (FKL2) /ADAGIO1 (ADO1), containing a PAS domain. Contributing to the plant fitness (carbon fixation, biomass) by influencing the circadian clock period. The F–box component of an SCF complex implicated in the degradation of TOC1.	Y2H	Park et al, 2011
EIP11/RAD23C	At3g02540	RAD23 proteins play an essential role in the cell cycle, morphology, and fertility of plants through their delivery of UPS (ubiquitin/26S proteasome system) substrates to the 26S proteasome.	Y2H	Park et al, 2011
TPL	At1g15750	TOPLESS/WUS–interacting protein 1. Involved in transcriptional repression of root–promoting genes in the top half of the embryo during the transition stage of embryogenesis.	Y2H	Causier et al, 2011
TPR3	At5g27030	Topless–related protein 3	Y2H	Causier et al, 2011
MSI1	AT5G58230	PRC2 component. Implicating in gametophyte and seed development,	Pull–down	Bouveret et al, 2006; Calonje et al, 2008
AtRING1a	AT5G44280	PRC1 Ring finger protein	Pull–down	Bratzel et al, 2010
AtRING1b	AT1G03770	PRC1 Ring finger protein	Pull–down	Bratzel et al, 2010
AtBMI1a	AT1G06770	PRC1 Ring finger protein	Pull–down	Bratzel et al, 2010
AtBMI1b	AT2G30580	PRC1 Ring finger protein	Pull–down	Bratzel et al, 2010

EMF1 might be a putative PRC2 member as well. Firstly, Weak *emf1* mutant phenocopies *emf2*, indicating both might have similar role in developmental regulation. **Secondly**, pull–down assay showed that EMF1 can interact with MSI1 through the region M1 (amino acids 337–617) and Ct (amino acids 866–1096), whereas no interact with other EMF2–PRC2 members, such as EMF2, CLF and FIE (Calonje et al., 2008). In fact, MSI1 is also implicated in other PRC2 complexes, like VRN2–PRC2 and FIS2–PRC2. In addition, MSI1 can interact with PRC1 member LHP1 to form a positive feedback loop to recruit PRC2 to H3K27me3–carried chromatin (Derkacheva et al., 2013). MSI1 can form a complex with RBR1 that is required for activation of the imprinted genes FIS2 and FWA (Jullien et al., 2008). Moreover, MSI1 can together with FASCIATA1 (FAS1) and FAS2 forms chromatin assembly factor 1 (CAF–1) complex (Kaya et al., 2001). But according to the mutant phenotype displayed by PRC or CAF components, EMF1 is more inclined to a PcG protein. **Thirdly,** EMF1 function mainly cooperates with PRC2. Among all the genes marked by H3K27me3 in WT, over 40% display reduced H3K27me3 in *emf1*, less than PRC2 mutants *emf2* (Calonje et al., 2008; Kim et al., 2012). So, besides classical PRC2 components, EMF1 is also required for H3K27me3 deposition. EMF1 binding pattern across euchromatin covers the entire transcription unit with the peak

around the transcriptional start site, displaying strong correlation with global H3K27me3 profile (Kim et al., 2012). Genes with high/low H3K27me3 deposition often exhibit high/low enrichment level of EMF1 binding. The EMF1–bound genes with high H3K27me3 are always involved in meristem, leaf, vascular, root, flower, seed development and all sorts of phytohormone response. However, The EMF1–bound genes absent of H3K27me3 markers tend to be actively transcribed genes, and always participate in cellular organization and biogenesis, cytosol and chloroplast. For instance, many photosynthesis–related genes are down–regulated in *emf1* (Kim et al., 2010), suggesting EMF1 might promote their expression mainly in other indirect ways. **Finally,** EMF1 can counteract TrxG function. ULTRAPETALA1 (ULT1) can interact with ARABIDOPSIS TRITHORAX1 (ATX1), which belongs to trithorax group (TrxG) and catalyzes lysine–3 on histone 4 (H3K4me3), opposite to PcG with H3K27me3 activity (Saleh et al., 2007). Removal of ULT1 activity which leads to late flowering and production of extra sepals and petals can rescue the early flowering and curly leaf of PRC2 mutant *clf* (Carles et al., 2009). Although *ult1* cannot rescue *emf1* phenotype at least at very early germinating stage, it can rescue both the *clf*–like phenotype and most of the misregulated gene expression in leaf primordia–specific LFY::asEMF1 silencing line, consistent with the major role of ULT1 in flower development (Pu et al., 2013). So, ULT1 can antagonize EMF1 action mainly in floral transition stage, not in germinating and vegetative stage. ULT1 is upregulated in *emf1* and *emf2* mutants (Kim et al., 2012). Moreover, EMF1 can bind the chromatin of ULT1 and ULT2 (Pu et al., 2013), indicating EMF1 directly repressing ULT1 expression.

CONCLUSIONS

EMF genes were firstly characterized to associate with shoot maturation and development through genetic screening (Yang et al., 1997). Disruption of EMF loci results in extremely early flowering, due to absence of vegetative development. Transcriptomic analysis shows that EMF proteins maintain vegetative development mainly through suppressing the misexpression of reproductive–related genes, including flowering and seed maturation genes. Since EMF2 as a well–known PRC2 component is highly conserved in animal and plant kingdom, naturally, EMF1 has similar mutant phenotype and was thought to link to PcG function. However, EMF1 homolog only exists in plant, more precisely, in angiosperm, not found in other organisms. Certainly, animal and plant harbor so different life styles that even the homologs might evolve divergent functions, or either of them might directly evolves new components to accomplish species–specific behaviors and events. There are several important lines of evidence supporting the role of EMF1 as a PcG function. Firstly, EMF1 can produce a global influence on gene expression, consistent with epigenetic regulation. Secondly, EMF1 is required for H2Aub modification of PRC1, and EMF1 can interact with PRC1 Ring–finger proteins. Thirdly, EMF1 function cooperates with PRC2 and H3K27me3, and EMF1 can interact with PRC2 component MSI1. But there are still plenty of important questions on EMF1 need to answer, such as lacking strong evidence of in vivo protein interaction assay and further detection of precisely biochemical/regulatory function. Maybe, high throughput interactome analysis and suppressor screening can provide some better choices for deeply investigation on EMF1.

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