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Groucho/Tup1 family co-repressors in plant development

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Transcription repression is emerging as a key regulatory mechanism underlying cell fate specification and body patterning in both animals and plants. In animals and fungi, the Groucho (Gro)/Tup1 family co-repressors generate the repressed chromatin state in genetic loci that control major developmental decisions ranging from dorsal–ventral patterning to eye development. In higher plants, information about the Gro/Tup1 co-repressors is beginning to emerge. Several recent publications have revealed both conserved and unique structural and mechanistic features of plant Gro/Tup1 co-repressors, including LEUNIG (LUG), TOPLESS (TPL) and WUSCHEL-INTERACTING PROTEINS (WSIPs). These co-repressors regulate key developmental processes in floral organ identity specification, embryo apical-basal fate determination, and stem cell maintenance at the shoot apex.

Introduction: Gro/Tup1 family co-repressors in animals and fungi

Transcription repression is an important regulatory strategy that inhibits the expression of key regulatory genes, the inappropriate expression of which often leads to abnormal development. As with all co-repressors, *Drosophila* Groucho (Gro) and its mammalian homolog Transducin-like enhancer of split (TLE) lack intrinsic DNA-binding ability and are recruited by sequence-specific DNA-binding transcription factors to regulate target gene expression [1]. Gro/TLE proteins are characterized by an N-terminal glutamine (Q)-rich domain and C-terminal WD-repeats (Figure 1a). Each WD-repeat motif consists of ~40 amino acid residues with characteristically located tryptophan (W) and aspartate (D) residues [2]. The ‘β-propeller’ structure of the WD-repeats mediates their interactions with transcription factors such as Engrailed and Dorsal [3,4]. By contrast, the Q-rich domain is primarily involved in homo-tetramerization of the co-repressors [1]. The Q-rich and WD-repeat domains are separated by a less-conserved region implicated in transcription repression and nuclear localization [1].

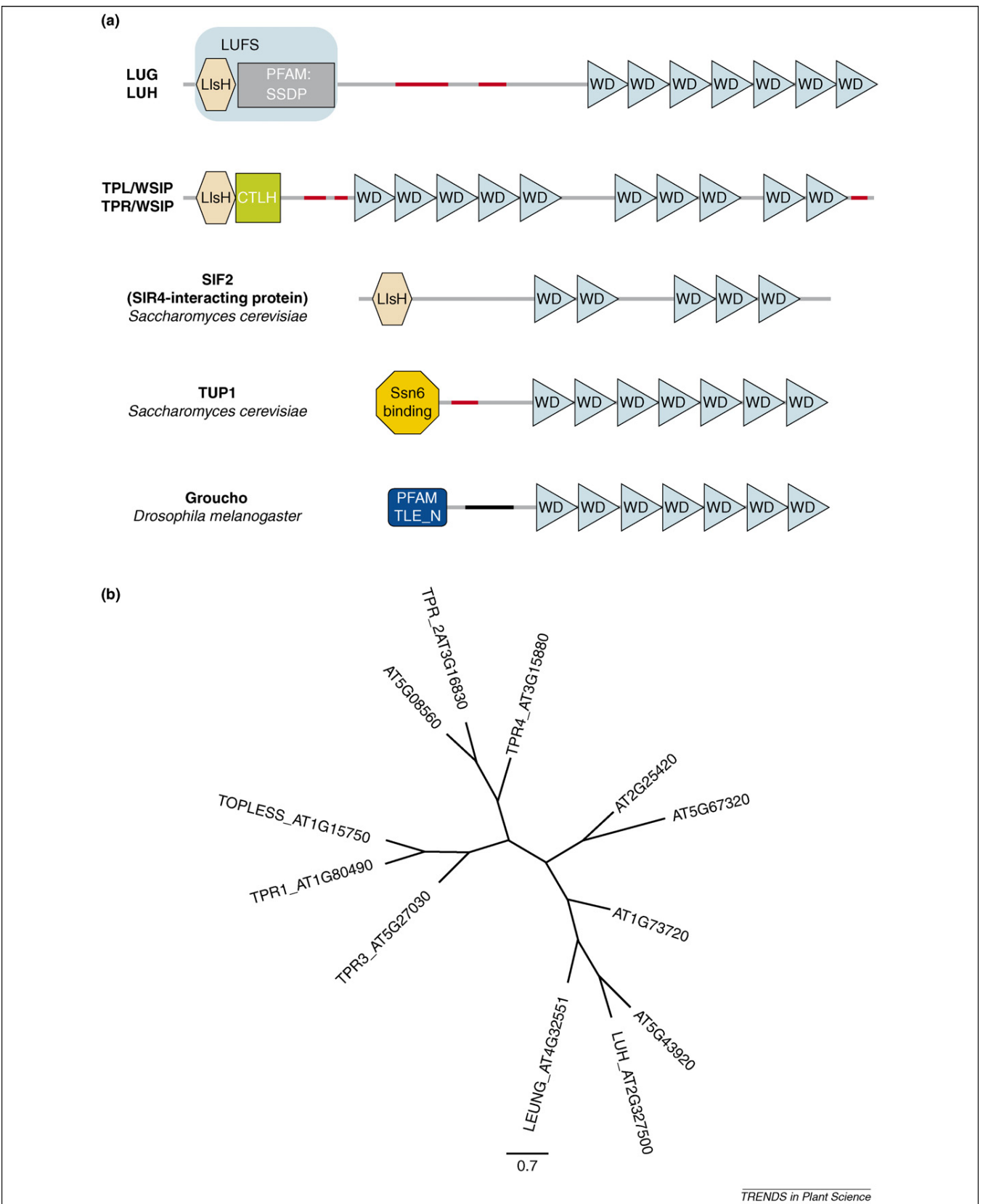
In *Drosophila*, Gro can be recruited by several transcription factors, including the basic helix–loop–helix (bHLH) repressor Hairy, the Rel-family protein Dorsal, and the homeodomain protein Engrailed. In doing so it is able to regulate diverse developmental processes, including lateral inhibition, segmentation, sex determination, dorsal–ventral and terminal pattern formation,

and eye development [5]. Gro interacts directly with short peptide motifs (e.g. Trp-Arg-Pro-Trp) present in these DNA-binding transcription factors. The mammalian TLE proteins interact with the transcription factors AML1 and AML2 (acute myeloid leukemia 1 and 2) to regulate hematopoiesis and osteoblast differentiation. Similarly, TLE interacts with the transcription factor LEF1 (lymphocyte enhancer factor 1) to regulate Wnt signaling during cell fate determination [6,7].

Other than the C-terminal WD-repeats, there is no significant sequence homology between *Saccharomyces cerevisiae* Tup1 and *Drosophila* Gro. However, Tup1 and Gro are regarded as functional analog, owing to their similar domain organization and mechanisms of repression [1]. Unique to Tup1 is the presence of an N-terminal domain (Figure 1a) required for its tight association with Ssn6 (suppressor of snf1), a Q-rich protein with 10 tetrapeptide repeats that function to mediate protein–protein interactions [8]. When tethered to reporter genes by LexA, Tup1 is capable of repressing genes in the absence of Ssn6, but Ssn6 is not capable of repressing genes in the absence of Tup1, suggesting that Ssn6 mainly functions as an adaptor protein between the Tup1 repressor and particular DNA-binding transcription factors [9–11]. In a similar fashion to *Drosophila* Gro, yeast Tup1–Ssn6 co-repressor complex represses genes involved in diverse pathways, including glucose metabolism, DNA damage repair, mating-type switch, and anaerobic respiration [12]. As with other co-repressors, the specificity of repression is determined by its interaction with sequence-specific DNA-binding proteins, including Mig1 (multicopy inhibitor of GAL), Crt1 (constitutive RNR transcription) and α2 [13].

Tup1–Ssn6 mediates repression by multiple mechanisms [12]. First, both Tup1 and Ssn6 were found to physically interact *in vivo* with multiple histone deacetylases (HDACs; Box 1), including Rpd3 (reduced potassium dependency), Hos1 (HAD one similar) and Hos2 [14]. By recruiting HDACs, Tup1–Ssn6 is able to deacetylate histones at target promoters, which in turn stabilizes the interaction between the histones and the Tup1 complex, creating a self-reinforcing repressive state and an effective long-range repression [5,12,15] (Box 1). Second, nucleosome repositioning is observed in genes that are subject to Tup1 repression, limiting the accessibility of promoter and/or enhancer elements to the transcription activation machinery [12]. Third, Tup1–Ssn6 interacts with several components of the Mediator complex [12]

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Figure 1. Domain organization of Gro-Tup1 family proteins. **(a)** Comparisons of the protein domain organization of *Arabidopsis* LUG and LUG HOMOLOG (LUH), *Arabidopsis* TPL-TPR-WSIP, yeast SIF2 and Tup1, and *Drosophila* Groucho (Gro). Glutamine (Q)-rich regions are in red, and the proline (P)-rich region is in black. The LUFs domain in LUG and LUH consists of LisH followed by a second conserved domain (the Pfa domain SSDP). *Drosophila* Groucho and mammalian TLE (not shown) also possess a conserved N-terminal domain (the Pfa domain TLE_N), which is Q-rich and is involved in oligomerization. **(b)** Neighbor-joining phylogenetic tree showing the predicted relationship among *Arabidopsis* Gro/Tup1 family proteins. TOPLESS is identical to WSIP1, and TPR4 is identical to WSIP2. The phylogram was generated using ClustalX 1.81 and was drawn using FigTreeV1.0 (<http://tree.bio.ed.ac.uk/software/figtree/>).

Box 1. Basic concepts in chromatin modification, and mechanisms of repression**Histone deacetylases (HDACs) and histone acetyltransferases (HATs)**

HDACs and HATs are enzymes that influence transcription by selectively deacetylating or acetylating the ϵ -amino groups of lysine residues that are located near the amino termini of core histone proteins. Chromatin acetylation by HATs correlates with transcriptional activity, whereas histone deacetylation by HDACs correlates with gene silencing. There are three classes of HDACs. Examples of class I, II and III HDAC genes in yeast include *RPD3*, *Hda1* and *Sir2*, respectively. Trichostatin A (TSA) is a potent inhibitor of class I and II HDACs [52].

Long- and short-range repression

Repressors can be classified into long- or short-range repressors. Long-range repressors are thought to make a promoter resistant to the influence of all enhancers that could be located kilobases away from the co-repressor binding sites. By contrast, short-range co-repressors such as CtBP (C-terminal binding protein of E1A) [76] work by blocking the function of DNA-bound activators located in close proximity to the co-repressor binding sites [5]. Gro/Tup1 are considered long-range repressors. They probably achieve their long-range effect by spreading themselves along chromatin owing to their ability to recruit HDACs and also to bind hypo-acetylated histones. A second long-range mechanism might involve the formation of a DNA loop that brings together the repressors and the basal machinery of transcription [5]. It should be noted that, in yeast, genes and enhancers are packed together to a greater degree. Therefore, the long-range effect of Tup1 in yeast is merely a few hundred bases, compared with several kilobases in higher eukaryotes.

Transcription Mediator complex

This is a protein complex that transmits signals from transcription factors to the RNA polymerase II initiation complex. Thus, the Mediator complex is recognized as an additional level of transcriptional regulation in eukaryotes [77]. The Mediator complex consists of 25–30 proteins that are conserved throughout eukaryotes [78]. Biochemical purification of the *Arabidopsis thaliana* Mediator complex identifies 21 conserved and 6 *Arabidopsis thaliana*-specific Mediator subunits, including SWP (STRUWWELPETER) and PFT1 (PHYTOCHROME AND FLOWERING TIME 1) [79].

Yeast- and plant-based repression assays

These assays are useful for characterizing candidate repressors or co-repressors. Two to five *GAL4* binding sites (2XUAS–5XUAS) are inserted upstream of constitutive promoters (e.g. *GCN4* in yeast and 35S in plants [31,80]), which drive the expression of reporter genes such as *lacZ* (encoding β -galactosidase), *GUS* (encoding β -glucuronidase) and *LUC* (encoding luciferase). Putative repressor genes fused to the *GAL4* DNA-binding domain are introduced into yeast or plant repression assay systems containing the respective reporter genes described above. Binding of *GAL4*-candidate repressors to the respective reporter genes will reduce reporter gene expression.

(Box 1). By interacting with Mediator complex subunits, Tup1 competes with co-activators for binding to the Mediator components, thus blocking interactions between activators and the Mediator. Similar repression mechanisms were also reported for animal Gro and TLE [5].

Despite extensive studies of the Gro/Tup1 family co-repressors in animal and fungal development, little is known about Gro/Tup1 family co-repressors in higher plants. This review summarizes recent discoveries of this class of co-repressors in regulating fundamental aspects of higher plant development.

Plant Gro/Tup1 co-repressors

In *Arabidopsis*, the Gro/Tup1-like proteins constitute a small family of ~13 members (<http://smart.embl-heidelberg.de/>; Figure 1b). Among them, the functions of LEUNIG (LUG), TOPLESS (TPL), TOPLESS-RELATED (TPR) and WUSCHEL-INTERACTING PROTEINS (WSIPs) are being characterized [16–18]. On the basis of phylogenetic analysis, they can be grouped into two separate subclasses, represented by TPL/TPR/WSIP and LUG/LUH (Figure 1b). Interestingly, all *Arabidopsis* and plant Gro/Tup1-like proteins contain a dimerization motif, the LisH (lissencephaly homology) domain, at the N terminus (Figure 1a). In this respect, the plant Gro/Tup1 proteins are more similar to yeast SIF2p (sucrase-isomaltase foot-print) (Figure 1a), which also contains the N-terminal LisH and C-terminal WD-repeats and is an integral component of the Set3 complex. This complex consists of an assembly of proteins with some homology to the human SMRT (silencing mediator of retinoid acid and thyroid hormone receptor) and NCoR (nuclear receptor co-repressor) complexes [19,20], the repressor activities of which are also dependent on HDACs [21].

In *Arabidopsis*, the TPL/TPR/WSIP and LUG/LUH subclass proteins differ in two aspects of their domain organization. First, TPL/TPR/WSIP proteins have centrally located WD-repeats in addition to the C-terminal WD-repeats (Figure 1a). Second, TPL/TPR/WSIP proteins contain a CTLH (C-terminal to LisH) domain (Figure 1a), whereas LUG/LUH proteins lack a CTLH domain but possess a conserved LUFs (LUG, LUH, Flo8, SSDP) domain at their N termini (Figure 1a). Both CTLH and LUFs are protein–protein interaction domains. By interacting with different protein partners (see below), CTLH and LUFs might contribute to the functional diversification between these two subclasses of co-repressors.

The function of these plant Gro/Tup1-like proteins as global regulators of gene expression has, for LUG at least, been demonstrated through genetic and genomic studies. *lug* mutants exhibit defects in gynoecium development, female and male fertility, leaf and floral organ shape, leaf development, and vasculature [22–26]. In *Antirrhinum majus*, mutants of *STYLOSA*, an ortholog of *LUG*, also showed hypersensitivity towards auxin and polar auxin inhibitors, suggesting reduced auxin transport [27]. Genome-wide array studies identified LUG-regulated target genes in abiotic and biotic stress response, meristem function, and transport [28]. In this review, we highlight the studies of *LUG*, *TPL* and *WSIP* in flower development, embryo apical-basal fate determination and stem cell maintenance, respectively.

Flower development

Arabidopsis LUG was the first Gro/Tup1-like co-repressor identified in plants, owing to its role as a transcription regulator of the floral homeotic gene *AGAMOUS* (*AG*) [17,22]. In wild type *Arabidopsis*, *AG* mRNA is expressed only in the inner two whorls of the flower [29,30]. In *lug* loss-of-function mutants, *AG* was ectopically expressed in the outer two whorls of the flower [22] (Figure 2ab), converting sepals into carpelloid floral organs and reducing the number of petals and stamens (Figure 2c). Therefore,

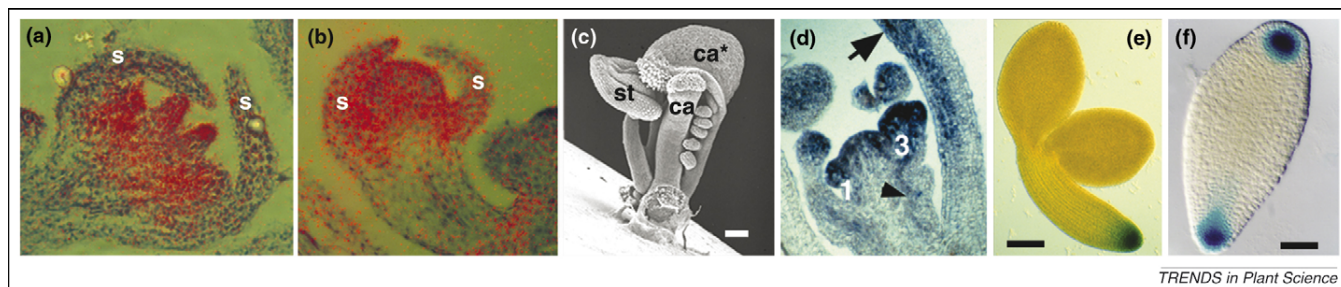


Figure 2. *lug* and *tpl* mutant phenotypes. (a) *In situ* hybridization showing *AG* mRNA expression in the inner two whorls of a developing wild type flower. (b) *In situ* hybridization showing ectopic *AG* mRNA expression in all four whorls (including sepals) of a *lug* mutant flower. (c) Scanning electron microscopic photo showing carpelloid first whorl organs and reduced floral organ number in a *lug* mutant flower. The first whorl carpel is labeled *ca** to distinguish it from the fourth whorl carpel (*ca*). (d) *In situ* hybridization showing *LUG* mRNA in all four whorls of floral organ primordia. Numbers indicate the stage of respective floral meristem development. *LUG* mRNA is also detected in vascular tissues (arrowhead) and in the adaxial side of the cauline leaf (arrow). (e) In a wild type embryo, the root-specific marker *LENNY* is only expressed in the root pole, as shown by the blue staining. (f) In a *tpl-1* mutant embryo at 29°C, *LENNY* expression is found at both poles (shown by blue staining), indicating a transformation from shoot to root fate. Abbreviations: *ca*, carpel; *st*, stamen; *s*, sepal. Image (d) is reproduced, with permission, from Ref. [17]. Images (a–c) are reproduced, with permission, from Ref. [22]. Images (e–f) are reproduced, with permission, from Ref. [58].

LUG might function as an outer-whorl-specific repressor of *AG* transcription. *LUG* mRNA is, however, detected in all four floral whorls and in vegetative tissues (Figure 2d). One explanation is that *LUG* activity might be regulated post-transcriptionally. Alternatively, other inner-whorl-specific factors might counter *LUG* activity specifically in the inner two whorls to relieve the repression of *AG*.

LUG works together with SEUSS

The N-terminal LUFs domain of *LUG* is required for transcription repression and for direct interaction with *SEUSS* (*SEU*) [31]. *SEU* is a Q-rich protein with a centrally positioned dimerization domain also present in the LIM-domain-binding (*Ldb*) family of transcriptional co-regulators in mammals and *Drosophila* [32]. The *LUG*(LUFs)–*SEU* interaction is supported by a parallel study in *Drosophila* and mouse in which the LUFs domain of the single-stranded DNA-binding protein (SSDP) directly interacted with the *Drosophila* and mouse *Ldb1*-type proteins [33,34]. Furthermore, yeast two-hybrid interactions between the *Antirrhinum* orthologs of *LUG* and *SEU* [27] suggest that *LUG* and *SEU* form an evolutionarily conserved co-repressor complex in plants, analogous to the yeast Tup1–Ssn6 complex.

By fusing *LUG* to the DNA-binding domain of *GAL4* or *LexA* and testing the *LUG*–BD fusion construct in yeast and plant cell assays (Box 1), it was demonstrated that *LUG* exhibited a strong repressor activity by significantly reducing reporter gene expression [31]. However, no repressor activity was detected for *SEU* [31]. It was proposed that *SEU* acted as an adaptor protein, bridging the interaction between *LUG* and specific DNA-binding transcription factors in a manner analogous to *Ssn6*.

Recruitment of LUG–SEU to specific target sequences

How are *LUG*–*SEU* complexes recruited to the *AG* cis-regulatory elements? It was shown that the second intron of *AG* drove β -glucuronidase (*GUS*) reporter expression in the inner two whorls, a pattern identical to that of the endogenous *AG* mRNA [35–37]. Two transcription factors, *LEAFY* (*LFY*) and *WUSCHEL* (*WUS*), activate *AG* transcription by binding to this *AG* intron [36,38,39]. When introduced into *lug* mutants, the *AG* intron-driven *GUS* was expressed ectopically in all four floral whorls and

precociously at earlier stage floral meristems than in wild type [35,37], indicating that *LUG* represses *AG* through this intron. Chromatin immunoprecipitation (ChIP) showed that *SEU* specifically associates with the 3' half of the *AG* second intron [40], which contains two MADS (MCM1, *AG*, *DEF*, *SRF*)-box protein binding sites (CARG boxes) in addition to the *LFY* and *WUS* binding sites. The existence of two MADS-box binding sites suggests the possibility that *SEU* and *LUG* directly interact with MADS-box proteins bound to the 3' end of the second intron of *AG*. Thus, *LUG* seems to exert its repressor effect over a long range, several kilobases downstream from the *AG* promoter.

Four MADS-box proteins, *APETALA1* (*AP1*), *SEPAL-LATA3* (*SEP3*), *AGAMOUS-LIKE 24* (*AGL24*) and *SHORT VEGETATIVE PHASE* (*SVP*), were recently implicated in recruiting *SEU* to *AG* [40,41]. *AP1* and *SEP3* were previously shown to activate floral organ identity genes (e.g. *AG*) [42–44], and the C-terminal domains of both *AP1* and *SEP3* were shown to exhibit strong transcriptional activation function [45,46]. Hence, the recruitment of *SEU* by *AP1* and *SEP3* to repress *AG* was, at first, surprising. Using a transient plant gene expression system, the *AG* 3' intron-driven reporter was shown to be activated by *AP1* or *SEP3* [40], suggesting that *AP1* and/or *SEP3* bind to the two CARG boxes at the 3' end of *AG* intron II. However, when *SEU* and *LUG* were simultaneously introduced into the same transient gene expression system together with *AP1* or *SEP3*, *AP1* or *SEP3* acted as repressors, indicating that a direct interaction with *LUG*–*SEU* converts *AP1* and *SEP3* from activators to repressors [40]. The fact that *AP1* has a negative role in transcription is also supported by a recent report wherein *AP1* directly repressed a group of flowering time genes to promote flower fate [47]. In a different study, *AP1*–*AGL24* or *AP1*–*SVP* dimers were shown to interact with *SEU*–*LUG* in yeast [41]. Previous studies only identified a role for *AGL24* and *SVP* in the regulation of flowering time and the determination of floral meristem identity [48–51]. Their roles in *AG* repression were, however, further supported by *svp;agl24* double and *svp;agl24;ap1* triple mutants with floral phenotypes similar to those of *lug;seu* double mutants [41].

Previously, it was suggested that *AG* was constitutively expressed by default in all four floral whorls and was

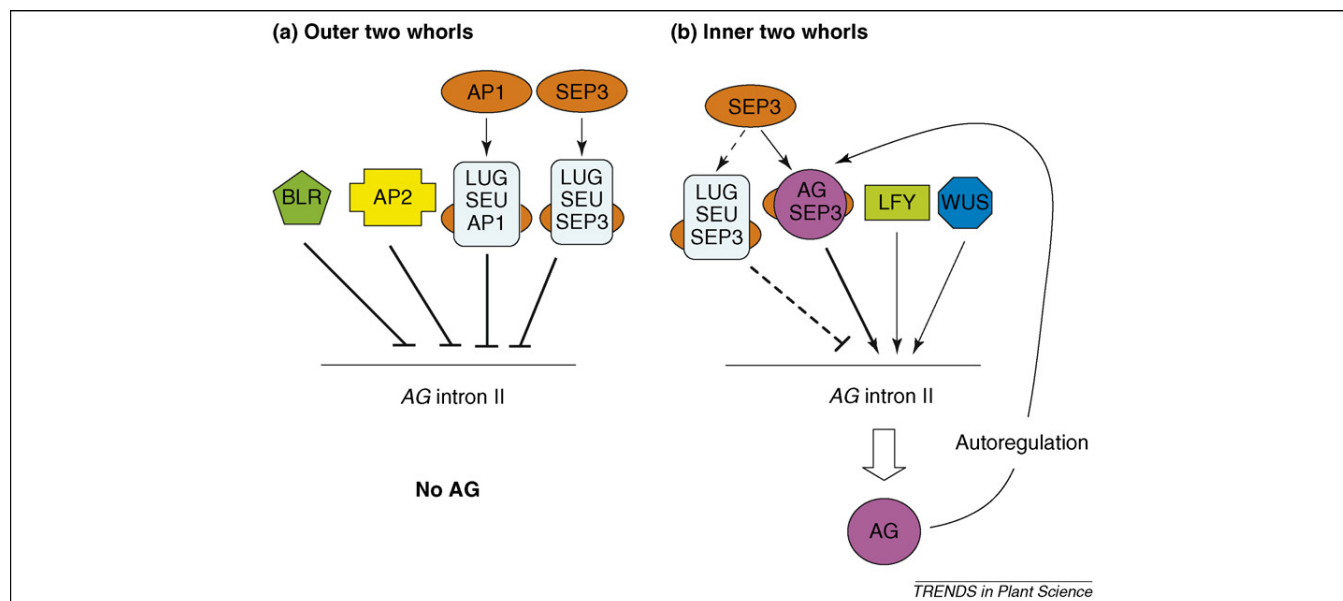


Figure 3. A model of how the inner-two whorl-specific expression of *AG* might be achieved. **(a)** In the outer two whorls, negative regulatory activities conferred by SEU–LUG–AP1 and SEU–LUG–SEP3 impinge upon the second intron of *AG* to prevent *AG* transcription. AP2 (APETALLA2) and BLR (BELLRINGER) are additional outer whorl-specific negative regulators of *AG* [74,75]. **(b)** In the inner two whorls, LFY and WUS in addition to positive autoregulation by AG–SEP3 antagonize the negative effect of SEU–LUG–SEP3 to promote *AG* transcription. Arrows leading from AP1 or SEP3 to respective LUG–SEU–AP1, LUG–SEU–SEP3 or AG–SEP3 complexes indicate the incorporation of these MADS box proteins to the respective protein complexes. Dotted lines indicate weak effects. Thick lines indicate strong effects. Adapted, with permission, from Ref. [40].

turned off in the outer two whorls by LUG–SEU [17,22,31]. However, this model did not explain why the LUG–SEU co-repressors, although present in all four floral whorls, only turned off *AG* expression in the outer two whorls. The revelation that AP1 and SEP3 were involved in recruiting LUG–SEU suggested a new model [40] (Figure 3). In this model, LUG–SEU turns off *AG* in all four whorls, and this negative regulation is antagonized by activating functions specific to the inner two whorls (e.g. the activities of LFY, WUS and the positive autoregulatory AG–SEP3 complexes), leading to inner whorl-specific activation of *AG*.

Mechanisms of LUG-mediated repression: recruiting HDACs and Mediator components

How does LUG repress transcription? Trichostatin A (TSA), a potent and specific inhibitor of HDAC activity [52], was able to efficiently abolish the repressor activity of LUG in the transient gene expression system [31], indicating that HDAC activity is required for LUG-mediated repression. The *Arabidopsis* genome contains four class 1 HDACs (HDA6, HDA7, HDA9 and HDA19) [53], among which mutants of *hda19* (also named *HD1*, *HDA1* and *RPD3A*) display phenotypic similarities to *lug* [54,55]. In support of this, HDA19 was shown to directly and specifically interact with LUG *in vitro* [28]. Thus, recruiting HDA19 to reversibly modify histones is one repression mechanism used by LUG.

Recently, an HDAC-independent mechanism involving the Mediator complex (Box 1) was described. LUG activity was impaired in yeast strains lacking the Mediator component yMED14, and a direct physical interaction was detected between LUG and AtMED14 (also named STRUWWELPETER [SWP]) [28,56]. *swp* mutants showed abnormal shoot apical meristem (SAM) development [56].

By contrast, *lug* mutants did not show obvious abnormality in SAM development. It remains to be determined whether *lug* and *swp* interact genetically. *Arabidopsis* CDK8 (also known as AtCDK8 and HEN3), another Mediator component, was previously shown to regulate *AG* [57]. Both LUG and SEU interact with AtCDK8 in yeast and *in vitro* [28]. The interaction between LUG–SEU and Mediator complex components suggests that LUG–SEU uses a second mechanism through direct regulation of RNA polymerase II activity.

Together, the results summarized above provide us with important mechanistic insights into how LUG co-repressors control domain-specific expression of *AG*. By forming a co-repressor complex with SEU, which directly interacts with MADS box transcription factors that bind to the *AG* second intron, LUG is recruited to the *AG cis*-regulatory element and exerts its repressor effect on *AG* through at least two different mechanisms: the recruitment of HDACs and the interaction with Mediator complex components.

Embryo apical-basal fate determination

Plant embryos develop along a polar axis, with a root on one end and a shoot on the other. A defective *TOPLESS* (*TPL*) gene, encoding another *Arabidopsis* Gro/Tup1-type co-repressor, causes plant embryos to develop two oppositely oriented root poles [18,58] – hence the gene's name. The double-root phenotype is only seen in *tpl-1* mutants at a high temperature (29°C). At 29°C, although *WUS* mRNA, which serves as a shoot meristem-specific marker, accumulates normally in the *tpl-1* globular stage embryos, its accumulation becomes absent by the transitional stage of *tpl-1* embryos [18]. Root-specific markers such as *SCARECROW* and *LENNY* are ectopically expressed at the apical pole [58] (Figure 2ef). This striking shoot-to-root identity transformation phenotype in *tpl-1* mutants

indicates the existence of a master regulatory network that can switch completely between apical and basal fates.

Interestingly *tpl-1* is a dominant-negative mutation, which, at 29°C, interferes with the function of four other *Arabidopsis* TPL homologs, namely TOPLESS RELATED (TPR) 1, 2, 3 and 4 [18] (Figure 1b). Loss-of-function *tpl* alleles or intragenic suppressor mutations of *tpl-1* (predicted to reduce or abolish TPL gene function) cause no mutant phenotype, indicating that the four TPRs can substitute for TPL. The functional redundancy between TPL and these four TPRs is further supported by *tpl-2; tpr1-1; tpr3-1; tpr4-1* quadruple mutants carrying a TPR2 RNA interference (RNAi) transgene. These mutants displayed a phenotype similar to that of *tpl-1*. It was proposed that, in transition stage embryos, TPL and TPR proteins repress the expression of root-promoting genes in the top half of the embryo, thereby enabling proper differentiation of the shoot pole.

Through genetic approaches, both HDACs and histone acetyltransferases (HATs) (Box 1) were shown to function with TPL [18]. T-DNA, the transfer-DNA from *Agrobacterium tumefaciens*, was inserted into the *HDA19* locus. At 29°C, this T-DNA-induced *hda19* mutant displayed *tpl*-like phenotypes. At a reduced temperature of 24°C, *hda19* exhibited a milder phenotype but could enhance the effect of *tpl-1*. Mutations in *Arabidopsis* histone acetyltransferase *GCN5* [also known as *HAG1* (H antigen gene)] suppressed *tpl-1* mutants at 29°C. Triple mutants of *tpl-1; hda19-1; hag1-3* exhibited phenotypes similar to single *hda19* mutants, indicating that *hag1-3* can suppress *tpl-1* mutants even in the absence of *HDA19*. These data suggest that TPL recruits HDA19 to deacetylate histones in the target chromatin.

The targets of TPL regulation are currently under intensive investigation. Auxin transport or signaling, the key determinants of embryo patterning [59,60], are the prime suspects. During the earliest stages of embryo formation, a predicted flux of auxin is established. This first flux directs auxin towards the apical cell, but then the flux switches, directing auxin away from the apical cell and towards the embryonic base. The polarity of this second outward flux parallels, and is thought to determine, the embryonic shoot-root polarity. Auxin signaling involves both auxin response factors (ARFs), a family of transcription factors that activate or repress expression of auxin response genes, and the auxin (AUX) proteins [also known as IAA (indole acetic acid) proteins], a family of transcriptional repressors. AUX/IAAs contain an EAR (ERF-associated amphiphilic repression) motif that confers transcription repression activity [61]. AUX/IAAs bind to ARFs, inhibiting ARF-activated transcription. The recent discovery of a direct interaction between the CTLH domain of TPL and the EAR domain of IAA12 indicated a crucial role for TPL in mediating the inhibitory effect that AUX/IAAs have on ARF-regulated transcription [62].

These studies suggest that TPL and closely related co-repressor proteins control a crucial developmental cell fate switch at early stages of embryogenesis by repressing the transcription of genes involved in auxin signaling. In

contrast to LUG, which interacts with SEU through its N-terminal LUFs domain, TPL interacts directly with IAA12 through its N-terminal CTLH domain. Nevertheless, both LUG and TPL depend on HDACs for their repressor activities.

Stem cell maintenance

One of the most significant features of plant development is its continuous production, elaboration and modulation of new organs throughout its life span, an ability conferred by meristems. Shoot apical meristem (SAM) development gives rise to all of the above-ground structures. Within SAMs, the stem cell proliferation must be exactly balanced with the rate of daughter-cell differentiation [63–65]. WUS maintains the stem cell pool within the SAMs and is expressed in a small group of cells, the organizing center, within the L3 layer of the SAM [66,67]. WUS signals the stem cells lying above the organizing center to express CLAVATA3 (CLV3), the putative ligand of the CLV1 and CLV2 receptors. Activated CLV1 and CLV2 receptors in turn inhibit WUS expression. The CLV–WUS negative feedback loop ensures homeostasis in the stem cell pool. However, the mechanism by which WUS maintains the stem cell pool or prevents the differentiation of stem cells is currently unclear.

WUS and its orthologs ROSULATA (ROA) in snapdragon (*A. majus*) and TERMINATOR (TER) in petunia (*Petunia hybrida*) [16,68] possess three short conserved C-terminal motifs: an acidic domain, a WUS box (TLPLFPMH), and an EAR-like domain [16]. The existence of the acidic domain is consistent with a function in transcriptional activation [69]. Although the function of the WUS box is unknown, the EAR-like domain, in a similar fashion to the EAR domain in IAA proteins, is involved in transcriptional repression [61]. Because these three motifs are required for complementing *wus-1* mutants, they are suspected to mediate essential protein–protein interactions. A yeast two-hybrid screen identified two related proteins, WUS-interacting protein 1 and 2 (WSIP1 and WSIP2), which might interact with the EAR-like domain in WUS. Interestingly, WSIP1 and WSIP2 are identical to TPL and TPR4, respectively [16] (Figure 1b), suggesting that the repression of root-fate by TPL/WSIP is intimately tied to the maintenance of the stem cell pool in the shoot pole during embryogenesis. Given that the EAR domain of IAA12 interacted with TPL and that the EAR-like domain of WUS probably contributes to its interaction with WSIP, the involvement of TPL/WSIP proteins in mediating the repressor activity of EAR and EAR-like domains emerges as a general theme.

What then are the genes regulated by WUS? In a microarray experiment, 44 genes repressed by WUS were identified [70]. They include cytokinin-inducible genes of the *ARABIDOPSIS RESPONSE REGULATOR* (ARR) family. WUS was shown to directly bind to the *ARR7* promoter, and an ectopic *ARR7* activity caused a *wus*-like phenotype of early meristem termination. Thus, WUS might maintain the stem cell pool by recruiting TPL/TPR/WSIP co-repressors to repress cytokinin synthesis or signaling.

Concluding remarks

In summary, the field of co-repressor research is emerging as an important area in plant biology. The plant Gro/Tup1 co-repressors regulate diverse developmental pathways through interactions with pathway-specific DNA-binding factors. The similar domain organization of plant Gro/Tup1 co-repressors and their animal and fungal homologs might underlie their similar modes of action, including histone deacetylation and inhibition of RNA pol II activity. TPL/TPR/WSIP and LUG differ in the N-terminal subdomains (see Figure 1a), which might account for their different interacting partners (EAR domain-containing proteins and SEU, respectively) and different regulatory targets.

The plant Gro/Tup1 co-repressors, as with their fungal and animal counterparts, are global regulators controlling many aspects of plant development. Current and future efforts in identifying direct targets of these co-repressors will benefit from an array of techniques, including ChIP, ChIP-chip [71], ChIP-seq [72], and transcriptome profiling combined with inducible systems such as glucocorticoid receptor fusions [73]. The ability to identify downstream regulatory targets and the corresponding DNA-binding factors that recruit these co-repressors will provide important insights into how these global co-repressors achieve their regulatory specificity in target selection. Co-repressors are integral components of larger multi-protein complexes. Future biochemical purification of the co-repressor complex in plants will be necessary to uncover as yet unidentified complex components, to facilitate their further functional characterization, and to enable cross-kingdom comparisons of co-repressor complexes. These future studies will answer fundamental questions about higher plant development, such as how cell fates and organ identities are properly specified, how body patterning is established, and how the stem cell pool is maintained.

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References

- Chen, G. and Courey, A.J. (2000) Groucho/TLE family proteins and transcriptional repression. *Gene* 249, 1–16
- Neer, E.J. *et al.* (1994) The ancient regulatory-protein family of WD-repeat proteins. *Nature* 371, 297–300
- Pickles, L.M. *et al.* (2002) Crystal structure of the C-terminal WD40 repeat domain of the human Groucho/TLE1 transcriptional corepressor. *Structure* 10, 751–761
- Tolkunova, E.N. *et al.* (1998) Two distinct types of repression domain in engrailed: one interacts with the groucho corepressor and is preferentially active on integrated target genes. *Mol. Cell. Biol.* 18, 2804–2814
- Courey, A.J. and Jia, S. (2001) Transcriptional repression: the long and the short of it. *Genes Dev.* 15, 2786–2796
- Daniels, D.L. and Weis, W.I. (2005) Beta-catenin directly displaces Groucho/TLE repressors from Tcf/Lef in Wnt-mediated transcription activation. *Nat. Struct. Mol. Biol.* 12, 364–371
- Levanon, D. *et al.* (1998) Transcriptional repression by AML1 and LEF-1 is mediated by the TLE/Groucho corepressors. *Proc. Natl. Acad. Sci. U.S.A.* 95, 11590–11595
- Blatch, G.L. and Lassle, M. (1999) The tetratricopeptide repeat: a structural motif mediating protein-protein interactions. *Bioessays* 21, 932–939
- Keleher, C.A. *et al.* (1992) Ssn6-Tup1 is a general repressor of transcription in yeast. *Cell* 68, 709–719
- Tzamarias, D. and Struhl, K. (1995) Distinct TPR motifs of Cyc8 are involved in recruiting the Cyc8-Tup1 corepressor complex to differentially regulated promoters. *Genes Dev.* 9, 821–831
- Tzamarias, D. and Struhl, K. (1994) Functional dissection of the yeast Cyc8-Tup1 transcriptional co-repressor complex. *Nature* 369, 758–761
- Malave, T.M. and Dent, S.Y. (2006) Transcriptional repression by Tup1-Ssn6. *Biochem. Cell Biol.* 84, 437–443
- Smith, R.L. and Johnson, A.D. (2000) Turning genes off by Ssn6-Tup1: a conserved system of transcriptional repression in eukaryotes. *Trends Biochem. Sci.* 25, 325–330
- Davie, J.K. *et al.* (2003) Tup1-Ssn6 interacts with multiple class I histone deacetylases *in vivo*. *J. Biol. Chem.* 278, 50158–50162
- Davie, J.K. *et al.* (2002) Histone-dependent association of Tup1-Ssn6 with repressed genes *in vivo*. *Mol. Cell. Biol.* 22, 693–703
- Kieffer, M. *et al.* (2006) Analysis of the transcription factor WUSCHEL and its functional homologue in antirrhinum reveals a potential mechanism for their roles in meristem maintenance. *Plant Cell* 18, 560–573
- Conner, J. and Liu, Z. (2000) LEUNIG, a putative transcriptional corepressor that regulates AGAMOUS expression during flower development. *Proc. Natl. Acad. Sci. U.S.A.* 97, 12902–12907
- Long, J.A. *et al.* (2006) TOPLESS regulates apical embryonic fate in *Arabidopsis*. *Science* 312, 1520–1523
- Cockell, M. *et al.* (1998) Sif2p interacts with Sir4p amino-terminal domain and antagonizes telomeric silencing in yeast. *Curr. Biol.* 8, 787–790
- Cerna, D. and Wilson, D.K. (2005) The structure of Sif2p, a WD repeat protein functioning in the SET3 corepressor complex. *J. Mol. Biol.* 351, 923–935
- Yoon, H.G. *et al.* (2003) Purification and functional characterization of the human N-CoR complex: the roles of HDAC3, TBL1 and TBLR1. *EMBO J.* 22, 1336–1346
- Liu, Z. and Meyerowitz, E.M. (1995) LEUNIG regulates AGAMOUS expression in *Arabidopsis* flowers. *Development* 121, 975–991
- Liu, Z. *et al.* (2000) Regulation of gynoecium marginal tissue formation by LEUNIG and AINTEGUMENTA. *Plant Cell* 12, 1879–1892
- Franks, R.G. *et al.* (2006) SEUSS and LEUNIG regulate cell proliferation, vascular development and organ polarity in *Arabidopsis* petals. *Planta* 224, 801–811
- Chen, C. *et al.* (2000) LEUNIG has multiple functions in gynoecium development in *Arabidopsis*. *Genesis* 26, 42–54
- Cnops, G. *et al.* (2004) The rotunda2 mutants identify a role for the LEUNIG gene in vegetative leaf morphogenesis. *J. Exp. Bot.* 55, 1529–1539
- Navarro, C. *et al.* (2004) Molecular and genetic interactions between STYLOSA and GRAMINIFOLIA in the control of *Antirrhinum* vegetative and reproductive development. *Development* 131, 3649–3659
- Gonzalez, D. *et al.* (2007) The transcription corepressor LEUNIG interacts with the histone deacetylase HDA19 and mediator components MED14 (SWP) and CDK8 (HEN3) to repress transcription. *Mol. Cell. Biol.* 27, 5306–5315
- Yanofsky, M.F. *et al.* (1990) The protein encoded by the *Arabidopsis* homeotic gene AGAMOUS resembles transcription factors. *Nature* 346, 35–39
- Drews, G.N. *et al.* (1991) Negative regulation of the *Arabidopsis* homeotic gene AGAMOUS by the APETALA2 product. *Cell* 65, 991–1002
- Sridhar, V.V. *et al.* (2004) Transcriptional repression of target genes by LEUNIG and SEUSS, two interacting regulatory proteins for *Arabidopsis* flower development. *Proc. Natl. Acad. Sci. U.S.A.* 101, 11494–11499
- Franks, R.G. *et al.* (2002) SEUSS, a member of a novel family of plant regulatory proteins, represses floral homeotic gene expression with LEUNIG. *Development* 129, 253–263
- Chen, L. *et al.* (2002) Ssdp proteins interact with the LIM-domain-binding protein Ldb1 to regulate development. *Proc. Natl. Acad. Sci. U.S.A.* 99, 14320–14325
- van Meyel, D.J. *et al.* (2003) Ssdp proteins bind to LIM-interacting cofactors and regulate the activity of LIM-homeodomain protein complexes *in vivo*. *Development* 130, 1915–1925

- 35 Sieburth, L.E. and Meyerowitz, E.M. (1997) Molecular dissection of the AGAMOUS control region shows that *cis* elements for spatial regulation are located intragenically. *Plant Cell* 9, 355–365
- 36 Busch, M.A. *et al.* (1999) Activation of a floral homeotic gene in *Arabidopsis*. *Science* 285, 585–587
- 37 Deyholos, M.K. and Sieburth, L.E. (2000) Separable whorl-specific expression and negative regulation by enhancer elements within the AGAMOUS second intron. *Plant Cell* 12, 1799–1810
- 38 Lohmann, J.U. *et al.* (2001) A molecular link between stem cell regulation and floral patterning in *Arabidopsis*. *Cell* 105, 793–803
- 39 Parcy, F. *et al.* (1998) A genetic framework for floral patterning. *Nature* 395, 561–566
- 40 Sridhar, V.V. *et al.* (2006) APETALA1 and SEPALLATA3 interact with SEUSS to mediate transcription repression during flower development. *Development* 133, 3159–3166
- 41 Gregis, V. *et al.* (2006) AGL24, SHORT VEGETATIVE PHASE, and APETALA1 redundantly control AGAMOUS during early stages of flower development in *Arabidopsis*. *Plant Cell* 18, 1373–1382
- 42 Weigel, D. and Meyerowitz, E.M. (1993) Activation of floral homeotic genes in *Arabidopsis*. *Science* 261, 1723–1726
- 43 Castillejo, C. *et al.* (2005) A new role of the *Arabidopsis* SEPALLATA3 gene revealed by its constitutive expression. *Plant J.* 43, 586–596
- 44 Ng, M. and Yanofsky, M.F. (2001) Activation of the *Arabidopsis* B class homeotic genes by APETALA1. *Plant Cell* 13, 739–753
- 45 Honma, T. and Goto, K. (2001) Complexes of MADS-box proteins are sufficient to convert leaves into floral organs. *Nature* 409, 525–529
- 46 Cho, S. *et al.* (1999) Analysis of the C-terminal region of *Arabidopsis thaliana* APETALA1 as a transcription activation domain. *Plant Mol. Biol.* 40, 419–429
- 47 Liu, C. *et al.* (2007) Specification of *Arabidopsis* floral meristem identity by repression of flowering time genes. *Development* 134, 1901–1910
- 48 Michaels, S.D. *et al.* (2003) AGL24 acts as a promoter of flowering in *Arabidopsis* and is positively regulated by vernalization. *Plant J.* 33, 867–874
- 49 Yu, H. *et al.* (2004) Repression of AGAMOUS-LIKE 24 is a crucial step in promoting flower development. *Nat. Genet.* 36, 157–161
- 50 Hartmann, U. *et al.* (2000) Molecular cloning of SVP: a negative regulator of the floral transition in *Arabidopsis*. *Plant J.* 21, 351–360
- 51 Lee, J.H. *et al.* (2007) Role of SVP in the control of flowering time by ambient temperature in *Arabidopsis*. *Genes Dev.* 21, 397–402
- 52 Vigushin, D.M. *et al.* (2001) Trichostatin A is a histone deacetylase inhibitor with potent antitumor activity against breast cancer *in vivo*. *Clin. Cancer Res.* 7, 971–976
- 53 Pandey, R. *et al.* (2002) Analysis of histone acetyltransferase and histone deacetylase families of *Arabidopsis thaliana* suggests functional diversification of chromatin modification among multicellular eukaryotes. *Nucleic Acids Res.* 30, 5036–5055
- 54 Tian, L. *et al.* (2003) Genetic control of developmental changes induced by disruption of *Arabidopsis* histone deacetylase 1 (AtHD1) expression. *Genetics* 165, 399–409
- 55 Tian, L. and Chen, Z.J. (2001) Blocking histone deacetylation in *Arabidopsis* induces pleiotropic effects on plant gene regulation and development. *Proc. Natl. Acad. Sci. U.S.A.* 98, 200–205
- 56 Autran, D. *et al.* (2002) Cell numbers and leaf development in *Arabidopsis*: a functional analysis of the STRUWWELPETER gene. *EMBO J.* 21, 6036–6049
- 57 Wang, W. and Chen, X. (2004) HUA ENHANCER3 reveals a role for a cyclin-dependent protein kinase in the specification of floral organ identity in *Arabidopsis*. *Development* 131, 3147–3156
- 58 Long, J.A. *et al.* (2002) Transformation of shoots into roots in *Arabidopsis* embryos mutant at the TOPLESS locus. *Development* 129, 2797–2806
- 59 Vieten, A. *et al.* (2007) Molecular and cellular aspects of auxin-transport-mediated development. *Trends Plant Sci.* 12, 160–168
- 60 Jenik, P.D. and Barton, M.K. (2005) Surge and destroy: the role of auxin in plant embryogenesis. *Development* 132, 3577–3585
- 61 Ohta, M. *et al.* (2001) Repression domains of class II ERF transcriptional repressors share an essential motif for active repression. *Plant Cell* 13, 1959–1968
- 62 Szemenyei, H. *et al.* (2008) TOPLESS mediates IAA12/BODENLOS transcriptional repression during embryogenesis in *Arabidopsis*. *Science* DOI: 10.1126/science.1151461
- 63 Gross-Hardt, R. and Laux, T. (2003) Stem cell regulation in the shoot meristem. *J. Cell Sci.* 116, 1659–1666
- 64 Baurle, I. and Laux, T. (2003) Apical meristems: the plant's fountain of youth. *Bioessays* 25, 961–970
- 65 Williams, L. and Fletcher, J.C. (2005) Stem cell regulation in the *Arabidopsis* shoot apical meristem. *Curr. Opin. Plant Biol.* 8, 582–586
- 66 Mayer, K.F. *et al.* (1998) Role of WUSCHEL in regulating stem cell fate in the *Arabidopsis* shoot meristem. *Cell* 95, 805–815
- 67 Schoof, H. *et al.* (2000) The stem cell population of *Arabidopsis* shoot meristems is maintained by a regulatory loop between the CLAVATA and WUSCHEL genes. *Cell* 100, 635–644
- 68 Ferrario, S. *et al.* (2006) Control of floral meristem determinacy in petunia by MADS-box transcription factors. *Plant Physiol.* 140, 890–898
- 69 Ma, J. and Ptashne, M. (1987) A new class of yeast transcriptional activators. *Cell* 51, 113–119
- 70 Leibfried, A. *et al.* (2005) WUSCHEL controls meristem function by direct regulation of cytokinin-inducible response regulators. *Nature* 438, 1172–1175
- 71 Bulyk, M.L. (2006) DNA microarray technologies for measuring protein-DNA interactions. *Curr. Opin. Biotechnol.* 17, 422–430
- 72 Mardis, E.R. (2007) ChIP-seq: welcome to the new frontier. *Nat. Methods* 4, 613–614
- 73 Wagner, D. *et al.* (1999) Transcriptional activation of APETALA1 by LEAFY. *Science* 285, 582–584
- 74 Bao, X. *et al.* (2004) Repression of AGAMOUS by BELLRINGER in floral and inflorescence meristems. *Plant Cell* 16, 1478–1489
- 75 Jofuku, K.D. *et al.* (1994) Control of *Arabidopsis* flower and seed development by the homeotic gene APETALA2. *Plant Cell* 6, 1211–1225
- 76 Chinnadurai, G. (2002) CtBP, an unconventional transcriptional corepressor in development and oncogenesis. *Mol. Cell* 9, 213–224
- 77 Taatjes, D.J. *et al.* (2004) Regulatory diversity among metazoan co-activator complexes. *Nat. Rev. Mol. Cell Biol.* 5, 403–410
- 78 Struhl, K. (2005) Transcriptional activation: mediator can act after preinitiation complex formation. *Mol. Cell* 17, 752–754
- 79 Backstrom, S. *et al.* (2007) Purification of a plant mediator from *Arabidopsis thaliana* identifies PFT1 as the Med25 subunit. *Mol. Cell* 26, 717–729
- 80 Saha, S. *et al.* (1993) New eukaryotic transcriptional repressors. *Nature* 363, 648–652

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