MODES OF TRANSCRIPTIONAL REGULATION

Regulation of transcription in plants: mechanisms controlling developmental switches

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Abstract | Unlike animals, plants produce new organs throughout their life cycle using pools of stem cells that are organized in meristems. Although many key regulators of meristem and organ identities have been identified, it is still not well understood how they function at the molecular level and how they can switch an entire developmental programme in which thousands of genes are involved. Recent advances in the genome-wide identification of target genes controlled by key plant transcriptional regulators and their interactions with epigenetic factors provide new insights into general transcriptional regulatory mechanisms that control switches of developmental programmes and cell fates in complex organisms.

Pluripotent cell
An undifferentiated cell that has the potential to adopt different identities. In plants, pluripotent cells are found in meristems and there are stem cell-like populations in shoots, roots and leaves.

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Organ development in plants is a continuous and flexible process, which is not restricted to the embryonic phase[1]. This plasticity in plant development is linked to the presence of pluripotent cells residing in meristems, which acquire different 'identities' that depend on their position in the plant and its developmental phase, thus leading to the production of different organ types. The switch from a vegetative shoot apical meristem to an inflorescence meristem, as well as the establishment of floral meristem and floral organ identities, are among the most well-studied examples of developmental switches in plants (FIG. 1), particularly in the model species *Arabidopsis thaliana*. These meristem switches are controlled by multiple environmental and internal input pathways. Switches in cell identity not only have a role in major phase transitions but also occur during embryonic patterning and more locally during organ differentiation. Positional information plays a crucial part in cell fate specification, and the plasticity of plant development is at least partially linked to the ability of cells to differentiate or dedifferentiate, depending on external signals and cell-to-cell communication, which are mediated by hormones and other mobile signalling molecules[2]. Cell-extrinsic signals have also been shown to have a role in cell specification and lineage commitment in animal development[3,4].

Pioneered by research in *Drosophila melanogaster*[5], signalling cascades that control developmental switches in animals and plants have been shown to converge at the level of gene regulation; transcription factors can change entire developmental programmes, resulting in switches of cell and organ identities. Ectopic expression of key regulatory transcription factors can cause reprogramming of cell fate in animals, resulting in dedifferentiation or conversion of one partially or fully differentiated cell type into another[3]. In addition, switches in cell identity and differentiation state are also regulated at the level of chromatin structure. Accordingly, histone-modifying enzymes as well as ATP-dependent nucleosome-remodelling enzymes have been shown to control these processes in plants and animals[6,7].

The first glimpse of how developmental switches are controlled in plants came in the early 1990s when key regulatory genes that control the transition from vegetative to reproductive growth were identified in the model species *A. thaliana* and *Antirrhinum majus*. These regulatory genes encode transcription factors that specify the identities of floral meristems and organs[8]. Genetic and molecular studies revealed antagonistic genetic interactions between transcription factors that specify alternative meristem identities, as well as cooperation and reinforcement among factors involved in the same identity switch. These studies also uncovered roles for heteromeric protein complexes in cell and organ identity specification. For example, the crucial role of heteromeric protein interactions of floral 'master regulators' was consolidated in 2001 (REFS 9,10) when the 'floral quartet model' was proposed[11]. According to this model, floral organ identity regulators of the MADS-box family assemble into organ-specific quarternary transcription factor complexes, thereby obtaining their regulatory specificity. Since then, many interactions between transcription factors that act in diverse developmental processes have

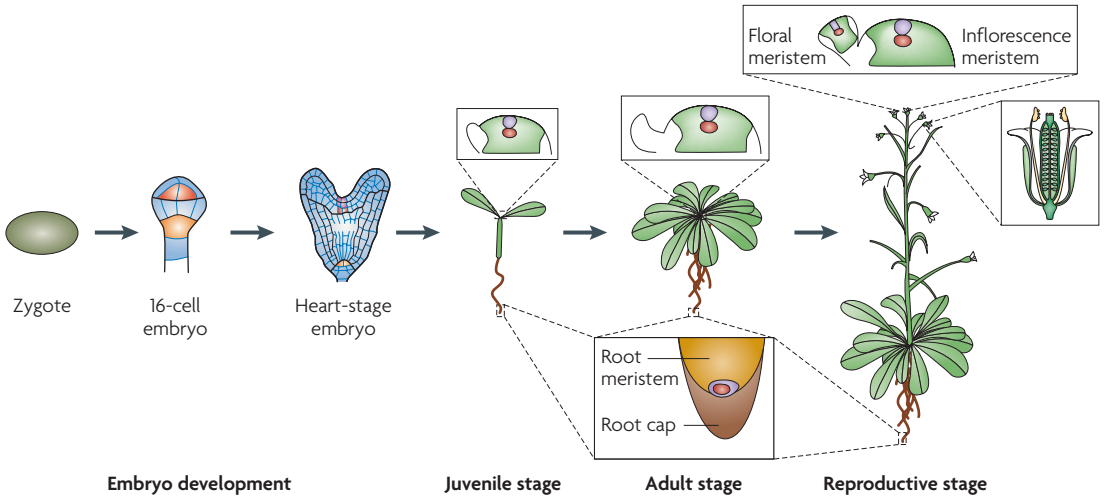


Figure 1 | **Phase changes in plant development.** Root and shoot meristems are established during embryo development, which occurs in the seed. The first shoot meristem marker to be expressed is WUSCHEL (WUS, shown in red) in the 16-cell embryo. WUS expression defines the 'organizing centre' of the shoot meristem, which is a group of cells just below the stem cells. The hypophysis (orange) will give rise to the quiescent (organizing) centre of the root meristem. In the heart-stage embryo, the shoot and root meristem are already established. After germination, which occurs after the heart stage, the shoot apical meristem produces leaves. In the shoot meristem (upper boxes), the meristem region is shown in green, the stem cell marker CLAVATA 3 in purple and WUS expression in red. The first leaves have a juvenile appearance and the leaves produced later gradually develop more adult characteristics[168]. The change from adult to reproductive stage is more dramatic in flowering plants. In *Arabidopsis thaliana*, it is triggered by floral induction pathways that converge on the upregulation of SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1 and other factors in the shoot meristem, which triggers conversion from a vegetative to an inflorescence meristem identity[169]. The inflorescence meristem produces floral meristems at its flanks, in which separate stem cell pools are established. Floral meristem identity genes repress inflorescence meristem identity genes and activate the expression of floral organ identity genes. The floral meristem then produces the different types of floral organs (right box): sepals and petals in the outer two whorls, respectively, as well as stamens (male reproductive organs) and carpels (female reproductive organs) in the inner two whorls. After floral organ initiation, stem cell activity in the floral meristem ceases and undifferentiated cells are only maintained in specific regions in the carpels. These cells give rise to ovules, in which the zygote is formed after fertilization of the egg cell by the male gamete (not shown). In the root meristem, the stem cells surround the quiescent centre (red oval). In contrast to the shoot meristem, root stem cell identity is controlled at a single-cell level: asymmetric cell divisions give rise to a daughter cell that remains a stem cell and another daughter cell that differentiates. During development, lateral roots are formed formed by the *de novo* formation of meristems, which initiate from lateral root founder cells in the pericycle.

Meristem
A tissue in plants consisting of pluripotent cells. In apical meristems, cell-to-cell signalling establishes and maintains a zone that contains the stem cells, which is separated from the peripheral zone in which differentiation is eventually initiated. Other types of meristems give rise to the vasaculture and epidermis, or enable secondary growth.

Shoot apical meristem
The meristem that forms all major above-ground plant organs. It is established during embryogenesis. During plant development, it changes from a juvenile to a vegetative state, and then to inflorescence and floral identity.

Inflorescence meristem
A type of shoot apical meristem that gives rise to floral meristems at its flanks.

Floral meristem
A meristem that gives rise to the floral organs: sepals, petals, stamens and carpels.

Histone-modifying enzyme
An enzyme that can modify specific sites in histones, for example, by adding or removing a chemical group. Common modifications are methylation, acetylation, ubiquitylation, sumoylation, phosphorylation and proline isomerization.

Nucleosome-remodelling enzyme
An enzyme that can establish, remove, or change the positions of nucleosomes on the DNA.

MADS-box family
A family of transcription factors that is present in all major groups of eukaryotes. The family is named after the founding members MCM1 from *Saccharomyces cerevisiae*, AGAMOUS from *Arabidopsis thaliana*, DEFICIENS from *Antirrhinum majus* and SRF from humans.

been identified in different plant species, mainly using the yeast two-hybrid system. However, how transcription factors function and interact *in planta*, what their target genes are and how regulatory specificity is determined could not be resolved by these classical types of studies.

Genome-wide approaches for target gene identification provided new insights into mechanisms of gene regulation by transcriptional key regulators. Several studies used inducible versions of transcription factors and the global analysis of gene expression changes on induction[12–16]. Recent genome-wide analyses of the *in vivo* DNA-binding sites of transcription factors using chromatin immunoprecipitation (ChIP) techniques, such as ChIP–seq and ChIP–chip[17], have been powerful for identifying potential direct target genes.

In this Review, we first introduce recent findings on the direct downstream targets of key regulatory transcription factors that control developmental switches in plants, and how autoregulation and cross-regulation in transcriptional regulatory networks control developmental switches. We then discuss the emerging concept that developmental transitions are regulated by the interplay of transcription factor complexes and proteins that modulate chromatin structure. This provides insights into the mechanisms underlying the action and functional specificity of different transcription factor complexes and chromatin regulators in the control of developmental switches in plants.

Global gene regulation by transcription factors

During developmental transitions and cell fate specification in higher eukaryotes, changes in the expression of many genes need to be coordinated to initiate the correct differentiation programmes and to suppress earlier or inappropriate programmes. This is the task of transcriptional regulators. The term master regulator was first introduced in 1985 and applied to regulators of mating type in yeast[18]. It was later adopted for transcriptional regulators in metazoan[19] and plant[20] development. Initially, master regulators were thought to control a limited set of 'second-level' transcription factors, which in turn regulate the expression of downstream target genes that are more involved in cellular responses, such as the cell cycle, metabolic processes and intracellular signalling processes[21,22] (FIG. 2). The structure of regulatory networks became more complex than initially anticipated after

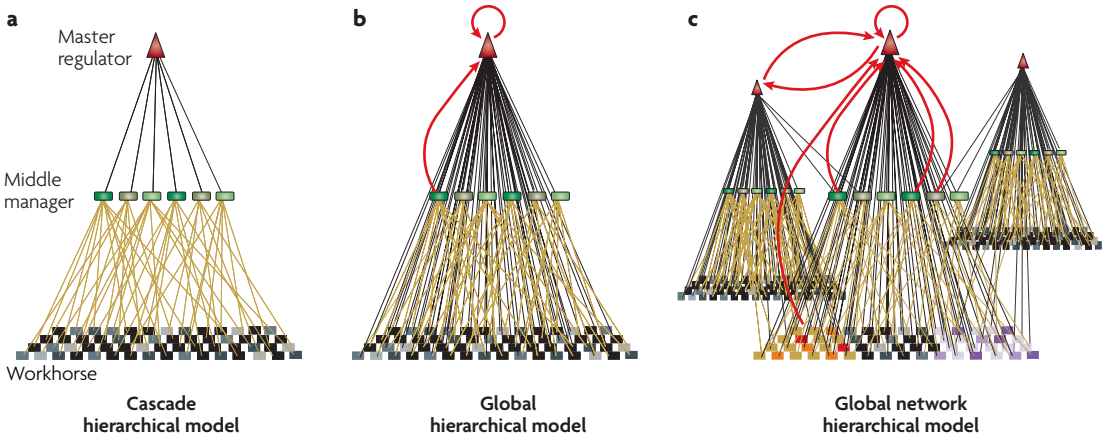


Figure 2 | **Evolving models of the action of developmental master regulators. a** | The classical model suggested a strictly hierarchical network, in which master regulators activate a (small) set of second-level regulatory genes ('middle managers'), which control the expression of genes that produce distinct morphologies ('workhorses'); for example, genes that encode enzymes and structural proteins. **b** | Identification of downstream targets of individual transcription factors suggested a more complex network, in which key transcriptional regulators can control genes at different levels in the hierarchy. The regulators at the top of the hierarchy seem to directly regulate hundreds of genes, in combination with other transcriptional regulators. Also, the factors at the top of the hierarchy can be regulated by their own targets or can autoregulate (red arrows). **c** | Recent genome-wide identification of targets of multiple transcription factors suggests complex combinatorial regulation of subsets of downstream targets, as well as multiple cross-regulatory loops. Note that transcription factors control genes that can function upstream or downstream in the developmental pathway.

ChIP–seq
(Chromatin immunoprecipitation followed by next-generation sequencing). A technique that is used to identify the *in vivo* DNA-binding sites of proteins. After crosslinking of proteins to DNA, isolation and fragmentation of the chromatin, genomic regions that are bound by the protein of interest are isolated using specific antibodies. The immunoprecipitated DNA is then sequenced.

ChIP–chip
(Chromatin immunoprecipitation followed by microarray). DNA associated with a protein of interest, isolated by chromatin immunoprecipitation, is hybridized to genomic-tiling arrays to identify DNA-binding sites of the protein.

Direct target gene
A gene whose expression is controlled by a particular transcription factor through direct binding of the factor to *cis*-regulatory elements of that gene.

Autoregulation
A mechanism in which a molecule (such as a transcription factor) regulates its own production. This process can involve interactions with other molecules.

Floral pathway integrator
A protein that can integrate the inputs of the different environmental and internal floral induction pathways and transmit the information to their downstream targets, such as floral meristem identity genes, at the shoot apex. Their combined action controls flowering time. The transcriptional regulators SOC1, LFY, FT and FD are 'classical' floral pathway integrators.

Perianth
The sterile organs in the outer whorls of a flower. Many flowering plant species such as *Arabidopsis*, *Petunia* and *Antirrhinum* have a perianth that is differentiated into sepals and petals.

target genes of transcription factors were identified[22–24] and, in particular, when the results of genome-wide target gene identification approaches became available, as master regulators directly control more genes than previously expected. In addition to the second-level transcription factors, master regulators control genes that encode structural proteins, other signalling molecules (for example, those involved in hormonal pathways) and enzymes. Most likely, subsets of these transcriptional cascades are regulated by different combinations of transcription factors, some of which are also regulated by the master regulator, resulting in a more complex transcriptional network (FIG. 2c). In this model, combinatorial transcription factor interactions can create flexibility and specificity in the regulation of subsets of target genes. The transcriptional cascades are also characterized by multiple feedback and feedforward loops[25,26], creating a more 'democratic' network structure[27].

Orchestration of gene expression during developmental switches. Genome-wide DNA-binding maps and direct target genes of several key regulatory transcription factors that have roles in plant development have been reported recently (Supplementary information S1 (table)). The results shed light on global regulatory networks that control switches in meristem and organ identity, as well as local networks that specify cell fate and terminal differentiation during organ development.

Stem cell identity in plants and animals is controlled by an interplay between intercellular signalling and transcriptional regulation[4]. For example, the homeobox transcription factor WUSCHEL (WUS) has an instructive role in stem cell identity specification in embryos and in the maintenance of stem cell niches in shoot meristems[4]. Analysis of direct WUS target genes revealed multiple regulatory links to hormonal signalling, cell division control, as well as feedback control of the receptor complex that restricts stem cell proliferation and WUS expression[28]. These findings emphasize the complexity of intercellular communication and transcriptional feedback in stem cell specification in plants.

Transcription factors control developmental phase switches by orchestrating gene expression changes in meristems to repress previous developmental programmes and establish new ones. Thus, they modulate shoot growth and specify which types of lateral organs are produced. Several transcription factors have been described that function as key switches in meristem identity during the transition from vegetative to reproductive growth (FIG. 3). The switch from vegetative to inflorescence meristem identity is controlled by floral pathway integrators, which integrate the input of different environmental and internal signalling pathways. Floral integrators activate the expression of floral meristem identity genes in the flanks of the inflorescence meristem. The MADS-domain transcription factor APETALA1 (AP1) establishes floral meristem identity and consequently plays a part in the initiation of perianth organs in the flower (for review, see REF. 8). *AP1* functions partially redundantly with a closely related gene, *CAULIFLOWER*[29]. AP1 has approximately 2,000 genomic binding sites in the earliest stages of floral meristem initiation[16], and at this developmental stage it acts mostly as a transcriptional repressor to downregulate the previous developmental programme of the meristem. Among its many targets, AP1 downregulates its own activators[16,30], such as FD[31], SQUAMOSA PROMOTER BINDING PROTEIN-LIKE 9 (REF. 32), as well as the floral integrator SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1 (SOC1). However, it also upregulates

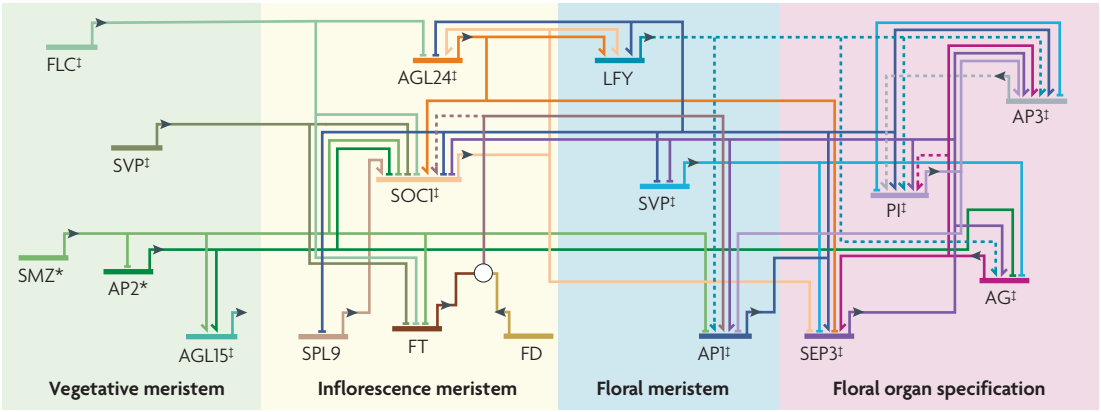


Figure 3 | **A regulatory network of the transition to flowering.** As an illustrative example of a developmental regulatory network, this figure summarizes major regulators that act as suppressors or activators in the sequential developmental stages from a vegetative meristem to a floral meristem, from which floral organs develop. All the factors shown are transcription factors, except FLOWERING LOCUS T (FT), which is a mobile protein produced in leaves and is transported to the shoot apical meristem where it interacts with the bZIP transcription factor FD[31,170] to initiate flowering. The factors are shown in the pathway at the position they initially act, for example, APETALA 1 (AP1) is required for floral meristem and organ identity specification. Various external (for example, photoperiod and temperature) and internal (autonomous pathway, age and hormones) conditions regulate the transition from vegetative to inflorescence meristem. These inputs into the network are combined at the level of the so-called floral pathway integrators (SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1 (SOC1), AGAMOUS-LIKE 24 (AGL24), FT and LEAFY (LFY)), and ultimately regulate the expression of the floral meristem identity genes (*AP1*, *SHORT VEGETATIVE PHASE* (*SVP*) and *LFY*). Subsequently, *SEPALLATA 3* (*SEP3*) is activated by AP1 and SEP3 functions in the flower as a main component of protein complexes that specify floral organ identities. Other floral organ identity genes (*PISTILLATA* (*PI*), *AP3* and *AGAMOUS* (*AG*)) are also controlled by the meristem identity factors AP1 and LFY. For activation of the class B identity genes *AP3* and *PI*, LFY requires its co-regulator UNUSUAL FLORAL ORGANS[171] (not shown). The SVP node is duplicated in the figure because of its dual role in floral transition and meristem specificiation. CAULIFLOWER was not represented in the network owing to its largely redundant function with AP1. Dashed arrows show interactions that have been proposed based on genetic and/or *in vitro* DNA-binding studies but to date have not been confirmed as a direct interaction by *in planta* chromatin immunoprecipitation. In this figure, we focus on major transcriptional regulators acting in floral transition and flower development that are also mentioned in the text. For a more detailed overview of the signalling pathways controlling floral transition, see REF. 172. *AP2 family member. ‡MADS family member. FLC, FLOWERING LOCUS C; SMZ, SCHLAFMUETZE; SPL9, SQUAMOSA PROMOTER BINDING PROTEIN-LIKE 9.

genes such as the floral master regulator *LEAFY* (*LFY*), which acts together with AP1 in floral meristem identity specification[16,33–35] (FIG. 3). Later in floral meristem development, AP1 activates the expression of genes that are involved in downstream processes, such as floral organ identity specification and organ initiation, probably together with its MADS family dimerization partner SEPALLATA3 (SEP3). SEP3 interacts with many MADS-domain transcription factors[9,36], and it mediates the formation of higher-order complexes that may form DNA loops (BOX 1). SEP3 is a global regulator of floral organ identities in an apparently redundant fashion with three other SEP paralogues[37]. SEP3 also binds thousands of regions in the genome[38]. Floral organ identity regulators such as SEP3 and AGAMOUS (AG) are also expressed at later stages of flower development and modulate aspects of organ differentiation, which is reflected in the large range of functions of their target genes[12,13,38,39].

Another example of a transcription factor that plays a part in identity specification in the meristem is AP2, which is the founding member of the plant-specific AP2-like transcription factor family. Besides its role as a repressor of floral transition, AP2 promotes sepal and petal identity specification in the outer two whorls of the flower and it acts in stem cell maintenance by promoting WUS expression[40–43]. Approximately 2,000 genomic AP2-binding sites have been identified, and it can repress the floral transition by direct transcriptional repression or indirect repression through the activation of other repressor genes or microRNAs (miRNAs), and so forms a network with other key regulators[43]. AP2 is a member of a subfamily of AP2-like genes whose partially redundantly acting members are regulated by miR172 (REF. 44) (BOX 2). For example, its paralogue SCHLAFMUTZE functions as a repressor of the transition to flowering and shares more than one-quarter of its potential direct target genes with AP2 (REFS 43,45,46).

Meristem and organ identity genes establish basic developmental decisions in meristems, whereas lateral organ development is triggered by transcription factors that repress meristematic fate and activate differentiation programmes[47,48]. During organ growth, local switches in individual cell fates lead to organ patterning and terminal cell differentiation. The specification of cellular identities during organ growth requires a fine balance of cell specification versus maintenance of cell division potential[49]. Accordingly, cell cycle control genes are not only targeted by meristem regulators but are also identified among the direct targets of transcription factors that specify trichomes and the guard cells of stomata in the

Trichome
An epidermal outgrowth (hair) that can have different structures and functions. In *Arabidopsis thaliana*, trichomes are unicellular.

Stoma
A pore found in the epidermis of leaves and in several other above-ground plant organs. Stomata are surrounded by pairs of specialized epidermal cells called guard cells.

Box 1 | **Combinatorial interactions of MADS-domain proteins and promoter structure**

The spatiotemporal regulation of gene expression during eukaryotic development is controlled by a complex interplay among *cis*-regulatory modules in core promoters and enhancers. The current model of enhancer action involves the binding of activating transcription factors to enhancer sequence elements, subsequent recruitment of additional co-activators and the formation of loops that bring these factors close to the core promoter, thus leading to activation of transcription by RNA polymerase II (REF. 135).

Although DNA-binding sites of transcription factors are enriched in the proximal promoter regions of genes, they can also occur at greater distances upstream, in introns or even downstream of target genes. Surprisingly, many target gene loci of MADS-domain proteins contain multiple binding sites for the same factor, sometimes covering large regions upstream and/or downstream of the gene[16,38,136]. The binding of transcription factors to multiple sequence elements suggests that transcriptional control can be a quantitative process that enables the modulation of transcriptional activity by varying the number of transcription factor molecules that are independently bound to DNA[137,138]. Alternatively, multiple binding sites could be required to allow a conformational change of the DNA that allows the formation of DNA loops. Recent models support a role for chromatin structure and the formation of large DNA loops in repression of transcription by Polycomb group complexes and for the recruitment of genomic loci to RNA polymerase complexes[139], which may be located in so-called 'transcription factories' (REFS 140,141).

DNA looping at short distances has been observed for MADS-domain protein complexes that are composed of two dimers that bind two binding sites *in vitro*[142,143]. Such *in vitro* DNA-binding studies have shown that a minimum distance between the binding sites is required[143] and that the formation of heteromeric higher-order complexes can stabilize the binding to DNA[142]. Although loop formation has only been tested for DNA sites that lie within approximately 100 bp of each other *in vitro*, loops could potentially bridge larger distances *in vivo*. The flexibility of the DNA that allows looping depends on the nucleosome density of the chromatin. The optimal separation distances for looping-mediated interactions *in vivo* is estimated to be several tens of kb in condensed chromatin fibres[144]. Accordingly, nucleosome density around transcription factor-binding sites needs to be low to facilitate looping at shorter distances. Besides the distance between interacting sites, the helical phasing of the DNA is also crucial to ensure that DNA-bound proteins are orientated so that they can interact with each other[143].

Characteristics of loop formation could contribute to the functional specificity of different MADS-domain protein complexes, as could differences in DNA bending by MADS dimers[145,146]. Thus, reorganization of promoter structure by MADS-domain transcription factors is one possible mechanism by which these proteins control the expression of genes that are tightly regulated during development. Future research is required to discover whether MADS-domain proteins form DNA loops *in vivo* and what the regulatory effects of such loops are.

epidermis of above-ground organs. The R2R3 MYB transcription factor FOUR LIPS seems to repress several core cell cycle genes[50] to control the formation of stomata, and the interacting transcription factors GLABRA1 (GL1) and GL3 initiate endoreduplication and thereby terminal differentiation of trichomes by the activation of cell cycle modulators[15]. GL1 and GL3 both have several hundred genomic binding sites but they share very few potential direct target genes, likely reflecting additional independent functions and interaction partners. The tight links between cell division and cell fate specification are also reflected in the target genes of the interacting GRAS transcription factors SHORTROOT and SCARECROW (SCR). These factors together control the formative cell divisions that generate the ground tissue in the root, and were found to activate specific core cell cycle genes[51].

Combinatorial interactions between transcription factors. In genome-wide target gene analyses, many regulatory links can be found among transcription factors that function in the same or related developmental pathways, which emphasizes the importance of transcriptional networks in developmental switches and cell fate specification. Heteromeric protein interactions have been identified for transcription factors that control meristem identity switches[52,53], meristem functions[54,55], patterning and differentiation[9,56–62]. The consequences of protein interactions for the regulation of target gene expression are just starting to be elucidated.

Transcription factor interactions potentially influence DNA-binding site selection *in vivo*. This has been suggested to play a part in target gene selection of SEP3 complexes, based on genome-wide binding data in wild-type plants and in a floral homeotic mutant[38]. Combinatorial interactions can also influence associations with co-factors that affect the transcriptional response. For instance, interactions of AP1 with its MADS-protein interaction partners SOC1, SVP and AGL24 have been proposed to be involved in gene repression through the recruitment of co-repressors in floral meristem initiation[53,63]. By contrast, the AP1–SEP3 heterodimer seems to preferentially activate genes in floral organogenesis[16].

Combinatorial interactions also have a role in trichome development. According to the current model, trichomes are specified by a transcription factor complex consisting of different types of regulators[58]: TRANSPARENT TESTA GLABRA 1, which is a WD40 domain protein; GL1, which is a MYB protein; and GL3 and/or ENHANCER OF GLABRA 3, which are basic helix-loop-helix (bHLH) proteins. This model also notes that this complex activates its own inhibitor, which can move to neighbouring cells and replace GL1 in the complex. This replacement renders the complex inactive in trichome specification and may thereby contribute to the regular spacing of trichomes in the leaf epidermis[58].

Although individual members of transcription factor complexes can potentially bind to target gene promoters on their own, all members of a complex are required to

Homeotic mutant
A mutant in which one organ type is replaced by a different organ type.

Box 2 | **Regulation of developmental switches and cell fate specification by plant microRNAs**

In plants, microRNAs (miRNAs) control major developmental phase transitions and cell fate specification in different tissues. One important feature is their ability to target several, often evolutionarily related, transcription factors simultaneously. Another emerging feature is their ability to move between cells, thereby controlling cell fate in a non-cell-autonomous manner.

For example, the juvenile-to-adult phase transition in *Arabidopsis thaliana* is regulated by the sequential action of miR156 — which targets ten SQUAMOSA PROMOTER-BINDING- LIKE (SPL) transcription factors — and miR172, which targets five APETALA2 (AP2) transcription factors. miR156 and miR172 show complementary temporal expression patterns both in *A. thaliana* and in maize[32,147,148]. During the juvenile phase, miR156 functions to repress the transition to the adult vegetative phase. By contrast, miR172 shows increased expression in the adult phase. Two targets of miR172, TARGET OF EAT 1 and TARGET OF EAT 2, repress floral transition and adult characteristics of leaf morphology. To trigger the transition from the juvenile to adult phase, two targets of miR156, SPL9 and SPL10, directly activate the expression of a *MIR172* locus, and also negatively regulate their own expression by direct upregulation of miR156. This negative regulation can be overcome by input from the photoperiodic flowering pathway, which results in a rapid increase in SPL expression. In addition to the activation of miR172, SPL transcription factors directly contribute to the transition to flowering by activation of floral pathway integrators and floral meristem identity genes[32,149].

miRNAs also participate in the cross-antagonistic regulation of transcription factors that specify different cell fates. For instance, radial patterning of the shoot and abaxial–adaxial patterning of the leaf are regulated by the miR165–166 group, which target members of the HD-ZIP family of transcription factors[150,151]. Data from root development show that miR165–166 expression is upregulated by the interacting GRAS transcription factors SHORTROOT (SHR) and SCARECROW (SCR) in the endodermis, it then moves to the central part of the stele where it forms a gradient to repress HD-ZIP family members in a dose-dependent manner[152]. Different levels of HD-ZIP activity seem to specify different cell fates in the vasculature. This movement of miR165–166 is opposite to that of SHR, which is produced in the central vascular tissue and moves to endodermal cells, where it is transported to nuclei by its interaction partner SCR. This bidirectional cell signalling is reminiscent of the morphogen gradients that establish and consolidate cell fate decisions in *Drosophila melanogaster* embryo development[153]. Another example of the involvement of a miRNA in plant development is the establishment of boundaries between meristem growth and organogenesis by miR164 and its targets[154,155].

Gradients of miRNAs also influence organ patterning in the flower. In addition to its role in floral transition in *A. thaliana*, miR172 accumulates in the centre of floral meristems, where it restricts the accumulation of AP2 and thereby specifies the boundary between perianth and reproductive organs[44,156]. In *Antirrhinum majus* and *Petunia hybrida*, the conserved miR169 is required for threshold-dependent activation of MADS-box genes that specify reproductive organ identities. This miRNA accumulates throughout the floral meristem, and targets and modulates the steady-state expression levels of NF-YA genes, which are activators of reproductive MADS-box gene expression[157].

More roles of miRNAs and other small RNAs in hormonal responses and morphogenesis have been described, and have been reviewed comprehensively elsewhere[158,159].

control the expression of a specific set of target genes. Small changes in the composition of these protein complexes may drastically affect binding to the promoters of target genes or the transcriptional response and thereby promote or repress a specific developmental programme. At another level, protein interactions can alter transcription factor localization and general activity[52,57,64]. Understanding the dynamics of transcription factor interactions and their transcriptional output is a major challenge for future studies.

Combinatorial regulation of gene expression may not require a strong direct interaction between transcription factors at target gene promoters, as proteins that bind individually or cooperate on DNA binding can influence gene expression in a combinatorial fashion. Identification of *cis*-regulatory modules that specify distinct spatiotemporal expression patterns — similar to those that have been identified in *D. melanogaster*[65] — is expected to contribute to our understanding of the combined action of different types of regulators of cell identity in plants.

Feedback and feedforward regulation. A remarkable feature of developmental regulatory networks in animals and plants is the presence of multiple, complex auto- and cross-regulatory loops among key transcriptional regulators[3]. Positive auto- and cross-regulatory loops coupled to protein dimerization or cooperative DNA binding can promote bistability[66] and, thereby, trigger threshold-dependent genetic switches. In addition, feedback loops mediated by heteromeric protein complexes can confer robustness to developmental programmes[67,68]. Cross-antagonism of competing transcription factors has been proposed to be a driving force of cell lineage specification in animals[3]. Also, in plants, negative cross-regulation between antagonistically acting transcription factors involved in identity specification seems to be a common theme. Examples include the genetic antagonism between transcription factors specifying the root and the shoot pole in the embryo[69]; between floral meristem and inflorescence meristem identity genes[35]; the mutual repression of genes maintaining meristem identity and genes triggering differentiation[70,71]; and the mutual repression of genes specifying abaxial and adaxial identities[72]. Remarkably, coordinated regulation by miRNAs and their target transcription factors participates in cross-antagonistic loops during developmental transitions and cell type specification (BOX 2).

In positive autoregulatory loops, gene products activate the expression of their corresponding genes to ensure maintenance of gene expression. A classical

Stele
The central part of the root, mainly consisting of the vasculature and the surrounding pericycle.

example is the autoregulation by obligate heterodimers of class B homeotic proteins that specify petal and stamen identities[73]. Also, other transcription factors specifying meristem and organ identities can control their own transcription[13,38]. Cross-regulatory interactions between proteins can be accompanied by direct protein interactions, particularly among members of the MADS-box transcription factor family. The requirement for two or more proteins to interact with each other to establish a positive feedback loop ensures that a specific developmental programme is only activated in the presence of all involved factors.

A common feature of key transcriptional regulators that function in the same developmental switch is that they can upregulate each other's expression, for example, SOC1 and AGL24 (which interact at the protein level)[52,74,75] or AP1 and LFY[16,35]. Reinforcing positive feedforward loops are found during meristem identity specification as well as linking meristem and organ identity genes. When one or more floral meristem or inflorescence meristem identity genes become mutated, the balance in the regulatory network becomes disturbed, and the meristem can switch from floral meristem or inflorescence meristem identity back to a more vegetative state during development[76,77], often depending on the environmental conditions. This process is called floral reversion.

Another type of regulation found among transcription factors controlling developmental switches are negative feedback loops of consecutively acting proteins. These loops ensure the suppression of the preceding programme once the activator of the next programme is induced. This ensures that a switch in developmental programmes is established and reversion to the previous stage is blocked. Examples include negative feedback loops during establishment of the floral meristem[16,30]. Negative feedback loops can also enhance robustness to fluctuations in protein levels, for instance in stem cell homeostasis[78].

Interplay with chromatin-associated proteins

The transcriptional activity of eukaryotic genes is determined by the combined action of transcription factors and chromatin-modifying proteins. Accordingly, histone-modifying enzymes and ATP-dependent nucleosome-remodelling complexes as well as transcription factors have been shown to coordinate differentiation programmes during plant and animal development. Several major classes of chromatin-organizing proteins are conserved between plants and animals (see Further information for a link to the Chromatin Database,). Linked to the evolution of complex body plans and developmental programmes in plants and animals, chromatin-associated protein families have expanded and diversified independently in these two groups of higher eukaryotes[79–81]. Global changes in chromatin structure have been associated with the initiation of differentiation or reprogramming, and local chromatin changes have been associated with cell fate specification in developing organs[82]. However, the regulatory interplay between transcription factors and chromatin regulators in the control of developmental switches is only starting to be unravelled. Below, we give examples of regulatory and molecular links between transcription factors and chromatin regulators in the control of developmental switches and differentiation in plants. These examples show that the dynamic activities of chromatin organizers and transcription factors are interconnected and also shed light on the molecular action of target gene control by transcription factors.

Regulatory interplay of chromatin organizers and transcription factors. Chromatin-associated proteins are involved in the dynamic spatiotemporal organization of gene expression, as well as in the maintenance of expression states across cell divisions ('cellular memory'). The role of chromatin organizers in coordinating developmental switches and cellular specification can often be related to their role in the repression or activation of transcription factors that control cell identity.

Nucleosome remodelling and assembly complexes have broad roles during plant development (Supplementary information S2 (table)), as nucleosomes modulate the accessibility of DNA to transcription factors and the basic transcriptional machinery. During DNA replication, proper nucleosome deposition is essential for maintaining the correct expression of patterning genes. Mutants of core components of the nucleosome assembly factor CAF1 (*fas1* and *fas2*) show deregulation of the stem cell factors WUS and SCR in the shoot and root meristems, respectively, and CAF1 is also involved in other aspects of organ development and differentiation[83–85]. Nucleosome sliding by the SWI/SNF-type remodellers BRAHMA and SPLAYED has roles in meristem maintenance, major developmental phase transitions and the regulation of floral organ identity genes[86–91]. Deposition of the histone variant H2A.Z by an *A. thaliana* SWR1 homologue mediates the developmental response to changes in temperature, for instance by triggering floral induction through regulation of the floral inducer *FLOWERING LOCUS T* (*FT*)[92].

At the local level of a single cell, the end of mitosis and beginning of G1 of the next cell cycle are important times for cell fate decisions, as this is when cell fate regulators are induced or remain switched off[7]. Accordingly, study of the *GL2* locus revealed local changes in chromatin organization from a closed to an open state associated with *GL2* activation, which triggers the differentiation of non-hair cells in the root epidermis[93]. Correct nucleosome deposition during mitosis by CAF1 (REF. 93) and changes in the histone modification state of the *GL2* promoter region[94] are associated with coordinated activation of *GL2* in non-hair root cells.

Histone-modifying enzymes are involved in providing the cellular memory of gene expression states, and are therefore important in cell fate changes. Histone acetylation is a dynamic and reversible modification, and high levels of acetylation are correlated with activation of gene expression through changes in DNA accessibility. Acetylated histones are also recognized by certain types of nucleosome remodellers. The broad roles of histone acetylation in cell fate control are reflected in, for example, mutants of the histone acetyltransferase GCN5, which

Floral reversion
The reversion of a meristem from a reproductive state back to a vegetative state, caused by mutations in regulatory genes. A floral meristem can revert to an inflorescence meristem or an inflorescence meristem can revert to a vegetative meristem. Reversion leads to the formation of shoots instead of flowers and 'aerial' rosettes instead of shoots.

forms SAGA-like complexes in *A. thaliana* (for a review, see REF. 95). Among other functions, GCN5 is required for PLETHORA-mediated root stem cell regulation[96], for restricting the expression domains of *WUS* and of floral organ identity genes (possibly indirectly), as well as for cell differentiation in the leaf and root epidermis[95,97].

Trimethylation of histone H3 lysine 27 (H3K27me3) labels transcriptionally repressed genes and thereby creates cellular memory. The Polycomb-group (PcG) protein complex PRC2 induces H3K27me3 (REF. 98), a mark that is recognized by PRC1 in animals, which then confers mitotically stable gene repression. A functional equivalent to PRC1 in plants seems to be a complex containing LIKE HETEROCHROMATIN PROTEIN 1 (LHP1; for a review, see REF. 80). Also, EMBRYONIC FLOWER 1 is a candidate for PRC1-like function in plants[99]. PcG complexes are required for the repression of meristem factors in differentiating organs[100] and for the repression of genes that trigger switches in meristem and organ identity (Supplementary information S2 (table))[80]. Their role seems to be similar to that in animals[101], in which PcG proteins contribute to maintenance of pluripotency, but can also promote cellular differentiation. Plant PcG protein complexes prevent precocious expression of reproductive identity genes during the vegetative phase and in the switch to reproductive phase, and they also inhibit the expression of floral repressors on receipt of environmental stimuli. Genetic and biochemical studies support the existence of several PRC2-like complexes that have partially overlapping biological functions in plants[102]. PcG repression might also create a threshold for transcriptional activation that ensures that genes are not switched on under low inductive conditions; this suggests a more dynamic role in the regulation of at least some of their targets[103].

In animals, the repressive action of PcG complexes is counteracted by trithorax group (TrxG) proteins[104]. These proteins induce trimethylation of histone H3 lysine 4 (H3K4me3)[105], a mark that is mostly found around the start site of transcriptionally active genes. In animal stem cells, genes can be poised for activation by the co-occurrence of H3K27me3 and H3K4me3, but this 'bivalent' marking remains to be investigated further in plants[106]. The TrxG homologue ATX1 (REFS 107,108), which has histone methyltransferase activity[109], and related proteins[108] have been associated with TrxG-like functions in *A. thaliana*. Two other factors have been suggested to act as TrxG proteins: the SAND-domain transcriptional regulator ULTRAPETALA1, which interacts with ATX1 (REF. 110) and the (CHD)-type nucleosome remodellers PICKLE (PKL) and PICKLE-RELATED 2 (REF. 101) (BOX 3). Loss of PKL results in reduced expression of many PcG target genes[101] and PKL has multiple roles in development, for example, as a repressor of embryonic traits and probably in the activation of floral organ identity genes. In line with a TrxG-like function of PKL, floral organ identity genes seem to be directly antagonistically regulated by PKL and PcG proteins. At a mechanistic level, in the absence of PcG proteins, PKL might shift nucleosomes in target gene core promoters and thereby allow transcriptional activators to access their binding sites.

(CHD)-type nucleosome remodeller
An ATP-dependent chromatin-remodelling factor of the chromodomain/helicase/DNA-binding domain (CHD) subfamily. These remodellers usually function as part of multisubunit complexes. In mammals and flies, they are involved in transcriptional repression by nucleosome remodelling and histone deacetylation. They have also been shown to be involved in transcriptional activation.

Vernalization
The induction of the transition from vegetative to reproductive plant growth by a prolonged period of cold (winter).

Chromatin-modifying enzymes and core members of nucleosome-remodelling complexes often have broad expression patterns during plant development. However, changes in the expression of specific subunits of chromatin-organizer complexes can drastically change their activity. For instance, the vernalization-induced addition of a single subunit is required for efficient repression of the floral repressor *FLOWERING LOCUS C* (*FLC*)[111]. Genes encoding certain members of PcG protein complexes are themselves under epigenetic control, which suggests that cross-regulatory mechanisms can control chromatin-organizer activity[101,112].

Protein interactions between transcription factors and chromatin organizers. Most chromatin organizers have broad functions in growth and differentiation of plants as revealed by pleiotropic mutant phenotypes, whereas transcription factors orchestrate specific switches in development. However, if chromatin regulators control partially different sets of target genes during distinct developmental phases, how are they recruited to the correct genomic loci? Currently, the most widely accepted view is that they are recruited by tissue-specific transcription factors (for reviews, see REFS 6,113) and/or RNA molecules (for a review, see REF. 114).

All except three loci in the transcription factor network shown in FIG. 3 have been found to be epigenetically regulated[115]. Therefore, the involvement of chromatin organizers in the expression of transcription factors seems likely to be important to establish the negative and positive regulatory interactions among the transcriptional regulators that control developmental switches in plants. Also, several protein interactions between plant transcription factors and histone-modifying enzymes have been described. For example, MADS-box transcription factors that control the transition to flowering interact with chromatin regulators[116,117]: SVP can interact with the PcG-like protein LHP1; and SOC1, AGL24 and AGL15 can interact with a member of the Sin3–histone deacetylase complex[116,117]. The repressive activities of complexes consisting of transcription factors and chromatin modifiers were shown to prevent the premature activation of the floral organ identity factor SEP3 in early floral meristems, thus inhibiting premature differentiation of cells in the floral meristem[116]. These interactions could also play a part in the repression of target genes during floral meristem initiation by AP1 (REFS 53,63), as AP1 has been shown to interact with SVP, SOC1 and AGL24 (REFS 53,63,118) (BOX 3). In addition, AP1 gradually downregulates the expression of SVP, SOC1 and AGL24, thereby eventually releasing the inhibitory effects of these proteins on *SEP3* and possibly other target genes[16].

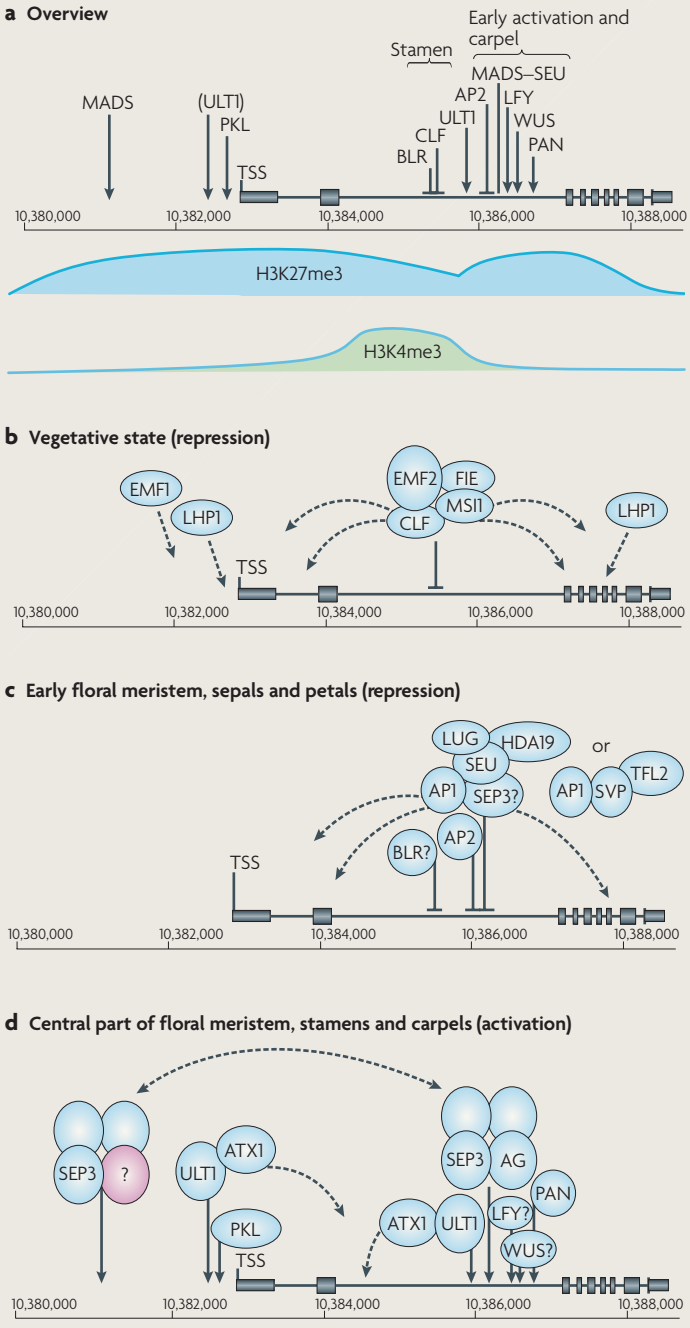
A second mechanism by which MADS-domain protein complexes can repress the expression of target genes by chromatin modification is through their interaction with a general co-repressor complex consisting of SEUSS (SEU) and LEUNIG (LUG)[53,119]. SEU shares homology with transcriptional co-regulators in animals[120] and LUG is similar to Groucho-like co-repressors in animals and yeast[121,122]. These co-factors do not

possess a DNA-binding domain and are recruited to their regulatory targets by interacting with DNA-bound transcription factors. LUG directly interacts with components of the Mediator complex and with the class 1 histone deacetylase HDA19, and the repressor activity of LUG depends on histone deacetylase activity[123]. SEU–LUG transcriptional co-repressor complexes could thus mediate target gene repression through histone modifications, which seems to be a general mechanism of eukaryotic co-repressor function[124]. The best-studied target of a MADS–co-repressor complex is the *AG* locus[119] (BOX 3).

Groucho-type co-repressors have various roles in cell fate specification and developmental patterning. For example, LUG and its close homologue LEUNIG HOMOLOG also interact with YABBY transcription factors that specify the abaxial cell identities and morphology of developing organs[125,126]. Another Groucho-type co-repressor, TOPLESS (TPL), seems to form a complex with the Aux/IAA factor IAA12 (also known as BODENLOS) and is essential for specification of the shoot pole by repression of root identity genes in embryos[127]. Similar to LUG, TPL represses transcription through HDA19 activity[128]. Thus, general co-repressors

Box 3 | **Interplay between transcription factors and chromatin-regulatory proteins in the regulation of *AGAMOUS* expression**

a | Most regulatory elements that control *AGAMOUS* (*AG*) expression are located in the second intron of the gene[160] (introns as a single line; exons as bars; TSS, transcriptional start site). Early studies identified two enhancers (brackets): one that acts in stamens and another that acts in early activation and in carpels[161]. *AG* is repressed in vegetative tissues, in early floral meristems and the outer whorl perianth organs. *AG* is activated at later stages of floral meristem development in the central part that gives rise to the stamens and carpels. **b** | In vegetative tissues, *AG* is stably repressed by the action of a PRC2-like Polycomb group (PcG) complex that contains CURLY LEAF (CLF), FERTILISATION-INDEPENDENT ENDOSPERM (FIE), EMBRYONIC FLOWER 2 (EMF2), MULTICOPY SUPPRESSOR OF IRA1 (MSI1)[100] and possibly other, yet-to-be identified components. The PRC2 complex catalyses trimethylation of histone H3 at lysine 27 (H3K27me3). The *AG* locus is also targeted by the putative PRC1 analogues LIKE HETEROCHROMATIN PROTEIN 1 (LHP1)[162,163] and EMF1 (REF. 99) (for a review, see REF. 80). **c** | In early floral meristems, and in petals and sepals, *AG* is repressed by the combined action of several transcription factors. The MADS-domain proteins AP1 and SEP3 can interact with the co-regulator SEUSS (SEU), which forms a complex with LEUNIG (LUG)[119] to recruit histone deacetylase 19 (HDA19)[123]. A MADS-domain protein complex containing AP1 and SHORT VEGETATIVE PHASE (SVP) has been proposed to prevent precocious *AG* activation in the early stages of meristem development through the recruitment of LHP1 (REF. 63). The transcription factor APETALA 2 (AP2) is also required for *AG* repression in young floral meristems as well as in petals and sepals[40,43]. Also, the BELL1-like homeobox protein BELLRINGER (BLR) is required for *AG* repression in inflorescence and early floral meristems[164]. BLR binds to sequence elements overlapping with the region most strongly bound by CLF *in vitro*; however, *in vivo* binding has not been shown so far. Other factors have been described as *AG* repressors; however, it is not known whether this regulation is direct. **d** | At the stage when organ identities are being determined, *AG* expression is activated in parts of the floral meristem that develop into stamens and carpels. SEP3 activates *AG* expression and may also interact with the AG protein, thus conferring a positive autoregulatory control of *AG* expression[13,38]. In wild-type inflorescences, SEP3 has two binding sites in the *AG* locus[38]: one binding site is located upstream of the TSS and another is present in the second intron at about 4 kb distance from the TSS. This creates the possibility that a loop between these two sites might be formed by higher-order complexes containing SEP3 and AG (dashed arrow in panel **d**), ultimately leading to the recruitment of activating factors close to the TSS. In floral stem cells, potential TrxG proteins like ULTRAPETALA 1 (ULT1), and possibly also PICKLE (PKL), contribute to the early activation of *AG*[101,110]. The bZIP transcription factor PERIANTHIA (PAN) contributes to *AG* activation in specific regions of the floral meristem[165,166]. Also, the floral meristem identity factor LEAFY (LFY) and the stem cell regulator WUSCHEL (WUS) have been proposed the contribute to *AG* activation[167]. However, binding of these transcription factors to the *AG* locus has not been shown *in vivo* so far.



Sequential ChIP
The identification of DNA-binding sites that are common to two proteins (for example, two types of modified histones or transcription factors). It involves two rounds of immunoprecipitation using separate antibodies against the proteins of interest.

seem to be major components of the antagonistic relationships between transcription factors that specify cell fates during plant development. Although some interactions of transcription factors and chromatin modifiers in target gene repression have been described, the relationship between these two types of regulators in gene activation during plant development is much less well understood. One example that suggests combined action of transcription factors and chromatin regulators is the activation of *KNUCKLES* by AG, which apparently requires rounds of cell division after initial AG binding to the locus[129]. A related mechanism requiring rounds of cell divisions might partly account for the observed delay of gene activation during early floral meristem development. Understanding the molecular dynamics of epigenetic changes and the role of transcription factors in epigenetic programming and reprogramming in plants remains a major challenge for the future.

Conclusions and perspectives

Recent technological advances have enabled us to study the action of transcription factors in the direct regulation of gene expression. This has led to improved understanding of developmental processes in plants and animals. Transcription factor networks are highly organized: complex regulatory loops between transcription factors and the combinatorial actions of these factors control the expression of target genes. Regulatory loops in transcription factor networks ensure sharp transitions in developmental programmes, and feedforward and autoregulatory loops also allow stable maintenance of developmental programmes.

Developmental switches require changes in chromatin organization. Recent results indicate that transcription factors and chromatin regulators act in union in the control of gene expression during development. However, many questions in this field remain to be answered. For example, how generally important are direct protein interactions between transcription factors and chromatin regulators? Although at least some plant transcription factors interact with histone-modifying proteins to repress gene expression, the role of such interactions in gene activation remains to be elucidated. Dissection of the transcriptional responses linked to *cis*-regulatory elements and chromatin status at specific genomic loci, as done for the *FT* locus recently[130], is one step towards unravelling the molecular mechanisms of target gene regulation by transcription factors and chromatin regulators. Transcription factors that regulate developmental transitions directly control the expression of many genes, only a fraction of which are also targets of the PcG–TrxG system. Thus, it is possible that there are multiple mechanisms by which these transcription factors can control the expression of individual genes.

The first global comprehensive studies of *in vivo* DNA-binding sites and target genes of key developmental transcription factors have provided a wealth of information about the topology of transcriptional networks. However, how combinatorial interactions between proteins affect DNA binding and transcriptional regulation needs to be studied further. To decipher this regulatory code, more systematic studies of transcription factor-binding sites and transcriptional responses are needed. Technically challenging *in vivo* approaches such as sequential ChIP[131] and/or the use of mutants in which one of the interacting factors is eliminated can shed light on the role of combinatorial transcription factor interactions. The protein complex assembly on target gene promoters and the transcriptional output of these genes are expected to be highly dynamic and cell-type specific, and approaches to analyse gene regulation in a cell-type specific manner have just started to be used[51,132,133]. To understand the nature of combinatorial interactions, one challenge is to identify the interaction partners of endogenously expressed transcription factors *in planta*. Both biochemical and sophisticated imaging-based technologies offer the potential to study the developmental dynamics of protein interactions.

The important role for the higher-order organization of chromatin in the control of eukaryotic gene expression has also emerged in recent years. Biochemical techniques to study chromatin loops such as chromosome conformation capture-based and ChIP-based technologies are just starting to be applied in plants[134]. In the future, these methods will help us to understand the dynamics of chromatin organization during developmental switches, as well as the role of key regulatory transcription factors in this process.

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