Development 137, 3153-3165 (2010) doi:10.1242/dev.030049

KNOX genes: versatile regulators of plant development and diversity

Angela Hay* and Miltos Tsiantis*

Summary

Knotted1-like homeobox (KNOX) proteins are homeodomain transcription factors that maintain an important pluripotent cell population called the shoot apical meristem, which generates the entire above-ground body of vascular plants. KNOX proteins regulate target genes that control hormone homeostasis in the meristem and interact with another subclass of homeodomain proteins called the BELL family. Studies in novel genetic systems, both at the base of the land plant phylogeny and in flowering plants, have uncovered novel roles for KNOX proteins in sculpting plant form and its diversity. Here, we discuss how KNOX proteins influence plant growth and development in a versatile context-dependent manner.

Key words: Evolution, KNOX, Plant development

Introduction

The maize *knotted1* (*kn1*) gene was isolated two decades ago through transposon tagging in a mutant with striking 'knotted' leaves, and revealed that the predicted gene product encoded a member of the homeodomain superfamily of transcriptional regulators (Fig. 1) (Vollbrecht et al., 1991). This discovery generated considerable excitement among developmental biologists, not least because the isolation of animal homeobox genes a few years earlier had revolutionized our understanding of the molecular basis of metazoan development and evolution. The cloning of the first member of a KNOX gene family in maize led to a similar explosion of new research in plant development (Hake et al., 1995). Today, a substantial body of information exists on the function of KNOX proteins in both model and non-model plants. These studies have revealed parallels with the mechanistic action of animal TALE (see Glossary, Box 1) homeodomain proteins, and have helped us to understand how these proteins influence plant development and to unravel key aspects of the logic that underpins cell fate allocation and tissue differentiation in the 'green branch' of the tree of life.

The discovery of KNOX genes provided the first molecular insights into the function of the shoot apical meristem (SAM; see Glossary, Box 1). The nuclear expression of KN1 protein in the maize shoot is confined to meristem cells and is excluded from leaf founder cells, thus providing the earliest marker for meristem versus lateral organ (see Glossary, Box 1) cell fate (Fig. 1C) (Smith et al., 1992). The SAM is established at the shoot pole during embryogenesis and harbours a stem cell population that allows continued organogenesis throughout the life of a plant (Fig. 1A). This continuous development contrasts with how animals develop and allows for plasticity in plant form, such that plants (which are sessile organisms) can readily modify their development in response to environmental cues. KN1 activity is required to prevent pluripotent cells in the maize SAM from adopting differentiated cell fates, as does the related protein SHOOT MERISTEMLESS (STM) in *Arabidopsis*, such that *stm* and *kn1* loss-of-function mutants fail to establish and maintain a SAM (Fig. 1D; Fig. 3A,B) (Long et al., 1996; Vollbrecht et al., 2000).

KNOX genes comprise a small family of TALE homeobox genes that are found in all green plant lineages (Table 1) and fall into two subclasses on the basis of sequence similarity within the homeodomain, intron position, expression pattern and phylogenetic analysis (Kerstetter et al., 1994; Mukherjee et al., 2009). Class I KNOX genes are most similar to *kn1* and are expressed in overlapping domains within the SAMs of both monocot and eudicot plants (see Glossary, Box 1) (Hake et al., 2004; Jackson et al., 1994). Class II KNOX genes form a monophyletic group that is distinct from Class I genes, and show diverse expression patterns and few known functions (Zhong et al., 2008). We therefore focus this review on Class I KNOX genes.

Although the first plant homeobox gene was cloned over 20 years ago, only recently are we beginning to understand how KNOX genes function in diverse developmental contexts, and how these functions relate to developmental transitions during land plant evolution. In this review, we discuss the functions of Class I KNOX proteins during development and the advances in understanding of KNOX gene regulatory networks, including upstream regulators, protein partners and downstream effectors of KNOX function. We highlight the discovery of a novel class of KNOX genes that lack a homeobox and insights into KN1 protein trafficking in plants (Box 2). We also discuss recent work in the unicellular green alga *Chlamydomonas reinhardtii* that suggests ancestral KNOX genes controlled diploid development and diversified in land plants in order to control multicellular body plans (Lee et al., 2008). Finally, we consider how evolutionary changes in KNOX gene regulation may have influenced plant diversity.

Class I KNOX genes in meristem and compound leaf development

The *Arabidopsis* genome contains four Class I KNOX genes: *STM, BREVIPEDICELLUS* (*BP*), *Kn1-like in Arabidopsis thaliana2* (*KNAT2*) and *KNAT6* (Table 2). These genes participate in various developmental processes during the plant life cycle; however, their expression in specific domains of the SAM maintains the activity of the SAM and of its lateral organ and stem boundaries throughout development (Fig. 2). *STM* is the first KNOX gene expressed during early embryogenesis and its expression marks the entire SAM (Long et al., 1996). *KNAT6* is expressed in the embryonic SAM once bilateral symmetry is established and later marks the SAM boundaries (Belles-Boix et al., 2006). *BP* shares aspects of both these gene expression patterns in the SAM during post-embryonic development (its embryonic expression marks the

Plant Sciences Department, University of Oxford, South Parks Road, Oxford OX1 3RB, UK.

*Authors for correspondence (angela.hay@plants.ox.ac.uk; miltos.tsiantis@plants.ox.ac.uk)

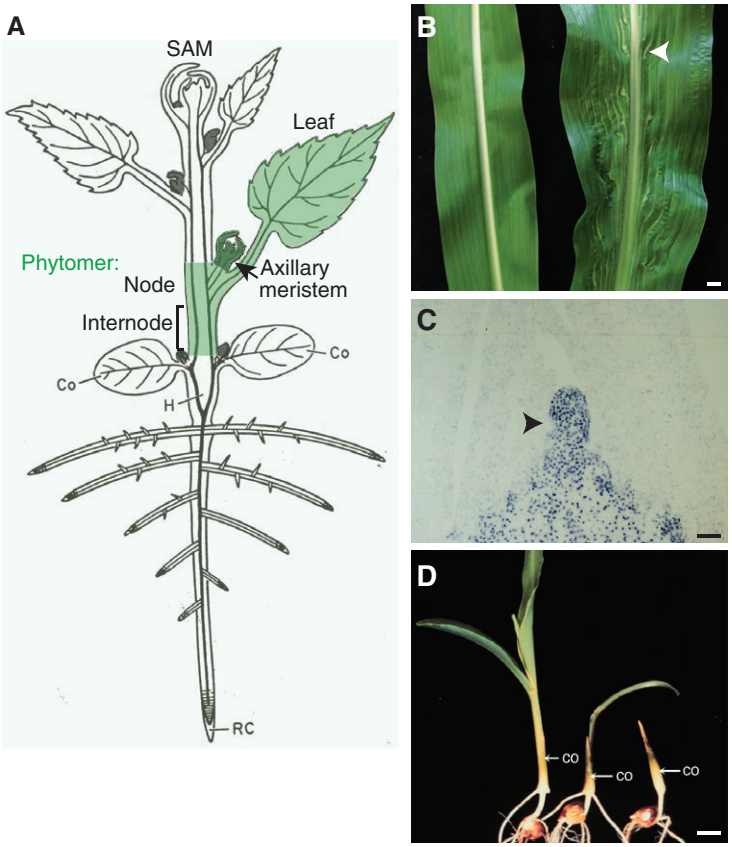


Fig. 1. *knotted1*: the first homeobox gene identified in plants. (**A**) Diagram of a typical vascular plant, showing the shoot apical meristem (SAM) located at the shoot apex and a phytomer (green) that includes a leaf and axillary meristem at a single node, and an associated internode (bracketed). Abbreviations: Co, cotyledons; H, hypocotyl; RC, root cap. (**B**) A wild-type maize leaf blade (left) and a *Kn1-N* leaf blade (right). Dominant *Kn1-N* mutants express *kn1* inappropriately in leaves and alter leaf development such that proximal cell fates, such as ligule, differentiate in distal positions in the blade (arrowhead), and cells overgrow to form knots. (**C**) KN1 protein has a nuclear localization in a maize SAM and is absent from leaves and leaf founder cells (arrowhead). (**D**) A wild-type maize seedling (left), compared with two shootless *kn1-e1* loss-of-function mutants (right). Scale bars: 1 cm in B,D; 50 μm in C. (A) Modified, with permission, from from Tsiantis and Hay (Tsiantis and Hay, 2003).

hypocotyl), whereas *KNAT2* is expressed during embryogenesis and marks the base of the SAM (Byrne et al., 2002; Dockx et al., 1995). Genetic redundancy masks the contribution of *KNAT6* and *BP*, which act, in addition to *STM*, to maintain SAM activity and organ separation (Belles-Boix et al., 2006; Byrne et al., 2002).

The architecture of *bp* mutants differs markedly from wild-type plants owing to their reduced height, irregularly shortened internodes and reduced apical dominance (see Glossary, Box 1; Fig. 3C,D) (Byrne et al., 2003; Douglas et al., 2002; Smith and Hake, 2003; Venglat et al., 2002). Plant architecture consists of repeating modules produced at the SAM called phytomers, each containing an internode, leaf and axillary meristem (Fig. 1A). In *Arabidopsis* inflorescences, growth of the leaf is suppressed such that only the floral meristem develops. *BP* expression at the boundary of the inflorescence SAM might, therefore, regulate cell allocation between floral primordia and internodes, explaining why the flowers are positioned aberrantly along *bp* mutant stems. Defective internode patterning is also observed in rice plants in which the related gene *Oryza sativa homeobox15* (*OSH15*) is mutated (Sato et al., 1999). However, the flower stalks, or pedicels, are specifically shortened and curved downwards in *bp* mutants, owing to defects in cell division, cell elongation and cell differentiation (Douglas et al., 2002; Venglat et al., 2002), and it remains unclear which aspects of BP function are shared between this tissue and the SAM. *KNAT6* and *KNAT2* are part of a segmental chromosomal duplication in *Arabidopsis*, and single mutations in these genes do not affect shoot development (Belles-Boix et al., 2006; Byrne et al., 2002). However, *KNAT6* and *KNAT2* gene expression is expanded in *bp* mutants, and mutations in each of these genes show antagonistic genetic interactions with *bp* mutants, suggesting that the role of BP in inflorescence development is mediated at least in part by repression of *KNAT6* and *KNAT2* (Fig. 2) (Ragni et al., 2008).

In model organisms with simple leaves (see Glossary, Box 1), such as *Arabidopsis*, maize and tobacco, KNOX gene expression is confined to the shoot meristem and stem, and leaf development is dramatically altered by the ectopic expression of KNOX genes in leaves (Lincoln et al., 1994; Sinha et al., 1993; Vollbrecht et al., 1991). For example, transgenic expression of any Class I KNOX gene ectopically in the leaves of *Arabidopsis* can dramatically alter the simple leaf margin to produce a highly lobed shape (Fig. 3E,F) (Shani et al., 2009). These studies

Table 1. Class I KNOX genes grouped according to similarity in function, expression and sequence

Arabidopsis	Maize	Rice	Tomato
SHOOTMERISTEMLESS (*STM*)	*knotted1* (*kn1*)	*Oryza sativa homeobox1* (*OSH1*)	*Tomato Kn2* (*TKn2*)/*LeT6*
BREVIPEDICELLUS (*BP*)	*rough sheath1* (*rs1*)	*OSH15*	*TKn1*
	gnarley1 (*gn1*)		
kn1-like in Arabidopsis thaliana2 (*KNAT2*)	*liguless3* (*lg3*)	*OSH6*	
KNAT6	*liguless4a* (*lg4a*)	*OSH71*	
	liguless4b (*lg4b*)		

The relationship between gene function and orthology is unclear and not indicated here. Tomato contains two additional Class I KNOX genes, *TKn3* and *TKn4*, for which data are not yet available.

Box 1. Glossary

Adaxial
The side of a lateral organ that initiates next to the meristem; the abaxial side initiates away from the meristem.

Apical dominance
Where growth of the apical meristem suppresses the growth of axillary meristems and hence suppresses branching.

Apical meristem
Stem cell-containing structures that reside at the growing apex of the root or shoot of a plant where organogenesis occurs.

Awn
A slender bristle-like structure found on the spikelets (structures that contain the flowers) of many grasses.

Auxin
A class of plant hormones, typified by indole-3-acetic acid.

Compound leaf
A leaf shape where the lamina is divided into individual leaflets borne on a supporting rachis.

Convergent evolution
The independent evolution of the same biological trait in different lineages; **parallelism** is used to describe instances where the convergent trait has the same genetic basis.

Cytokinins
Plant growth hormones with an adenine-type structure.

Eudicot (Eudicotyledonous plants)
The largest group of angiosperms; characterized by two cotyledons (seed leaves) and tricolpate pollen.

Gametophyte
The plant generation that has a haploid set of chromosomes and produces gametes, which fuse to produce a sporophyte.

Gibberellins
Tetracyclic diterpene acids that act as plant growth hormones.

Internode
A portion of a plant stem between adjacent nodes where leaves are attached.

Lamina
The usually flattened parts of a leaf on either side of the midvein.

Lateral organ
Organs produced from the shoot apical meristem, such as leaves and putatively homologous organs such as cotyledons, bracts and floral organs.

Leaf founder cells
A group of cells that encompass several layers of the shoot apical meristem and from which a leaf primordium is derived.

Ligule
An epidermal fringe of leaf tissue typically found in grasses. In maize, it marks the junction between proximal sheath tissue and distal blade tissue.

Monocot (monocotyledonous plants)
Angiosperms with one cotyledon (seed leaf).

Petal spur
An outgrowth of a petal in which nectar collects.

Simple leaf
A leaf shape where the lamina is undivided.

Sporophyte
The diploid form in plants that undergo an alternation of generations. It results from a union of haploid gametes and meiotically produces haploid spores that grow into the gametophyte generation.

TALE (three amino acid loop extension)
A conserved superclass of homeobox genes characterized by an extension of three amino acids between helices 1 and 2 of the homeodomain. Animal TALE proteins include the pre B cell homeobox1 and *Caenorhabditis elegans* homeobox20 (PBC) family (Exd in *Drosophila* and Pbx proteins in vertebrates) and the myeloid ecotropic viral integration site (MEIS) family (Hth in *Drosophila*, and Meis and Prep proteins in vertebrates).

suggested that ectopic KNOX gene expression within a simple leaf development programme produced differential growth at the leaf margin, owing to the creation of novel ‘meristem-leaf’ boundaries. The ectopic initiation of shoot meristems in transgenic tobacco and *Arabidopsis* leaves supported this idea (Chuck et al., 1996; Sinha et al., 1993). Strikingly, this correlation between the expression of KNOX genes in the leaf and a more complex leaf shape was shown to hold for tomato and many other plant species with naturally compound leaves (also known as dissected leaves; see Glossary, Box 1) (Bharathan et al., 2002; Hareven et al., 1996). It is now clear that KNOX gene expression is reactivated following leaf initiation in order to facilitate leaflet formation in both tomato and the *Arabidopsis* relative *Cardamine hirsuta* (Hay and Tsiantis, 2006; Shani et al., 2009). Gain of KNOX function in these species where KNOX genes are part of a compound leaf development programme can produce a striking reiteration of leaflets upon leaflets (see Fig. 3G,H) (Hareven et al., 1996; Hay and Tsiantis, 2006). Therefore, Class I KNOX genes have specific functions in compound leaf development that are distinct from their ability to induce shoot meristem formation.

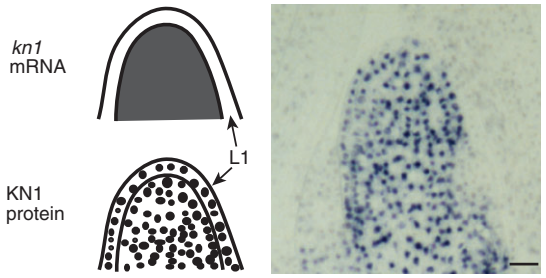
KNOX-BELL protein interactions

KNOX proteins interact with another group of TALE proteins, the BEL1-like homeodomain family (BELL or BLH, see Table 2), in a highly connected, complex network that determines not only high-affinity KNOX target selection but also their subcellular localization (Bellaoui et al., 2001; Bhatt et al., 2004; Cole et al., 2006; Hackbusch et al., 2005; Smith et al., 2002). These interactions depend on the KNOX MEINOX domain, and each homeodomain of the two proteins binds to target DNA as a protein heterodimer (Box 3). Such regulatory interactions are reminiscent of those between different TALE proteins in animals, indicating that they might have an ancient origin. In *Drosophila*, for example, nuclear translocation of the MEIS-class TALE protein Homothorax depends on its interaction with the PBC-class TALE protein Extradenticle (see Glossary, Box 1) (Rieckhof et al., 1997). MEIS proteins in animals and KNOX proteins in plants share a MEINOX domain, which mediates their TALE protein interactions (Burglin, 1997). However, plants do not have easily recognizable PBC proteins, and this role of selectively interacting with KNOX proteins to regulate their nuclear translocation and target affinity is fulfilled by BELL proteins (Bellaoui et al., 2001).

This protein interaction network also includes *Arabidopsis thaliana* OVATE family proteins (AtOFP), which negatively control the activity of TALE protein dimers by causing their relocalization from the nucleus, where they are functional, to the cytoplasmic space (Box 3) (Hackbusch et al., 2005). Genetic evidence for AtOFP regulation of KNOX-BELL activity in *Arabidopsis* embryo sac development came recently from an analysis of a gain-of-function BLH1 allele called *eostre* (Pagnussat et al., 2007). The *eostre* phenotype depends on the Class II KNOX gene *KNAT3* and is partially phenocopied by loss of *AtOFP5* function, suggesting that repression of KNAT3-BLH1 dimer activity by AtOFP5 is essential for embryo sac development.

TALE protein interactions are selective between specific members of the KNOX and BELL protein families, and these interactions are required for high-affinity DNA binding (Smith et al., 2002). Different combinations of KNOX/BELL transcription factors may, therefore, regulate different downstream genes. Genetic analyses in *Arabidopsis* indicate that the formation of different heterodimers of STM or BP with the BELL protein

Box 2. KNOX protein trafficking



Knots form in dominant *Kn1* mutant leaves in response to a signal that originates from internal leaf tissues and moves to outer epidermal cells, where it triggers aberrant proliferation (Hake and Freeling, 1986). The KN1 protein itself was a good candidate for this signal because KN1 protein could be detected in the outer L1 layer of the maize shoot apical meristem (SAM), where *kn1* mRNA was not expressed (see accompanying box figure) (Jackson et al., 1994). It is now clear that Knotted1-like homeobox (KNOX) proteins can signal between cells by directly trafficking through intercellular channels that are specific to plant cells called plasmodesmata (Lucas et al., 1995). KN1 is also able to transport its own mRNA, and trafficking of both protein and mRNA is mediated by a signal contained within the homeodomain (Kim et al., 2005). Movement protein binding protein 2C was recently identified as a protein that interacts with the KN1 homeodomain and regulates the cell-to-cell trafficking of KN1 by sequestering the protein on microtubules (Winter et al., 2007). Thus, the homeodomain of KN1 determines not only its DNA target specificity but also its cellular destination. (Scale bar: 20 μm.)

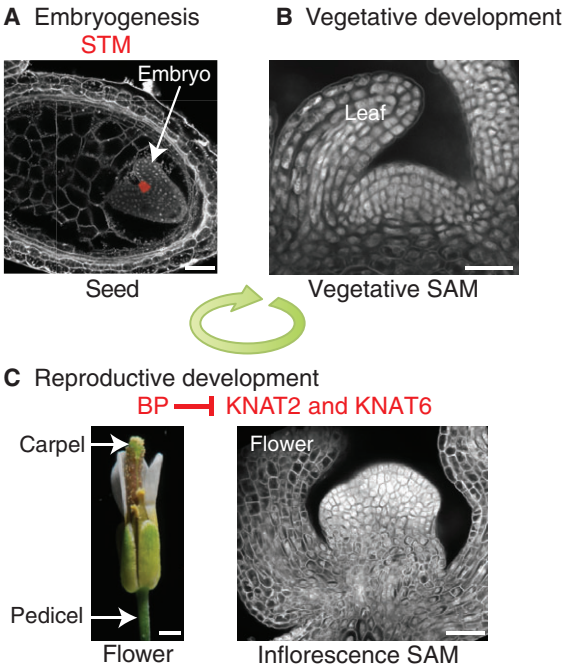


Fig. 2. Class I KNOX genes function throughout the *Arabidopsis* life cycle. (**A-C**) *SHOOTMERISTEMLESS* (*STM*), *BREVIPEDICELLUS* (*BP*) and *Kn1-like in Arabidopsis thaliana6* (*KNAT6*) function to maintain the activity of the shoot apical meristem (SAM) and its lateral organ and stem boundaries throughout embryogenesis, and vegetative and reproductive development. Knotted1-like homeobox (KNOX) proteins act as heterodimers with the BELL proteins BELLRINGER (BLR), ARABIDOPSIS THALIANA HOMEOBOX1 and POUND-FOOLISH throughout the *Arabidopsis* life cycle. *STM*, *BP, KNAT2, KNAT6* and *BLR* also function during development of the carpel and fruit. Stage-specific functions of individual KNOX genes are indicated in red. (A) *STM* has a role in establishing the embryonic SAM, and (C) BP represses *KNAT2,* and *KNAT6* gene expression during reproductive development to regulate pedicel and fruit development. Scale bars: 25 μm; 0.5 cm in flower image. Green arrow indicates progress through the life cycle from embryogenesis to vegetative and then reproductive development.

BELLRINGER (BLR) plays a role in maintaining the SAM and in patterning the inflorescence and fruit (Fig. 2) (Byrne et al., 2003; Roeder et al., 2003; Smith and Hake, 2003). Additional STM-BELL dimers are likely to promote SAM activity, as triple mutants of the BELL genes *BLR*, *POUND-FOOLISH*, *ARABIDOPSIS THALIANA HOMEOBOX1* are shootless (Rutjens et al., 2009). This phenocopying of *stm* mutants could indicate that these BELL proteins regulate the nuclear localization of STM in a redundant manner (Box 3).

KNOX gene regulation: an abundance of repressors

Establishing a boundary between KNOX-expressing and non-expressing cells in the SAM is crucial for initiating lateral organs, whereas KNOX gene expression outside the SAM disrupts lateral organ development, causing transformations of cell fate and organ shape (Hake et al., 2004). Establishing such a boundary in the SAM, therefore, requires the repression of KNOX gene expression in leaf founder cells, and maintaining this repression regulates leaf development in many plants, such as in maize and *Arabidopsis*. Negative regulators of KNOX genes have been identified, mostly through studying recessive mutants that phenocopy the effects of KNOX overexpression in the leaf (Fig. 4). These regulatory pathways all act to maintain the repression of KNOX genes during lateral organ development and suggest that as yet unidentified processes might control the repression of KNOX activity in founder cells.

The first upstream regulator of KNOX genes was identified from a recessive maize mutant *rough sheath2* (*rs2*), which has leaves that resemble those of dominant Knox mutants

Table 2. KNOX and BELL genes in green plant genome sequences

	Eudicots		Monocots		Lycophytes	Bryophytes	Green algae
Gene class	*Arabidopsis*	Poplar	Maize	Rice	*S. moellendorffii*	Moss	*C. reinhardtii*
KNOX I	4	9	9	8	3	3	0
KNOX II	4	6	4	4	2	2	1
BELL	13	19	17	14	2	4	1

Number of KNOX Class I, KNOX Class II, and BELL genes (not including pseudogenes) identified in the sequenced genomes of *Arabidopsis thaliana*, *Populus trichocarpa*, *Zea mays*, *Oryza sativa*, *Selaginella moellendorffii*, *Physcomitrella patens* and *Chlamydomonas reinhardtii*. Data sourced, with permission, from Meuherjee et al. (Mukherjee et al., 2009).

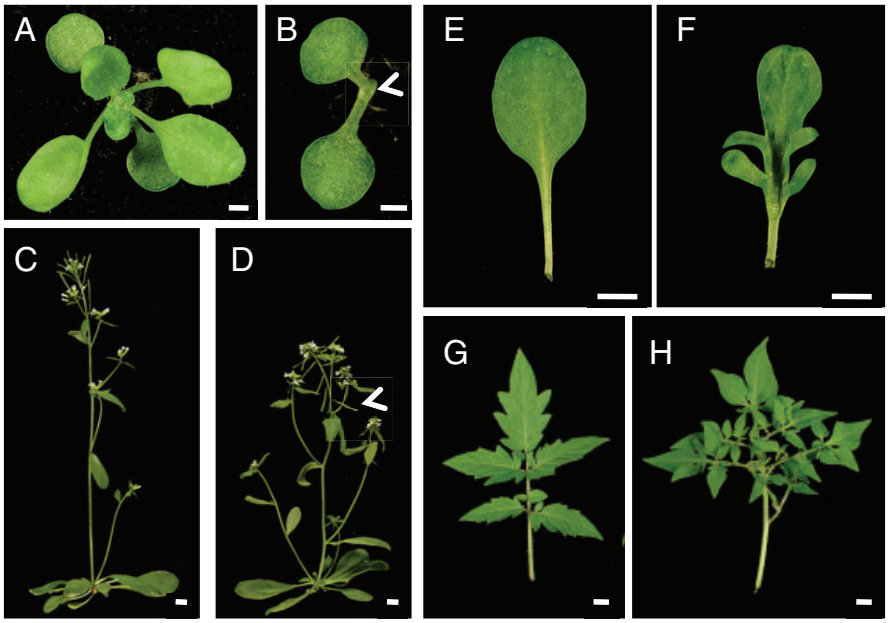
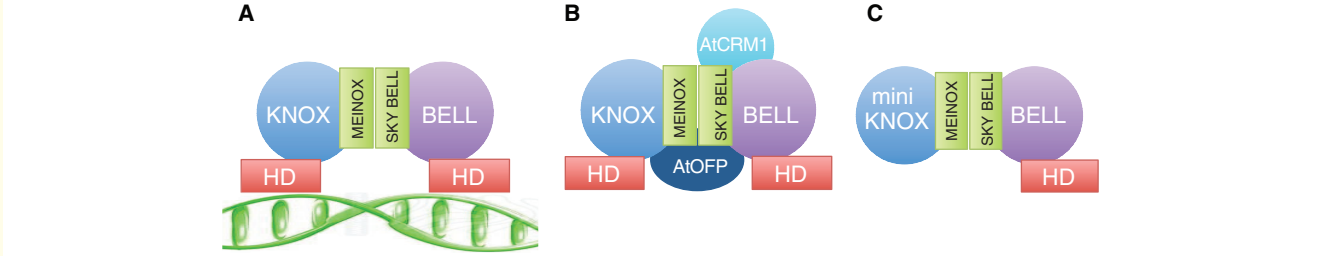


Fig. 3. Class I KNOX genes function in shoot development and compound leaf development. (**A**) A wild-type *Arabidopsis* seedling has cotyledons and leaves, which are initiated from the shoot apical meristem (SAM). (**B**) A *shootmeristemless* (*stm*) mutant seedling has cotyledons but fails to produce leaves because it lacks a SAM (arrowhead). (**C**) A wild-type *Arabidopsis* plant. (**D**) A *brevipedicellus* (*bp*) mutant plant is short, with reduced apical dominance and downward-pointing fruits. (**E**) A wild-type *Arabidopsis* leaf has an entire shape. (**F**) A *35S::BP Arabidopsis* leaf, where *BP* is broadly expressed, has a lobed shape. (**G**) A wild-type tomato leaf has leaflets arranged along a rachis. (**H**) A tomato leaf of a *Tkn2* gain-of-function allele *Mouse ears* has additional leaflets arranged on leaflets. Scale bars: 0.5 cm.

(Schneeberger et al., 1998). *rs2* encodes a myb-type protein that represses KNOX expression in the lateral organs of several plant species with both simple leaves (such as maize, *Arabidopsis* and snapdragon) and compound leaves (such as pea and the *Arabidopsis* relative *C. hirsuta*). Collectively, these are called ARP proteins [ASYMMETRIC LEAVES1 (AS1) in *Arabidopsis*, Rough sheath2 in maize, PHANTASTICA in snapdragon] (Byrne et al., 2000; Hay and Tsiantis, 2006; Tattersall et al., 2005; Timmermans et al., 1999; Tsiantis et al., 1999). In these plants, ARP and KNOX gene expression distinguishes leaf founder cell from meristem cell fate in the shoot apex. Although mutations in ARP genes cause ectopic KNOX expression in lateral organs, the boundary between KNOX-expressing and non-expressing cells in the SAM is maintained (Schneeberger et al., 1998). Expression of the KNOX genes *BP*, *KNAT2* and *KNAT6* is reactivated in leaf primordia of *Arabidopsis as1* mutants, producing asymmetric leaves with lobed margins and occasional shoot meristems (Byrne et al., 2000; Ori et al., 2000). Transgenic expression of each of these KNOX genes is sufficient to elicit similar alterations in *Arabidopsis* leaf patterning (Shani et al., 2009), and loss of all

Box 3. Novel KNOX proteins lack a homeobox



A new class of mini Knotted1-like homeobox (KNOX) proteins that lack a homeodomain were recently discovered in *Arabidopsis* and tomato, and are conserved in eudicots but not in monocots (Kimura et al., 2008; Magnani and Hake, 2008). These proteins, called KNATM in *Arabidopsis* and PETROSELINUM (PTS) in tomato, selectively interact with BELL proteins and affect their availability to form active DNA-binding complexes (see accompanying box figure). For example, mutations that result in gain of *PTS* function or loss of function of the *BELL* gene *BIPINNATA* (*BIP*) increase the complexity of the compound tomato leaf, resembling the effects of KNOX overexpression [remarkably, the *Pts* allele described here underlies natural variation in tomato leaf shape, originating in *Solanum galapagense*; a species collected by Charles Darwin in the Galapagos Islands (Kimura et al., 2008)]. Therefore, BIP-KNOX dimers antagonize KNOX function but can be outcompeted by BIP-PTS dimer formation. Similarly, in *Arabidopsis* the BIP homologs SAWTOOTH1 (SAW1) and SAW2 form dimers with KNOX and KNATM proteins, and both *saw1;2* mutants and *KNATM* overexpression increase the margin complexity of *Arabidopsis* leaves (Kumar et al., 2007; Magnani and Hake, 2008). Ectopic KNOX expression in *saw1;2* leaves suggests that these KNOX co-factors may also regulate KNOX gene expression to create a feedback loop that influences the phenotypic readout of KNOX activity. In the box figure, (**A**) KNOX (blue) and BELL (purple) proteins are shown to interact through MEINOX, SKY and BELL domains (green). The homeodomain (HD, red) of each protein binds to DNA. (**B**) *Arabidopsis* OVATE family proteins (AtOFP) interact with both BELL and KNOX proteins and repress heterodimer activity by relocating KNOX-BELL dimers to the cytoplasm. BELL protein localization also involves a nuclear exclusion mechanism [conserved with animal MEIS-PBC proteins (Rutjens et al., 2009)], in which nuclear export signals located in the BELL domain interact with the CRM1/Exportin-1 receptor AtCRM1 to result in BELL nuclear export. These interactions are disrupted by KNOX-BELL dimer formation, and hence KNOX-BELL dimers accumulate in the nucleus. (**C**) Mini-KNOX proteins interact with BELL proteins through a MEINOX domain.

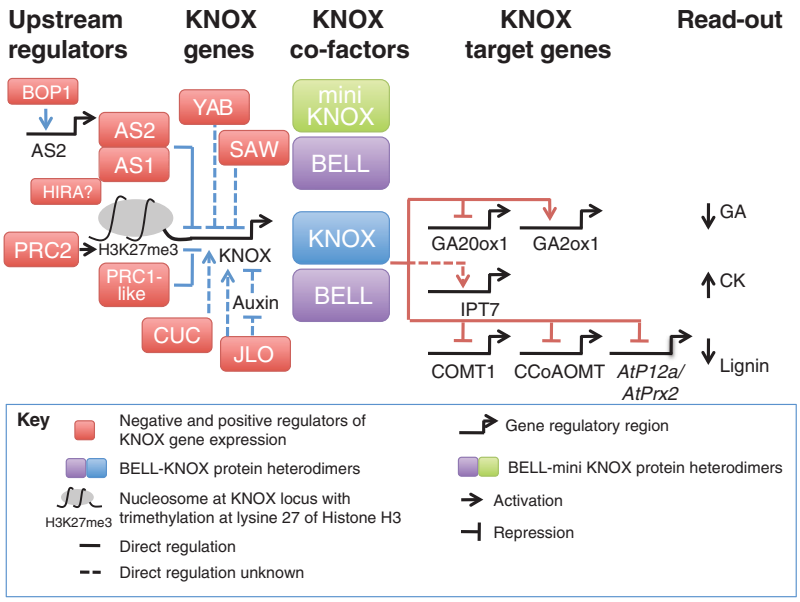


Fig. 4. KNOX gene regulatory network. Upstream regulators control the expression of Knotted1-like homeobox (KNOX) genes. KNOX proteins function as heterodimers with BELL protein co-factors to activate or repress target genes, thus producing a cellular read-out. The mechanistic basis for KNOX gene regulation is either direct, mediated through chromatin modifications, or is unknown (figure key). KNOX proteins directly bind to promoters of the biosynthetic gene GA 20-oxidase1 (*GA20ox1*) and the catabolic gene GA 2-oxidase1 (*GA2ox1*) to reduce gibberellin (GA) levels, and the lignin biosynthetic genes caffeic acid-O-methyltransferase1 (*COMT1*), caffeoyl-CoA *O*-methyltransferase (*CCoAOMT*) and Arabidopsis thaliana peroxidase12a (*AtP12a*) to reduce lignin levels. KNOX proteins activate the biosynthetic gene *ISOPENTENYL TRANSFEREASE7* (*IPT7*) to increase cytokinin (CK) levels. KNOX proteins probably regulate these target genes as KNOX-BELL heterodimers, and this activity is antagonized by the interaction of mini-KNOX proteins with BELL partners (see key). Abbreviations: AS1, ASYMMETRIC LEAVES1; BELL, BEL1-like homeodomain family; BOP1, BLADE-ON-PETIOLE1; CUC, CUP-SHAPED COTYLEDON; H3K27me3, trimethylation of histone H3 at lysine 27; HIRA, histone regulatory protein A; JLO, *JAGGED LATERAL ORGANS*; PRC2, polycomb repressive complex 2; SAW, SAWTOOTH; YAB, YABBY.

three KNOX genes rescues many of these defects in the *as1* mutant (Ikezaki et al., 2010). ARP proteins are, therefore, required to repress KNOX expression during leaf development.

The mechanistic basis for this regulation in *Arabidopsis* is beginning to shed light on how ARP proteins maintain a KNOX repressive state. AS1 forms a heterodimer with the LATERAL ORGAN BOUNDARIES (LOB) domain protein ASYMMETRIC LEAVES2 (AS2) in the adaxial (see Glossary, Box 1) domain of the *Arabidopsis* leaf, and genetic epistasis indicates that these genes function together (Ori et al., 2000; Xu et al., 2003). The BTB-POZ domain BLADE-ON-PETIOLE proteins (BOP1 and BOP2) are active specifically at the adaxial base of lateral organs, where BOP1 directly activates *AS2* transcription (Jun et al., 2010). This AS1-AS2 protein dimer directly binds the promoters of *BP* and *KNAT2*, possibly as a repressive chromatin complex through recruitment of the histone chaperone histone regulatory protein A (HIRA) (Fig. 4) (Guo et al., 2008; Phelps-Durr et al., 2005).

Consistent with an epigenetic mode of KNOX gene repression, a second pathway confining KNOX activity to meristems requires the polycomb-group proteins CURLY LEAF (CLF), SWINGER (SWN) and FERTILISATION INDEPENDENT ENDOSPERM (Katz et al., 2004; Schubert et al., 2006). The SET domains of CLF and SWN have histone methyltransferase activity, which facilitates the maintenance of silenced target gene expression states through cell divisions. Recent work has shown how the combined actions of two polycomb repressive complexes (PRCs) repress KNOX transcription (Xu and Shen, 2008). CLF-containing PRC2 marks KNOX genes by trimethylation of histone H3 at lysine 27 (H3K27me3) (Schubert et al., 2006). A PRC1-like complex, which contains the chromodomain protein LIKE HETEROCHROMATIN PROTEIN1 and two RING domain proteins, then binds to these methylated histones, resulting in the transcriptional repression of KNOX genes (Fig. 4) (Xu and Shen, 2008).

YABBY (YAB) proteins define a seed plant-specific family that promotes lamina outgrowth (see Glossary, Box 1) and represses KNOX expression in *Arabidopsis* leaves (Fig. 4). *YAB* gene expression is confined to lateral organs, and the cumulative loss of *YAB* function results in the progressive loss of lamina growth and in the reactivation of *STM*, *BP* and *KNAT2* expression in *fil;yab3* leaves (Kumaran et al., 2002). These leaves produce ectopic shoot meristems, associated with KNOX expression; however, similar to *as1* mutants, the repression of KNOX genes is maintained in leaf founder cells. Genetic evidence does suggest, however, that regulating this boundary of KNOX-expressing and non-expressing cells in the SAM involves antagonism between meristem determinants, such as *STM*, and lateral organ determinants such as *YAB*, *AS1* and *AS2* genes. Genetic interactions result in the suppression of *stm* mutants by *fil;yab3* or by *as1* or *as2*, indicating that cells that lack *STM* in the SAM boundary region are consumed by lateral organ production in the absence of *YAB*, *AS1* or *AS2* function (Byrne et al., 2000; Kumaran et al., 2002). Although it is not yet clear what component of YAB activity is mediated by KNOX repression, these observations strengthen the notion that restricting KNOX expression to the SAM is a prerequisite for normal leaf development in *Arabidopsis*.

Another mechanistic link between KNOX downregulation and sites of lateral organ initiation involves repression of KNOX expression by concentration maxima of the phytohormone auxin (see Glossary, Box 1) in the periphery of the SAM (Fig. 4). These auxin maxima are established by action of the PINFORMED1

(PIN1) auxin efflux transporter in *Arabidopsis* (Benkova et al., 2003; Reinhardt et al., 2003). *PIN1* and KNOX transcripts are expressed in mutually exclusive domains in the SAM, and compromising PIN1 activity or auxin signaling results in ectopic expression of the KNOX gene *BP* in *Arabidopsis* leaves (Hay et al., 2006). Loss of *BP* function can partially rescue the loss of lateral organs in *pin1* mutants, suggesting that failure to repress *BP* expression in the boundary of the SAM in *pin1* mutants could antagonize lateral organ formation (Hay et al., 2006). Furthermore, maize apices grown in the presence of an auxin transport inhibitor fail to repress KNOX genes or to initiate leaves at the shoot apex (Scanlon, 2003). Thus, it will be important to understand whether repression of KNOX gene expression in leaf founder cells is a direct read-out of auxin signaling. One possibility is that AUXIN RESPONSE FACTORs (ARFs) repress KNOX transcription because ectopic KNOX expression causes floral organ defects in *arf6 arf8* double mutants (Tabata et al., 2010).

Despite mounting evidence for antagonism between KNOX and auxin activities, the proportion of auxin action in lateral organ formation that depends on KNOX repression at the SAM boundary is unclear. The boundary regulator CUP-SHAPED COTYLEDON2 (CUC2) initially activates *STM* expression and later overlaps with *STM* in a region of repressed growth and low auxin activity at the SAM boundary (Fig. 4) (Heisler et al., 2005). Failure to initiate cotyledons in *pin1;pinoid* embryos depends on *STM* and *CUC1,2* function, and eliminating STM activity in *pin1;pid;stm* triple mutants recovers cotyledons (Furutani et al., 2004). The similarity of these genetic interactions between KNOX and auxin transport-related genes in the embryo, vegetative and reproductive shoots indicates that antagonism between auxin and KNOX genes might operate in multiple contexts throughout *Arabidopsis* development to promote organ initiation and the associated elaboration of organ boundaries (Fig. 2) (Furutani et al., 2004; Hay et al., 2006). A role for combined regulation of KNOX and auxin activities in boundary delimitation was also suggested by analysis of the boundary-expressed LOB domain gene *JAGGED LATERAL ORGANS* (*JLO*), which is sufficient to activate *STM* and *BP* expression and repress PIN auxin efflux transporters (Fig. 4) (Borghi et al., 2007).

This progress highlights two avenues of future research. First, isolation of higher-order KNOX-repressive complexes and analysis of their dynamic nature during development and the cell cycle should help to explain how these repressive pathways are integrated. Second, the precise phenotypic relevance of KNOX repression by different pathways will need to be resolved in order to understand which KNOX-repressive pathways have a clear contribution to cell-fate decisions and growth regulation. For example, although the mechanistic basis of PRC-mediated KNOX silencing is being clarified, it is unclear to what extent the mutant phenotypes of such chromatin repressors are attributable to mis-regulation of KNOX versus other target genes.

Hormones as KNOX downstream effectors: a balancing act

The few downstream targets of KNOX transcription factors identified thus far are beginning to shed light on how these proteins influence the balance between differentiation and replenishment of cells in the SAM. KNOX targets modulate the abundance of gibberellins (GAs) and cytokinins (CKs) (see Glossary, Box 1), and of the secondary cell wall polymer lignin (Fig. 4). CKs activate cell division, whereas GAs promote cell elongation, which is a cell differentiation process – as is lignin deposition – and KNOX proteins regulate the balance between GAs and CKs in meristem and leaf founder cells. In the SAM, KNOX proteins raise CK levels by activating the transcription of *ISOPENTENYL TRANSFEREASE7* (*IPT7*) and lower GA levels by directly inhibiting the biosynthetic gene GA 20-oxidase1 (*GA20ox1*) and activating the catabolic gene GA 2-oxidase1 (*GA2ox1*) (Bolduc and Hake, 2009; Jasinski et al., 2005; Sakamoto et al., 2001; Yanai et al., 2005). Genetic analyses demonstrate that this high CK:low GA ratio is important for KNOX function – to prevent cell differentiation and thus maintain pluripotent cell fate in the SAM. For example, *stm* mutant phenotypes are suppressed by elevating CK biosynthesis and are enhanced by either elevating GA activity or by reducing CK biosynthesis, and ultimately the combination of elevated GAs and reduced CKs is sufficient to phenocopy *stm* mutant phenotypes in wild-type *Arabidopsis* (Jasinski et al., 2005; Yanai et al., 2005). Conversely, the absence of KNOX expression in lateral organ founder cells likely correlates with a low CK:high GA ratio and with the competence to differentiate (Jasinski et al., 2005; Yanai et al., 2005).

One implication of the fact that modulating GA and CK pathways is largely sufficient to account for STM function in the SAM is that only a few target genes might mediate the function of this protein. This mirrors the scenario in *Drosophila*, in which HOX proteins have been shown to exert their function in some contexts by regulating only a few crucial targets (Lovegrove et al., 2006). Genome-wide target identification is required to evaluate this hypothesis and should help to reveal how distinct KNOX proteins exert their effects in diverse developmental contexts.

How these hormone signals are integrated with developmental processes operating in the SAM is less clear. For example, STM not only implements a high CK:low GA regime but also prevents cell differentiation by repressing leaf determinants such as *AS1* and *AS2* (Byrne et al., 2000; Byrne et al., 2002). The mechanistic basis for this repression is unlikely to be direct, so it will be interesting to know whether these different aspects of STM activity are independent or whether *AS1* and *AS2* respond to, and influence, hormone activity.

Evolving KNOX functions: KNOX genes and the rise of the sporophyte

As land plants evolved from green algae, the dominant phase of the life cycle changed from a haploid gametophyte to a diploid sporophyte (see Glossary, Box 1; Fig. 5). In seed plants, the haploid stage of the life cycle is reduced to few-celled gametophytes that produce male and female gametes, while morphological diversity resides in the sporophyte. However, land plants evolved from a group of green algae in which the diploid stage of the life cycle comprised merely the unicellular zygote. Mechanisms that control multicellular development in land plants, therefore, either evolved from genes directing unicellular development in the sporophyte or were recruited from existing genetic toolkits that controlled multicellular morphology in the gametophyte. Evidence exists for the recruitment of gametophyte genes into the sporophyte (Menand et al., 2007); however, genetic analyses in green algae and basal land plants suggest that ancestral KNOX genes controlled diploid development and diversified in land plants to control multicellular body plans.

A leafy shoot develops in the gametophyte of the moss *Physcomitrella patens* – a model organism for basal land plants (shown in Fig. 5). Given the role of KNOX genes in shoot development, it was surprising to find that Class I and II KNOX genes are neither expressed nor functional in the moss gametophyte, acting instead during sporophyte development

(Sakakibara et al., 2008; Singer and Ashton, 2007). Genetic control of the shoot system in haploid moss and in diploid flowering plants might, therefore, represent convergent evolution (see Glossary, Box 1). This suggests that the regulatory relationships between KNOX transcription factors and their downstream effectors in higher plants could be later innovations that were absent in basal land plant lineages. Indeed, *P. patens* KNOX deletion mutants show no alteration in *IPT*, *GA20ox* or *GA2ox* gene expression, suggesting that KNOX proteins in moss do not regulate these hormone pathways (Sakakibara et al., 2008). This contrasts with the biochemical activities of KNOX proteins, which might be conserved across land plants despite target gene divergence. For example, KNOX genes from both moss and the fern *Ceratopteris richardii*, where expression is also restricted to the sporophyte, elicit a similar spectrum of phenotypes to *Arabidopsis* KNOX genes when overexpressed in *Arabidopsis* (Sakakibara et al., 2008; Sano et al., 2005). These experiments highlight potential pitfalls for interpreting gene transfer experiments between different developmental contexts across very distant lineages if genetic tools are not available in both organisms to aid the understanding of endogenous gene function. Studies to identify KNOX target genes in moss will provide an interesting future direction for investigating the extent of divergence and conservation of the KNOX pathway, and how this relates to morphological transitions during land plant evolution.

If KNOX proteins have only diploid-specific functions, does this relate back to a history in regulating diploid development in algal ancestors? The discovery of interacting TALE proteins that are necessary and sufficient for zygote formation in the unicellular green alga *C. reinhardtii* suggests that this could be the case (shown in Fig. 5). Two haploid mating types in *C. reinhardtii* each contribute a different TALE protein, which interact and translocate to the nucleus in a diploid zygote – one is a Class II KNOX protein, the other a BELL protein (Lee et al., 2008). This *BELL* gene belongs to a lineage lost in land plants, despite an overall expansion of TALE genes during land plant evolution, which potentially allowed new interactions between BELL and KNOX proteins (see Table 2). An attractive correlation therefore arises between the diversification of KNOX/BELL genes and morphological diversity of plant sporophytes. For example, new TALE heterodimers in ancestral green plant lineages might have modified zygote behavior to allow a series of mitoses that produced a multicellular sporophyte. Exploiting the regulatory complexity created by multiple KNOX-BELL interactions could have facilitated the explosion of body plan complexity, which occurred as plants colonized terrestrial ecosystems. This hypothesis predicts that loss of TALE activity in land plants might condition zygotic defects, reflecting conservation of ancestral TALE gene functions to promote zygote formation. However, KNOX expression has not been detected in the *Arabidopsis* zygote – in fact, *STM* first acts at the globular stage of embryo development when the sporophyte comprises 32 cells (Long et al., 1996). Further studies are required, therefore, to map diversification of KNOX and BELL gene families to major morphological transitions during land plant evolution.

Diversifying KNOX function: *cis* regulatory evolution within a repressive landscape

Divergent Class I KNOX expression among species closely correlates with leaf shape such that in simple-leafed species, such as *A. thaliana* and maize, KNOX expression is confined to the meristem while KNOX proteins accumulate in compound leaves

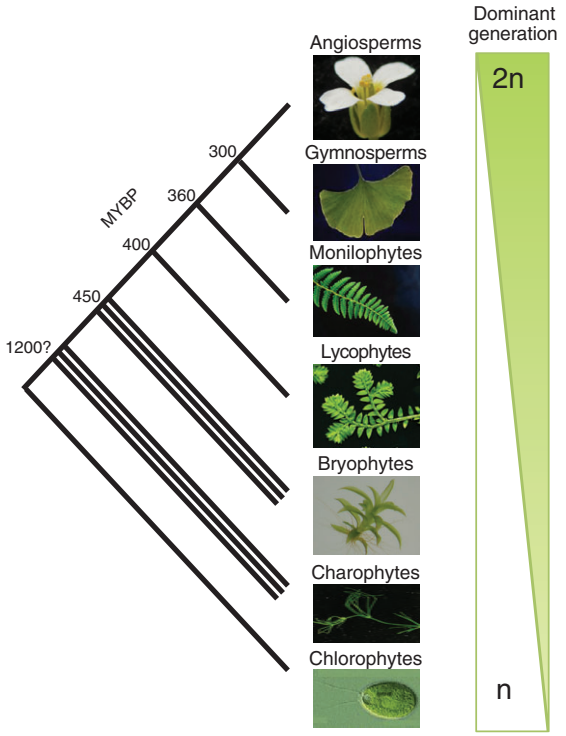


Fig. 5. KNOX genes function in the diploid generation of all major green plant lineages. Knotted1-like homeobox (KNOX) genes regulate diploid development in the two major green plant lineages: chlorophytes (green algae) and streptophytes (charophyte algae and land plants). Chlorophytes include the unicellular green alga *Chlamydomonas reinhardtii* (see figure). Charophytes include the green alga *Chara*, which is the sister taxa to land plants (see figure). Within land plants, vascular plants are distinct from bryophytes [liverworts (not shown), mosses (see figure) and hornworts (not shown)], forming a monophyletic group containing seed plants [gymnosperms and angiosperms (see figure)], monilophytes [such as ferns (see figure) and horsetails (not shown)] and lycophytes (see figure), each forming monophyletic groups within that clade. Both charophytes and bryophytes are not monophyletic groups. Estimated dates for nodes [in millions of years before present (MYBP)] are modified from Bowman et al. (Bowman et al., 2007). (Right) A diagram summarizing the transition from an ancestral haploid (n) dominant life cycle to a diploid (2n) dominant life cycle during land plant evolution, with the diploid-specific functions of KNOX genes indicated in green.

of many species (Bharathan et al., 2002). KNOX expression in leaves was independently recruited to control compound leaf development in multiple seed plant lineages, although legume species (including pea) are notable exceptions (Bharathan et al., 2002; Hofer et al., 2001) in which compound leaf development is independent of KNOX function. Ten years after KNOX overexpression was first shown to increase tomato leaf complexity, genetic evidence was provided that KNOX activity is necessary and sufficient for leaflet formation in *C. hirsuta* (Hay and Tsiantis, 2006). More recently, activity of the tomato Class I KNOX protein Tomato knotted 2 (Tkn2) was perturbed by means of translational fusion to a 12 amino acid EAR repressor motif of the *Arabidopsis SUPERMAN* gene termed SDRX, which confirmed a role for Tkn2 in tomato leaflet initiation (Shani et al., 2009).

These studies raise the question of what processes are responsible for KNOX genes being expressed in leaves of some species but not others. Parallel genetic studies in the closely related

species *Arabidopsis* and *C. hirsuta* offer a useful approach for investigating the mechanistic basis for such evolutionary diversification in KNOX gene expression. KNOX promoter swaps between *Arabidopsis* and *C. hirsuta* showed that *STM* and *BP* promoters directed reporter expression in a pattern characteristic of the native species (Hay and Tsiantis, 2006). Thus, differences in the *cis*-regulation of KNOX genes contribute to species-specific leaf shapes. It will, therefore, be interesting to identify the precise KNOX gene regulatory elements responsible for leaf expression in *C. hirsuta* and other species. Phylogenetic foot-printing of *STM* regulatory sequences, sampled from species with both simple and dissected leaves, identified the K-box as a conserved regulatory element required for repression of *STM* in the simple leaves of *A. thaliana* and tobacco (Uchida et al., 2007). Notably, this defined the first cis-regulatory element to mediate KNOX repression. Conservation of this K-box between *A. thaliana* and *C. hirsuta*, however, makes the precise significance of this element for elaboration of divergent leaf morphologies unclear.

The importance of cis-regulatory changes in KNOX loci for transitions in leaf form follows the logic that mutations in the cis-regulatory regions of developmental patterning genes are likely to underlie most of phenotypic evolution (Carroll, 2005). Extending the comparative genetic approach used between *C. hirsuta* and *Arabidopsis* to tomato relatives, in which KNOX regulation of leaf form evolved independently, should provide a broader picture of cis-regulatory evolution and should allow the individual sequence elements that control KNOX expression and leaf form to be functionally dissected.

Changes in KNOX gene expression between species could also arise by diversification of repressors, such as the ARP proteins. However, AS1 expression and function is conserved between *Arabidopsis* and *C. hirsuta*, such that, while AS1 prevents KNOX expression in *Arabidopsis* leaves, its repressive function defines the correct domain of *BP* expression within the context of the *C. hirsuta* leaf (Hay and Tsiantis, 2006). Transgenic complementation of *Arabidopsis as1* mutants by the gene *SkARP1* from the lycophyte *Selaginella kraussiana* suggests that the ability to repress KNOX expression might be an ARP function that is ancestral to vascular plants (Harrison et al., 2005). Isolation of KNOX repressors from *C. hirsuta* and tomato should help clarify to what degree novel versus conserved repressors of KNOX genes were used during evolution to produce compound leaves. For example, the *CLAUSA* and *TRIPINNATE* gene products repress KNOX transcripts in the adaxial domain of tomato leaves and regulate leaflet number (Jasinski et al., 2007). Thus, it is tempting to speculate that many avenues exist via which evolutionary change in either the trans-regulatory landscape or cis-regulatory elements can reconfigure KNOX expression. Modularity in KNOX regulation is also likely to circumvent the potentially pleiotropic effects of KNOX gene expression in lateral organs. Thus, a challenge for the future is to understand how evolutionary tinkering with the KNOX gene regulatory network has produced morphological diversity in different lineages.

Understanding KNOX function: from genes to modules

How does our understanding of KNOX gene function in meristem development relate to the formation and delimitation of leaflets in compound leaf development? Recent work suggests that this process reflects the function of different KNOX developmental modules that control primordium initiation, boundary formation and meristem maintenance in the context of both pluripotent cells of the SAM and in differentiating cells of the compound leaf.

KNOX-PIN-auxin

Leaflet formation from the leaf margin in *C. hirsuta* and tomato unfolds through a series of events similar to that of leaf formation at the periphery of the SAM (Barkoulas et al., 2008; Koenig et al., 2009). For example, PIN1 action facilitates the generation of auxin maxima sequentially along the leaf margin at sites where KNOX expression is repressed. These auxin maxima recruit cell-division centres at the leaf margin, resulting in outgrowth of individual lateral leaflets in the context of repressed growth elsewhere on the rachis. On the one hand, KNOX overexpression causes ectopic auxin maxima within leaflets, resulting in ectopic leaflet formation; on the other hand, exogenous auxin application can repress KNOX expression in the leaf (Barkoulas et al., 2008). These data suggest the following sequence of events: KNOX expression facilitates the formation of auxin maxima, which feed back to repress KNOX expression, thus allowing leaflet outgrowth. Whether such a feedback between KNOX/PIN1/auxin operates both in a compound leaf and in the context of the SAM, and its mechanistic basis, remains an interesting subject for future research.

KNOX-CUC

Genetic analysis in four distantly related eudicot species showed that CUC gene expression defines the distal boundary of leaflet initiation on the leaf rachis and is required for leaflet formation (Berger et al., 2009; Blein et al., 2008). CUC activity promotes KNOX expression within the leaf and vice versa, forming a positive-feedback loop that also functions during SAM and lateral organ boundary development (Blein et al., 2008; Takada et al., 2001).

KNOX-GA

KNOX activity ensures that low GA levels are maintained in the SAM, which favours meristem activity over cell differentiation. This KNOX-GA module also operates in tomato leaves where expression of the GA biosynthetic gene *LeGA20ox1* is repressed in response to *Tkn2* overexpression, and the effects of *Tkn2* overexpression are suppressed by constitutive GA signaling (Hay et al., 2002; Jasinski et al., 2008). Thus, antagonism between KNOX and GA activities in the tomato leaf regulates the correct pattern of leaflet formation.

In each of these regulatory modules, KNOX activity appears to influence the duration and direction of growth, while repressing tissue differentiation. Therefore, detailed, quantitative descriptions of growth will be useful in order to understand the role of KNOX proteins in different contexts. This may help to tease apart the degree to which genetic interactions, described above, reflect the integration of KNOX activity in specific modules versus the indirect consequences of KNOX expression on cellular differentiation. KNOX modules probably regulate meristem activity across all seed plants, and the repeated deployment of these modules to regulate compound leaf morphology is likely to reflect evolutionary parallelism (see Scotland, 2010). In some instances, however, the regulation of different morphologies by KNOX modules may reflect underlying homologies between structures. For example, tuber development in potato is regulated in part by an antagonistic interaction between KNOX and GA, probably reflecting the fact that tubers are modified shoots (Chen et al., 2004).

The action of KNOX proteins in these growth-regulating modules also provides a unified framework with which to interpret other striking morphologies caused by novel patterns of KNOX expression. For example, ectopic expression of the *kn1* orthologue in barley produces *Hooded* florets, a trait selected in some barley varieties in which the awn (see Glossary, Box 1) is transformed into reiterative inflorescence axes (Müller et al., 1995; Williams-Carrier et al., 1997). Similarly, ectopic KNOX expression in two snapdragon mutants *Hirzina* and *Invaginata* produces novel structures that resemble petal spurs (see Glossary, Box 1) (Golz et al., 2002). In both examples, ectopic KNOX expression leads to the formation of new tissue-organizing centres that can direct altered cell division and growth, which might reflect redeployment of KNOX modules in novel contexts. Whether selection has fixed evolutionary changes in KNOX expression that cause divergent morphologies in nature is an exciting issue that can be approached using population genetics methodologies empowered by an increasing wealth of genomic resources available in multiple taxa.

A matter of context: competence to respond to KNOX expression

The diverse effects of ectopic KNOX expression in lateral organs also highlight the fact that KNOX gene action is highly context and dose dependent. For example, dominant *Mouse ear* (*Me*) and *Curl* (*Cu*) mutations cause aberrant transcription of the tomato *Tkn2* gene, yet *Me* leaves vary between highly ramified vegetative leaves and bladeless reproductive leaves, while the growth of *Cu* leaves is severely arrested (Parnis et al., 1997). Variation in the expression domain of ARP repressors during development may influence these phenotypes; however, ARP expression has been reported to be both absent and present in the SAM of tomato so this issue requires further investigation (Kim et al., 2003; Pien et al., 2001).

Inducible KN1 expression in *Arabidopsis* has provided some insight into such phenotypic variation, as increased KN1 dose produces a qualitative increase in leaf phenotype from mild indentations at the leaf margin, to highly lobed margins, to strong growth arrest (Hay et al., 2003). This study also defined a window of competence during *Arabidopsis* leaf development in which only proliferating, but not differentiating, tissues could respond to KN1 activity. A recent study elegantly demonstrated the importance of developmental timing for the phenotypic output of KNOX overexpression in *Arabidopsis* and tomato leaves, which we further discuss in the next section (Shani et al., 2009). This work raises the issue of what the molecular basis of a differential competence to respond to KNOX expression could be.

Genetic analyses in *Arabidopsis* and tomato have identified two such mechanisms that influence the competence of shoot tissue to respond to KNOX activity. Studies in *Arabidopsis* suggest that KNOX-independent regulation of common target genes might modulate the competence of leaf tissue to respond to ectopic KNOX expression. For example, *as1* and *as2* mutant phenotypes are enhanced by mutations in *PICKLE* (*PKL*) and *SERRATE* (*SE*) genes, which encode a chromatin-remodeling protein and a miRNA biogenesis component, respectively [(Ori et al., 2000) and references therein]. KNOX genes are regulated normally in both *pkl* and *se* mutant leaves; however, expression of the KNOX target gene *GA20ox1* is repressed in the leaves of both mutants, indicating that PKL, SE and KNOX activities converge on at least one common target gene (Grigg et al., 2005). In the case of SE, this convergence could reflect its repression of a small family of microRNA-targeted *Class III HD-ZIP* genes, which promote meristem activity in common with KNOX genes (Grigg et al., 2005; McConnell et al., 2001). For example, *se* mutants showed heightened responses to KNOX activity that reflected elevated levels of *HD-ZIP III* expression, and *HD-ZIP III* gain-of-function mutants had reduced *GA20ox1* expression, thus mimicking the effects of KNOX overexpression (Grigg et al., 2005).

A second mechanism highlights the regulation of tissue differentiation as a key determinant for tissue response to KNOX gene expression. The *Lanceolate* (*La*) mutation in tomato produces miRNA-insensitive TEOSINTE BRANCHED1/CYCLOIDEA/PROLIFERATING CELL FACTOR 4 (TCP4) transcripts during leaf development, which cause precocious cell differentiation and prevent leaflet formation (Ori et al., 2007). KNOX expression is unaffected in *La* mutants, but KNOX gain-of-function effects are suppressed, suggesting that TCP-mediated control of cellular differentiation affects the competency of leaf tissue to respond to KNOX activity. Investigating the molecular underpinnings of this regulation and understanding how TCP and KNOX activities are coordinated during compound leaf development are interesting avenues for future research.

The maturation schedule revisited: KNOX genes as developmental timekeepers

Almost 20 years ago, an influential review article described a conceptual model for maize leaf development based on the perturbations caused mainly by dominant KNOX mutants (Freeling, 1992). Freeling hypothesized that a leaf primordium progresses through a series of defined developmental stages to form distinct tissue types – sheath, ligule (see Glossary, Box 1), auricle and blade – along the proximal-to-distal axis of the maize leaf. He termed this developmental trajectory a maturation schedule. Ectopic expression of KNOX genes in dominant mutants caused tissue transformations that he interpreted as developmental delays, whereby cells failed to progress through subsequent phases of the maturation schedule (Muehlbauer et al., 1997). For example, ectopic *kn1* expression along the lateral veins in *Kn1* mutants retarded this tissue development such that ligule differentiated in place of blade (Fig. 1B). Freeling's view that ectopic KNOX activity influenced the temporal progression of a leaf cell through developmental states has provided a valuable framework for conceptualizing compound leaf development, where KNOX proteins are active in endogenous, rather than ectopic, contexts.

Leaf development can be divided into three stages termed leaf initiation (I), primary morphogenesis (PM) and secondary morphogenesis (SM) (Fig. 6). The analysis of transcriptome dynamics during *Arabidopsis* leaf maturation described a sequential schedule of developmental steps operating during these stages and correlated expression signatures with each stage (Efroni et al., 2008). Using the promoters of such stage-specific genes to drive KNOX expression in the tomato leaf suggested that KNOX proteins act to prolong PM, thus allowing leaflet initiation (Fig. 6A) (Shani et al., 2009). Cell populations that occupy distinct positions along the maturation schedule may respond differently to KNOX expression, partly owing to their proliferative state and partly owing to receiving different tissue maturation signals from surrounding cells as the primordium grows. For example, KNOX overexpression during PM, but not SM, in tomato caused reiterative leaflet initiation (Shani et al., 2009). Here, KNOX activity retained cells in a divisive state for

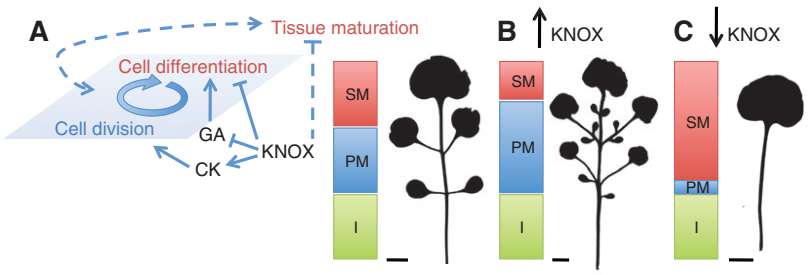


Fig. 6. A model of compound leaf development: KNOX proteins regulate a maturation schedule. (A-C) Compound leaf development is summarized as three continuous stages: initiation (I, green), primary morphogenesis (PM, blue) and secondary morphogenesis (SM, red). (**A**) Knotted1-like homeobox (KNOX) activity regulates the transition from PM to SM, such that tissue maturation is repressed until the appropriate developmental time (broken blue bar). During PM, KNOX proteins activate cytokinin (CK) levels, and repress gibberellin (GA) levels and additional processes such as lignin biosynthesis, which act at the cellular level to activate cell division and repress cell differentiation, in order to allow leaflet initiation. The balance between cell division and differentiation also influences tissue maturation and vice versa (broken blue arrow). (**B**) Increasing KNOX activity delays the transition from PM to SM, allowing iterations of the leaflet initiation programme. (**C**) Reducing KNOX activity precociously shifts SM forward in the maturation schedule, such that leaflets fail to initiate. *C. hirsuta* leaf silhouettes of the following genotypes are shown from left to right for illustrative purposes: wild type, dexamethasone-induced *35S::Kn1-GR* and *STM* RNAi [as described by Hay and Tsiantis (Hay and Tsiantis, 2006)]. The CaMV *35S* promoter, rather than stage-specific promoters, perturbs KNOX expression in these transgenic lines. Scale bars: 1 cm.

longer periods of time, permitting prolonged tissue responses to signals that promote leaflet formation (Fig. 6B). Reduction of KNOX activity during I in tomato, by expressing a KNOX-*SRDX* gene fusion, prevented leaflet initiation (Shani et al., 2009). In this case, reduced KNOX activity resulted in faster cessation of cell division, abbreviating PM to result in precocious tissue maturation, as observed in *C. hirsuta STM* RNAi leaves (Fig. 6C) (Hay and Tsiantis, 2006). Leaflet initiation was also blocked for different reasons by KNOX overexpression in tomato during I (Shani et al., 2009). Here, KNOX activity retained cells in a state in which they are not yet competent to respond to leaflet-promoting signals.

It is tempting to speculate that KNOX proteins retard progression from divisional growth to differentiation programmes by elevating CK and repressing GA levels, hence ultimately influencing the timing at which cells exit the cell cycle (Fig. 6A). This idea is consistent with observations in tomato where elevated GA signaling antagonizes KNOX action and results in faster growth rates during PM and in consequent reductions in leaflet number (Jasinski et al., 2008). It will be important to understand how KNOX activity in compound leaves integrates into the pathways that are shared by all leaves, which control the progression of a plant along a maturation schedule, in order to disentangle the influence of KNOX action on cell fate, tissue maturation and the direction and duration of cellular growth.

Conclusions

Twenty years on from cloning the first KNOX gene, we have a robust framework within which to understand how KNOX proteins control plant development. However, important gaps in our knowledge remain. First, the identification of genome-wide targets of distinct Class I KNOX proteins and their co-factors is required to understand the basis of context-specific KNOX activity in different tissues. Second, the identification of those targets that are able to account for the bulk of function of individual proteins will require in depth genetic analyses to overcome redundancy. Third, the implementation of quantitative and dynamic phenotyping frameworks to analyse KNOX function will help to explain how these proteins control growth and differentiation. In this context, it will be useful to devise in vivo assays to quantify KNOX contributions to distinct developmental processes and to exploit computational models to understand the logic of increasingly complex genetic circuitries. Finally, the development of genetic tools in diverse plant taxa will be needed to fully understand the role of KNOX genes in morphological evolution. This, in turn, will help to elucidate how the balance of conservation versus divergence in a fundamental developmental pathway sculpts plant form.

Acknowledgements

We thank S. Hake, Y. Yasumura and C. Canales for kindly providing images, and R. Scotland and D. Bailey for discussions. M.T. received support for this work from the Gatsby Charitable Foundation and Biotechnology and Biological Sciences Research Council. M.T. is a recipient of a Royal Society Wolfson Merit Award and A.H. is a recipient of a Royal Society University Research Fellowship.

Competing interests statement

The authors declare no competing financial interests.

References

Barkoulas, M., Hay, A., Kougioumoutzi, E. and Tsiantis, M. (2008). A developmental framework for dissected leaf formation in the Arabidopsis relative Cardamine hirsuta. *Nat. Genet.* **40**, 1136-1141.

Bellaoui, M., Pidkowich, M. S., Samach, A., Kushalappa, K., Kohalmi, S. E., Modrusan, Z., Crosby, W. L. and Haughn, G. W. (2001). The Arabidopsis BELL1 and KNOX TALE homeodomain proteins interact through a domain conserved between plants and animals. *Plant Cell* **13**, 2455-2470.

Belles-Boix, E., Hamant, O., Witiak, S. M., Morin, H., Traas, J. and Pautot, V. (2006). KNAT6: an Arabidopsis homeobox gene involved in meristem activity and organ separation. *Plant Cell* **18**, 1900-1907.

Benkova, E., Michniewicz, M., Sauer, M., Teichmann, T., Seifertova, D., Jurgens, G. and Friml, J. (2003). Local, efflux-dependent auxin gradients as a common module for plant organ formation. *Cell* **115**, 591-602.

Berger, Y., Harpaz-Saad, S., Brand, A., Melnik, H., Sirding, N., Alvarez, J. P., Zinder, M., Samach, A., Eshed, Y. and Ori, N. (2009). The NAC-domain transcription factor GOBLET specifies leaflet boundaries in compound tomato leaves. *Development* **136**, 823-832.

Bharathan, G., Goliber, T. E., Moore, C., Kessler, S., Pham, T. and Sinha, N. R. (2002). Homologies in leaf form inferred from KNOXI gene expression during development. *Science* **296**, 1858-1860.

Bhatt, A. M., Etchells, J. P., Canales, C., Lagodienko, A. and Dickinson, H. (2004). VAAMANA-a BEL1-like homeodomain protein, interacts with KNOX proteins BP and STM and regulates inflorescence stem growth in Arabidopsis. *Gene* **328**, 103-111.

Blein, T., Pulido, A., Vialette-Guiraud, A., Nikovics, K., Morin, H., Hay, A., Johansen, I. E., Tsiantis, M. and Laufs, P. (2008). A conserved molecular framework for compound leaf development. *Science* **322**, 1835-1839.

Bolduc, N. and Hake, S. (2009). The maize transcription factor KNOTTED1 directly regulates the gibberellin catabolism gene ga2ox1. *Plant Cell* **21**, 1647-1658.

Borghi, L., Bureau, M. and Simon, R. (2007). Arabidopsis JAGGED LATERAL ORGANS is expressed in boundaries and coordinates KNOX and PIN activity. *Plant Cell* **19**, 1795-1808.
Bowman, J. L., Floyd, S. K. and Sakakibara, K. (2007). Green genes-comparative genomics of the green branch of life. *Cell* **129**, 229-234.
Burglin, T. R. (1997). Analysis of TALE superclass homeobox genes (MEIS, PBC, KNOX, Iroquois, TGIF) reveals a novel domain conserved between plants and animals. *Nucleic Acids Res.* **25**, 4173-4180.
Byrne, M. E., Barley, R., Curtis, M., Arroyo, J. M., Dunham, M., Hudson, A. and Martienssen, R. A. (2000). Asymmetric leaves1 mediates leaf patterning and stem cell function in Arabidopsis. *Nature* **408**, 967-971.
Byrne, M. E., Simorowski, J. and Martienssen, R. A. (2002). ASYMMETRIC LEAVES1 reveals knox gene redundancy in Arabidopsis. *Development* **129**, 1957-1965.
Byrne, M. E., Groover, A. T., Fontana, J. R. and Martienssen, R. A. (2003). Phyllotactic pattern and stem cell fate are determined by the Arabidopsis homeobox gene BELLRINGER. *Development* **130**, 3941-3950.
Carroll, S. B. (2005). Evolution at two levels: on genes and form. *PLoS Biol.* **3**, e245.
Chen, H., Banerjee, A. K. and Hannapel, D. J. (2004). The tandem complex of BEL and KNOX partners is required for transcriptional repression of ga20ox1. *Plant J.* **38**, 276-284.
Chuck, G., Lincoln, C. and Hake, S. (1996). KNAT1 induces lobed leaves with ectopic meristems when overexpressed in Arabidopsis. *Plant Cell* **8**, 1277-1289.
Cole, M., Nolte, C. and Werr, W. (2006). Nuclear import of the transcription factor SHOOT MERISTEMLESS depends on heterodimerization with BLH proteins expressed in discrete sub-domains of the shoot apical meristem of Arabidopsis thaliana. *Nucleic Acids Res.* **34**, 1281-1292.
Dockx, J., Quaedvlieg, N., Keultjes, G., Kock, P., Weisbeek, P. and Smeekens, S. (1995). The homeobox gene *ATK1* of *Arabidopsis thaliana* is expressed in the shoot apex of the seedling and in flowers and inflorescence stems of mature plants. *Plant Mol. Biol.* **28**, 723-737.
Douglas, S. J., Chuck, G., Dengler, R. E., Pelecanda, L. and Riggs, C. D. (2002). KNAT1 and ERECTA regulate inflorescence architecture in Arabidopsis. *Plant Cell* **14**, 547-558.
Efroni, I., Blum, E., Goldshmidt, A. and Eshed, Y. (2008). A protracted and dynamic maturation schedule underlies Arabidopsis leaf development. *Plant Cell* **20**, 2293-2306.
Freeling, M. (1992). A conceptual framework for maize leaf development. *Dev. Biol.* **153**, 44-58.
Furutani, M., Vernoux, T., Traas, J., Kato, T., Tasaka, M. and Aida, M. (2004). PIN-FORMED1 and PINOID regulate boundary formation and cotyledon development in Arabidopsis embryogenesis. *Development* **131**, 5021-5030.
Golz, J. F., Keck, E. J. and Hudson, A. (2002). Spontaneous mutations in KNOX genes give rise to a novel floral structure in Antirrhinum. *Curr. Biol.* **12**, 515-522.
Grigg, S. P., Canales, C., Hay, A. and Tsiantis, M. (2005). SERRATE coordinates shoot meristem function and leaf axial patterning in Arabidopsis. *Nature* **437**, 1022-1026.
Guo, M., Thomas, J., Collins, G. and Timmermans, M. C. (2008). Direct repression of KNOX loci by the ASYMMETRIC LEAVES1 complex of Arabidopsis. *Plant Cell* **20**, 48-58.
Hackbusch, J., Richter, K., Muller, J., Salamini, F. and Uhrig, J. F. (2005). A central role of Arabidopsis thaliana ovate family proteins in networking and subcellular localization of 3-aa loop extension homeodomain proteins. *Proc. Natl. Acad. Sci. USA* **102**, 4908-4912.
Hake, S. and Freeling, M. (1986). Analysis of genetic mosaics shows that the extra epidermal cell divisions in *Knotted* mutant maize plants are induced by adjacent mesophyll cells. *Nature* **320**, 621-623.
Hake, S., Char, B. R., Chuck, G., Foster, T., Long, J. and Jackson, D. (1995). Homeobox genes in the functioning of plant meristems. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* **350**, 45-51.
Hake, S., Smith, H. M., Holtan, H., Magnani, E., Mele, G. and Ramirez, J. (2004). The role of knox genes in plant development. *Annu. Rev. Cell Dev. Biol.* **20**, 125-151.
Hareven, D., Gutfinger, T., Parnis, A., Eshed, Y. and Lifschitz, E. (1996). The making of a compound leaf: Genetic manipulation of leaf architecture in tomato. *Cell* **84**, 735-744.
Harrison, C. J., Corley, S. B., Moylan, E. C., Alexander, D. L., Scotland, R. W. and Langdale, J. A. (2005). Independent recruitment of a conserved developmental mechanism during leaf evolution. *Nature* **434**, 509-514.
Hay, A. and Tsiantis, M. (2006). The genetic basis for differences in leaf form between Arabidopsis thaliana and its wild relative Cardamine hirsuta. *Nat. Genet.* **38**, 942-947.
Hay, A., Kaur, H., Phillips, A., Hedden, P., Hake, S. and Tsiantis, M. (2002). The gibberellin pathway mediates KNOTTED1-type homeobox function in plants with different body plans. *Curr. Biol.* **12**, 1557-1565.
Hay, A., Jackson, D., Ori, N. and Hake, S. (2003). Analysis of the competence to respond to KNOTTED1 activity in Arabidopsis leaves using a steroid induction system. *Plant Physiol.* **131**, 1671-1680.
Hay, A., Barkoulas, M. and Tsiantis, M. (2006). ASYMMETRIC LEAVES1 and auxin activities converge to repress BREVIPEDICELLUS expression and promote leaf development in Arabidopsis. *Development* **133**, 3955-3961.
Heisler, M. G., Ohno, C., Das, P., Sieber, P., Reddy, G. V., Long, J. A. and Meyerowitz, E. M. (2005). Patterns of auxin transport and gene expression during primordium development revealed by live imaging of the Arabidopsis inflorescence meristem. *Curr. Biol.* **15**, 1899-1911.
Hofer, J., Gourlay, C., Michael, A. and Ellis, T. H. (2001). Expression of a class 1 knotted1-like homeobox gene is down-regulated in pea compound leaf primordia. *Plant Mol. Biol.* **45**, 387-398.
Ikezaki, M., Kojima, M., Sakakibara, H., Kojima, S., Ueno, Y., Machida, C. and Machida, Y. (2010). Genetic networks regulated by ASYMMETRIC LEAVES1 (AS1) and AS2 in leaf development in Arabidopsis thaliana: KNOX genes control five morphological events. *Plant J.* **61**, 70-82.
Jackson, D., Veit, B. and Hake, S. (1994). Expression of maize *KNOTTED1* related homeobox genes in the shoot apical meristem predicts patterns of morphogenesis in the vegetative shoot. *Development* **120**, 405-413.
Jasinski, S., Piazza, P., Craft, J., Hay, A., Woolley, L., Rieu, I., Phillips, A., Hedden, P. and Tsiantis, M. (2005). KNOX action in Arabidopsis is mediated by coordinate regulation of cytokinin and gibberellin activities. *Curr. Biol.* **15**, 1560-1565.
Jasinski, S., Kaur, H., Tattersall, A. and Tsiantis, M. (2007). Negative regulation of KNOX expression in tomato leaves. *Planta* **226**, 1255-1263.
Jasinski, S., Tattersall, A., Piazza, P., Hay, A., Martinez-Garcia, J. F., Schmitz, G., Theres, K., McCormick, S. and Tsiantis, M. (2008). PROCERA encodes a DELLA protein that mediates control of dissected leaf form in tomato. *Plant J.* **56**, 603-612.
Jun, J. H., Ha, C. M. and Fletcher, J. C. (2010). BLADE-ON-PETIOLE1 coordinates organ determinacy and axial polarity in arabidopsis by directly activating ASYMMETRIC LEAVES2. *Plant Cell* **22**, 62-76.
Katz, A., Oliva, M., Mosquna, A., Hakim, O. and Ohad, N. (2004). FIE and CURLY LEAF polycomb proteins interact in the regulation of homeobox gene expression during sporophyte development. *Plant J.* **37**, 707-719.
Kerstetter, R., Vollbrecht, E., Lowe, B., Veit, B., Yamaguchi, J. and Hake, S. (1994). Sequence analysis and expression patterns divide the maize *knotted1*-like homeobox genes into two classes. *Plant Cell* **6**, 1877-1887.
Kim, J. Y., Rim, Y., Wang, J. and Jackson, D. (2005). A novel cell-to-cell trafficking assay indicates that the KNOX homeodomain is necessary and sufficient for intercellular protein and mRNA trafficking. *Genes Dev.* **19**, 788-793.
Kim, M., McCormick, S., Timmermans, M. and Sinha, N. (2003). The expression domain of PHANTASTICA determines leaflet placement in compound leaves. *Nature* **424**, 438-443.
Kimura, S., Koenig, D., Kang, J., Yoong, F. Y. and Sinha, N. (2008). Natural variation in leaf morphology results from mutation of a novel KNOX gene. *Curr. Biol.* **18**, 672-677.
Koenig, D., Bayer, E., Kang, J., Kuhlemeier, C. and Sinha, N. (2009). Auxin patterns Solanum lycopersicum leaf morphogenesis. *Development* **136**, 2997-3006.
Kumar, R., Kushalappa, K., Godt, D., Pidkowich, M. S., Pastorelli, S., Hepworth, S. R. and Haughn, G. W. (2007). The Arabidopsis BEL1-LIKE HOMEODOMAIN proteins SAW1 and SAW2 act redundantly to regulate KNOX expression spatially in leaf margins. *Plant Cell* **19**, 2719-2735.
Kumaran, M. K., Bowman, J. L. and Sundaresan, V. (2002). YABBY polarity genes mediate the Repression of KNOX Homeobox Genes in Arabidopsis. *Plant Cell* **14**, 2761-2770.
Lee, J. H., Lin, H., Joo, S. and Goodenough, U. (2008). Early sexual origins of homeoprotein heterodimerization and evolution of the plant KNOX/BELL family. *Cell* **133**, 829-840.
Lincoln, C., Long, J., Yamaguchi, J., Serikawa, K. and Hake, S. (1994). A Knotted1-like homeobox gene in Arabidopsis is expressed in the vegetative meristem and dramatically alters leaf morphology when overexpressed in transgenic plants. *Plant Cell* **6**, 1859-1876.
Long, J. A., Moan, E. I., Medford, J. I. and Barton, M. K. (1996). A member of the KNOTTED class of homeodomain proteins encoded by the SHOOTMERISTEMLESS gene of Arabidopsis. *Nature* **379**, 66-69.
Lovegrove, B., Simoes, S., Rivas, M. L., Sotillos, S., Johnson, K., Knust, E., Jacinto, A. and Hombria, J. C. (2006). Coordinated control of cell adhesion, polarity, and cytoskeleton underlies Hox-induced organogenesis in Drosophila. *Curr. Biol.* **16**, 2206-2216.
Lucas, W. J., Bouche-Pillon, S., Jackson, D. P., Nguyen, L., Baker, L., Ding, B. and Hake, S. (1995). Selective trafficking of KNOTTED1 homeodomain protein and its mRNA through plasmodesmata. *Science* **270**, 1980-1983.
Magnani, E. and Hake, S. (2008). KNOX lost the OX: the Arabidopsis KNATM gene defines a novel class of KNOX transcriptional regulators missing the homeodomain. *Plant Cell* **20**, 875-887.
McConnell, J. R., Emery, J., Eshed, Y., Bao, N., Bowman, J. and Barton, M. K. (2001). Role of PHABULOSA and PHAVOLUTA in determining radial patterning in shoots. *Nature* **411**, 709-713.

Menand, B., Yi, K., Jouannic, S., Hoffmann, L., Ryan, E., Linstead, P., Schaefer, D. G. and Dolan, L. (2007). An ancient mechanism controls the development of cells with a rooting function in land plants. *Science* **316**, 1477-1480.
Muehlbauer, G. J., Fowler, J. E. and Freeling, M. (1997). Sectors expressing the homeobox gene *liguleless3* implicate a time-dependent mechanism for cell fate acquisition along the proximal-distal axis of the maize leaf. *Development* **124**, 5097-5106.
Mukherjee, K., Brocchieri, L. and Burglin, T. R. (2009). A comprehensive classification and evolutionary analysis of plant homeobox genes. *Mol. Biol. Evol.* **26**, 2775-2794.
Müller, K., Romano, N., Gerstner, O., Garcia-Maroto, F., Pozzi, C., Salamini, F. and Rohde, W. (1995). The barley *Hooded* mutation caused by a duplication in a homeobox gene intron. *Nature* **374**, 727-730.
Ori, N., Eshed, Y., Chuck, G., Bowman, J. L. and Hake, S. (2000). Mechanisms that control knox gene expression in the Arabidopsis shoot. *Development* **127**, 5523-5532.
Ori, N., Cohen, A. R., Etzioni, A., Brand, A., Yanai, O., Shleizer, S., Menda, N., Amsellem, Z., Efroni, I., Pekker, I. et al. (2007). Regulation of LANCEOLATE by miR319 is required for compound-leaf development in tomato. *Nat. Genet.* **39**, 787-791.
Pagnussat, G. C., Yu, H. J. and Sundaresan, V. (2007). Cell-fate switch of synergid to egg cell in Arabidopsis eostre mutant embryo sacs arises from misexpression of the BEL1-like homeodomain gene BLH1. *Plant Cell* **19**, 3578-3592.
Parnis, A., Cohen, O., Gutfinger, T., Hareven, D., Zamir, D. and Lifschitz, E. (1997). The dominant developmental mutants of tomato, *Mouse-Ear* and *Curl*, are associated with distinct modes of abnormal transcriptional regulation of a *Knotted* gene. *Plant Cell* **9**, 2143-2158.
Phelps-Durr, T. L., Thomas, J., Vahab, P. and Timmermans, M. C. (2005). Maize rough sheath2 and its Arabidopsis orthologue ASYMMETRIC LEAVES1 interact with HIRA, a predicted histone chaperone, to maintain knox gene silencing and determinacy during organogenesis. *Plant Cell* **17**, 2886-2898.
Pien, S., Wyrzykowska, J. and Fleming, A. J. (2001). Novel marker genes for early leaf development indicate spatial regulation of carbohydrate metabolism within the epical meristem. *Plant J.* **25**, 663-674.
Ragni, L., Belles-Boix, E., Gunl, M. and Pautot, V. (2008). Interaction of KNAT6 and KNAT2 with BREVIPEDICELLUS and PENNYWISE in Arabidopsis inflorescences. *Plant Cell* **20**, 888-900.
Reinhardt, D., Pesce, E., Stieger, P., Mandel, T., Baltensperger, K., Bennett, M., Traas, J., Friml, J. and Kuhlemeier, C. (2003). Regulation of phyllotaxis by polar auxin transport. *Nature* **426**, 255-260.
Rieckhof, G., Casares, F., Ryoo, H. D., Abu-Shaar, M. and Mann, R. S. (1997). Nuclear translocation of extradenticle requires homothorax, which encodees an extradenticle-related homeodomain protein. *Cell* **91**, 171-183.
Roeder, A. H., Ferrandiz, C. and Yanofsky, M. F. (2003). The role of the REPLUMLESS homeodomain protein in patterning the Arabidopsis fruit. *Curr. Biol.* **13**, 1630-1635.
Rutjens, B., Bao, D., van Eck-Stouten, E., Brand, M., Smeekens, S. and Proveniers, M. (2009). Shoot apical meristem function in Arabidopsis requires the combined activities of three BEL1-like homeodomain proteins. *Plant J.* **58**, 641-654.
Sakakibara, K., Nishiyama, T., Deguchi, H. and Hasebe, M. (2008). Class 1 KNOX genes are not involved in shoot development in the moss Physcomitrella patens but do function in sporophyte development. *Evol. Dev.* **10**, 555-566.
Sakamoto, T., Kamiya, N., Ueguchi-Tanaka, M., Iwahori, S. and Matsuoka, M. (2001). KNOX homeodomain protein directly suppresses the expression of a gibberellin biosynthetic gene in the tobacco shoot apical meristem. *Genes Dev.* **15**, 581-590.
Sano, R., Juarez, C. M., Hass, B., Sakakibara, K., Ito, M., Banks, J. A. and Hasebe, M. (2005). KNOX homeobox genes potentially have similar function in both diploid unicellular and multicellular meristems, but not in haploid meristems. *Evol. Dev.* **7**, 69-78.
Sato, Y., Sentoku, N., Miura, Y., Hirochika, H., Kitano, H. and Matsuoka, M. (1999). Loss-of-function mutations in the rice homeobox gene OSH15 affect the architecture of internodes resulting in dwarf plants. *EMBO J.* **18**, 992-1002.
Scanlon, M. J. (2003). The polar auxin transport inhibitor N-1-naphthylphthalamic acid disrupts leaf initiation, KNOX protein regulation, and formation of leaf margins in maize. *Plant Physiol.* **133**, 597-605.
Schneeberger, R., Tsiantis, M., Freeling, M. and Langdale, J. A. (1998). The *rough sheath2* gene negatively regulates homeobox gene expression during maize leaf development. *Development* **125**, 2857-2865.
Schubert, D., Primavesi, L., Bishopp, A., Roberts, G., Doonan, J., Jenuwein, T. and Goodrich, J. (2006). Silencing by plant Polycomb-group genes requires dispersed trimethylation of histone H3 at lysine 27. *EMBO J.* **25**, 4638-4649.
Scotland, R. W. (2010). Deep homology: a view from systematics. *BioEssays* **32**, 438-449.
Shani, E., Burko, Y., Ben-Yaakov, L., Berger, Y., Amsellem, Z., Goldshmidt, A., Sharon, E. and Ori, N. (2009). Stage-specific regulation of Solanum lycopersicum leaf maturation by class 1 KNOTTED1-LIKE HOMEOBOX proteins. *Plant Cell* **21**, 3078-3092.
Singer, S. D. and Ashton, N. W. (2007). Revelation of ancestral roles of KNOX genes by a functional analysis of Physcomitrella homologues. *Plant Cell Rep.* **26**, 2039-2054.
Sinha, N. R., Williams, R. E. and Hake, S. (1993). Overexpression of the maize homeo box gene, *KNOTTED-1*, causes a switch from determinate to indeterminate cell fates. *Genes Dev.* **7**, 787-795.
Smith, H. M. and Hake, S. (2003). The interaction of two homeobox genes, BREVIPEDICELLUS and PENNYWISE, regulates internode patterning in the Arabidopsis inflorescence. *Plant Cell* **15**, 1717-1727.
Smith, H. M., Boschke, I. and Hake, S. (2002). Selective interaction of plant homeodomain proteins mediates high DNA- binding affinity. *Proc. Natl. Acad. Sci. USA* **99**, 9579-9584.
Smith, L. G., Greene, B., Veit, B. and Hake, S. (1992). A dominant mutation in the maize homeobox gene, *Knotted-1*, causes its ectopic expression in leaf cells with altered fates. *Development* **116**, 21-30.
Tabata, R., Ikezaki, M., Fujibe, T., Aida, M., Tian, C. E., Ueno, Y., Yamamoto, K. T., Machida, Y., Nakamura, K. and Ishiguro, S. (2010). Arabidopsis auxin response factor6 and 8 regulate jasmonic acid biosynthesis and floral organ development via repression of class 1 KNOX genes. *Plant Cell Physiol.* **51**, 164-175.
Takada, S., Hibara, K., Ishida, T. and Tasaka, M. (2001). The CUP-SHAPED COTYLEDON1 gene of Arabidopsis regulates shoot apical meristem formation. *Development* **128**, 1127-1135.
Tattersall, A. D., Turner, L., Knox, M. R., Ambrose, M. J., Ellis, T. H. and Hofer, J. M. (2005). The mutant crispa reveals multiple roles for PHANTASTICA in pea compound leaf development. *Plant Cell* **17**, 1046-1060.
Timmermans, M. C., Hudson, A., Becraft, P. W. and Nelson, T. (1999). ROUGH SHEATH2: a Myb protein that represses knox homeobox genes in maize lateral organ primordia. *Science* **284**, 151-153.
Tsiantis, M. and Hay, A. (2003). Comparative plant development: the time of the leaf? *Nat. Rev. Genet.* **4**, 169-180.
Tsiantis, M., Schneeberger, R., Golz, J. F., Freeling, M. and Langdale, J. A. (1999). The maize rough sheath2 gene and leaf development programs in monocot and dicot plants. *Science* **284**, 154-156.
Uchida, N., Townsley, B., Chung, K. H. and Sinha, N. (2007). Regulation of SHOOT MERISTEMLESS genes via an upstream-conserved noncoding sequence coordinates leaf development. *Proc. Natl. Acad. Sci. USA* **104**, 15953-15958.
Venglat, S. P., Dumonceaux, T., Rozwadowski, K., Parnell, L., Babic, V., Keller, W., Martienssen, R., Selvaraj, G. and Datla, R. (2002). The homeobox gene BREVIPEDICELLUS is a key regulator of inflorescence architecture in Arabidopsis. *Proc. Natl. Acad. Sci. USA* **99**, 4730-4735.
Vollbrecht, E., Veit, B., Sinha, N. and Hake, S. (1991). The developmental gene *Knotted-1* is a member of a maize homeobox gene family. *Nature* **350**, 241-243.
Vollbrecht, E., Reiser, L. and Hake, S. (2000). Shoot meristem size is dependent on inbred background and presence of the maize homeobox gene, *knotted1*. *Development* **127**, 3161-3172.
Williams-Carrier, R. E., Lie, Y. S., Hake, S. and Lemaux, P. G. (1997). Ectopic expression of the maize *kn1* gene phenocopies the *Hooded* mutant of barley. *Development* **124**, 3737-3745.
Winter, N., Kollwig, G., Zhang, S. and Kragler, F. (2007). MPB2C, a microtubule-associated protein, regulates non-cell-autonomy of the homeodomain protein KNOTTED1. *Plant Cell* **19**, 3001-3018.
Xu, L. and Shen, W. H. (2008). Polycomb silencing of KNOX genes confines shoot stem cell niches in Arabidopsis. *Curr. Biol.* **18**, 1966-1971.
Xu, L., Xu, Y., Dong, A., Sun, Y., Pi, L. and Huang, H. (2003). Novel as1 and as2 defects in leaf adaxial-abaxial polarity reveal the requirement for ASYMMETRIC LEAVES1 and 2 and ERECTA functions in specifying leaf adaxial identity. *Development* **130**, 4097-4107.
Yanai, O., Shani, E., Dolezal, K., Tarkowski, P., Sablowski, R., Sandberg, G., Samach, A. and Ori, N. (2005). Arabidopsis KNOXI proteins activate cytokinin biosynthesis. *Curr. Biol.* **15**, 1566-1571.
Zhong, R., Lee, C., Zhou, J., McCarthy, R. L. and Ye, Z. H. (2008). A battery of transcription factors involved in the regulation of secondary cell wall biosynthesis in Arabidopsis. *Plant Cell* **20**, 2763-2782.