Title

Diverse functions of KNOX transcription factors in the diploid body plan of plants

Authors

Katsutoshi Tsuda and Sarah Hake

Affiliation

Plant Gene Expression Center, U.S. Department of Agriculture-Agricultural Research Service, Plant and Microbial Biology Department, University of California at Berkeley, Berkeley, California 94720, USA

Corresponding author: Sarah Hake

e-mail: hake@berkeley.edu

Telephone: (510)-559-5907

Fax: (510)-559-5678

Abstract

KNOX genes were initially found as shoot meristem regulators in angiosperms. Recent studies in diverse plant lineages however, have revealed the divergence of *KNOX* gene function during the evolution of diploid body plans. Using genomic approaches, class I *KNOX* transcription factors have been shown to regulate multiple hormone pathways including auxin and brassinosteroid as well as many transcription factors that play important roles

in plant development. Class I KNOX proteins appear to be activators, whereas class II proteins act as repressors in transcriptional regulation of their target genes.

Introduction

The shoot system of vascular plants, which accounts for the major biomass on the planet, has dramatically diversified during the evolution of plant lineages. Shoots initiate from the shoot apical meristem (SAM), an indeterminate structure harboring pluripotent stem cells. *KNOTTED1-like homeobox (KNOX)* genes are crucial regulators of SAM activity in vascular plants [1,2]. In this review, we will focus on recent studies that highlight the changes of *KNOX* gene function in the course of evolution of plant body plans, and those that reveal *KNOX* downstream gene networks in angiosperm meristems at a genome-wide level.

Diverse functions of *KNOX* genes in the plant lineage

Maize *knotted1 (kn1)* was the first homeobox gene described in plants [3,4]. *kn1* belongs to the three amino acid loop extension (TALE) homeodomain superfamily, which is present in all major eukaryote lineages [5,6]. In plants, TALE homeobox genes are classified into two families, *knotted1-like homeobox (KNOX)* and *BELL1-like homeobox (BELL or BLH)*. Both families are found in all plant lineages, however, the number of gene family members has increased during the evolution of multicellular diploid bodies [7]. Species in green and red algal lineages have only a single *KNOX* gene and one or two *BLH* genes. In land plants, multiple genes are found in both *KNOX* and *BLH*

families and *KNOX* family members have diversified into two classes (class I and II) [7,8] (Figure 1). A third class of *KNOX* genes, class M, which lacks the HD, is found only in dicot species so far [9,10]. Thus, *KNOX* subfamilies have emerged during the course of evolution in plant lineages.

The dominant generation in the life cycle of plants shifted from haploid to diploid during evolution. Along with this shift, *KNOX* gene function has diverged, but so far is specific to the diploid generation. In the unicellular green alga *Chlamydomonas reinhardtii*, the major generation in the life cycle is haploid. Upon nitrogen starvation, haploid cells initiate gametogenesis, and plus and minus gametes express the BELL protein Gamete-specific *plus1* (Gsp1) and *KNOX* protein Gamete-specific *minus1* (Gsm1), respectively. These proteins form a heterodimer upon gamete fusion and initiate the zygotic developmental program that includes formation of a thick cell wall for survival under unfavorable environments [11] (Figure 1). Similarly, TALE-HD proteins regulate mating cell type differentiation and zygotic gene expression in fungi [12,13]. Therefore, these studies suggest the ancestral function of *KNOX* and BELL proteins in the plant lineage was to regulate sexual and zygote development.

Bryophytes, the basal land plant lineage, develop multicellular diploid bodies. However, the haploid generation is still dominant over the diploid generation. The haploid gametophytes produce shoot structures from an indeterminate apical cell. The diploid sporophytes form on the gametophytes with a limited growth potential, developing an organ that forms haploid spores through meiosis (Figure 1). In the moss *Physcomitrella patens*, both class I and II *KNOX* genes are predominantly expressed in sporophytes. Mutations in

Class I *KNOX* genes cause a slight reduction of cell population suggesting they are involved in cell proliferation [14]. On the other hand, class II *KNOX* genes are required to repress gametophyte developmental program in sporophytes. Disruption of class II genes results in the formation of gametophyte-like structures in sporophytes without meiosis [15]. Thus, class II gene function in moss is reminiscent of the ancestral homologs in algae to regulate the haploid to diploid developmental transition.

In vascular plants, the diploid generation is dominant over the haploid. Upon fertilization, the zygote does not immediately undergo meiosis but continues to develop higher orders of shoot and root systems (Figure 1). In angiosperms, class I *KNOX* genes play essential roles in the establishment and maintenance of the SAM, which initiates all above ground organs such as leaves, stems, branches and flowers. They are expressed in indeterminate tissues including all shoot meristems and immature stems, but down-regulated on the flank of the shoot meristems (i.e., the Plastochron 0 (P0) leaf position) where lateral organs initiate. The loss-of-function mutants fail to maintain shoot meristems [16-18]. In addition, class I *KNOX* genes are involved in distinct aspects of developmental processes, such as leaf dissection in compound leaf species, internode elongation, inflorescence architecture and establishment of vascular connections between parasitic plants and their hosts [19-24]. Thus, the class I *KNOX* gene regulatory module has been recruited to various developmental processes during the evolution of shoot systems.

Class II genes in angiosperms are expressed in differentiating organs including leaves, stems, flowers and roots [8]. In *Arabidopsis*, the class II

genes *knotted-like from Arabidopsis thaliana3* (*KNAT3*), *KNAT4* and *KNAT5* have redundant and important functions in lateral organ differentiation [25]. In addition, another *Arabidopsis* class II gene *KNAT7* represses secondary cell wall biosynthesis in interfascicular fibers of inflorescence stems [26,27]. Collectively, angiosperm class I gene function is slightly reminiscent to that of moss homologs because both are involved in cell proliferation, whereas class II genes have been neofunctionalized and are involved in differentiation in angiosperms.

KNOX downstream pathways

Studies in the last decade identified several genes downstream of class I KNOX proteins in angiosperm. Tobacco NTH15 directly represses *GA20ox*, a gene encoding the enzyme that catalyzes the limiting step of gibberellin (GA) biosynthesis [28]. In maize, KN1 directly activates *ga2ox1*, which inactivates bioactive GAs [29]. On the other hand, cytokinin (CK) biosynthetic genes are activated by KNOX proteins in *Arabidopsis* and rice [30-32]. Thus, class I KNOX genes lower GA levels and activate CK accumulation. In addition, it was also reported that KNOX genes repress the biosynthesis of lignin, a major secondary cell wall component in *Arabidopsis* and maize [33,34] (Figure 2).

Recently, a genome-wide view of the downstream network was uncovered in maize and rice using chromatin immunoprecipitation followed by next generation sequencing (ChIP-seq) combined with transcriptome analyses [35,36]. In both cases, nearly 5,000 putative direct targets were identified. KN1 and its rice ortholog *Oryza sativa* homeobox1 (*OSH1*)

preferentially bind 5' of transcription start sites, but also bind to exon, intron and intergenic regions. The motif discovery analyses in these studies revealed that the most enriched motif in KNOX binding regions is highly degenerated and contains two GA cores separated by 3 base pairs (bp) with G enriched in between. Among their putative targets, the most enriched functional category is transcription factors (TFs) such as homeobox, ARF, AUX-IAA, AP2/EREBP, bHLH, YABBY, MADS, NAC, SPB, TCP and WRKY [35] (Figure 2). OSH1 directly activates class I *KNOX* genes, including itself, and this positive autoregulation is required for SAM maintenance [18]. *OSH1* overexpression also up-regulates a rice *BLH* gene *qSH1*, which encodes a putative cofactor of class I KNOX proteins. Thus, a critical *KNOX* gene function is to activate its own expression and that of cofactors. In *Arabidopsis*, the class I KNOX protein SHOOT MERISTEMLESS (STM) directly activates *CUPSHAPED COTYLEDON1* (*CUC1*), which is essential for organ boundary formation [37]. KN1 and OSH1 also target *CUC* genes. These results may explain the boundary defects observed in *KNOX* gain and loss of function mutants [36,38-40]. KN1 and OSH1 targets include TFs that are involved in leaf and flower development such as HD-ZIPIII, YABBY and MADS-box families. Although functional relationships between KNOX proteins and these TFs are yet to be studied, this finding suggests that KNOX proteins play roles not only in SAM maintenance, but also in lateral organ development.

Hormonal pathways

The KN1 ChIP-seq study uncovered the prevalence of hormonal pathways as another prominent downstream category [35]. Strikingly, KN1 binds to loci that

encode various components of hormonal pathways not only for GA and CK, but also for auxin, brassinosteroid (BR), ethylene and abscisic acid (ABA). Even in the GA and CK pathways, many genes that were previously unlinked to *KNOX* functions, such as the receptors and signaling genes, were bound by KN1 proteins. Among these hormones, the auxin pathway is the most dramatically modulated in transcriptome analyses in *kn1* loss-of and gain-of function mutants. KN1 directly binds auxin pathway genes at all levels, including biosynthesis, catabolism, transport, perception and signal transduction. In the *Kn1-N* dominant mutant, which ectopically expresses KN1 in leaves, the expression of an auxin signaling reporter *DR5:RFP* was increased in leaves compared to wild type. This result suggests that KN1 has a positive impact on auxin signaling [35] (Figure 2).

BR, a growth-promoting phytohormone involved in diverse aspects of plant growth and development [41], was also identified as an important downstream target of KNOX proteins in rice. Inducible overexpression of *OSH1* caused BR deficiency, whereas *osh1* loss-of-function mutants showed phenotypes indicative of BR overproduction [36]. ChIP-seq analysis revealed that OSH1 directly binds to several BR pathway genes such as biosynthesis, catabolism, perception and signaling. Among them, three BR catabolism enzyme genes, *CYP734A2*, *CYP734A4* and *CYP734A6*, were rapidly up-regulated after *OSH1* induction (Figure 2). RNAi knockdown of these three genes occasionally caused shoot growth arrest with abnormally vacuolated cells, suggesting that BR catabolism is important to prevent premature cell differentiation in the SAM.

Transcriptional regulation by KNOX proteins

How do KNOX proteins regulate target gene expression? In *Arabidopsis* and tomato, overexpression of class I KNOX proteins fused with repressor domains caused dominant negative phenotypes, similar to loss-of-function phenotype [42,43]. In addition, a time course transcriptome experiment showed that a large fraction of differentially expressed genes was up-regulated in response to the induction of OSH1 overexpression in rice [36]. Thus, two lines of evidence indicate that class I members globally act as activators (Figure 3). However, it was shown earlier that a tobacco class I KNOX protein NTH15 directly represses *GA20ox* in the SAM [28]. Therefore, class I KNOX proteins can also act as repressors. The molecular mechanism of activation versus repression is still an open question.

For class II KNOX proteins, only a single direct downstream target has been reported so far. *Arabidopsis* KNAT7, which inhibits the biosynthesis of secondary cell walls in interfascicular fibers of the stem, forms a heterodimer with BLH6, and directly represses *REVOLUTA/INTERFASCICULAR FIBERLESS1 (REV/IFL1)* [44]. Therefore, class II proteins act as repressors. Importantly, the occupancy of KNAT7 on *REV/IFL1* promoter requires BLH6, suggesting that the formation of the heterodimer is essential for their functional binding to targets in vivo.

Another interesting finding from recent studies is that class I and class II KNOX proteins have antagonistic functions. Class I KNOX proteins are specifically expressed in shoot meristems, and their ectopic expression in leaves causes leaf serration, similar to loss-of-function mutants of class II genes. Class II KNOX genes and their putative BLH cofactor SAW2 are

normally expressed in developing leaves but not in the SAM. Importantly, ectopic expression of class II *KNOX* genes and *SAW2* in the SAM resulted in failure of SAM formation/maintenance, similar to *stm* mutants [25]. Because loss-of-function mutant phenotypes of class I and II genes were additive and the expression pattern of class I genes was not altered in class II mutants, it is unlikely that both classes of *KNOX* genes negatively regulate each other. In addition, mutation in *CUC2*, which plays a major role in leaf serration downstream of class I *KNOX* genes, suppressed leaf serration phenotype of class II *KNOX* mutants [45]. These results suggest that both classes of *KNOX* genes have similar downstream targets, but have opposite transcriptional regulatory functions, i.e., STM binds and activates a set of targets for functional meristems while the class II genes may repress this same set (Figure 3).

Conclusion

Functional studies in various plant species reveal that the *KNOX* gene family had been subdivided and neofunctionalized during the evolution of plant lineages. They invariably play important roles in the diploid generation while a role for the haploid generation has not been well documented. These facts raise the possibility that *KNOX* genes have contributed to the innovation of a diploid body plan from unicellular zygotes to multicellular shoot systems. Genome-wide studies opened our view of the downstream networks of class I *KNOX* proteins, that include a much larger number of putative direct targets than previously anticipated. Therefore, a current model of *KNOX* downstream targets, which includes transcription factors and multiple hormonal pathways,

is likely to be part of more complex regulatory networks. In addition, recent genetic and molecular studies of class II *KNOX* genes suggest they have antagonistic function against class I *KNOX* genes in angiosperm. What downstream targets do they share? What happens when they bind their target loci? Does a single *KNOX* protein regulate a target differently depending on cofactors or the developmental context? To answer these questions, the exact combinations and spatio-temporal distribution of *KNOX*-BLH heterodimers in vivo will need to be determined. The identification of other cofactor proteins as well as their genome-scale occupancy is also important. These studies will provide further insights for the function of *KNOX* gene family.

Acknowledgements

This work was supported by NIFA Grant 2012-67014-19429 (to S.H.) and JSPS Postdoctoral Fellowships for Research Abroad (to K.T.).

References

1. Hake S, Smith HMS, Holtan H, Magnani E, Mele G, Ramirez J: The role of *KNOX* genes in plant development. *Annu. Rev. Cell Dev. Biol.* 2004, 20:125-151.
2. Hay A, Tsiantis M: *KNOX* genes: versatile regulators of plant development and diversity. *Development* 2010, 137:3153-3165.
3. Hake S, Vollbrecht E, Freeling M: Cloning Knotted, the dominant morphological mutant in maize using *Ds2* as a transposon tag. *EMBO Journal* 1989, 8:15-22.
4. Vollbrecht E, Veit B, Sinha N, Hake S: The developmental gene Knotted-1 is a member of a maize homeobox gene family. *Nature (London)* 1991, 350:241-243.
5. Derelle R, Lopez P, Le Guyader H, Manuel M: Homeodomain proteins belong to the ancestral molecular toolkit of eukaryotes. *Evol Dev* 2007, 9:212-219.
6. Bertolino E, Reimund B, Wildt-Perinic D, Clerc RG: A novel homeobox protein which recognizes a TGT core and functionally interferes with a retinoid-responsive motif. *J Biol Chem* 1995, 270:31178-31188.

7. Mukherjee K, Brocchieri L, Burglin TR: A comprehensive classification and evolutionary analysis of plant homeobox genes. . Mol. Biol. Evol. 2009, 26:2775-2794.
8. Kerstetter R, Vollbrecht E, Lowe B, Veit B, Yamaguchi J, Hake S: Sequence analysis and expression patterns divide the maize knotted1-like homeobox genes into two classes. Plant Cell 1994, 6:1877-1887.
9. Kimura S, Koenig D, Kang J, Yoong F-Y, Sinha N: Natural Variation in Leaf Morphology Results from Mutation of a Novel KNOX Gene. Current biology : CB 2008, 18:672-677.
10. Magnani E, Hake S: KNOX lost the OX: the Arabidopsis KNATM gene defines a novel class of KNOX transcriptional regulators missing the homeodomain. Plant Cell 2008, 20:875-887.
11. Lee JH, Lin H, Joo S, Goodenough U: Early sexual origins of homeoprotein heterodimerization and evolution of the plant KNOX/BELL family. Cell 2008, 133:829-840.
12. Johnson AD: Molecular mechanisms of cell-type determination in budding yeast. Curr Opin Genet Dev 1995, 5:552-558.
13. Kues U, Richardson WV, Tymon AM, Mutasa ES, Gottgens B, Gaubatz S, Gregoriades A, Casselton LA: The combination of dissimilar alleles of the A alpha and A beta gene complexes, whose proteins contain homeo domain motifs, determines sexual development in the mushroom Coprinus cinereus. Genes Dev 1992, 6:568-577.
14. Sakakibara K, Nishiyama T, Deguchi H, Hasebe M: Class 1 KNOX genes are not involved in shoot development in the moss Physcomitrella patens but do function in sporophyte development. Evol Dev 2008, 10:555-566.
15. Sakakibara K, Ando S, Yip HK, Tamada Y, Hiwatashi Y, Murata T, Deguchi H, Hasebe M, Bowman JL: KNOX2 genes regulate the haploid-to-diploid morphological transition in land plants. Science 2013, 339:1067-1070.
16. Long JA, Moan EI, Medford JI, Barton MK: A member of the KNOTTED class of homeodomain proteins encoded by the SHOOTMERISTEMLESS gene of Arabidopsis. Nature 1996, 379:66-69.
17. Vollbrecht E, Reiser L, Hake S: Shoot meristem size is dependent on inbred background and presence of the maize homeobox gene, knotted1. Development 2000, 127:3161-3172.
18. Tsuda K, Ito Y, Sato Y, Kurata N: Positive autoregulation of a KNOX gene is essential for shoot apical meristem maintenance in rice. Plant Cell 2011, 23:4368-4381.
19. Bharathan G, Goliber TE, Moore C, Kessler S, Pham T, Sinha NR: Homologies in leaf form inferred from KNOX1 gene expression during development. Science 2002, 296:1858-1860.
20. Hareven D, Gutfinger T, Parnis A, Eshed Y, Lifschitz E: The making of a compound leaf: Genetic manipulation of leaf architecture in tomato. Cell 1996, 84:735-744.
21. Alakonya A, Kumar R, Koenig D, Kimura S, Townsley B, Runo S, Garces HM, Kang J, Yanez A, David-Schwartz R, et al.: Interspecific RNA interference of SHOOT MERISTEMLESS-like disrupts Cuscuta pentagona plant parasitism. Plant Cell 2012, 24:3153-3166.

22. Testone G, Condello E, Verde I, Nicolodi C, Caboni E, Dettori MT, Vendramin E, Bruno L, Bitonti MB, Mele G, et al.: The peach (*Prunus persica* L. Batsch) genome harbours 10 KNOX genes, which are differentially expressed in stem development, and the class 1 KNOPE1 regulates elongation and lignification during primary growth. *J Exp Bot* 2012, 63:5417-5435.
23. Li Y, Pi L, Huang H, Xu L: ATH1 and KNAT2 proteins act together in regulation of plant inflorescence architecture. *J Exp Bot* 2012, 63:1423-1433.
24. Hay A, Tsiantis M: A KNOX family TALE. *Curr Opin Plant Biol* 2009, 12:593-598.
25. Furumizu C, Alvarez JP, Sakakibara K, Bowman JL: Antagonistic roles for KNOX1 and KNOX2 genes in patterning the land plant body plan following an ancient gene duplication. *PLoS Genet* 2015, 11:e1004980.
26. Li E, Bhargava A, Qiang W, Friedmann MC, Forneris N, Savidge RA, Johnson LA, Mansfield SD, Ellis BE, Douglas CJ: The Class II KNOX gene KNAT7 negatively regulates secondary wall formation in *Arabidopsis* and is functionally conserved in *Populus*. *New Phytol* 2012, 194:102-115.
27. Gong SY, Huang GQ, Sun X, Qin LX, Li Y, Zhou L, Li XB: Cotton KNL1, encoding a class II KNOX transcription factor, is involved in regulation of fibre development. *J Exp Bot* 2014, 65:4133-4147.
28. Sakamoto T, Kamiya N, Ueguchi-Tanaka M, Iwahori S, Matsuoka M: KNOX homeodomain protein directly suppresses the expression of a gibberellin biosynthetic gene in the tobacco shoot apical meristem. *Genes & development* 2001, 15:581-590.
29. Bolduc N, Hake S: The Maize Transcription Factor KNOTTED1 Directly Regulates the Gibberellin Catabolism Gene *ga2ox1*. *Plant Cell* 2009, 21:1647-1658.
30. Jasinski S, Piazza P, Craft J, Hay A, Woolley L, Rieu I, Phillips A, Hedden P, Tsiantis M: KNOX action in *Arabidopsis* is mediated by coordinate regulation of cytokinin and gibberellin activities. *Curr Biol.* 2005, 15:1560-1565.
31. Yanai O, Shani E, Dolezal K, Tarkowski P, Sablowski R, Sandberg G, Samach A, Ori N: *Arabidopsis* KNOXI proteins activate cytokinin biosynthesis. *Curr Biol.* 2005, 15:1566-1571.
32. Sakamoto T, Sakakibara H, Kojima M, Yamamoto Y, Nagasaki H, Inukai Y, Sato Y, Matsuoka M: Ectopic expression of KNOTTED1-like homeobox protein induces expression of cytokinin biosynthesis genes in rice. *Plant Physiol* 2006, 142:54-62.
33. Mele G, Ori N, Sato Y, S. H: The knotted1-like homeobox gene *BREVIPEDICELLUS* regulates cell differentiation by modulating metabolic pathways. *Genes Dev.* 2003, 17:2088-2093.
34. Townsley BT, Sinha NR, Kang J: KNOX1 genes regulate lignin deposition and composition in monocots and dicots. *Front Plant Sci* 2013, 4:121.
35. Bolduc N, Yilmaz A, Mejia-Guerra MK, Morohashi K, O'Connor D, Grotewold E, Hake S: Unraveling the KNOTTED1 regulatory network in maize meristems *Genes & Development* 2012, 26:1685-1690.
36. Tsuda K, Kurata N, Ohyanagi H, Hake S: Genome-wide study of KNOX regulatory network reveals brassinosteroid catabolic genes

- important for shoot meristem function in rice. *Plant Cell* 2014, 26:3488-3500.
37. Spinelli SV, Martin AP, Viola IL, Gonzalez DH, Palatnik JF: A mechanistic link between STM and CUC1 during Arabidopsis development. *Plant Physiol* 2011, 156:1894-1904.
 38. Kerstetter RA, Laudencia-Chingcuanco D, Smith LG, Hake S: Loss of function mutations in the maize homeobox gene, knotted1, are defective in shoot meristem maintenance. *Development* 1997, 124:3045-3054.
 39. Clark SE, Jacobsen SE, Levin JZ, Meyerowitz EM: The CLAVATA and SHOOT MERISTEMLESS loci competitively regulate meristem activity in Arabidopsis. *Development* 1996, 122:1567-1575.
 40. Belles-Boix E, Hamant O, Witiak SM, Morin H, Traas J, Pautot V: KNAT6: an Arabidopsis homeobox gene involved in meristem activity and organ separation. *Plant Cell* 2006, 18:1900-1907.
 41. Clouse SD, Sasse JM: BRASSINOSTEROIDS: Essential Regulators of Plant Growth and Development. *Annu Rev Plant Physiol Plant Mol Biol* 1998, 49:427-451.
 42. Shani E, Burko Y, Ben-Yaakov L, Berger Y, Amsellem Z, Goldshmidt A, Sharon E, Ori N: Stage-specific regulation of Solanum lycopersicum leaf maturation by class 1 KNOTTED1-LIKE HOMEODOMAIN proteins. *Plant Cell* 2009, 21:3078-3092.
 43. Markel H, Chandler J, Werr W: Translational fusions with the engrailed repressor domain efficiently convert plant transcription factors into dominant-negative functions. *Nucleic Acids Res.* 2002, 30:4709-4719.
 44. Liu Y, You S, Taylor-Teeple M, Li WL, Schuetz M, Brady SM, Douglas CJ: BEL1-LIKE HOMEODOMAIN6 and KNOTTED ARABIDOPSIS THALIANA7 Interact and Regulate Secondary Cell Wall Formation via Repression of REVOLUTA. *Plant Cell* 2014, 26:4843-4861.
 45. Hasson A, Plessis A, Blein T, Adroher B, Grigg S, Tsiantis M, Boudaoud A, Damerval C, Laufs P: Evolution and diverse roles of the CUP-SHAPED COTYLEDON genes in Arabidopsis leaf development. *Plant Cell* 2011, 23:54-68.
 46. Smith HMS, Boschke I, Hake S: Selective interaction of plant homeodomain proteins mediates high DNA-binding affinity. *Proc Natl Acad Sci U S A.* 2002, 99:9579-9584.

Reference annotation

** [15]

The authors found that deletion of class II KNOX genes in *Physcomitrella patens* resulted in formation of haploid gametophyte-like structures from diploid sporophytes without meiosis, indicating that these genes repress a haploid developmental program in the diploid phase. This study suggested

that class II KNOX genes have played an essential role in the evolution of haploid/diploid generation in land plants.

**[25]

The authors found that class II KNOX genes are important for organ differentiation of all above ground organs, distinct from class I gene function, showing an example of neofunctionalization of duplicated genes. In addition, they showed that class II KNOX genes act antagonistically against class I genes in shoot meristems, suggesting that both subclasses might share similar targets but have distinct regulatory activities on target expression.

**[35]

The authors performed ChIP-seq and transcriptome analysis for maize KN1 and found that it directly regulates many developmental regulators including phytohormones and transcription factors. The auxin pathway was highlighted as a major downstream pathway targeted by KN1. This study demonstrated the orchestrating role of KN1 in the regulation of a large developmental gene network.

*[36]

The authors observed an induced overexpression phenotype of rice class I KNOX gene *OSH1*, and found that *OSH1* represses the BR pathway. Using ChIP-seq and a time-course transcriptome analysis, they showed that *OSH1* directly activate BR catabolism genes that are important to prevent premature

differentiation of shoot meristems, indicating that BR catabolism is one of critical downstream pathway of class I KNOX genes.

*[44]

The authors showed that the *Arabidopsis* KNAT7-BLH6 complex directly represses *REV/IFL1* in interfascicular fibers to prevent secondary cell wall biosynthesis. Importantly, BLH6 is required for the KNAT7 occupancy in the target promoter, showing that KNOX-BLH heterodimerization is important for their functional binding in vivo. This is the first report of direct targets of class II KNOX-BLH heterodimer.

Figure 1

Figure 2

Figure 3

Figure 1. Diversification of *KNOX* gene functions in diploid developmental programs during the evolution of plant lineages. Left; phylogeny of plant lineages. Middle; examples of life cycles in the unicellular green alga *Chlamydomonas reinhardtii* (Chlorophytes), the moss *Physcomitrella patens* (Bryophytes), and the flowering plant *Zea mays* (Angiosperms). Diploid bodies are colored in green. Right; functions of *KNOX* genes in diploid developmental programs in each organism.

Figure 2. A model of class I *KNOX* downstream pathways in angiosperm shoot meristems. *KNOX* expression domain in the shoot meristem is colored with light blue.

Figure 3. Antagonistic functions of class I and II *KNOX* proteins. Class I and II *KNOX* proteins are likely to act as activators and repressors, respectively, and may share common downstream targets.