Class III Homeodomain-leucine Zipper Genes in Plant Development

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Abstract: The homeodomain-leucine zipper (HD-Zip) genes containing both a homeodomain and a leucine zipper motif encode plant-specific transcription factors, which belong to the homeobox gene supper family. HD-Zip genes are believed to have key roles in various aspects of plant development. These transcription factors, especially the class III HD-Zip genes, involve in apical embryo patterning, postembryonic meristem initiation, organ polarity and vascular development. According to sequence conservatism, HD-Zip genes can be subdivided into four subfamilies named HD-Zip I, II, III and IV. The class III HD-Zip transcription factors have become the hotspot for studying plant development in recent years. This review focuses on what is known about the function of class III HD-Zip transcription factors and of the encoded proteins.

Key words: Homeodomain-leucine Zipper (HD-Zip), plant development, transcription factor

INTRODUCTION

The homeobox, a DNA sequence motif, was first identified in a set of Drosophila development genes (McGinnis et al., 1984; Scott and Weiner, 1984) and subsequently shown to be present in evolutionary distant organisms, including animals, fungi and plants. In the plant there is a kind of special homeobox genes, which encode transcription factors including a homeodomain structurally characterized by a leucine zipper domain tightly linked to the homeodomain, which called homeodomain-leucine zipper (HD-Zip)(Daima et al., 1995; Carabelli et al., 1993; Mattsson et al., 1992; Schena and Davis, 1992, 1994; Sessa et al., 1994; Söderman et al., 1994). These genes have so far been found only in plants and have key roles in development of plants. According to sequence conservation, HD-Zip genes can be subdivided into four subfamilies, named HD-Zip I, II, III and IV (Sessa et al., 1994).

Functional data available on a subset of the class II genes and I have shown a number of them to be involved in developmental reprogramming in response to changes in environmental conditions (Anna et al., 2004). A number of HD-Zip I proteins have been suggested to be dependent on ABA-signaling for their transcriptional regulation. The class I genes ATHB5, ATHB6 and ATHB7 are suggested to regulate aspects of the plant response to ABA, which are implicated in the plant response to water deficit as deduced from their transcriptional induction by water deficit conditions or ABA treatment (Söderman et al., 1996, 1999; Lee and Chun, 1998; Johannesson et al., 2003). ATHB5 also has a role in the signaling pathway that mediates the inhibitory effect of ABA on growth during early seedling establishment. The class I gene ATHB16 is thought to mediate blue-light responses (Wang et al., 2003) and ATHB13 is suggested to have a role in sucrose signaling (Hanson et al., 2001). The class II genes ATHB2 and ATHB4 are essential for the shade avoidance response (Carabelli et al., 1996; Steindler et al., 1999; Ohashi et al., 2001). Two HD-Zip genes (CPF H-1 and CPF H-2, class II) isolated from C. plantagineum involved in regulation of dehydration responses through different branches of the dehydration-induced signaling network, ABA-independent or ABA-
dependent. The function of Athb-10/GL2 (HD-Zip I) might be different in the two organs, acting as a positive regulator of trichome formation in the shoot and a negative regulator of hair formation in the root (Amna et al., 2004; Cristina et al., 1996; Rerie et al., 1994).

The function of class III HD-Zip genes are the most complicated among these four groups of HD-Zip family, which involve in post embryo development and have an important role in regulating apical embryo patterning, postembryonic meristem initiation, vascular development and leaf polarity (Kirsten et al., 2005; Prigge et al., 2005). There is a dramatically progress in this research area, a number of genes have been isolated. We try to introduce some recently reported certain functions of this gene subfamily.

**STRUCTURE OF CLASS III HD-ZIP**

HD-Zip belongs to homeodomain gene super family, which contains a homebox, a 180 bp consensus DNA sequence, encodes a 60 amino acid protein motif, the homeodomain, which folds into a characteristic DNA-binding structure composed of three K-helices separated by a loop and a turn. Hence, the homeodomain allows the sequence-specific recognition of other genes by homeodomain containing proteins, which act as transcription factors, regulating the expression of the target genes. DNA recognition is established by helix III, which lies within the major groove of DNA and by the N-terminal fixable arm and the loop between helices II and I. Most DNA sequences that are bound efficiently by homeodomains contain the ATTA (TAAT in the complementary strand) core, which interacts with the highly conserved amino acids. HD-Zip III genes are highly conserved in land plants; >50% of the full-length amino acid sequence is conserved between the moss PpHB10 protein and each of the Arabidopsis HD-Zip III proteins (Sakakibara et al., 2001). HD-Zip proteins may have redundant functions, such that one family member might fully or partially substitute for the loss of function of another in specific cells or tissues. Whereas highly conserved, the nonequivalence of HD-Zip III gene function is suggested by the retention of gene pairs from ancient duplication events.

**ARABIDOPSIS HD-ZIP III GENES**

*Arabidopsis* homeobox genes have been identified in studies using mutants, degenerate oligonucleotides deduced from conserved sequences, differential screening or binding to known promoters. According to sequence conservation, plant homeboxes can be subdivided into different families, each comprising several members, till now there were 93 homeobox genes have been submitted to genebank (http://www.ncbi.nlm.nih.gov/). Among these genes, REVOLUTA (REV), INTERFASCICULAR FIBERLESS1 (IFL1), PHAVOLUTA (PHV), PHABULOSA (PHB) and CORONA (CNA) have a close relationship, which belong to HD-Zip III subfamily. Theses genes play overlapping and divergent roles in Arabidopsis development (Prigge et al., 2005).

**HD-ZIP III GENES REGULATE APICAL EMBRYO PATTERNING**

During plant zygotie embryogenesis the cell divisions relatively evenly distributed within the embryo. The apical part of the embryo will become the shoot and the basal part the root. Postembryonic growth, however, is quite different between the two kingdoms. At this stage, plant growth is highly polarized with cell proliferation occurring almost exclusively at the two ends of the longitudinal axis: the root tip and the shoot tip. The seedling, therefore, no longer has a single ‘anterior-posterior’ axis. Instead it has two, oppositely orientied apical basal axes, with the base of both at the root-shoot junction (Jurgens, 2003).

In Arabidopsis three mutants rev phb phv have been discovered and their functions are indicated to be involved in apical embryo patterning. Genetic analysis revealed that REVOLUTA (REV), PHAVOLUTA (PHV) and PHABULOSA (PHB) play the key, overlapping roles in two major processes during embryogenesis: the establishment of apical bilateral symmetry and the establishment of the shoot apical meristem (SAM) (Prigge et al., 2005). Rev phb double mutant plants usually displayed a shoot meristemless phenotype, characterized by the normal production of all embryonic structures, with the exceptions that the SAM was absent and cotyledons were occasionally absent or display patterning defects (Prigge et al., 2005) and no further postembryonic growth occurred in the double mutants. It is suggests that REV is required for adventitious shoot formation. Mutations in the CNA gene similarly enhanced the apical patterning defect of rev phb embryos such that the triple mutant developed a radially symmetric apical structure similar to that of the rev phb phv triple mutant. This indicates The CNA gene also plays a role in apical embryo patterning.

**HD-ZIP III GENES REGULATE POSTEMBRYONIC MERISTEM INITIATION**

A central feature of plant development is the continuous generation of organs throughout the plant's
lifespan. The capacity to generate new aboveground organs post-embryonically is a property of shoot meristems. Within shoot meristems reside stem cells that are maintained at a constant number while giving rise to organ primordia and ultimately all of the differentiated cells of organs and tissues (Steeves and Sussex, 1989). In this way, shoot meristems have the capacity to balance perpetual differentiation of cells while replenishing the pool of undifferentiated, pluripotent cells. Genetic screens have identified several key regulators of shoot meristem development (Barton and Peathig, 1993; Clark et al., 1993; Laux et al., 1996; Pogany et al., 1998; Yu et al., 2000). The WUSCHEL (WUS) gene encodes a homeodomain protein, which is an important regulator of stem cell identity (Mayer et al., 1998; Schoof et al., 2000). Loss-of-function wus mutants fail to organize functional shoot meristems. After germination, wus mutants sporadically generate adventitious shoots, which form only a few organs before termination (Endrizzi et al., 1996; Laux et al., 1996). Expression of WUS within the meristem appears to be sufficient for establishing stem cell identity. When WUS was ectopically expressed, transgenic seedlings accumulate undifferentiated stem cells (Schoof et al., 2000).

Three CLAVATA genes (CLV1, CLV2 and CLV3) promote the differentiation of stem cells. Loss-of-function CLV1, CLV2 and CLV3 mutants accumulate undifferentiated cells in shoot and floral meristems, resulting in meristems that are significantly larger than the wild type and in flowers with increased numbers of floral organs (Clark et al., 1993, 1995; Jeong et al., 1999). The CLV1, CLV2 and CLV3 loci encode signal transduction components: a receptor kinase (Clark et al., 1997), a receptor-like protein (Jeong et al., 1999) and a small secreted protein (Fletcher et al., 1999), respectively. WUS is a key target of the CLV signal transduction pathway (Brand et al., 2000; Schoof et al., 2000). In wild-type plants, the domain of WUS expression is normally restricted to a small, centrally located subset of cells beneath the three outermost cell layers (Mayer et al., 1998; Schoof et al., 2000). In clv3 mutant meristems, the WUS expression domain expands laterally and apically into the topmost cells of the L3 layer (Brand et al., 2000; Schoof et al., 2000). Conversely, plants overexpressing CLV3 recreate the wus phenotype and do not appear to express WUS mRNA (Brand et al., 2000), indicating that the CLV signaling pathway limits stem cell number by restricting the size of the WUS expression domain. Overexpression of WUS through promoter fusions can also lead to ectopic stem cell accumulation (Schoof et al., 2000). Interestingly, transcripts of CLV3 are found on the periphery of stem cell masses formed by WUS overexpression, whereas in the meristems of wild-type plants, CLV3 expression is restricted to the center of the shoot meristem (Fletcher et al., 1999; Schoof et al., 2000). These expression analyses indicate that while the CLV signaling pathway targets WUS and restricts its activity, WUS activity is also sufficient to induce transcription of CLV3. This regulatory feedback loop may act to maintain strict control of the number of stem cells. Organogenesis at the shoot meristem requires a delicate balance between stem cell specification and differentiation. In Arabidopsis thaliana, WUSCHEL (WUS) is a key factor promoting stem cell identity, whereas the CLAVATA (CLV1, CLV2 and CLV3) loci appear to promote differentiation by repressing WUS expression. In a screen for mutations modifying clv1 mutants, a novel regulator named CORONA (CNA) has been identified, which controls meristem's development. clv cna double mutants develop massively enlarged apices that display early loss of organogenesis, misexpression of WUS and CLV3 and eventual differentiation of the entire apex. The CNA gene was isolated by positional cloning and found to encode a class III homedomain leucine zipper protein. A missense mutation resulting in the dominant-negative cna-1 allele was identified in a conserved domain of unknown function and a likely null allele was shown to display a similar but weaker phenotype. CNA is expressed in developing vascular tissue, diffusely through shoot and flower meristems and within developing stamens and carpels. Analysis of WUS expression in wild type, clv, clv and cna plants revealed that contrary to current models, WUS is neither necessary nor sufficient for stem cell specification and that neither WUS nor CLV3 is a marker for stem cell identity. It is suggested that CNA functions in parallel to the CLV loci to promote organ formation (Kirsten et al., 2005). Besides CAN there are several other genes also regulating postembryonic meristem initiation. REV is required for the formation of lateral shoot meristems (LSM) and floral meristems (FM) as well as adventitious shoots (Otsuga et al., 2001; Prigge et al., 2005). rev mutants are characterized by rosette and cauline leaves with barren axils and flowers lacking full meristematic activity, although these phenotypes are variably expressive (Otsuga et al., 2001; Prigge et al., 2005; Talbert et al., 1995). The PHV gene appears to play a lesser role in LSM function. CNA and ATHB8 play roles antagonistic to REV, PHB and PHV in the formation of LSM, with CNA and ATHB8 promoting meristem activity (Prigge et al., 2005).
HD-ZIP III GENES REGULATE LEAF POLARITY

Based on gain-of-function alleles, HD-Zip III Genes are most important for patterning in lateral organs, especially *PHB* and *PHV*. Gain-of-function mutations in HD-Zip III Genes affected a sterol-binding domain that is conserved in these proteins, suggesting that they act as receptors for a sterol signal from the meristem (McConnell et al., 2001). Subsequently, however, these mutations were shown to prevent microRNA (miRNA)-directed mRNA cleavage (Bartel, 2004) that restricts HD-Zip expression to the adaxial side of the leaf primordium (Tang et al., 2003). A loss of miRNA-mediated negative regulation could account for both the spatial expansion and the increase in expression levels, although positive autoregulation cannot be discounted (McConnell et al., 2001). The control of polarity in the leaves and stem vasculature by the same mechanism (i.e., by HD-Zip genes that are downregulated on the abaxial side by miRNAs and by *KANADI* genes) has been confirmed (Emery et al., 2003; McHale and Koning, 2004).

ROLES OF HD-ZIP III GENES IN VASCULAR DEVELOPMENT

Vascular development involves the formation of provascular cells that give rise to the procambium and after specific events of coidifferentiation, to both conducting tissues (Steeves and Sussex, 1989). The histological analysis of transgenic plants suggests that *ATHB-8* is likely to act as a differentiation-promoting transcription factor regulating the activity of procambial and cambial cells. The expression of *ATHB-8* is modulated by auxin. As a positive regulator, auxin activates the provascular cells, which stimulate the expression of the *ATHB-8* gene and subsequently, cell division and cyto-differentiation toward the formation of the vascular tissue. Through the transgenic tobacco experiment (Baima et al., 1995), it is suggested that *ATHB-8* is involved in revascularization processes caused by wounding.

CONCLUSIONS

In the plant lifespan post-embryogenesis is important and complex, which relates to a precise order of events ensuring the correct relative positioning of embryonic organs the shoot and root stem-cell systems (i.e., meristems), cotyledons and the hypocotyl and the correct arrangement of different cell types within each organ. Apical embryo patterning, postembryonic meristem initiation, leaf polarity and vascular development have been found to be regulated by a series of genes and plant hormones, among which HD-Zip III genes are research hot spots in last three years. Class III HD-Zip genes are found to be involved in the processes during embryogenesis: the establishment of apical bilateral symmetry and the establishment of the shoot apical meristem (SAM). *CAN* (Class III HD-Zip) can promote differentiation by repressing *WUS* expression and keep the balance between stem cell specification and differentiation and regulates the postembryonic meristem initiation. Class III HD-Zip transcription factors also involve in auxin mediated polarity. Class III HD-Zip genes downregulated on the abaxial side by miRNAs and by *KANADI* genes can specify adaxial development. Finally *ATHB-8* modulated by auxin, as a differentiation-promoting transcription factor regulates the activity of procambial and cambial cells and promotes formation of the vascular tissue.

By suppression subtractive hybridization (SSH), we isolated a class III HD-Zip gene *GbHBI* (NCBI AY996446). Transformation study showed that it could partially recover the type till mutant of *Arabidopsis*, suggesting that *GbHBI* has a role in vascular development (data not shown). Although dramatically progress has been made in this area, there is still a long way to unveil the precise mechanism of class III HD-Zip genes in plant development.

ACKNOWLEDGMENTS

This research is financially supported by the National Basic Research Program (973) of China (No.2004CB117300) and China Ministry of Education.

REFERENCES


