[Plant Signaling & Behavior 4:2, 86-88; February 2009]; ©2009 Landes Bioscience

Mini-Review

Structure and function of homodomain-leucine zipper (HD-Zip) proteins

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Abbreviation: HD-Zip, homeodomain-leucine zipper; ABA, abssisic acid; SAM, shoot apical meristem

Key words: homeodomain-leucine zipper, development, structure, function, signaling, embryogenesis

Homeodomain-leucine zipper (HD-Zip) proteins are transcription factors unique to plants and are encoded by more than 25 genes in *Arabidopsis thaliana*. Based on sequence analyses these proteins have been classified into four distinct groups: HD-Zip I–IV. HD-Zip proteins are characterized by the presence of two functional domains; a homeodomain (HD) responsible for DNA binding and a leucine zipper domain (Zip) located immediately C-terminal to the homeodomain and involved in protein-protein interaction. Despite sequence similarities HD-ZIP proteins participate in a variety of processes during plant growth and development. HD-Zip I proteins are generally involved in responses related to abiotic stress, abscisic acid (ABA), blue light, de-etiolation and embryogenesis. HD-Zip II proteins participate in light response, shade avoidance and auxin signalling. Members of the third group (HD-Zip III) control embryogenesis, leaf polarity, lateral organ initiation and meristem function. HD-Zip IV proteins play significant roles during anthocyanin accumulation, differentiation of epidermal cells, trichome formation and root development.

Structure of HD-Zip Proteins

Homeodomain leucine zipper (HD-Zip) proteins are transcription factors classified into four different groups based on gene structure, presence of unique domains and function.[1] Unique features of all HD-Zip members are the presence of a homeodomain (HD) and a leucine zipper motif (Zip). The HD domain is involved in DNA binding whereas the Zip domain is involved in protein homo and heterodimerization.[2] Functional studies using truncated proteins have shown the requirement of the Zip motif for the DNA binding ability of the HD domain.[3]

In Arabidopsis the HD-Zip class I comprises seventeen members encoding proteins of similar size (~35 kDa) including a well conserved HD domain and a less conserved Zip motif. PCR-assisted binding site selection and footprinting assays showed the ability of HD-Zip I proteins to recognize and bind the pseudopalindromic sequence CAAT(A/T)ATTG.[4] The affinity, but not the specificity, of protein-DNA interaction is affected by sequences located at the N-terminal of the HD-Zip I proteins.[5]

HD-Zip class II is composed of nine members (*ATHB2*/*HAT4*, *ATHB4*, *HAT1-HAT3*, *HAT9*, *HAT14*, *HAT17* and *HAT22*) in Arabidopsis. All these members are characterized by the presence of a third domain, known as CPSCE, located downstream of the Zip motif and involved in cellular redox status perception.[6] HD-ZipII proteins are able to recognized DNA regions with the pseudopalindromic sequence CAAT(C/G)ATTG. Only five members are included in the HD-Zip class III in Arabidopsis (*ATHB8*, *PHAVOLUTA*/*ATHB9*, *PHABULOSA*/*ATHB14*, *CORONA*/*ATHB15* and *REVOLUTA*/*IFL1*). Members of this group also share three additional domains: a MEKHLA domain possibly involved in oxygen redox and light signaling,[7] a START domain motif with putative lipid binding capability,[8] and a SAD domain which is a transcriptional activation domain.[9] In vitro experiments have shown that, *ATHB9*, a member of this class, has high affinity for the pseudopalindromic sequence GTAAT(G/C)ATTAC.[10]

HD-ZipIV comprises sixteen members in Arabidopsis including *GLABRA 2*.[1] Despite lacking a MEKHLA domain, members of this group have a START and SAD motifs. HD-Zip IV proteins target the sequence which is characterized by TAAA core.[11]

Biological Functions of HD-Zip Proteins

Members of HD-Zip class I are generally involved in abiotic stress responses such as water and light stress[12,13] Expression studies reveal that *ATHB12* and *ATHB7* are upregulated by water-limiting conditions and applications of ABA.[14] *ATHB6*, another member of this class is a crucial regulator in the ABA signal pathway possibly by interacting with *ABI1*.[15] Evidences that HD-Zip class I proteins might be implicated in ABA responses have also been shown in *Picea glauca* (white spruce) where *PgHZ1* increases ABA sensitivity and promotes embryogenesis in vitro.[16] Other members of this class, including *ATHB52*, are affected by light conditions and play a key role during photomorphogenesis and de-etiolation.[17]

Proteins of class II are mainly involved in phototropism and auxin responses as revealed by expression and transformation studies.[18] In Arabidopsis shade avoidance response is regulated by *ATHB-2* through complex mechanisms involving three distinct phytochromes. Hypocotyl elongation, similar to that observed in wild type plants grown under far red light conditions, was induced in Arabidopsis seedlings overexpressing *ATHB-2*. By contrast an opposite phenotype

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Submitted: 12/19/08; Accepted: 12/23/08

was observed in plants with reduced *ATHB-2* expression. A model explaining these phenotypes in relation to auxin distribution has been reviewed by Morelli and Ruberti.[19] Tissue elongation was also induced by high expression of *HAT-2*, an auxin-inducible gene of the same class II.[20] Response to dark and light conditions also involves *HAHB-10*, a sunflower gene with high similarity to *ATHB-2*. Ectopic expression of *HAHB-10* in Arabidopsis produced a variety of phenotypic deviations, including dark cotyledons, planar leaves, reduced life cycle and accelerated flowering.[21]

Members of HD-Zip class III play an important role during morphogenesis. Three proteins of this class, *REV*, *PHB* and *PHV*, control the pattern of apical formation during embryo development.[22] Mutant analyses demonstrated the role of *REV* and *PHB* in regulating shoot apical meristem (SAM) maintenance and lateral organ initiation.[23] Regulation of these morphogenic events might be caused by changes in auxin flow, since several members of HD-Zip class III have been implicated in events leading to changes of polar auxin transport.[24] Several studies have elucidated the mode of action of *REV*, *PHB* and *PHV*, together with *KANADI* in controlling abaxial-adaxial patterning of lateral organs.[25] The abaxilation process and phloem differentiation are initiated by *KANADI* which also represses the expression of *REV*, *PHB* and *PHV*. This repression is gradually released and expression of these three genes inhibits *KANADI* through feedback mechanisms and results in the adaxilation of the lateral organ and xylem formation.[25] Another member of this class, *ATHB-8* is also implicated in vascularization.[26] Production of xylem is significantly increased in Arabidopsis plants overexpressing this gene suggesting a possible role for *ATHB-8* in inducing xylem element differentiation.[26] This control however only occurs in the presence of specific cues since a reciprocal phenotype was not observed in lines with low *ATHB-8* expression.

The expression of several members of HD-Zip class IV is often restricted in the outer cells of plant organs where they regulate processes such as epidermal fate, trichome formation and anthocyanin accumulation.[23,27,28] The lack of epidermal cell identity in leaves of *pdf2/atml1* double mutants argues for their involvement in epidermal identity acquisition. These genes might work in redundant fashion as denoted by the high degree of similarity. Proper trichome formation is also under the control of several members of this class. Genetic analyses revealed that *hdg11/hdg12* double mutants produce highly branched trichomes. Further studies have revealed that HD-Zip IV members act redundantly with each other during developmental events.[29] Another gene of this class *ANL2* is also involved in biosynthesis of anthocyanin in subepidermal tissues and cellular organization of primary root.[28]

Conclusions

HD-Zip proteins are transcription factors unique to plants characterized by a homeodomain and a leucine zipper motif. Despite these structural similarities HD-Zip proteins participate in diverse and sometimes overlapping events ranging from stress responses to morphogenesis and development. Genetic analyses have revealed that their functions rely on the activation of downstream target genes the majority of which remain unknown. Elucidation of these downstream events will be key in understanding the role played by this important class of transcription factors.

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