Molecular Plant • Volume 6 • Number 6 • Pages 1758–1768 • November 2013 REVIEW ARTICLE

Z-Box Binding Transcription Factors (ZBFs): A New Class of Transcription Factors in *Arabidopsis* Seedling Development

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ABSTRACT One set of genes encoding diverse groups of transcription factors that interact with the Z-box (ATACGTGT; a potential Z-DNA forming sequence) is called ZBFs (Z-box Binding Factors). *ZBFs* include *ZBF1*, *ZBF2*, and *ZBF3*, which encode ZBF1/MYC2 (bHLH), ZBF2/GBF1 (bZIP), and ZBF3/CAM7 (Calmodulin) proteins, respectively. With several recent reports, it is becoming increasingly evident that ZBFs play crucial roles in *Arabidopsis* seedling photomorphogenesis. ZBFs integrate signals from various wavelengths of light to coordinate the regulation of transcriptional networks that affect multiple facets of plant growth and development. The function of each ZBF is qualitatively and quantitatively distinct. The *zbf* mutants display pleiotropic effects including altered hypocotyl elongation, cotyledon expansion, lateral root development, and flowering time. In this inaugural review, we discuss the identification, molecular functions, and interacting partners of ZBFs in light-mediated *Arabidopsis* seedling development.

Key words: photomorphogenesis; transcription factor; Z-box; G-box; MYC2/ZBF1; GBF1/ZBF2; CAM7/ ZBF3.

INTRODUCTION

Plants are sensitive to altered quality, intensity (quantity), and duration of light. To cope with the fluctuating light environments, plants are evolved with intrinsic signaling processes that can absorb light, and subsequently integrate into the system for growth and development. The light signals are perceived by different types of specialized photoreceptors: far-red light and red light absorbing phytochromes (phyA, and phyB to phyE), blue light (BL) and UV-A light absorbing cryptochromes (cry1 to cry3) and phototropins (phot1 and phot2), and UV-B light absorbing UVR8 photoreceptor (Cashmore et al., 1999; Schepens et al., 2004; Jiao et al., 2007; Chen and Chory, 2011; Rizzini et al., 2011). Light has a major role to play at the early stage of development such as seed germination to appearance of the first pair of true leaves. Seedlings survive on reserved food material and gradually become photoautotrophic at this stage of development. If light is a limiting factor at this stage, the seedlings exhibit etiolated or skotomorphogenic growth, which is characterized by elongated hypocotyl, closed and small cotyledons (photosynthetically inactive or less active) (Jiao et al., 2007; Chen and Chory, 2011). In contrast, in the presence of light, seedlings follow deetiolated or photomorphogenic growth characterized by short hypocotyl with open, expanded, and photosynthetically active cotyledons, and well-developed root system (Bae and Choi, 2008; Arsovski et al., 2012).

Genome-wide gene expression studies have demonstrated the occurrence of large-scale (nearly 33%) transcriptional reprogramming during transition from skotomorphogenesis to photomorphogenesis (Ma et al., 2001; Tepperman et al., 2001). This global change in transcription profile is preceded by the perception of light through photoreceptors and subsequently transducing the signal to downstream key transcription factors (TFs). These TFs in turn help the regulation of downstream TFs and regulatory genes, which are directly or indirectly associated with various light-dependent physiological and metabolic processes (Jiao et al., 2007; Chen and Chory, 2011). In order to understand the transcriptional regulatory networks, it is indeed important to investigate the function of individual TFs and the downstream molecules associated with it. Furthermore, it is important to determine the molecular and functional relationships among these TFs. Analyses of the promoters of light-regulated genes have identified several commonly occurring *L*ight *R*esponsive *E*lements (LREs) such as G, GATA, GT1 and Z-box. These LREs have been reported to play crucial role in light-mediated activation of transcription (Ha and An, 1988; Donald and Cashmore, 1990; Gilmartin et al., 1990; Puente et al., 1996; Ang et al., 1998;

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doi:10.1093/mp/sst140, Advance Access publication 24 October 2013

Received 1 September 2013; accepted 28 September 2013

Arguello-Astorga and Herrera-Estrella, 1998; Chattopadhyay et al., 1998a, 1998b; Zhou, 1999; Tepperman et al., 2001; Yadav et al., 2002; Castillon et al., 2007; Leivar and Quail, 2011; Gangappa et al., 2013).

Z-box LRE has been found to be present in *CAB1* (which encodes for *chlorophyll a/b binding protein1*), a light-regulated gene in *Arabidopsis* (Ha and An, 1988). Mutation and deletion analysis of *CAB1* promoter have led to the conclusion that Z-box is essential for the light-mediated expression of *CAB1* (Ha and An, 1988). Later, it has been shown that Z-box as a single LRE or paired with another LRE responds to different wavelengths of light (Puente et al., 1997; Yadav et al., 2002). The involvement of cryptochromes and phytochromes has been shown be critical for the light-mediated repression of Z-box-containing promoters (Yadav et al., 2002). Additionally, downstream signaling components such as HY5 and COP1 have been shown to regulate the expression of the Z-box-containing promoters (Yadav et al., 2002). Although the Z-box binding factor (ZBF) activity was reported in 2002 (Yadav et al., 2002), the identification of several ZBFs in *Arabidopsis* has been reported in 2005 (Yadav et al., 2005). Thus far, three such ZBFs (ZBF1, ZBF2, ZBF3) have been shown to bind to Z- and G-box LREs present in *CAB1* and *RBCS-1A* promoters, respectively (Yadav et al., 2005; Mallappa et al., 2006; Kushwaha et al., 2008). Importantly, these ZBFs were found to play crucial roles in light-mediated seedling development in *Arabidopsis* (Yadav et al., 2005; Mallappa et al., 2006; Kushwaha et al., 2008). This review summarizes in brief the identification of ZBFs and their physiological roles in light-mediated seedling development in *Arabidopsis* (Figure 1).

IDENTIFICATION OF Z-BOX BINDING FACTORS (ZBFS)

To identify ZBFs, a ligand-binding screen was set up in which a cDNA expression library was screened using the dimeric Z-box (Yadav et al., 2005). This particular screen resulted in the identification and cloning of several *ZBFs* and, among those, three ZBFs have thus far been functionally characterized: ZBF1 (At1g32640), ZBF2 (At1g36730), and ZBF3 (At3g43810). The specificity of ZBFs binding to the Z-box LRE was established when tertiary screening plaques were blotted on to the membrane and cut into two halves; one half was probed with the Z-box and the other half was probed with other LREs. The strong binding activity found with the Z-box LRE but not with other LREs such as GT1 and GATA demonstrated that ZBFs specifically interact with the Z-box LRE (Yadav et al., 2005; Mallappa et al., 2006; Kushwaha et al., 2008) (Table 1). Hence ZBF1, ZBF2, and ZBF3 are bona fide Z-box binding factors present in *Arabidopsis*. It should be noted here that ZBF1, ZBF2, and ZBF3 were previously reported as MYC2, GBF1, and CAM7, respectively (Schindler et al., 1992; Abe et al., 1997; McCormack et al., 2005).

MYC2/ZBF1 was previously identified from ligand-binding screen by two independent groups and designated as RAP1 (de Pater et al., 1997) and AtMYC2 (Abe et al., 1997, 2003). Various studies revealed that RAP1/AtMYC2 protein interacted with the G-box in pea (*Pisum sativum*) lectin promoter (de Pater et al., 1997) and CACATG sequence, a dehydration-responsive *cis*-acting element in *rd22* promoter (Abe et al., 2003). Subsequently, the AtMYC2 homolog of tomato, LeMYC2, has been shown to interact with AAACGTG element present in *LAP2* promoter (Boter et al., 2004). A number of G-box variants are also recognized by MYC2/ZBF1 (Dombrecht et al., 2007) (Table 1). GBF1/ZBF2 protein was earlier shown to interact with the G-box LRE (Giuliano et al., 1988). In fact, GBF1/ZBF2 is a member of G-box binding factor (GBF) family and forms G-box binding heterodimers with other family members (Schindler et al., 1992). CAM7/ZBF3 is a Ca^{+2} binding calmodulin (CaM) protein, and is a unique member of the seven-member calmodulin family in *Arabidopsis* (McCormack et al., 2005). Identification of CAM7 as ZBF provided the first evidence of calmodulin protein working as a transcriptional regulator (Kushwaha et al., 2008). Due to sequence similarity of the Z-box with G-box LRE and previous reports available, it was further tested by DNA–protein interaction studies whether MYC2/ZBF1, GBF1/ZBF2, and CAM7/ZBF3 could also bind to the G-box LRE. It has been shown that these proteins indeed bind to the G-box LRE present in *RBCS-1A* minimal promoter (Yadav et al., 2005; Mallappa et al., 2006; Kushwaha et al., 2008) (Table 1).

DIFFERENTIAL EXPRESSION OF *ZBFS* AND PROTEIN STABILITY

MYC2 equally expresses in light- or dark-grown seedlings (Yadav et al., 2005). The level of expression of MYC2 is similar in RL and BL; however, in FR, the expression is lesser in comparison to other wavelengths of light (Yadav et al., 2005). Expression in different developmental stages as obtained from digital Northern data (www.geneinvestigator.com) suggests that MYC2 belongs to moderately expressed gene category, and its expression is low in early seedling stage and mature seeds as compared to other developmental stages such as juvenile or reproductive stage (Figure 2) (Zimmermann et al., 2004). Transcription analyses in different tissue types suggest that *MYC2* expresses in all major tissue types (Yadav et al., 2005). The digital Northern blot data obtained from the Genevestigator database further support these observations (Figure 3) (Zimmermann et al., 2004). However, jasmonic acid or abscisic acid (ABA) treatment further increases *MYC2* expression (Anderson et al., 2004; Boter et al., 2004). Recently, it has been reported that MYC2 protein follows rhythmic expression, and its expression is dramatically elevated at the end of the day (Shin et al., 2012). Similarly to transcript level, MYC2 protein level also increases with JA treatment (Shin et al., 2012). Recently, it has been shown that TIME FOR COFFEE (TIC), a circadian clock component, negatively regulates MYC2 protein stability. MYC2 has also been shown to undergo proteasomal degradation, since the application of proteasomal inhibitors increase the stability of MYC2 protein at night (Shin et al., 2012).