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Review

The zinc finger network of plants

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Abstract. The zinc finger domain enables different proteins to interact with or bind DNA, RNA, or other proteins, and is present in the proteomes of many different organisms. Proteins containing zinc finger domain(s) were found to play important roles in eukaryotic cells regulating different signal transduction pathways and controlling processes, such as development and programmed cell death. There are many types of zinc finger proteins, classified according to the number and order of the Cys and His residues that bind the Zinc ion. Among these, the C2H2-type zinc finger proteins, with 176 members in *Arabidopsis thaliana,* constitute one of the largest families of transcriptional regulators in plants. They are mostly plant-specific and contain a conserved QALGGH sequence within their zinc finger domain. Recent studies revealed that C2H2 zinc finger proteins could function as key transcriptional repressors involved in the defense and acclimation response of plants to different environmental stress conditions. Here we highlight recent functional characterization studies of different C2H2 proteins in *Arabidopsis*, and suggest that many of these proteins function as part of a large regulatory network that senses and responds to different environmental stimuli.

Keywords. C2H2, zinc finger, EAR motif, abiotic stress, *Arabidopsis.*

Introduction

Zinc finger proteins play a critical role in many cellular functions, including transcriptional regulation, RNA binding, regulation of apoptosis, and protein-protein interactions. They are classified into several different types including C2H2, C2C2, C2HC, C2C2C2C2, and C2HCC2C2 based on the number and order of the Cys and His residues that bind the Zinc ion in the secondary structure of the finger [1–3]. Among the different zinc finger types, C2H2-type zinc finger proteins are one of the best studied and most abundant in eukaryotes [4]. According to *in silico* analysis, ~3% of all genes in mammals, ~0.8% of all genes in *Saccharomyces cerevisiae* and ~0.7% of all genes in *Arabidopsis* encode C2H2-type zinc finger proteins [5]. Even though many of these proteins are thought to mainly bind DNA, some are also thought to bind RNA and protein, and a subclass of zinc finger proteins is thought to specifically bind RNA [6]. In this review we focus on the C2H2 class of plant zinc finger proteins and present new findings regarding their role in regulating signal transduction events in plants.

The C2H2-zinc finger motif was first discovered in the *Xenopus* oocytes transcription factor TFIIIA about 17 years ago [7]. Early studies suggested that it associates with 5S rRNA within a 7S particle in *Xenopus* [8], but later studies suggested that it might

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bind to DNA and regulate the expression of the 5S rRNA gene [9]. Nowadays, some C2H2-zinc finger proteins are still referred to as TFIIIA-type zinc finger proteins. The first report of C2H2-zinc finger proteins in plants occurred in 1992 for the DNA-binding protein of petunia, ZPT2–1 (previously named as EPF1) [10]. Soon after, WZF1 was reported in wheat as a DNA-binding zinc finger protein that interacts with a *cis* element of histone genes [11]. Subsequently, many other TFIIIA-type zinc-finger proteins have been reported from different plant species including wheat, petunia, *Arabidopsis* and rice.

C2H2-type zinc finger proteins contain one of the best-characterized DNA-binding motifs found in eukaryotes. This motif consists mostly of about 30 amino acids and includes two conserved Cys and two conserved His residues bound to one zinc ion tetrahedrally, and is represented as $CX_{2-4}CX3FX5$-$LX2HX_{3-5}H$ (see example in Fig. 1) [12]. Each finger forms two β strands and one α helix. A recent *in silico* analysis revealed that there are 176 C2H2-type zinc finger proteins in *Arabidopsis thaliana* with only 33 of them conserved with other eukaryotes and 81 % of them are plant specific [5]. Two main structural features, found in most of the C2H2-type plant zinc finger proteins, distinguish them from other eukaryotes [13]: (i) In multiple fingered plant C2H2-type proteins, the zinc finger domains are separated by long spacers that vary in length and sequence from protein to protein [14], whereas in yeast and animals, the C2H2-type fingers are mostly clustered and separated by a short spacer (six to eight amino acids) known as an H-C link [1]; and (ii) most of the plant zinc finger proteins have an invariant QALGGH motif in the zinc finger helices, while animal and yeast lack this motif [13]. *In vitro* binding analysis revealed that the conserved QALGGH motif in plants plays a critical role in DNA binding activity. It has been shown that each amino acid of this conserved sequence is essential for the DNA-binding activity of C2H2-type zinc finger proteins [15]. Thus, substitution of any of the A, L, G, G or H residues of the first finger of the ZPT2–2 protein resulted in a complete loss of DNA-binding ability of ZPT2–2, whereas substitution of the Q residue significantly reduced DNA-binding ability of ZPT2–2 [15]. Another study performed with substituting the second G residue of the SUPERMAN protein, which contains only one C2H2-type zinc finger, to D resulted in loss of function of SUPERMAN [16].

Englbrecht et al. [5] used different criteria, including zinc finger position and sequence, to divide all *A. thaliana* C2H2-type zinc finger proteins into three different sets (A, B and C), each divided into different subsets (e.g., C1, C2 and C3), which in turn are divided into different families and subclasses [5]. Pair-wise distance analysis revealed that A1 and C1 family members have smaller pair-wise distances than C2 and C3 subsets [5]. Many members of subset C2 and subset C3 are involved in ancient cellular pathways such as RNA metabolism, whereas almost all members of A1 and C1 families are plant and *Arabidopsis* specific and are involved in processes such as development and stress responses, suggesting that C2 and C3 subsets are evolutionary older than A1 and C1 families [5]. Among the plant-specific C2H2-type zinc finger proteins, A1 with 24 members and C1 with 64 members are the largest and evolutionary youngest families [5]. A1 family members consist of tandemly organized zinc finger domains, whereas C1 family members have either one isolated or two to five dispersed zinc finger domains [5].

C2H2-type zinc finger proteins play a crucial role in many metabolic pathways as well as in stress response and defense activation in plants. Recent studies emphasized the importance of C2H2-type zinc finger proteins with a putative repression activity to the defense and stress response of plants. Most of these proteins are thought to acquire their repression activity *via* their ethylene-responsive element-binding factor (ERF)-associated amphiphilic repression (EAR) domain (described below). Recent studies performed with the C1 C2H2-type zinc finger family suggested that these proteins play key roles in different developmental pathways, as well as in the defense and stress response pathways of *Arabidopsis*. These are described in detail below.

C1 family

The C1 family of plant zinc finger proteins contains 64 members and is one of the largest and evolutionary youngest zinc finger families [5]. C1 family members contain either one isolated or two to five dispersed C2H2-type zinc fingers (indicated by the acronym 'i'; e.g., C1–2i), and are classified according to their number of zinc fingers; 1i donates one finger, 2i two fingers, etc. [5]. The C1 subclasses include C1–1i (33 members), C1–2i (20 members), C1–3i (8 members), C1–4i (2 members) and C1–5i (1 member) [5]. Among these subclasses, members of C1–1i and C1–2i are some of the most investigated plant C2H2-type zinc finger proteins.

C1–1i subclass

C1–1i with 33 members is the largest subclass of the C1 family. Members of C1–1i have only one C2H2-

type zinc finger domain, most of which contain the conserved QALGGH motif [5]. In recent years, many members of the C1–1i subclass have been investigated. One of these proteins is Telomerase Activator 1 (TAC1, At3g09290). Studies showed that in the presence of endogenous auxin TAC1 can induce telomerase expression in non-cycling cells [17]. A later study showed that BT2, a calmodulin-binding protein, is also required for TAC1-related telomerase expression [18]. Recent studies suggested that TAC1 might play a role in the auxin-signaling pathway involved in telomerase induction. Another C1–1i subclass member investigated is Glabrous Inflorescence Stems (GIS, At3g58070). GIS plays a role in shoot maturation in *Arabidopsis* [19]. It plays a role in trichome initiation downstream of the giberellin (GA)-signaling pathway during inflorescence development [19]. Cytokinin-induced trichome initiation requires two other members of the C1–1i subclass: ZFP8 (At2g41940) and GIS2 (At5g06650) [20]. Both proteins are also required for giberellin-induced trichome initiation, which is interesting because several papers have suggested that giberellin and cytokinin work antagonistically [20]. Even though GIS, GIS2 and ZFP8 seem redundant in function, they are all regulated differentially during giberellin and cytokinin signaling. Other redundantly working members of C1–1i subclass include JAGGED (At1g68480) and NUBBIN (At1g13400). Both proteins play a role in microsporangia growth of anthers and the valves that are close to the apical region of gynoecium that encloses the ovules [21]. They play a role in specifically defining cell layer numbers and differentiation of adaxial cell types of the carpel walls of gynoecium [21]. One of the most investigated C1–1i subclass member is SUPERMAN (SUP, At3g23130). It has been proposed that SUP maintains the boundary between the third and fourth whorls of the flower [16]. SUP can bind to DNA through its zinc finger domain and two basic regions that surround the domain, suggesting that SUP acts as a transcription factor [22]. Later studies showed that other SUP-like proteins play a role in the development of *A. thaliana.* One of these proteins is RABBIT EARS (RBE, At5g06070). RBE has been proposed to play a role in early development of the organ primordia of the second whorl and maintain the boundaries of homeotic gene expression between whorls [23, 24]. Several studies have suggested that RBE might play a role as a repressor and obtain this ability through its EAR domain [24]. KNUCKLES (KNU, At5g14010), also encodes a SUP-like protein suggested to a play role as a transcriptional repressor of cellular proliferation [25].

C1–3i, C1–4i and C1–5i subclasses

Subclass C1–3i consists of eight C2H2-type zinc finger proteins all with three dispersed zinc finger domains [5]. Among them only Zat1 (At1g02030) has been previously characterized [26]. C1–4i subclass has two members with four dispersed zinc finger domains and C1–5i has only one member, which has five dispersed zinc fingers [5]. To the best of our knowledge, the function(s) of both C1–4i and C1–5i subclass members are unknown at present.

C1–2i subclass

The C1–2i subclass contains 20 members including Zat5, Zat6, Zat7, Zat8, Zat10, Zat11, Zat12, Zat13, Zat14, Zat15, Zat16, Zat17, Zat18, AZF1, AZF2, AZF3, At5g04390, At1g02040, At2g26940, and At4g04404 that show extensive homology at their first and second zinc finger domains (Fig. 1) [5]. Most of these proteins were isolated by homology-based cloning [26], and all members consist of two dispersed C2H2-type fingers [5, 26].

Eighteen of the C1–2i subclass members contain the invariant QALGGH motif in both their zinc finger helices (Fig. 1) [5]. However, it is unclear at this point whether members that lack this motif are different in their function from members that contain it. Other than the zinc finger domains, most members also share several putative nuclear localization sequences and an EAR motif [L/FDLNL/F(x)P] that is thought to have an active repression activity and is found at the C terminus of the proteins (Fig. 1C) [5, 26]. A neighbor joining tree analysis performed for the different C1–2i members revealed that several of these members could be the result of recent gene duplication (e.g., Zat10 and Zat6, Zat11 and Zat18, and Zat 7 and Zat8; Fig. 1D).

The EAR motif was first identified in the AP2/ERF domain proteins [27]. AP2/ERF (or ERF proteins) domain proteins are plant-specific transcription factors that consist of a DNA-binding domain named the ERF domain [28–30]. ERF proteins bind to the core sequence of a conserved ethylene-responsive element (GCC box) that is found in the promoters of many defense and stress response genes [31, 32]. Many genes that encode ERF proteins are thought to play a role in plant growth, development and response to biotic or abiotic stresses [27, 32]. *In silico* analysis identified over 124 genes that contain the ERF domain in plants [33].

Homology studies showed that there are two different classes of ERF proteins: class I ERFs and class II ERFs [30]. Class I ERF proteins act as activators of

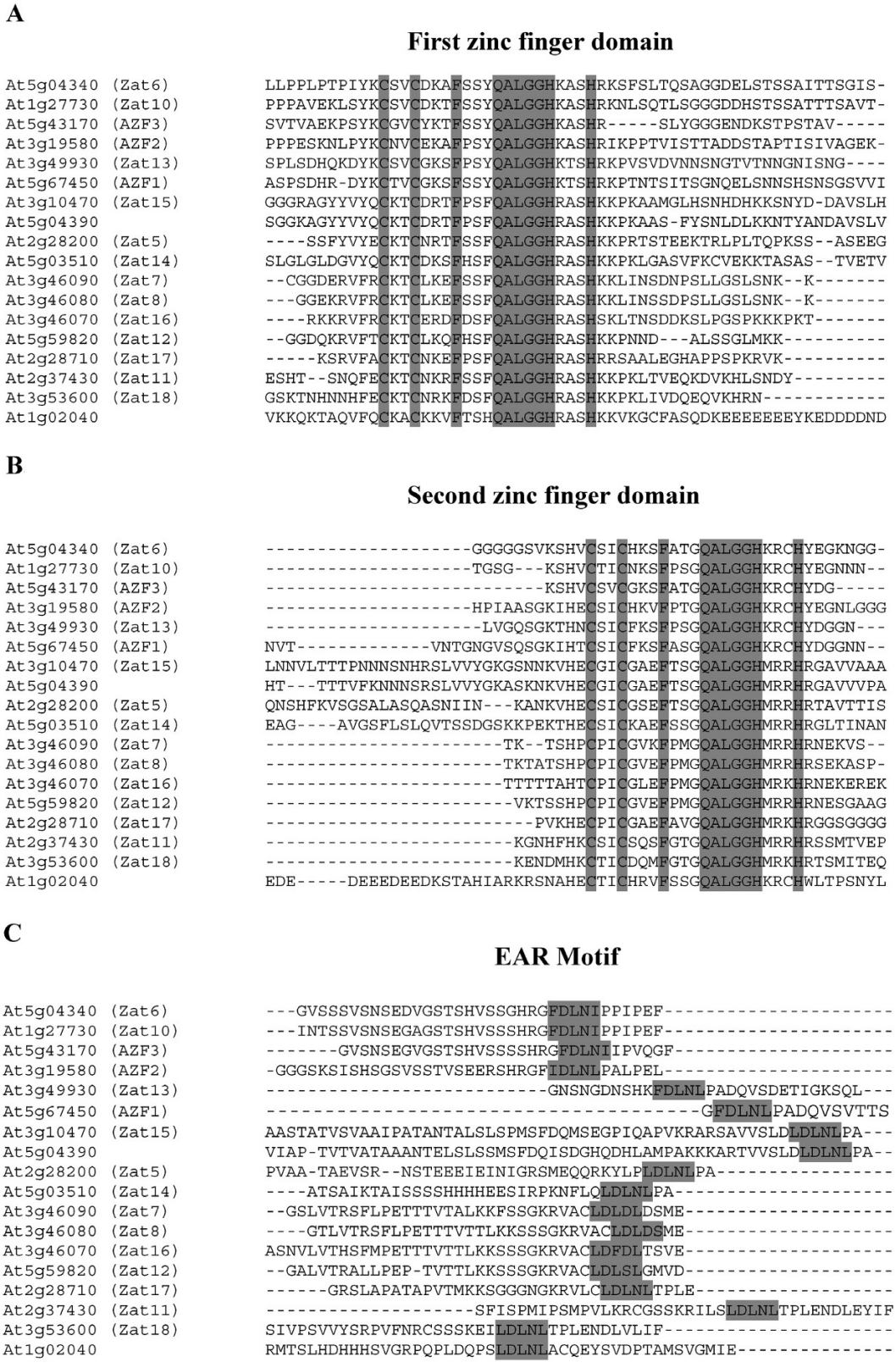

Figure 1. Conserved sequences in the first and second zinc fingers, and the core ethylene-responsive element-binding factor (ERF)-associated amphiphilic repression (EAR) motif of C1–2i subclass representatives. (*A*, *B*) Conserved sequences within the zinc finger domains of C1–2i subclass representatives. (*C*) EAR core sequence of C1–2i subclass representatives. (*D*) A neighbor joining tree of C1–2i *Arabidopsis* proteins. Alignments were performed with ClustalW (http://www.ebi.ac.uk/Tools/clustalw/ index.html).

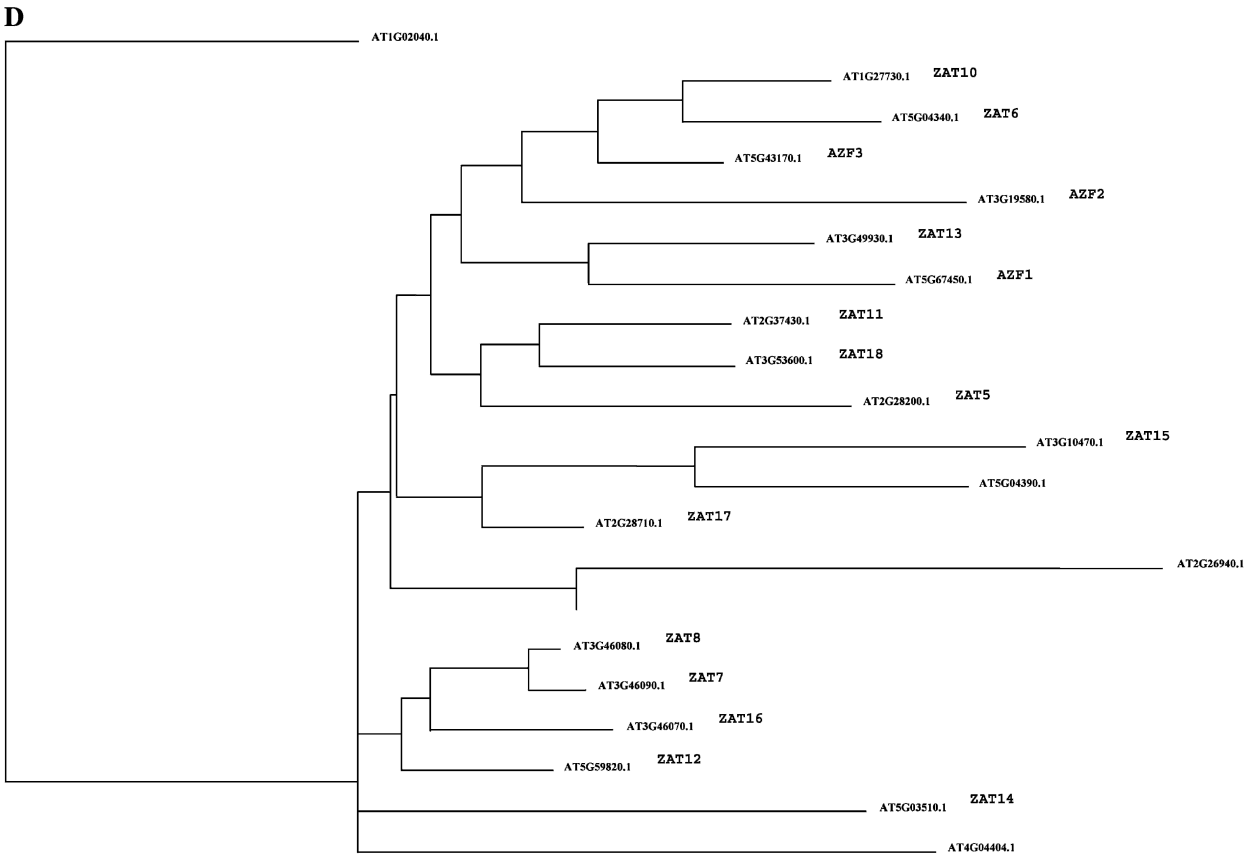

Figure 1. *Continued.*

transcription [27]. Members of class I ERFs include tobacco ERF2, ERF4 and JERF1, *Arabidopsis* AtERF1, AtERF2, AtERF5, ERF1, CBF1, DREB1 and DREB2, periwinkle ORCA2 and ORCA3, and tomato Pti4 [27, 30, 34–40]. Class II ERFs include NtERF3, AtERF3, AtERF4, AtERF7 and LeERF3b [27, 30, 41, 42]. This class of ERFs is thought to play a role as active repressors [27]. Active repressors include an independent repressor domain that represses transcription directly by chromatin modifications such as histone deacetylation or methylation, whereas passive repressors do not include an independent repressor domain and repress transcription indirectly by either DNA-protein or protein-protein interactions [32, 43]. Studies revealed that class II ERF repressors contain a conserved motif 'L/FDLNL/F(x)P', the EAR motif [27]. Mutation in the EAR motif of ERF3 abolished its repression activity, as tested with reporter gene expression [27].

Recent studies suggested that EAR-motif containing repressors play a key role in plant defense and stress-response mechanisms by transcriptional repression of different defense or stress-response-related genes in the absence of stress [32]. For instance, the EAR repressor AtERF4 negatively regulates the expression of PDF1.2 that encodes an antifungal peptide belonging to the family of plant defensins by modulating ethylene and jasmonic acid responses [44, 45]. Another EAR repressor, NIMIN1, represses the expression of the pathogenesis-related PR-1 gene that encodes a defense protein induced in response to pathogens or salicylic acid in plants [32]. In accordance, overexpression of the NIMIN1 protein in *Arabidopsis* resulted in suppression of PR-1 expression and elevated pathogen susceptibility, whereas suppression of NIMIN1 resulted in constitutive expression of PR-1 after salicylic acid treatment [46]. It has also been shown, by fusion of the EAR motif to different DNA-binding domains, that it could actively repress transcription of several genes *in vivo* [47, 48]. These result suggested that EAR motif-containing C2H2-type zinc finger proteins could act as repressors in plants [27, 32]. Key members of this group include Zat6, Zat7, Zat10/STZ, Zat12, AZF1, AZF2, and AZF3, which are C1–2i subclass members. Several of these proteins are thought to play a role in the response of plants to different biotic and abiotic insults. In accordance with this hypothesis, transcriptome profiling analyses has shown that the steady-state transcript level of many of these zinc finger

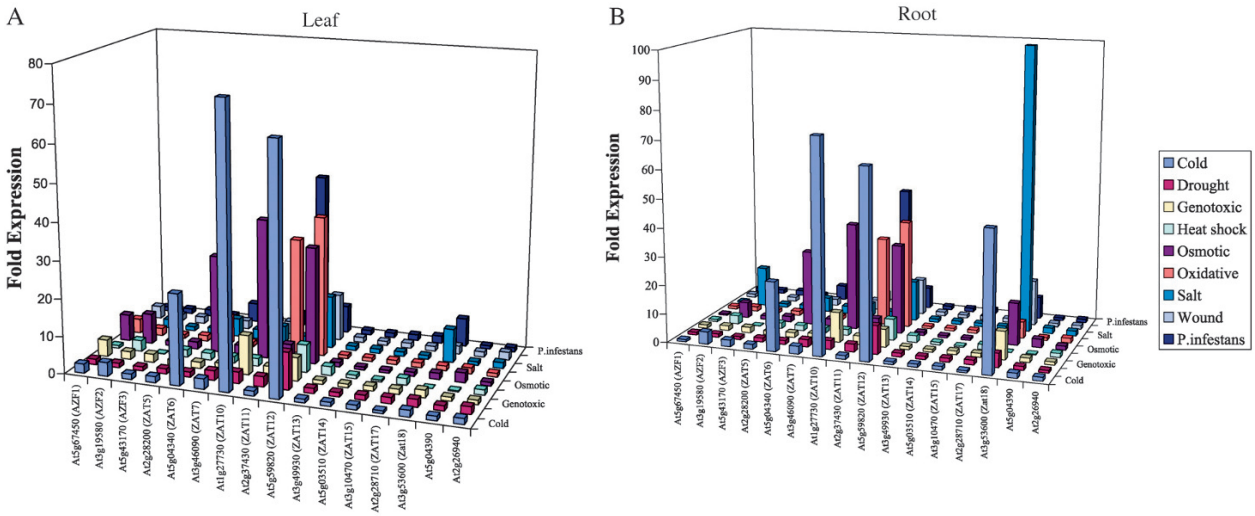

Figure 2. *In silico* DNA chip analysis of selected C1–2i subclass representatives in response to different biotic or abiotic stresses. (Left) Relative expression of C1–2i subclass members in leaves of *Arabidopsis* exposed to different stresses. (Right) Relative expression of C1–2i subclass members in roots of *Arabidopsis* exposed to different stresses. *In silico* analysis of DNA chip data obtained from (https://www.genevestigator.ethz.ch) was conducted according to [53]. Due to tandem duplication of Zat7 [5], ATH1 252567_at was used to measure its expression.

proteins is elevated during different stress conditions (Fig. 2). Genetic and biochemical studies of these proteins are described below.

Zat10/STZ

Zat10 was first identified as a cDNA that rescues yeast calcineurin null mutants [49]. Calcineurin is Ca^{2+}/calmodulin-dependent protein that plays a role in modulating ion channels required for tolerance to Na^+ and Li^+ ions [50]. In accordance, expression of Zat10/STZ in salt-sensitive yeast cells rescued the phenotype of these cells [49]. Moreover, it also enhanced the tolerance of wild-type yeast cells to high concentrations of Na^+ and Li^+ [49].

Zat10 is a TFIII-type stress-response protein consisting of two C2H2-type zinc fingers. Because of Zat10's high structural similarity with different ZPT2-related proteins, it was thought to bind to DNA in a similar manner. ZPT2-related proteins bind to two tandemly repeated AGT core sequences separated by 10 bp [51]. Investigations showed that Zat10 recognizes either AGT and ACT core sequences separated by 3 bp, or ACT and AGT core sequences separated by 4 bp [14]. The DNA binding preference of Zat10 is slightly different from that of ZPT2-type and can be summarized as A[G/C]T-X_{3-4}-A[G/C]T [14]. Expression studies showed that Zat10 is expressed in all parts of *Arabidopsis* in response to different stresses including salinity, high light and cold [14, 26, 49, 52, 53].

Zat10 contains an EAR motif at its C terminus, suggesting that Zat10 might play role as repressor. First evidence for Zat10's repression activity was revealed in 2001 [27]. Both full-length Zat10 and the repressor domain of Zat10 that includes the EAR motif repressed transcription of a luciferase reporter gene through interacting with EP2-type promoter that was shown to bind ZPT2-type proteins [27]. Full-length Zat10 and its repression domain were also shown to repress the transcription of luciferase when fused to AtERF5, a class I ERF protein [27]. Soon after, another study showed that Zat10 could bind to the RD29A promoter and repress its transcription [54]. RD29A is a classical stress-response gene and this finding suggested that Zat10 could regulate RD29A transcription during stress [54].

Recent studies suggested that Zat10 plays a dual role in the response of plants to abiotic stress. Transgenic plants that constitutively express Zat10 were found to be more tolerant to drought stress, osmotic stress, and salt and heat stresses [14, 53]. Interestingly, Zat10-knockout and RNAi lines were also more tolerant to osmotic and salinity stresses [53]. Overexpressing Zat10 enhanced transcription of ascorbate peroxidase 1 and 2 (APX 1, 2) and iron superoxide dismutase 1 (FSD1), which are known to play a role in scavenging reactive oxygen species in plant [53]. Zat10 might enhance the transcription of these genes by directly activating their transcription, or repressing a repressor of these genes. Taken together these data suggest that Zat10 is required for stress tolerance, and possibly plays a dual role as both an activator and a repressor of stress-response genes. Even though different studies revealed that the EAR motif of Zat10 can repress the

expression of different reporter genes *in vivo* [14, 27, 54], to the best of our knowledge, direct genetic evidence for the role of this domain in the Zat10-controlled stress-response pathway(s) of *Arabidopsis* has not been presented.

Recent studies showed that overexpression of CBF3 (C-repeat binding factor 3, also known as dehydration-responsive element-binding protein 1A or DREB1A) in *Arabidopsis* resulted in enhanced expression of Zat10 [55]. CBF3 is a member of the C-repeat binding factor (CBF) regulon that plays a role in cold acclimation by activating expression of COR (cold responsive) genes [56]. Moreover, a decrease in CBF3 expression, as a result of ice1 (inducer of CBF expression 1) mutation, resulted in a decrease in Zat10 expression in response to cold [56]. Transient expression assays showed that Zat10 can suppress the expression of RD29A, which is regulated by the CBF regulon, suggesting that Zat10 might act downstream of the CBF regulon and play a role in the regulation of a subset of COR genes [54, 56]. Interestingly, studies suggested that Zat12 acts as a negative regulator of the CBF regulon, therefore functioning upstream to Zat10 that is regulated by the CBF regulon [57]. Transcriptome analysis revealed that the expression patterns of Zat10 and Zat12 are similar during several different stresses (Fig. 2) [53]. Both transcripts are elevated in response to cold stress, salinity, UV-B, oxidative stress, osmotic stress and genotoxic stress (Fig. 2). These findings could suggest that Zat10 and Zat12 function in a coordinated manner during different stresses.

Zat12

Zat12 was first identified by homology cloning [26]. It consists of two C2H2-type zinc finger domains with a 22-amino acid inter-finger region, and belongs to the C1–2i subclass [5]. Following its initial identification, Zat12 was found to be a light-stress-response protein [58]. Later studies suggested that Zat12 is involved in the cold and oxidative stress response of *Arabidopsis* [57, 59, 60]. Zat12 is required for the expression of the defense enzyme cytosolic ascorbate peroxidase 1 (APX1) during oxidative stress [59]. It is also required for the expression of two important oxidative stress response proteins: Zat7 and WRKY25 in *Arabidopsis* during oxidative stress [59]. These data suggest that Zat12 expression is essential for reactive oxygen metabolism in *Arabidopsis*.

Transgenic plants that constitutively express Zat12 are more tolerant to high light, osmotic and oxidative stresses, and Zat12 antisense and knockout plants are more sensitive to light, osmotic stress and salinity [58–60]. Moreover, expression of Zat12 in transgenic plants was found to elevate the expression of 42 different transcripts involved in the response of plants to high light and osmotic stress [60]. Trancriptome analysis showed that Zat12 expression is elevated in response to many different abiotic stresses (Fig. 2). Nevertheless, Zat12 gain- and loss-of-function studies suggested that Zat12 is required for stress tolerance in only a few of these stresses [60]. Because of the extensive overlap between the transcriptome of plants subjected to hydrogen peroxide stress and the transcriptome of plants expressing Zat12 [60], it was suggested that Zat12 expression might be associated with the response of plants to reactive oxygen species accumulation during abiotic stresses [60].

Transcriptome analysis of the cold response in *Arabidopsis* suggested that 302 genes are up-regulated and 212 genes are down-regulated in response to low temperature [57]. Most of the genes highly regulated by low temperature were assigned to two main regulons: the CBF regulon and a regulon controlled by Zat12 [57]. Moreover, it was suggested that Zat12 negatively regulates the CBF regulon [57].

Zat12 contains an EAR motif at the C terminus and this motif might function as a repressor domain [5, 27, 57, 60]. It has been suggested that Zat12 suppresses the expression of the key cold-stress-response transcription factors CBF1, CBF2 and CBF3 in response to stress [57]. Microarray analysis revealed that overexpression of Zat12 resulted in the repression of several transcripts [57, 60]. Moreover, Zat12 loss-of-function lines showed enhanced tolerance to heat stress, suggesting that Zat12 might function as repressor [60]. Even though several lines of evidence suggest that Zat12 has a repression activity, most likely through its EAR motif, at present direct function-structure studies that support this hypothesis have not been reported.

Zat7

Zat7 is C2H2-type zinc finger protein consisting of two zinc fingers with a conserved QALGGH sequence and an EAR motif [5]. Zat7 was initially identified as an oxidative stress-response protein in knockout APX1 plants subjected to internal oxidative stress [59]. Transgenic plants that constitutively express Zat7 have enhanced tolerance to salinity stress and the EAR motif of Zat7 has been shown to be required for this tolerance [61]. Moreover, in contrast to transgenic plants that constitutively express Zat7 and show enhanced tolerance to salinity, transgenic plants that constitutively express a Zat7 protein that lacks a functional EAR motif showed enhanced sensitivity to salinity [61]. Yeast-two-hybrid experiments suggested that the EAR motif is also required for protein-protein interactions [61]. These studies showed that Zat7 interacts with stress-response and defense-relat-

ed proteins such as the transcription factor WRKY70 and the miRNA transport protein HASTY through its EAR domain [61]. Interestingly, the expression of Zat7, WRKY70 and HASTY is up-regulated in knockout APX1 plants that are more tolerant to salinity stress, suggesting that these three proteins are part of a salinity stress signaling pathway [61]. Transgenic plants that constitutively express Zat7 showed enhanced tolerance to cold stress but increased sensitivity to osmotic stress [61]. These findings could suggest a complex mode of regulation for zinc finger proteins during different stresses.

Previous studies suggested that constitutive expression of EAR motif-containing zinc finger proteins, such as Zat10, Zat7 or Zat12, resulted in growth suppression of plants. Because plants with suppressed growth are typically more tolerant to abiotic stresses, it was suggested that growth suppression in these transgenic plants might be the reason for their enhanced tolerance to different abiotic stresses [53, 57]. Nevertheless, the studies of Ciftci-Yilmaz et al. [61] clearly demonstrated that growth suppression in Zat7-overexpressing plants could be distinguished from enhanced tolerance to stress. These studies also suggested that the EAR motif is not responsible for growth suppression in transgenic plants expressing Zat7 [61].

AZF1, AZF2, AZF3

AZF1 (*Arabidopsis* zinc-finger protein 1), AZF2, and AZF3 were first identified by homology cloning [52]. They all contain two canonical C2H2-type zinc fingers separated by a long spacer, and a conserved EAR motif [5, 52]. AZFs show similarity to Zat10 both structurally and functionally [5, 14, 52]. Similar to Zat10, AZF2 also binds to two canonical A[G/C]T sequences [14]. Transient expression analysis revealed that all AZFs have repression activity possibly through their EAR domain [14]. Expression analysis indicated that AZF1 and AZF3 expression is mainly restricted to roots, whereas AZF2 is expressed at various levels at all organs of *Arabidopsis* with high expression in roots [14, 52].

All AZFs are involved in the water-stress response of *Arabidopsis* [52]. AZF1 responds rapidly to salt and cold stresses but appears to be abscisic acid (ABA) independent (Fig. 2) [14, 52]. AZF2 contains an ABA-responsive element (ABRE) in its promoter region [14, 52], is strongly induced by ABA and salt, and likely to be ABA dependent [14, 52]. Interestingly, all AZFs are induced by ethephon, which produces ethylene and hydrogen peroxide [14, 62].

Zat6

Zat6 expression is enhanced in response to cold and osmotic stresses (Fig. 2); nevertheless, it is not clear whether Zat6 function is required for tolerance to these stresses. Recent studies suggested that Zat6 is involved in root development and phosphate homeostasis in *Arabidopsis* [63]. Enhanced expression of Zat6 in *Arabidopsis* resulted in repression of primary root growth and a subsequent change in phosphate acquisition, whereas suppressing Zat6 expression resulted in lethality [63]. Moreover, enhanced expression of Zat6 repressed the expression of several phosphate response genes such as At4 and Pht1;1 during phosphate starvation [63]. It is possible that the EAR domain of Zat6 is involved in transcriptional repression during phosphate starvation and development of primary root growth.

Conclusion

With 176 members, the H2C2-zinc finger protein family constitutes one of the largest families of transcriptional regulators in *Arabidopsis* [5]. To date, many different studies have shown that C2H2-zinc finger proteins are required for key cellular processes including transcriptional regulation, development, pathogen defense, and stress responses. A recent study of the *Oryza sativa* (rice) C2H2-type zinc family identified 189 members of this family and demonstrated that at least 26 of them respond to different environmental stresses [64]. Interestingly, the expression of 12 rice C2H2 proteins is up-regulated in response to different environmental stresses, as well as during different phases of reproduction. Recent genetic studies pointed to possible interactions between different zinc finger proteins during stress. Expression of Zat7 in transgenic plants, for example, enhanced the tolerance of plants to cold stress, but decreased the tolerance of plants to osmotic stress [61]. In contrast, expression of Zat7 proteins with a mutated or truncated EAR domain in transgenic plants had no effect on abiotic stress tolerance [61]. Could Zat7 interact with other zinc finger proteins during osmotic stress and disrupt their function? In addition to possible protein-protein interactions between different zinc-finger proteins, a cascade of zinc finger proteins could be activated during stress. Thus, for example, during oxidative stress Zat12 is required for the expression of Zat7 [59], and during cold stress Zat12 functions upstream to Zat10 [56]. Could different zinc finger proteins interact with each other in a hierarchical or a combinatorial manner to regulate transcription? What then is the order of regulation? Future studies

attempting to address these questions could shed much needed light on the mode of action of different zinc finger proteins and how they regulate different process in plants. The *in silico* analysis shown in Figures 1D and 2 reveals some very interesting relationships between different C1–2i members. Not all members appear to respond to different biotic and abiotic insults, demonstrating a high degree of specificity for different Zat proteins. Zat6 and Zat10 that appear to be highly related and may be the result of recent gene duplication (Fig. 1D), appear to respond in a similar manner to the different stresses studied in leaves and roots (Fig. 2). In contrast, Zat10 and Zat12 that appear to respond similarly to different stresses (Fig. 2) are much less similar and appear to be more distinct (Fig. 1D).

In addition to studying the basic functions of different zinc finger proteins, applied applications of different zinc finger proteins should be considered. Thus, different zinc finger proteins or domains of different proteins, such as the EAR domain, could be used to alter development, or enhance the tolerance of transgenic plants to different stress conditions. Nevertheless, before such applications can be considered, the exact mode of action of different zinc finger proteins should be elucidated because simply overexpressing them in transgenic plants could result in deleterious side effects, including decreased tolerance to other unrelated stresses, or suppressed growth. Although this review has mainly focused on the C1 family, other families of zinc finger proteins have been recently reported to be involved in basic processes in plants, e.g., the A3 subclass AtCZS protein was reported to play a role in chromatin modifications in *Arabidopsis* [65].

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