1 Review
2 Arginine-rich motif-tandem CCCH zinc finger proteins in plant stress responses and post-
3 transcriptional regulation of gene expression

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stress response, seed germination

Abbreviations: ABA, abscisic acid; ARE, AU-rich element; AtTZF, *Arabidopsis*
thaliana tandem zinc finger protein; GA, gibberellic acid; MAPK, mitogen-activated
protein kinase; MARD1, Mediator of ABA-Regulated Dormancy 1; PB, processing body;
RD21A, Responsive to Dehydration 21A; RR-TZF, arginine-rich motif-tandem CCCH
zinc finger proteins; SG, stress granule; TTP, tristetraprolin protein;

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1
2 **Abstract**

3 Tandem CCCH zinc finger (TZF) proteins are evolutionarily conserved regulators of
4 gene expression at the post-transcriptional level. TZFs target AU-rich RNA elements at
5 3' un-translated region and recruit catabolic machineries to trigger mRNA degradation.
6 The plant TZF families are over-represented by a class of proteins with a unique TZF
7 domain preceded by an arginine-rich motif (RR-TZF). RR-TZF proteins are mainly
8 involved in hormone- and environmental cues-mediated plant growth and stress
9 responses. Numerous reports have suggested that RR-TZF proteins control seed
10 germination, plant size, flowering time, and biotic and abiotic stress responses via
11 regulation of gene expression. Despite growing genetic evidence, the underlying
12 molecular mechanisms are elusive. This review outlines the highly conserved roles of
13 plant RR-TZFs in various stress responses and the potential involvements of RR-TZF
14 proteins in transcriptional and post-transcriptional regulation of gene expression. The
15 dynamic subcellular localization of RR-TZF proteins, implication of predominant
16 protein-protein interactions between RR-TZF proteins and stress response mediators and
17 future directions of this research field are also discussed.

1. Tandem CCCH Zinc Finger Proteins (TZFs)

Gene expression in eukaryotes is extensively regulated at the post-transcriptional level, in which RNA binding proteins have major roles in modulating mRNA processing, transport, localization, stability, and translation [1]. Zinc finger proteins are a large family of metalloproteins involved in both transcriptional (e.g., CCHH-type) and post-transcriptional (e.g., CCCH-type) modulation of gene expression [2]. This review discusses recent development in understanding the mechanisms by which a unique family of RNA binding zinc finger proteins affects plant growth, stress responses, and post-transcriptional regulation of gene expression.

Tandem CCCH Zinc Finger proteins (TZFs) are conserved from yeast to metazoans. The typical TZF proteins are characterized by a TZF domain consisting of two identical CCCH motifs separated by a spacer of 18 amino acids. Tristetraprolin (TTP), a prototype of mammalian TZFs, triggers mRNA degradation by targeting AU-Rich Elements (AREs) in the 3' un-translated region of mRNAs encoding cytokines and many other regulatory proteins [3]. Although plants also contain proteins with an evolutionarily conserved TZF motif, most of them are divergent from the consensus CCCH spacing pattern, and are preceded by arginine-rich (RR) domains [4, 5]. It is currently unclear to what extent the mechanism is shared by plant RR-TZF proteins and mammalian TZFs in RNA targeting and post-transcriptional regulation of gene expression.

2. Plant RR-TZF proteins

Genes encoding plant RR-TZF proteins have been systematically identified from
 genomes of various plant species. For example, Arabidopsis and rice contain 11 (*AtTZFs*)
 and 9 (*OsTZFs*) members of *RR-TZFs*, respectively [6]. A more recent report has
 revealed that *RR-TZF* genes are widespread in the plant kingdom, including in lower
 plants such as moss *Physcomitrella patens* and alga *Chlamydomonas reinhardtii* [7]. The
RR-TZF genes have been further classified into 5 groups (I to V) using 461 sequences
 identified from various plant genomes. The largest group (RR-TZF I) comprises genes
 encoding the proteins homologous to Arabidopsis AtTZF7-11, characterized by two
 ankyrin protein repeats at the N-termini that may be involved in protein-protein
 interactions. The second group (RR-TZF II) includes genes encoding smaller proteins
 that are homologous to Arabidopsis AtTZF1-5 without ankyrin repeats. RR-TZF II is
 further divided into two subgroups. Ila group genes (*AtTZF1/2/3* like) occur throughout
 plant kingdom even in green algae, I Ib group genes (*AtTZF4/5* like) are only found in
 angiosperm dicot plants and are missing in monocot plants, indicating a more recent
 evolution [7].

Numerous genetic and phenotypic studies have shown that plant RR-TZF proteins are
 potent regulators of hormone-mediated growth and stress responses [4]. Plant *RR-TZF*
 families contain a much higher number of genes than those in other organisms, due to
 tissue-specific expression and functional redundancy. Nevertheless, the majority of
 Arabidopsis *RR-TZF* genes has been functionally characterized (Table 1). For example,
 AtTZF1 is a positive regulator for sugar and abscisic acid (ABA) response, and a
 negative regulator for gibberellic acid (GA) response, in part by modulating the

1 expression of genes involved in these pathways. Compared to the wild type, AtTZF1
 2 overexpression plants are more compact and late flowering, and have superior cold and
 3 drought stress tolerance. Conversely, loss-of-function plants are more susceptible to
 4 abiotic stresses [8]. *AtTZF1* expression is induced by salt (NaCl). Overexpression of
 5 *AtTZF1* resulted in enhanced tolerance to high salt, due to stronger maintenance of ionic
 6 balance and reduced oxidative and osmotic stress responses [9]. In contrast, *AtTZF1*
 7 expression is de-repressed by sugar starvation and *AtTZF1* overexpression plants are
 8 hypersensitive to sugars [8]. It is conceivable that AtTZF1 may serve as a crosstalk point
 9 in integrating both nutrient and stress signals and modulating downstream gene
 10 expression at the post-transcriptional level.
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 12 Consistent with the findings in Arabidopsis, the rice homolog *OsTZF1* is induced by
 13 drought, high salt, H₂O₂, ABA, methyl jasmonate, and salicylate [10]. The rice OsTZF1
 14 enhances stress responses by suppressing the expression of salt inducible genes and genes
 15 involved in reactive oxygen species generation. Overexpression of *OsTZF1* in rice caused
 16 delayed seed germination and retarded seedling growth, reminiscent of the effects of
 17 overexpression of *AtTZF1* in Arabidopsis [10]. The expression of *AtTZF2* and *AtTZF3* is
 18 also induced by various abiotic stresses. They act as positive regulators for ABA,
 19 oxidative, and salt stress responses, and as negative regulators for stress hormone methyl
 20 jasmonate-induced senescence [11, 12]. Similarly, rice OsTZF2 delays methyl jasmonate-
 21 induced leaf senescence [13]. The available results so far indicate that functions of RR-
 22 TZF proteins are conserved between monocot and dicot plants.
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1 Some molecular mechanisms have been revealed for the positive roles of RR-TZF
2 proteins in ABA responses. For example, *ABA insensitive 2 (ABI2)* encodes a negative
3 regulator of ABA responses. The expression of *ABI2* was down regulated in *AtTZF3*
4 overexpression plants and up regulated in antisense plants compared to the wild type,
5 suggesting that the positive effects of *AtTZF3* on ABA responses are mediated through
6 the repression of *ABI2* [12]. More mechanistic insights for the roles of RR-TZF proteins
7 in ABA and GA responses have been obtained from the characterization of dicot-specific
8 RR-TZF IIb proteins (*AtTZF4/5*-like). Genetic analyses have revealed that the seed-
9 specific *AtTZF4/5* are negative regulators for phytochrome- and GA-mediated promotion
10 of seed germination. *AtTZF4/5* overexpression plants are late germination, while
11 knockout plants display early and light-independent germination. *AtTZF4/5* affect seed
12 germination by differential regulation of genes involved in both ABA and GA
13 metabolism and response [14, 15]. For example, *AtTZF4/5* up regulate ABA biosynthesis
14 and GA catabolic genes, but down regulate ABA catabolic and GA biosynthesis genes,
15 thus enhancing ABA accumulation and reducing GA accumulation. It is currently unclear
16 though if *AtTZF4/5* directly or indirectly regulate these genes via transcriptional, post-
17 transcriptional, or both mechanisms.

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19 Most of the plant mRNAs accumulate after seed imbibition during germination and
20 seedling growth. It is rather unusual that the mRNAs of *AtTZF4/5* accumulate at the
21 highest levels in dry seeds, and decrease gradually after imbibition in the early phase of
22 seed germination [15, 16]. The downward mRNA accumulation pattern is also observed
23 for *AtTZF1/2/3*, suggesting that they might share some common functions in suppressing

1 seed germination. Indeed, AtTZF3 was found to negatively affect seed germination under
 2 salt stresses. Similar results have been found in durum wheat and moss homologs of
 3 *AtTZF1/2/3*, suggesting that salt stress-enhanced *RR-TZF* mRNA accumulation is
 4 conserved across plant kingdom [7].
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 6 There are various possibilities for the decline of *AtTZF4* and 5 transcript levels during
 7 seed germination, including transcriptional repression and/or RNA degradation.
 8 Curiously, the transcription factor Phytochrome-Interacting factor 3-Like 5 (PIL5) and
 9 ABA Insensitive 3 (ABI3) collaboratively activate the expression of *AtTZF4* by directly
 10 binding to its promoter in imbibed seeds [16]. This is inconsistent with the fact that
 11 *AtTZF4* mRNA levels actually decline during seed germination. Nonetheless, it raises a
 12 possibility that *AtTZF4* mRNA degradation might exceed the rate of production during
 13 seed germination. Interestingly, a second report from the same group indicates that ABI3
 14 interacts with ABI5 and DELLA-domain containing proteins and activates *AtTZF4*
 15 expression in imbibed seeds when under high-temperature stresses. These findings are
 16 more in line with the idea that stress-induced mRNA production compensates for the
 17 decay of *AtTZF4* mRNA during seed germination [17]. It is possible that stress enhances
 18 *RR-TZF* mRNA accumulation by two different mechanisms: delaying mRNA
 19 degradation [15] and activating transcription [7]. In contrast to *AtTZF4/5*, the cotton
 20 homolog Zinc Finger Protein 1 (GhZFP1) enhances not only salt stress tolerance, but also
 21 disease resistance caused by a fungal pathogen [18]. *GhTZF1* is also induced by osmotic
 22 and salt stresses. Overexpression of *GhTZF1* enhanced drought tolerance and delayed

1 drought-induced senescence by suppressing the expression of genes involved in reactive
2 oxygen species generation [19].

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4 The RR-TZF group I members, AtTZF7/9/10/11 work redundantly in maintaining
5 vegetative growth, activating oxidative stress tolerance, and preventing stress-induced
6 transition to flowering [20]. *AtTZF10* and *11* are also up regulated by salt and the
7 encoded proteins are positive regulators of salt stress tolerance [21]. In contrast to all
8 characterized AtTZFs, AtTZF9 is additionally involved in initiating a full Pathogen-
9 Associated Molecular Pattern (PAMP)-triggered immune response [22]. Together,
10 aforementioned genetic and phenotypic analyses overwhelmingly support the notion that
11 plant RR-TZFs have pivotal roles in various stress responses, particularly those related to
12 oxidative and salt stresses [4].

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14 **3. RR-TZF proteins in processing-bodies (PBs) and stress granules (SGs)**

15 The nucleocytoplasmic shuttling protein TTP is localized in Processing-Bodies (PBs) and
16 Stress Granules (SGs) in cytoplasm. PBs and SGs are aggregations of cytoplasmic
17 messenger ribonucleoprotein complexes involved in post-transcriptional regulation and
18 epigenetic modulation of gene expression [23]. PBs are mRNA decay complexes
19 enriched with translation-repressed mRNAs and mRNA silencing and degradation
20 machineries [24], whereas SGs are aggregates of stalled pre-initiation complexes mainly
21 acting as mRNA storage sites [25]. TTP recruits and activates decapping and 5'-to-3'
22 mRNA decay machineries that degrade targeted mRNAs [26, 27]. Remarkably, TTP also
23 recruits exosomes that degrade targeted ARE-containing mRNAs via 3'-to-5' [28].

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6 2 Arabidopsis AtTZF1 was the first RR-TZF found that traffics between cytoplasmic foci
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8 3 and nuclei. AtTZF1 co-localizes with both PB and SG markers in cytoplasmic foci,
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10 4 indicating an evolutionarily conserved subcellular localization mechanism [6]. All 11
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12 5 members of Arabidopsis RR-TZF proteins have been detected in cytoplasmic foci [29].
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14 6 However, different studies have shown variable results for subcellular localization of
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16 7 plant RR-TZF proteins. For example, Arabidopsis AtTZF1 [9], AtTZF11 [21] and cotton
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18 8 GhZFP1 [18] were found solely in the nuclei when ectopically expressed in onion
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20 9 epidermal cells; AtTZF2 and 3 were found in cytoplasm in tobacco epidermal cells [11];
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22 10 AtTZF4 was found in the nuclei in Arabidopsis root cells [14]; AtTZF7 was detected in
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24 11 both cytoplasm (non-foci) and nuclei in Arabidopsis leaf cells [20]; and OsTZF2 was
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26 12 localized in the nuclei in rice root cells [13]. These results seem to be inconsistent at the
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28 13 first glance. However, PB and SG assembly is not spontaneous. It is triggered by specific
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30 14 cues and orchestrated by particular signal transduction pathways [23]. In plants, it
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32 15 appears that RR-TZFs is routinely localized in PBs and SGs when AtTZF1 [6], AtTZF4-6
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34 16 [15], AtTZF9 [22], and OsTZF1 [10] were transiently expressed in protoplasts. The
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36 17 reasons for this consistent PB/SG localization pattern in protoplasts are unknown, as the
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38 18 cues and transduction pathways have not been elucidated. One possible cause might be
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40 19 the use of high-concentration osmotic reagents, such as mannitol, sorbitol, or salt solution
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42 20 in maintaining protoplast turgor pressures.
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46 22 Conditional assembly of PBs and SGs is also observed in intact plants. AtTZF1-
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48 23 associated cytoplasmic foci (putative PBs and SGs) were only found in meristems,
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1 stomata, and wounded or MeJA-treated tissues, even when the *CaMV 35S* promoter was
2 used to drive constitutive and strong expression in transgenic Arabidopsis plants [6].
3 Similarly, OsTZF1-associated cytoplasmic foci were assembled in transgenic rice roots
4 only when treated with ABA or high salts [10]. To argue that plant RR-TZF associated
5 cytoplasmic foci are not assembled due to artifacts of over-expression, AtTZF6 is
6 localized to cytoplasmic foci in embryos under methyl jasmonate treatment when
7 expressed by using native *AtTZF6* promoter [15]. Together, these results indicate that the
8 assembly of RR-TZF associated PBs and SGs in plants requires post-translational
9 signaling mechanisms that are as dynamic as those in animals and yeast [23].

11 **4. TZFs and RR-TZFs in mRNA metabolism**

12 The mechanisms by which TTP promotes mRNA degradation in animals have been
13 studied extensively [30]. In contrast, post-transcriptional regulation of gene expression
14 mediated by plant RNA binding proteins, including RR-TZFs, is poorly understood [31].
15 AtTZF1 binds to both DNA and RNA in vitro [6] and differentially affects the expression
16 of specific genes [8]. AtTZF1 was predicted to bind mainly to non-consensus AREs, as
17 plant RR-TZFs' RNA-binding structures are different from those in TTP [32].
18 Subsequent RNA-binding analyses revealed that AtTZF1 could indeed bind a consensus
19 ARE, but with much weaker affinity than TTP. This is consistent with the prediction that
20 AtTZF1's optimal RNA binding sites are probably not classical AREs. In contrast to TTP
21 in which the TZF motif is sufficient to bind AREs, both RR and TZF motifs are required
22 for AtTZF1 to bind AREs. Furthermore, AtTZF1 targets AREs at 3'UTR and promotes
23 mRNA degradation [5]. Like AtTZF1, AtTZF9 [22] and rice OsTZF1 [10] also bind

1 specific RNA elements. It has not been determined if these bindings can result in mRNA
2 degradation. In an attempt to determine if AtTZF2 and 3 could bind potential target
3 RNAs, they displayed RNase activities. While it was proposed that these nucleolytic
4 activities might be involved in controlling RNA turnover, their specificities were not
5 determined [11]. Together, there has only been one line of evidence from AtTZF1 to
6 suggest that plant RR-TZFs are also involved in post-transcriptional regulation of gene
7 expression.

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9 Mammalian TZF proteins are nucleocytoplasmic shuttling proteins. Nuclear TTP acts as
10 a co-repressor of multiple steroid receptors in cancer cells (Barrios-García et al., 2016).
11 Both AtTZF1 and OsTZF1 are also detected in nuclei, but neither act as transcriptional
12 activators or repressors [10, 33]. Curiously, separate reports have shown that some plant
13 RR-TZF proteins do act as transcription factors. For example, AtTZF1 transcriptionally
14 activates a GAL4 reporting system in yeast cells, although it remains undetermined if the
15 same were true in plant cells [9]. AtTZF7 acts as a transcriptional activator by binding to
16 so-called BOXS2 elements in the promoters of several floral integrator genes [20].
17 Finally, rice OsTZF9 targets a promoter region of seed storage protein glutelin gene
18 *GluB-1* and down regulates its expression [34]. Together, these results suggest that plant
19 RR-TZFs might act as transcription factors in nuclei or as RNA regulators in the
20 cytoplasm. Whether or not any given RR-TZFs have dual activity in transcription and
21 RNA regulation has yet to be determined.

22 23 **5. RR-TZF protein-protein interaction**

1 It is well documented that TTP recruits RNA decay machineries to PBs and promotes
 2 degradation of target mRNAs [26, 27, 35]. TTP is present in the same protein complex
 3 with mRNA decapping enzymes, a deadenylase called Carbon Catabolite Repressor 4,
 4 and a 5'-to-3' exoribonuclease in co-immunoprecipitation assays [26, 35]. A component
 5 of human exosome is also co-purified with TTP [35]. These results suggest that TTP may
 6 directly or indirectly interact with components of RNA degradation machineries. While
 7 N-terminal domain of TTP had been thought to be involved in the interaction with
 8 mRNA decay enzymes [35], a more recent report indicated that the C-terminal domain of
 9 TTP was responsible for a directly interaction with a deadenylase complex component
 10 [36]. This raises a possibility that TTP N-terminus might only be indirectly involved in
 11 protein-protein interaction. This notion is supported by a recent report indicating that
 12 another protein (CIN85) directly interacts with TTP via its C-, but not N-terminal domain
 13 [37]. In addition to the C-terminal mediated interactions, TTP also interacts with other
 14 proteins via its central TZF domain. For example, TTP interacts with AUFp45 (an ARE
 15 binding protein) and its related protein laAUF1 via TZF motif [38]. TTP also inhibits
 16 poly(A)-tail synthesis of ARE-containing mRNAs by competing with the interaction
 17 between poly(A)-binding protein nuclear 1 and poly(A) polymerase via TZF motif [39].
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 19 Some preliminary analyses have been carried out using yeast two-hybrid assays to
 20 determine if plant RR-TZFs also interact with components of RNA decay machineries.
 21 Curiously, AtTZFs do not interact directly with decapping complex components [40].
 22 This could be due to low level of conservation in amino acid sequences or structures
 23 between TTP and AtTZFs. Arguably, it is actually unclear if TTP directly interacts with

1 any of the decapping components, although they are present in the same protein complex
 2 [35]. TTP also interacts with a cytoplasmic [37] and a nuclear poly(A)-binding protein
 3 [39], respectively, via its TZF domain. Again, AtTZFs cannot interact with a poly(A)-
 4 binding protein (PABP8) [40]. This could be due to the difference in the CCCH spacing
 5 pattern in TZF motif between AtTZFs and TTP [6]. Conversely, Arabidopsis PABP8
 6 might not share enough similarity with human poly(A)-binding proteins for a conserved
 7 protein-protein interaction [41]. It is also possible that AtTZFs might interact with other
 8 unidentified PABPs or have no interaction at all with any PABPs. More protein-protein
 9 interaction screens in the future may provide a better answer for whether or not plant RR-
 10 TZFs could directly interact with components of mRNA decay machineries. In addition,
 11 purification of RR-TZF protein complexes using chromatography would reveal if any of
 12 the mRNA decay enzymes were in the same complex, albeit the association might be
 13 indirect.

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 15 Based on limited reports to date, plant RR-TZFs appear to preferentially interact with
 16 stress response regulators. For example, cotton GhZFP1 interacts with Responsive to
 17 Dehydration protein 21A and Pathogenesis-Related protein 5. The effects of GhTZF1 in
 18 enhancing salt stress tolerance and fungal disease resistance may be mediated through
 19 these protein-protein interactions, respectively [18]. These physical interactions are
 20 mainly mediated through both TZF motif and the N-terminal region [18]. In addition,
 21 Mitogen-activated Protein Kinase 3 and 6 (MPK3 and MPK6) direct interact with and
 22 phosphorylate AtTZF9 in the nucleus. AtTZF9 binds RNA and mediates Pathogen-
 23 Associated Molecular Pattern (PAMP)-triggered immune response, and MPK3 and

1 MPK6 are activated by PAMP. Thus, it has been postulated that plant defense signaling
2 may involve AtTZF9-mediated post-transcriptional control that requires MPK-dependent
3 post-translational modification [22]. However, it remains to be determined how AtTZF9-
4 MPK interactions in the nucleus could potentially impact post-transcriptional regulation
5 that is largely occurred in the cytoplasm. One possibility would be that AtTZF9 also
6 interacts with MPKs in PBs and SGs.

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8 Using yeast two-hybrid (Y-2-H) library screens, 35 putative AtTZF5 interacting proteins
9 have also been identified [40]. The interactions with Mediator of ABA-Regulated
10 Dormancy 1 (MARD1) and Responsive to Dehydration 21A (RD21A) have been
11 confirmed by bimolecular fluorescence complementation and co-immunoprecipitation
12 analyses. Interestingly, MARD1 and RD21A also interact with seed-specific AtTZF4 and
13 6, but not with the more ubiquitously expressed AtTZF1. This is intriguing because the
14 protein-protein interaction is mediated through TZF motif, which is conserved in
15 AtTZF1. It is possible that AtTZF1 contains a unique domain that affects protein
16 folding/structure and hence prevents its interaction with MARD1 and RD21A. The
17 interaction between AtTZF5 and MARD1 or RD21A appears to take place in PBs and
18 SGs, because all interacting components co-localize with PB and SG markers,
19 respectively [40]. Like *attzf4*, *5* and *6*, *mard1* mutants also displayed phytochrome-
20 independent and early seed germination. This once again raises a possibility that
21 AtTZF4/5/6 and MARD1 work in concert in mediating post-transcriptional regulation in
22 seed germination response.

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6. Outlooks

Functional characterization of plant RR-TZFs has been progressed rapidly in recent years [4]. Numerous results support the notion that RR-TZFs are powerful switches in mediating plant growth, development, and particularly stress responses. It has been widely speculated that post-transcriptional regulation of gene expression underlies the effects of RR-TZFs in various biological processes [4]. However, only limited protein-RNA interaction analyses have been performed to date and most of them are conducted using non-specific RNA substrates. A few potential mRNA targets have been speculated from expression profiling results and their interactions with RR-TZFs have been confirmed in vitro [10]. Nevertheless, only one report has shown that RR-TZFs promote mRNA degradation. Unfortunately, a generic ARE element, but not a native mRNA target was used as a reporting construct in this study [5]. Therefore, it is urgent to address the following questions to advance the research of plant RR-TZFs:

1. What are the in vivo RNA targets of RR-TZF proteins? This will tell us if RR-TZFs target mRNAs or non-coding sequences and hence providing clues of their potential roles in regulation of gene expression.
2. Do RR-TZF proteins target unique RNA elements other than consensus AREs? This is important to know because RNA recognition elements are gateways to elucidate the functional relationships between RR-TZF proteins and target genes. These RNA elements are excellent tools to search additional plants genomes and identify potential target genes.
3. Can RR-TZF proteins promote the degradation/stabilization of target mRNAs? From this we will better understand the potential roles of RR-TZF proteins in specific growth, stress or hormone signal transduction responses.

1 4. By what mechanisms do RR-TZFs affect mRNA half-lives? It is important to
2 determine if RR-TZFs use conserved mechanisms to control mRNA metabolism, given
3 current results do not support the idea that they interact with mRNA degradation
4 machineries directly.

5 5. Could RR-TZFs also affect transcription and translation? Based on the results obtained
6 so far, some RR-TZF proteins are apparently transcription factors. It will be interesting to
7 find out if they can also affect translation.

8 6. What are the roles of PBs and SGs in RR-TZFs mediated post-transcriptional
9 regulation? Current models in other organisms support a potential pivotal role of PBs and
10 SGs in post-transcriptional regulation [23]. Hence it is critical to determine to what extent
11 that PB and SG assembly is required for RR-TZFs mediated post-transcriptional
12 regulation of gene expression.

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14 An obvious starting point to address these questions is to perform genome-wide RNA-
15 immunoprecipitation followed by high throughput RNA sequencing (RNA-seq) to
16 identify RNA targets [42]. As this technique has not been fully adopted in plant research,
17 some modifications may be required to optimize the technology. The RNA elements in
18 direct contact with RR-TZFs would still be unknown even if the target genes had been
19 revealed genome-wide. The next step would be to perform more advanced experiments to
20 narrow down RR-TZFs' RNA recognition elements using high throughput genome-wide
21 approaches, such as UV CrossLinking ribonucleoprotein Immuno-Precipitation (CLIP)
22 [43]. This would facilitate the identification of minimal RNA elements necessary for RR-
23 TZFs to bind. Although these methods are technically challenging, they are highly

1 efficient in mapping RNA recognition elements. For example, a newly developed High-
2 Throughput Sequencing of RNAs by CLIP (HITS-CLIP/CLIP-seq) method successfully
3 identified A/U-rich elements around polyadenylation sites of thousands of transcripts
4 bonded to heterogeneous ribonucleoprotein (hnRNP) A1-like protein 1 (HLP1) in
5 Arabidopsis [44]. In conclusion, in a relatively short history, RR-TZFs have emerged as
6 powerful new players in mediating plant hormone and stress signal transduction. It is
7 conceivable that RR-TZFs may work in concert with interacting protein partners that
8 orchestrate growth and stress signaling by modulating gene expression at post-
9 transcriptional level in PBs and SGs. Genome-wide identification of RR-TZFs' RNA
10 targets and interacting partners should provide new insights into the interface between
11 plant signal transduction and RNA regulation.

12

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Figure legend

Figure 1. Putative molecular mechanisms for plant RR-TZF proteins mediated growth and stress responses. RR-TZF proteins may act as transcription factors in the nucleus and/or post-transcriptional regulators in processing body (PB) and stress granules (SG). The transcription of *RR-TZF* genes and assembly of PBs and SGs are induced by specific stress cues. In the nucleus, RR-TZF proteins bind specific cis-elements (e.g., BOXS2) in the promoters and activate floral integrator genes, or they bind unidentified cis-elements and repress seed storage protein genes. In PBs/SGs, RR-TZF proteins bind target mRNAs at U-rich elements and recruit MARD1 (Mediator of ABA-Regulated Dormancy 1), RD21A (Responsive to Dehydration 21A), or PR5 (Pathogenesis-Related Gene 5), and possibly mRNA decay machinery to trigger mRNA degradation. RR-TZF proteins also interact with MPKs (Mitogen Activated Protein Kinases) in the nucleus to facilitate mRNA regulation mediated by PBs/SGs (indicated by a dashed line). ^{m7}G, mRNA cap. AAAA, mRNA poly(A) tail.

Table 1. *Arabidopsis thaliana* arginine-rich motif-tandem CCCH zinc finger (RR-TZF) proteins

Genes (accession number)	mRNA accumulation induced by	Subcellular localization	Positive regulators for	Negative regulators for	Key references	Paralogs in other plant species	Key references
AtTZF1 (At2g25900)	ABA, salt, sugar depletion	PB/SG, nucleus	ABA, sugar, and salt stress tolerance responses	GA responses	Han et al., 2014; Lin et al., 2011	Rice OsTZF1	Jan et al., 2013; Zhang et al., 2012
AtTZF2 (At2g19810)	ABA, cold, H ₂ O ₂ , osmotic stress, salt	cytoplasm, cytoplasmic foci	ABA, oxidative, and salt stress tolerance responses	MeJA responses	Huang et al., 2011; Huang et al., 2012; Lee et al., 2012;	Rice OsTZF2	Kong et al., 2006
AtTZF3 (At4g29190)	ABA, cold, H ₂ O ₂ , osmotic stress, salt	cytoplasm, cytoplasmic foci	ABA, oxidative, and salt stress tolerance responses	MeJA responses	D'Orso et al., 2015; Huang et al., 2011; Huang et al., 2012; Lee et al., 2012;		
AtTZF4 (At1g03790)	ABA	PB/SG, nucleus	ABA responses	GA- and phytochrome- mediated seed germination responses	Bogamuwa & Jang, 2013; Kim et al., 2008	cotton GhTZF1	Kong et al., 2006; Zhou et al., 2014
AtTZF5 (At5g44260)	ABA	PB/SG, nucleus	ABA responses	GA- and phytochrome- mediated seed germination responses	Bogamuwa & Jang, 2013	cotton GhTZF1	Kong et al., 2006; Zhou et al., 2014
AtTZF6 (At5g07500)	ABA	PB/SG, nucleus	ABA responses	GA- and phytochrome- mediated seed germination responses	Bogamuwa & Jang, 2013		
AtTZF7 (At2g41900)		cytoplasm, cytoplasmic foci, nucleus	vegetative growth and abiotic stress tolerance responses	stress-induced transition to flowering	Blanvillain et al., 2011	wheat TaZnFP	Min et al., 2013
AtTZF8 (At5g12850)		cytoplasmic foci				wheat TaZnFP	Min et al., 2013
AtTZF9 (At5g58620)		PB/SG, nucleus	vegetative growth and both abiotic and biotic stress tolerance responses	stress-induced transition to flowering	Blanvillain et al., 2011; Maldonado- Bonilla et al., 2014		
AtTZF10 (At2g40140)	salt	cytoplasmic foci	vegetative growth and abiotic stress tolerance responses	stress-induced transition to flowering	Blanvillain et al., 2011; Sun et al., 2007		
AtTZF11 (At3g55980)	salt	cytoplasmic foci, nucleus	vegetative growth and abiotic stress tolerance responses	stress-induced transition to flowering	Blanvillain et al., 2011; Sun et al., 2007		

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m⁷G, mRNA cap. AAAA, mRNA poly(A) tail.