CURRENT REVIEW

From Pioneers to Team Players: TGA Transcription Factors Provide a Molecular Link Between Different Stress Pathways

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The plant immune system encompasses an arsenal of defense genes that is activated upon recognition of a pathogen. Appropriate adjustment of gene expression is mediated by multiple interconnected signal transduction cascades that finally control the activity of transcription factors. These sequence-specific DNA-binding proteins act at the interface between the DNA and the regulatory protein network. In 1989, tobacco TGA1a was cloned as the first plant transcription factor. Since then, multiple studies have shown that members of the TGA family play important roles in defense responses against biotrophic and necrotrophic pathogens and against chemical stress. Here, we review 22 years of research on TGA factors which have yielded both consistent and conflicting results.

The pioneer: Tobacco TGA1a.

After the discovery of the flow of genetic information from DNA to RNA to protein in the middle of the last century, the question how this fundamental process is controlled was of major interest in molecular biology. Initiation of transcription is an essential step in gene expression and the prokaryotic lac operon constituted a paradigm for how sequence-specific DNA binding proteins can control the activity of a promoter. While the sequence of the *lacI* gene was released in 1978 (Farabaugh 1978), the sequences of the first yeast transcription factors (GAL4, GCN4) became known in 1984 (Laughon and Gesteland 1984; Penn et al. 1984). Whereas these were cloned by complementation of the corresponding mutants, isolation of regulatory factors from higher eukaryotes was more difficult. Nevertheless, sequences of the first human transcription factors (e.g., glucocorticoid receptor, cJUN, and SP1) became known between 1985 and 1988 (Hollenberg et al. 1985; Kadonaga et al. 1987; Ryder et al. 1988). Isolation strategies that were based on the specific DNA-binding activities (Singh et al. 1988) were instrumental for the cloning of the first plant transcription factor in 1989 (Katagiri et al. 1989).

Thus, the characterization of essential *cis* elements in the plant viral *Cauliflower mosaic virus* (CaMV) 35S promoter was an important prerequisite (Lam et al. 1989). At that time,

viral promoters that were efficiently recognized by the plant transcriptional machinery were believed to bind host transcription factors with high efficiency. Two functionally important TGACG motifs were identified between base pair positions –63 and –83. This sequence was subsequently called *activation sequence-1* (*as-1*). In tobacco nuclear extracts, an *as-1*-binding protein complex was identified that was named activation sequence factor-1 (ASF-1). In order to clone *as-1*-binding proteins, a tobacco cDNA expression library was screened with radioactively labeled oligonucleotides encoding multiple TGA CG motifs. In 1989, this strategy culminated in the first report describing the amino-acid sequence of TGA1a (Katagiri et al. 1989).

The primary structure of TGA1a revealed homology to the basic domain leucine zipper (bZIP) motif found in transcription factors from other kingdoms (e.g., human cJUN and CREB, yeast GCN4). In TGA1a, the basic DNA-binding domain consists of 20 amino acids with six arginine and four lysine residues. The following 21 amino acids contain three leucine residues in every seventh position. Such a bZIP element adopts an uninterrupted alpha-helical conformation with the leucine side chains forming a hydrophobic interface for dimerization (Ellenberger et al. 1992; Landschulz et al. 1988). Accordingly, bZIP transcription factors bind as dimers to palindromic sequences with each DNA-binding domain recognizing one half site. Sequence alignment of TGA1a with the ten *Arabidopsis* TGA factors reveals a conserved domain structure that extends beyond the bZIP element (Fig. 1). The bZIP region is preceded by a variable N-terminal domain ranging from 45 to 187 amino acids. In contrast, the 244 to 263 amino-acid domain following the bZIP element is more conserved. Two glutamine-rich sequences (Q1, eight glutamines in 29 residues and Q2, six glutamines in 25 residues) were already highlighted in the first publication on TGA1a (Katagiri et al. 1989) and are found in all members of the *Arabidopsis* TGA family.

In the years following the discovery of TGA1a, several in vitro studies documented that TGA factors bind to variants of the palindromic sequence TGAC/GTCA, the minimal requirement being an intact TGACG motif (Izawa et al. 1993; Katagiri et al. 1992; Schindler et al. 1992). The activation domain of TGA1a was mapped to the 87-amino acids long N terminus (Neuhaus et al. 1994). The basic DNA-binding domain encodes a bipartite nuclear localization domain (van der Krol and Chua 1991). Weak dimerization due to the short zipper domain (only three leucine residues as compared with five in cJUN) is compensated by a C-terminal dimerization domain, which at

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the same time interferes with formation of mixed dimers between different TGA proteins (Katagiri et al. 1992).

In parallel to studies focusing on the properties of the TGA1a protein, different laboratories aimed to unravel their function in planta. Expression of a potato TGA factor with an altered DNA-binding domain (Lex-PG13) in tobacco led to reduced ASF-1 activity, indicating that ASF-1 consists of TGA factors that form inactive dimers with Lex-PG13 through the ZIP region (Rieping et al. 1994). Subsequent studies found that ASF-1 consists predominantly of TGA2.2 and contains only a small amount of TGA1a (Kegler et al. 2004; Niggeweg et al. 2000). Interfering with ASF-1 activity correlated with compromised expression of an *as-1*-driven reporter construct (Rieping et al. 1994). This result provided the first loss-of-function evidence that TGA factors are involved in activating gene expression from the *as-1* element.

The presence of TGACG motifs in the promoters of several plant *glutathione S-transferase* genes (Ellis et al. 1993; van der Zaal et al. 1996) gave rise to the hypothesis that TGA proteins might activate plant stress-responsive genes. Chimeric promoters consisting of either one or multiple *as-1*- or *as-1*-like elements upstream of a minimal *CaMV* 35S promoter are responsive to multiple chemical stress cues including the plant defense hormones salicylic acid (SA) and jasmonic acid (JA), the auxin-related herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) and H₂O₂ (Liu and Lam 1994; Qin et al. 1994; Xiang et al. 1996). Chemicals structurally related to SA or 2,4-D (3-hydroxybenzoic acid and 2,6-D, respectively) do not induce TGA-mediated gene expression (Niggeweg et al. 2000). Still, the coding regions of *as-1*-containing target genes and the high concentrations of chemicals that must be used for induction (Pascuzzi et al. 1998) argue for a role of TGA factors in the detoxification of these compounds. Moreover, *as-1*-driven promoters are active in root tips (Benfey et al. 1990; van der Zaal et al. 1991), which correlates with the expression domain of *TGA1a* and the related gene *PG13* (Klinedinst et al. 2000). It was discussed that the high abundance of TGA factors in meristems would serve to activate genes that counteract the

oxidative stress occurring in rapidly dividing cells (Zhang and Singh 1994).

Since transcript levels of *TGA1a* were unaffected under inducing conditions, it was hypothesized that TGA activity is regulated at the posttranscriptional level (Liu and Lam 1994). Electrophoretic mobility shift analyses (EMSA) of ASF-1 or recombinant TGA1a in the presence of tobacco extracts suggested that inhibitory proteins would interfere with DNA binding of TGA factors in the noninduced state. Stress-induced phosphorylation of either TGA factors or these inhibitory proteins would lead to the dissociation from this inhibitory complex (Johnson et al. 2001; Jupin and Chua 1996; Pascuzzi et al. 1998; Stange et al. 1997). Since *as-1*-driven transcription and ASF-1 binding activity to *as-1* are induced by the protein synthesis inhibitor cycloheximide (Liu and Lam 1994), it was speculated that the hypothetical repressive proteins are subject to a rapid turnover. Up to now, these proteins have not been identified.

After the advent of *Arabidopsis thaliana* as one of the prime model systems for plant molecular biology, analysis of the TGA family was mainly performed in *Arabidopsis*. The release of the *Arabidopsis* genome sequence unraveled ten members of the TGA family falling into five clades (Jakoby et al. 2002). Clade I comprises TGA1 (At5g65210) and TGA4 (At5g10030), which are most similar to tobacco TGA1a (Fig. 1). Clade II consists of three closely related factors TGA2 (At5g06950), TGA5 (At5g06960), and TGA6 (At3g12250), which contain shorter N termini than the other TGA factors. TGA3 (At1g22070) and TGA7 (At1g77920) constitute clade III. PERIANTHIA (At1g68640) and TGA9 and TGA10 (At1g08320, At5g06839) form the remaining two clades, respectively.

From a pioneer with uncertain functions to central players of the plant immune system: Clade II TGA factors are essential regulators of systemic acquired resistance.

In 1998 and 1999, two independent lines of evidence pointed at TGA factors being essential regulators of the SA-induced defense network. First, a TGACG motif (*LS7*) was found to be

Fig. 1. Primary structure of TGA transcription factors. The founding member TGA1a from tobacco and the 10 *Arabidopsis* TGA factors are displayed. Different domains are indicated by different colors. Glutamine-rich domains are denoted Q1 and Q2. Numbers in the domains of TGA1a and in the N termini indicate the number of amino-acid residues. Numbers below the domains indicate the range of the size of the individual domains in different TGA factors. Balls mark the position of cysteine residues; functionally important cysteines are marked in red. Green triangles denote a STDxDT motif that can be phosphorylated.

stringently required for the activation of the *PATHOGENESIS-RELATED-1 (PR-1)* gene in *Arabidopsis* seedlings treated with SA or its analogue 2,6-dichloroisonicotinic acid (INA) (Lebel et al. 1998). Second, clade II TGA factors (TGA2, TGA5, TGA6) were identified as interaction partners of the ankyrin repeat protein NPR1 (NONEPRESSOR OF PR GENES 1 [Zhang et al. 1999]). NPR1 is required for expression of *PR-1* and for the establishment of the SA-dependent defense response called systemic acquired resistance (SAR) (Zhang et al. 2003). The finding that TGA factors act at the interface between the non-DNA-binding protein NPR1 and the *PR-1* promoter established them as part of a hormone-controlled signaling cascade that is efficient against biotrophic pathogens. Since clade I TGA factors did not interact with NPR1 in the yeast two-hybrid system (Zhou et al. 2000) and since clade II TGA factors constitute the main component of the *Arabidopsis* ASF-1 complex in leaf nuclear extracts (Lam and Lam 1995), subsequent analyses focused on TGA2, TGA5, and TGA6. Due to the short genomic distance between *TGA2* (*At5g06950*) and *TGA5* (*At5g06960*), reverse genetics exploiting knock-out mutants was hampered for a time. Therefore, expression of a dominant negative TGA protein in transgenic plants was chosen as an alternative strategy to prove that TGA factors and NPR1 act in the same pathway. The C-terminal domain of TGA2, which interacts with NPR1 but cannot bind to DNA, interferes with INA-induced *PR-1* expression, most likely by sequestering NPR1 into an inactive complex. Like *npr1*, plants expressing this protein show an enhanced disease susceptibility phenotype (Fan and Dong 2002). A fast neutron deletion mutagenesis-based reverse genetics system was deployed to isolate the *tga2-1 tga5-1* deletion line (Li et al. 2001). Crossing of *tga2-1 tga5-1* with *tga6-1* yielded a mutant that displays an *npr1*-like phenotype with respect to compromised SAR and lack of *PR-1* induction after treatment with the SA analogue INA (Zhang et al. 2003). Although TGA2, TGA5, and TGA6 act redundantly with respect to SAR establishment, they exert different functions in the noninduced state. TGA2 suppresses basal levels of *PR-1* expression whereas TGA6 functions as an activator if it

is overexpressed (Kesarwani et al. 2007). The basal transcriptional activation observed in the *tga2-1 tga5-1 tga6-1* mutant is due to the activity of TGA3, as revealed by analysis of the *tga2-1 tga3-1 tga5-1 tga6-1* quadruple mutant (Kesarwani et al. 2007). Susceptibility against virulent pathogens is increased in *npr1* but not in *tga2-1 tga5-1 tga6-1*, indicating that NPR1 interacts with other factors in basal resistance.

An area of debate: Mechanistic aspects of the regulation of NPR1- and TGA-dependent gene expression under inducing and noninducing conditions.

Whereas genetic data convincingly demonstrate that the NPR1-TGA interaction is required for SAR establishment, studies on mechanistic aspects have yielded conflicting results. According to the current model, the majority of NPR1 is located as a multimer in the cytosol of unchallenged plant cells (Mou et al. 2003). As soon as SA levels increase, a redox change in the cell leads to the reduction of critical cysteine residues in NPR1. Subsequently, monomeric NPR1 translocates into the nucleus, in which it interacts with TGA factors (Fig. 2A). Early studies implicated NPR1 as an enhancer of the binding of TGA factors to the DNA. One line of evidence was based on a chimeric transcription factor containing the DNA-binding domain of yeast transcription factor GAL4 instead of the bZIP domain of TGA2 (Fan and Dong 2002). Cellular extracts obtained from uninduced seedlings expressing TGA2-GAL4 show lower binding activity to GAL4 motifs in EMSA than do cellular extracts from seedlings grown on INA. Importantly, no GAL4 binding activity to its recognition site was detected when the construct was expressed in the *npr1-2* mutant background. Consistently, *Arabidopsis* nuclear ASF-1 was reported to bind to a TGACG-containing DNA probe in an SA- and NPR1-dependent manner (Johnson et al. 2003). However, another study reported a constitutively enhanced ASF-1 activity when using cellular extracts from the *npr1* mutant for EMSA (Despres et al. 2000). Nevertheless, data published in the same study supported the idea that NPR1 would enhance the DNA binding of TGA proteins using

Fig. 2. Working models of TGA factors and their interacting proteins at promoters activated by different signal transduction cascades. **A**, Cytokinin-enhanced salicylic acid (SA)-induced expression of defense genes. Elevated SA levels lead to a change of the cellular redox state, resulting in the reduction of critical cysteines of NPR1 and translocation of the protein into the nucleus (Mou et al. 2003). TGA transcription factors TGA2, TGA3, TGA5, and TGA6 can recruit the transcriptional coactivator NPR1 to the promoters of SA-induced genes (like *PR-1*). In cytokinin-rich tissues, TGA3 recruits phosphorylated (blue triangle) response regulator ARR2, leading to hyperactivation of some SA genes (Choi et al. 2012). TGA2 and possibly TGA5 and TGA6 are located in the cytosol and the nucleus (Cheng et al. 2009). **B**, Xenobiotic-induced expression of detoxification genes. Class II TGA factors are required for the activation of xenobiotic-inducible genes like *CYP81D11*. The GRAS protein SCL14, which is activated by a yet-unknown mechanism, is required as a transcriptional coactivator (Fode et al. 2008). SCL14, TGA2, and possibly, TGA5 and TGA6 are located in the cytosol and the nucleus (Cheng et al. 2009). **C**, Jasmonic acid and ethylene (JA/ET)-induced expression of defense genes. Induction of JA/ET genes depends on class II TGA factors if JA and ET are applied at the same time. Under these conditions, JA-induced MYC2 dampens expression levels (Lorenzo et al. 2004), potentially by triggering the expression of GRX480/ROXY19, as deduced from the observations that ectopically expressed GRX480/ROXY19 suppresses *PDF1.2* expression (Ndamukong et al. 2007) and that JA induction of GRX480/ROXY19 depends on MYC2 (Koster et al. 2012). If SA is applied, *PDF1.2* expression is further down-regulated, potentially due to a higher repressive efficiency of GRX480/ROXY19 under reducing conditions. If TGA factors and MYC2 are missing (low GRX480/ROXY19 levels), wild-type *PDF1.2* transcript levels are observed after JA/ET treatment, but the responsiveness to SA is abolished (Zander et al. 2010).

in vitro translated proteins. The NPR1-TGA-DNA complex was not detected in EMSA, suggesting that NPR1 would serve to facilitate DNA binding of TGA factors rather than forming a stable complex. This interpretation is consistent with the finding that in vitro translated NPR1 binds preferentially to recombinant TGA proteins that are not in their DNA binding-competent conformation (Johnson et al. 2008). In support of the above-mentioned results, chromatin immunoprecipitation studies provided evidence for SA-induced, NPR1-dependent recruitment of TGA factors to the *PR-1* promoter (Johnson et al. 2003). While these data were obtained with antibodies against the N termini of TGA2 and TGA3, immunoprecipitation of a tagged TGA2 protein expressed under the control of the *CaMV 35S* promoter revealed constitutive binding of TGA2 to the *PR-1* promoter region, independently of SA and NPR1 (Rochon et al. 2006). In vitro data suggest that the N terminus of TGA2 mediates multimerization of TGA2 at the *PR-1* promoter; this multimer was proposed to dissociate upon association with NPR1 with TGA2 (Boyle et al. 2009). Assuming that the antibody against the N terminus of TGA2 might not recognize TGA2 in such a repressive complex, chromatin immunoprecipitation with this antibody might have failed to detect TGA2 at the inactive *PR-1* promoter (Johnson et al. 2003). However several discrepancies have remained. First, chromatin immunoprecipitation experiments with the antibody against the tagged TGA2 should have yielded reduced TGA2 levels at the activated promoter. Second, the results of the EMSA experiments implicate induced recruitment of TGA2. Therefore, the question of whether TGA factors require NPR1 for binding to their target promoters or whether NPR1 leads to the dissociation of a large fraction of TGA2 at the activated promoter is not yet resolved. However, it is generally accepted that NPR1 functions as a transcriptional coactivator (Spoel et al. 2009), with SA potentially binding to its transcriptional activation domain (Wu et al. 2012).

The observation that the *PR-1* promoter shows elevated basal expression levels in the *tga2-1 tga5-1 tga6-1* mutant raised the question of whether TGA factors could also act in a negative way, at least on this promoter. Since mutation of the *as-1*-like element leads to enhanced *PR-1* expression levels (Pape et al. 2010), it seems plausible that TGA factors can exert their repressive activity in *cis*. However, this would require at least some basal levels of promoter occupancy by TGA factors, as suggested in some studies (Boyle et al. 2009; Rochon et al. 2006). Based on the observation that *PR-1* transcript levels are increased in the *npr3 npr4* double mutant (Zhang et al. 2006), it has been suggested that NPR1-related proteins NPR3 and NPR4 might form a repressive unit by binding to clade II TGA factors in the absence of SA at the *PR-1* promoter (Pape et al. 2010). Recent results have shown that NPR1 levels increase in the absence of NPR3 and NPR4 (Fu et al. 2012), which would also explain the elevated *PR-1* transcript levels in *npr3 npr4*. This explanation can be reconciled with the finding that basal activity in the *tga2-1 tga5-1 tga6-1* mutant depends on TGA3 (Kesarwani et al. 2007). However, when inferring molecular mechanisms from the analysis of *PR-1* expression in different mutants, it has to be considered that the *PR-1* promoter is directly and indirectly regulated by the NPR1/TGA complex. Even a *PR-1* promoter derivative lacking the *as-1*-like element responds to INA in an NPR1-dependent manner (Pape et al. 2010). Presumably, SA-inducible WRKY transcription factors, many of them being under the direct control of the TGA/NPR1 complex (Wang et al. 2006), activate the promoter. The *PR-1* promoter had originally been chosen for the analysis because it displays low background levels and high induced expression that is most likely due to direct and indirect effects of repressing and activating proteins.

Clade I TGA factors become interesting again: The redox state of *Arabidopsis* TGA1 is modulated by SA.

Since the redox state of the cell is altered after *Pseudomonas* infections or treatment with INA (Mou et al. 2003) and since TGA factors interact with glutaredoxins (GRX) (Li et al. 2009; Ndamukong et al. 2007; Zander et al. 2012), they appear to be likely targets of a redox modification process. Clade II TGA factors contain only one cysteine (Fig. 1), which may or may not be oxidized. However, Cys-260 and Cys-266 in the Q1 region of TGA1 form a disulfide bridge (Despres et al. 2003). These cysteines are conserved in TGA4 and tobacco TGA1a. When they are mutated to asparagine and serine, respectively, TGA1 can interact with NPR1 in a yeast two-hybrid system, which is not the case for the wild-type protein. The relevance of these data is supported by the observation that the disulfide bridge in TGA1 is reduced in SA-treated *Arabidopsis* leaf cells. Moreover, an in planta two-hybrid protein interaction system revealed that nuclear localized GAL4-NPR1 interacts with TGA2 but not with TGA1, unless SA is added (Despres et al. 2003; Rochon et al. 2006).

Although the biochemical data suggest a functional context of the SA-induced and redox-controlled TGA1-NPR1 interaction, Clade I TGA-mediated defense reactions are largely NPR1-independent (Shearer et al. 2012). The *tga1-1 tga4-1* double mutant displays enhanced susceptibility to *Pseudomonas syringae* pv. *maculicola* ES4326 (Kesarwani et al. 2007) and *Hyaloperonospora arabidopsidis* Emwa1 (Shearer et al. 2012). However, TGA1 and TGA4 control basal resistance independently from NPR1. Moreover, they are not involved in the establishment of SAR. Still, basal *PR-1* transcript levels are enhanced in the *tga1-1 tga4-1* mutant (Lindermayr et al. 2010). Surprisingly, these are not fully reverted to wild-type levels by a *CaMV35S:TGA1* construct. Replacement of Cys-260 and Cys-266 and two other cysteines by serine residues does not convincingly alter the low efficiency of the wild-type protein to suppress basal *PR-1* transcript levels. Since *PR-1* expression was only monitored under noninducing conditions, conclusions on the potential of these residues to function in an SA and NPR1-regulated process cannot be drawn. Neither wild-type TGA1 nor any of the mutated proteins complement the *eds* phenotype of the *tga1-1 tga4-1* mutant.

Connecting SA with cytokinin: Clade III TGA factors.

Clade III encompasses TGA3 and TGA7. Like the *tga1-1 tga4-1* double mutant, the *tga3-1* mutant is more susceptible to *Pseudomonas syringae* pv. *maculicola* ES4326 (Kesarwani et al. 2007). Although TGA3 and NPR1 physically interact in the yeast two-hybrid system (Zhou et al. 2000), TGA3 acts partially independent from NPR1, as revealed by the higher susceptibility of the *tga3-1 npr1-1* double mutant as compared with the respective single mutants.

The phytohormone cytokinin supports SA-mediated responses. Based on the observations the *tga3-1* mutant fails to confer cytokinin-enhanced resistance against *Pseudomonas syringae* pv. *tomato* DC3000 and that TGA3 recruits cytokinin-activated transcription factor ARR2 to SA-responsive defense promoters through direct protein-protein interactions, it has been concluded that TGA3 provides a molecular link between the SA and the cytokinin pathways (Choi et al. 2011).

Moreover, TGA3 plays a role in the regulation of long-distance cadmium transport. The *tga3-2* mutant accumulates higher amounts of cadmium in the root and lower amounts in the shoot when compared with wild-type plants (Farinati et al. 2010). Ectopically expressed TGA3 from *Brassica juncea* restores the transport to the shoot and confers higher cadmium tolerance. Consistently, basal and cadmium-induced transcript levels of transporters *AtMRP3* and *AtPDR8* are reduced in

tga3-2. DNA binding of TGA3 is enhanced in vitro by calmodulin (Szymanski et al. 1996), but it is not yet known whether this is relevant for either of the two known functions of TGA3 or whether it plays a role in a not yet identified functional context.

So far, no information on TGA7 is yet available, so that a potential function can only be inferred from its expression pattern (Zimmermann et al. 2004). In contrast to other TGA factors, it is highly expressed in the xylem or under conditions that induce xylem differentiation.

Playing in different teams: Clade II TGA factors regulate the detoxification pathway.

The discovery that clade II TGA factors play a central role in SAR was a major breakthrough in understanding SAR establishment; still, the question on the regulation of TGA function within the SA- and 2,4-D-activated stress pathways had remained unanswered. Not only in tobacco, but also in *Arabidopsis*, many *glutathione S-transferase* and *glucosyl-transferase* genes are induced by SA, 2,4-D, JA, and H₂O₂ (Chen and Singh 1999; Wagner et al. 2002). Induction by SA is NPR1-independent but requires clade II TGA factors (Blanco et al. 2005, 2009). In roots, TGA4 acts as a negative regulator of this response (Foley and Singh 2004). In contrast to the NPR1-dependent genes, which respond to a reducing environment after SA treatment or accumulation, the NPR1-independent genes seem to be activated through reactive oxygen species (Garreton et al. 2002). As glutathione *S*-transferases and glucosyltransferases are part of the detoxification pathway, their regulation may be viewed in this context. Along with these lines of evidence, microarray analysis after treatment of *Arabidopsis* plants with reactive oxidized fatty acids (e.g., phyto-prostanes) revealed that TGA factors are required for the expression of 60% of the induced genes, most of them being related to detoxification, stress responses, and secondary metabolism (Mueller et al. 2008). Consistently, many safener-induced genes are controlled by clade II TGA factors but not by NPR1 (Behringer et al. 2011). A yeast protein interaction screen yielded the GRAS protein SCL14, which functions as a coactivator of clade II TGA factors at a subset of promoters that are induced by different chemicals like SA, 2,4-D, or 2,3,5-triiodobenzoic acid. Chromatin immunoprecipitation experiments indicated that the TGA/SCL14 complex is bound to these promoters already in the noninduced state (Fode et al. 2008). Whether SCL14 is activated by redox-mediated changes like NPR1 or whether other activation steps are involved remains to be investigated (Fig. 2B).

Playing in different teams: Clade II TGA transcription factors are essential activators of JA/ethylene (ET)-induced defense responses.

Ten years after having discovered that clade II TGA factors are important for the establishment of the SA-dependent defense response SAR, it was reported that they are also required for the activation of the JA/ET-dependent defense pathway (Zander et al. 2010). Consistently, the *tga2-1 tga5-1 tga6-1* mutant displays increased disease symptoms after infection with the necrotrophic fungus *Botrytis cinerea*. Transcriptional activation of *PLANT DEFENSIN (PDF1) 1.2*, a marker gene of the JA/ET response, depends on TGA factors after application of the ET precursor 1-aminocyclopropane-carboxylic acid, either alone or in combination with JA, and after *Botrytis* infection (Fig. 2C). TGA6, which is redundant to TGA2 and TGA5 with respect to INA-induced *PR-1* expression (Zhang et al. 2003), cannot support activation of *PDF1.2*. Since SA suppresses the JA/ET pathway, TGA factors were considered to be candidates for functioning as the molecular link between the SA and the

JA/ET pathway. The analysis of the contribution of clade II TGA factors to the SA antagonism was hampered by the fact that induction of *PDF1.2* by the combined treatment with JA and ET is already compromised in the *tga2-1 tga5-1 tga6-1* mutant. In order to avoid this dilemma, the *tga2-1 tga5-1 tga6-1* mutant was crossed with the *jin1-1* mutant, which lacks the negative regulator of the JA/ET response MYC2. The *jin1-1* allele restores JA/ET-induced transcription of the *PDF1.2* gene in the *tga2-1 tga5-1 tga6-1* background, indicating that the function of TGA factors within the JA/ET pathway is to counteract the negative effect of MYC2. Importantly, the SA antagonism is compromised in the *jin1-1 tga2-1 tga5-1 tga6-1* quadruple mutant, documenting that TGA factors integrate the SA signal. Since the negative effect of SA on the JA/ET pathway is NPR1-independent (Leon-Reyes et al. 2009), other TGA-interacting proteins have to be postulated that would facilitate and modify the positive function of TGA factors. Candidates are GRX, small heat-stable oxidoreductases that transfer electrons from glutathione to oxidized cysteine residues. All 21 land plant-specific GRX (called ROXY) interact with TGA2 (Zander et al. 2011). A particularly strong candidate for mediating the SA-JA/ET cross-talk is GRX480/ROXY19, which is induced by JA and SA and interferes with *PDF1.2* expression when constitutively expressed in transgenic plants (Ndamukong et al. 2007; Zander et al. 2011). Thus, GRX might modulate the redox state of TGA factors or other proteins resulting in suppression of the JA/ET defense pathway.

TGA factors are not required if *PDF1.2* is induced by exogenous JA in the absence of elevated ET levels. Still, inhibition of JA-induced *PDF1.2* expression by SA depends on clade II TGA factors and can be suppressed by ectopically expressed GRX480/ROXY19 (Ndamukong et al. 2007; Leon-Reyes et al. 2010). Under these conditions, SA-induced expression of GRX480/ROXY19 is partially NPR1-dependent, which is also the case for the SA-JA antagonism. Up to now, there is no loss-of-function evidence for the role of GRX480/ROXY19 in regulating the cross-talk between the SA and the JA/ET defense pathway. Whether this is due to the redundancy of the GRX or another TGA-dependent mechanism exists has remained elusive.

The developmental role of TGA factors: Defense responses and flower organ formation evolved from a common regulatory module.

TGA factor PERIANTHIA (PAN) plays a role in flower development (Chuang et al. 1999). PAN is a repressor of petal formation and its mutation causes the development of one additional organ in the first two whorls. Like the other TGA factors (Ndamukong et al. 2007), PAN interacts with ROXY-type GRX (Li et al. 2009). Since *roxy1* mutants have, on average, 2.5 petals, whereas *pan* has five, it can be assumed that ROXY inactivates PAN. When cysteine residue 340 in PAN is mutated into a serine, the resultant protein cannot complement the *pan* phenotype, indicating that PAN is only active when this cysteine is oxidized. Oxidized PAN in *roxy1* would lead to the strong suppression of petal number. Consistently, the *pan roxy1* phenotype is identical to the *pan* phenotype. However, direct biochemical evidence for redox modification of PAN is still missing.

Plants lacking the basic leucine-zipper transcription factors TGA9 and TGA10 have defects in male gametogenesis that are similar to those observed in *roxy1 roxy2* mutants. Since TGA9 and TGA10 directly interact with ROXY proteins in yeast and in plant cell nuclei, it is suggested that the activity of TGA9 and TGA10 is controlled through ROXY-mediated modifications involving the conserved cysteine in Q1 (Fig. 1) As already observed for TGA1 and TGA4 (Lindermayr et al.

2011), the cDNAs for TGA9 and TGA10 are unable to complement the *tga9 tga10* phenotype, so that mutations of critical cysteines could not be tested (Murmu et al. 2010).

Interestingly, PAN interacts with two NPR1-like genes, *BLADE-ON-PETIOLE1* (*BOP1*) and *BOP2* (Hepworth et al. 2005). Mutants *bop1 bop2* and *pan* share a pentamerous arrangement of first whorl floral organs. These data indicate that BOP proteins control patterning via direct interactions with TGA transcription factors, which are, in turn, connected to the action of GRX. Thus, the regulatory triad involving TGA factors, NPR-like proteins, and GRX has been duplicated, with one copy serving defense responses in leaves and the other copy functioning in the context of flower organ formation.

Not yet established: Functional relevance of posttranslational modifications, protein stability, and subcellular localization of TGA factors.

As outlined above, redox-modifications might control the activity of TGA factors, although in vivo evidence for functional relevance of such modifications is missing for members of clades I, II, and III. Protein phosphorylation constitutes another way of regulating transcription factor activity. In tobacco protein extracts, SA-induced DNA binding of tobacco TGA2.2 was sensitive to phosphatase treatment (Jupin and Chua 1996; Niggeweg et al. 2000). Further studies suggested that a casein protein kinase 2-related protein is involved in the activation of the *as-1*-binding activity of tobacco proteins in either whole cell or nuclear extracts (Stange et al. 1997; Hidalgo et al. 2001). Taken together, these results suggest that phosphorylation of either TGA factors or interacting proteins leads to increased DNA binding. Another study demonstrated SA-induced phosphorylation at serine and threonine residues in the N terminus of TGA2 (Fig. 1) (Kang and Klessig 2005). However, this modification negatively regulates TGA2 DNA-binding activity. The phosphorylated sequence STDxDT is conserved in tobacco TGA2.2, hinting at a potential relevance. When expressed in transgenic *Arabidopsis* plants, TGA2-green fluorescent protein (GFP) and mTGA2-GFP lacking the putative phosphorylation sites activate *PR-1* to the same levels (Kang and Klessig 2005). Still, it might be worthwhile to revisit the importance of these residues for other TGA-mediated stress-regulated genes.

Moreover, it remains to be investigated whether regulation of protein turnover plays a role in the control of TGA factor activity (Pontier et al. 2002). TGA factors interact with the SA receptor proteins NPR3 and NPR4 (Fu et al. 2012; Zhang et al. 2006), which recruit interacting proteins into CUL3-Rbx1 E3 ubiquitin ligase complexes and might thus regulate SA-dependent TGA degradation. Finally, TGA2 is detected both in the cytosol and the nucleus (Cheng et al. 2009). Whether TGA2 forms already a complex with NPR1 or SCL14 in this compartment is not known. The importance of the cytosolic fraction is an issue that remains to be addressed.

TGA-interacting proteins: An overview.

As outlined in this review, several TGA-interacting proteins are known to modulate their activity. Further potential regulators have been identified in a systemic yeast two-hybrid assay, where a collection of approximately 8,000 open reading frames representing about 30% of the predicted protein-coding genes of the *Arabidopsis* genome were tested (The Arabidopsis Interactome Mapping Consortium 2011). This analysis included TGA factors TGA1, TGA2, TGA3, TGA5, and TGA6. The interactions with NPR1, NPR3, NPR4, and GRX ROXY1 and GRX480/ROXY19 were confirmed for at least two of the five TGA factors. Moreover, an interaction with the NPR1-interacting small protein NIMIN1 (Weigel et al. 2005) was demonstrated for TGA2 and TGA6. Not-yet-described interactions between single TGA factors and other proteins like, for example, a Myb-like helix-turn-helix transcription factor (At2g38300), an E3 ligase (At2g25700), a serine threonine kinase (At3g12200) or the TFIID-related protein ENHANCED ETHYLENE RESPONSE 4 (At1g25490) were found. In yet another study, TGA5 and TGA6 were listed to interact with proteins related to the corepressor TOPLESS (Causier et al. 2011). The functional relevance of these interactions might be worthwhile investigating.

Moreover, TGA factors have been shown to interact with other transcription factors. For instance, SA-induced DOF transcription factors (Kang and Singh 2000) and the ET-inducible GCC box-binding protein AtEBP/RAP2.3 (At3g16770) (Buttner and Singh 1997) were found in protein interaction screens using TGA4 (OBF4) as a bait. In addition, TGA4 interacts with CONSTANS, a transcriptional activator of the Flowering Locus T (Song et al. 2008). These factors might enhance binding of TGA transcription factors to TGACG motifs in

Table 1. Function of TGA factors

TGA factors	Function ^a	Interacting protein	Reference
Clade I (TGA4)	Repression of <i>GST6</i> expression in roots		Foley and Singh 2004
Clade I (TGA1/4)	Activation of basal resistance Activation of defense responses elicited by the constitutively active mutant R protein SNC1		Kesarwani et al. 2007 Shearer et al. 2012
Clade II (TGA 2)	Negative regulation of basal <i>PR-1</i> transcript levels		Kesarwani et al. 2007
Clade II (TGA2/5)	Activation of JA/ET-induced <i>PDF1.2</i> expression		Zander et al. 2010
Clade II (TGA2/5/6)	Activation of <i>PR-1</i> expression after INA treatment Activation of early SA-induced NPR1-independent genes Activation of detoxification genes SA-mediated suppression of the JA/ET pathway	NPR1 SCL14 GRX480/ROXY19	Zhang et al. 2003 Blanco et al. 2005 Müller et al. 2008; Fode et al. 2008 Ndamukong et al. 2007; Zander et al. 2010
Clade III (TGA3)	Activation of basal <i>PR-1</i> transcript levels in the absence of class II TGA factors Activation of basal resistance Regulation of long-distance cadmium transport Enhancement of SA-dependent defense responses after cytokinin treatment	ARR2	Kesarwani et al. 2007 Kesarwani et al. 2007 Farinati et al. 2010 Choi et al. 2011
PAN	Repressor of formation of a fifth petal	ROXY1	Chuang et al. 1999
Clade IV (TGA9/10)	Anther development	ROXY1/2	Murmu et al. 2010

^a Functions as determined by loss of function analysis of single, double, and triple *Arabidopsis* mutants are summarized. JA = jasmonic acid, ET = ethylene, and SA = salicylic acid.

their vicinity, in which they might either activate or repress transcription. Several reports have documented a stimulating activity of the *as-1* element in different promoter contexts. For instance, although the isolated *as-1* element cannot mediate light-responsive gene expression, it is necessary to support light-induced gene expression from a chimeric promoter consisting of four light-responsive *cis* elements fused to the *CaMV* 35S core promoter (Lam and Chua 1990). Likewise, although the *as-1* element cannot mediate ET-induced gene expression, it is important for ET-induced gene expression in the context of the *PDF1.2* promoter (Zander et al. 2010). In the context of the *CYP81D11* promoter, it is essential for JA induction (Koster et al. 2012). Being the target of SA signaling, TGA factors can thus connect the SA pathway with the JA/ET pathway. It can be assumed that more examples for such interplays will be discovered when further exploring the function of TGA factors.

In conclusion, members of the TGA family of transcription factors regulate gene expression in response to many environmental and developmental cues (Table 1). Depending on the promoter context they can recruit different transcriptional co-activators, such as SA-activated NPR1 or SCL14. Moreover, they can enhance the activity of other transcription factors at a variety of promoters. All TGA factors interact with ROXY-type GRX and all 21 *Arabidopsis* ROXY interact with *Arabidopsis* TGA2. This interaction and the interaction with NPR-type proteins have been conserved after numerous rounds of gene duplications that have separated NPR, TGA, and ROXY regulatory modules into those that are involved in flower development and those regulating defense responses. It will be fascinating to elucidate whether TGA factors are direct substrates of ROXY or whether TGA interacting proteins are subject to ROXY-mediated redox-modulations, or both.

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