Auxin-induced Stress Potentiates \textit{trans}-activation by a Conserved Plant Basic/Leucine-zipper Factor\textsuperscript{*}

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The expression of nuclear genes in response to cellular and environmental cues is largely regulated by \textit{trans}-acting factors and their cognate \textit{cis}-elements. In plants, auxin hormones (e.g., indole-3-acetic acid) promote growth and affect a number of cellular processes. Auxins are believed to mediate at least some of their effect by enhancing the expression of specific genes through target \textit{cis}-elements (1, 2). The prototype for one class of auxin-responsive element is \textit{activation sequence-1 (as-1)},\textsuperscript{1} which was first identified by Lam \textit{et al.} (3) in the cauliflower mosaic virus (CaMV) 35 S promoter. Homologs of \textit{as-1} (e.g., \\textit{ocs} and \\textit{nos}), moreover, occur in the promoters of plant-transforming T-DNA genes of agrobacteria (4–6). Apart from regulating these genes in plants, a broader role for \textit{as-1} elements has been suggested by their functional presence in plant glutathione \textit{S}-transferase genes (7–9). For example, auxin-responsive expression of at least one member of the tobacco GST gene family, \textit{GNT35}, is mediated by \textit{as-1} (8, 9). Unlike other auxin-responsive elements, \textit{as-1} requires physiologically high (i.e., micromolar) concentrations of auxins to activate transcription, suggesting that this response may involve chemical stress. This view is further supported by the observation that biologically inactive analogs of auxins are also strong inducers of \textit{as-1}-dependent activity.

Multiple TGA factors that bind selectively to \textit{as-1} and related motifs have been cloned from several plant species, and some or all of these factors are likely to mediate \textit{as-1}-dependent transcription. Based on their amino acid homology, at least three subclasses of TGA factors occur in plants, with multiple members in each class. Heterodimerization between these factors may generate new combinations with potentially distinct functional properties (10). Posttranscriptional regulation may also determine the contribution of each factor to transcription (11). To date, the only TGA factor that is known to activate transcription is TGA1a of tobacco (12). Although originally suggested to be a plant homologue of CREB, TGA1a lacks several distinguishing features of this animal bZIP transcription factor (13, 14).

Although little is known about how and where TGA1a functions in plants, its evolutionary conservation implies an important regulatory role in gene expression. To test this view, \textit{trans}-dominant inhibitors of TGA1a and of its homologue PG13 have been developed and expressed in plants (10, 15). These inhibitors are believed to act by forming inactive “heterodimers” with their endogenous counterparts, thus markedly reducing their availability and activity. Although the PG13 and TGA1a inhibitors decreased \textit{as-1} binding activity, they had little or no effect on either plant growth or development. One explanation for these findings is that the residual \textit{as-1} binding activity that survives inhibition is sufficient for normal functions, or that \textit{as-1} acts in conjunction with other \textit{cis}-elements in most cellular promoters. Recent efforts to completely disrupt the functions of individual TGA factors by homologous recombination may provide alternative approaches for investigating \textit{in planta} contributions by these factors (16).

Work described here is part of an ongoing effort to elucidate the molecular mechanism(s) by which \textit{as-1}-dependent transcription is regulated in response to environmental cues. Toward this goal, we chose to study the functional properties of TGA1a as it is highly conserved in plants, suggesting an important role in \textit{as-1}-dependent transcription. In addition, TGA1a is a member of a large group of bZIP transcription factors, the activity of which in many cases is governed by cellular and environmental cues (17–19). To investigate functional properties of this tobacco factor, we employed a homologous transient transfection assay with protoplasts, an approach that has been previously used to characterize \textit{as-1}-

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\textsuperscript{1} The abbreviations used are: \textit{as-1}, \textit{activation sequence-1}; 2,4-D, 2,4-dichlorophenoxyacetic acid; 2,3-D, 2,3-dichlorophenoxyacetic acid; PCR, polymerase chain reaction; CAT, chloramphenicol acetyltransferase; GST, glutathione \textit{S}-transferase; CaMV, cauliflower mosaic virus; SA, salicylic acid; MJ, methyl jasmonate; bZIP, basic/leucine-zipper; bp, base pair(s); BY, Bright Yellow; NT, amino-terminal; CT, carboxyl-terminal; TTBS, Tris-buffered saline plus 0.5% Tween-20.
dependent transcription (20). By co-transfection and gain-of-function assays, we show here that trans-activation by TGA1a is stimulated by auxins and their analogs via chemical stress.

**EXPERIMENTAL PROCEDURES**

**Construction of Effector and Reporter Plasmids**—An oligonucleotide containing the −90 to +4 bp region of the CaMV 35 S promoter was ligated into *HindIII* and *XbaI* sites of pUC19 containing bacterial chloramphenicol acetyltransferase (CAT) coding sequence (21) to create the −90-CAT reporter. The −76 to −4 bp PCR fragment of the 35 S promoter through a 142 bp DNA fragment by PCR amplification of TGA1a cDNA clone (13). In each case, the 5′-terminal sequences for the FLAG-epitope tagged factors with translational kit (Promega). pMON effector DNAs were made by excising constructs for transient transfection studies were sequenced and analyzed in-frame into the Bluescript SKII(−) vector (Promega). G418 chimeric factors were created with an EcoRI containing PCR product that encodes for the first 147 amino acids of the yeast GAL4 factor. This fragment was inserted into the EcoRI site adjacent to the FLAG epitope. Effector constructs for transient transfection studies were sequenced and analyzed for expression of full-length protein with a coupled transcription/translation kit (Promega). pMON effector DNAs were by excising sequences for the CAT-reporter tagged factors with BamHI and Xbal, filling in the ends with Klenow DNA polymerase, and ligating the blunt-ended fragments into the vector pMON 999 at Klenow-modified BglII and EcoRI sites.

**Plant Cell Cultures**—Tobacco variety Bright Yellow 2 (BY-2) suspension cultures were maintained at 26 °C, 150× 150× 150 mm and 3% sucrose in Murashige and Skoog’s medium supplemented with 3% sucrose, B5 vitamins, and 0.9 μM of 2,4-dichlorophenoxyacetic acid (2,4-D) as described (24). Cells were subcultured weekly by transferring 10 ml of a mid-log phase culture to 100 ml of fresh medium. The packed cell volume of the suspension culture at transfer was ≈30%.

**Preparation of Tobacco Protoplasts**—For transfection experiments, BY-2 cultures were cultured for 4 days, harvested by centrifugation at 500 × g for 5 min, washed twice with auxin-free medium, and incubated in the same medium for 2 days. To prepare protoplasts, −20 ml (packed volume) of BY-2 cells were washed twice with 0.4 M mannitol and incubated in 50 ml of 1% (w/v) Cellulase Onozuka RS and 0.1% Pectolyase Y-23 (Kurabo) in 0.25 M mannitol, 0.1% Pectolyase Y-23 (Kurabo) in 0.4 M mannitol, pH 5.5, for 60–90 min at 28 °C. Every 30 min of incubation, the cells were gently aspirated and expelled with a 25-gauge needle. Protoplasts were collected by centrifugation at 3000 g for 5 min and lysed in one volume of 2% (v/v) paraformaldehyde in PBS containing 0.3 M mannitol. Fixed protoplasts were washed with PBS, collected on glass slides, and air dried. After blocking with 3% (w/v) nonfat powdered milk (Bio-Rad), the protoplasts were incubated in 1 μg/ml of anti-FLAG (M2) monoclonal antibody for 2 h at 4 °C. The protoplasts were washed with PBS, incubated with 2 μg/ml of anti-FLAG (M2) monoclonal antibody for 1 h, followed by incubation on ice with 10 μl (packed volume) of GammaBind Sepharose (Amersham Pharmacia BioTech) at 4 °C for 30 min with gentle shaking. The beads were recovered by centrifugation, washed three times with 1 ml each of RIPA buffer and resuspended in 20 μl of Laemmli loading buffer (Bio-Rad). The protein samples were fractionated by SDS-PAGE. Gels were treated with Enhance, as described by the manufacturer (NEN Life Science Products), and then analyzed by fluorography.

**Immunofluorescent Staining**—After incubation for 2 h, transfected protoplasts were harvested by centrifugation at 500 × g and fixed for 1 h at 20 °C in 2% (w/v) paraformaldehyde in PBS containing 0.3 M mannitol. Fixed protoplasts were washed with PBS, collected on glass slides, and air dried. After blocking with 3% (w/v) nonfat powdered milk (Bio-Rad), the protoplasts were incubated in 1 μg/ml of anti-FLAG (M2) monoclonal antibody in PBS overnight at 4 °C. After 4 h in PBS, the protoplasts were washed with PBS, mounted in Fluoromount-G medium (Southern Biotechnology Associates), and photographed under UV light.

**RESULTS**

**TGA1a Potentiates Transcription through as-1**—To investigate basal and signal-responsive properties of TGA1a, one or more effector and reporter genes (Fig. 1) were transiently transfected into protoplasts of tobacco BY-2 suspension cells. Changes in reporter gene activity were monitored by analyzing the activity of the encoded CAT enzyme. To minimize potential effects of physiological heterogeneity on the results, only protoplasts from cells grown to mid-log phase were used in these studies. To evaluate whether as-1-dependent transcription is regulated by auxins in transfected BY-2 tobacco protoplasts, we fixed and immunolabeled versus (−90-CAT) of the 35 S promoter of CaMV, which contains a single as-1 element centered at −72 bp from the start site of transcription, to the CAT reporter gene. This truncated 35 S promoter has previously been shown to confer basal and auxin-induced transcription through as-1 in transgenic tobacco (9, 27). To determine whether as-1 is required for transcription of the −90-CAT reporter, we studied the activity of a mutant version of this promoter (−76-CAT) that specifically lacks the upstream TGAC motif of as-1. Prior studies have shown that this mutation inhibits binding by
by elements other than moter (CHS-CAT), the activity of which in tobacco is regulated auxin, we analyzed the behavior of a chalcone synthase pro-
cDNA were also fused in-frame to a sequence that encodes for the octapeptide epitope (Kodak). Wild-type or mutant versions of TGA1a sensus sequence for translational initiation (23) upstream of the FLAG end of each cDNA was fused to a 30-bp sequence containing the con-
3 bp) CaMV 35 S promoter, which contains a single
as-1 (21, 29).

FIG. 1. Effecter and reporter constructs. Structural features of TGA1a: NT domain (amino acids 1–86), bZIP region (amino acids 87–142), and CT domain (amino acids 143–373). Acidic and glutamine-rich sequences and the dimer stabilization domain are as indicated. a, effecter DNAs. cDNAs encoding the TGA1a proteins were inserted between the −645 to +4 bp region of the CaMV 35 S promoter and the nos 3′-polyadenylation/termination sequences in pMON 999. The 5′-end of each cDNA was fused to a 30-bp sequence containing the consensus sequence for translational initiation (23) upstream of the FLAG octapeptide epitope (Kodak). Wild-type or mutant versions of TGA1a cDNA were also fused in-frame to a sequence that encodes for the 147-amino acid DNA binding domain of the yeast GAL4 transcription factor. b, reporter DNAs. All promoters were inserted upstream of a bacterial CAT gene and agrobacterial nos 3′-polyadenylation/termination sequences (21–22). The following promoters, the nucleotide end points of which are indicated in the figure, were studied: a −90 (to +4 bp) CaMV 35 S promoter, which contains a single as-1 element and TATA-box (3); a −76 (to +4 bp) CaMV 35 S promoter, which lacks the upstream TGAC motif of as-1; CHS, a chalcone synthase promoter (Chs15) from fenchbean (21) that contains a single G-box, H-box, and TATA box element; and GAL, a synthetic promoter with nine GAL4 elements fused to the TATA-box of the −40 to +4 bp CaMV 35 S promoter (22).

TGA1a (11, 13) and as-1-dependent transcription (3, 9, 27, 28). To determine the specificity of the transcriptional response to auxin, we analyzed the behavior of a chalcone synthase promoter (CHS-CAT), the activity of which in tobacco is regulated by elements other than as-1 (21, 29).

As expected, expression of CHS-CAT (Fig. 2, lanes 1–3) and
−76-CAT (lanes 4–6) in transfected protoplasts was unresponsive to the synthetic auxin 2,4-D. In contrast, expression of −90-CAT, which contains as-1, was induced >7-fold by this compound (lanes 7–9). Having shown that auxin induces as-1-dependent transcription in BY-2 protoplasts, presumably through endogenous TGA factors, we next examined whether TGA1a, when overexpressed, could enhance this transcriptional response. At the highest amount of effector DNA tested (80 μg), the activity of −76-CAT was unresponsive to TGA1a (lanes 10–12). In contrast, TGA1a enhanced basal and auxin-responsive expression of −90-CAT (lanes 13–15). Although this factor had only a modest effect on −90-CAT basal activity (compare lanes 7 and 13), the response to auxin was augmented >600% by TGA1a (compare lanes 8 and 14), resulting in a higher overall fold induction (compare lanes 9 and 15).

One possible explanation for the selective effect of TGA1a on auxin-responsive transcription might be that the synthesis or degradation of the factor is governed by auxin. To test this point, we analyzed the relative amounts of (FLAG epitope-tagged) TGA1a from control and auxin-treated protoplasts. Western immunoblot analysis with an α-FLAG antibody of total protein extracts revealed that FLAG-TGA1a accumulated in transfected protoplasts to a similar degree in the absence and presence of auxin (data not shown). Alternatively, differences between basal and auxin-induced activities might reflect a change in the subcellular compartmentalization of TGA1a. To test this hypothesis, protoplasts were fixed and analyzed by immunofluorescent staining with α-FLAG antibody for the presence of FLAG-TGA1a. This factor was localized almost exclusively to nuclei, as determined by its co-localization with 4,6-diamidino-2-phenylindole (DAPI) fluorescent staining, in both control and auxin-treated protoplasts (data not shown).

Sequences That Flank the bZIP Domain of TGA1a Confer Basal and Auxin-responsive Activation—One means by which auxin could govern the expression of the −90-CAT reporter is by altering the affinity of TGA1a for as-1. If this were so, then
TGA1a might not be expected to confer auxin-responsive activation through a heterologous DNA binding domain and target cis-element. To test this hypothesis, we fused TGA1a to a 147-amino acid region of the yeast GAL4 factor, which confers binding to the GAL4 element, homodimerization, and nuclear import (30). In tobacco protoplasts, as shown here (Fig. 3) and elsewhere (22), this truncated GAL4 factor is relatively inactive on a minimal promoter that contains multiple GAL4 cis-elements (GAL-CAT), and was unaffected by auxin (Fig. 3, lanes 1–3). By contrast, GAL4-TGA1a markedly enhanced the activity of GAL-CAT in auxin-treated, but not control, protoplasts. Because the basal activity of GAL4-TGA1a was below that of GAL4 alone, we surmised that the presence of multiple DNA binding, dimerization, and nuclear import sequences in the chimeric factor interferes with its activity, expression, or stability. This notion was tested by removing the bZIP domain of TGA1a to create GAL4-TGA1aΔbZIP. In protoplasts, GAL4-TGA1aΔbZIP showed significantly higher basal and auxin-responsive trans-activation than those seen with GAL4-TGA1a (Fig. 3, lanes 4–5 versus lanes 7–8). In vivo labeling experiments confirmed that this difference is due, at least in part, to the higher accumulation of GAL4-TGA1aΔbZIP versus GAL4-TGA1a (data not shown).

Basal trans-activation by TGA1a—By GAL4 gain-of-function assays similar to those described above, we identified the contribution of specific portions of TGA1a to basal and signal-responsive trans-activation. Earlier work has shown that the first 80 amino acids of TGA1a contain multiple acidic residues that are essential for trans-activation in vitro and in vivo (12, 28). To confirm these findings and to determine whether amino-terminal residues contribute to the response to auxin, we fused the GAL4 binding domain to the first 86 amino-terminal (NT) residues of TGA1a to create GAL4-TGA1a-NT. This chimeric factor stimulated GAL-CAT reporter activity to a similar degree in the presence or absence of 2,4-D (Fig. 3), implying that amino-terminal residues are not likely to be a direct regulatory target of auxin.

The fact that GAL4-TGA1a-NT showed considerable basal activity when compared with GAL4-TGA1aΔbZIP suggested that the NT domain in the latter factor is latent or repressed. In vivo labeling of these two factors revealed that their relative accumulation was similar, either in the presence or absence of auxin (Fig. 4, lanes 3 and 4 versus lanes 7 and 8), at an effector concentration (40 μg) that maximized GAL-CAT reporter activity (Fig. 5). Differences between the basal activities of GAL4-TGA1a-NT and GAL4-TGA1aΔbZIP thus result from intrinsic transcriptional properties of these factors.

To further delineate sequences required for basal trans-activation, the carboxyl-terminal (CT) region (amino acids 142–373) of TGA1a was fused to GAL4 to create GAL4-TGA1a-CT. Basal activity of GAL4-TGA1a-CT was comparable to that of GAL4-TGA1aΔbZIP (Fig. 3). However, correcting for the fact that GAL4-TGA1a-CT was expressed nearly 4 times more than GAL4-TGA1aΔbZIP (Fig. 4), the basal activity of the CT domain is likely to be similar to background levels seen with GAL4 alone.

Results with the CHS-CAT reporter (Table I) showed that the GAL4 chimeric factors do not generally effect RNA polymerase II-dependent transcription or have cytotoxic effects. These data imply that GAL4 chimeric factors affected gene expression through the GAL4 element and not by an effect on CAT reporter enzyme activity or cell viability.

The Carboxyl-terminal Domain of TGA1a Mediates the Response to Auxin—trans-activation by GAL4-TGA1a-CT under basal conditions was stimulated ~6-fold by auxin (Fig. 3). At saturating amounts of effector DNAs, GAL4-TGA1a-CT had less than half the activity of GAL4-TGA1aΔbZIP (Fig. 5). This result was not simply due to relative differences in the accumulation of these factors, as the amount of GAL4-TGA1a-CT was ~4 times higher than that observed with GAL4-TGA1aΔbZIP (Fig. 4) at the highest amount of effector DNA tested. These data imply that NT and CT domains in TGA1a may both be required for achieving maximal trans-activation by this factor in response to auxin.

**Chemical Cues That Induce Transcription through as-1 and**

**FIG. 3.** Basal and signal-responsive activation domains of TGA1a. Protoplasts were co-transfected with GAL-CAT and the following effector constructs: the GAL4 binding domain alone (GAL4), GAL4 fused to either wild-type TGA1a (GAL4-TGA1a) or a mutant version of TGA1a that lacks the bZIP domain (GAL4-TGA1aΔbZIP), or GAL4 fused to the amino-terminal (GAL4-TGA1a-NT) or carboxyl-terminal (GAL4-TGA1a-CT) regions of TGA1a. Transfected protoplasts were incubated in protoplast culture medium with or without 50 μM 2,4-D and analyzed for CAT activity. Basal (open columns) and auxin-responsive (solid columns) gene activity is expressed as percentage of conversion of CAT substrate to its acetylated forms. Fold induction (hatched columns) is the ratio of auxin-responsive to basal activities. The mean and S.E. of three experiments are shown. The absence of error bars reflects a S.E. of <1.0.

**FIG. 4.** Differential expression in vivo of chimeric TGA1a factors. Protoplasts were transfected with 40 μg of pMON vector or GAL4 effector DNAs and incubated for 20 h without (−) or with (+) 50 μM 2,4-D (auxin) and [35-S]methionine. Protein extracts from protoplasts were subsequently analyzed by immunoprecipitation, SDS-polyacrylamide gel electrophoresis, and autoradiography for the presence of FLAG-tagged factors. Arrows in the autoradiograph indicate labeled immunoprecipitated factor.
from the 2,4-D and its inactive analog 2,3-D to stimulate transcription possibility, we tested the ability of the biologically active auxin to involve chemical stress cues (8, 20). To further investigate this possibility, we extended these findings by demonstrating that transiently expressed TGA1a can activate transcription only at concentrations that inhibited cell growth, thus implying that these transcriptional responses to auxin are mediated by chemical stress.

**FIG. 5.** GAL-CAT activity is saturable by chimeric TGA1a factors. Protoplasts were transfected with the GAL-CAT reporter and 0–80 μg of effector DNAs: GAL4-TGA1a-NT (open squares), GAL4-TGA1aΔbZIP (closed squares), GAL4-TGA1a-CT (open circles), and GAL4-TGA1a (closed triangles). Transfected protoplasts were incubated in protoplast culture medium containing 50 μM 2,4-D for 20 h and analyzed for CAT activity, which is expressed as percentage of conversion of CAT substrate to its acetylated forms. The mean of three experiments is shown.

| Effector       | % of basal (S.D.) | % of auxin (S.D.) |
|----------------|-------------------|-------------------|
| GAL4          | 100               | 100               |
| GAL4-TGA1aΔbZIP| 100.5 (8.5)       | 91 (1.0)          |
| GAL4-TGA1a-NT | 85.6 (3.5)        | 97.5 (6.5)        |
| GAL4-TGA1a-CT | 99 (2.6)          | 113 (1.7)         |

**TABLE I** trans-activation by chimeric GAL4 factors is promoter-specific

2,4-D over a range of concentrations. To determine the concentrations of 2,4-D that promote or inhibit growth, auxin-starved BY-2 cells were incubated with 2,4-D over a range of concentrations for 1 week, after which time the fresh weight of the cells was determined. The optimal growth of the culture under these conditions required 0.1 μM 2,4-D, a concentration that has little effect on the activities of either the –90-CAT reporter or GAL4-TGA1aΔbZIP (Fig. 6). Significantly, 2,4-D augmented transcription only at concentrations that inhibited cell growth, thus implying that these transcriptional responses to auxin are mediated by chemical stress.

**FIG. 6.** Auxin promotes as-1-dependent transcription and GAL4-TGA1aΔbZIP trans-activation via chemical stress. Protoplasts were transfected with either the –90-CAT reporter alone (open diamond) or GAL4-TGA1aΔbZIP and GAL4-CAT (open circles), incubated for 20 h in protoplast culture medium containing 0–100 μM 2,4-D, and then analyzed for CAT activity. Reporter gene activity is expressed as the percentage of conversion of CAT to its acetylated forms. Mitogenic or cytotoxic activities of 2,4-D on BY-2 cells were measured by plotting the fresh weight of the culture after a 1-week incubation in medium containing 0–100 μM of the auxin (closed triangles). The mean and S.E. of two experiments are shown. The absence of error bars reflects a S.E. of <1.0.

**DISCUSSION**

In plants, as-1 and related elements have been shown to confer transcription in response to such diverse cues as auxins, salicylic acid, methyl jasmonate, heavy metals, wounding, and xenobiotic stress. Little is currently known about the individual contributions of as-1 binding TGA factors to these transcriptional responses. Of these factors, only TGA1a has been shown to activate plant transcription in vivo (28). Results here extend these findings by demonstrating that transiently expressed TGA1a can activate as-1-dependent transcription in response to auxin.

As in plants, auxin induces a change in the activity of TGA factors of BY-2 protoplasts to enhance transcription from a truncated –90 (to +4 bp) 35 S promoter that contains a single as-1 element centered at –72 bp (3). Moreover, as-1 is the primary determinant of this truncated promoters activity in tobacco root and suspension culture cells (9, 32). In tobacco leaves (27) and roots (data not shown), as-1 confers transcription from the –90 promoter in response to exogenous auxin. In BY-2 protoplasts, basal and auxin-responsive transcription from –90-CAT requires as-1, as evidenced by the fact that a mutant version of this promoter (–76-CAT) that lacks the upstream TGAC motif of this element was inactive (Fig. 2).

Having shown that the –90-CAT reporter gene was selectively activated by auxin, we next examined whether TGA1a

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might potentiate the activity of this promoter. Although transiently expressed TGA1a enhanced basal and auxin-responsive transcription through as-1, it had a more pronounced effect on the latter activity. The ability of TGA1a to differentially affect −90-CAT activity was explored by a number of ways. First, reporters that lack as-1 (e.g., −76-CAT and CHS-CAT) were unresponsive to transiently expressed TGA1a, thus demonstrating that the affect of TGA1a on −90-CAT activity is through as-1. Results here with CHS-CAT also show that a G-box element that shares homology in its core motif to as-1, but binds poorly to TGA1a in vitro (13, 33), is unresponsive to this factor.

We also explored the possibility that TGA1a might be sequestered in the cytoplasm and subsequently mobilized to the nucleus in response to auxin. This type of regulation is observed with the stimulus-coupled nuclear import of NF-κB and STAT transcription factors in animal cells (17). By immunofluorescent staining, we observed that (epitope-tagged) TGA1a is predominantly localized to the nucleus in control and auxin-treated protoplasts. Because no change in the cellular amount of this factor was found in response to auxin, we conclude that TGA1a is constitutively present in the nucleus and that its transcriptional activity is latent in the absence of a stimulus.

If binding to as-1 is regulated by auxin, we reasoned that a chimeric fusion protein of TGA1a and the heterologous DNA binding domain of the yeast GAL4 transcription factor might promote constitutive, rather than signal-responsive, transcription. Contrary to this expectation, we found that GAL4-TGA1a conferred both activities. This prompted us to investigate whether as-1-specific binding, and perhaps other bZIP-encoded functions, are required for trans-activation by this factor. We found that the activity of a mutant form of GAL4-TGA1a that lacks the bZIP domain (i.e., GAL4-TGA1aΔbZIP) was enhanced by auxin, thus demonstrating that bZIP-encoded functions (e.g., as-1-specific binding, dimerization, and nuclear import) in TGA1a are not the primary regulatory target of this stimulus. Removal of the bZIP domain from GAL4-TGA1a was also found to increase its overall basal and auxin-responsive activities. Although the reason for this effect is unknown, we speculate that the presence of multiple and distinct DNA binding domains in GAL4-TGA1a may result in a chimeric factor that is functionally unstable or that binds to plant as-1 elements, thus decreasing its effective concentration on the GAL-CAT reporter. Data from in vivo labeling experiments favor the former possibility, because the accumulation of GAL4-TGA1a is at least one-fourth that of GAL4-TGA1aΔbZIP at effector DNA concentrations that maximize transcription.

Trans-acting factors stimulate gene expression by overcoming rate-limiting steps in the recruitment of RNA polymerase II to the promoter. Within these factors, domains that are rich in acidic, glutamine, proline, or isoleucine residues commonly mediate trans-activation or repression. The NT region of TGA1a contains an acidic-rich region (amino acids 18–66) that promotes trans-activation in vitro (13) and in vivo (28). Experiments here confirm and extended these results by showing that the first 84 residues of the amino terminus of TGA1a, when fused to the DNA binding domain of the heterologous GAL4 protein, confer basal trans-activation. Moreover, the activity of the NT domain is constitutive and unaffected by auxin, in keeping with our findings that the response to auxin is largely mediated by the carboxyl-terminal domain of TGA1a. We were surprised to find that at saturating amounts of each factor, the basal activity of GAL4-TGA1a-NT is markedly higher than that of GAL4-TGA1aΔbZIP (Fig. 3). This difference suggests that CT residues 143–373, which flank the bZIP domain, might inhibit the basal activity of the NT region. We speculate that auxin somehow relieves this repression, thus maximizing trans-activation. This notion is also consistent with results here showing that maximal activation by GAL4-TGA1aΔbZIP and GAL4-TGA1a-NT are quite similar in the presence of auxin. In regard to the potential regulatory role of the CT domain on NT activity, we note that trans-activation by an animal bZIP factor termed ATF-2 is regulated through intramolecular interactions (34).

Evidence here indicates that the carboxyl-terminal region of TGA1a may have multiple roles in transcription. First, acidic and glutamine residues make up over one-third of a 50-amino acid region within the CT domain (Fig. 1), thus implying the presence of a second activation domain in TGA1a. Consistent with this view, we found that the CT domain of TGA1a alone can confer basal and auxin-responsive transcription through the GAL4 binding domain, albeit at lower levels than those seen with TGA1aΔbZIP. Further mutagenesis and gain-of-function assays may help to identify specific residues in the CT domain that contribute to these individual activities.

How might auxin affect the trans-activation potential of the CT domain? Prior studies have identified a “dimer stabilization domain” (amino acids 178–373) within the CT of TGA1a. Although this domain enhances the ability of TGA1a to dimerize and bind as-1, it is not essential for either of these activities (35). We suggest that this domain may reversibly bind to an additional factor that mediates transcription, a situation that is analogous to that observed with other bZIP factors (36, 37). Experiments here indicate that under basal conditions this mediator may bind the CT domain of TGA1a to repress trans-activation through the NT domain. This inhibition is apparently relieved by auxin. We are further exploring this notion that a regulatory protein differentially associates with TGA1a in response to auxin.

Diverse plant hormones such as auxins, SA, and MJ, in addition to a number of their biologically inactive analogs, stimulate transcription through as-1 type elements (2, 20). We found that 2,3-D, a biologically inactive analog of the auxin 2,4-D, stimulated as-1-dependent gene expression and the trans-activation potential of TGA1a. Lack of correspondence between the biological activity of auxins or their analogs and their ability to induce transcription has been interpreted as evidence of a response to general chemical stress (20). To further explore the nature of the auxin pathway involved, we examined whether mitogenic or toxic properties of 2,4-D mediated its affect on as-1-dependent transcription. As transcription was only induced by concentrations of 2,4-D that inhibited cell-growth, we suggest that this response involves an inhibitory chemical stress pathway. Although benzyl isothiocyanate, dimethyl fumarate, and hydroquinone induce the transcription of animal genes through chemical stress (38), we found that 2,3-D, a biologically inactive analog of the auxin 2,4-D, stimulated as-1-dependent gene expression and the trans-activation potential of TGA1a. Lack of correspondence between the biological activity of auxins or their analogs and their ability to induce transcription has been interpreted as evidence of a response to general chemical stress (20). To further explore the nature of the auxin pathway involved, we examined whether mitogenic or toxic properties of 2,4-D mediated its affect on as-1-dependent transcription. As transcription was only induced by concentrations of 2,4-D that inhibited cell-growth, we suggest that this response involves an inhibitory chemical stress pathway.
possibility that transcription factors that control the expression of GST genes may be evolutionarily conserved.

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