The Plant Cell, Vol. 3, 1167-1175, November 1991 © 1991 American Society of Plant Physiologists

An Auxin-Responsive Promoter Is Differentially Induced by Auxin Gradients during Tropisms

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We constructed a chimeric gene consisting of a soybean small auxin up RNA (SAUR) promoter and leader sequence fused to an *Escherichia coli* β-glucuronidase (GUS) open reading frame and a 3′ untranslated nopaline synthase sequence from *Agrobacterium tumefaciens*. This chimeric gene was used to transform tobacco by Agrobacterium-mediated transformation. In R2 etiolated transgenic tobacco seedlings, GUS expression occurred primarily in elongation regions of hypocotyls and roots. In green plants, GUS was expressed primarily in the epidermis and cortex of stems and petioles, as well as in elongation regions of anther filaments in developing flowers. GUS expression was responsive to exogenous auxin in the range of 10^{-8} to 10^{-3} M. During gravitropism and phototropism, the GUS activity became greater on the more rapidly elongating side of tobacco stems. Auxin transport inhibitors and other manipulations that blocked gravitropism also blocked the asymmetric distribution of GUS activity in gravistimulated stems. Light treatment of dark-grown seedlings resulted in a rapid decrease in GUS activity. Light-induced decay in GUS activity was fully reversed by application of auxin. Taken together, our results add support for the formation of an asymmetric distribution of auxin at sites of action during tropism.

INTRODUCTION

Tropism is asymmetric growth or curvature of plant organs in response to a stimulus such as gravity, light, or touch. The most widely studied tropisms are gravitropism and phototropism, which are stimulated by gravity and light, respectively. The mechanisms involved in growth curvature or tropisms have been debated for more than half a century but are still largely unresolved. A large amount of experimental evidence suggests that tropisms result from an asymmetric distribution of auxin on the opposing sides of an organ, which are proximal and distal to a stimulus such as gravity or unilateral light (Briggs, 1963; Harrison and Pickard, 1989; Evans, 1991). A theory, referred to as the Cholodny-Went theory, that was formulated some 60 years ago, proposes that tropism or growth curvature is initiated by the directional longitudinal and lateral transport of auxin (Wilkins, 1984). This transport would create an asymmetric distribution of auxin that, in turn, would result in asymmetric growth or curvature of an organ. Because auxin is a hormone involved in cell elongation (Evans, 1985), an asymmetric distribution of auxin would promote asymmetric growth. The Cholodny-Went theory has been challenged because the magnitude of and/or kinetics for the auxin asymmetry, as measured by bioassay or analytical techniques, is widely held to be insufficient to promote the differential growth observed (Firn and Digby, 1980; Trewavas, 1981; Evans, 1991).

We have recently shown that a class of auxin-responsive mRNAs called small auxin up RNAs (SAURs) is most strongly expressed in elongating regions of hypocotyls and epicotyls (McClure and Guilfoyle, 1987, 1989a; Gee et al., 1991). These mRNAs are symmetrically distributed in epidermal and cortical cells of hypocotyls when seedlings are grown in the normal vertical orientation but become asymmetrically distributed in the lower and upper halves of the hypocotyl within 20 min after seedlings are reoriented to a horizontal position (i.e., a position that promotes gravitropic curvature) (McClure and Guilfoyle, 1989a). Here, we demonstrated that a SAUR promoter fused to a GUS reporter gene drives the expression of GUS in an asymmetric manner during gravitropism and phototropism. We showed that auxin transport inhibitors or other manipulations that prevent auxin transport inhibited both asymmetric growth and asymmetric GUS expression. Because the SAUR promoter responds only to active auxins in a dose-dependent fashion (McClure and Guilfoyle, 1987), our results suggest that an active pool of auxin becomes asymmetrically distributed during both gravitropism and phototropism. Furthermore, our results suggest that the asymmetry of auxin in an active pool that develops during tropisms may be greater than previous measurements

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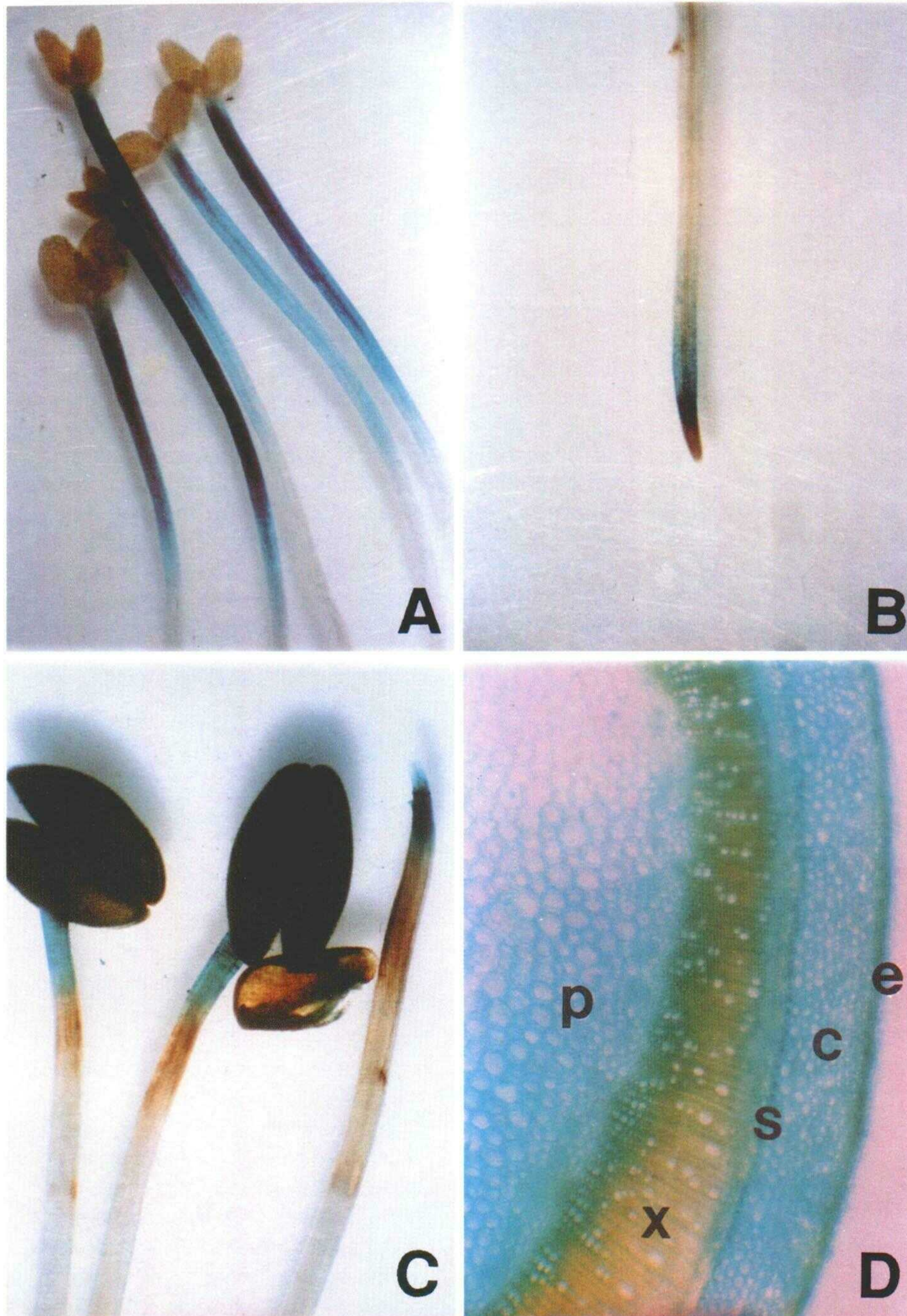

Figure 1. Histochemical Staining for GUS in Organs and Tissues of Transgenic Tobacco Seedlings and Plants.

(A) Dark-grown, 10-day-old seedlings.
(B) Root of a young plant.
(C) Anther filaments.
(D) Cross-section through a stem of a young plant. e, epidermis; c, cortex; s, starch sheath; x, xylem; p, pith.

have indicated and, therefore, may be sufficient for the differential growth observed. We conclude that the SAUR promoter, which is exquisitely sensitive and specific for measuring the localization and degree of the auxin response system, provides a molecular probe that helps clarify the physiological role of auxin in tropic responses.

RESULTS

Tissue-Specific and Organ-Specific Expression of the GUS Fusion Gene

As shown in Figure 1, histochemical staining for GUS activity revealed that the SAUR promoter was most active in a variety of elongating regions of organs, including hypocotyls, roots, and anther filaments of transgenic tobacco plants. Within these elongating organ regions, the greatest amount of GUS expression was detected in epidermis, cortex, and a layer of cells just internal to the cortex, referred to as the starch sheath. Some expression was also detected in developing xylem cells and within the pith. This localized expression driven by the SAUR promoter is in general agreement with tissue print and in situ hybridization studies conducted in soybean seedlings that used a SAUR antisense RNA probe (McClure and Guilfoyle, 1989a; Gee et al., 1991).

Auxin Dose Response in Transgenic Tobacco

Young transgenic tobacco seedlings that had been grown in the light were used to determine the range of auxin concentrations that could induce the SAUR promoter. Figure 2 shows that as little as 10^{-8} M exogenous indoleacetic acid (IAA) caused an increase in GUS expression in transgenic tobacco seedlings. Increased expression of GUS was observed over several orders of log increase in IAA concentration, up to an optimal concentration of 10^{-5} M. Further increases in auxin concentration resulted in less than optimal GUS expression. Thus, our results showed that the SAUR promoter in transgenic tobacco is responsive to auxin concentrations ranging from, at least, 10^{-8} to 10^{-3} M of externally applied IAA.

GUS Expression during Gravitropic Curvature of Transgenic Tobacco Stems

When 3- to 4-week-old transgenic tobacco plants were placed horizontally, the stems bent upward in a negative gravitropic response. As shown in Figure 3, histochemical staining revealed that an asymmetric distribution of GUS activity occurred during gravitropic bending. A larger amount of GUS activity was detected on the bottom, more rapidly elongating side of the stem compared to the top side of the stem. We have tabulated all of our data in terms of percent GUS activity on the lower versus the upper side of the stem. This type of tabulation was necessary because the absolute amounts of GUS activity in stems varied from plantlet to plantlet, even though the plantlets were clonal (i.e., derived from the same R2 transgenic plant by explanting stem segments). Table 1 summarizes the quantitative assays for GUS activity on the bottom and top side of the stem. These data indicated that a gradual increase in GUS activity occurred on the bottom side of the stem over a 5-hr gravitropic response. A greater than fourfold difference in GUS activity was detected on the bottom versus the top side of the stem after 5 hr of gravistimulation. Both the gravitropic curvature and asymmetric distribution of GUS activity were blocked if plants were treated with either of the auxin transport inhibitors, 2, 3, 5-triiodobenzoic acid (TIBA) or N-(1-naphthyl)phthalamic acid (NPA).

To provide further correlations between expression from the auxin-inducible SAUR promoter and gravitropic bending, we performed a number of experimental manipulations that perturbed or modified the gravitropic response in tobacco seedlings. Data provided in Table 2 indicated that removal of either leaves or apical portions of the tobacco plants, which are thought to be sources of auxin, resulted in both decreased gravitropic curvature and asymmetric distribution of GUS activity. Removal of both apices and leaves resulted in a more dramatic reduction of gravitropism and asymmetric distribution of GUS activity. If 5 μM IAA was added to the cut apex, a modest restoration of

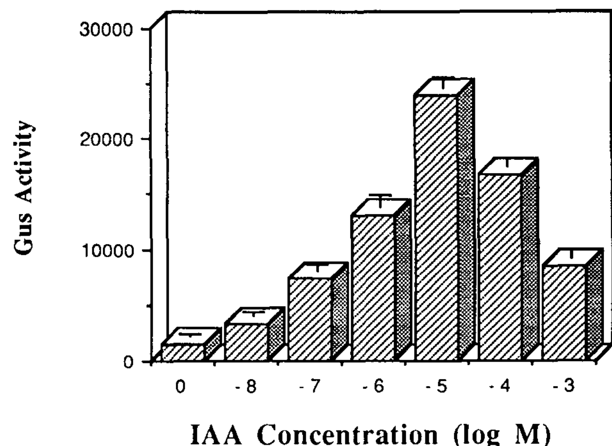

Figure 2. Auxin Dose Response for SAUR Promoter-Driven GUS Expression.

Ten-day-old, light-grown transgenic tobacco seedlings were incubated in different concentrations of IAA for 24 hr and then GUS activity was determined fluorometrically with tissue homogenates. GUS activity units are pmol $min^{-1}mg^{-1}$ protein.

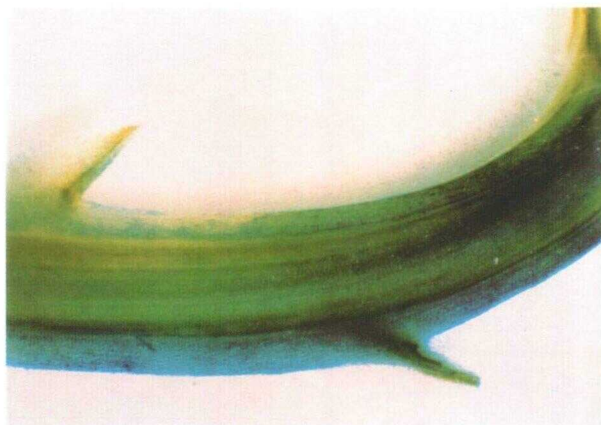

Figure 3. Histochemical Staining for GUS in the Stem of a Transgenic Tobacco Plant after 4 Hr of Gravistimulation.

A 4-week-old tobacco plant was grown in light/dark cycles as described in Methods and then placed in darkness for 24 hr prior to carrying out the gravitropic experiment. Gravistimulation was carried out in the dark, and the stem was bisected and stained for GUS activity.

gravitropic bending and asymmetric distribution of GUS activity was observed. This restoration of the gravitropic and GUS responses was more dramatic if the applied auxin concentration was raised to 50 μM. If plants were submerged in a solution of 10 μM IAA, gravitropic curvature and asymmetric distribution of GUS activity were reduced compared to submerged plants that had not been exposed to auxin. The gravitropic and asymmetric GUS responses in the submerged plants were further reduced by increasing the auxin concentration to 50 μM.

GUS Expression during Phototropic Curvature of Transgenic Tobacco Stems

When 3- to 4-week-old transgenic tobacco explants were placed in the dark for 24 hr and were then exposed to a unilateral light source, the stems bent toward the light over a period of several hours. Table 3 provides quantitative information on the GUS activity on the sides of the stem proximal to and distal from the light source. There was a gradual change in GUS activity so that more GUS activity was detected on the more rapidly elongating, distal side compared to the proximal side over 12 hr of unilateral light exposure. By 12 hr of phototropic curvature, twice as much GUS activity was detected on the side of the stem distal to the unilateral light source, compared to the illuminated side. Because the plants were pretreated for 24 hr in the dark prior to applying the unilateral light source, we concluded that there was actually a decrease in GUS activity on the lighted side of the stem during the phototropic curvature (see results below on seedlings grown under light and dark conditions). Although the absolute decrease in GUS activity on the lighted side is impossible to assess because of the variation in GUS activity within individual plants, the adverse effect of light on GUS activity, described below, supports our contention that light inhibits GUS activity on the photostimulated side of the stem. In any case, it is clear that GUS activity on the faster growing side of the stem is greater in the gravitropically versus the phototropically stimulated plants, and this difference in GUS activity is reflected in the degree and rate of curvature observed with these two tropisms. Application of the auxin transport inhibitor TIBA blocked both phototropic curvature and asymmetric distribution of GUS activity. Although these results suggest that auxin transport is at least partially responsible for the asymmetry of GUS expression observed, it is possible that light-induced auxin destruction might also be involved in the development of this asymmetry.

Figure 4A shows that, similar to the effect of unilateral light on the expression of the SAUR promoter-GUS fusion gene, we have observed that exposure of dark-grown seedlings to light resulted in a rapid loss of GUS activity in transgenic tobacco seedlings. A significant loss in GUS activity was observed within 4 hr after seedlings were exposed to light. After 8 hr of light exposure, the GUS activity was only 30% of that observed in seedlings that had not been exposed to light. It is unlikely that the loss

Table 1. Kinetics for Shoot Bending and Expression of GUS during Gravitropism

Gravity Stimulation (hr)	Curvature (degrees)	GUS Activity (% in lower side)
0	0.0	50.4 ± 2.7
1.0	0.3 ± 1.3	55.6 ± 1.5
1.5	3.6 ± 1.5	59.5 ± 2.1
2.0	20.5 ± 2.2	66.5 ± 3.4
3.0	34.3 ± 4.8	72.7 ± 4.2
5.0	45.9 ± 4.1	83.3 ± 5.8
5.0[a]	42.5 ± 4.4	80.6 ± 6.7
5.0[a] (1 mM TIBA)	2.3 ± 1.5	53.1 ± 4.6
5.0[a] (1 mM NPA)	3.5 ± 1.3	52.6 ± 3.1

After gravistimulation for the time indicated, the bending portion of the stem was removed and bisected with a razor blade into upper and lower halves. After removal of the pith from each half, the tissues were homogenized, and GUS activity was determined fluorometrically. The percent of total GUS activity in the lower half compared to that in the upper half is tabulated.

[a] In these experiments, plants were submerged in potassium phosphate buffer (pH 6.0) so that the auxin-transport inhibitors TIBA or NPA could be uniformally applied.

Table 2. Effects of Different Manipulations on Shoot Bending and GUS Activity during Gravitropism

Treatment	Curvature (degrees)	GUS Activity (% in lower side)
Control	43.7 ± 5.1	76.8 ± 2.7
Removal of leaves[a]	28.3 ± 2.4	67.0 ± 1.5
Removal of apex[b]	12.5 ± 1.8	56.3 ± 2.0
Removal of leaves and apex	3.6 ± 1.2	52.5 ± 1.4
Removal of leaves and apex Addition of 5 μM IAA[c]	12.9 ± 2.5	58.7 ± 2.4
Removal of leaves and apex Addition of 50 μM IAA[c]	20.3 ± 3.5	68.5 ± 2.9
Submerged Control[d]	41.3 ± 4.2	76.2 ± 2.3
Submerged + 10 μM IAA[d]	23.5 ± 3.1	67.1 ± 2.7
Submerged + 50 μM IAA[d]	3.5 ± 1.4	57.3 ± 2.0

GUS activity was determined in the lower and upper halves of the stem as described in Table 1.
[a] All leaves below the shoot tip were removed from the plants.
[b] The 4 mm shoot tip was removed from the plants.
[c] Agar blocks (0.8%) containing IAA in 10 mM potassium phosphate buffer were attached to the severed apex surface at the onset of gravistimulation.
[d] Plants were submerged as described in Table 1.

in GUS activity resulted from light inactivation of GUS because we have also observed that SAUR mRNA levels rapidly decayed when dark-grown soybean seedlings were exposed to light (this was likely due to shut down of the SAUR promoter) (Figure 4B) and because GUS activity was rapidly restored if transgenic tobacco seedlings that had been exposed to light were treated with exogenous auxin (Figure 4C). Because the dose responses and levels of auxin-induced GUS expression were nearly identical in the seedlings grown under light and dark conditions, it is clear that light does not alter the seedlings' apparent sensitivity to auxin. These results suggest that the SAUR promoter is inactivated in the presence of light and that this inactivation is likely due to a decrease in auxin concentration within the tissues that express the SAUR genes.

DISCUSSION

Results presented here are consistent with our contention that the SAUR promoter responds to intracellular or extracellular changes in auxin concentration within active pools (i.e., sites of action). First, the SAUR promoter was activated in an auxin concentration-dependent fashion and was extremely sensitive to the application of exogenous auxin, with as little as 10^{-8} M IAA inducing expression from this promoter. Second, auxin transport inhibitors blocked asymmetric expression of GUS driven by the SAUR promoter under gravistimulation and photostimulation. Third, removal of internal auxin supply (i.e., stem apices and leaves) blocked expression driven by the SAUR promoter, and exogenous application of auxin to stem apices substituted for the normal sources of auxin supply. Fourth, uniform external application of auxin (i.e., plants submerged in auxin) blocked asymmetric expression of GUS driven by the SAUR promoter under gravistimulation, presumably by preventing the asymmetric distribution of auxin. Fifth, exposure of etiolated seedlings to light resulted in reduced expression from the SAUR promoter. We suggest that this light effect results from a decrease in active pools of auxin in the tissues where SAURs are expressed. This decrease might be brought about by a block in the directed transport of auxin, the conversion of auxin to inactive metabolites, the sequestration of auxin to inactive compartments, or the photodestruction of auxin. Because exogenous application of auxin restored the activity of the SAUR promoter in seedlings exposed to light, the promoter appears to be activated by the same concentrations of auxin under both light and dark conditions. This latter experiment also indicates that when the SAUR promoter is dramatically shut down in the light, sensitivity to auxin is apparently not involved because addition of auxin fully restores the activity of the promoter in a dose-dependent manner, which parallels the dose response of dark-grown seedlings.

Based on the results presented here, we propose that SAUR promoter-GUS fusion reporter gene expression provides a more accurate assessment of relative changes in auxin concentrations that occur during tropisms and other auxin-induced growth responses than assessments based on bioassay or analytical procedures. Although expression of the fusion gene did not allow us to determine the

Table 3. Kinetics for Shoot Bending and Expression of GUS during Phototropism

Unilateral Light Stimulation (hr)	Curvature (degrees)	GUS Activity (% in nonilluminated side)
0	0.0	50.4 ± 2.5
2.5	0.2 ± 1.0	53.8 ± 1.8
5	6.7 ± 1.2	57.5 ± 2.1
8	12.5 ± 1.9	60.9 ± 3.0
12	29.5 ± 2.2	65.0 ± 2.5
8[a]	10.3 ± 1.5	60.0 ± 2.6
8[a] (+ 1 mM TIBA)	−0.2 ± 0.4	49.4 ± 1.7

GUS activity was determined in the illuminated and nonilluminated halves of the stem as described in Table 1.
[a] In these experiments, plants were submerged as described in Table 1.

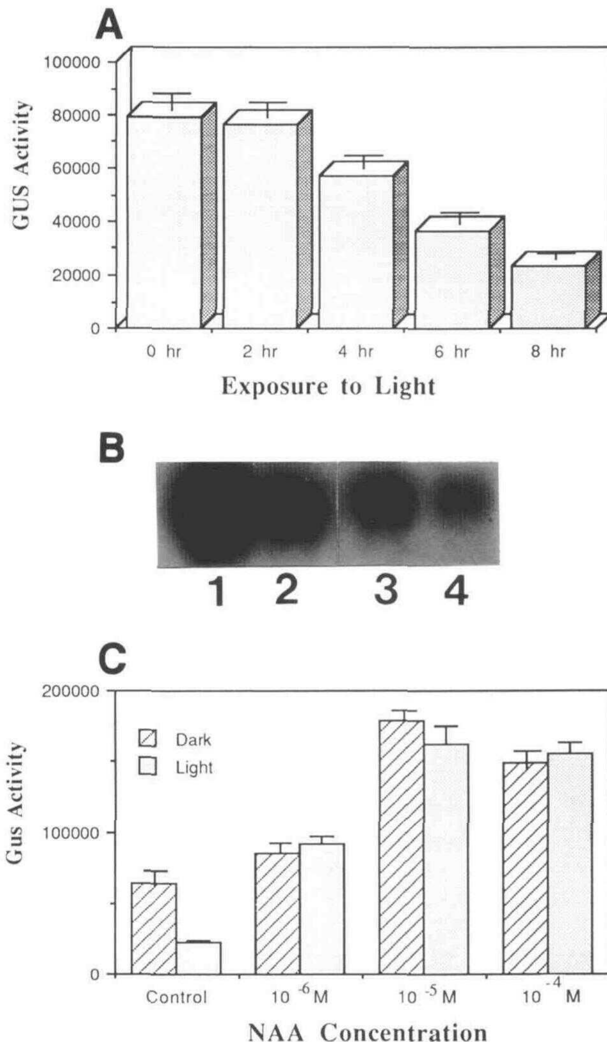

Figure 4. *Inhibition of GUS Activity and Decay in SAURs Resulting from Light Exposure.*

(A) Kinetics for the light inhibition of GUS activity in dark-grown transgenic tobacco seedlings.

(B) Kinetics for the light-induced decay of SAURs in dark-grown soybean seedlings. Lane 1, dark-grown seedlings treated for 3 hr with 50 μM 2,4-D; lane 2, dark-grown seedlings with no 2,4-D treatment; lane 3, dark-grown seedlings transferred to light for 3 hr and no 2,4-D treatment; lane 4, dark-grown seedlings transferred to light for 6 hr and no 2,4-D treatment.

(C) Reversal of light inhibition of GUS activity in transgenic tobacco seedlings treated with auxin. Seedlings were treated in the light or dark for 8 hr with the concentrations of α-naphthaleneacetic acid (NAA) indicated. Dark refers to dark-grown plants. Light refers to dark-grown plants that were exposed to light for 8 hr in the presence or absence (control) of NAA.

GUS activity was determined fluorometrically with tissue homogenates in **(A)** and **(C)**, and GUS activity units are pmol $min^{-1}mg^{-1}$ protein.

absolute levels of active pools of auxin on opposing sides of gravistimulated or photostimulated organs, it did allow us to monitor a change in the relative concentration of auxin (as assayed with the SAUR promoter) at a site of action in tissues that express SAURs. The limitations and inaccuracies of bioassays have been thoroughly addressed previously (Firn and Digby, 1980; Trewavas, 1981), and analytical procedures developed to measure auxin concentrations are limited by amounts of material required for analysis and problems inherent with disruption of tissues, such as distinguishing between active auxin pools and inactive pools. The sequestration of auxins into various cellular and extracellular compartments makes it nearly impossible to determine the concentration of auxin within a site of hormone action by bioassay or analytical applications. On the other hand, the SAUR promoter-GUS fusion reporter system used here is exquisitely responsive to only active pools of auxin that are compartmentalized at an intracellular or extracellular (i.e., this refers to the plasma membrane) site of auxin action. Furthermore, our results suggest that the relative levels of auxin that trigger responses (in our case, increased transcription from the SAUR promoter) on opposing sides of an organ during tropisms are likely to be greater than previous estimates that were based on analytical or bioassay techniques. Based on the dose response of the SAUR promoter and the relative promoter activity on opposing sides of tropically stimulated stems, we suggest that auxin concentrations at a site of action might differ by 10-fold or greater during tropic curvature. Such a large difference would not be detected by analytical methods or bioassays due to the complex distribution of auxin within intracellular and extracellular pools. Regardless of the absolute concentration difference for auxin on the opposing sides of the stem undergoing gravitropism, this difference is sufficient to drive the expression of the SAUR promoter in an asymmetric fashion (McClure and Guilfoyle, 1989a; this paper). Thus, there is no a priori reason to believe that such a concentration difference could not also drive asymmetric growth during tropisms.

Although it might be argued that we cannot accurately monitor changes in auxin concentration with the SAUR promoter because of unknown transduction processes that operate between "the auxin receptor" and the SAUR promoter, we would argue, based on our results and in the absence of evidence for changes in transduction pathways during tropisms, that the SAUR promoter is indeed sensing changes in auxin concentration. Because of the possible complexity of the signal transduction processes from auxin perception to activation of the SAUR promoter and production and/or stability of the GUS enzyme, we cannot rule out the possibility that additional factors besides auxin gradients may play a role in the asymmetric distribution of GUS activity during tropisms. However, it is worth noting that bioassays, which have been routinely used to monitor changes in hormone concentrations, must

involve a considerably more complex array of signal transduction events than a SAUR promoter-driven GUS assay.

Our results suggest that an asymmetric distribution of auxin develops prior to and during phototropism and gravitropism and that this asymmetry is probably greater than estimates reported previously. Furthermore, our results suggest that it is likely that directed transport of auxin is responsible for the asymmetric distribution of this hormone because auxin transport inhibitors block both the tropisms and the asymmetric distribution of GUS activity. Our results are consistent with the theory that auxin concentrations (at least auxin concentrations in active pools) change during tropisms because auxin transport inhibitors block both tropisms and asymmetric distribution of GUS. Although our results on gravitropism support the Cholodny-Went theory, our results on phototropism do not distinguish whether auxin gradients develop exclusively from auxin transport or whether auxin destruction might also be involved (Briggs, 1963). The decreased expression of GUS in light-grown seedlings and the dose-dependent increase in GUS expression in these seedlings, compared with dark-grown seedlings, after exposure to external auxin (Figure 4), suggest that auxin concentration in active pools, not auxin sensitivity, regulates the SAUR promoter.

Our evidence for auxin asymmetry in tropistic responses rests on the assumption that SAUR promoter-GUS fusion gene expression is a reliable reporter for auxin concentrations at sites of auxin action. We have previously demonstrated (1) that SAUR gene expression is transcriptionally regulated by a variety of active auxins but not by inactive analogs (McClure and Guilfoyle, 1987; McClure et al., 1989); (2) that SAUR mRNAs accumulate within 2.5 min after auxin is applied (McClure and Guilfoyle, 1987) and turn over rapidly (McClure and Guilfoyle, 1989b; Franco et al., 1990); (3) that other plant hormones (i.e., including cytokinins, abscisic acid, gibberellic acid, and ethylene) and a variety of other chemical and environmental agents do not activate or inactivate transcription of the SAUR genes (McClure and Guilfoyle, 1987); (4) that expression of SAURs is largely restricted to rapidly elongating regions of organs and tissues thought to be involved in the cell extension responses (McClure and Guilfoyle, 1987, 1989a; Gee et al. 1991; this paper); and (5) that an asymmetric distribution of SAURs is detected before any observable gravitropic curvature is observed in soybean hypocotyls (McClure and Guilfoyle, 1989a).

Although changes in auxin sensitivity during tropisms are not completely ruled out by our results, we suggest that changes in sensitivity need not be invoked if auxin concentrations change by 10-fold or greater at a site of auxin action during tropisms. Because sensitivity to hormones has not yet been defined or substantiated in biochemical or molecular terms, sensitivity to auxin during tropisms remains to be rigorously demonstrated. In any case, our results suggest that auxin transport, which presumably produces an auxin gradient, is involved in driving asymmetric expression of the SAUR promoter in auxin-responsive tissues during tropisms. We argue that if an auxin gradient is produced during tropisms that is sufficient to drive asymmetric expression of an auxin-responsive promoter, then such a gradient may be sufficient to drive the asymmetric growth observed during tropic bending.

In addition to providing some support for the Cholodny-Went theory, our results also indicated that the GUS reporter gene could be used to monitor fairly rapid changes in gene expression in certain tissues of transgenic plants. We have shown that, at least in some tissues and organ regions, GUS activity (i.e., presumably GUS protein) was relatively unstable. This instability of GUS was especially apparent in the dark-grown tobacco seedlings that had been exposed to light. Because the GUS activity in these seedlings was fully restored to levels observed in dark-grown plants following auxin application, the decay in GUS activity in the light was most likely due to a repression or shut down of transcription of the SAUR promoter in the light, followed by a gradual decay of the GUS protein. We have not investigated whether the GUS protein turns over more rapidly in the light than the dark. Based on quantitative assays, we believe that the difference in GUS activity on the bottom and top side of a gravistimulated stem was largely due to increased synthesis of GUS on the bottom side. On the other hand, we believe that the difference in GUS activity on the nonilluminated and illuminated side of photostimulated stems was due, at least in part, to loss of GUS activity on the illuminated side. However, this loss in GUS activity must have involved a change in auxin distribution on the illuminated and nonilluminated sides because the auxin transport inhibitor TIBA blocked the asymmetric distribution of GUS as well as the bending of the stem toward the light. Because of the quantitative differences in the levels of GUS activity observed during gravitropism and phototropism, our results suggest that the mechanism for auxin redistribution may be somewhat different for these two tropisms. In both cases, however, our results suggest that an asymmetric distribution of auxin within active pools occurs rapidly during tropisms and this asymmetry is revealed by differential expression of GUS driven by the SAUR promoter.

METHODS

Plant Material

Three independent transgenic R2 tobacco plants (*Nicotiana tabacum* cv Xanthi-nc) with a single copy of the fusion gene were used in all of the experiments. Seven- to 10-day-old light-grown R2 seedlings, which were germinated and grown on moistened sand at 25°C, were used to determine the auxin dose response, and dark-grown seedlings of the same age were used to determine the effect of light treatments on GUS activity. The light sources for unilateral and direct room light experiments were

fluorescent lamps (200 μmol m^{-2} sec^{-1}). Explants (i.e., stem cuttings) from transgenic R2 tobacco plants were grown on agar media containing 30 g/L sucrose and MS salts (Sigma Chemical Company) at 25°C with a daylength of 14 hr. The explants were grown for 3 to 4 weeks until plants reached a height of 3 to 4 inches and had formed roots. Three to six individual plantlets, propagated from a single R2 plant, were used for each treatment, and results are given as a mean of replicates with standard deviation. Although different R2 transgenic plants gave similar qualitative results, it was necessary to use a single R2 parent for generating explants used in tropic experiments to control for quantitative differences in expression levels of GUS in different transgenic plants. All experiments were repeated at least once with seedlings or plantlets derived from a second independent R2 transgenic plant. Gravitropic experiments were conducted in complete darkness. For experiments with auxin and auxin transport inhibitors, untreated as well as treated plants were submerged in 10 mM potassium phosphate buffer (pH 6.0) within an aerated plexiglass chamber. After treatments, tropically stimulated plant shoots were traced onto pieces of paper, and the degree of bending was measured through the stem axis by using a protractor. Immediately after treatments or manipulations, seedlings or excised tissues to be used for fluorometric GUS assays were frozen in liquid nitrogen and stored at −80°C.

Construction of Fusion Genes and Plant Transformation

An 832-bp fragment of the SAUR 10A (McClure et al., 1989) promoter and 5′ leader was amplified by polymerase chain reaction and fused to a GUS open reading frame in pEBGUS (Hagen et al., 1991), which was derived from pAGUS1 (Skuzeski et al., 1990). The promoter was fused to the GUS open reading frame at the start site of translation, and the GUS open reading frame was followed by a nopaline synthase (NOS) 3′ untranslated sequence (Hagen et al., 1991). The SAUR/GUS/NOS fusion was cloned and excised as a PstI/EcoRI fragment. The isolated fragment was made blunt-ended with mung bean nuclease and ligated into pMON505 at its unique EcoRV site (Horsch and Klee, 1986). The pMON construct was mobilized into the binary vector *Agrobacterium tumefaciens* pTi37-SE using the triparental-mating method and transformed into tobacco by the leaf disc method described by Horsch et al. (1985). Transformed shoots were regenerated and rooted in Magenta boxes (Horsch et al., 1985; Rogers et al., 1987), and the plantlets were transferred to soil and grown in growth chambers at 25°C with a 14-hr daylength.

RNA and DNA Isolation and Hybridization

RNA was isolated from soybean hypocotyls, subjected to electrophoresis on agarose gels, and blotted to nylon membranes, as described previously (Franco et al., 1990).

GUS Assay and Histochemical Staining

Fluorometric and histochemical staining for GUS activity were conducted as described by Jefferson (1987) and Hagen et al. (1991).

ACKNOWLEDGMENTS

We thank Xiangyang Shi for technical assistance, Dr. Jim Skuzeski for providing a GUS/NOS fusion construct, pAGUS1, Dr. Christopher Brown for constructing the SAUR 10A promoter/pEBGUS fusion, and Monsanto Chemical Company for providing pMON505. This work was supported by Grant DCB 8904493 from the National Science Foundation to T.J.G. and G.H. This is paper No. 11,428 of the Journal Series of the Missouri Agricultural Experiment Station.

Received August 9, 1991; accepted September 20, 1991.

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