

Tissue-Specific and Organ-Specific Expression of Soybean Auxin-Responsive Transcripts GH3 and SAURs

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We used in situ hybridization to localize two classes of auxin-regulated transcripts, GH3 and SAURs, within organs and tissues of soybean seedlings and flowers. GH3 transcripts occurred in the inner cortex and protoxylem ridges of roots and were expressed transiently during flower and pod development. SAUR transcripts were expressed in the epidermis, cortex, and starch sheath of epicotyls and immature hypocotyls. SAUR transcripts became more abundant on the bottom side of hypocotyls that were undergoing gravitropic curvature. SAURs were also expressed in developing xylem elements of the hypocotyl hook. When soybean organ sections were treated with 50 micromolar 2,4-dichlorophenoxyacetic acid (2,4-D), GH3 transcripts became more abundant in the vascular regions of all organs analyzed. High levels of GH3 transcripts were also found in developing palisade mesophyll cells of leaves, cotyledons, and flowers treated with 2,4-D. SAUR transcripts became more abundant in the epidermis, cortex, starch sheath, and pith of epicotyls and hypocotyls after 2,4-D treatment. Our results showed that a variety of tissues and cell types express auxin-responsive transcripts and that different tissues respond rapidly to exogenous auxin by expressing different hormone-responsive genes.

INTRODUCTION

When plants or excised plant organs are treated with auxin, a variety of responses such as stem or coleoptile elongation, cambial cell division, and xylem differentiation are observed, depending on the organ or organ region examined. All of these auxin-induced growth and developmental alterations involve changes in gene expression (reviewed by Guilfoyle, 1986; Theologis, 1986; Hagen, 1987; Key, 1989). A number of mRNAs and/or genes have been identified that increase rapidly after auxin application to plants, excised plant organs, or cultured plant cells (Walker and Key, 1982; Hagen et al., 1984; Theologis et al., 1985; McClure and Guilfoyle, 1987; van der Zaal et al., 1987; Alliotte et al., 1989; Takahashi et al., 1989). Expression of some of these genes occurs either before or concomitant with auxin-induced cell extension or cell division.

Our laboratory has described two classes of auxin-inducible transcripts identified by cDNA clone probes. The first class includes GH1, GH2, GH3, and GH4, which were isolated from soybean hypocotyls that had been sprayed with the synthetic auxin 2,4-D and harvested 2 hr later (Hagen et al., 1984). This auxin treatment ultimately leads to an increase in girth of the hypocotyls and roots, owing to radial cell expansion and cambial cell division within mature regions of these organs (reviewed by Key, 1969;

Guilfoyle, 1986). The GH transcripts and/or polypeptides have been shown to increase in abundance after auxin treatment in a variety of plant organs (Hagen et al., 1984; Hagen and Guilfoyle, 1985; Wright et al., 1987). The second class of auxin-responsive mRNAs, the SAURs (small auxin-up RNAs), is a family of highly homologous sequences (e.g., 6, 10a, and 15) that are most strongly expressed in the zone of cell elongation in the soybean hypocotyl (McClure and Guilfoyle, 1987) and on the bottom side of gravistimulated hypocotyls (McClure and Guilfoyle, 1989a). Both of these classes of auxin-responsive mRNAs are transcriptionally induced by auxins within 5 min to 15 min after hormone application.

We used in situ hybridization to localize GH3 and SAUR transcripts in soybean seedlings and flowers. We used probes for these rapidly induced auxin-responsive mRNAs to estimate the proportion of cells and tissues that have auxin receptors and to address the following questions. Are GH3 and SAUR transcripts expressed in the same or different tissues? Does exogenous auxin alter the pattern of tissue-specific gene expression or simply elevate the levels of expression in those same tissues that express in the absence of added auxin? Are these auxin-responsive mRNAs, which have been previously analyzed in hypocotyls, expressed in other organs, including roots, leaves, petioles, and flowers, and, if so, at what developmental stages are they expressed? Are these mRNAs detected

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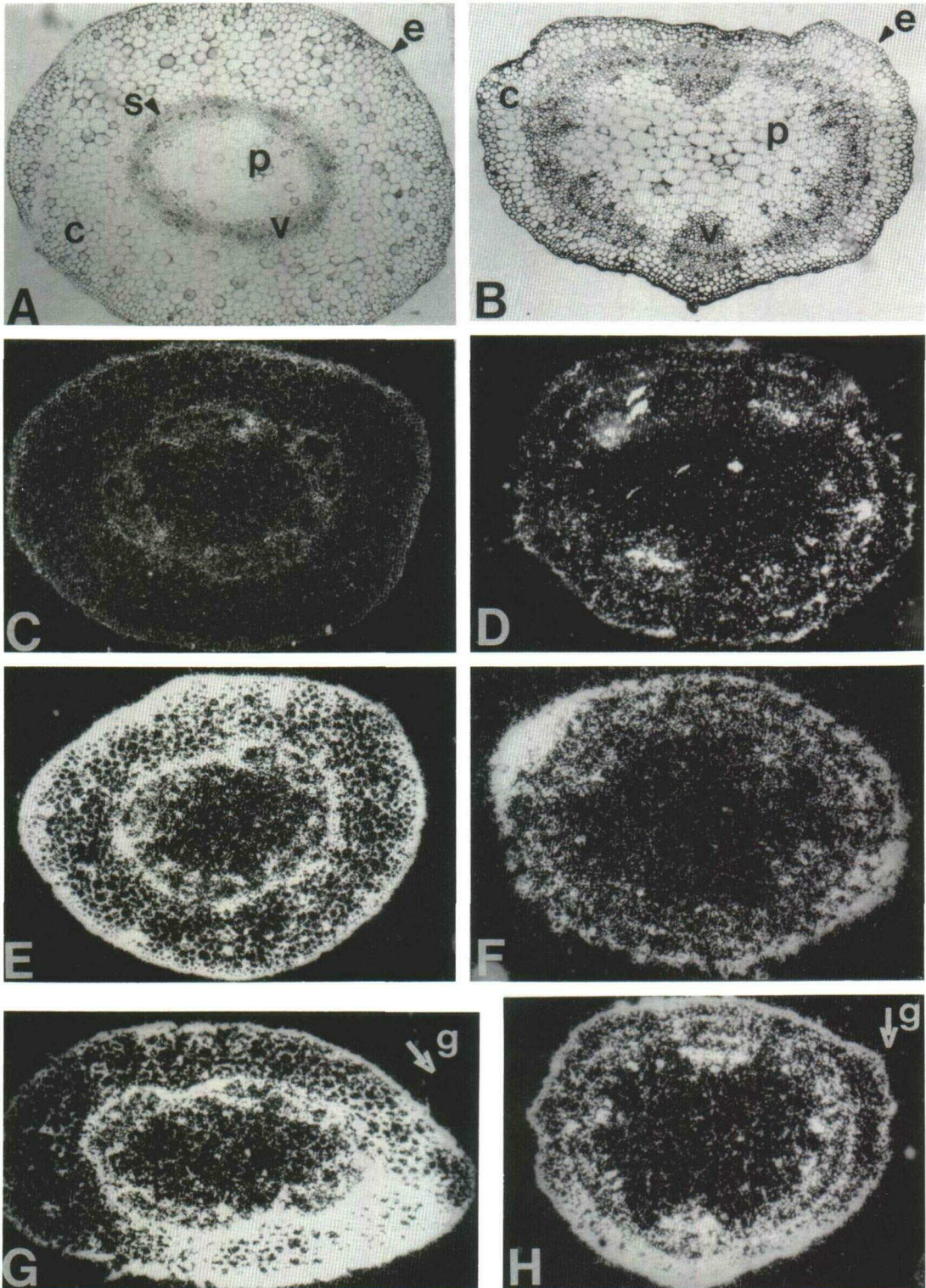


Figure 1. Localization of SAUR Transcripts in Soybean Hypocotyls and Epicotyls That Had not Been Exposed to Exogenous Auxin.

in specific cell types during auxin-regulated processes such as cell extension, gravitropism, and xylogenesis? Are these mRNAs expressed in both external and internal tissues of elongating hypocotyls and epicotyls? Results from this work demonstrated that GH3 and SAUR transcripts show different patterns of expression within various organs and tissues and that both transcripts rapidly increase in abundance in specific tissues and/or organs after plants or plant sections are treated with auxin. Our results indicated that different tissues respond to auxin by activating different types of genes and that the GH3 and SAUR auxin-regulated gene products are likely involved in different auxin-mediated cellular responses. Furthermore, our results showed that most cells in most plant organs rapidly respond to exogenous auxin and that GH3 and SAUR transcripts display both unique and overlapping patterns of tissue-specific gene expression. These results suggested that multiple types of auxin receptors and/or signal transduction pathways are operative in plants and that these receptors and/or pathways may coexist in some of the same cells and tissues, whereas other tissues may contain one or the other receptor and/or pathway.

RESULTS

Localization of SAURs in Untreated Soybean Seedlings

In situ hybridization was carried out to localize the GH3 and SAUR transcripts in various organs and organ regions of the etiolated soybean seedling and soybean flowers. When soybean seedlings are grown in the dark, the hypocotyl undergoes hyperextensive growth over a 7-day period, and the epicotyl emerges from the cotyledons at about day 5 or 6 and shows a similar hyperextensive growth. SAUR transcripts were observed in the elongating region of hypocotyls in 3-day-old or 4-day-old seedlings and the elongating region of epicotyls in 7-day-old seedlings, as shown in Figure 1. In both organs, expression of

SAURs occurs in the endodermis, cortex, and epidermal tissues, but vascular tissues show little, if any, SAUR expression. In epicotyls, SAUR transcripts are most abundant in the cortical regions adjacent to major vascular bundles (Figure 1F).

When hypocotyls or epicotyls of etiolated seedlings are placed horizontally for 60 min, gravitropic curvature occurs because of more rapid cell elongation on the bottom compared with the top side of the hypocotyl (McClure and Guilfoyle, 1989a, 1989b). In situ hybridization revealed that the SAURs become more abundant within the epidermis and cortex on the bottom side relative to the top side of both hypocotyls and epicotyls undergoing gravitropic bending (Figures 1G and 1H).

Low levels of SAUR expression were observed in the basal hypocotyl of 3-day-old but not 4-day-old seedlings (data not shown). It is likely that as the basal hypocotyl becomes further removed from the elongating region during the third and fourth day of seedling growth, the SAURs are not synthesized and decay in the more mature basal regions.

In situ hybridization revealed that the meristematic hook region of soybean hypocotyls contains low levels of SAURs compared with the elongation region (data not shown), confirming previous results obtained by RNA blot hybridization (McClure and Guilfoyle, 1987). SAURs were detected by in situ hybridization, however, within developing xylem cells of the hook, as shown in Figure 2. Localization of SAUR transcripts to developing xylem elements does not result from artifactual association of SAURs with xylem cell walls because hybridization to mature xylem elements, which contain no cytoplasm, was not observed. In addition, sense RNA probes did not hybridize with the developing xylem elements (data not shown).

A variety of other organs were examined for SAUR transcripts by using in situ hybridization. No hybridization was detected in mature roots, plumules, or cotyledons of young etiolated seedlings or in mature flowers (data not shown).

Figure 1. (continued).

- (A) Transverse section of hypocotyl stained with toluidine blue.
 - (B) Transverse section of epicotyl stained with toluidine blue.
 - (C) Hypocotyl transverse section hybridized to SAUR sense probe.
 - (D) Epicotyl transverse section hybridized to SAUR sense probe. Xylem elements appear white because of refraction of their thick cell walls and not because of hybridization with the sense RNA probe.
 - (E) Hypocotyl transverse section hybridized to SAUR antisense probe.
 - (F) Epicotyl transverse section hybridized to SAUR antisense probe.
 - (G) Gravitropically stimulated hypocotyl transverse section hybridized to SAUR antisense probe. The direction of the gravity vector, *g*, is indicated.
 - (H) Gravitropically stimulated epicotyl transverse section hybridized to SAUR antisense probe. The direction of the gravity vector, *g*, is indicated.
- Seedlings in (A) to (F) were vertically maintained throughout growth, whereas seedlings in (G) and (H) were placed horizontally for 60 min before fixation of the hypocotyls. Hybridizations are shown in dark-field microscopy. e, epidermis; c, cortex; p, pith; v, vascular cylinder; s, starch sheath.

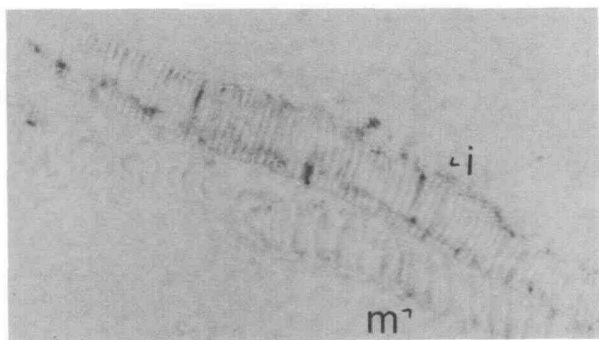


Figure 2. Hybridization of the SAUR Antisense Probe to a Developing Xylem Element.

A mature (m) and a developing (immature, i) xylem element from the hook region of a soybean hypocotyl are shown in bright-field microscopy. The dark silver grains are visible along the length of the developing xylem element.

Localization of GH3 mRNA in Untreated Soybean Seedlings and Flowers

The GH3 mRNA shows a considerably different distribution from that of the SAURs in young soybean seedlings and flowers. This transcript was not detected in untreated cotyledons, basal or elongating hypocotyls, epicotyls, or plumules (data not shown). Figure 3 shows that a small amount of GH3 hybridization was observed in mature regions of soybean roots. In this organ, GH3 mRNA was detected in the endodermal and inner cortex regions. The GH3 mRNA appeared to be most abundant in the protoxylem ridges where cell divisions give rise to more xylem and lead to secondary root formation. The dense cytoplasm and small size of the cells within the protoxylem might, however, contribute to the more intense hybridization signal within this tissue compared with that in the adjacent cells with larger vacuoles.

Results shown in Figure 4 indicate that GH3 is most highly expressed in developing organs of flowers and pods (relative to other vegetative organs) in plants that have not been exposed to exogenous auxin. GH3 transcripts were detected in the lower placenta and trichomes of ovaries and in the ovule integuments of mature flowers (Figure 4C). GH3 transcripts were not observed in floral buds at the early stages of floral development (Figure 4D), but transcripts were detected in the vascular regions of the receptacle at about 9 days to 12 days before anthesis (DBA) (Figure 4E). At about 6 DBA to 8 DBA, GH3 expression was detected in the ovary and petals as well as the receptacle (Figure 4F). GH3 transcripts appear in the ovule at about the time when the ovule and carpel vascular tissues become united (approximately 2 DBA to 6 DBA; Figure 4G; Carlson and Lersten, 1987). At the time of

anthesis and fertilization, GH3 transcripts are most abundant in the ovule and ovary (Figure 4H). After fertilization and during the early stages of embryogenesis, GH3 transcripts begin to decay in the ovary and embryo (Figure 4I), and by 10 days to 14 days after anthesis, GH3 transcripts are no longer detectable in the embryo and surrounding tissues (data not shown).

Tissue-Specific and Organ-Specific Auxin Induction of SAURs

To determine whether the same or different organs and tissues produce auxin-inducible transcripts when seedlings or organ sections are exposed to exogenous auxin, we analyzed a variety of auxin-treated organs and organ regions by *in situ* hybridization. Figure 5 shows induction of SAURs in elongating hypocotyl and epicotyl sections that have been preincubated for 4 hr in phosphate/sucrose buffer and then treated with phosphate/sucrose buffer in the absence or presence of 50 μ M 2,4-D for 1 hr. In these excised organ sections, exogenous auxin caused an increase in abundance of the SAURs in the same tissues (i.e., the epidermis, cortex, and starch sheath) that express SAURs in the absence of exogenous auxin. Lower levels of SAURs were detected in the pith of auxin-induced hypocotyls and epicotyls. In hypocotyl hooks, auxin-induced SAUR expression could be detected only in the epidermis, cortex, and pith of the basal region that joins the elongating region of the hypocotyl and in developing xylem cells in the upper part of the hook (data not shown).

When excised plumules were treated with 2,4-D, a slight amount of SAUR hybridization was observed within most, if not all, cells of the plumule, whereas SAUR transcripts were not detected in untreated plumules (data not shown). In flowers, a low level of SAUR expression was detected in sepals, but not other organs, after auxin treatment (data not shown). Cotyledons, mature root sections, and basal hypocotyl sections from 4-day-old seedlings did not show an accumulation of SAUR transcripts after auxin treatment (data not shown).

Tissue-Specific and Organ-Specific Auxin Induction of GH3 mRNA

Whereas strong auxin induction of SAUR expression is limited primarily to elongation zones of hypocotyls and epicotyls, Figure 6 shows that GH3 transcripts are induced by auxin in a wide variety of different organs including cotyledons, hypocotyls, epicotyls, roots, and flowers. Induction of GH3 gene expression by exogenous auxin within these various organs showed a tissue specificity that is also clearly distinct from SAUR gene expression. In excised, incubated epicotyls and hypocotyls, GH3 mRNA showed the greatest amount of auxin induction within the

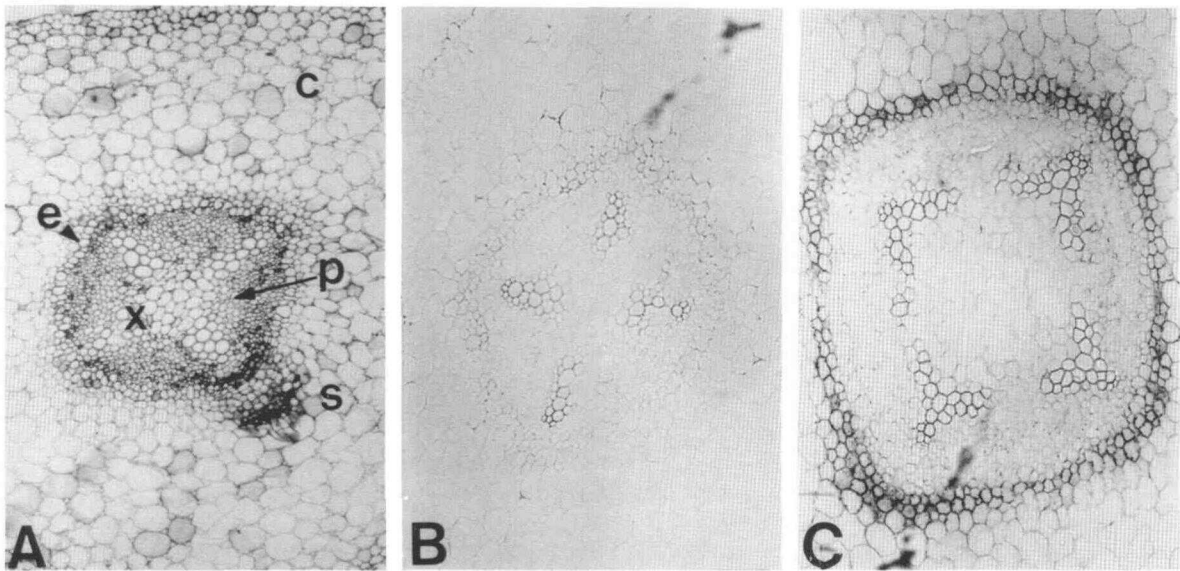


Figure 3. Localization of GH3 Transcripts in Roots That Had not Been Exposed to Exogenous Auxin.

(A) Transverse section of a root stained with toluidine blue.

(B) Root transverse section hybridized to GH3 sense RNA probe.

(C) Root transverse section hybridized to GH3 antisense RNA probe.

Hybridization is shown in bright-field microscopy. c, cortex; e, endodermis; x, xylem; p, phloem; s, secondary root.

vascular tissues (Figures 6A, 6B, and 6C). Lower amounts of auxin-induced GH3 transcripts occurred in the epidermis, cortex, and pith. However, this apparent lower induction of transcripts by auxin outside of the vascular cylinder may reflect the larger vacuole to cytoplasm volume of the cortex and pith. Within the vascular cylinder, cells in the phloem, xylem and cambium showed induction of GH3 mRNA in response to exogenous auxin. Like the SAURs, GH3 is expressed in developing xylem elements (data not shown), but GH3 is also expressed in other developing and mature cell types in the vascular tissue. The results shown in Figure 6 are from organs treated for 1 hr with 2,4-D, but we have also analyzed organs that were treated for longer times with auxin because GH3 mRNA continues to increase in abundance for several hours after hormone addition (Hagen et al., 1984). Longer incubation periods with auxin revealed that the tissue distribution of GH3 remained identical to that observed at 1 hr after auxin treatment, but the abundance of the GH3 transcript continued to increase in these tissues for at least 4 hr after auxin application (data not shown).

We also analyzed elongating and basal hypocotyl regions from intact seedlings that were sprayed with 2.5 mM 2,4-D. Under these conditions, auxin causes a herbicidal response that results in massive cellular proliferation within the vascular cambium and swelling of the epidermal and cortical cells (Key, 1969; Guilfoyle, 1986). Hypocotyl tissues analyzed 8 hr after this auxin application showed the

same relative levels of tissue-specific GH3 gene expression as that observed with excised, incubated hypocotyl sections (data not shown).

Roots accumulated GH3 transcripts in response to exogenous auxin in essentially the same tissues as in hypocotyls and epicotyls (i.e., vascular tissues; Figure 6D). However, auxin induction of GH3 mRNA within the stele of the root appeared to be much stronger in the pericycle, endodermis, and protoxylem ridges compared with the phloem and cambium. No hybridization with the GH3 antisense RNA probe was detected in excised root sections or excised hypocotyl and epicotyl sections that were incubated in the absence of auxin, using the same exposure times as shown in Figure 6 (data not shown).

Figure 7A shows that in the excised, incubated plumules, exogenous auxin induces accumulation of GH3 transcripts in the vascular bundles, but also in cells destined to become palisade mesophyll tissue (Lersten and Carlson, 1987). No hybridization was detected in excised plumules that were incubated in the absence of auxin (data not shown). Cotyledons also showed strong induction of GH3 mRNAs in vascular tissues after auxin application (data not shown). Less, but significant, auxin induction of GH3 mRNA occurred in most other tissue types within plumules and cotyledons.

Figure 7B shows that in excised, incubated mature flowers, leaf-like structures such as the sepals and petals showed strong auxin-induced GH3 gene expression pri-

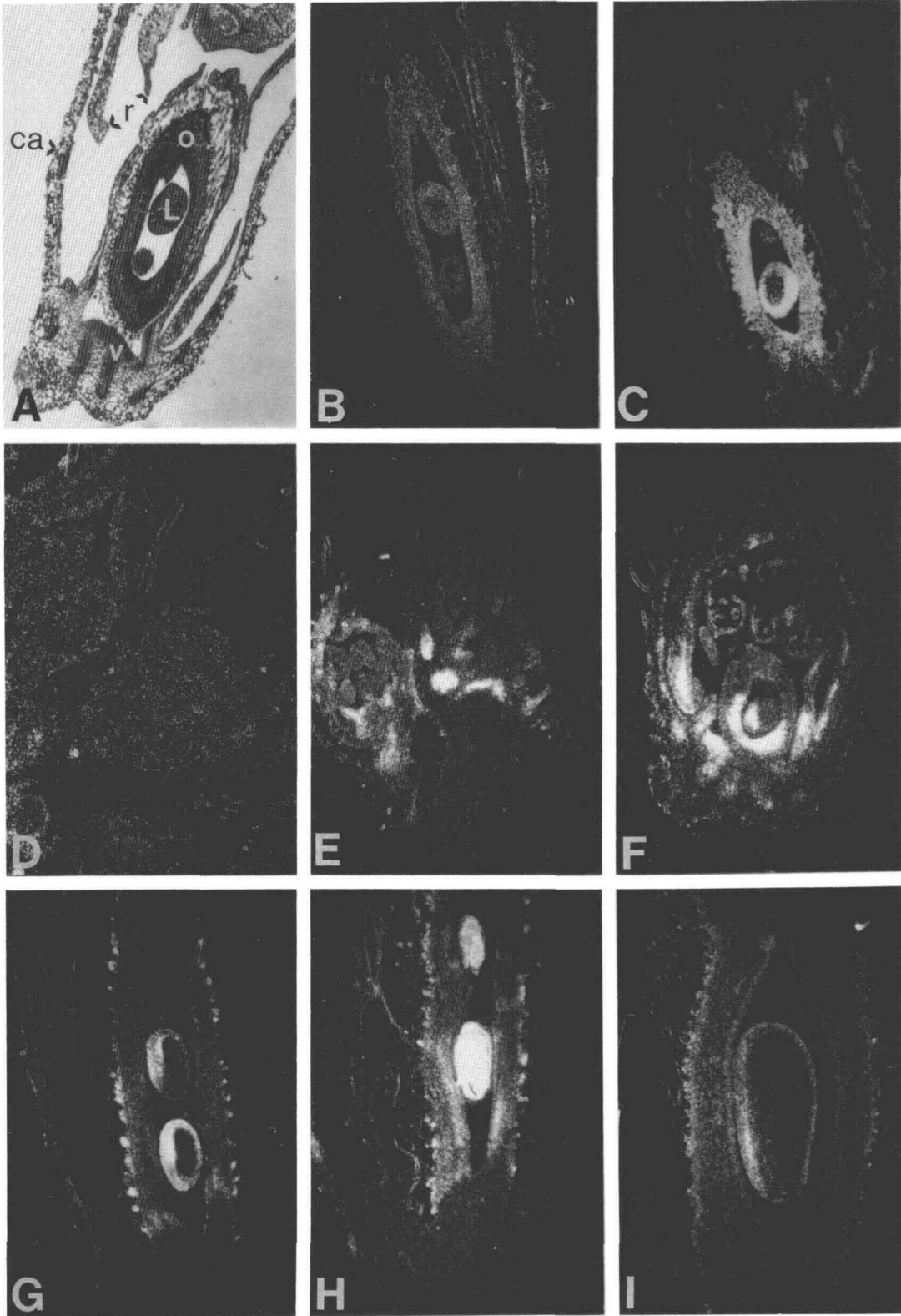


Figure 4. Expression of GH3 mRNA during Flower and Pod Development.

marily in vascular tissues. Auxin also induced GH3 gene expression in the inside layer of cells of the banner petal. The vascular tissues of most other floral organs, including peduncles, ovules, ovary, and stamens, showed expression of GH3 mRNA after application of auxin. Whereas the ovules and ovary wall of freshly harvested flowers expressed GH3 transcripts in the absence of added auxin (Figure 4), GH3 mRNA became more abundant in the ovules and ovaries of excised flowers that had been incubated for 1 hr with 2,4-D (data not shown). At the stage of floral development (at the position where the flower was sectioned) shown in Figure 7, no hybridization to any floral organ was detected in excised flowers that were incubated in the absence of auxin (data not shown).

In addition to the organs described above, exogenous auxin induced GH3 gene expression in a variety of other organs of the plant such as lateral shoot buds and petioles (data not shown). In these latter organs, GH3 gene expression was again localized primarily to vascular tissues.

DISCUSSION

The Auxin-Induced Transcripts GH3 and SAUR Display Distinctly Different Patterns of Expression

We have shown that two classes of auxin-responsive mRNAs, GH3 and SAUR, show strikingly different patterns of expression in a variety of soybean organs and tissues (Table 1). For the most part, the organ-specific and tissue-specific SAUR gene expression was maintained after exogenous auxin application. Auxin elevated the abundance of the mRNAs in organ regions and tissues that normally express SAURs. On the other hand, little, if any, expression of GH3 was observed in untreated soybean seedlings, except in the root. After auxin administration, however, GH3 mRNA was detected primarily within the vascular tissue but also in epidermis, cortex, and pith of a variety of organs including hypocotyl, epicotyl, plumule, cotyledon, and root.

In general, SAUR transcripts are most abundant in tissues that are elongating or are programmed to elongate

in response to auxin. The observation that SAURs accumulate on the lower, more rapidly growing side of horizontally placed seedlings (McClure and Guilfoyle, 1989a; Figure 1) provides further correlation between the expression of SAURs and auxin-induced cell elongation. (This correlation is made under the assumption that auxin concentrations or sensitivities change on the upper and lower side of the hypocotyl during gravitropism.) It is important to note, however, that SAURs accumulate in a number of hypocotyl and epicotyl cell or tissue types (e.g., inner cortex, starch sheath, and pith) that are not thought to be primary participants in auxin-induced cell elongation (Kutschera and Briggs, 1987; Kutschera et al., 1987; Dietz et al., 1990). Our observations on tissue-specific SAUR expression add support to the belief that these mRNAs are regulated by endogenous auxin in the intact seedling because SAURs are expressed in epidermal and cortical cells of elongating hypocotyls and epicotyls of intact seedlings, disappear when these organs sections are depleted of auxin (by extended incubation of excised sections in incubation media lacking exogenous auxin), and rapidly reappear in the same tissues after excised organs are exposed to auxin. This is an important observation because it indicates that added auxin specifically activates expression of the SAURs in a manner that duplicates the pattern seen with intact seedlings (where exogenous auxin is withheld).

The GH3 cDNA clone was selected from a library derived from seedlings treated with an auxin concentration that would lead to a cell division response (Hagen et al., 1984); however, *in situ* hybridization revealed that this transcript was not restricted to dividing cells or cells with the potential to divide. In addition, auxin inducibility of GH3 showed a much broader range of organ and tissue expression than is observed in normal, untreated tissues. Because GH3 was expressed in some of the same organ regions as SAURs after auxin addition but was not detected in these SAUR-expressing tissues without exogenous auxin, it is possible that higher concentrations of auxin are required for GH3 gene expression compared with SAUR gene expression in epidermal and cortical cells. Because GH3 expression is difficult to detect in most organs of young seedlings and older plants, the endogenous concentrations

Figure 4. (continued).

- (A) Longitudinal section of a flower during anthesis, stained with toluidine.
 - (B) Flower section hybridized to GH3 sense RNA probe.
 - (C) Flower section hybridized to GH3 antisense RNA probe.
 - (D) Flower primordia (approximately 20 DBA) section hybridized to GH3 antisense RNA probe.
 - (E) Two flower bud sections (about 9 DBA and 12 DBA) hybridized to a GH3 antisense RNA probe.
 - (F) A flower bud section (about 3 DBA) hybridized to a GH3 antisense RNA probe.
 - (G) Flower ovary section [2 days to 3 days after anthesis (DAA)] hybridized to a GH3 antisense RNA probe.
 - (H) Developing pod section (4 DAA to 5 DAA) hybridized to GH3 antisense RNA probe.
 - (I) Developing pod (6 DAA to 8 DAA) hybridized to a GH3 antisense RNA probe.
- Hybridizations are shown in dark-field microscopy. ca, calyx; r, corolla; v, vascular bundle; o, ovary; L, ovule.

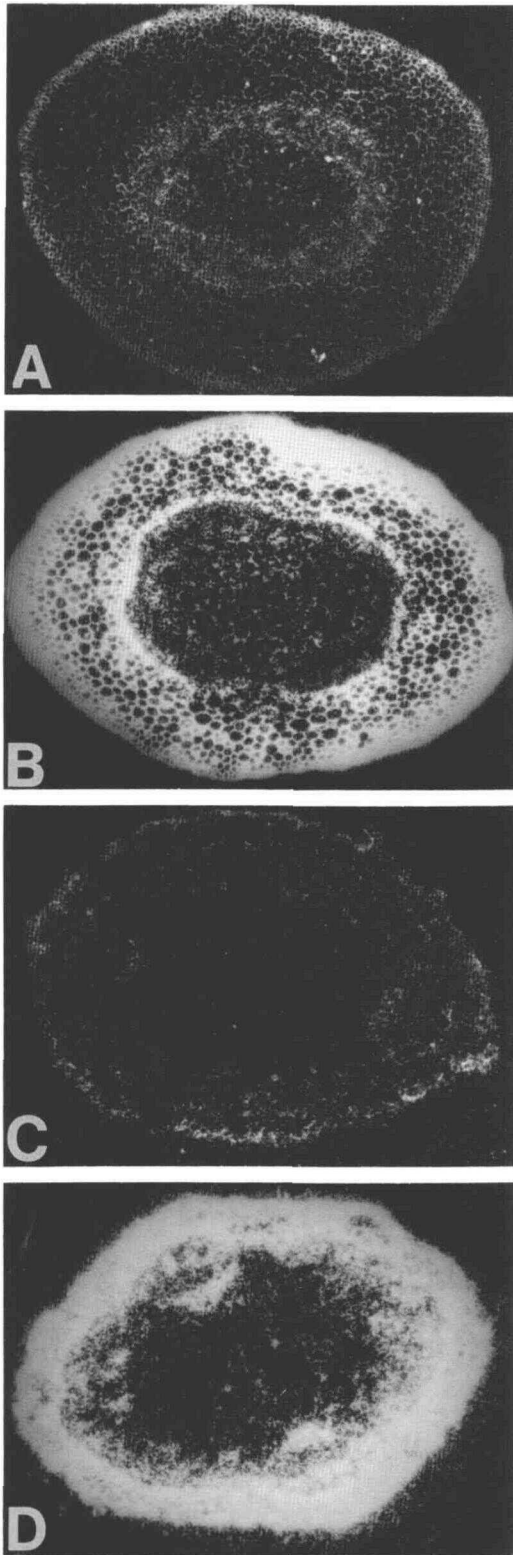


Figure 5. Induction of SAURs in Hypocotyls and Epicotyls after Treatment with 2,4-D.

of auxin in most regions of the plant must be insufficient to induce expression of this gene. Both nuclear run-on experiments with soybean (Hagen and Guilfoyle, 1985) and β -glucuronidase expression, driven by the GH3 promoter, in transgenic tobacco (T. Guilfoyle, unpublished results) indicate that auxin concentrations as low as 0.1 μ M are sufficient to activate transcription of the GH3 gene. It is not clear why floral organs express GH3 transiently during floral development or why this expression is high in the vascular tissues of the receptacle at early stages of floral development and high in the ovules and ovary at later stages of floral development. It remains to be determined whether GH3 expression in flowers is regulated by changes in internal auxin concentration or auxin gradients.

In contrast to the SAURs, which reach maximal auxin-induced levels within 30 min to 60 min after hormone application (McClure and Guilfoyle, 1987), GH3 transcripts continue to accumulate for several hours after seedlings or excised organ sections are exposed to exogenous auxin (Hagen et al., 1984). Because expression of the GH3 and SAUR genes appears to be spatially and temporally distinct, these auxin-induced gene products are probably not part of a common sequence of events in auxin action or auxin-regulated processes. Thus, GH3 and SAUR gene products are likely involved in different auxin-mediated cellular processes, although there may be some overlap or interaction of auxin-induced events. Such an interaction could be the case during xylogenesis, in which both GH3 and SAURs are expressed. In total, our results suggest that different tissues within an organ display different mechanisms for rapidly responding to a single hormone, in this case auxin.

Are There Multiple Types of Auxin Receptors?

Our results indicate that most organs and tissues in the plant respond to auxin by expressing one or both of two different types of auxin-responsive genes. The activation of these different genes by auxin must involve different mechanisms, as evidenced by the auxin inducibility of GH3

(A) Transverse section of a hypocotyl that had been incubated without 2,4-D.

(B) Transverse section of a hypocotyl that had been incubated with 2,4-D.

(C) Transverse section of an epicotyl that had been incubated without 2,4-D.

(D) Transverse section of an epicotyl that had been incubated with 2,4-D.

One-centimeter-long excised organ segments were preincubated for 4 hr in a buffer (McClure and Guilfoyle, 1987) and then incubated for an additional hour in fresh buffer with or without 50 μ M 2,4-D. After incubation, organ sections were fixed, sectioned, and hybridized with a SAUR antisense probe. Hybridizations are shown in dark-field microscopy.

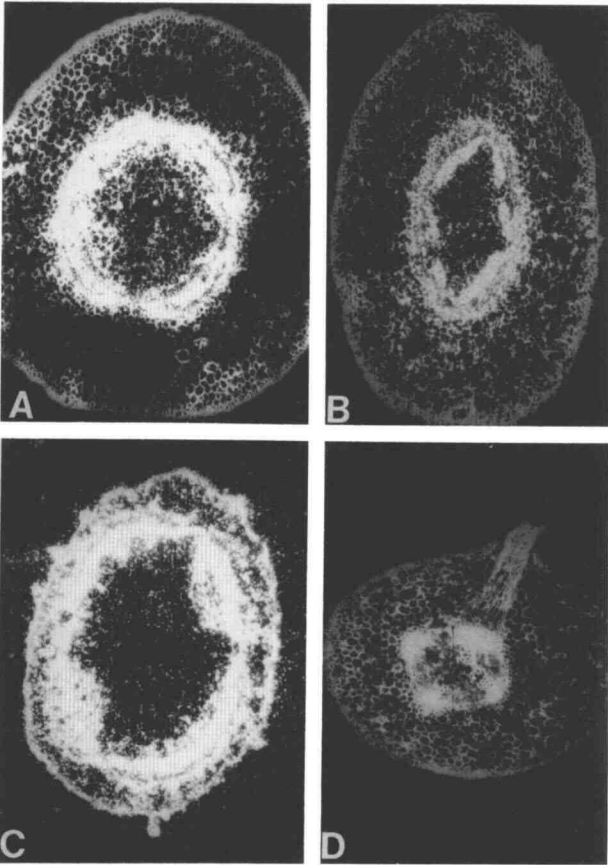


Figure 6. Induction of GH3 Transcripts in Hypocotyls, Epicotyls, and Roots after Treatment with 2,4-D.

(A) Transverse hypocotyl section from the elongating zone of a 4-day-old etiolated seedling.

(B) Transverse hypocotyl section from the mature or basal zone of a 4-day-old etiolated seedling.

(C) Transverse epicotyl section from the elongation zone of a 7-day-old etiolated seedling.

(D) Transverse root section from the mature zone of a 4-day-old etiolated seedling. A secondary root extends past the epidermis of the primary root.

One-centimeter-long excised organ segments were preincubated in buffer and then incubated in fresh buffer with 50 μ M 2,4-D for an additional hour. After incubation, segments were fixed, sectioned, and hybridized with a GH3 antisense RNA probe. Hybridizations are shown in dark-field microscopy.

and SAURs among different organ and tissue types. This suggests that specific tissues in different organs might contain one or more different types of auxin receptors, auxin transduction pathways, or auxin-responsive transcription factors. It is also possible, however, that tissue-specific differences in DNA methylation or mRNA turnover could influence tissue specificity of auxin-induced gene expression.

It is clear from our studies that most, if not all, cell, tissue, and organ types in soybean seedlings must possess auxin receptors and/or mechanisms to respond rapidly to localized changes in auxin concentration. Because of the very rapid transcriptional responses (i.e., within 5 min after auxin stimulation) of both SAUR and GH3 genes (Hagen et al., 1985; McClure et al., 1989) and because protein synthesis inhibitors do not block this transcriptional response (Hagen et al., 1985; McClure and Guilfoyle, 1987; Franco et al., 1990), it is reasonable to assume that transcriptional activation is tightly coupled to auxin perception (i.e., auxin receptors and signal/transduction) and represents a primary response to auxin. Although putative auxin receptors have been localized to epidermal cells in maize coleoptiles (Lobler and Klambt, 1985; Klambt, 1990), our results suggest that epidermal cells are unlikely to be the sole site of auxin receptors or molecules involved in auxin-stimulated transduction processes. It is possible that more than one type of auxin receptor may exist, and while some of these may be membrane associated, others may be localized in the cytoplasm or nucleus (Napier and Venis, 1990; Vesper and Kuss, 1990). In addition, it is possible that a single cell or tissue type might possess more than one type of auxin receptor. The latter possibility is suggested from our observation that epidermal, cortical, and endodermal cells of elongating hypocotyl and epicotyl regions respond to auxin by expressing both SAURs and GH3, whereas other tissues and organs express only GH3.

Although different types of auxin receptors or auxin transduction pathways may play a role in organ-specific and tissue-specific gene expression, the presence or absence of specific transcription factors could also participate in selective auxin-induced gene expression in different organs and tissues. Genes for GH3 and SAURs have been isolated and sequenced (McClure et al., 1989; G. Hagen, unpublished results). Comparison among the 5' flanking regions of these genes, as well as additional auxin-responsive genes (Ainley et al., 1988; Czarnecka et al., 1988; An et al., 1990; Conner et al., 1990; Maurel et al., 1990; Takahashi et al., 1990), does not reveal any strikingly similar sequence motifs, suggesting that auxin-inducible elements may be quite variable or difficult to discern by simple computer analysis. On the other hand, there may be multiple types of auxin-inducible elements that bind to different types of auxin-responsive transcription factors.

Another possible means of tissue-specific regulation could result from different stabilities of transcripts in different tissues. In the case of SAURs, gravitropic experiments have indicated that these RNAs turn over very rapidly, with a half-life of about 5 min to 10 min (McClure and Guilfoyle, 1989a, 1989b). Franco et al. (1990) showed that a labile protein may be involved in SAUR transcript stability because protein synthesis inhibitors (i.e., cycloheximide, emetine, anisomycin, and puromycin) cause SAUR accumulation in the absence of transcriptional stimulation. The work of Franco et al. (1990) showed, however, that protein

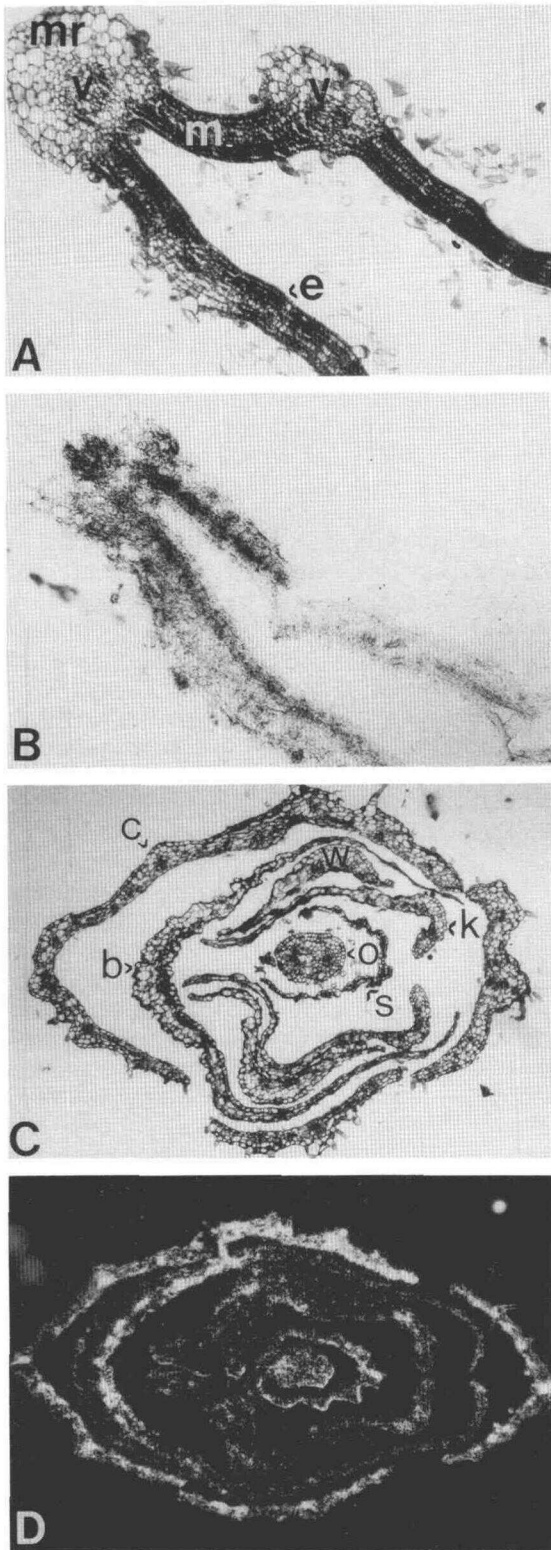


Figure 7. Induction of GH3 Transcripts in Plumules and Flowers Treated with 2,4-D.

synthesis inhibitors cause SAURs to accumulate in the same tissue-specific and organ-specific manner as auxin (which activates transcription of the SAUR genes), suggesting that RNA turnover may alter transcript levels but not govern tissue specificity.

Conclusions

Our results demonstrate that auxin-induced gene expression has a complex pattern of tissue specificity, with different auxin-responsive mRNAs displaying different patterns of expression. Auxins do not act on single cell or tissue types; in fact, most, if not all, tissues within an organ respond to auxin by activating SAUR and/or GH3 gene expression. Even in elongating hypocotyl or epicotyl sections, where endogenous, as well as exogenous, auxin is thought to act on the epidermis and outer cortical cells, a wide variety of tissue types express auxin-responsive genes, including external and internal tissues. Although some of these genes are expressed primarily in epidermis and cortex, other genes are expressed primarily in vascular tissues after auxin application. Our results suggest that one or more types of auxin receptors are present in internal as well as external tissues and that at least some of these receptors are not restricted to specific organs or organ regions. Our results show that expression of auxin-responsive genes is controlled at discrete developmental stages within specific tissue types (e.g., SAUR expression is largely restricted to epidermis and cortex of hypocotyls and epicotyls that are in the cell expansion stage of development, and GH3 expression is restricted largely to specific tissues within organs of the developing flowers in the absence of added auxin). Finally, our results show that certain auxin-responsive genes (i.e., SAURs) are regulated tightly and expression is restricted to a few tissue types at specific developmental stages (e.g., elongation zones of hypocotyls or epicotyls), and these restrictions are maintained even if the organs are exposed to exogenous auxin. In contrast, other auxin-responsive genes (i.e., GH3) are regulated tightly in the whole plant, but exposure of

(A) Transverse section of an etiolated plumule (primary leaf) stained with toluidine blue.

(B) Transverse section of an etiolated plumule hybridized with a GH3 antisense RNA probe. Hybridization is shown in bright-field microscopy.

(C) Transverse section of a mature flower (day of flowering) stained with toluidine blue.

(D) Transverse section of a mature flower (day of flowering) hybridized with a GH3 antisense RNA probe. Hybridization is shown in dark-field microscopy.

Excised organs were preincubated for 4 hr in buffer and then incubated for an additional hour with 50 μ M 2,4-D. mr, midrib; v, vascular tissue; m, mesophyll; e, epidermis; c, calyx; o, ovary; s, staminal column; b, banner petal; w, wing petal; k, keel petal.

Table 1. Summary of Tissue-Specific Expression of GH3 and SAURs

Plant Organ	GH3		SAURs	
	-Auxin	+Auxin	-Auxin	+Auxin
Root	V, C	V > E, C, P	ND	ND
Basal hypocotyl	ND	V > E, C, P	ND	[E, C]
Elongating hypocotyl	ND	V > E, C, P	E, C	E, C
Apical hypocotyl	ND	V(X), E, C, P	V(X)	V(X)
Epicotyl	ND	V > E, C, P	E, C	E, C
Cotyledon	ND	V > T	ND	ND
Plumules	ND	V > T	ND	[T]
Flower				
Receptacle	V	V > T	ND	ND
Ovule	I	I	ND	ND
Ovary	T	T	ND	ND
Pistil	ND	T	ND	ND
Stamen/anther	ND	V > T	ND	ND
Petals	V > T	V > T	ND	ND

Expression in floral organs is limited to specific developmental stages. Abbreviations used are: V, vascular; V(X), developing xylem elements; E, epidermis; C, cortex; P, pith; I, integument; T, all tissues; [] = low levels; ND = not detected.

plants or plant parts to exogenous auxin results in activation of these genes within the vascular tissues of most, if not all, plant organs.

METHODS

Plant Material

Soybean (*Glycine max*, var Williams 82) seedlings were germinated in a 50% vermiculite/50% perlite mixture in the dark at 28°C to 30°C for 4 days or 7 days. Flowers were from plants grown in the greenhouse. For in situ hybridizations of untreated seedlings, organs were excised and fixed directly in 1.5% glutaraldehyde, 50 mM cacodylate (pH 7), or 75 mM Pipes (pH 7). Fixed tissue was embedded in paraffin. For auxin induction studies, organs were excised and incubated in 2% sucrose, 10 mM potassium phosphate buffer (pH 6) with shaking for 4 hr to remove endogenous auxins, and then treated in fresh buffer alone or with 50 μ M 2,4-D for 1 hr before fixation.

For gravitropic experiments with hypocotyls, pots containing intact seedlings were placed horizontally in the dark. After 0 min, 30 min, 60 min, or 90 min, hypocotyls were sectioned in dim light and fixed. For gravitropic experiments with epicotyls, 7-day-old, etiolated seedlings were excised 1 cm below the cotyledons and placed vertically in 0.8% agarose in complete darkness for 20 hr. Seedlings were placed horizontally for 60 min, and epicotyls were sectioned in dim light and fixed. Hypocotyls and epicotyls were sectioned at an angle so that their top and bottom orientation with respect to the gravity vector could be determined after fixation of the tissue.

RNA Probes

RNA probes were prepared using T7 RNA polymerase with SAUR or GH3 cDNA sequences cloned behind the T7 promoter (Franco et al., 1990). SAUR probes included nearly full-length cDNAs 6B, 10A5, and 15A (McClure et al., 1989). ³⁵S-labeled probes used for in situ hybridizations were further processed by alkaline hydrolysis (Cox and Goldberg, 1989), Sephadex G-50 (Pharmacia, Piscataway, NJ) filtration, and phenol/chloroform extraction.

In Situ Hybridization

In situ hybridizations were carried out as described by Cox and Goldberg (1989). Ten-micron sections of fixed and embedded tissue were mounted on slides that had been dipped in poly-L-lysine. Prehybridization treatments included proteolysis with 200 μ g/mL proteinase K at 37°C for 30 min, and incubation in 0.2 M triethanolamine and acetic anhydride for 10 min at room temperature. Tissue was then treated with hybridization solution containing 50% formamide, 0.3 M NaCl, and 5 \times 10⁶ cpm/mL of sense or antisense RNA probes that had been hydrolyzed to an average length of 150 bp. After hybridization at 42°C for 18 hr, sections were treated with ribonuclease A (50 μ g/mL) for 30 min at 37°C and then washed at 60°C in 0.1 \times SSC. After drying, slides were dipped in 50% liquid photographic emulsion (Kodak NTB2), exposed at 4°C, and developed in half-strength D19 developer (Eastman Kodak, Rochester, NY).

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