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Rapid Redistribution of Auxin-Regulated RNAs During Gravitropism

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Gravitropism, the bending of plants in response to gravity, is caused by differential growth rates on two sides of a responding organ. The general belief, although somewhat controversial, is that auxins play a major role in gravitropism by controlling the rate of cell extension. The tissue print technique was used to ascertain the distribution of auxin-regulated RNAs during the gravitropic response of soybean hypocotyls. In vertically oriented seedlings, auxin-regulated RNAs are symmetrically distributed in the elongating region of the hypocotyl. In horizontally orientated seedlings the distribution becomes asymmetrical within 20 minutes and the greatest asymmetry coincides with the onset of rapid bending. The results provide a clear correlation between the dynamic expression of genes under auxin control and a morphogenetic phenomenon traditionally known as an auxin response.

GRAVITY IS AMONG THE MOST IMportant determinants of plant growth. The gravitropic response results in the familiar pattern of plant growth (that is, aerial portions grow up and roots grow down). This differential growth response is thought to be mediated by the plant hormone auxin, since there is a large body of evidence that connects auxin with plant cell extension. Several groups have demonstrated that exogenously supplied auxin causes the accumulation of specific RNAs concomitant with (*1–3*) or prior to (*4*) auxin-induced cell extension. These studies suggest that at least part of the growth-controlling action of auxin is mediated by auxin-regulated gene expression. We have characterized a family of auxin-regulated RNAs from soybean that begin to accumulate within 2.5 min after application of auxin (*4*). We report here that gravistimulation rapidly alters the distribution of these RNAs in responsive organs and that the presence of the RNAs is highly correlated with cell extension. These results suggest that auxin-regulated gene expression is involved in the response of plants to gravity.

In excised elongating hypocotyl sections (EHSs), application of exogenous auxin results in a two- to fourfold enhancement of the rate of cell extension after a lag of about 15 min (*5*). We used the soybean hypocotyl system to identify cloned cDNAs corresponding to RNA species that rapidly and specifically accumulate in response to auxin (*3, 4*). Among these RNAs are a group of small auxin up RNAs (SAURs) that accumulate very rapidly after application of exogenous auxin to excised EHS (half-maximal accumulation after about 10 min; steady-state induction of 18- to 48-fold over controls within 30 to 60 min after application of 50 μ*M* 2,4-dichlorophenoxyacetic acid). The SAURs consist of three closely related families of RNAs of about 550 nucleotides. The 6, 10A, and 15 families show essentially identical RNA induction kinetics and auxin specificity with only subtle differences in other response parameters (*4*). Five SAUR genes have recently been sequenced, and at least three of the genes are transcriptionally regulated in the EHS (*6*).

We used the tissue print technique (*7*) modified for the detection of RNAs with ^{35}S-labeled antisense RNA probes to investigate the distribution of the SAURs during gravitropism. To increase the autoradiographic signal, we used a mixed SAUR probe (*8*) that contains four cDNAs. This probe detects only auxin inducible RNAs, whereas the RNA detected by the control clone [clone 5A (*9*)] is not auxin inducible (Fig. 1).

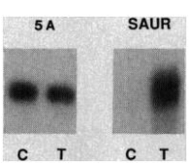

Fig. 1. Auxin inducibility of RNAs. RNA blot (*13*) of total RNA extracted from excised soybean EHS incubated for 4 hours (C) in the absence of auxin or (T) in the presence of 2,4-dichlorophenoxyacetic acid (50 μ*M*) for an additional hour. RNAs were detected with ^{32}P-labeled antisense RNA probes from cDNA clone 5A (*9*) or with a mixture of four SAUR cDNAs (*8*) as shown. RNA 5A consists of approximately 450 bases. The doublet band detected by the mixed SAUR probe consists of the 560-base RNAs hybridizing to the clone 6 family and the 530-base RNAs hybridizing to both the clone 10A and 15 families (*4*). Clone 6 family probes also detect a 1100-base auxin-inducible RNA (*4*) that is not visible in this exposure. Plant materials, growth conditions, RNA extraction procedures, and blotting methods have been described (*4*).

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The phenomenon of gravitropism allows experimental manipulation of the rate of cell extension within the soybean hypocotyl without the application of exogenous auxin. Soon after a seedling is moved to a horizontal positon, the rate of extension increases on the lower side. This differential growth response continues until the apex regains a vertical orientation and symmetrical growth resumes (Fig. 2). Under the conditions used in this study, the gravitropic growth response is evident in most seedlings after 45 min of horizontal gravistimulation. A symmetrical distribution of SAUR hybridization about the major axis of the hypocotyl in seedlings incubated vertically is shown in Fig. 2. This distribution is maintained for at least 10 min of horizontal incubation. Seedlings incubated horizontally for 20 min show a decreasing SAUR hybridization signal on the upper right side of the seedling and an increase in signal on the lower side. By 45 min of gravistimulation, when hypocotyl bending is first clearly apparent, SAUR hybridization is weak on the upper side and a very strong signal is observed in the lower cortex. After 90 min in the horizontal position most seedlings have bent about 45 degrees and the strongest SAUR hybridization signal is along the lower side of the curved hypocotyl where cell extension is greatest. By 180 min, the apices of the seedlings regain a vertical position. In these seedlings SAURs are symmetrically distributed in the vertically oriented portion of the hypocotyl while retaining a strongly asymmetric distribution in the horizontally oriented segment. The kinetics of this redistribution are similar to those previously reported for the auxin-induced accumulation of SAURs in excised EHS (that is, a steady state appears to have been reached after 45 min of gravistimulation). However, the decay of the SAUR hybridization signal on the upper side of the hypocotyl by 45 min is faster than the decay of the signal in the excised organs. In excised EHSs, the minimum SAUR hybridization signal was observed after 4 hours of incubation in the absence of auxin (*4*), suggesting that these sequences turn over more rapidly under the gravistimulation conditions used here.

Comparison of the autoradiographic and India ink–stained images (Fig. 2) shows that the differential SAUR hybridization signal is not due to uneven tissue printing. In other control experiments, we found that sense strand RNAs do not hybridize to tissue prints of uninduced or auxin-induced seedlings (*10*), and that the distribution of a non–auxin-inducible RNA (5A) is not altered during gravitropism (Fig. 2). Other experiments have shown that in gravistimulated organs, redistribution of the SAURs results in greater concentration on the lower side, regardless of the position of the cotyledons (*10*).

The results presented here show that the redistribution of the SAURs, induced by gravistimulation, occurs before the onset of gravitropism (as observed with the unaided eye). The distribution of the SAURs indicates where the enhanced rate of cell extension will occur. Tissue printing shows that the SAURs are expressed in the cortex and epidermal layers of the hypocotyl. In situ hybridization studies can be used to define the exact cell types that express the SAURs in gravistimulated and unstimulated organs. The kinetics of the SAUR redistribution are in accord with our earlier results, which show that accumulation of these RNAs begins before auxin-induced cell extension in excised EHSs and that expression is strongest in the rapidly elongating regions of soybean stems (the EHS and the apical portion of the epicotyl) (*4*). Therefore, the expression of the SAUR genes is highly correlated with cell extension. However, the fungal toxin fusicoccin, which also induces cell extension in these organs, does not cause accumulation of the SAURs (*4*). Thus, any role of these sequences in cell extension could be specific to auxin-induced cell extension. Our results suggest that auxin-regulated gene expression has a role in the gravitropic response. This may be due to a redistribution of endogenous auxin (*11*), or to altered sensitivity to auxin in gravistimulated tissues (*12*), or to some other mechanism.

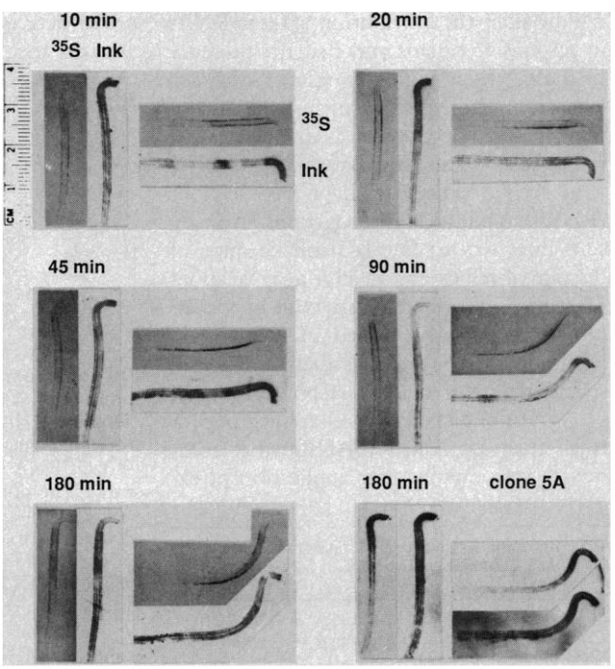

Fig. 2. Time course of SAUR redistribution in response to gravity. At each time point, examples of vertical control and horizontally gravistimulated seedlings are shown in their respective orientations. For each seedling a tissue print autoradiogram (^{35}S) and a stained image (Ink) is shown. India ink stains proteins and complex carbohydrates and produces an image of the tissue print; this indicates that all regions of the hypocotyl are uniformly blotted. For comparison with the SAUR hybridization patterns a second set of 180-min control and gravistimulated seedlings hybridized with the non–auxin-responsive clone 5A is shown at the lower right. Under the incubation conditions used here, the negative gravitropic response is visible after about 45 min of gravistimulation (*14*).

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8. The mixed SAUR probe contains equal amounts (by weight) of clones 6B, 6G, 10A5, and 15C. Clones 6B23 (491 bp) and 10A51 (493 bp) are identical in sequence to the sequenced genes 6B and 10A5 (*6*), clones 6G196 (477 bp) and 15C21 (459 bp) are 96% identical to genes 6B and 15A, respectively. All cDNAs were cloned into a derivative of pIBI25 (IBI) and linearized to produce antisense RNA probes in in vitro transcription reactions.
9. The cDNA clone 5A was selected as a control clone and represents an abundant, non–auxin-responsive RNA. Clone 5A421 (404 bp) was used to generate antisense RNA probes with T7 RNA polymerase as recommended by the enzyme supplier (BRL).
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14. Etiolated soybean (*Glycine max* cv. Wayne) seedlings were grown for 3 to 4 days in the dark as described (*4*). Gravistimulation experiments were conducted under dim red light (15W Kodak safelight GBX-2 at 1.5 to 2.0 m). Seedlings were cut above the root-shoot transition zone to a length of 5 to 6 cm. Rootless seedlings were incubated in humid 80 by 100 mm petri dishes filled to about 1.5 cm with 0.8% agarose. Seedlings receiving gravistimulation were all orientated with the cotyledons down. After incubation, the petri dishes were flooded with ice water and were removed to room light. After removal of the cotyledons, tissue prints (7) were prepared by sectioning the seedlings with a razor blade and pressing the freshly cut surface onto a nylon membrane (Zetaprobe). After air drying, the blots were prewashed, prehybridized, and hybridized to ^{35}S-labeled antisense RNA probes synthesized by T7 RNA polymerase-driven in vitro transcription in the

presense of [^{35}S]uridine 5′-(α-thio) triphosphate (>1200 Ci/mmol) (DuPont, Biotechnology Systems) at 5 × 10^7cpm/ml. Hybridization and washing conditions were as recommended by the membrane supplier. Before autoradiography, tissue prints were stained with India ink (*7*). Autoradiograms were made on Kodak Tmax 400 film exposed for 10 days at −70°C and developed in Tmax developer for 11 min at 24°C. A detailed description of the tissue print and autoradiography methods used in this study will be presented elsewhere (B. A. McClure and T. Guilfoyle, in preparation).

15. Tissue localization of RNAs by hybridization of antisense probes to tissue prints was suggested by J. E. Varner. We thank C.S. Brown for reading of this manuscript. Supported by a contribution from the Missouri Agricultural Experiment Station, Journal Series Number 10636, and by NSF grant DCB 8517676.

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Gating of Retinal Transmission by Afferent Eye Position and Movement Signals

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Vision in most vertebrates is an active process that requires the brain to combine retinal signals with information about eye movement. Eye movement information may feed forward from the motor control areas of the brain or feed back from the extrinsic eye muscles. Feedback signals elicited by passive eye movement selectively gate retinal outflow at the first relay, the dorsal lateral geniculate nucleus. The gating predominantly facilitates retinogeniculate transmission immediately after eye movement and inhibits transmission when a new steady-state eye position is achieved. These two gating effects are distributed in a complementary fashion across the dorsal lateral geniculate nucleus such that the spatiotemporal activity profile could contribute to object detection and localization.

THE EXTRAOCULAR MUSCLES (EOM) of mammals are richly innervated by stretch receptors (*1, 2*) that, when activated, produce phasic bursts of activity (*2, 3*) that may interact with visual signals in neurons in brain regions that receive monosynaptic retinal input (*4*). Interaction of phasic eye movement signals from the EOM with retinal signals has also been reported in higher centers such as visual cortex (*5*). However, information is not available on the effects of EOM-mediated tonic eye position changes and phasic eye movements on early visual processing in functionally identified neurons with respect to their positions on the retinal map. Thus, it is not known how the nervous system could use such information to facilitate spatial localization of objects. This is of particular interest in light of reports that feed-forward signals may be sufficient to signal eye position during performance of certain oculomotor tasks (*6*).

We have examined the effects of passive changes in eye position and eye movement on retinogeniculate transmission in the cat. General surgical methods, anesthesia, and electrophysiological recordings were as described (*7, 8*). The experimental procedure is shown in Fig. 1. The same visual stimulus was repeatedly delivered to the right eye while the steady-state position or time of a brief phasic movement of the left eye was randomly varied by a computer (*9*). The left eye was prevented from receiving visual stimulation by occlusion with an opaque contact lens and an intravitreal injection of tetrodotoxin (TTX) (*10*).

Extracellular recordings made with fine-tipped micropipettes (*7*) were obtained from 209 single X or Y (*11*) neurons of the A layer of the left dorsal lateral geniculate nucleus (LGN_d) (contralateral to the eye being visually stimulated but ipsilateral to the eye being moved). Thus, LGN_d layer A received a modulated retinal signal only from the stationary eye. Two types of experiments were performed: a tonic eye position experiment in which the steady-state position of the left eye was varied (n = 133 neurons) and a phasic eye movement experiment in which a brief movement of the left eye occurred at various times before or after the visual stimulus onset (n = 76 neurons). A subset of the neurons that showed significant changes in their visual response after eye movements (n = 12) was also tested for

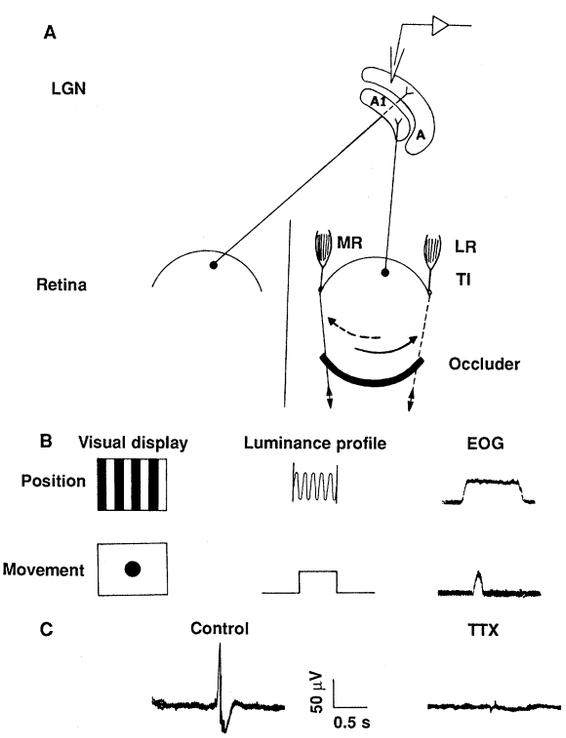

Fig. 1. Experimental procedure. (**A**) Schematic top view of the cat's eyes and relevant projection of the retinas to the LGN (animal looking toward bottom of figure). The locations of the medial rectus (MR) and lateral rectus (LR) muscles and their tendons of insertion (TI) on the globe of the left (moving) eye are indicated. The right eye, which remained stationary and received visual stimulation, was fitted with a contact lens. By application of the correct spectacle lens, the right retina was made conjugate with a display monitor positioned at a viewing distance of 57 cm. The left eye, which was moved but received no visual stimulation, was fitted with an opaque contact lens occluder and received an intraocular injection of TTX in most cases. (**B**) Visual stimuli (delivered to right eye) and eye movement signals (of left eye). For the eye position experiment, the visual display (a drifting grating pattern) and electrooculogram (EOG) indicating a steady-state change in eye position are indicated. For the eye movement experiment, the visual display (an abrupt onset circular spot positioned within the receptive field of the LGN_d neuron) and brief (75 ms) phasic eye movement are indicated. Neuronal responses were collected for six eye positions or ten times of eye movement in a randomized, interleaved fashion. Blocks of five trials were collected for each eye position or time of movement in an interleaved fashion. The new position or time was selected by the computer with a new randomization from a table of preselected values during a 1-s pause. During the pause period, the visual display remained spatially and temporally homogeneous. (**C**) Visually evoked potential recorded from optic nerve of left (moving) eye before and after TTX injection into vitreous humor.

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