

The ashfall, which was preceded by a massive dark cloud, began about 2:00 p.m. and continued until about 2:00 a.m. the next morning. The mass/area ratio of the ash that fell on Moscow was 2.29 kg/m<sup>2</sup>, and that of the ash that fell on Pullman was 2.14 kg/m<sup>2</sup>. The ash samples from Moscow that we examined were untreated, whereas ash samples from Pullman were dried at 100°C before being analyzed. Because of settling, measurements of ash thickness differ, depending on when they were made. Initial thicknesses have ranged from about 1.00 cm in Moscow to over 1.25 cm just north of Pullman. Although precise values are not yet available, the initial thickness increased northward to a maximum of about 7.00 cm along an east-northeast axis about halfway between Pullman and Spokane, Washington.

The first ash to fall was visibly darker than the later ash, and observers noted that the change occurred abruptly between 5:15 and 5:30 p.m. (Fig. 1). The mass of the pale ash is between two and four times that of the dark ash. The two ashes have similar particle sizes, with most particles ranging between 10 and 100 μm. But smaller fragments are present; air pollution data (1) indicate significant quantities of particles between 0.1 and 1.0 μm in the air up to a week after the main ashfall.

The dark ash is composed predominantly of plagioclase crystals (strongly zoned around 50 mole percent anorthite), glass which varies from colorless to dark brown and which usually includes abundant microlites and composite lithic fragments of plagioclase and glass. Crystals of titanium-rich magnetite make up 5 to 10 percent (by volume), and crystals of basaltic hornblende form about 1 percent by volume. Occasional crystals of orthopyroxene are present.

The pale ash is composed of about 80 percent clear glass, typically vesicular with ragged outlines and largely free of microlites. Plagioclase is the principal crystalline phase in this ash as well, but smaller crystals of iron oxide and hornblende are present. Neither quartz nor potassium feldspar have been observed under the optical microscope, but quartz or cristobalite and possibly very small grains of biotite have been detected with the electron microprobe.

Nine ash samples have been analyzed for major elements by x-ray fluorescence (XRF) methods (2). The exact location and accumulation period of each sample is recorded with the chemical composition in Fig. 1. The analyses are strongly bimodal. The first three samples collected (dark ash) have virtually identical

compositions (Table 1, column A); the next two samples were collected over the period of abrupt change, and they are mixtures of the two ash types (Table 1, column B); the last four samples collected (pale ash) are virtually identical, and their average composition is recorded in Table 1, column C, together with the "standard deviation" of the four measurements for each oxide. Columns D and E of Table 1 give the compositions of the glass in the dark and pale ashes, respectively, analyzed on an electron microprobe (3).

The bimodal nature of the ash implies two separate source materials, probably associated with separate explosions. This conclusion is strengthened by the quite distinct glass compositions in the two ashes. The dark ash in bulk appears to represent silicic andesite, with abundant plagioclase both as phenocrysts and as groundmass, and may be derived from a part of the old volcanic cone dispersed

in the first explosion. The pale ash in bulk has the composition of rhyodacite and may represent new magma. If so, it was mainly liquid when it erupted explosively with only minor amounts of crystalline plagioclase and possibly iron oxide and hornblende.

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## Gravitropism in Plant Stems May Require Ethylene

**Abstract.** *Two inhibitors of ethylene synthesis in plants (cobaltous ion and aminoethoxyvinylglycine) and two inhibitors of ethylene action (silver ion and carbon dioxide) significantly delay the gravitropic response (upward bending) of mature dicot stems laid on their sides. This evidence suggests that ethylene may be required in the gravitropic response of shoots.*

Our evidence that ethylene plays a role in stem gravitropism (an upward bending of stems laid on their sides) could lead to resolution of some of the objections presently being raised against the Cholodny-Went theory, a model of gravitropic mechanism formulated in 1926 (1). Statoliths (now thought to be amyloplasts, each containing two or more starch grains) fall toward the bottom of gravity-sensitive cells called statocytes; this is gravity perception. According to the model, this settling leads to a redistribution of the plant growth hormone auxin (indole-3-acetic acid) toward the bottom of the statocytes and ultimately toward the cells on the lower side of a stem turned on its side, causing increased growth of these lower cells and thus an upward bending of the stem; this is the expression of the perceived gravity stimulus (2). Other plant-stem growth hormones, the gibberellins, have also been implicated (3), and it has been recognized that stems will bend even when leaves or apical meristems, or both, have been removed (4).

In 1976, Digby and Firn (5) reviewed several papers on plant gravitropism and concluded that the Cholodny-Went model did not adequately agree with the

facts. For one thing, evidence seems to suggest that stem bending occurs before sufficient time has elapsed for movement and action of auxin, and gibberellins require even more time than auxins to induce cell elongation (6). They also concluded that too little auxin accumulates on the lower side to cause the observed unequal growth. The observed differences in the concentration of auxins and gibberellins on the upper and lower sides of gravitropically responding stems could be consequences of the bending rather than its cause.

There are a few hints in the literature that ethylene might be required in gravitropism. It has long been known, for example, that relatively high concentrations of ethylene can redirect the gravitropic response of shoots so that they grow horizontally rather than vertically (7). Chadwick and Burg (8) reported that ethylene, produced by treatment with low concentrations of indole-3-acetic acid, was responsible for inhibition of root growth and proposed that ethylene was essential for gravitropic curvature in roots. They showed that CO<sub>2</sub>, a widely used inhibitor of ethylene action in plants (9), retarded gravitropic response of pea and lima bean roots but not pea

shoot sections. Zobel (10) observed that treatment of a mutant diagravitropic (horizontally growing) tomato with minute quantities of ethylene normalized shoot gravitropic response. Jackson (11) showed that this mutant has a modified response mechanism to gravity and to ethylene rather than an abnormally slow rate of ethylene synthesis. Wright *et al.* (12) measured increases in auxin followed by threefold increases in ethylene evolution from excised grass nodes (*Echinochloa colonum*) placed horizontally; they concluded that the ethylene was symptomatic rather than causal. In related studies, we (13) have shown that gravity perception (rather than mechanical stresses) in plants turned on their side and rotated on a clinostat apparently leads to ethylene evolution, accounting for the severe downward bending or twisting of leaves. Others (14) have also implicated ethylene in the clinostat response.

In spite of these clues to ethylene involvement, it is not suggested in reviews on gravitropism (2) or on ethylene action in plants (9) that ethylene might play an essential role in gravitropism. As a gaseous hormone, ethylene is difficult to study. But its release could in some way be triggered by the gravity perception mechanism, directly causing an increased growth of lower stem cells or an inhibition of upper cells rather than influencing the distribution of auxin as has often been suggested for clinostat epinasty (15).

We have strong circumstantial evidence that ethylene is required in stem gravitropism. As shown in Fig. 1, two inhibitors of ethylene synthesis [ $\text{Co}^{2+}$  and aminoethoxyvinylglycine (AVG)] and two inhibitors of ethylene action ( $\text{Ag}^+$  and  $\text{CO}_2$ ) (16) slow the rate of gravitropic bending of cocklebur (*Xanthium strumarium* L.) stems laid on their sides. Although there is sometimes a delay in the initiation of bending, most of our results show a reduced rate of bending so that the time required to reach a given curvature is longer for plants treated with the ethylene inhibitors than for comparable controls. In general, we find  $\text{Co}^{2+}$  to be only marginally effective (maximum delays to reach a bending of about  $60^\circ$  from the horizontal of only 2 hours), but AVG, a powerful inhibitor of ethylene synthesis, is dependably effective, sometimes delaying the time required to reach  $60^\circ$  by as much as 12 hours. Silver ion is also highly effective (our maximum, 10 hours), although in our experience usually not as effective as AVG. Carbon dioxide, long used as an inhibitor of ethylene action (9), has been quite effective in

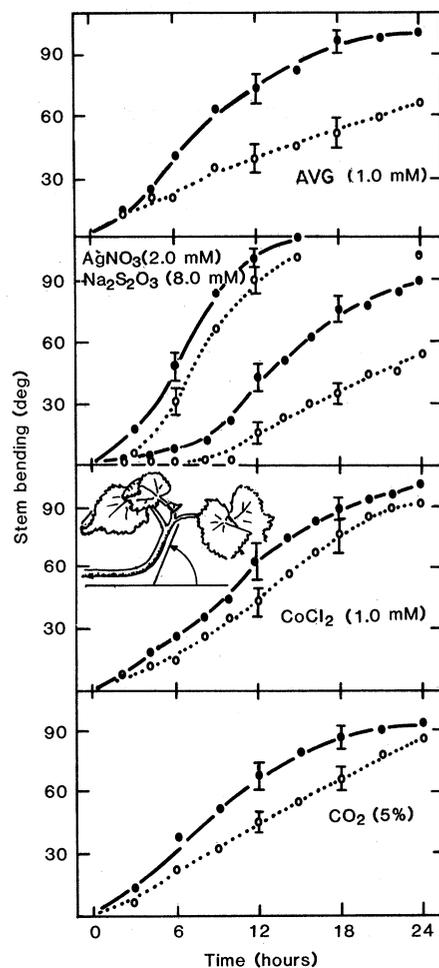


Fig. 1. Effects of various ethylene inhibitors on gravitropic bending of cocklebur (*Xanthium strumarium*) stems placed on their sides, expressed as the amount of bending from the horizontal, as illustrated. Solid curves on each graph represent control plants, and vertical bars represent the standard error of the mean. All leaves older than the youngest fully expanded one were removed. About 30 minutes before the stems were laid on their sides, upper stems and all leaves were dipped in solutions of  $\text{AgNO}_3$  (complexed with  $\text{Na}_2\text{S}_2\text{O}_3$ ),  $\text{CoCl}_2$ , or AVG, to which one drop of Tween 20 per 100 ml of solution had been added as a wetting agent. Control plants were dipped in suitable solutions ( $\text{Na}_2\text{S}_2\text{O}_3$  for  $\text{AgNO}_3 + \text{Na}_2\text{S}_2\text{O}_3$ ;  $\text{MgCl}_2$  for  $\text{CoCl}_2$ ; and none for AVG), which in separate experiments had been found to be without effect on bending. Plants in Plexiglas cylinders were exposed to constantly flowing air or 5 percent  $\text{CO}_2$  (20 percent  $\text{O}_2$ , 75 percent  $\text{N}_2$ ) beginning 30 minutes before the plants were turned onto their sides and lasting until bending was complete. All experiments were conducted in the dark, at approximately  $25^\circ\text{C}$ . Measurements were made under a dim green safelight. Each point for AVG and  $\text{CO}_2$  represents three plants, each point for  $\text{Ag}^+$  five plants, and each point for  $\text{Co}^{2+}$  five plants. All experiments were repeated several times. The actual bending times of control plants vary from experiment to experiment because of plant age (older plants bending more slowly) and other factors. The graph for  $\text{AgNO}_3 + \text{Na}_2\text{S}_2\text{O}_3$  illustrates this. In the experiment at the left we used young, rapidly growing plants; in the experiment at the right we used older, more slowly growing plants.

some of our experiments (6-hour delay with cocklebur and 8-hour delay with tomato) but almost without effect in others. We have successfully repeated our experiments with tomato (*Lycopersicon esculentum* Mill., var. Bonny Best) and with castor bean (*Ricinus communis* L.).

In view of these results, we suggest that ethylene should be considered to exert a prime role in the mechanism of stem gravitropism. We are testing the hypothesis that gravitropic bending in dicot stems is caused by an ethylene (or other) inhibition of the cells on the top of a dicot stem laid on its side, although we have no idea how ethylene (or other inhibitors) might be redistributed in response to gravity. Inhibitory activity on the top of horizontal stems has been reported (17), and our preliminary data strongly suggest that curvature is caused more by an inhibition of top cells than by a promotion of bottom ones. In preliminary experiments with clinostated tomatoes, we have induced stem curvatures by treating one side of a stem with ethephon (which releases ethylene), an approach that fails with auxins applied to intact dicot stems (5). Measurements should be made of ethylene evolution from different parts of plant stems as a function of time after changing the plant's orientation with respect to gravity. Such studies could lead to a thoroughly revised model of how stems respond to gravity.

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## J Genes for Heavy Chain Immunoglobulins of Mouse

**Abstract.** A 15.8-kilobase pair fragment of BALB/c mouse liver DNA, cloned in the Charon 4A $\lambda$  phage vector system, was shown to contain the  $\mu$  heavy chain constant region ( $C_{H\mu}$ ) gene for the mouse immunoglobulin M. In addition, this fragment of DNA contains at least two J genes, used to code for the carboxyl terminal portion of heavy chain variable regions. These genes are located in genomic DNA about eight kilobase pairs to the 5' side of the  $C_{H\mu}$  gene. The complete nucleotide sequence of a 1120-base pair stretch of DNA that includes the two J genes has been determined.

An important feature of the immune system is its ability to generate, from a relatively small amount of genetic material, antibody molecules with many different antigen binding specificities. Antibody molecules contain heavy and light chains, each of which contains an amino terminal variable (V) region and a carboxyl terminal constant (C) region. The antigen binding site is formed by the V

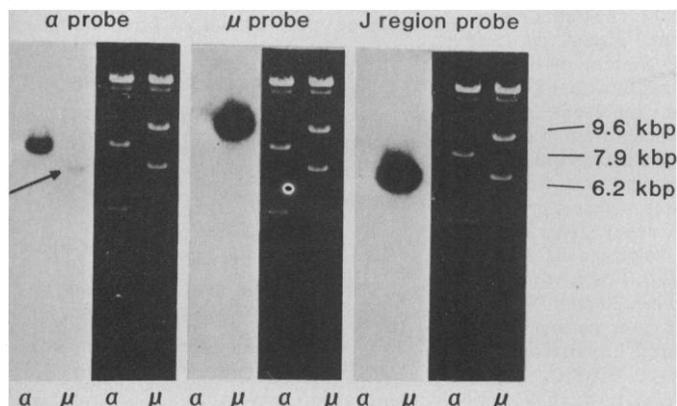
regions of both heavy and light chains (1). The C genes for the heavy chains and the two types of light chains,  $\kappa$  and  $\lambda$ , are all on separate chromosomes and have separate V region gene repertoires (2).

The mechanism for generating diversity of V regions for light chains in mouse has been thoroughly studied at the DNA level. These V regions are coded by two gene segments, V and J,

which are joined by recombination at the DNA level to produce the completed V gene. The VJ and C genes are subsequently joined at the RNA level by splicing of the messenger RNA (mRNA) precursor (3).

The mechanism for generating diversity of V regions for heavy chains is not as well characterized at the DNA level as that for light chains. In mouse there are at least eight heavy chain constant region ( $C_H$ ) types ( $\mu$ ,  $\delta$ ,  $\gamma_3$ ,  $\gamma_1$ ,  $\gamma_{2a}$ ,  $\gamma_{2b}$ ,  $\alpha$ , and  $\epsilon$ ) (4), and all are encoded in the same chromosomal region (2). Analysis of protein sequences suggests that the V regions of heavy chains may be coded in separate V and J segments of DNA as the light chains are, and some protein V region sequences suggest that a third "D" segment may occur between V and J (5). In this report we present DNA sequence data establishing that heavy chain J regions are indeed encoded separately from the V and C regions. We have identified and sequenced two J genes and have shown that in germ-line DNA they are located about eight kilobase pairs (kbp) to the 5' side of the  $C_{H\mu}$  gene.

The general approach used in these studies was to isolate DNA clones for the heavy chain genes from shotgun collections made from mouse DNA ob-



**Fig. 1.** Southern hybridization analysis of genomic  $C_{H\alpha}$  and  $C_{H\mu}$  genes. The two clones, Ch4A142.4 and Ch4A142.7, were digested with Eco RI and assayed by electrophoresis in triplicate on agarose gels in the channels designated  $\alpha$  and  $\mu$ , respectively. Photographs of the ethidium bromide-stained gels are shown in the right portion of each panel. In the left portion of each panel are autoradiographs of hybridization with three probes: (i) the VJ $\alpha$  plasmid, p $\alpha$ (J558)<sup>13</sup>; (ii) the  $\mu$  plasmid, p $\mu$ (3741)<sup>9</sup>; and (iii) the J region plasmid, pN12. The arrow indicates the positive hybridization of Ch4A142.7 to the J segment seen with normal exposure with the VJ $\alpha$  plasmid as probe. The other two panels are overexposed to show that there is no hybridization of either  $\mu$  or J coding sequences to Ch4A142.4. By plaque hybridization experiments it was also shown that neither  $\gamma_{2b}$  nor  $\gamma_{2a}$  [which shares enough sequence homology to  $\gamma_{2b}$  that its presence would be shown with that  $\gamma_{2b}$  cDNA probe (26)] occur within 15 kbp of the 5' side of the cloned  $C_{H\alpha}$  gene (data not shown).

**Fig. 2.** Physical map of the genomic DNA insert in Ch4A142.7. The upper line shows the genomic DNA insert of Ch4A142.7 with the four domains of the  $C_{H\mu}$  gene indicated as black boxes. Sizes of fragments are indicated below the line in base pairs. The size of the dashed Hind III fragment varies in different clones due to a tendency of this region of DNA to delete. The size of the interval marked  $\times$  is 4450 bp in Ch4A142.7 but is about 7400 in genomic DNA. The lower line shows a more detailed map of the 1011-bp subfragment that was sequenced. The dots and arrows show the strategy used in sequencing. Fragments were end-labeled with <sup>32</sup>P at the dots, and the length and direction of sequence obtained is shown by the arrows. The restriction maps were obtained by electrophoretic analysis of DNA on agarose and acrylamide gels of single or multiple restriction endonuclease digestions. Southern hybridization analysis was used to identify fragments containing  $C_{H\mu}$  or J<sub>H</sub> sequences.

