

1.5 bars, the model yields a  $\text{CH}_4/\text{N}_2$  mixing ratio near 0.03.

We have used the TCM for a preliminary CO analysis; the results are encouraging, although not yet entirely satisfactory. This TCM provides an improved fit to the observed band structure over that for the RLM and yields a preliminary CO column abundance of  $a = 12$  m-amagats above the dense cloud and a  $\text{CO}/\text{N}_2$  mixing ratio of  $1.5 \times 10^{-4}$  if the dense cloud is at 1.5 bars.

A considerable amount of work, including taking proper account of the temperature dependence of  $\text{CH}_4$  absorption in the  $6300\text{-cm}^{-1}$  region, remains to be done before we can precisely estimate the amount of CO in Titan's atmosphere. A refined atmospheric model appropriate to this region is needed to improve the fit of the synthetic line profiles to the observations, and perhaps better observations above our present signal-to-noise level of 40 may be necessary. We believe that we have established the abundance of CO in Titan's atmosphere to within a factor of 3.

The steady-state CO abundance predicted by the photochemical model used to explain the  $\text{CO}_2$  detection is  $\text{CO}/\text{N}_2 \approx 1.1 \times 10^{-4}$  (7). Our result appears consistent with this prediction. The steady-state CO abundance is independent of the influx of  $\text{H}_2\text{O}$  (assumed to be the source of the OH radical required in the key reactions producing  $\text{CO}_2$ ), if that influx is greater than about 0.3 of the value associated with meteoritic infall on Earth (7). Our abundance determination may support the photochemical model suggested; but, in any event, it confirms the proposed identification of  $\text{CO}_2$  (7). It now seems unlikely that it can provide any information on the primordial CO abundances or direct support of the clathrate model. An unambiguous detection of  $^{36}\text{Ar}$  remains the best evidence for the clathrate capture process.

The addition of 50 to 150 parts per million of CO to Titan's atmosphere, with the concomitant implication of active OH, makes this atmosphere even more interesting as a natural laboratory for testing ideas about chemistry on prebiological Earth. Recent thinking has suggested that early Earth may have had an atmosphere that was less reducing than the traditional Miller-Urey model (10). Titan departs most drastically from this revised Earth model in being far too cold to permit the presence of liquid water. Nevertheless, atmospheric chemical reactions taking place on Titan today may resemble some of those that occurred on early Earth. Of particular interest is the issue of preferential pathways: Are some routes toward chemical

synthesis favored over others? The Titan surface (95 K) serves as a cold trap for any complex organic aerosols that precipitate from the atmosphere; and, with the presence of both HCN and CO established, these compounds should include many of biological interest (11).

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## Reversible Loss of Gravitropic Sensitivity in Maize Roots After Tip Application of Calcium Chelators

**Abstract.** *The application of calcium chelating agents (EDTA or EGTA) to the tips of maize roots caused a loss of gravitropic sensitivity. When the chelator was replaced with calcium chloride, gravitropic sensitivity was restored. Asymmetric application of calcium chloride near the tip of a vertical root caused curvature toward the calcium source. When the calcium was applied to the upper surface of the tip of a root oriented horizontally, the root curved upward even though control roots exhibited strong downward curvature. Application of calcium chloride to the tips of decapped roots, which are known to be gravitropically insensitive, did not restore gravitropic sensitivity. However, asymmetric application of calcium chloride near the tips of decapped roots caused curvature toward the calcium source. Calcium may play a key role in linking gravity detection to gravitropic curvature in roots.*

Within 10 to 15 minutes after maize seedlings are oriented horizontally, the primary roots begin to exhibit positive gravitropism. The gravity-detection mechanism is located within the root cap even though the asymmetric growth which causes curvature occurs in the elongation zone behind the root cap (1, 2). Although there is evidence that gravity detection in root cap cells involves the dense amyloplasts (2), there is little information on the link between gravity detection and the induction of asymmetric growth in the elongation zone. Recently, calcium has been implicated in gravitropic responses in plants. Calcium moves toward the upper side of gravistimulated hypocotyls and coleoptiles (3), and the amyloplasts in the root cap carry substantial amounts of calcium (4).

Calcium is required for the transport of auxin, a hormone that may mediate asymmetric growth in gravitropism (5).

We have examined the effect of calcium depletion and replenishment on gravitropic sensitivity in maize roots. We find that calcium chelators (EDTA or EGTA) applied to the root cap cause loss of gravitropic sensitivity and that sensitivity can be restored if the chelator is replaced with  $\text{CaCl}_2$ . The experiments were done on 3-day-old maize seedlings, and the test chemicals were applied in agar blocks placed directly on the root (6).

When 50 mM EDTA (7) was applied to the tip of the root and the root was immediately oriented horizontally and maintained in that orientation for 180 minutes, no gravitropic curvature oc-

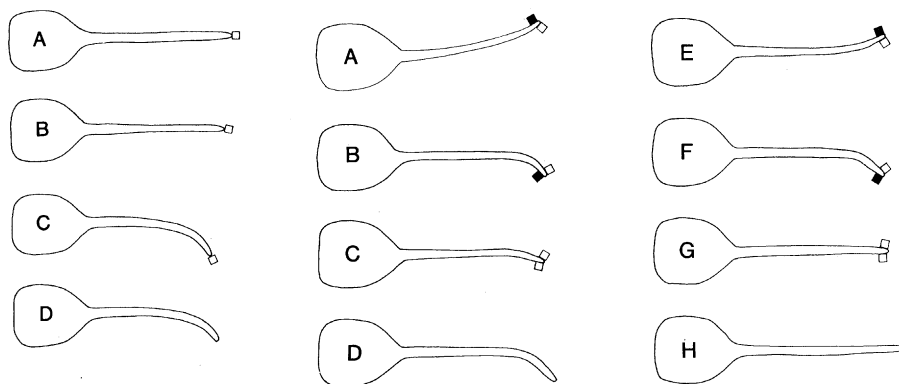


Fig. 1 (left). Reversible suppression of gravitropism in maize roots by tip application of EDTA. Each diagram shows the degree of curvature 180 minutes after the seedling had been oriented horizontally. (A) EDTA (50 mM) was applied to the root tip and the root was oriented horizontally for 180 minutes. (B) EDTA was replaced after 90 minutes with a plain agar block. (C) EDTA was replaced after 90 minutes with an agar block containing 10 mM  $\text{CaCl}_2$ . (D) Control. Fig. 2 (right). Induction of curvature by asymmetric application of  $\text{CaCl}_2$ : (A–D) intact roots; (E–H) decapped roots; (D) intact control (no EDTA or  $\text{CaCl}_2$  applied); (H) decapped control (no EDTA or  $\text{CaCl}_2$  applied). Each diagram shows the curvature 180 minutes after the seedling had been oriented horizontally. In all cases except (D) and (H), the root tip was exposed to EDTA (50 mM for intact roots, 25 mM for decapped roots) for the first 90 minutes. The EDTA-containing agar block was then removed, and for the last 90 minutes an agar block containing 10 mM  $\text{CaCl}_2$  (■) was applied near the tip on either the top (A and E) or the bottom (B and F) of the root and a plain agar block (□) was applied to the opposite side. In (C) and (G), the EDTA-containing block was removed from the tip at 90 minutes and plain agar blocks were applied to both the top and bottom of the root tip.

curved (Fig. 1A) even though the root continued to grow (8). Control roots showed a gravitropic response during this time (Fig. 1D). If, after 90 minutes, the EDTA was replaced by 10 mM  $\text{CaCl}_2$ , gravitropism occurred during the next 90 minutes (Fig. 1C). Replacing the EDTA with a plain agar block after 90 minutes failed to restore gravitropic sensitivity during the next 90 minutes (Fig. 1B) (8). Neither  $\text{MgCl}_2$  nor  $\text{MnCl}_2$  was an effective substitute for  $\text{CaCl}_2$ .

These results indicate that calcium may play a role either in gravity detection or in the gravitropic growth response. In order to test the possibility that gravity-induced gradients of calcium at the root tip might induce curvature, we tested whether asymmetric application of calcium to the tip of a root might induce curvature. First we treated the roots with EDTA by placing an agar block containing 50 mM EDTA at the root tip and maintaining it there for 90 minutes. The block was then removed, and an agar block containing 10 mM  $\text{CaCl}_2$  was placed within 2 mm of the tip on the top or bottom of the root (9). In both intact and decapped roots,  $\text{CaCl}_2$  application on the upper surface or on the lower surface caused the root to curve toward the calcium source (Fig. 2) (10).

The results in Fig. 2 indicate that a calcium gradient near the root tip can induce gravitropic-like curvature in roots that have been desensitized to gravity either through cap removal or through

the application of EDTA. We also tested whether asymmetric application of calcium might induce curvature in roots held vertically. In both intact and decapped roots, asymmetric application of  $\text{CaCl}_2$  caused curvature toward the calcium source, whereas asymmetric application of EDTA caused curvature away from the point of application (Fig. 3).

Calcium gradients may play a role in root gravitropism. This idea is supported by recent experiments by dela Fuente and Leopold (11), who found that unilateral application of the calcium ionophore A23187 to the apical 5 mm of vertical maize roots causes curvature toward the ionophore. However, neither our data

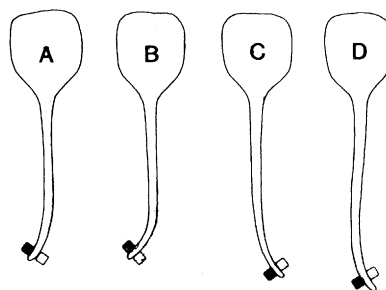


Fig. 3. Induction of curvature in vertical roots by asymmetric application of  $\text{CaCl}_2$  or EDTA: (A and C) intact roots; (B and D) decapped roots. An agar block containing either 10 mM  $\text{CaCl}_2$  (A and B) or 50 mM EDTA (C and D) (■) was applied to the left side of the root tip, and a plain agar block (□) was applied to the opposite side. Each diagram shows the degree of curvature 90 minutes after the application of the agar blocks.

nor the findings of dela Fuente and Leopold provide evidence that natural calcium gradients occur in gravistimulated roots. If such gradients do occur, the question remains as to how gravistimulation leads to calcium redistribution and how asymmetric distribution of calcium at the root tip might lead to asymmetric growth in the elongation zone.

The possibility that a calcium gradient at the root tip might indirectly lead to asymmetric growth in the elongation zone needs to be considered. Since calcium is necessary for auxin transport (5) and auxin has been reported to induce calcium efflux from plant cells (12), it may be that calcium gradients can modify the auxin distribution in the root. Alternatively, calcium redistribution near the root tip may be related to rapid changes in the pattern of electrical currents in gravistimulated roots (13), with the electrical pattern somehow influencing the growth pattern of the root.

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7. EGTA was at least as effective as EDTA.
8. If the EDTA was left on the root tip, the growth rate began to decline after about 4 hours. Curvature in roots treated with EDTA for 90 minutes began about 150 minutes after removal of the EDTA.
9. To determine whether asymmetric application of calcium to the root surface resulted in a gradient of calcium within the root,  $\text{CaCl}_2$  labeled with  $^{45}\text{Ca}$  was applied to one side. We determined the distribution of labeled calcium in the root after 45 minutes by slicing the root longitudinally and determining the labeled calcium in each half of the apical 2 mm. In all cases (six experiments with 25 roots each) there was an approximately twofold (average, 1.9) gradient of labeled calcium across the root tip.
10. The ability of magnesium and manganese to substitute for calcium was also tested. In decapped roots,  $\text{MgCl}_2$  and  $\text{MnCl}_2$  were without effect. In intact roots,  $\text{MgCl}_2$  and  $\text{MnCl}_2$  sometimes caused curvature, but the effect was not as strong as that obtained with  $\text{CaCl}_2$ .
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