CII protein inserted into the Cla I site of pBR322. The prokaryotic expression vector pCO-5 is identical to vector pJL6 of Lautenberger and his co-workers (*26*); pJL6 was not made available by those authors despite its previous publication. The 589-bp Hpa II fragment of BPV (nucleotide numbers 80 to 669) was cloned into the regenerated Cla I site of pCO-5. The resultant construct, pCO6-14, encodes the first 13 amino acids of the phage CII gene fused to BPV coding sequences, which begin three amino acids upstream from the predicted AUG of E6 and continues past the stop codon of the ORF. This DNA was then introduced into bacteria N6405, a $N^+$ RR1 strain of *E. coli* that contains a lysogenic temperature-sensitive $P_L$ repressor. Proteins were extracted by lysing thermally induced (90 minutes) bacteria bCO-5 (control) or bCO6-14 (E6 insert) in 50 m*M* tris, *p*H 8, 1 m*M* EDTA, and lysozyme (0.25 mg/ml) for 30 minutes at 4°C. $MgCl_2$ and NaCl were added to attain a final concentration of 6 m*M* and 0.2*M*, respectively, and DNase I was added to attain a final concentration of 0.1 mg/ml, for 30 minutes at 4°C. Bacterial proteins were extracted with nonionic detergents (0.5 percent NP-40 and 0.5 percent deoxycholate); insoluble proteins, including the E6 fusion protein, were subsequently solubilized with 0.5 percent SDS.


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# A Pea Mutant for the Study of Hydrotropism in Roots


Abstract. *Plant roots grow in the direction of increasing soil moisture, but studies of hydrotropism have always been difficult to interpret because of the effect of gravity. In this study it was found that roots of the mutant pea 'Ageotropum' are neither gravitropic nor phototropic, but do respond tropically to a moisture gradient, making them an ideal subject for the study of hydrotropism. When the root caps were removed, elongation was not affected but hydrotropism was blocked, suggesting that the site of sensory perception resides in the root cap.*



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Roots grow in response to water gradients (*1*). Usually the gradient is vertical; that is, desiccation occurs by evaporation at the soil surface and gravity tends to pull water to lower levels. Garwood and Williams (*2*) showed that in a light alluvial soil beneath a pasture, the water potential decreased from −2 bars in April to −15 bars in July at a depth of 30 cm. However, at a depth of 60 cm, water potential decreased from −1 bar in April to only −4 bars in July. Thus, during the summer, a period of considerable root growth, there was a water gradient of 14 bars between 30 and 60 cm.

Roots appear to grow toward the region of greater water potential. Klepper *et al.* (*3*) monitored the growth of cotton roots during a period of soil desiccation. Before desiccation, the water potential was the same (about −0.5 bar) from 23 to 173 cm below the surface. After 13 days of desiccation, the water potential at 173 cm was still about −1 bar, but at 23 cm it was about −9 bars, establishing an 8-bar gradient. During the experiment the density distribution of the root system changed from being highest at about 20 cm deep and lowest at about 180 cm to the reverse. (Care must be taken in interpreting such results. They could indicate a greater amount of growth in moist areas or the death of roots in drier areas, instead of directed growth.)

The mechanism of hydrotropism [as Sachs (*4*) called the directed growth of roots in a moisture gradient] has been studied at least since the early 19th century. Dutrochet (*5*) suggested that roots may bend in response to moisture gradients in the root environment, but was unable to demonstrate such a response experimentally. Sachs (*4*) described a simple experiment in which he planted pea seeds in a freely hanging cylinder of damp sawdust held together by a zinc mesh screen. The roots penetrated and grew through the sawdust (positive gravitropism); however, when they grew out of the lower surface, they bent around and grew back toward the wet substratum, thus overcoming the force of gravity. Sachs observed that thigmotropism also plays a role in the directional growth of roots. If roots encounter a unilateral impediment or are rubbed on one side, they will bend in the direction of the mechanical perturbation (*6*).

It is likely that both thigmotropism and gravitropism interact with hydrotropism and therefore interfere with the study of hydrotropism. Mechanical stimuli can easily be avoided, but gravity is ubiquitous (at least on Earth). Therefore a root system lacking the gravitropic reaction would be an important tool in the study of root hydrotropism.

The roots and shoots of the pea mutant 'Ageotropum' are gravitropically unresponsive when the plant is grown in darkness (*6, 7*). However, when the plants are illuminated, the shoots become gravitropic, bending upward regardless of the direction of illumination. The photoactivation of gravitro-

pism in the shoots is mediated by phytochrome (*8*).

When this phenomenon was originally reported (*6*), it was noted that the roots, some of which grew up out of the soil and into the light, promptly bent around and grew back into the soil. This was interpreted as the activation of gravitropic responsiveness by the light. However, we have found that neither white light, red light, nor far red light has any potentiating effect on gravitropism of 'Ageotropum' roots (*9*). We must also point out that this response could have been a negative phototropic effect.

Accordingly, we studied the spectral response of 'Ageotropum' plants and found that, while the shoot shows phototropism in response to blue light, the root does not (Fig. 1). Furthermore, roots from plants growing in the dark bent downward to the soil after they emerged. 'Ageotropum' roots do not have chlorophyll, do not perform photosynthesis, and are not phototropic. Nor are they gravitropically potentiated through phytochrome (red and far red light have no effect on oriented root growth). Therefore, it was necessary to search for some other mechanism for the downward bending of the roots when they grow up out of the soil.

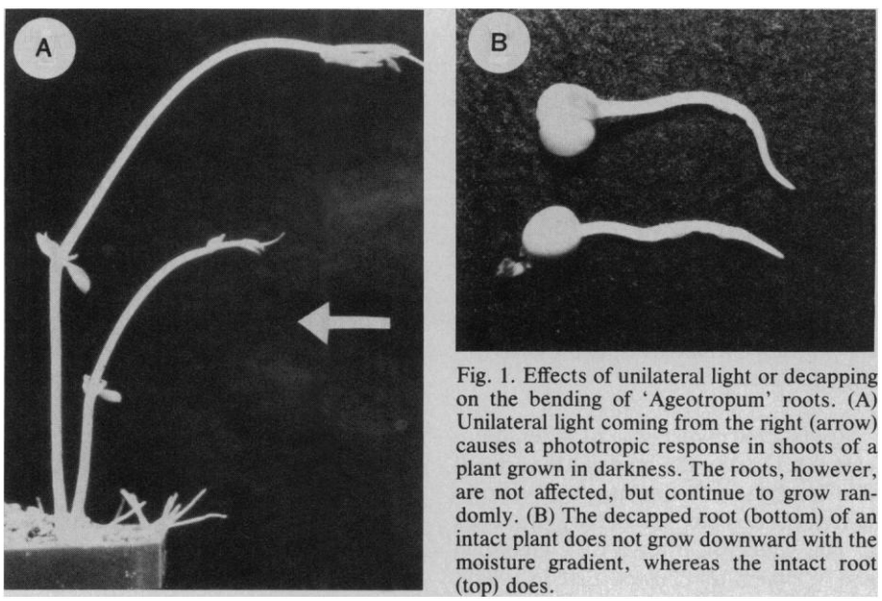


Fig. 1. Effects of unilateral light or decapping on the bending of 'Ageotropum' roots. (A) Unilateral light coming from the right (arrow) causes a phototropic response in shoots of a plant grown in darkness. The roots, however, are not affected, but continue to grow randomly. (B) The decapped root (bottom) of an intact plant does not grow downward with the moisture gradient, whereas the intact root (top) does.

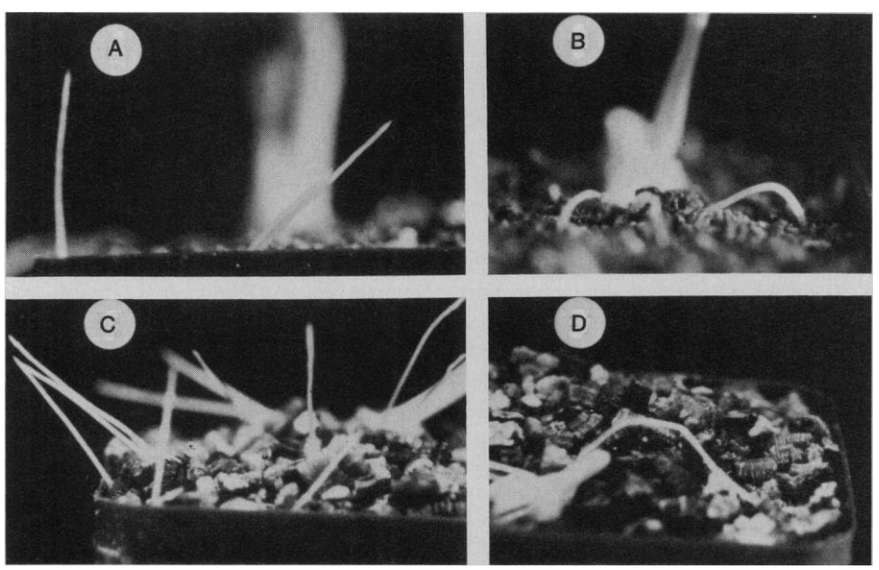


Fig. 2. Effect of a moisture gradient on the downward bending of roots of 'Ageotropum' grown in light (A and B) or in darkness (C and D). The plants were grown in the absence (A and C) of a moisture gradient (air RH, 95 to 98 percent) or in the presence (B and D) of a moisture gradient (air RH, 80 to 85 percent).

Because of the known hydrotropic capacity of plant roots, it was appropriate to examine hydrotropism as a possible mechanism. Glass chambers were assembled with appropriate inlets and outlets for the flow of air (0.33 m/sec, a rate that did not mechanically perturb the plants). The relative humidity (RH) of the air in the chamber was controlled by (i) filtering inlet air through $CaCl_2$ (45 percent RH), (ii) letting room air enter the chamber (60 percent RH), (iii) lining the chamber with chromatography paper standing in 1-cm-deep water in the bottom of the chamber to increase humidity by evaporation (80 to 85 percent RH), (iv) bubbling the air through ion-free water before it entered the chamber (90 to 95 percent RH), or a combination of (iii) and (iv) (95 to 98 percent RH). The air temperature was held at 24°C and the soil in which the plants were growing was kept thoroughly wetted.

In all cases some roots grew up out of the soil, but at less than 65 percent RH the growing regions of these roots quickly died. Whether plants were brought into the light or kept in the dark, roots held at 75 to 82 percent RH bent downward and grew back into the soil (Fig. 2, B and D). However, roots growing into air with 85 to 90 percent RH bent only slightly toward the substratum. If the roots grew up into air with 95 to 98 percent RH, they continued to grow upward, reaching lengths of 4 cm or more (Fig. 2, A and C). These results have been duplicated in humidity-controlled plant growth chambers at the Kennedy Space Center. Both primary and lateral roots were capable of hydrotropism.

To understand the mechanism of hydrotropism in roots, it is necessary to know the location of the sensory system. Darwin (*6*) covered the apical 1 or 2 mm of roots with a hydrophobic mixture of olive oil and lampblack and found that they no longer responded to a moisture gradient. 'Ageotropum' root tips have normal-appearing root caps 1 to 2 mm long (*10*). Decapped or complete primary roots were laid horizontally on an agar block covered with wet filter paper. The roots, still attached to the plant, were positioned with their tips projecting just past the edge of the agar block, and the preparation was held in the light at 80 percent RH. At that time and after 12 hours of incubation, the length and curvature of the roots were measured with the WEEDWATCHER II program of the DARWIN image analyzer (*11*). The experiment was repeated three times with five roots each. After 12 hours, control roots had bent down 32° ± 5° (mean

± standard error), whereas decapped roots bent only 9° ± 2° (Fig. 1). Decapping had no effect on the potential for root growth, since the controls and decapped plants grew 0.80 ± 0.25 and 0.90 ± 0.26 mm, respectively. Thus, because the plant does not respond to gravity, the root cap seemed to be the site of hydrotropic sensory perception.

We suggest the following mechanism for the downward bending of emergent 'Ageotropum' pea roots. When the seed is planted, the radical and then the root grows in whatever direction it was pointed at planting. As the secondary roots appear, they also grow in random directions. By chance, some of these roots grow upward and emerge from the soil into the air (and into the light if the plants are in an illuminated area). As soon as the root cap has emerged, a sensory system in the cap detects a moisture gradient between the wet soil and the drier air. It responds to this gradient by bending and growing back into the soil. The fact that it bends downward when exposed to the light is purely fortuitous, since its behavior is the same in the dark as it is in the light as long as the moisture gradient is present. The moisture gradient of 80 percent RH (air) to about 99 percent RH (water-saturated soil) is great enough to induce hydrotropism.

Thus a model system is now available to study hydrotropism in roots, unimpeded by complications involving gravitropism, phototropism, or phytochrome. This mutation should provide a probe for the elucidation of the mechanism of hydrotropism in plant roots.

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# Transcription of Class III Genes Activated by Viral Immediate Early Proteins


Abstract. *The adenovirus E1A and pseudorabies virus immediate early (IE) proteins induce transcription from transfected viral and nonviral genes transcribed by RNA polymerase II (class II genes). These proteins have now been shown also to activate transcription of transfected genes transcribed by RNA polymerase III (class III genes). As previously observed for class II genes, this stimulation of class III gene transcription was much greater for transfected genes than for the major endogenous cellular class III genes. Extracts made from cell lines stably expressing a transfected pseudorabies virus IE gene were 10 to 20 times more active in the in vitro transcription of exogenously added class III genes than extracts of the parental cell line. These results indicate that the E1A and IE proteins stimulate the expression of class III genes by a mechanism similar to the mechanism for stimulation of class II gene transcription by these proteins.*



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Genetic studies have led to the discovery of viral proteins that induce viral gene transcription. In the adenoviruses, which have a duplex DNA of about 36 kilobase pairs, the largest protein (289R) encoded in the leftmost transcription unit called E1A, stimulates transcription from five viral promoters transcribed by RNA polymerase II (*1*, *2*). In the herpesviruses, which have genomes of about 150 kbp, immediate early (IE) proteins are required for transcription of an even larger set of viral promoters (*3*).

The adenovirus E1A protein, unlike well-studied positive activators of transcription in prokaryotic systems such as the adenosine 3′5′-monophosphate (cyclic AMP) binding protein of *Escherichia coli* (*4*) or sigma-like factors of the *Bacillus subtilis* phage SPO1 (*5*), is not absolutely required for the transcription of the genes under its control. After infection of HeLa cells by an Ad5 E1A deletion mutant, d1312 (*6*), transcription from early viral promoters occurs, although it is greatly delayed compared to

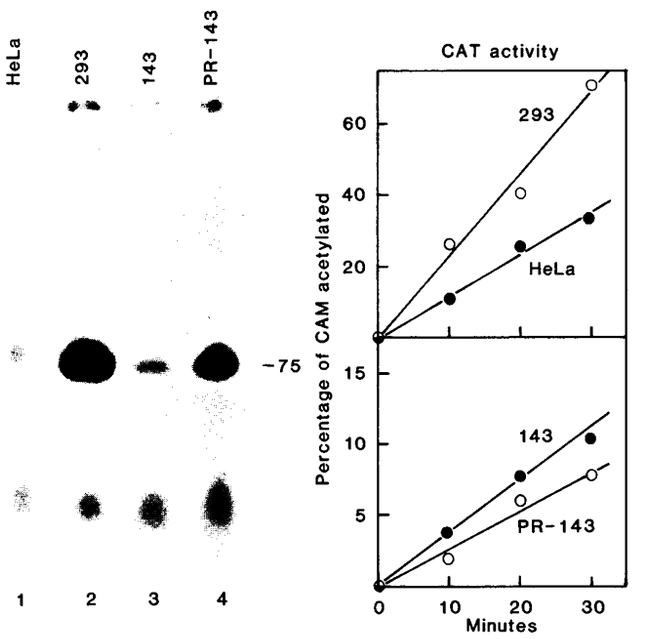


Fig. 1. Transcriptional activation of the Ad2 VA-I gene by viral early proteins. Two 100 m*M* plates of HeLa (lane 1), 293 (lane 2), 143 (lane 3), and PR-143 (line PR14) (lane 4) cells were cotransfected with 15 μg of a plasmid containing the adenovirus VA-I and VA-II genes (*28*) and 5 μg of the RSV-CAT plasmid (*23*). Total cytoplasmic RNA was harvested from one plate 48 hours after transfection (*26*), and 100 μg of RNA was analyzed by S1 nuclease analysis with a 96-bp 5′ end-labeled Bam HI–Sal I DNA fragment (*28*). Hybridized VA-I RNA protects a 75-nucleotide fragment from digestion by S1. The remaining 100 m*M* plate from each transfection was harvested and analyzed for CAT activity as described (*23*). The percentage of conversion of [$^{14}$C]chloramphenicol (CAM) to the acetylated form at 10, 20, and 30 minutes of reaction catalyzed by 300 μg of protein extract is shown at the right.