

How Roots Respond to Gravity

A century ago botanists discovered that a root turned on its side curves downward rapidly in response to gravity, but the mechanism underlying this behavior is only now beginning to be understood

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In gravity-free outer space there is no up or down. When a plant is moved from some arbitrarily designated vertical position to a horizontal one, the stem and the root continue to grow straight; they do not return to their initial positions. On the earth, plants behave very differently. They exhibit gravitropism (which used to be called geotropism): their parts reorient in response to being "gravistimulated," or repositioned with respect to gravity. After a vertically oriented plant is turned on its side the shoot curves upward, away from the direction of gravity's pull. The root, which is the subject of our investigations, curves downward, in the direction of gravity. In many instances a root begins to reorient within 10 to 30 minutes of being gravistimulated.

Although a casual observer might suspect that such downward curvature by roots is merely a search for water or a passive response to gravity, neither is the case. Roots of some plants do seek water, but gravity has an independent effect on the direction of root growth. Moreover, growing roots, which are strong enough to penetrate packed soil, are certainly strong enough to resist the direct pull of gravity; root gravitropism arises from some other mechanism.

Botanists have attempted to uncover this mechanism since before the time of Charles Darwin, whose 1881 book *The Power of Movement in Plants* included detailed descriptions of gravitropism. It is nonetheless only with the advent of the U.S. space program, and a consequent increase in funding for research into the effects of gravity and zero gravity, that investigators have made significant progress in describing the processes that underlie gravitropism in roots.

Gravitropism can be divided into three phases: perception, transduction and response. In roots the perception, or initial detection, of gravity appears to occur in the cap, which is the terminal half millimeter of the root. The response—an altered growth pattern leading to downward curvature—takes place somewhat

behind the cap, in the "elongating zone," where, as the name implies, cells elongate. Transduction, the intermediate (and most mysterious) phase, almost certainly involves some kind of communication between the cap and the elongating zone, but the exact nature of the interaction between the two regions is only now beginning to be deciphered.

Although the root's precise gravity-perceiving mechanism is not understood, even Darwin recognized that the root cap is the probable site of such perception. The cap, he wrote in 1881, "having the power of directing the movements of the adjoining parts, acts like the brain of one of the lower animals; the brain being seated within the anterior end of the body, receiving impressions from the sense-organs, and directing the several movements." He was among the first investigators to find that removal of the root cap eliminates the ability of the root to respond to gravity. Other investigators have since confirmed the observation and have also shown that replacing an excised cap with one from another root restores gravitropism.

Most workers consider the gravity-detecting portion of the cap to be the central region called the columella, which consists of cells rich in dense amyloplasts: organelles that are filled with starch grains. In vertically oriented roots the amyloplasts reside at the lower end of each columella cell, toward the root tip. Within seconds after roots are gravistimulated, amyloplasts in the columella fall from their former position and rapidly settle along the new lower wall of each cell.

There is substantial evidence that amyloplast displacement constitutes the initial detection of gravity. For example, experimentally treated roots lacking amyloplasts do not respond to gravity. In some cases tilting a plant on its side for a mere 12 seconds tells the plant that it has been reoriented; amyloplasts appear to be the only organelles in the root cap that are significantly displaced within such a short period. In roots

made nonresponsive to gravity by the removal of the root cap, recovery of responsiveness to gravity correlates with the formation and settling of new amyloplasts; de-capped roots regain gravitropic sensitivity in from 14 to 22 hours, the time it takes for new amyloplasts to form and settle in certain cells near the tip of the decapped root.

If amyloplast settling is in fact the root's gravity-perceiving mechanism, it is probably the only step of the gravitropic response in which gravity directly pulls down some target (in this case the amyloplasts); the other phases seem to be mediated by chemical activity. Before discussing these other phases we should note that a laboratory-developed mutant of one type of plant has been described that lacks amyloplasts in its cap yet still has a nearly normal gravitropic response. This finding suggests that gravitropism can take place in the absence of amyloplasts. It also suggests that amyloplasts may not be the initial gravity detector in roots or that, in the absence of amyloplasts, roots can activate an as yet unknown alternative gravity-detecting mechanism.

Whatever mechanism accounts for the perception of gravity, the ultimate effect of gravity detection—root curvature—clearly results from asymmetric growth in the elongating zone. When a root is oriented vertically, it grows uniformly on all sides. In contrast, when a root is turned horizontally, the upper side grows faster than the lower side; slower growth at the bottom makes the root curve downward.

To discover the factor that directly alters the growth pattern of a gravi-stimulated root, we and other investigators sought to determine the precise changes in growth along the upper and lower sides of the root. We considered many possible permutations. For instance, does the growth rate along the upper side increase while the rate along the lower side remains constant? Does the rate on the lower side decrease while the rate on the top increases? Does the growth rate on both sides increase, with the top rate increasing more than the bottom, or does the growth rate on both sides decrease, with the bottom rate decreasing more than the top?

Amy J. Nelson, a graduate student working with one of us (Evans), determined the typical growth pattern of gravistimulated corn roots. She put young seedlings in a chamber that had uniformly high humidity,

thereby providing the moisture needed for normal root growth. In this experiment, as in the others we shall discuss, each seedling was placed in an environment that eliminated the possible effects of an uneven distribution of moisture, light, nutrients or temperature on the direction of root growth; this ensured that any curvature in the root could be attributed to gravity alone. Nelson positioned the white root of each seedling in front of a white background and put small black beads along the root surface, which is naturally sticky. A television camera focused on the marked root then sent signals to a computer. The computer tracked the gradual movement of the beads as the root grew, calculating the growth rate of various parts of the root before and during the response to gravity.

Nelson found that gravistimulation causes a reduction in the growth rate on both the upper and the lower surfaces of the root, but the greatest reduction occurs along the lower surface, from two to three millimeters behind the cap. Other workers employing different test methods with different kinds of plants have similarly found that retarded growth at the lower side of the root causes downward curvature in gravistimulated roots.

What could account for the slowing of growth along the lower side? The growth of plant cells is controlled largely by hormones, which suggests that there is an increase in the concentration of some growth-inhibiting hormone along the lower side of the elongating zone. One hypothesis for root gravitropism suggests that the growth inhibitor in question is the hormone abscisic acid, which forms naturally in plant roots. When abscisic acid is applied in large doses to the outside of roots, it is absorbed and inhibits growth. In roots of certain corn seedlings that do not exhibit gravitropism in the dark, illuminating the root cap induces gravitropism within an hour, during which time the rate of synthesis of abscisic acid in the root cap has also been found to increase.

In spite of these findings, more recent evidence has led us to conclude that the abscisic acid hypothesis is incorrect. One of us (Evans) has shown that the hormone inhibits root elongation only when it is applied to roots at concentrations significantly higher than are thought to occur naturally.

What is perhaps more significant, another of us (Moore) has found that roots of corn seedlings grown in the presence of an inhibitor

of abscisic acid synthesis have undetectable levels of abscisic acid but nonetheless curve downward in response to gravistimulation. Similarly, roots of a corn mutant not capable of synthesizing abscisic acid respond to gravity. Moreover, one of us (Evans) has found that roots immersed in a high concentration of abscisic acid curve downward after being gravistimulated. The latter finding is significant because it is unlikely that a subtle gravity-induced gradient of the hormone could by itself induce curvature when the entire root is immersed in saturating levels of the substance.

Strong evidence suggests that the hormone auxin controls gravitropic root curvature. Auxin, which like abscisic acid occurs naturally in roots, is a powerful inhibitor of their growth, even at concentrations from 100 to 1,000 times lower than those at which abscisic acid is effective. In roots made nonresponsive to gravity (by removal of the root cap), auxin applied in a small dose to one side of the elongating zone can induce the roots to curve toward the side where the auxin has been applied—a finding that suggests a subtle, physiological increase of auxin at the lower side of a horizontal root should be sufficient to cause downward curvature.

After radioactively labeled auxin was applied uniformly to the elongating zone of a gravistimulated root, the labeled hormone moved toward the lower side of the root, suggesting that at least some of the naturally occurring auxin moves in such a way that it collects at the bottom of the elongating zone. Moreover, chemicals that interfere with the cell-to-cell movement of auxin in roots, thereby presumably impeding the increased deposition of auxin in the usual area of gravitropic curvature, overcome the root's ability to curve in response to gravity.

To demonstrate more conclusively that auxin accounts for downward curvature in gravistimulated roots, it would be necessary to show directly that natural auxin within the root becomes concentrated at the bottom of the elongating zone just before the onset of gravitropic curvature. Such evidence has not yet been found.

If the settling of amyloplasts in the columella of the root cap accounts for the perception of gravity, and if an increase of auxin at the lower side of the elongating zone accounts for the ultimate response of the root, what ties these events together? That is, what constitutes the mysterious transduction phase

of gravitropism?

On the basis of recently collected data we propose that the settling of amyloplasts triggers the release of calcium ions (Ca^{2+}) from organelles along the lower side of the columella cells. In turn the released calcium activates transport systems that move calcium and auxin downward from cell to cell toward the lower side of the root cap. The calcium at the bottom of the cap then facilitates the movement of auxin from the cap back along the lower side of the elongating zone.

We first suspected that the release and movement of stored calcium might be an important initial step in the transduction phase of root gravitropism after several papers published in the 1960s and 1970s reported that calcium appears to move from cell to cell when shoots respond to gravity. These experiments showed that calcium migrates toward the top surface of a gravistimulated shoot before the shoot curves upward.

To test our idea Konrad M. Kuzmanoff and Timothy J. Mulkey, graduate students working for one of us (Evans), applied EDTA, a chemical that binds and immobilizes calcium, to the caps of corn roots. To our surprise, the EDTA-treated roots, which continued to grow at their normal rate, became totally unresponsive to gravity. Removing EDTA and replacing it with calcium restored the roots' gravitropic responsiveness. Kuzmanoff and Mulkey hence provided strong evidence that free and mobile calcium in the root cap is essential for root gravitropism. Their observation has since been strengthened by other studies showing that a variety of treatments preventing calcium movement can all abolish gravitropism.

Taking up where Kuzmanoff and Mulkey left off, June S. Lee, who at the time was visiting Ohio State University from Kang Reung National University in Korea, determined the probable direction of calcium movement within gravistimulated roots. First he studied roots that were not gravistimulated and discovered that calcium has a powerful influence on their direction of growth. When he applied calcium to the root cap unevenly, the root curved toward the area of greatest calcium concentration. In fact, when calcium was applied continuously to one side of the cap of a vertically oriented root, the root eventually curved through a complete 360-degree loop. Knowing that gravistimulated roots curve downward, Lee concluded that

such curvature may result from an excess of calcium at the bottom of the cap, perhaps deposited by the downward movement of calcium.

Next Lee showed that calcium could in fact move downward within the cap. He applied radioactive calcium evenly across corn roots, oriented the roots either vertically or horizontally and measured the movement of the radioactive ions. In vertically oriented roots the radioactive calcium remained uniformly distributed. In horizontally oriented roots, on the other hand, the radioactive calcium moved toward the lower side of the root. This directional movement of calcium was particularly strong in the root cap.

Results from a completely different kind of study lend support to the proposal that calcium moves downward in gravistimulated roots. By placing special microelectrodes close to a vertically oriented root of cress, H. M. Behrens and his co-workers at the University of Bonn showed that an electric current flowed in a symmetrical pattern along the root surface and into the root near the tip. When the workers put the root in a horizontal position, they found that the current pattern became asymmetric; in particular, the current along the upper side of the cap flowed out through the top, whereas the current along the lower side flowed into the cap. They found evidence that the current was carried by a flow of hydrogen ions (H^+).

Thomas Bjorkman and A. Carl Leopold of Cornell University recently confirmed these observations in studies of corn roots. They found that from two to six minutes after the roots are oriented horizontally the direction of the current in the root cap changes: it moves upward and out through the top of the cap. Bjorkman and Leopold speculate that the upward flow of hydrogen ions could indirectly reflect a flow of calcium ions to the lower side of the cap; to maintain electrical neutrality, the cap would have to balance such calcium movement with a counterflow of other positive charges, such as hydrogen ions. (The amount of upward current measured by Bjorkman and Leopold is consistent with our estimates of the calcium that moves to the bottom of the root cap.) If the upward current does in fact reflect calcium migration, the timing of calcium movement suggests that the migration results from amyloplast settling; the calcium movement immediately follows settling, and it precedes downward curvature of the root.

Just how amyloplast settling triggers calcium movement and how calcium comes to collect along the lower side of the root cap is not clear, but Barbara G. Pickard of Washington University in St. Louis has proposed a reasonable hypothesis, one that is consistent with the results of Bjorkman and Leopold. In columella cells, amyloplasts displaced by gravistimulation fall onto the endoplasmic reticulum, a complex of calcium-rich membranes and vesicles. Pickard suggests that the pressure of the amyloplasts on the endoplasmic reticulum causes calcium ions to escape from the complex, resulting in a localized elevation of the calcium level along the lower side of the cells.

We propose an addition to this model. We suspect that when the calcium level reaches a certain threshold, the ions activate calmodulin: a small protein known to be a powerful activator of many enzymes important to cellular function, not only in plants but also in animals and even in some microorganisms. Once it is activated by calcium, calmodulin appears to stimulate calcium movement by "turning on" calcium pumps, which are large enzymes thought to traverse the cell membrane. The activated pumps at the lower side of the cells then excrete excess calcium, which eventually accumulates along the lower side of the root cap.

The calmodulin required by this model does appear to be available in the root cap. Charles L. Stinemetz, a graduate student working with one of us (Evans), verified its presence in corn roots and showed that the concentration of calmodulin is four times as great in the cap as it is in the elongating zone. Lending further credence to the potential role of calmodulin, Stinemetz also found that root-cap calmodulin may be necessary for root gravitropism. When he applied calmodulin inhibitors to the caps of roots, the inhibitors strongly retarded gravitropic curvature.

In order to explain the remaining steps of the gravitropic response—the downward movement of auxin in the root cap and the calcium-enhanced movement of auxin to the lower side of the elongating zone—we must first describe what is thought to be the usual pattern of auxin movement through the root. In roots that are vertically oriented auxin moves toward the root cap through the core of the elongating zone. Some of the auxin traveling into the cap is either metabolized or moves through the tip into the growth medium. At the same time some auxin branches off toward the

sides of the cap, where it is redirected up into the elongating zone by cells along the outer margins of the root. The symmetry of movement causes the vertical root to grow straight.

In gravistimulated roots the pattern of auxin movement in the root cap changes. On the basis of recent data collected by one of us (Hasenstein), we propose that calcium-activated cal-modulin in the columella cells activates not only calcium pumps in the lowermost part of the cell membrane but also auxin pumps. Such auxin pumps are known to exist in plant-cell membranes. We think these pumps transport auxin out through the lower side of the columella cells, so that much of the auxin entering the root cap from the elongating zone travels downward to the lower side of the cap instead of dividing symmetrically between the upper and lower sides. We further propose that the elevated concentration of calcium in cells along the lower side of the root cap then somehow enhances the rate at which auxin in the lower part of the root cap travels back into the lower side of the elongating zone.

We shall now return to the question posed more than a century ago: By what mechanism does a horizontally oriented root respond to gravity? We suggest that gravity pulls on amyloplasts in the columella cells of the root cap. The amyloplasts, falling onto the endoplasmic reticulum at the lower side of the cells, cause calcium levels in the cytoplasm to rise, thereby activating calmodulin, which in turn activates calcium and auxin pumps in the membrane at the lower side of the cells. The pumps force calcium and auxin out through the lower side of the cells, leading ultimately to an accumulation of calcium and auxin at the lower side of the root cap. The elevated concentration of calcium in this part of the cap then enhances the loading of auxin into the pathway that carries auxin back toward the lower side of the elongating zone. There the auxin markedly inhibits growth and causes the root to curve downward in the direction of gravity.

It is not surprising that the downward pattern of growth produced by gravitropism almost always leads the root toward a supply of moisture and nutrients. Our recent findings and those of other botanists confirm Darwin's suggestion that this self-preserving behavior of the root (or, in his words, the "radicle") is controlled by the cap. Indeed, investigators of root gravitropism, and of other root behavior, have reason today to echo Darwin's 1881

assessment that "there is no structure in plants more wonderful, as far as its functions are concerned, than the tip of the radicle