

### Research Offers New Biochemical Insights into How Plants Perceive and Respond to Gravity

The ability of plants to generate oxygen, water, and food will make them an indispensable part of life support systems on future long-duration human space missions. The work of NASA-supported investigator B.W. Poovaiah is providing new insights into the fundamental biochemical processes by which plants respond to changes in environmental conditions such as light, touch, and gravity—insights that may also be important to efforts to grow plants in microgravity.

Plants, like humans and other animals, respond to changes in their environment—to light and darkness, warmth and cold, moisture and aridity. Although they cannot change their location, they *can* change their direction of growth to gain access to more favorable environmental conditions. Plants also respond to gravity—the roots "know" that they are supposed to grow down and the shoots up. Yet much remains unknown about precisely how all of this is accomplished—how plants perceive and respond to environmental signals, including gravity.

The same qualities that make plants essential to life on Earth—their ability to cleanse water, produce food, and turn carbon dioxide into oxygen—will also make them vital on long-term human exploratory missions in space. But before plants can be used for life support in space or

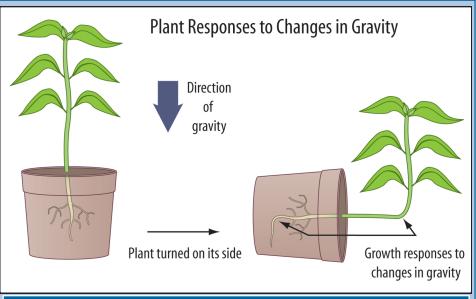
on other planets, it's necessary to understand how they will adapt to the condition of microgravity, since the force that directs roots down and shoots up—gravity—is no longer present.

NASA-supported investigator B.W. (Joe) Poovaiah, Ph.D., professor of plant molecular biology and physiology at Washington State University in Pullman, Washington, and his colleagues are trying to shed light on the complex chain of biochemical and molecular events involved in the perception and response of plants to gravity. They have focused on calcium, which is thought to play a central role in plants' processing of the gravity signal, and on proteins with which calcium interacts. Their studies take advantage of an array of molecular biological techniques and use a variety of plant species, including genetically altered plants.

# Environmental Changes Trigger Changes in Calcium and Calmodulin

In the 1970s, Poovaiah and his associates observed that changes in environmental conditions such as light, touch, gravity, and temperature alter the concentration of free calcium in the cellular fluid of plant roots and other organs. These alterations in calcium concentration resulted in changes in plant development. Investigators elsewhere have recently demonstrated that Earth's gravitational field does, indeed, induce changes in calcium concentration in cellular fluid.

More recently, Poovaiah's laboratory has shown that alterations in calcium concentration are detected by a protein called calmodulin, which attaches itself to the calcium molecule (a process known as binding).



When a plant is turned on its side, it undergoes a series of biochemical changes that leads to changes in the direction of growth. Roots bend down towards gravity (this is called positive gravitropism, since the growth is towards gravity). Shoots grow upward, away from gravity (negative gravitropism). Growth responses to light (phototropism) are distinct from responses to gravity. Responses to light may complement or counteract the gravity response, depending on the direction of light and gravity. Calmodulin is a multifunctional protein, present in all plants and animals, that interacts with many other proteins.

The binding of calmodulin to calcium creates a new, combined calcium/calmodulin molecule, which begins sending signals to numerous other proteins, creating a signaling cascade and altering the activity of the other proteins. The alterations may enhance or inhibit the action of the affected proteins.

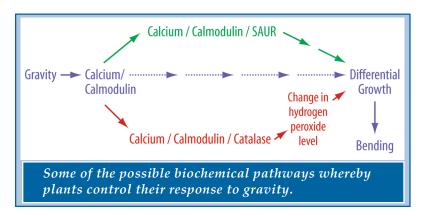
Poovaiah and his team have identified eight genes that control the production of calmodulin. They have shown that some of these genes recognize and respond to environmental changes. They have now embarked on an effort to pinpoint the influence of each of the proteins in the calcium/calmodulin signaling cascade on plants' gravitysensing processes.

#### Calcium Interacts with Auxin and Regulates Hydrogen Peroxide

Prior research by Poovaiah's group and others strongly suggested that calcium interacts with auxin, a hormone-like chemical that regulates many processes in plant growth and development. However, the mechanism of the interaction between calcium and auxin remains controversial.

Auxin regulates a family of genes called *SAURs*. These genes generate proteins that are thought to play a role in plants' ability to change their direction of growth in response to gravity (by, for example, starting to grow upright again soon after being flattened in a heavy rainstorm). Poovaiah's group has shown that these SAUR proteins bind to the calcium/calmodulin molecule. "These results provide direct molecular and biochemical evidence that calcium/calmodulin is involved in plants' response to gravity," explains Poovaiah.

More recently, experiments by Poovaiah and his associates have provided evidence that calcium/calmodulin is also involved in regulating cellular levels of hydrogen peroxide. Hydrogen peroxide is a highly reactive molecule that can cause cellular damage when present at higher than normal levels. Recent research elsewhere has shown that hydrogen



peroxide is also a signaling molecule involved in plants' adaptation to environmental changes, including gravity.

## Uniquely in Plants, Calcium/Calmodulin Binds to Catalase

Because hydrogen peroxide performs these two divergent roles—signaling and causing cellular damage—its levels in cells must be stringently controlled. A major antioxidant enzyme called catalase controls the level of hydrogen peroxide by breaking it down into oxygen and water. Poovaiah and his associates have recently shown that, uniquely in plants, calcium/calmodulin binds to catalase. The activated calcium/calmodulin/catalase complex breaks down hydrogen peroxide more rapidly, reducing cellular levels of hydrogen peroxide.

"These results suggest that stimulation of catalase activity by calcium/calmodulin reduces hydrogen peroxide levels in the cell," says Poovaiah. He hypothesizes that a change in hydrogen peroxide level alters the gravitational response—in this case, a reduction in hydrogen peroxide has the effect of stopping the normal downward gravitational response of a plant's root. When the reduction ceases, normal downward bending occurs.

### Adding Pieces to the Puzzle

Taken together, the findings that (1) auxin-regulated SAUR proteins involved in plant responses to gravity bind to calcium/calmodulin, and that (2) calcium/calmodulin stimulation of catalase reduces cellular levels of hydrogen peroxide, suggest that auxin, calcium, and hydrogen peroxide are linked in an intricate chemical network to control the direction of plant growth in response to gravity, says Poovaiah.

This work adds more pieces to the complex puzzle of plants' perception of and response to gravity, he adds. "If we are going to grow plants in space, we

will have to teach them to recognize 'up' and 'down' in the absence of gravity. These findings bring us one step closer to understanding how to do that."

#### References

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