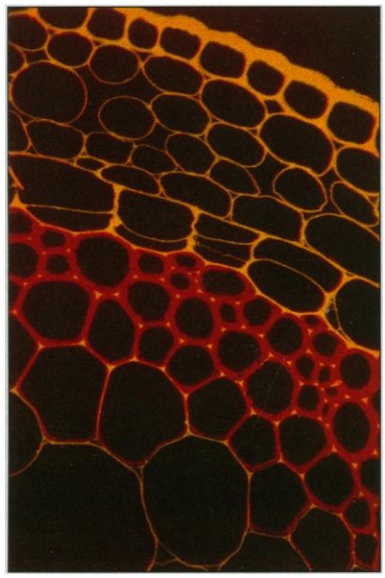
Plant cell walls, once thought to be inert boxes, are revealed as powerful signalers that determine the fate of cells during development

When Walls Can Talk, Plant Biologists Listen

Since the 17th century, when British scientist Robert Hooke trained an early microscope on a piece of tree bark and saw cork, biologists have known that plant cells are surrounded by rigid walls. Today, the glossary of any biology textbook will describe a traditional, narrow role for walls, depicting them as cellulose boxes that passively encase the active, living material of the cell. But over the past few years, a steady stream of research has begun to contradict this humdrum picture.

The new work shows that far from being inert, cell walls play active roles in determining plant cell fates. Walls contain a gallery of carbohydrates and proteins—whose identities are still largely mysterious—that appear to "talk" to other molecules, both inside and outside the cell. Researchers are trying to track down these messenger molecules and are also exploring just what information they convey. So far, most of the chatter seems to be related to development, as walls direct some cells to become roots while ordering others to develop into shoots and leaves.



Staining the walls. The amount of cellulose (red) and pectin (yellow) varies in the cell walls of an *Arabidopsis* stem.

"The last few years have seen a revolution in how we think about cell walls," says Roger Pennell, a plant biologist at Ceres Inc. in Malibu, California. "They're not static, rigid, impermeable things. They're very dynamic, very porous, very malleable, and they broadcast signals to the cells they contact directly as well as from one cell to another."

Indeed, many researchers now liken the wall to the extracellular matrix (ECM) of animal cells, which engages in a constant dialogue with cells, affecting many aspects of their lives, from cell division to differentiation. Like the ECM, the cell wall consists largely of load-bearing fibers tethered together in a watery gel. And the components of both the cell wall and the ECM are originally secreted by cells; then, once positioned in the wall or ECM, the molecules "talk back" to the cells and their neighbors. "The fact that, historically, people called it a wall generated a psychological barrier that probably wasn't based in reality," says Keith Roberts, a plant cell biologist at the John Innes Centre in Norwich, U.K.

The new respect given to cell walls was evident at the 8th International Cell Wall Conference in Norwich. The first such meeting 20 years ago mustered only 30 scientists. But this year 400 scientists turned out, and an additional 100 were turned away because of lack of space.

Determining destiny

Plant pathologists have long known that fragments of the cell wall can influence cell behavior when plants are fighting off microbes. Then the first inklings that cell walls might regulate normal development in healthy plants appeared in the late 1980s, when plant developmental biologist Ralph Quatrano, currently at Washington University in St. Louis, and colleagues were studying embryos of the brown alga *Fucus*. The group found that enzymatic removal of the cell wall destroys the ability of the embryo to "remember" where its rhizoid—the alga's version of a root—is supposed to grow. Once the walls were gone, exposure to light caused the rhizoids to regrow in new directions, suggesting that the wall stored signals needed to direct root growth.

This finding was extended in 1994, when plant biologists Fred Berger, Colin Brownlee, and Alison Taylor of the Marine Biological Association in Plymouth, U.K., made a serendipitous observation while studying two-celled *Fucus* embryos. At this stage, the fates of both cells are determined—one is destined to form the rhizoid and the other to develop into leaflike fronds. The researchers were studying whether the two cells differed in how they moved ions across the cell membrane, and the cell wall hindered their access to the membrane. So they used a laser to slice a hole in the wall of the rhizoid-forming cell, letting the cell inside ooze out for study. Then they tossed away the remaining cell and empty cell wall. But one day, on a whim, Berger saved the leftovers.

He saw the intact cell—which had been destined to become fronds—divide and multiply. The cell walls of its descendants eventually touched the empty rhizoid cell wall—and these cells then changed identities, maturing into rhizoid cells. "One day I saw an embryo with a rhizoid," says Berger, who is currently at the Institut National de Recherche Agronomique in Lyon, France. "The next day there were 20 [such] embryos, and I realized something interesting was going on." Follow-up experiments confirmed that the empty cell wall could in fact determine the identity of the cells that touched it.

Next, the researchers studied cells already specialized to form either rhizoids or fronds and found that once liberated from the wall, such cells could give rise to both types of cells. That suggests that the *Fucus* embryo cells didn't contain the information needed to specify their own destinies—but their cell walls did. "As soon as you remove a cell from its wall, it goes back to the zygotic state—it has the potential to become anything," says Berger.

In work on roots reported earlier this year in *Current Biology*, Berger has extended these findings to the more evolutionarily advanced experimental plant *Arabidopsis*. During normal *Arabidopsis* development, epidermal cells—those that form the outer layer of the root—sit above an inner, or cortical, layer of root cells. Not all the epidermal cells grow root hairs, however; only those sitting on the junction between two cortical cells do that. Berger wanted to find out whether this ability was due to a cell's lineage—whether it came from certain root-hair–forming pre-

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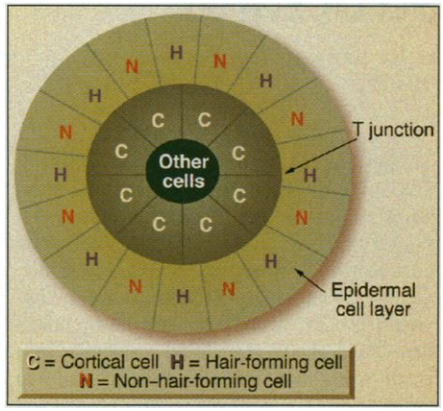
decessors—or to its position. By studying cases where the plane of cell division goes awry and by moving cells around, he and his colleagues showed that it was in fact a cell's position—touching cortical cell junctions or not—that determined its fate.

The source of this fate-determining signal turned out to be the cortical cell wall, as the researchers showed by killing selected cortical cells, leaving only their walls. Cells touching the junction between the former cortical cells still became root hairs even though the cortical cells themselves were gone. "The only structure that's left is the actual skeleton of the cortical cells themselves," says Berger. "The most logical explanation is that the messengers [specifying cell fate] are in place within the [cortical cell] wall." Roberts agrees: "The cell's fate changes depending on what wall the cell touches. This work makes a very persuasive case that some immobilized component in the wall is what's required."

This result shows that the cell wall sends messages that help determine the identity of neighboring cells as well as of the cell it encloses. It also implies that the composition of the cortical cell wall varies, with some as-yet-unknown molecular signal occupying certain patches of the wall—near cortical cell junctions, for example.

In fact, over the last 10 years, other researchers have been finding just such variations in cell walls through monoclonal antibody studies. Researchers have long known that the wall contains a wide variety of carbohydrates and proteins, some of which are attached to two of the wall's major components, hemicellulose and pectin. Few of these molecules have been identified yet, but the antibodies have shown that wall components vary among different cell types, at different times in development, and even between different locations around a single cell. "It's clear that the plant cell is investing a tremendous amount of metabolic energy in synthesizing components that get put into walls at specific times and places, and it doesn't need to do that if the purpose is to make a box just to hold the plant cell," says Michael Hahn, a plant biochemist at the Complex Carbohydrate Center at the University of Georgia, Athens.

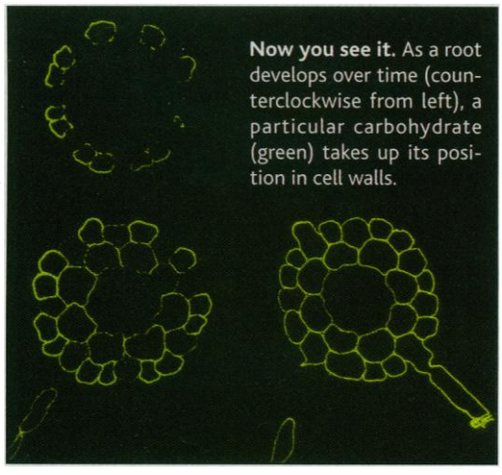


Touchy business. As shown in this root cross section, epidermal cells that grow root hairs touch the junction between two cortical cell walls.

Messengers of fate?

The next step is to try to pin down the identities of these suspected signaling molecules. Researchers have long known of one, the so-called S locus glycoprotein, which helps the female parts of crucifers such as broccoli reject pollen from plants that are too closely related, and they're hot on the trail of others, most of which seem to be important to development.

Last December in *The Plant Cell*, for example, Pennell and colleagues reported a cell wall molecule—probably a kind of pectin—identified because it binds to an antibody called JIM8, and linked to differentiation. In culture, undifferentiated cells whose walls contain this JIM8 antigen can give rise to complete embryos, while cells with walls that don't express it remain stuck in an unspecialized state. This is consistent with the idea that the JIM8 antigen helps transform cells into embryos.



Now you see it. As a root develops over time (counterclockwise from left), a particular carbohydrate (green) takes up its position in cell walls.

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While Pennell works to better characterize this antigen and its function, other researchers are studying a class of cell wall molecules—originally noted for their structural role—that also appears to be important in organ formation. As a plant cell grows, its volume balloons by 10- to 1000-fold, and the wall must grow proportionately, while remaining strong enough to counter up to 5 atmospheres of pressure from inside the cell.

One class of proteins that helps promote cell wall expansion is the expansins, identified by Daniel Cosgrove, a plant biologist at Pennsylvania State University in University Park, and his colleagues in 1992. The latest research shows that these molecules can also trigger the formation of leaves.

Last year Cris Kuhlemeier, a molecular geneticist at the University of Bern in Switzerland, and his colleagues found that by applying expansin-soaked beads to the growing tip of a tomato stem, they could elicit the production of leaflike structures. Moreover, other components of the plant recognized the expansin-induced leaf as "real": After the team induced a leaflike structure to grow in this way, other leaves formed in a pattern that mimics normal growth, appearing at regular intervals around the stem relative to the new "leaf" (*Science*, 30 May 1997, p. 1415). "By changing the mechanical properties of the cell wall with expansin, you can start a developmental program," says Kuhlemeier. "That makes the cell wall a decisive element in the whole process of how plants turn from undifferentiated cells into leaves."

Kuhlemeier's most recent work suggests that expansin performs this function normally, not just when applied by scientists. In a paper in last month's *Plant Cell*, the group showed that the expansin is being made at just the right place and time to stimulate cell growth. They studied the messenger RNA (mRNA) that encodes the expansin protein and found that in healthy tomato plants its highest concentrations appear in the exact position where the leaf grows. And the mRNA accumulates before a hint of a leaf appears. "It predicts where the leaf will form," says Kuhlemeier. "That makes it very plausible that what we showed by external application is really what's going on in vivo."

At the moment, however, no one knows exactly what the many molecules in cell walls are doing. Although Berger's experiments reveal what "message" walls are conveying, for example, he has not yet identified the molecular messenger. And although Pennell seems to have his hands on an intriguing molecule, its function is still unclear. "Much of the work is descriptive and correlative rather than showing a clear causative chain of events," says Roberts.

One problem, he notes, is that many of the wall molecules are carbohydrates, which are much more difficult to work with than proteins. As a result, he says, "it will take a lot of time, effort, and maybe new [methods] to nail down what the signaling molecules are. People are very excited, though. They've been gingered up to a state of expectation with all these molecules that are specific to particular walls. Then these results of Berger's come and make people think, 'Maybe it's really true. We better go after these molecules.'" After more than 100 years, cell walls' image has finally gotten a whole lot livelier.

–EVELYN STRAUSS

Evelyn Strauss is a science writer in San Francisco.