PLANT BIOLOGY

Plants, Like Animals, May Make Use of Peptide Signals

In any community, good communication is crucial for coordinating activities. But, like a housing development in which individual units are separated by high fences, plants seem to have an internal communications problem: Their constituent cells are encased within rigid walls. For many years, plant biologists assumed that those walls might prevent plant cells from using one of the most versatile types of signals—the peptides and proteins that regulate embryonic development, cell growth, and many other activities in animal cells. These signaling molecules, the prevailing wisdom held, were just too big to penetrate the plant cell wall.

But recent evidence suggests that plants may actually make wide use of peptide signaling, both in embryonic development and later in life when they rally their cells to defend against pathogens and insect pests. Just a few weeks ago, a team of researchers from the Netherlands and Germany reported work indicating that a peptide called ENOD40 is a key regulator of the formation of nitrogen-fixing root nodules in legumes (*Science*, 19 July, p. 370).

ENOD40 is only the second peptide messenger found in plants, but several labs have found additional evidence of peptide signaling: numerous plant genes coding for proteins that look like the peptide receptors carried by animal cells. The latest of these genes is described on page 1406 by Philip Becraft of Iowa State University in Ames and his colleagues. Identified because it causes abnormal seed and leaf formation in corn when it is mutated, the gene codes for a protein resembling the receptor for tumor necrosis factor (TNF), a protein that helps regulate inflammatory responses in mammals.

No one has yet conclusively put together a peptide signaling pathway in plants. Still, the recent discoveries are helping convince researchers that these pathways may be important. Says Clarence Ryan of Washington State University in Pullman, whose team in 1991 discovered the first plant peptide messenger, systemin, which helps fight off insect pests, "When you find one, it's unusual, but when you find another, it suggests there might be a lot more out there." And Joseph Ecker of the University of Pennsylvania, who studies signaling in plants by a very simple molecule, the gas ethylene, predicts that "a lot more peptide hormones will come out. The difficulty lies in finding them."

If peptide signaling in plants is as widespread as these early results indicate, the work would provide a new view of plant biology. Up to now, communication in plants has been thought to be only the work of small, readily diffusible molecules, such as ethylene or the plant hormone auxin, and materials exchanged through plasmodesmata, channels through the cell wall that connect adjacent cells. Animal-cell communication also makes use of small molecules, and in some cases, direct cell contacts called gap junctions, but for plants these were supposed to be enough, Becraft says: "I don't think that people really thought ... that there would be any need for protein signaling [in plants]. But the more we learn about plants the more things they have in common with animals."

PHOTOS BY BECRAFT *ET AL.*

***Crinkly* clue.** A mutation causing crinkled corn leaves and a defective seed covering pointed to a possible peptide receptor.

Evidence that plant cells communicate with peptides has been slow in coming at least partly, Ryan says, because biologists lack convenient assays for purifying peptides in the very low concentrations likely to be present in plant tissues. He notes that isolating systemin was a formidable task, requiring nearly 28 kilograms of tomato leaves and assays on some 30,000 tomato plants to produce 1 microgram of the peptide. Indeed, in the more recent work, researchers didn't try to isolate the peptides or their receptors; instead they began with the genes and worked backward to their functions.

The ENOD40 peptide, for example, is the product of one of the genes turned on in legume roots early in the formation of nitrogen-fixing nodules (hence the name "*ENOD*," for early nodulation gene). The gene looked interesting, says Henk Franssen of the Agricultural University in Wageningen, Netherlands, a member of the team that pinned down the function of its peptide, because it becomes active before the cells that form the nodule start dividing. That suggested that it might somehow be involved in initiating the cell divisions.

In the 19 July *Science* paper, Franssen, Ton Bisseling, and their colleagues in Wageningen, in collaboration with Karin van de Sande, Katharina Pawlowski, and their colleagues at the Max Planck Institute for Plant Development Research in Cologne, Germany, confirmed that suspicion. When the researchers transferred *ENOD40* into tobacco plants, they found that the resulting plants grew one or two extra shoots in addition to the main shoot. That suggested that the gene somehow releases cells from the growth inhibition that prevents such extra shoot formation—and subsequent work with cultured tobacco cells confirmed that assumption. The researchers found that while high concentrations of auxin inhibit the growth of normal plant cells, they had no effect on cells carrying a transferred *ENOD40* gene. "It turned out that this gene confers tolerance to high auxin concentrations," Franssen says. Its activation in response to infection by nitrogen-fixing bacteria might then allow the nodule-forming cells to begin their divisions.

Researchers had thought that the RNA made by *ENOD40* was the active product, because the gene's sole potential protein-coding region, or "open reading frame" (ORF), is very short—long enough to make a peptide of only 12 or 13 amino acids. Normally in higher organisms, such short peptides are made as part of larger precursors that have to be cut to release the active segments. But Franssen and his colleagues found that the portion of the gene containing the small ORF was sufficient to confer the auxin tolerance, as was the totally synthetic peptide. "This strongly suggests that the peptide is indeed the active part of the gene," Franssen concludes. And because tobacco turned out to have its own version of *ENOD40*, the peptide may be a signal in nonlegumes, too.

Genes also provided the starting point for tracking down the putative receptors for peptide signals. Becraft, who works with Donald McCarty of the University of Florida, Gainesville, and Philip Stinard of the U.S. Department of Agriculture's Agricultural Research Service in Urbana, Illinois, has been studying a mutation in a corn gene called *crinkly4* (*cr4*)—so named because an abnormal outer cell layer, or epidermis, causes the leaves of affected plants to crinkle. In addition, the outer seed layer, which is called the aleurone, fails to develop properly.

Normally, the aleurone begins forming

on the side of the seed that contains the embryo and then spreads to cover the whole thing, but in the mutants aleurone formation stops after covering only about half the seed. "This suggested that we were ... interrupting some signaling event involved in propagating the aleurone," says McCarty.

The current work, in which the team cloned *cr4*, supports that idea. The gene's sequence reveals that its protein has the overall structure characteristic of the receptor kinases seen in animal cells. Such receptors—which are so called because their inner segment is a kinase, an enzyme that can add phosphates to other proteins—often transmit developmental and other types of regulatory signals.

JUNE NASRALLAH/CORNELL UNIVERSITY

Ovule bound. Pollen tubes are growing in a crucifer pistil, but peptide signaling may prevent this if self-pollination occurs.

The portion of the CR4 kinase that probably protrudes from the cell and binds to some signaling molecule proved to be particularly interesting, Becraft says: It looks like the corresponding part of the receptor that, on animal cells, binds tumor necrosis factor. And because TNF is a polypeptide, containing some 157 amino acids, that similarity suggests that CR4 also binds a peptide. Becraft cautions, however, that until that peptide is found it's just "a hint at a possible peptide signal."

Other systems are providing similar hints, as several additional receptor kinase candidates have been identified in plants. Two of these so far have also been linked to plant development. One is encoded by the *Erecta* gene of *Arabidopsis thaliana*, which helps determine the shape of the plant's leaves and flower clusters and has been cloned by Keiko Torii of the University of Tokyo, Norihiro Mitsukawa of the Mitsui Plant Biotechnology Research Institute in Tsukuba, Japan, and their colleagues. (The results appear in the April issue of *Plant Cell.*) And in as-yet-unpublished work Steve Clark of the University of Michigan, Ann Arbor, has traced another developmental mutation in *Arabidopsis* (*Clavata 1*)—this one resulting in an abnormally thick stem and extra flower parts—to a gene encoding a protein that appears to be receptor kinase.

Still other plant receptor–like kinases have nondevelopmental functions. For example, the one encoded by a gene called *Xa21*, which was cloned last year by a team led by Pamela Ronald of the University of California, Davis, confers resistance to an important rice pathogen, the bacterium *Xanthomonas oryzae* pv. *oryzae* race 6 (*Science*, 15 December 1995, p. 1804). And a kinase encoded by a gene in the self-incompatibility (S) locus of plants in the crucifer family, which includes broccoli, cauliflower, and *Arabidopsis*, is needed to prevent the plants from self-pollinating. Work by June Nasrallah's team at Cornell University indicates that the S locus receptor kinase (SRK) detects a signal—presumably a peptide made by pollen—that tells the cells on the pistil, the female reproductive organ, to block pollen grains from the same plant from adhering and forming pollen tubes.

While all the other investigators who study possible peptide signaling in plants have identified only signaling peptides or receptors, never a matched pair, Nasrallah says she has a strong candidate for the SRK ligand. This is a protein, also encoded by a gene within the S locus, that is expressed only in the anther, the male organ that produces the pollen. "It has the right smell for a ligand," Nasrallah says, "but we're still in the process of determining whether it is."

Just how such a peptide signal might cross the cell-wall barrier is still a mystery, although molecular "ferries" may aid in the transport. The Nasrallah team has found that still a third gene in the crucifer S locus encodes a protein that accumulates in the cell wall and has a sequence very similar to that of the extracellular portion of SRK. She proposes that this protein may bind whatever ligand a pollen grain uses to signal its presence and shuttle it to SRK itself.

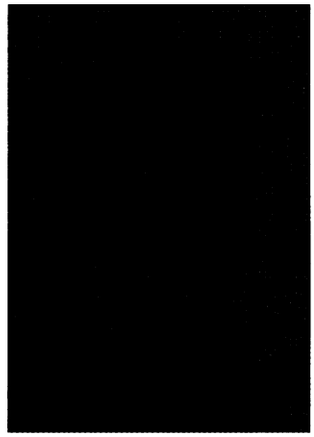
Further work will be needed to confirm that picture, and also to pin down the missing ligands for the other receptor kinases. But already it seems that investigators are on the verge of listening in on a new and unexpected language of plant-cell communication.

–Jean Marx

PLANT EVOLUTION

Probing Flowers' Genetic Past

Mother Nature did some major redecorating early in the Cretaceous period, about 120 million years ago. That's when the first angiosperms—plants whose reproductive structures are showcased inside flowers—appear in the fossil record. But while botanists have long believed that angiosperms evolved from simpler nonflowering plants, their understanding of the gene changes that gave modern angiosperms the ability to make petals and other floral organs has remained rudimentary. Now studies of a unique mutation in the weed *Arabidopsis thaliana* have uncovered what may be a living record of plants' leap from drabness to floral glory.



A. RAYU OF R MEDICAL PHOTOGRAPHY

Late bloomer. Plants without *SIN1 (right)* grow extra leaves and stems before flowering. Plant at left is normal.

Plant evolutionists believe this transition came about when some of the genes controlling the development of female ovules, or eggs, began moonlighting as flower-building genes, helping transform the seed-bearing leaves of earlier plants into leaflike floral organs such as sepals and petals. In that case, some of the genes that give form to ovules in modern angiosperms should retain this double duty, helping to switch on flower growth at the appropriate time spurring the growth of specific flower parts. But botanists hadn't found any examples of the hypothetical timekeeping genes—until the current work.

In the September issue of *Development*, a research team lead by Animesh Ray of the University of Rochester in New York reports that an *Arabidopsis* gene called *Short Integument* (*SIN1*) plays a key role in regulating both the development of ovules and the time of flowering. "We know so little about angiosperm evolution," says Detlef Weigel, a developmental geneticist at the Salk Institute in La Jolla, California. "If *SIN1* does turn out to be the first gene to act on both [ovules and flowering time], that would be very nice."

One part of *SIN1*'s activity—its role in ovule development—has been known since 1992, when breeding studies by Harvard University plant geneticist Robert Pruitt showed that plants in which both copies of the gene are mutated have poorly formed ovules, rendering them sterile. But Ray, a molecular biologist, suspected that the gene might be doing much more. He observed that these mutant specimens also have many more leaves, are taller, and take much longer to flower than normal plants. That indicated that the plants' meristems, regions of rapidly dividing cells at the tips of growing shoots that give rise to all of the plant's specialized