

Profile of Daniel J. Cosgrove

The proper development, growth, and functioning of plants rely on the relaxation and expansion of plant cell walls and the osmotic actions associated with such cell structure changes. Proteins known as expansins play a key role in cell wall expansion in certain plants. For example, to reach its target, microscopic maize pollen must travel over 6 inches through the narrow confines of corn silk, and expansins grease the pollen's pathways, helping it squeeze between tightly packed cell walls. Plant biochemist Daniel J. Cosgrove, elected to the National Academy of Sciences in 2005, led the team that first isolated expansins from cucumber seedlings in 1992, during his search for the protein that causes plant cell wall relaxation (1).

Since that time, Cosgrove has moved from cucumber seedlings to maize pollen and from enzyme assays to crystallography, all in an effort to determine how expansins work their magic. In his Inaugural Article in this issue of PNAS, Cosgrove, currently the Eberly Family Chair in Biology at Pennsylvania State University (Penn State, State College, PA), presents the crystal structure of EXPB1, an expansin from maize pollen, and its surprisingly long binding groove that spans two domains of the 27-kDa protein (2).

Precocious Torturing of Plants

Born in 1952 in Chicopee, MA, Cosgrove spent several years of his early childhood in Lima, Peru, where his father served as a military attaché. Plants interested Cosgrove from an early age. "I was one of those kids who tortured his mother's plants," he recalls. When Cosgrove was about 12 years old, he used his mother's prized begonias and African violets to separate plant pigments via paper chromatography. His mother encouraged his interests and gave him the microscope that she had used as a child. In high school, Cosgrove entered science fairs, read the journal *Science*, and even had a subscription to the *Arabidopsis Information Newsletter*—decades before *Arabidopsis* became an everyday word in the plant biology community. "I was one of those science nerds, I'm afraid," he says.

In college, Cosgrove majored in botany at the University of Massachusetts (Amherst, MA). He wanted to spend his junior year abroad but could not afford the costs, so he participated in an exchange program and studied at the University of Oregon (Eugene, OR). At Oregon, Cosgrove studied floral bud



Daniel J. Cosgrove

culture with Sandy Tepfer. "[Tepfer's] role was to give me the keys to the lab and set me free," says Cosgrove, who relished the independence. Returning to the University of Massachusetts for his senior year, Cosgrove undertook an honors project with William F. Thompson of North Carolina State University (Raleigh, NC), studying floral induction of epidermal strips of tobacco. Cosgrove graduated in 1974 with a bachelor's degree in botany.

For his doctoral studies, Cosgrove found himself drawn to multiple areas. "I wanted to combine physics with plant development," he says. "I applied to three places and picked the one that seemed to have the most faculty doing things that interested me." Cosgrove entered Stanford University (Stanford, CA) in 1974 and chose Paul Green as his graduate advisor. Green was one of the most influential people in Cosgrove's scientific development, with a unique way of thinking about science from a physical point of view. Cosgrove valued Green's experimental creativity and innovativeness. "Paul had a great way of looking at the big picture and tackling big problems," says Cosgrove.

For his thesis, Cosgrove decided to develop expertise unique to his department. "I wanted to pick a problem that was at the interface of two of the professors," he says. Inspired by both Green and Winslow Briggs, who studied photobiology, Cosgrove investigated a puzzling observation in the literature on photobiology. In the dark, seedlings grow stringy as their stems elongate, but within a few seconds of exposure to blue light, this elongation stops. "Everyone thinks of plants as doing things slowly," says Cosgrove, "but this is a case of plants responding in a few seconds." At the time, methods for record-

ing plant growth were rather primitive, what Cosgrove describes as "based on 1940's technology." He spent a year in the electrical engineering department at Stanford to learn about microcomputers and how to interface them with sensors and controllers for his research. Cosgrove then rebuilt all of the laboratory's equipment so that plant growth could be recorded by computer and the lights could be turned on and off automatically on a precise schedule, feats that he wrote up for publication in the microcomputer journal *Byte* (3).

An important step in the plant maturation process is the expansion of cells. Individual cells can increase their volume over 1,000-fold as water-filled vacuoles expand within the cell. Trees owe their great height to this process, which is made possible by yielding, or relaxation, of the normally rigid cell walls. When it came time to investigate how the plant cells could respond so quickly to light, Cosgrove encountered a surprise. "We suspected that there was a rapid membrane leakage, causing a drop in turgor pressure," he says, with reduced turgor pressure slowing wall expansion. But instead, he observed little change in turgor pressure (4). "All of the change was due to subtle stiffening of the cell walls," he says. This stiffening caused the cell walls to resist pressure and cease expansion. For his thesis, he detailed the mechanism, analyzed the kinetics and dynamics of cell wall elongation, and investigated which photoreceptors perceived blue light (4–7).

Poking, Prodding, and Stretching

Cosgrove earned his Ph.D. in biological sciences in 1980 and joined the laboratory of Robert Cleland at the University of Washington (Seattle, WA) for his first postdoctoral position. After a few months, however, Cosgrove was offered the opportunity to study with Ernst Steudle at the Kernforschungsanlage (Nuclear Research Center) in Juelich, Germany. Cosgrove accepted and moved to Germany, where he spent a year learning a new method for studying plant cells. The Steudle group had just developed a pressure microprobe for "poking plant cells and measuring their turgor pressure," says Cosgrove. In 1981, he returned to Cleland's laboratory in Seattle and used the new method to analyze the biophysical mechanism of

This is a Profile of a recently elected member of the National Academy of Sciences to accompany the member's Inaugural Article on page 14664.

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auxin-induced cell growth (8). The approach involved concomitant measurements of the biophysical parameters determining water flow and cell wall yielding at the cellular level.

In 1982, Cosgrove applied for and received an assistant professorship in biology at Penn State. He brought his expertise in plant-growth measurement and the pressure microprobe and, over the next 6 years, developed novel theory and techniques for measuring cell wall relaxation in living cells. Initially Cosgrove used the pressure microprobe to measure the pressure inside the plant cell. By manipulating the cell so that it cannot absorb water, he could study the dynamics of relaxation (9). The method disturbs the plant, however, because it requires poking a needle into the cell. "You have to have very good manual dexterity to do that," says Cosgrove. He therefore developed a noninvasive technique for measuring cell wall relaxation in living plants by sealing the growing portion of plants in a pressure chamber with a device for measuring growth. The water source and roots remain outside of the chamber. When pressure is applied just to the point where cells cannot take up water, the growth, or elongation, stops. As the cell walls relax, preparing for more growth, the cells' ability to suck up water greatly increases. As a result, the pressure applied must be increased. The changing measure of pressure needed to prevent water absorption provides a time course for cell wall relaxation (10, 11).

At the end of the 1980s, Cosgrove found himself with two potentially fruitful research paths. "At that point, there was a dichotomy," he says, with one path aimed at studying membrane properties regulating plant-growth responses, using the patch-clamp technique he learned while on a sabbatical at the University of Göttingen (Göttingen, Germany) from 1989 to 1990. The second avenue derived from a McKnight Foundation award and National Science Foundation Presidential Young Investigator funding that gave Cosgrove the freedom to try risky experiments. With the funding, he investigated "the mythical wall-loosening enzymes," as he describes them, which were hypothetical proteins purportedly enabling cell walls to relax. "People had talked about them for years," he says, "but nobody could point to specific examples."

With long-time technician Daniel Durachko, Cosgrove searched for extracts that could restore extensibility to cell walls *in vitro*. He and Durachko used cucumber seedlings because the cell walls of these seedlings showed particularly stable wall-extension processes,

something that Cosgrove had judged based on physiological properties (12). He recalls asking, "Let's look for a system where the wall-loosening process is highly active and stable." In other words, he wanted to study plants with fast and prolonged cell wall extension. "We were lucky and wise at the same time," he says. A rotation student visiting from another laboratory, Simon McQueen-Mason, worked out a simple method to extract proteins and reconstitute walls with extension activity. Entering what Cosgrove calls the protein phase, the group purified, isolated, and characterized the new protein, later named expansin (1, 13). Cosgrove, who was still doing benchwork at the time, expected that the wall extraction and reconstitution assay was going to be tricky, so he experimented with a few difficult techniques. McQueen-Mason, however, extracted the protein activity

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with a relatively straightforward procedure, leaving Cosgrove with a lesson: "Try the easy things first," he says. Purifying the protein was difficult, however, as they "were not abundant and were very sticky," says Cosgrove.

The time came to characterize expansin. Most researchers expected that the protein must be an enzyme that somehow cuts the cell wall polymers to induce relaxation. In terms of enzyme assays, however, "everything we tried was negative," says Cosgrove. He explains that the protein did not hydrolyze any of the cell wall polysaccharides (14). However, it did seem to act as a catalyst, causing the cell wall polymers to slip and slide as if lubricated. In the molecular phase, where Cosgrove's group cloned the first expansins, obtaining the sequence "confirmed for us that we weren't incompetent biochemists," he says. When his group ran the sequence through the Basic Local Alignment Search Tool (BLAST) database, all that showed up were a few expressed sequence tags (ESTs) of unknown function. This result meant that expansin was indeed a new protein (15), but unfortunately, the finding did not give Cosgrove any insight into what activity it might have. Nevertheless, he was happy to face a large unknown protein to study rather than be frustrated by the lack of information revealed by the sequence.

Allergen Comes in Handy

Cosgrove began to look for other methods to investigate how expansin might help cell walls expand. One hypothesis was that the protein aids in the dissociation of polymers, and assays measuring this activity were positive (16). In his PNAS Inaugural Article, Cosgrove worked with crystallographer Neela Yennawar and biochemist Lian-Chao Li to resolve the crystal structure of one expansin, EXPB1 (2). EXPB1 is in the EXPB family, the group of expansins that loosens the cell walls of the grass family with greatest effectiveness. Cosgrove used grass pollen from maize to isolate expansins because EXPB1 is easy to work with, although difficult to express in heterologous systems. "It is a well behaved protein in solution," he says. In addition, maize pollen is abundantly available: every summer Cosgrove and his students go out into the fields of Penn State's experimental farm and bag maize tassels from the maize demonstration plots. Tassels from a few hundred plants can yield about 1 kg of pollen after a few days of collection, which is enough for about a year's work.

In his Inaugural Article, Cosgrove and his group characterized EXPB1's structure to 2.7-Å resolution, helping to elucidate which parts of the protein bind to the cell wall. The protein's crystal structure revealed an unusual, highly conserved binding surface. The binding surface was unusual because it is very long and crosses two domains of the protein. It binds the cell wall polysaccharide xylan. Cosgrove speculates that upon binding, EXPB1 facilitates noncovalent rearrangement of the support networks in the cell wall, causing them to relax and then move as water enters the cell because of the relaxation.

Since the discovery of the first expansin in 1992, genomic studies have revealed numerous examples of expansin genes, and the field now recognizes two large families of expansins based on activity and sequence differences. For Cosgrove, finding so many expansins, as revealed by genomic sequencing, was a surprise. "Every week there was a new expansin gene discovered in *Arabidopsis*," he recalls, relating that *Arabidopsis* has 36 expansin genes and rice has nearly 60. A current realm of investigation for Cosgrove, with postdoctoral researcher Javier Sampedro and evolutionary biologist Claude de Pamphilis, focuses on why so many expansins exist and on determining their roles (17). At this point, Cosgrove explains, different cell types appear to have different expansins (18, 19).

In grass pollen, expansin is proposed to loosen the cell walls of the maternal plant. For maize pollen to reach its target, it must travel 6–9 inches through a narrow tube, corn silk.

These corn silks are actually passage-ways for the pollen to the ovary. With an expansin crystal structure in hand, Cosgrove can now test these hypothe-

ses by mutagenesis for protein structure and function analyses. By modifying certain residues, he plans to determine how the protein binds to the wall and causes wall relaxation.

A second avenue opened by this research is directly related to pollen's effect on human immune systems. Expansin from maize pollen is a major

allergen, and studying which surfaces are the most immunogenic could lead to the development of a peptide-based vaccine for grass-pollen allergies. Luckily for Cosgrove, he is not allergic to his research subject.

Tinsley H. Davis,
Freelance Science Writer

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