Extra DNA causes Mendel's peas to pucker

Genetic research has added a new wrinkle on an old pea. More than a century after Gregor Mendel crossed his round and wrinkled peas, British geneticists have cloned the enzyme-encoding gene that ultimately determines the shapes so painstakingly recorded by the Austrian monk. In pinpointing the gene's chromosomal location, or locus, they have discovered that the wrinkling trait stems from an extra piece of DNA, which prevents the gene from directing proper starch synthesis.

The new work, conducted at the John Innes Institute in Norwich, confirms that the chromosomal locus *r* houses the gene coding for the production of starch-branching enzyme 1 (SBE1). This enzyme, found in all plants, converts amylose, a simple starch of linear construction, into a "branched" starch called amylopectin.

Scientists have known for some time that the ratio of these starches in peas and other plants influences other elements of their composition, says Alison M. Smith, who coauthored the report in the Jan. 12 Cell. Round seeds (*RR* or *Rr*) contain a much higher ratio of amylopectin to amylose than do wrinkled seeds (*rr*), suggesting the enzyme doesn't function properly in wrinkled peas. When a plant's starch conversion is impaired, sucrose and water build up in the young seeds. Maturing seeds lose much of this water, and the shrinkage leaves them wrinkled.

When the researchers cloned the gene, they found it was always larger in wrinkled seeds than in round seeds. This, says Smith, indicates that wrinkled seeds carry a gene with an extra insertion of DNA that leaves the plants without an efficient means of starch conversion. The insertion occurs in the part of the gene that codes for the SBE1, thus garbling the DNA message for SBE1 production.

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The expression "as alike as two peas in a pod" doesn't always hold water. Geneticists have now traced the wrinkles to a DNA insertion that leads to water loss.

Now that scientists understand how the *r* locus influences starch production, they hope to modify the chemical structure of plant starches to improve the quality of prepackaged vegetables. Frozen-food packagers often add starch to maintain a vegetable's structure, Smith notes, but the food tends to lose water as it thaws, leaving consumers with a drippy mess. *– C. Decker*

Droopy plants drop hints of enzyme's role

Even at its death knell, after the last traces of chlorophyll and other vital chemicals have vanished, a withering plant keeps producing one compound – a red-pigmented enzyme called peroxidase. Since the turn of the century, scientists have studied this intriguing and easily detectable enzyme, which assists plants in wound healing, oxidation and cell elongation. But because a plant may contain up to 35 different types of peroxidase, each reacting with several plant compounds, researchers have been frustrated in their attempts to pin down the enzyme's fundamental role in plant development.

Now, with a bit of genetic sleight-of-hand, investigators have uncovered hints that the enzyme may play a part in plant aging and fertility.

In 1987, molecular biologist L. Mark Lagrimini of Ohio State University in Columbus cloned the gene that codes for one type of peroxidase found in tobacco plants. He and his colleagues at Ohio State and the University of Guelph in Ontario went on to combine a peroxidase gene with fragments of cauliflower mosaic virus, which stimulates overproduction of peroxidase. They inserted this gene-virus combination into two species of tobacco plants, which began producing up to 10 times the normal amount of peroxidase. Genetically altered plants containing at least twice the usual enzyme levels were outwardly identical to their normal relatives until flower buds appeared. Then the plants began to droop in sunlight, at first recovering during the night, but after several weeks wilting permanently.

Wilting and the stunted growth associated with it were more severe in plants with larger amounts of peroxidase, Lagrimini and his collaborators report in the January Plant Cell. None of the altered plants died prematurely, and all the leaves remained green, unlike those that droop from drought. But the wilting, combined with peroxidase's well-known ability to thrive in dying plants, suggests a link between peroxidase and plant aging, Lagrimini says.

More recent observations, he told Science News, indicate peroxidase action may concentrate in the roots. Genetically altered tobacco stems grafted onto normal roots no longer drooped, although normal stems grafted onto roots containing the altered gene still wilted.

But these observations fill in only one piece of the peroxidase puzzle. Other research by the same group suggests another role for the enzyme: regulating fertility. Tobacco plants altered to produce unusually low peroxidase levels grew normally but yielded about one-hundredth as much seed as unaltered controls. Lagrimini, who is now extending his peroxidase pursuit to tomato plants and sweet gum trees, says the enzyme's primary function remains unclear.

"Even after [publication of] three to four thousand papers on plant peroxidase, we're still ignorant about what it does," says Fred B. Abeles, a plant physiologist at the USDA's Appalachian Fruit Research Station in Kearneysville, W. Va. "But [Lagrimini's] work is a new strategy" for narrowing the possibilities, he says. *– R. Cowen*

If not cold fusion, try fracto-fusion?

Whatever its finale, the cold fusion story that began last March will color the history of science. Most of the drama has subsided, but a few researchers have carried on the investigations, continuing to observe phenomena they cannot explain (SN: 12/23&30/89, p.406). Several of these scientists are now exploring a theoretical concept dubbed fracto-fusion to explain at least some of the mystery observations.

Fracto-fusion describes what might happen when microcracks develop in metals containing deuterium or tritium. In this scenario, electrical charges along the cracks speed up deuterium nuclei within the voids, increasing the chances of the nuclei fusing together.

The latest indication that fracto-fusion may occur in some deuterium-loaded solids comes from scientists at Washington State University in Pullman and the Los Alamos (N.M.) National Laboratory. "We suggest that crack growth results in charge separation on the newly formed crack surfaces, which act like a miniature 'linear accelerator,'" the team writes in the January Journal of Materials Research.

Although the researchers report no direct evidence of fusion in their samples, University of Washington physicist J.

News of the week continued on p. 90

the paper was written, ETH's Christopher Y. Switzer has shown that installing xanthosine in the template directs the polymerases to faithfully incorporate kappa into the appropriate spots on product strands, Benner told Science News.

"The real goal here is to have an RNA molecule that's able to replicate itself," says Benner. That would be a first step toward such visionary goals as creating lifelike chemical systems in a test tube. Finding self-making molecules, which would catalyze their own copying without the help of specialized enzymes like polymerases, is the golden ring for a small community of origin-of-life researchers.

There may be some bugs with the new letters, cautions Leslie Orgel of the Salk Institute for Biological Studies in San Diego, in a commentary accompanying the research report. For one, he notes, the xanthosine base carries an extra bit of negative charge that could prevent the forming of double helices or other molecular arrangements important for biological activity.

Says Benner: "With one funny base in a backbone of normal bases, we don't see any problem." But he concedes that no one knows how longer chains with more funny bases will behave. "We have taken a step in a rather long series of steps that is necessary for getting something that is self-replicating."

Orgel questions the need to add letters to the genetic alphabet for the goal of building new catalytic RNA molecules. "Maybe four are enough [to work with]," he suggests. In addition, he says, chemists are getting so good at chemically synthesizing RNA that the use of harder-to-handle enzymes could soon become more trouble than it's worth.

But Benner suspects otherwise. By adding bases with different chemical features to the pool of ingredients for making RNA molecules, he says, researchers will have more to work with in designing catalysts for performing specific chemical transformations. Even with the Berkeley group's advances in making proteins with synthetic amino acids, deliberate engineering of catalytic RNA to perform specific molecular jobs may still emerge as an easier task, Benner asserts. Protein designers still do not understand how linear sequences of amino acids fold into the three-dimensional arrangements they must assume to function properly. "In RNA, we actually understand how this works," Benner says. Combining this understanding of structure with a larger variety of nucleotide building blocks will better enable researchers to understand and control RNA molecular structure and, therefore, to design RNA-based catalysts, he predicts.

Manipulating bases isn't the only way in which scientists have tweaked the structures of RNA and DNA. "People have tried to change everything," says Joyce of the Scripps Clinic. For instance, he notes, another ETH scientist has replaced the nucleotides' five-membered ribose sugar component with the six-member glucose. "Basically any sugar has been tried," says Joyce.

Stanley Miller of the University of California, San Diego, experiments with bases that have more open structures, lacking the rings characteristic of the bases in normal DNA and RNA. Others have tried changing the type of chemical bond that links the nucleotides. Benner, for instance, is building DNA- and RNA-style structures with sulfur-containing chemical bridges instead of the normal phosphorus-oxygen-phosphorus link. Orgel has tried using an amide linkage, the same type of chemical bond that links amino acids into proteins.

Driving many of these experiments is the scientific enigma of how life began. After scrutinizing the molecular biology of living organisms, many origin-of-life researchers have come to suspect that a so-called RNA world preceded the DNA-based life forms that have presided for more than 3 billion years (SN: 10/7/89,

Continued on p.94

News of the week continued from p.87

Thomas Dickinson says the experiments show unambiguously that a crucial condition for fracto-fusion – charge separation across microcracks in a solid – does occur. In addition, he notes, calculations reported by Japanese researchers suggest that a growing crack, even in an electrically conductive material, can outpace separated charges attempting to neutralize each other by speeding around the increasing perimeter of a crack.

In principle, the oppositely charged sides of a microcrack could create a strong electric field that would greatly accelerate positively charged particles, such as deuterium nuclei, in the gap. This would increase the probability of deuterium-deuterium fusion reactions, Dickinson's group suggests.

As early as 1986, Soviet scientists reported observing neutron emission when they violently crushed lithium deuteride in the presence of an ice made of heavy (deuterium-containing) water. In a letter published in the Nov. 16 Nature, they describe more recent experiments involving titanium chips and several deuterium-containing materials, including frozen heavy water and lithium deuteride. While vigorously milling the titanium and the deuterium sources, and for a few minutes after milling had ceased, the Soviet researchers detected neutrons emerging at up to seven times the levels measured for titanium chips or deuterium sources milled separately. They suggest that fracturing may play a role.

Dickinson's group placed successive slabs of hydrogen-loaded titanium, deuterium-loaded titanium and unloaded titanium in an apparatus that bends materials until they crack and finally break. During and shortly after fracture, the researchers recorded each specimen's emissions of positively charged particles and photons of various wavelengths. Though they had expected the two gas-loaded materials to yield similar results, they found that the deuterium-loaded specimens produced far stronger signals. "The differences in the fracto-emission between these two types of specimens were astounding," they report.

The identities and energies of the emitted particles and radiation remain unknown, and Dickinson wants to conduct follow-up experiments to answer those questions. He notes, however, that funding for cold fusion research has become scarce, especially since last November, when the Energy Department issued a report essentially writing off cold fusion claims as unfounded (SN: 7/28/89, p.78).

Like many physical scientists, Harold Furth, head of the Plasma Physics Laboratory at Princeton (N.J.) University, trains a critical eye on any proposed mechanism for cold fusion. Although Furth himself casually mentioned the possibility of fracto-fusion last May at a meeting of the American Physical Society, he says he now suspects the entire cold fusion drama sprang from misinterpretations of data and experimental errors. "I wouldn't rule out that this [fracto-fusion] is zilch," he told Science News.

In the midst of such skepticism, physicist Steven E. Jones of Brigham Young University in Provo, Utah, maintains that "fracto-fusion probably is the leading model right now." Jones headed one of the two independent research teams that initially announced the possibility of achieving cold fusion by using electrochemical processes to jam deuterium into metal rods (SN: 4/8/89, p.212). Although he admits that the evidence for fracto-fusion remains inconclusive, he and collaborators at Los Alamos are assembling a sophisticated apparatus that may help settle the issue. By injecting tritium into gas-loaded titanium samples and using hydraulic presses to squeeze and fracture the specimens, the team hopes to increase the probability of fusion by several orders of magnitude, Jones told Science News. If any fusion-produced neutrons do emerge, the apparatus should allow researchers to detect them and measure their energy, he says.

– I. Amato