

Tracking Corn Gene Regulation

Learning more about basic gene mechanisms

By Susan McGinley

Basic research delves into the fundamental processes that govern the way things work. When we know how something operates, we can use that knowledge to solve practical problems. Molecular geneticists in the College of Agriculture and Life Sciences are currently studying genes they've already identified in corn to learn more about how those genes are regulated and expressed. Their results, funded by a \$10.5 million grant from the NSF in 1999, will assist scientists around the world in breeding and improving crop plants.

"It's a tool-building project," says Vicki Chandler, a molecular geneticist in the Department of Plant Sciences. "We're getting the corn genes into the test tube so we can manipulate them in a variety of ways to get a better corn plant." This work builds on continuing results from a structural genetics project Chandler began last year to find all 50,000 genes in corn. About 10,000 genes have been identified so far.

"I study gene regulation, how appropriate genes are turned on in the leaves, the flowers, the roots, how genes are turned on in response to environmental signals," Chandler says.

This mechanism works at a very complex level. Chandler and Richard Jorgensen, also a molecular geneticist in plant sciences, plan to identify and functionally analyze the genes in both corn and Arabidopsis, a plant in the cabbage family, that contribute to chromatin level control of gene expression. Arabidopsis has a very small genome, making it simpler to sequence the entire genome, and a fast growth cycle, which makes it convenient to perform genetic experiments.

"We're using Arabidopsis as a starting point and applying it to understand our work with maize," Jorgensen says. Seventy percent of the Arabidopsis genome is currently known, compared to only 20% of corn genes.

Inside maize and other crop plants there are 2.5 billion base pairs of DNA sequences. What most people don't know is that only a small percentage of DNA is actually genes, probably much less than 10%, according to Jorgensen and Chandler. The corn genome project (see sidebar) takes advantage of the natural biology of transposable ele-

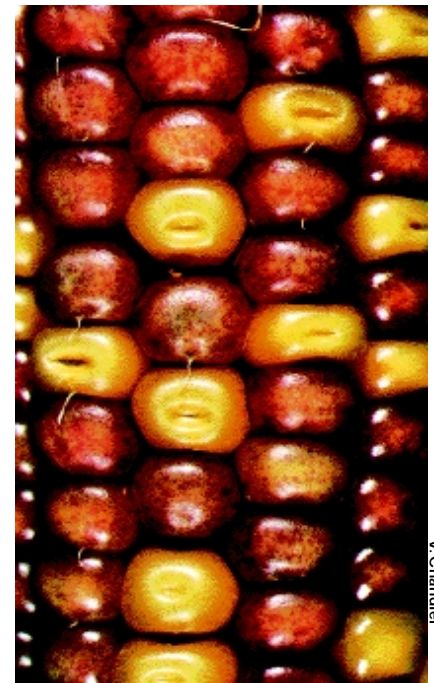
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ments to find all the genes in corn that are dispersed in the DNA sequence.

"We know that DNA is part of a higher order structure called chromatin," Chandler says. Chromatin consists of the DNA which encodes the genes, complexed with various proteins. "The DNA strands are not just laid out, they are wrapped around proteins, and that complex is coiled up inside the nucleus. It's a very complex machinery that interacts with the chromatin and activates genes at the right time."

The scientists use computer algorithms to identify the sequences of genes in the Arabidopsis and maize databases that interest them, and target them for mutations, which they induce in both Arabidopsis and maize, one gene at a time. Their method takes advantage of a natural gene silencing mechanism in plants that probably evolved as virus protection in plants. The genes they select for study may only be 1% of the full set of genes, but they are the ones that control the expression of the other 99%. In the laboratory, the researchers look at the way the mutations alter global gene expression. This is functional genomics.

Assisting Chandler and Jorgensen with the computer searches for genes, and with constructing databases to provide information to other scientists, are David Mount, a professor in the UA Department of Molecular and Cellular Biology; David Selinger, research scientist, Ritu Pandey, and Andreas Muller, post-doctoral research associates, both from Plant Sciences; and visiting scientist Ross Atkinson, from HortResearch in Auckland, New Zealand. Carolyn Napoli, a research scientist in the Department of Plant Sciences, along with three technicians, two undergraduates and two post-doctoral associates, is cloning the genes and making the mutant constructs. In addition, scientists from five



V.Chandler

other universities are collaborating on analyzing mutant areas of gene expression.

"We can effectively clone the gene using polymerase chain reaction (PCR) to construct an inverted repeat sequence of genes," Chandler says. "Then we use a relatively new technology on the clone sequences to target the gene in the plant that we want to mutate." They've characterized about 30 Arabidopsis genes so far, and plan to study 130 more Arabidopsis genes and 100 maize genes within the five-year time frame of the study.

"By doing that analysis we're determining the function of the normal gene by looking at what went wrong in the mutant, at what biological property is missing in the mutation," Jorgensen says.

As they determine the function of each targeted gene, this information is entered into a computer database and becomes accessible to plant breeders, plant genetic engineers and researchers in basic biology around the world who want to know more about how plants work. They can look up gene functions and select only the genes they need to perform certain operations in plants.

"We are really approaching the stage in biology where we can learn what we need to learn to solve our problems," Chandler says. ❖

Plant Genome Project for Reducing Salt and Drought Stress

A consortium of researchers led by Hans Bohnert, a UA biochemist, David Galbraith, professor, and Jian-Kang Zhu, associate professor, both from the Department of Plant Sciences, is attempting to map ALL the genes that enable plants to withstand drought and salinity. The NSF awarded the team an \$8.2 million, four-year grant in 1998 to address this goal.

This grant includes collaborative arrangements with two other universities, and builds on earlier work from the research team both in terms of using the latest techniques available to analyze genes in water-stressed and salt-stressed plants, and in developing novel techniques to chart changes in gene activities as a function of water and salt stress.

The research team employs plants for this work, including crops such as maize and rice, as well as the model species *Arabidopsis thaliana* and *Mesembryanthemum crystallinum* (ice plant). The team is also examining non-plant organisms, such



as bacteria, fungi, and yeast, to find out whether mechanisms of resistance to water and salt-stress in these organisms might also function in plants.

Thus far, the team has identified a large number of different genes whose activities are altered under water and salt stress, and is proceeding both to identify the functions of these genes, and to find out the effects of altering the normal activities of these genes. The team has also been instrumental in developing large populations of plants carrying mutations in these different genes, as well as a series of novel methods for analyzing coordinate regulation of these genes.

This work has implications for reducing crop losses resulting either from a lack of water or from long-term irrigation with water resulting in salinized soil. The project may help plant breeders who seek to engineer crops for both salt and drought tolerance. These problems affect countries in both the industrialized and developing nations.

Corn Gene Identification Project

In 1998 plant scientists from the UA and five other universities won a 5-year, \$12 million grant from the NSF to discover all 50,000 genes in corn, the nation's most important economic crop. The scientists are using a new method for discovering and sequencing genes in corn, and are sharing project findings and material resources with public and private researchers working to develop improved traits in corn and many other agronomically important grasses, such as wheat, barley, rice and oats. The corn genomics project is expected to lead to greater fundamental genetic understanding of cereals that worldwide contribute roughly 70 percent of the calories in the human diet.

"To this day, we've probably identified a quarter of those genes," says Vicki Chandler, plant geneticist and lead



"A genome is all of the chromosomes, or the full set of heritable traits in an organism."

researcher on the project at the UA. "The project is already having major benefits for plant research around the world. Thousands of people are requesting these genes." Under the direction of UA plant scientist David Galbraith, scientists have been preparing microscope slides of the new-found gene sequences to study the way the genes are expressed under different environmental, physiological or developmental conditions. They are making their slides, their gene libraries and seed containing the mutated genes available to the scientific community.