Gene Might Aid Plant Regeneration

Researchers seeking to genetically engineer new plant strains often run into a major obstacle: how to grow whole plants from cells into which they have introduced a new gene. So far, that's been much more of an art than a science, as researchers have had to resort to laborious trial-and-error methods to find just the right culture conditions. Now, a new development might help solve this hitherto intractable problem.

At the meeting, Qi-Wen Niu, a postdoc in Nam-Hai Chua's lab at Rockefeller University in New York City, reported that he and his colleagues have identified a gene in the model plant *Arabidopsis* that can, when overexpressed, cause ordinary somatic cells to develop into embryos. The result, Chua suggests, is like converting plant cells to stem cells. (The results also appeared in the May issue of *Plant Journal*.) The finding could help "transform cell culture studies from an empirical to a more targeted, experimental discipline," says Indra Vasil, a plant biologist at the University of Florida, Gainesville.

The gene, dubbed *pga6* (for *plant growth activation 6*), is not the first one found to have this ability. About 5 years ago, Tamar Lotan, then working in the laboratory of John Harada at the University of California, Davis, found that overexpression of the *lec1* gene has a similar effect, but the frequency of embryo production was low. In contrast, *pga6* triggers the formation of copious numbers of somatic embryos that develop into fertile plants.

Niu, Chua, and their colleagues, including Rockefeller's Giovanna Frugis and Jianru Zuo of the Institute of Genetics and Developmental Biology in Beijing, discovered *pga6* using a method called activation tagging. This involves introducing into plant cells foreign DNA containing a regulatory element known as a promoter that enhances gene activity in response to chemicals, the hormone estradiol in the Chua team's case.

The researchers then exposed cells containing the DNA to estradiol and looked for any changes produced when genes near the inserted promoters increased their activity. Some of the transformed cells produced numerous somatic embryos—an effect the group traced to the *pga6* gene. The researchers also showed that when they turned off *pga6* expression by removing estradiol from the culture medium, the somatic embryos germinated into morphologically normal and fertile plants.

Further work by Chua and his colleagues showed that *pga6* is identical to the *WUSCHEL* gene, which was originally identified about 5 years ago by Tom Laux's team at the University of Freiburg in Germany. Laux and his co-workers found that *WUSCHEL* is important for normal development of zygotic embryos—those produced sexually when pollen fertilizes a plant's ovules—but they did not find any relation between *WUSCHEL* and somatic embryo formation. Now that the Chua team has made the connection, plant researchers might be able to increase the efficiency of their genetic engineering efforts by temporarily upregulating the *pga6* gene in the plant cells they are transforming.

–ANNE SIMON MOFFAT

GENOME RESEARCH

NSF's Ark Draws Alligators, Algae, and Wasps

Evo-devo researchers are thrilled about NSF's new grants for genetic studies of neglected species, although there are too many species and not enough money

Scott Edwards treks around the world seeking songbirds. He's especially passionate about house finches, a focus of his ornithological research at the University of Washington, Seattle. So how did he end up last month as principal investigator on a genetics project involving a jumble of reptiles—including the relatively obscure (and nearly legless) worm lizard? The answer is simple: Edwards followed the rules set by the National Science Foundation (NSF), embracing species he doesn't normally study, and won a grant.

NSF launched a novel competition a year ago, attempting to boost genetic research on organisms not deemed to be models of human biology and thus not usually funded by biomedical science. The agency decreed that most of the projects should be broad, covering as many branches on the Tree of Life as possible. The call for breadth brought together extended families of organisms that don't always fraternize. In Edwards's case, after reviewers had trimmed his plan, the flightless emu was left as the lone bird to tag along with lizards, a turtle, and an alligator.

Contestants. The apple maggot fly didn't make the cut, but the American alligator was among 63 winners.

NSF's purpose in divvying up $6.4 million among 10 groups this way is to start building libraries of so-called bacterial artificial chromosomes (BACs) for detailed genetic studies of neglected species. BACs are useful for storing and cataloging DNA, essentially as bits of genomes that have been broken into manageable stretches, tagged, and inserted into bacteria. Biologists use BACs to examine genes, compare whole genomes of two or more organisms, and fill the gaps in existing gene sequences. Winners will use the money to pay for BAC libraries covering organisms of interest.

The NSF grant competition electrified a diverse set of biologists and yielded an array of potential new genome projects. The species that came flying, swimming, and slithering out of NSF's award chute last month range from Edwards's worm lizard (*Amphisbaenia alba*) to a butterfly (*Heliconius erato*) and a tiny crustacean with a transparent body (*Daphnia pulex*). A study of two ancient marine animals, led by Rob Steele of the University of California, Irvine, seeks to probe the development of the first multicellular organisms, looking in particular at how and when a family of regulatory genes, called *HOX* genes, evolved. Another, led by Dina Mandoli of the University of Washington, Seattle, will explore genetic diversity in a range of plants, and a third, led by Marian Goldsmith of the University of Rhode Island, Kingston, is comparing genes that govern wing patterning in two moths and a butterfly.

Still, $6.4 million goes only so far. Although NSF initially hoped to fund BAC libraries for 100 organisms—the cost of each depends on the size of its genome—money ran out after 63. Like garnering an invite to the Oscars, earning a spot on that final list demanded lots of politicking and a little luck, which sometimes failed to materialize.

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Artful gambits

The field of evolutionary and developmental biology—or "evo-devo," as it's often called—isn't accustomed to glamour contests. If there's one thing that irks the evo-devo community, it's the unending vanity of high-stakes biology. Being human, "we tend to devote our funds to the organism we care most about, which is us," says Jeanne Romero-Severson of Purdue University in West Lafayette, Indiana. Biologists interested in nonhuman evolution are used to being ignored. They aren't mobbed at cocktail parties, for example, especially if it emerges that their work revolves around an obscure species of beetle. Nor are they sought out by the National Institutes of Health (NIH) unless their research can be tied to saving lives. (NIH earlier this year pledged $7.5 million over 3 years for 30 BAC libraries, most of them mammals and all connected in some way to human health.)

With a mission to fund basic sciences, NSF was persuaded that coordinated genetic studies of overlooked species would provide valuable evolutionary insights. A workshop in September 2000 brought together the evo-devo set along with evolutionists studying the Tree of Life. The group resolved that creating BAC libraries—relatively cheap to assemble and useful to a broad swath of biologists—should mark the beginning of such efforts. BACs are good raw material for genetic maps and a starting point for whole-genome sequencing, says Richard Gibbs, director of the sequencing center at Baylor College of Medicine in Houston, Texas.

Once NSF set the rules for its BAC funding a year ago, biologists reached out to colleagues they chatted up regularly and in some cases forged new alliances. Jeffrey Feder, a biologist at the University of Notre Dame in Indiana who adores the black-and-white-striped apple maggot fly, jumped in with Romero-Severson, who's also passionate about flies, or Diptera, and David Stern of Princeton University in New Jersey, who's fascinated by a type of aphid. They focused on their favorites but concluded that NSF's rules demanded some affirmative action. In addition to six species of fly (including the apple maggot) and an aphid, they tossed in six other insects they had not studied, including a beetle, a wasp, and a grasshopper. Three weeks of slaving over the grant proposal met with bittersweet results: Five of their affirmative-action recipients were blessed with $700,000 in funding but not their precious Diptera or aphid. "That caused great consternation," says Feder, although he's still grateful. Romero-Severson adds: "The *Nasonia* [wasp] people are thrilled."

Edwards, the University of Washington ornithologist, also encountered partial disappointment. His grant proposal, which included four birds and six other species in the Reptilia class, was cut down in size, as were other awards. "The ornithologist side of me was a bit chagrined," because only one bird was approved, he says. But he admits that, genetically, birds seem relatively uniform, whereas reptiles "are a huge black box." Still, Edwards's $1.1 million award is the largest he's ever received; his giddiness eclipses any regrets.

Even those who lost out might agree that NSF faced a daunting task in selecting a handful of organisms from the millions of possible candidates. Indeed, researchers at the planning workshop questioned whether it made sense to follow the traditional grant process. One evolutionary biologist who attended, Greg Wray of Duke University in Durham, North Carolina, recalls suggestions that the entire community vote for its favorite organisms. Although he didn't consider that wholly unreasonable, he says, "you'd have to be careful you didn't get people stuffing the ballot boxes." NSF rejected these unorthodox ideas and in the end used its standard process, although it "was a little hurly-burly," says Wray, who also sat on the final review committee. "We had one [proposal] for 20 different fungi," he recalls, "and we were comparing [that] to proposals that were going to pick one really cool organism."

NSF's goal, says Judith Plesset, overseer of the grant competition, was to favor branches of the evolutionary tree for which few resources existed. She hinted at why Romero-Severson's flies were spurned, noting that "we have sequenced Diptera"—specifically, fruit flies, or *Drosophila melanogaster*, a model species used by labs worldwide. There was an exception: a $600,000 grant covering 11 strains of rice, deemed worth funding even though a rice genome has already been sequenced.

Species	No. of genomes	Size of grant
Flagellate and sea anemone	2	$66,000
Insects	5	$700,000
Flowering plants	5	$400,000
Moths and butterfly	3	$210,000
Reptilia	5	$1,100,000
Metazoa, incl. sponge and mollusks	11	$1,100,000
Crustaceans	3	$350,000
Fish	2	$150,000
Rice	11	$600,000
Green algae, seed, and nonseed plants	16	$1,810,000

Now that the grants are out, BAC makers are racing to keep up. Almost none of the new NSF awardees will actually build the BACs themselves. The money will flow instead from investigators to the handful of scientists nationwide who can build the libraries.

"Everybody wants a BAC library, and many people think it's easy to do," says Pieter de Jong, a renowned BAC builder at Children's Hospital and Research Center at Oakland, California, who's constructing libraries for both NIH and NSF. "Then they fall on their face." De Jong's lab has doubled in size, to 25 people, in the last year as orders continue flooding in. He has streamlined the process so that one worker can construct a library in about 3 months, extracting and purifying the DNA and keeping each BAC as large as possible—at least 150,000 bases in length, on average. Building so many simultaneously remains challenging. But de Jong and other BAC builders say they're thrilled by the renewed demand and will keep pace. Virtually all the biologists plan to apply the BACs in their own research, although they might need extra funding to do so. NSF mandates that the BACs be freely available for at least 5 years following their completion.

A shaky future

With NSF's $6 million in start-up money lingering like a sweet taste, grant recipients are hoping that their select species will enjoy more blessings later on. "We foresee that these [species] can be used as leverage" to persuade NSF to fund more genomics work, says Goldsmith, whose BAC grant totaled $210,000. But she worries that the NSF project might be over. "We're asking [Plesset] what's next, and she's saying, 'I don't have any more money.' " Plesset agrees that, for now, her treasure chest is empty. "At the moment, there's no commitment from the senior management at NSF to repeat this competition," she says.

That has some scientists grumbling about the program's stinginess: "The amount of money that the Enron guys walked away with is more than the NSF is able to devote to this," complains Thomas Kaufman, a developmental geneticist at Indiana University, Bloomington, who didn't participate in the grant competition.

And so, even as they revel in their windfall and dream about its future impact on the field, evo-devo researchers are ready to fight for more. "Our field is now sexy," proclaims Romero-Severson. And she, along with many others, doesn't want to see this summer fashion slip out of style. **–JENNIFER COUZIN**