DEVELOPMENTAL BIOLOGY

Dictyostelium Researchers Expect Gene Bonanza

Amoeba-like slime mold cells creeping along the forest floor may not seem much like a mammalian embryo. But if you are a developmental biologist, the analogy is not too far-fetched. The slime mold *Dictyostelium discoideum* has long intrigued biologists because its cells engage in many of the behaviors—migration, aggregation, and differentiation—seen during embryogenesis in much more complicated organisms, including mammals. But the creature has suffered from a drawback as a model for embryo formation: Researchers have had a hard time identifying and isolating the individual genes that would give them the keys to understanding the biochemical basis of those behaviors.

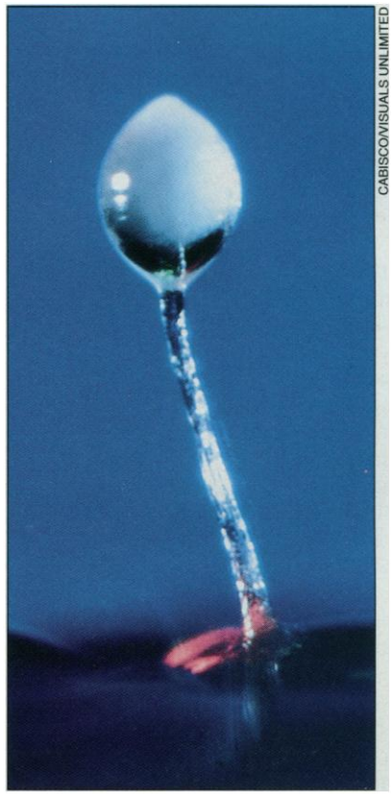
Now, that drawback has been removed. A method devised for *Dictyostelium* by developmental biologists Adam Kuspa and William Loomis of the University of California, San Diego (UCSD), is providing researchers with a highly efficient method of "tagging" the organism's developmentally important genes with foreign DNA. Once tagged, the genes can easily be isolated.

It's been only about 6 months since Kuspa and Loomis developed the method, which is based on a technique originally worked out in yeast about a year ago by Robert Schiestl and Thomas Petes of the University of North Carolina, Chapel Hill. But it's already sweeping the *Dictyostelium* community, where it's winning rave reviews. "It works like an absolute charm," says Rick Firtel, whose lab is also at UCSD. "This is the final method that *Dictyostelium* researchers have been waiting for to open things up for our organism," avers another researcher who's trying the technique, Jeff Segall of Albert Einstein College of Medicine in New York City.

Indeed, only about 15% of *Dictyostelium*'s estimated 300 developmental genes have been isolated so far. But thanks to the new technique, the researchers predict that practically all the rest should be in hand within the next few years. "It really does look like a bonanza," says Loomis. In addition to providing a wealth of information about development in *Dictyostelium* and perhaps in higher organisms as well, isolation of the genes may also benefit the Human Genome Project, Loomis says, by adding new genes to the databases to which genome project researchers turn when they seek to identify the genes that they sequence.

One of the principal reasons for developmental scientists' interest in model organisms such as *Dictyostelium* is that they, unlike more complicated forms of life, offer the ready opportunity to do standard genetic experiments—creating mutants that are defective in some aspect of development and then working backward to find the gene responsible. *Dictyostelium*'s life style in particular makes it well suited for the generation of developmental mutants. When food is plentiful, the cells live a free and independent life, during which their genes can be easily mutated simply by exposing the cells to appropriate mutagenic chemicals.

But when food gets scarce, the cells enter an easily observed developmental pathway. First, thousands of the cells stream together to form an aggregate resembling a tiny translucent slug that can migrate in response to stimuli such as light and warmth, leaving behind a trail of slime (hence the name slime mold). Ultimately the slug settles down, and the originally identical cells differentiate into either of two cell types: stalk cells, which form a slender, stem-like structure, and spore cells, which migrate to the top of the stalk and form a fruiting body that produces spores. When the spores disperse, they germinate and produce more of the amoeba-like, free-living cells.



CABISCO/VISUALS UNLIMITED

Peak of development. The fruiting body at the top of the *Dictyostelium* stalk makes spores.

Researchers trying to figure out the genetics controlling these behaviors expose free-living *Dictyostelium* cells to mutagens and clone them to produce large populations of genetically identical cells. Then, after starving the cloned cells to trigger their aggregation and differentiation, they can simply watch to see if any of the populations carry a mutation that alters one or another aspect of their development. In the 40 or so years that researchers have been working on *Dictyostelium*, they've created hundreds of developmental mutants this way, indicating that at least that many genes are involved. But there's just one problem. As Rob Kay of the Medical Research Council Laboratory in Cambridge, England, points out, "It's always been possible to isolate mutants very easily, but there's been no routine way of going from mutant to gene."

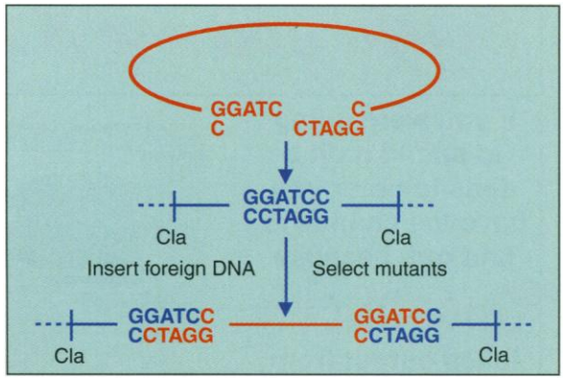
Dictyostelium lacks, for example, a system that has worked very well for identifying developmental genes in two other model organisms, the fruit fly *Drosophila melanogaster* and the roundworm *Caenorhabditis elegans*. These organisms have transposons, gene sequences that jump from place to place in the genome. If one lands in a developmental gene, it both disrupts the gene, creating a mutant, and tags it with a readily identifiable piece of DNA that can be used to pull out the gene for cloning. But that's not been possible with *Dictyostelium*. " '*Dicty*' has transposons," says Firtel, "but none we can make jump." And that's where the new method comes in. It provides an efficient means of tagging developmental genes, not with a transposon, but with a piece of foreign DNA.

The basis of the method is more than a little surprising, notes Jeff Williams of the Imperial Cancer Research Fund lab in London. To increase the frequency with which the foreign DNA inserts itself into the *Dictyostelium* genome, the DNA is transferred into the cells together with a restriction enzyme that can cut the host cell DNA at all sites having a particular short sequence of bases. The surprise, Williams says, is that the genome can recover from the damage. "I wouldn't have predicted that working in a million years. It's the sort of idea that if you suggested it to your grad students, they would laugh at you."

Indeed, that was pretty much Kuspa's reaction when Loomis originally brought up the idea in the summer of 1991. But that September the *Proceedings of the National Academy of Sciences* carried the paper in which Schiestl and Petes described their yeast results. In the course of research aimed at understanding how foreign DNA integrates into the yeast genome, the North Carolina workers had serendipitously found that adding a restriction enzyme increases the frequency of the insertion events about seven-fold. "Their paper gave us the confidence to try this," Loomis says. "Within a month we knew that it worked beautifully." (Kuspa and Loomis de-

scribe the method in the 15 September 1992 issue of *Proceedings*.)

Not only did the *Dictyostelium* cells not die, but the foreign DNA was apparently inserted into the sites cut by the restriction enzyme when they were repaired by the cell's normal DNA-repair machinery. The result: the frequency of integration of the foreign DNA was between 20 and 50 times higher than it was without the enzyme. The main requirement, Loomis says, is that the piece of foreign DNA be prepared so that the sequences at the ends match up with those at the ends of the cuts made by the restriction enzyme used.



Tagging a gene. A restriction enzyme such as *Bam*HI can aid gene tagging by cutting the *Dictyostelium* DNA so that a piece of foreign DNA *(red)* can insert. The tag with flanking cellular sequences can then be cut out with a different enzyme (here *Cla*) and cloned.

Since the *Dictyostelium* genome is small, about 40 megabases of DNA, with relatively little of the noncoding "junk" DNA that clutters up larger genomes, the high integration frequency means that the foreign DNA has a good chance of inserting itself into a developmental gene. When it does, the insertion both disrupts the gene's function, creating a mutant, and tags the affected gene. Using just one restriction enzyme (designated *Bam*HI), Loomis and Kuspa have already produced 50 developmental mutants of *Dictyostelium*, and isolated 16 of the corresponding genes.

Since there are several hundred restriction enzymes, each of which cuts at its own specific DNA sequences, it should be possible, Loomis says, to saturate the *Dictyostelium* genome with insertional mutations very quickly—and pick up the organism's entire repertoire of developmental genes. "Expect an explosion of papers in the next few years," predicts Segall. Indeed, some 15 other groups have already begun to apply the method, which Kuspa and Loomis have named REMI for "restriction enzyme mediated integration," to *Dictyostelium*.

Most of the 40 or so genes already implicated in *Dictyostelium* development were identified because the researchers had biochemical evidence that pointed to the genes' involvement. But with REMI, no prior knowledge is needed to identify genes, and that means, says Firtel, that "you can now get genes that you had no idea existed before." In fact, Loomis says that his group is already recovering genes that "we knew nothing about and wouldn't have predicted." The identification of all those genes should provide a detailed picture of the biochemical pathways that control migration and the various other aspects of *Dictyostelium* development—and perhaps provide clues to the comparable pathways in higher organisms.

And as a bonus, the identification of new *Dictyostelium* genes could be important for the Human Genome Project. As Williams points out, project participants "will sequence a lot of DNA never seen before." To find clues to the function of the newly discovered human genes, the sequencers will need to look for matching genes in databases compiled from other species.

The growing enthusiasm for REMI doesn't mean that the technique is perfect, however. It can't be used to identify genes that are essential for life, since any cell in which such a gene is knocked out will simply die. And it's not clear that it will be applicable to many other species beside *Dictyostelium*. It may be less effective, Loomis cautions, for organisms whose genomes contain a lot of junk DNA, decreasing the likelihood that an insertion will hit a coding region and cause an interesting mutation.

But for *Dictyostelium* fanciers, REMI seems ideal. And to increase the efficiency of their gene search—and avoid unnecessary competition—the researchers plan to coordinate their efforts. Loomis and Kuspa, who run what amounts to the *Dictyostelium* genome project and are physically mapping the organism's genome, will serve as a clearing house where researchers can send the genes they find. By comparing the genes' locations on the map, it will be possible to tell whether they are new or have been previously isolated and whether two researchers have come up with the same gene. If they have, Loomis says, "we'll tell them that they ought to talk to each other." That way the researchers could work together, or one might agree to back off in favor of the other. As Loomis says, "There are more than enough genes for all of us. For a period of 4 or 5 years, we can all be generous."

–Jean Marx

PLANETARY SCIENCE

Earth Gains a Retinue of Mini-Asteroids

Talk about rush hour traffic. Astronomers conducting the most powerful search ever for tiny objects passing near Earth have discovered a slew of house-sized asteroids whizzing along in our neighborhood. Indeed, astronomers David Rabinowitz, Tom Gehrels, and their colleagues at the University of Arizona reported at last week's Division of Planetary Science meeting in Munich that as many as 50 of the mini-asteroids must pass between Earth and the moon each day. That's 100 times more of these nearby asteroids than observations of larger bodies had implied—which leaves astronomers puzzling over their origins and wondering how often they wreak havoc on the ground.

The objects weren't detected before because they're too faint to show up on the photographic plates used in previous searches, leaving extrapolation from counts of larger, less numerous objects as the only means of estimating their abundance. But 2 years ago, Gehrels and his Arizona colleagues in the Spacewatch program, a vigil for asteroids passing near Earth, installed a sensitive charge-coupled-device camera on a 72-year-old telescope on Kitt Peak. Since then, the camera has revealed eight new asteroids between 5 and 100 meters in size passing nearby. Given that Gehrels and his colleagues had only imaged a tiny fraction of the sky for a small part of the time, they inferred that the skies near Earth must be teeming with asteroids.

The big question now is, where do they come from? One clue, says Rabinowitz, comes from the shapes of their orbits, which are unexpectedly similar to Earth's. Of the eight asteroids, two have orbits more like Earth's than any other known asteroid, another two never stray too far from Earth's orbit, and two more pass near Earth's orbit at their closest approach to the sun.

At first, the near-identity of some asteroid orbits with Earth's raised the possibility—ruled out as more discoveries came in—that some of these objects were actually the spent upper stages of rockets launched from Earth. But several speculative possibilities remain, says Rabinowitz. Earth's gravity might have captured the mini-asteroids as they swung in from the main asteroid belt. They might have been blasted off the moon by large impacts. Or, they may be the debris from the breakup of a larger object in an Earth-like orbit.

Wherever they come from, they are unsettlingly abundant. A meteor 40 meters or so in diameter exploded over Siberia in 1908, releasing energy equivalent to that of a 15-megaton nuclear bomb and leveling 1600 square kilometers of forest. Astronomers had previously calculated that a hit like that occurs every 200 to 300 years, on average. In light of the Spacewatch discoveries, researchers are now recalculating the odds. Celestial traffic accidents, it seems, could be all too frequent.

–Richard A. Kerr