Making Transgenic Mice: Is It Really That Easy?

The race is on to replicate one of the most exciting discoveries of the year, but no one has claimed to have crossed the finish line

THE EXPERIMENT DESCRIBED at its publication as possibly the "cold fusion" of biology continues to stand alone, as research groups scramble—so far apparently in vain—to repeat it.

Only 10 weeks ago, Corrado Spadafora and his colleagues at the University of Rome shocked biologists when they announced in the journal *Cell* that they had found a new, simple way to introduce foreign genes into mice. They had simply mixed mouse sperm with the foreign DNA, then used the sperm for in vitro fertilization, they said.

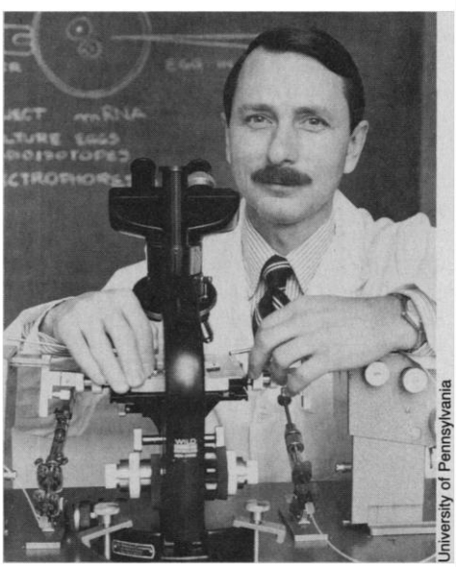
If true, the technique would greatly simplify the production of transgenic mice—a procedure becoming increasingly popular for studying the effects of genes on normal development or disease states. The current method requires the labor-intensive injection of DNA into the nuclei of individual fertilized eggs (see box), a technique that is not only difficult to master but requires expensive, specialized equipment.

Spadafora's discovery promised to change all that. Moreover, if it proved widely applicable to all mammalian species, it would not only aid the engineering of commercially important agricultural animals, but would raise the possibility of introducing genes into humans as well.

But was it true? In a "mini-review" in the same issue of *Cell*, well-known molecular biologist Max Birnstiel, director of the Institute of Molecular Pathology (IMP) in Vienna, and his colleague Meinrad Busslinger called the results "startling" but said they could not find a trivial error or flaw. Still, even though they had aided the Spadafora group with preparation of the manuscript, Birnstiel and Busslinger recommended "a hefty dose of skepticism" and compared the claim to cold fusion—an "ingeniously simple" technique, that, if proven true through independent replication, would be "a cornerstone in biology."

At the time, most researchers took a skeptical view of the paper. "It was a set of provocative results," says Richard Palmiter of the University of Washington. But, he adds, it seemed "too good to be true." Still, the potential significance of such a discovery forced research teams around the world to drop what they were doing and scurry to see if they could repeat Spadafora's experiment. Because the production of transgenic mice is a hurry-up-and-wait process, it has taken until the past few weeks for the first pups to be tested for DNA. What have the early results revealed?

In the 21 July issue of *Science*, Birnstiel and Busslinger announced in a letter to the editor that "at least one other group has been able to repeat Spadafora's experiments." Birnstiel told *Science* Editor Daniel Koshland that the success had been scored by Thomas Wilkie, a postdoctoral fellow at Caltech. But this optimism has proved premature, at best.



Keeping an open mind. *Biologist Ralph Brinster awaits clear confirmation.*

Wilkie told *Science* that he had called Spadafora for advice about some inconsistencies in his results after a quick survey of his mouse pups suggested that some had incorporated the foreign DNA. But follow-up analysis showed the first results to be false positives, a frequent occurrence in the rapid screening process.

The news is no more encouraging elsewhere. Most of the U.S. researchers attempting to replicate the work consider their results preliminary and as yet inconclusive, but no one contacted by *Science* admitted to having—or knowing of—a confirmation of the result.

Although researchers are reluctant to pass final judgment yet, Ralph Brinster of the University of Pennsylvania says their silence speaks for itself. "If there were a positive confirmation from anybody, I think it would have crept out," he says.

John Bishop, acting director of the new Unit for Animal Genome Research of the British Agriculture and Food Research Council in Edinburgh, says he is optimistic about the technique and expects to have results soon from his laboratory's efforts to repeat it. The Edinburgh group has also tried using the DNA-treated sperm to artificially inseminate mice—an approach that, if successful, would go one step further than Spadafora in streamlining the production of transgenic mice by eliminating the need for surgical implantation of the embryos.

Brinster, who, in the early 1980s pioneered the microinjection technique for producing transgenic mice, agrees that artificial insemination would be an "ideal" way to put foreign DNA into mice. But he is far more cautious than Edinburgh's Bishop: "I wouldn't even entertain the idea of doing that kind of experiment until I could see myself that the exact repeat [of Spadafora's technique] worked. And from the rumors, it appears there are going to be . . . problems."

If the process indeed fails to pan out, where might the problem be? In their letter to *Science*, Birnstiel and Busslinger offered one possible hang-up: they said Spadafora suspects that phenol red, a *p*H indicator frequently used in culture media, may inhibit the DNA uptake process. Spadafora did not use phenol red in the buffer—called Whittingham's Tyrode—in which he mixed the sperm and DNA. But a popular technical manual includes phenol red in its formula for the buffer, possibly misleading some experimenters. But phenol red cannot be the only impediment to success because several groups, including Brinster's and Elizabeth Lacy's at Memorial Sloan Kettering Cancer Center in New York, have been unable to reproduce Spadafora's results, even without phenol red.

Many people think there are flaws in the original paper itself. The DNA Spadafora introduced into the mice was the gene for a bacterial enzyme, chloramphenicol acetyl transferase, or CAT. Analysis of the DNA from a number of mice showed the gene was there, but had apparently undergone a rearrangement during transfer. But what is most troubling is that the same rearrangement seems to have arisen independently in five animals whose DNA is shown in the paper. That result seems like an artifact to most researchers, but it's not clear what the flaw

might be. Enzyme assays on tissues from the mice show that they really are making bacterial enzyme. That represents relatively irrefutable evidence that the mice indeed carry the CAT gene, which never appears naturally in mice.

Nevertheless, the *Cell* paper has "serious problems," according to Frank Costantini of Columbia University, who has been making transgenic mice since the early 1980s. If the paper is taken at face value, Costantini says, that would mean the gene had rearranged in many independent cases and could limit the applicability of the technique. Genes that get into mice in garbled form are likely to be of little use, even though in Spadafora's experiment the rearranged CAT gene still seems capable of producing CAT enzyme.

Many who read the paper question why the ability of sperm to pick up random DNA hasn't resulted in evolutionary chaos. In a recent interview with BBC radio, Spadafora suggested that sperm seem to be protected from invading DNA under normal conditions but that his procedure somehow destroys the protecting factors, allowing the DNA to penetrate.

Spadafora's experiment is not the first case of DNA uptake by sperm. In his letter to *Science*, Birnstiel mentioned an 18-year-old paper in the *Proceedings of the National Academy of Sciences* that reported that rabbit sperm could take up DNA and carry it into rabbit eggs. And a group at the University of Palermo published a report last April in *Cell Biology International Reports*, claiming to have used sea urchin sperm to transfer foreign DNA into sea urchin eggs. Neither report received widespread attention or scrutiny, nor reported the successful production of transgenic animals. But they raise the possibility that, if Spadafora's technique works for mice, it would work in a wide variety of species. Ralph Brinster, who has worked with mammalian eggs and embryos for 25 years, says that if the method were found to produce transgenic mice, he wouldn't be surprised if it worked for other species as well.

If Brinster's hunch were to prove correct, the technique could have a tremendous impact on the production of transgenic farm animals, whose opaque eggs make microinjection more problematic. But Palmiter is dubious: "If it were universally true and could make any animal transgenic with ease, then it would be a real boon. But they didn't demonstrate that."

If the method were universally applicable, it might also raise the issue of gene transfer into humans, forcing a debate over the ethics of altering the genes of future generations. But most researchers do not consider gene transfer into humans to be feasible, even if a method such as Spadafora's were to work. Such a procedure still would not overcome the fact that the introduced genes insert randomly into the DNA, where they may disrupt important genes—an acceptable risk in mice but not in humans.

Brinster, Costantini, and others agree that the greatest impact of the technique would be to open the door wide for new investigators to enter the field of transgenic research by eliminating the need to learn microinjection and to invest $40,000 in special equipment for manipulating the eggs.

These potential advantages are enough to continue to create excitement. Brinster says that because word is out that he is trying to repeat the experiments, he is getting calls from people considering entering the transgenic field. "They say, 'we don't want to invest in equipment until we know if this new technique works,' " he says.

But he is not ready yet to advise them on their shopping spree. He refuses to jump to conclusions before he has finished testing the method, using reagents he recently obtained from Spadafora. "[Spadafora] should be given every fair chance," he says. "I don't have any negative feelings about this—I think it's how science evolves. I always assume that the work published is true and that it can be repeated. If I have difficulty, [I get] in touch with the person."

Palmiter, who has collaborated with Brinster on transgenic mice for 9 years, agrees that judgment should not be passed on Spadafora prematurely. But, he says, "if it's not repeatable with his [DNA] and with his media, then we've tried our hardest. [Then] it's up to him to explain what he does that makes it work."

A mouse molecular genetics conference in Heidelberg later this month may become a forum on Spadafora's technique. Many transgenic mouse labs will be represented and are likely to come with reports of their latest results and the conditions they have tried. If the results are all negative, it will be up to Spadafora to suggest the next step.

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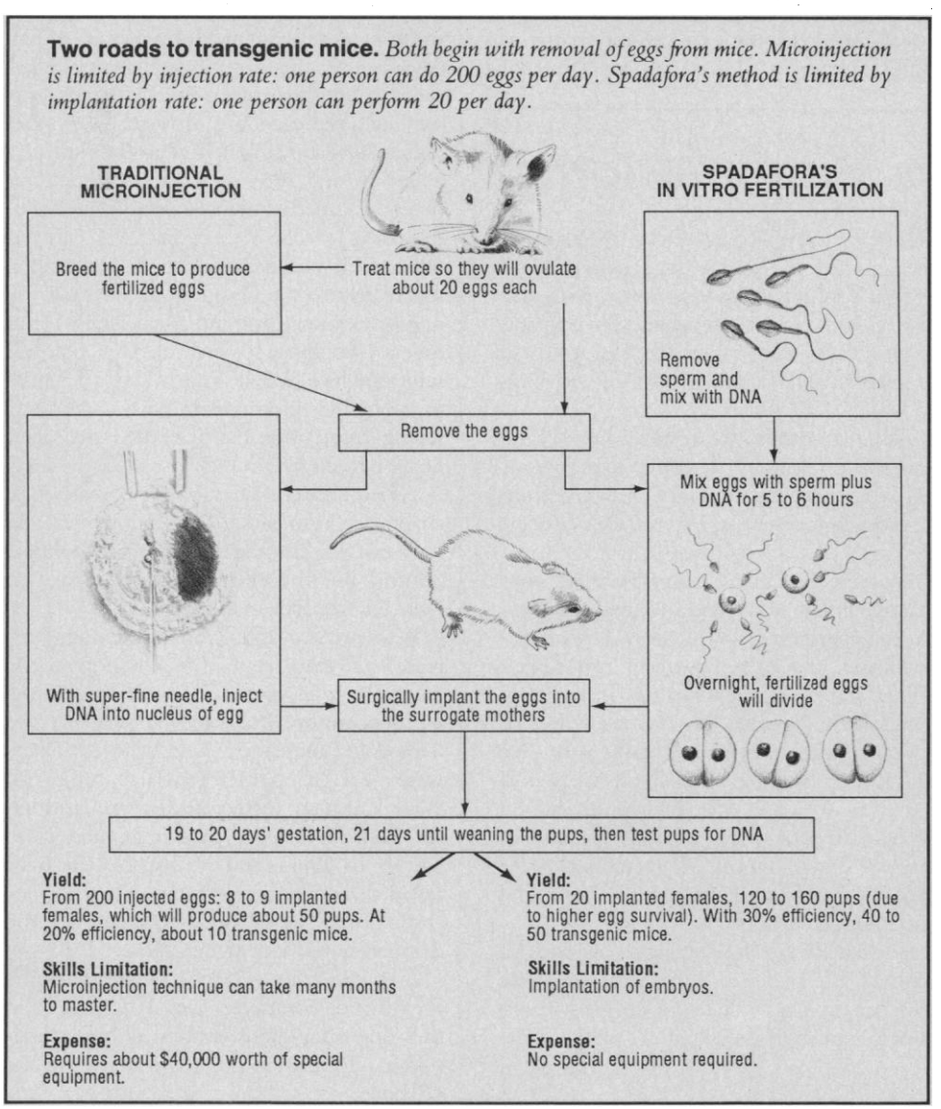
With reporting from Jeremy Cherfas.



Two roads to transgenic mice. *Both begin with removal of eggs from mice. Microinjection is limited by injection rate: one person can do 200 eggs per day. Spadafora's method is limited by implantation rate: one person can perform 20 per day.*