

## CLONING

## Mice Cloned From Cultured Stem Cells

In an experiment that links the hot-button fields of stem cells and cloning, scientists announced this week that they have cloned mice from embryonic stem (ES) cells kept in culture. These cells are capable of developing into any type of tissue but ordinarily can't grow into a complete organism on their own. The technique is still inefficient, but if researchers can improve it, it could provide an easier way to create genetically modified mice.

Although sheep, goats, cows, and mice have all been cloned, in most cases the genetic material came from cells that had not spent much time in culture. In contrast, researchers report in the current *Proceedings of the National Academy of Sciences* that they produced live-born mice from two ES cell lines that had each gone through more than 30 cell divisions in the lab. Although cells in culture are easy to work with, they often accumulate mutations. Those mutations can impede embryonic development, so scientists weren't sure whether long-cultured cell lines would be candidates for cloning.

In the hands of reproductive biologist Teruhiko Wakayama, who created the clones in the laboratory of Ryuzo Yanagimachi of the University of Hawaii, Honolulu, the cultured cells seemed to work as well as cells from a living animal. In collaboration with Peter Mombaerts of The Rockefeller University in New York City, the researchers extracted a nucleus from an ES cell and transferred it into a mouse egg that lacked a nucleus. Like all cloning, the procedure was hit or miss, however. In the team's most successful experiment, it took more than 1000 cloning attempts to obtain 13 mice that survived to adulthood, notes developmental biologist Davor Solter of the Max Planck Institute for Immunobiology in Freiburg, Germany.

If scientists could boost the technique's efficiency and use it to go directly from a genetically manipulated cell to a live animal, "that would be fantastic," says embryologist Brigid Hogan of Vanderbilt University in Nashville, Tennessee. Cloning mice from ES cells could streamline the rather cumbersome procedure used today to produce tens of thousands of so-called knockout mice a year.

Typically, scientists modify genes in an ES cell and then insert the cell into an already-developing embryo, producing a chimeric mouse that has descendants of the ES cell in

most of its tissues, including the reproductive organs. When the chimeras mate with normal mice, some of their offspring carry the mutation in all their cells. Those mice are then bred to create knockout mice. If scientists could clone mice directly from particular cells, they could create knockouts in one generation instead of three, Mombaerts says.

But at the moment, cloning mice is far too difficult to rival the traditional procedure, several mouse researchers say; although other labs have tried, no one else has reported cloning a mouse. "Whether [cloning] is going to be a better way of generating mice than chimeras remains an open question," says mouse geneticist Allan Bradley of Baylor College of Medicine in Houston, Texas. "I don't think we're going to drop everything overnight and start trying to clone ES cells." Indeed, Yanagimachi's team reports that they tried to clone mice from ES cells modified to contain a specific mutation, but they produced only one cloned knockout mouse that died soon after birth.

The new work does suggest that in some respects cloning may be easier technically than researchers had thought. Earlier reports seemed to suggest that successful cloning requires starting with cells at the resting phase of the cell cycle; Dolly the sheep was cloned from a so-called "quiescent," starved cell, and other cloning experiments have used cells in the resting phase. But ES cells don't survive long if they are starved, and they divide rapid-

wounds called calderas. The Galileo team hoped they also might find ribbons of fire called "lava fountains," similar to those that spurt skyward from long, narrow gashes in the Kilauea volcano on Hawaii. However, Galileo's camera hadn't yet pointed in the right place at the right time to see one.

That changed during a moment of "dumb luck" on 25 November as Galileo passed within 300 kilometers of Io, says Galileo team member Laszlo Keszthelyi of the University of Arizona, Tucson. The camera was aimed near Io's north pole at a nondescript volcanic



**Curtain of fire.** Heat from what may be a "lava fountain" on Io blinded Galileo's detector; the inset shows how the 25-kilometer-long fountain may have looked.

feature that the team now calls Tvashtar, after an Indian sun god. Heat flowing from a 25-kilometer-long stripe within Tvashtar's caldera was so intense that it blinded part of the camera's electronic detector and created a white blotch. However, details of parts of the blotch—especially its wavy top edge and a linear section in the middle—convinced Keszthelyi and his colleagues that they were seeing a curtain of lava at least 1.5 kilometers high. Key support for that scenario came from NASA's Infrared Telescope Facility on Mauna Kea, Hawaii, which happened to detect the Tvashtar hot spot from Earth just 3 hours later. According to planetary scientist John Spencer of Lowell Observatory in Flagstaff, Arizona, Tvashtar was so close to Io's horizon at the time that the telescope would not have seen the hot material unless it was jetting above the surface.

Still, the lava fountain scenario is "pretty speculative," says planetary scientist Gerald Schubert of the University of California, Los Angeles. "Because of the bleeding problem [in the image], it's very uncertain exactly what they're seeing," he says. But Spencer and his colleagues think they may be able to dispel those doubts by determining the three-dimensional shape of the hot spot from earlier observations of Io on the same day, when the view was more direct. —ROBERT IRION



**Father and son.** Hooper the mouse was cloned from the E14 embryonic stem cell line.

ly, so it is tough to catch one at the resting stage, says Mombaerts. The team found that cell stage didn't seem to matter: They produced live-born mice from cells in several stages of the cell cycle, including some that presumably had just finished replicating their DNA and were about to divide.

The next crucial step is to standardize the procedure. Mombaerts says he and Wakayama, who has recently moved to Rockefeller University, hope to do their part: They're planning a detailed guide on "how to clone a mouse"—a book that may make many researchers' lives a lot easier. —GRETCHEN VOGEL

CREDITS: (LEFT TO RIGHT) GALILEO SSI TEAM; WAKAYAMA ET AL. PNAS 96 (26), 14987 (1999)