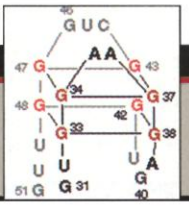
PAGE 1806
Squabble over salmon species



1809
How fragile X leads to retardation

BIOETHICS

Cloning Announcement Sparks Debate and Scientific Skepticism

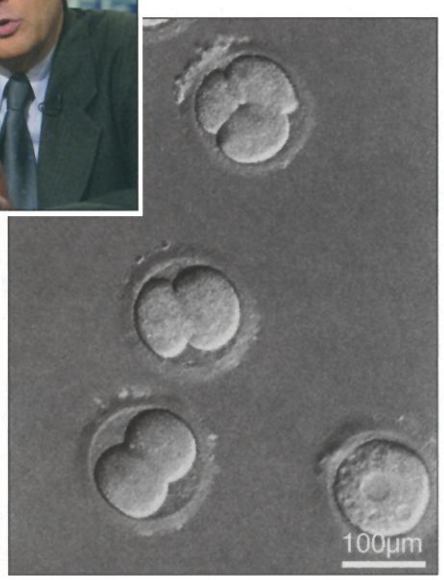
WASHINGTON, D.C., AND BERLIN—A small U.S. biotech firm made headlines around the world last week when it announced that it had cloned several human embryos for transplantation research. The breakthrough—as it was called by scientists who did the work at a privately held firm, Advanced Cell Technology (ACT) in Worcester, Massachusetts—prompted strong reactions. President George W. Bush denounced the research as unethical, European leaders discussed national controls on cloning, and the furor could spur efforts to pass a law in the United States that would ban research on human cloning. All this fuss over results whose scientific significance is questionable: Some scientists note that the six-cell clusters created by ACT barely qualify as embryos.

The experiments, according to ACT's chief executive Michael West, were designed to test a way of producing human embryos for use in transplantation therapy. The company is pursuing a vision known as "therapeutic cloning." The goal is to transfer genes from a patient into an experimental embryo that can generate healthy new stem cells. The hope is that the new cells might then be reimplanted in the patient without causing an immune reaction, to treat common illnesses.

A six-member ACT team, including West, scientist Jose Cibelli, and vice president Robert Lanza, claims to have completed the first step of this process in October, cloning human embryos for the first time. They describe their "protocols for the generation of human embryos" in an online publication called *e-biomed: The Journal of Regenerative Medicine*, posted on 26 November. On the same day, they published a first-person narrative in *Scientific American* and were profiled in a glowing account in *U.S. News & World Report*.

ACT launched its human cloning project in early 2000, advertising for egg donors in Boston newspapers. It convened an advisory board under ethicist Ronald Green of Dartmouth College in Hanover, New Hampshire, to review and approve the procedures. The scientists obtained 71 eggs from seven paid volunteers: women between 24 and 32 years old who already had given birth to at least one child.

ACT attempted several kinds of procedures. First, the team removed the nucleus from an unfertilized egg and replaced it with a nucleus taken from a skin cell of another adult donor—a procedure called nuclear transfer. None of the 11 eggs treated in this way divided. The team had better luck when it transferred nuclei from cumulus cells, support cells that surround developing eggs in the ovary. Three of four eggs in this experiment began dividing, but only one developed to the six-cell stage.



Clones & Co. CEO Michael West and human eggs after insertion of cumulus cell nuclei.

The team also attempted to trigger embryonic development in unfertilized eggs, a process called parthenogenesis, by exposing the eggs to a mix of chemicals known to prompt cell division (see image). Twenty of these 22 chemically activated eggs divided at least once. Six formed what Cibelli and his colleagues call a "blastocoele cavity," resembling the sphere of cells that a normal embryo forms a week after fertilization. However, they did not contain the crucial inner cell mass, a cluster of more than 100 cells that give rise to embryonic stem cells.

Many animal species can reproduce through parthenogenesis, but no mammals are known to do so. Because a parthenogenetically activated egg could never produce a full-term baby, some have speculated that stem cells derived from "parthenotes" might skirt the ethical questions that surround the use of human embryos. Bioethicists are skeptical. For example, Norman Fost of the University of Wisconsin, Madison, says he's not convinced there's a difference and notes that "the people who are against any use of human embryos in research are still going to be opposed."

The fact that none of the experimental embryos developed to eight cells suggests that the inserted nucleus wasn't working properly, says developmental biologist John Eppig of the Jackson Laboratory in Bar Harbor, Maine. In normal human embryos, the nucleus begins to express its genes between the four- and eight-cell stage. The failure to survive to eight cells "strongly suggests that you're not getting gene activation" in the transferred nucleus, he says. "And if you're not getting that, what have you got? Nothing." David Ayares of Edinburgh-based PPL Therapeutics, another company involved in animal cloning and attempts to generate human stem cell lines, agrees. "The fact that they only went to four cells implies incorrect gene activation. … Those embryos aren't going to go any further," he says.

West agrees that the data so far are "admittedly scant." Asked why he decided to go public at this time, he said: "We are asked all the time about where we are in our research; we made the decision that when we had enough reproducible data, we would publish." He added: "Our next goal is to create blastocysts," early-stage embryos that contain several hundred cells.

International reaction was mixed. The Vatican denounced ACT's research. Likewise, science ministers in Germany and France reaffirmed that such work was illegal in their countries. Earlier this month, France

CREDITS: (TOP TO BOTTOM) ALEX WONG/GETTY IMAGES; J. CIBELLI *ET AL.*, *THE JOURNAL OF REGENERATIVE MEDICINE*

and Germany issued a joint statement calling for an international ban on human reproductive cloning, while their legislatures are debating whether to allow work on embryonic stem cells. In the U.K., which has the most tolerant rules in Europe on the use of human embryos in research, legislators approved research on nuclear transfer to derive stem cells early this year. The House of Commons is planning to debate a bill that would explicitly outlaw the implantation of such an embryo. Lawmakers say ACT's announcement adds urgency to the debate.

The U.S. Senate, which is divided on the subject, has not yet voted on human cloning, although the House passed a bill in July that would make it illegal. If the House bill were in effect today, ACT might be prosecuted. The main effect of ACT's announcement, stem cell researcher John Gearhart of Johns Hopkins University told Reuters, was to scuttle backstage talks among congressional staffers on how to reach a compromise on the use of embryos in research. ACT's announcement, one House aide says, "has made everyone a little queasy."

–ELIOT MARSHALL AND GRETCHEN VOGEL

MOLECULAR IMAGING

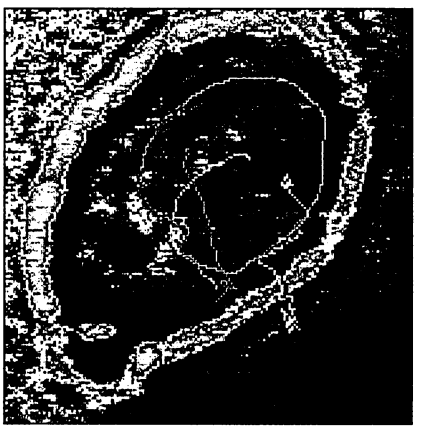
Virus Infects Cell: Live and Uncut

Reality TV has never been this good: After several brief kisses for its unwitting victim, a dazzling virus pushes inside the recumbent cell, while another radiant virus, unsuccessful in its flirtation, floats out of view. The camera pans to the cell interior, where glowing viruses glide along protein rails to the nucleus. There, some slip through nuclear pores, and others cruise through tunnels within the cell center.

Cut to laboratory: For the first time, researchers have viewed live scenes of viral infection. Their lens is an imaging technique that may open up gene therapy and antiviral research to highly detailed, blow-by-blow analysis.

On page 1929, a cadre of researchers led by physical chemist Christoph Bräuchle of Ludwig Maximilian University in Munich, Germany, reports having imaged—in real time—single adeno-associated virus (AAV) particles entering cells and moving into the nucleus. The closeup view was provided by a technique called single-molecule fluorescence spectroscopy, which until now had been used to view chemical reactions such as the enzymatic breakdown of adenosine triphosphate.

Although single-molecule imaging techniques have advanced significantly in the last few years (*Science*, 1 June, p. 1671), the technique had never before been used to watch a viral infection, says molecular virologist R. Jude Samulski, director of the University of North Carolina Gene Therapy Center in Chapel Hill. "This technique will be significant for helping us understand how the virus enters the cell," he says. "We're usually taking a picture after the event happened, but this is real time, the live story."



Caught in the act. Three viruses (yellow, green, and pink lines) infect one cell (outlined in yellow) and head straight to the nucleus (outlined in red).

CREDIT: G. SEISENBERGER *ET AL.*

The documentary approach revealed new information about the small virus, which gene therapy researchers are hotly pursuing as a gene delivery vector. Among their findings: AAV poked through the cell membrane in about 64 milliseconds, much faster than expected, and reached the nucleus in about 15 minutes. That's about an eighth of the time in which conventional cell culture methods, which rely on viral gene expression, can detect infection. Also, the researchers were surprised to see that some particles, after floating toward the nucleus, hopped aboard microtubule-based "tracks" on the nuclear surface and began to move in a straight line. Other viruses followed along the same tracks. Bräuchle suggests that the tracks are tube-shaped invaginations of the nuclear membrane, recently discovered structures never before known to ferry viruses.

Bräuchle's team pieced together its imaging system from commercially available equipment and customized it to circumvent obstacles such as a cell's inconvenient habit of autofluorescing, which would outshine the virus's signal. Bräuchle says conventional methods of measuring and imaging viral entry average the properties of a population and may introduce artifacts that affect infection in unpredictable ways. For example, to see where viruses are concentrated, scientists have had to coat each particle with more than 300 fluorescent molecules, which may get in the way of the virus's activities.

To minimize such interference, the researchers tagged individual viruses with one or two fluorescent molecules, each of which is about 1/25 the size of the virus. After using light microscopy to get a good picture of the mammalian cells lying on a microscope slide, the researchers infected each cell with 10 to 1000 viral particles, which Bräuchle says is much closer than typical cell cultures to normal conditions in the body. The molecules' glow lasted 1 to 10 seconds before it bleached out. This gave the researchers ample time to capture the movement of individual viral particles with snapshots every 40 milliseconds. "Because they're carrying little flashlights around, we can see where the virus is," says physical chemist Anne Myers Kelley of Kansas State University in Manhattan, an expert in single-molecule imaging who was not part of Bräuchle's team.

Bräuchle expects the technique to illuminate virus-host cell interactions for other types of viruses, as well as help screen antiviral drugs. "We can really see how drugs affect the uptake of virus into the living cell, at what stage of the infection pathway the drugs work, and to what extent [they interfere with infection]," Bräuchle says. The ability to view viral infection close up will ratchet up efforts to understand viral processes, says Samulski. "If we can understand processes at this level of detail, then we have to. When somebody breaks the mile record, it challenges everybody to run faster."

–MARY BECKMAN

Mary Beckman is a writer based in southeast Idaho.

RESEARCH COLLABORATIONS

Asian Astronomers Build Closer Ties

TOKYO—The vastness of space is bringing Asian astronomers a little closer together. Meeting earlier this month in Taipei, astronomers from China, Japan, Korea, and Taiwan moved ahead with cooperative plans on both regional and international projects.