Will Dolly Send in the Clones?

The first mammalian clone, produced from an adult sheep, took the world by storm, but leaves a rash of unanswered scientific questions in her wake

No longer will the name Dolly bring to mind Carol Channing or Barbra Streisand, leading ladies in the musical, "Hello, Dolly," or even the vivacious country western singer, Dolly Parton. Last week, a new Dolly—a 6-month-old lamb cloned from the udder cell of an adult ewe—made her debut. And even though animal scientists have been cloning sheep and cattle from embryos for a decade, the media went wild over Dolly, the first animal ever cloned from an adult cell.

The page-one headlines heralding Dolly's creation ignited worldwide concerns about the potential of this approach for cloning people. Within days, there were calls for ethics inquiries and new laws to ban human cloning (see sidebar). Concerns that human cloning would soon follow were further heightened when *The Washington Post* reported on 2 March that Don Wolf's team at the Oregon Regional Primate Research Center in Beaverton had cloned two rhesus monkeys, although from embryonic cells. But even as the media frenzy continues, researchers say it's still unclear how practical cloning of animals, let alone humans, will be.

As reported in the 27 February issue of *Nature*, Ian Wilmut and Keith Campbell at the Roslin Institute in Edinburgh, Scotland, cloned Dolly by transferring the nucleus from an udder cell into an egg whose DNA had been removed—an approach that could lead to flocks of prize animals with a genetic makeup guaranteed to match that of the adult donating the cell or of animals that produce valuable human proteins for therapeutic use.

But the procedure is quite inefficient. The Roslin group made 277 attempts in order to succeed with Dolly. And no one knows either how DNA from the udder cell was able to direct the development of an entire new organism, or whether the same will prove true in other species. "There are lots of questions to sit down and look at," says embryologist David Whittingham, director of the Medical Research Council's (MRC's) Experimental Embryology and Teratology Unit in London.

AP/NEWSFLASH

Clone craze. Dolly became an instant media star.

Until this report by Wilmut and Campbell, a great deal of evidence had indicated that while species ranging from frogs to mice to cattle and now monkeys can be cloned by transferring nuclei from embryonic cells, the DNA of older cells was irreversibly altered. Presumably, because of chemical changes and structural modifications, those genomes were supposed not to be "totipotent," that is, capable of supporting the development of all the different cell types needed to build an animal. For example, no one could get mice to develop reliably when they used nuclei from anything but one-, two-, or four-cell mouse embryos.

The trick behind the Roslin team's success, Wilmut says, was to make the DNA of donor cells behave more like the inactive DNA of a sperm or unfertilized egg. They did this by reducing the nutrient-laden serum supplied to the cells, in effect starving them into the dormant G0 or G1 stages of the cell cycle. The deprivation caused many genes to shut down and ensured that the DNA had not just replicated when it was transferred. The researchers then administered an electric current to fuse this donor cell with an egg whose own chromosomes had been extracted.

The fusion provided the egg with a full complement of new DNA and triggered the development of the egg. The first three divisions of the sheep egg replicate its DNA without expressing any of the new genes: Proteins and messenger RNAs already in the cytoplasm do all the work required for division. While the DNA goes along for the ride,

Cloning Sparks Calls for New Laws

The news of the first successful cloning of an adult mammal, a sheep (see main text), has sent ethical shock waves around the world. As a result, in many countries, officials and even some scientists are calling for new or strengthened legislation to outlaw human cloning, although at this early date, no concrete measures have been proposed.

In the United States, which currently has no law prohibiting the procedure, President Clinton announced an urgent inquiry into the potential ethical and legal implications by the National Bioethics Advisory Commission, which will report its conclusions by the end of May. Meanwhile, Clinton has banned federal funding for human cloning research and asked for a moratorium on nonfederally funded efforts. In addition, both houses of the U.S. Congress are holding hearings on the issue. In China, geneticist Zhang Jiaming, who was a delegate to last week's annual meeting of China's parliament, says he and other scientists in the legislature agree that new laws are needed to ban the cloning of humans.

Many European countries already have detailed legislation covering human embryo experiments, but even there, the cloning success throws up potential new problems. Take the United Kingdom, which passed some of the most comprehensive legislation in this area in 1990, but has now found that changes may be needed. "We have prohibited the transplant of nuclei into embryos, but in these sheep experiments, the nuclei were transferred into eggs," says Bea Heales, a policy manager at the Human Fertilization and Embryology Authority, which licenses research and treatment in Britain. "We may need a new policy that states such experiments in humans will not be licensed." And Germany's current law on human experimentation may also have a loophole that permits cloning, some legal experts say. At the European level, Jacques Santer, president of the European Commission, has ordered an inquiry.

Such is the scale of worldwide concern that moves have already begun to draw up international guidelines. The 40-nation Council of Europe, which includes countries outside the European Union, is currently developing a convention on human rights and bioethics. The council says work will start soon on a specific protocol on the protection of the embryo and the human fetus, which could include a ban on human cloning. "The cloning of an adult sheep may be an impressive scientific achievement, but it also demonstrates the need for firmer rules on bioethics," says Daniel Tarschys, secretary-general of the council.

–Nigel Williams

Wilmut says, it loses the proteins that came attached to it and takes up others from the cytoplasm. At the same time, it apparently becomes "reprogrammed" so that the embryo can develop normally.

Those multiple replications, and the several days it takes for them to occur, may be the reason nuclear transfer works in sheep but not very well in mice, suggests Richard Schultz of the University of Pennsylvania, whose own work focuses on gene expression in early development. In mice, all DNA remodeling takes place in the first cell division and the new DNA takes over by the two-cell stage, rather than in the eight-cell stage as in sheep. "Maybe in rodents there's just not enough time [for reprogramming]," he says. (In humans embryos, the new DNA apparently takes charge after the four-cell stage, in between mice and sheep or cows.)

Or it may be that Dolly's DNA didn't require much reprogramming. Her DNA came from cultured mammary cells, which are normally capable of developing into lactating tissue. Wilmut and his colleagues acknowledge that the collection of cells may have included a stem cell—an undifferentiated progenitor cell of many different tissue types—which has a higher developmental potential than an ordinary epithelial cell from the mammary gland. "The udder cells are a mixed population, and we don't know which are able to be totipotent," comments human geneticist Nick Hastie of the MRC's Human Genetics Unit in Edinburgh, U.K.

But assuming reprogramming did occur, its efficiency was low. The sole successful transfer out of 277 attempts "may say this is still a very difficult task in terms of successfully completing the reprogramming," Schultz points out.

Efforts to increase that success rate may run into another barrier, he adds: "We don't really know" how programming occurs normally during development. This makes understanding deprogramming difficult, although it likely involves reversal of chemical modifications, such as methylation and acetylation, that the DNA and its associated proteins undergo as cells take on specialized functions. Also, some reprogramming may occur when DNA is stripped of its old packaging proteins and repacked with new ones in the egg's cytoplasm—a process that also occurs with the DNA of a fertilizing sperm. "We need to spend a significant amount of effort in the near future in understanding that mechanism [of how the egg interacts with its new DNA]," says James Robl, a developmental biologist at the University of Massachusetts, Amherst. However, it may now be possible to study how that programming occurs by examining the molecular conversation that goes on between the egg and the transferred nuclei.

And the reprogramming is just one aspect of cloning that can go wrong. Many subtle differences exist between mammalian species in how they develop during those first few days. Not only do they differ in how quickly the new DNA takes charge, but they also vary in how they decide to implant in the uterus and develop a placental connection. These differences could make nuclear transfer from adult cells harder, if not impossible, in animals other than cows or sheep, suggests Zena Werb, a developmental cell biologist at the University of California, San Francisco. Also, in livestock, past efforts to clone embryonic cells have tended to produce oversized, delicate young that required extra care if they were to survive, notes embryologist George Seidel of Colorado State University in Fort Collins. The same may prove true of the new procedure.

But the tantalizing possibility of making identical copies of prized livestock, or even of animals used for research, will be too exciting to pass up, says Robl, who helped form a company 3 years ago to take advantage of these advances in cloning technology. Add to that the prospect of cloning genetically modified animals that can produce drugs or better milk, meat, or wool, Robl says, and "tomorrow, next year, this field is going to be so crowded."

Yet, even if few or none of the potential applications come to pass, Dolly will forever have her place in history. As Werb points out, this lamb's creation "is the category of experiment that bends your mind."

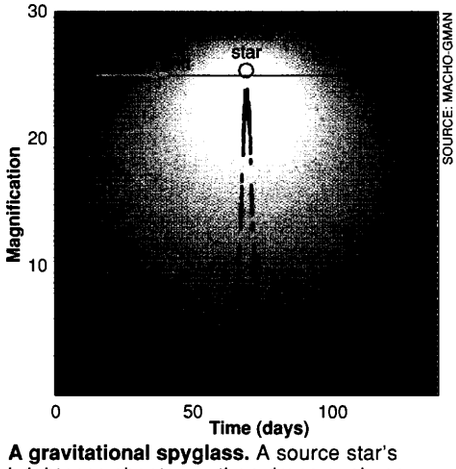
–Elizabeth Pennisi and Nigel Williams

ASTRONOMY

Farsighted Gravity Lens Sees Stars

Almost all stars are so distant that, even with the largest conventional telescopes, they appear as unresolved points of light, as featureless as the twinkling dots seen by an unaided observer on a clear night. Now, by using the gravity of one star as a huge magnifying glass, a team of astronomers has been able to make out features on the face of a second star located 30,000 light-years from Earth. The team found that the gravitational lens, which bends light rays as predicted by Einstein's theory of relativity, was aimed so precisely that it scanned across the face of the distant star, revealing details of its structure. "We have, in essence, obtained more than 8000 times better spatial resolution than the Hubble Space Telescope [HST]," says Andrew Becker of the University of Washington, Seattle, one of 57 astronomers from nine countries who collaborated in the study. The team announced its findings at a conference this week at the University of Notre Dame in Indiana.

Becker quickly adds that gravitational lensing requires the chance, near-perfect alignment of Earth and two stars, and so is much less versatile than the orbiting HST or conventional ground-based telescopes, which can be pointed anywhere in the sky. Still, says astronomer Virginia Trimble of the University of California, Irvine, who is not part of the collaboration, this use of gravity to peer at an object so far away "is obviously enormously exciting." The detail it reveals on distant stars, say other researchers, should help astronomers firm up computer models of how stars grow old and die.



A gravitational spyglass. A source star's brightness shoots up, then drops again as the gravity of a second star magnifies the signal through gravitational lensing.

The multinational collaboration that first noticed the event goes by the acronym MACHO, for Massive Compact Halo Object. MACHO's principal aim is to use gravitational lensing to search for dark blobs of matter, such as burnt-out stars or black holes, that might be swarming in a shadowy halo around our galaxy and making up most of its overall mass, as some theories predict. The project does this by constantly monitoring stars in a nearby galaxy called the Large Magellanic Cloud with a telescope at the Mount Stromlo Observatory in Australia, seeking sudden brightenings—a signal that a star has been magnified by the gravity of an unseen object in the halo. By keeping track of these events, MACHO hopes to estimate the overall amount of this kind of dark matter.

So far, MACHO has reported eight such brightenings, and is sitting on "a few" new ones for which the analysis hasn't been completed, says David Bennett, a team member at the University of Notre Dame. Settling the dark matter issue could take years, but while that program inches forward, the team also monitors stars near the Milky Way's more crowded central bulge. Here, the chance alignment of two stars and Earth in a straight line—the pre-