

## ANIMAL CLONING

# Bid for Better Beef Gives Japan a Leg Up on Cattle

Japanese researchers achieve high success rates in cloning cattle with techniques honed on efforts to help the livestock industry

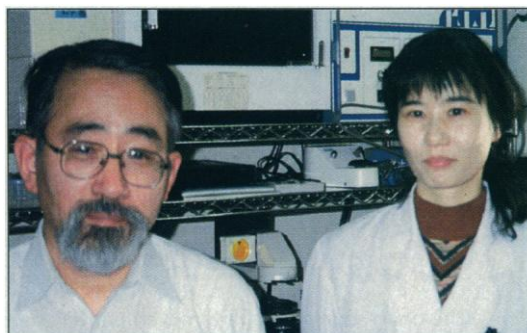
**NARA, JAPAN**—When British researchers announced in early 1997 that they had successfully cloned an adult sheep, the startling news touched off a global gabfest about the ethics, dangers, and possibilities of cloning adult mammals. For Yukio Tsunoda, however, Dolly's arrival was a call to action. Tsunoda, a professor of animal reproduction at Kinki University here, had spent more than a decade refining a cloning technique at the heart of the process that produced Dolly. And Tsunoda wasn't alone in his quest to emulate the work done at Scotland's Roslin Institute with adult sheep cells.

In the months following Dolly's arrival, at least seven Japanese groups set out to replicate the experiment in cattle. Their success has created a herd of clones stampeding out of livestock research centers. Five groups have reported the births of 19 calves cloned from adult cows, and so many more surrogate mothers are carrying cloned cow embryos that the Ministry of Agriculture, Forestry, and Fisheries has given up trying to track them all.

The first scientific paper resulting from these efforts appears on page 2095. In it Tsunoda's team reports cloning eight calves from cells taken from the oviducts and cumulus, the tissue that surrounds the oocytes, or egg cells, of a single adult cow. Closely following the nuclear transfer technique used to produce Dolly, the team starved the cells into quiescence, transferred their DNA-carrying nuclei into enucleated egg cells, and then reactivated them with an electric shock. Particularly significant is the group's success rate: Of 10 implanted embryos, eight were carried to term, although four died soon after birth. That rate is far higher than for any other group attempting to clone large mammals.

Although the self-effacing Tsunoda chalks up their success to "beginner's luck," most analysts point instead to Japan's extensive support for livestock research, a component of its international trade policy. Japan's longtime efforts to improve livestock management and breeding techniques gained a big boost in the early 1990s when the government bowed to heavy pressure

from the United States and other countries to gradually open its beef market to imports. In an attempt to gird local industry against the looming competition, the Agriculture ministry organized programs to train re-



**Cattle-cloning kings.** Yukio Tsunoda, left, and Yoko Kato in their lab at Kinki University.

searchers at prefectural livestock research centers in the latest biotechnological techniques. That early government investment gave the domestic livestock industry a leg up on the global competition and scientists valuable experience in cloning.

Before Dolly appeared, Japan's livestock researchers and their counterparts around the world were trying to clone animals by transferring the nuclei from very early embryo cells—before they have begun to differentiate into specialized cell types—into other embryos whose nuclei have been removed. The idea is to create many copies of an animal likely to have valuable traits. But there were major problems with the technique. Most of the fetuses grew to enormous sizes, resulting in difficult births. More importantly, the technique "never proved to be economical," says James Robl, a professor of veterinary and animal sciences at the

University of Massachusetts, Amherst.

Whereas those obstacles drove off researchers in the United States and Europe, Japanese scientists persisted. The result, says Akira Iritani, a professor of genetic engineering at Kinki University who is working on the cloning of rabbits, is close to 400 head of cattle born using this embryonic nuclear transfer technique. Many of those prefectural researchers trained under Tsunoda, who had pioneered nuclear transfer work in Japan in the mid-1980s.

Until the Dolly news broke, most researchers believed that DNA from adult mammalian cells could not develop into a complete embryo. Indeed, the Agriculture ministry even had a policy that discouraged the use of adult somatic cells. But once Dolly appeared, officials quickly revised their guidelines and in August 1997 approved attempts to clone adult animals. The early work with embryonic nuclear transfer put Japanese researchers in a good position. "Everyone [in the cattle-breeding industry] is very familiar with nuclear transfer techniques," says Chikara Kubota, who heads a cloning research group at Kagoshima Prefecture's Cattle Breeding Development Institute that followed Tsunoda's group in successfully bringing a fetus cloned from an adult cow to term.

Tsunoda was not surprised by the Dolly announcement. He had already spent years looking at various types of cell nuclei for totipotency—the ability of a cell to differentiate into all the types needed by an organism—and had discovered that a mouse could develop from cells taken from the trophoderm, the first differentiated cells in an embryo which eventually form the placenta. "So we thought somatic cells should have totipotency," he says.

When setting out to replicate the Dolly experiment with cattle, the group grew several oocytes in culture to the blastocyst stage and examined them for abnormalities in the number of chromosomes. When the first



**Mooove over.** These calves lead a herd of successful cloning efforts by teams of Japanese scientists.

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## NEWS FOCUS

batch of blastocysts proved normal, the researchers prepared a second batch of oocytes with transferred nuclei and cultured the developing embryo on mouse fibroblast cells for 8 or 9 days. They then selected 10 of the blastocysts and implanted two in each of five hosts. All five became pregnant and were cared for at the Ishikawa Prefectural Livestock Research Center, on the Japan Sea coast 300 kilometers west of Tokyo.

Two other groups started cloning efforts at about the same time, and more joined the chase once news of Tsunoda's pregnant cows spread. But Tsunoda's group won the race when one of the surrogate mothers delivered healthy twin calves after 243 days, only 37 days earlier than a normal gestation. The average weight at birth of the eight calves, all born naturally, was about 32 kilograms; the average for natural pregnancies is about 27 kilograms.

Despite Tsunoda's success, other groups have had problems with large calves, including one cloned from fetal cells that weighed in at a whopping 52 kilograms after a cesarean section. And although it won't be clear until their reports are published, few of the other groups seem to be matching Tsunoda's success rate. Kubota says only about 10% of his team's implanted embryos have been carried to term, and the Roslin Institute group produced only one live sheep from 13 transferred blastocysts.

Tsunoda believes that the most important factor in his high success rate may be his use of cumulus and oviductal cells, because of their role in reproduction. Other groups have used a variety of cells, including muscle and skin cells. Tsunoda is hoping to begin to clarify such issues through a systematic screening of cells. A second batch of cloned calves still in utero has been produced using cells from 20 different tissues, including the liver, kidney, and heart. Tsunoda anticipates results by spring.

Most research efforts outside Japan are likely to focus on cloning cattle from fetal rather than adult cells, says Will Eyestone, a reproductive physiologist working on transgenic animals at PPL Therapeutics Inc. in Blacksburg, Virginia. Embryonic cells containing fetal or embryonic DNA are believed to grow faster, and the resulting animals appear to live longer than if they had been cloned from adult cells, he notes. "It's a better way to go," adds Robl, if the goal is to modify the genetic makeup of the animals so that their milk contains drugs for use in humans. Using adult cells might offer an advantage in getting exact copies of cows or bulls that are particularly valuable for breeding purposes or for meat, however. But producing calves for agricultural purposes through cloning is not likely to prove economical in the United States. "We don't have a market for very high

premium beef," Robl says.

But Japan does. "The cost of agricultural products in Japan is high, but they still sell," says Tokyo University's Tomohiro Kono. Superpremium Matsuzaka beef roasts, for example, cost \$100 a pound, and those prices would support the expense of cloning prize beef cattle. But just how commercially important cloning might be is an open question. A genetically ideal calf is just the starting point for Matsuzaka beef. The animals are also fed beer and given daily massages as part of a regimen that results in fine flecks of fat uniformly scattered throughout the meat. "We have a long way to go to make [premium] beef inexpensive," says Hiroto Takahashi, an official in the Agriculture ministry's animal production division. "In other countries, there would be no meaning in producing [cattle] this way," he adds.

Given those limitations, some scientists feel that the research efforts should be focused on understanding the cloning mechanism itself. Kono, who uses rabbits to study that mechanism, says that while it was important to confirm the Roslin results in cattle, there is no need to have the efforts duplicated by so many groups. "It is a Japanese trait, [in which] everyone heads in the same direction," he says. "There isn't much originality in the research."

Tsunoda agrees that a lot of the work is redundant. But using different cells may help researchers clarify the mechanism through which cells are reprogrammed to start the development process anew. "Right now, what happens in cell reprogramming is a black box," he says. "We are at the starting point to study the reprogramming [of cells]."

—DENNIS NORMILE

## COMPUTER SCIENCE

# From Army of Hackers, an Upstart Operating System

The open-source software movement has developed a free computer operating system that is poised to compete with Microsoft's Windows

In the titanic struggle between Microsoft and the Justice Department, one of the software giant's chief defenses against the charge of monopoly-building is to argue that its lead in the operating-system market is vulnerable. New competitors, say the company, could challenge it at any time. That argument may seem laughable on the surface,

given that neither Apple nor IBM was able to best Microsoft in the operating system wars. But there is a competitor on the horizon—and if internal Microsoft documents are to be believed, the software Goliath may be showing some nervousness.

David in this case is called Linux ([www.linux.org](http://www.linux.org)), a simpler-to-use variant of the old standby UNIX. Already, Linux is the operating system of choice for Internet servers, the computers that route Internet traffic and host sites on the World Wide Web, and its use is growing rapidly for small-sized servers on local area networks. It also serves as the operating system on a cut-rate supercomputer at Los Alamos National Laboratory, called Avalon. And now, thanks to a massive effort by programmers around the world, all but a handful working for free, Linux is poised to make significant inroads in the workstation and desktop personal computer world, which is largely the domain of Microsoft and, to a lesser degree, of UNIX.

Linux, by all accounts, is stable, powerful, and fast—and it's free. Yes, free: Linux is the fruit of a kind of online commune, an intellectual descendant of the counterculture of the 1960s. And although free software is



**Supercomputing on the cheap.** The Linux operating system melds 140 desktop machines into the Avalon supercomputer, developed by Michael Warren and colleagues.

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