Dolly: a hard act to follow

Four years after Dolly's death, scientists are still amazed she was ever born. That's because the decade since the announcement of Dolly's birth has brought cloning researchers few triumphs and many frustrations. They may have learned a little more about the events that allow an egg to reprogramme the nucleus of an adult cell to produce a new organism. But these advances haven't led to big improvements in the cloning process, or yielded huge commercial payoffs.

In many ways, scientists are still fumbling in the dark, says Robert Lanza, vice-president for research and development at Advanced Cell Technology in Worcester, Massachusetts. "When Dolly was born, we thought that in a few years we would understand the magic in the egg that allows it to reprogramme a cell's DNA," Lanza says. "But cloning is still essentially a black box."

Frogs were cloned as early as 1952, but mammals proved too problematic until scientists at the Roslin Institute in Edinburgh cloned two sheep — Megan and Morag — from embryonic cells in 1995. But it was the group's creation of Dolly, cloned from an adult udder cell, that finally overturned the idea that in mammals, developed cells could not reverse their fate.

After Dolly, scientists thought they would soon be able to clone many other mammals. Some envisaged an industry of cloning applications, from the production of medicines in live bioreactors — cloned, genetically modified livestock — to the creation of herds of cloned food animals.

Scientists have made some progress towards potential applications. For instance, they have cloned transgenic pigs that might one day be used as organ donors, transgenic cows and goats that produce proteins that some humans lack — they have even cloned pets. But the low efficiency of the cloning process has stymied industrial development, and most companies set up to commercialize cloning have shut down.

Only 2–5% of cloned animal embryos grow into healthy offspring. This is slightly better than a decade ago — Dolly was the only lamb born from 277 cloned embryos — but it is still far below the efficiency demanded by industry.

There are several reasons why it has been difficult to figure out how cloning works. Early development is slightly different in every species, so cloning a new species is an arduous, trial-and-error process that requires scientists to use thousands of eggs. But eggs aren't always easy to obtain, especially for monkeys and humans, and no clones of either have been born. And even if eggs are available, some of scientists' traditional model species, such as mice, have proved extremely problematic.

> "I think we should be surprised that cloning works at all."

Yet some see a way ahead. Many of the abnormalities that doom cloned embryos are due to faulty epigenetics — a set of controls that silences or activates genes by chemically modifying the DNA or by binding it to certain proteins. Epigenetic processes guide young cells with limitless potential into restricted adult fates, by shutting down certain genes. During cloning, an adult nucleus is transplanted into an egg, which must then erase the adult genome's epigenetic marks, so it can re-express every gene necessary to build a new animal.

But requiring an egg to do this reprogramming on its own bypasses other natural mechanisms that normally help reset a cell's epigenetic programme. Perhaps it's not surprising that the egg doesn't always do a perfect job, says Ian Wilmut, Dolly's creator and director of the Scottish Center for Regenerative Medicine at the University of Edinburgh. "When you think about what we're asking the egg to do for us, in a way, I think we should still be surprised that cloning works at all," Wilmut says.

Rudolf Jaenisch, a biologist at the Massachusetts Institute of Technology's Whitehead Institute for Biomedical Research, is among those trying to decipher these epigenetic glitches. If they can understand what goes wrong with the reprogramming, they may be able to fix it — or, better yet, initiate the process themselves by adding chemicals or proteins to adult cell nuclei. This would allow scientists to bypass the need for eggs altogether.

The field is moving in this direction, trying to find ways to reprogramme cells without using eggs. Last summer, for instance, Shinya Yamanaka and Kazutoshi Takahashi from Kyoto University in Japan used a cocktail of proteins to turn adult mouse cells into more flexible cells that behave a lot like stem cells (K. Takahashi and S. Yamanaka *Cell* **126**, 663–676; 2006).

This molecular approach solves the efficiency problem and avoids society's ethical qualms about cloning (see page 800). "For many of us, that is the holy grail, and nuclear transplantation is an intermediate stage," says Alan Colman, chief executive of Singapore-based company ES Cell International and former research director at PPL Therapeutics, which contributed to the Dolly paper.

Egg-free approaches may also enable what many see as the most promising potential application of cloning: the creation of human embryonic stem cells, or cells made from them, that could be used to treat human disease.

Many scientists see human embryonic stem cells as having the most potential for the next decade, especially if scientists learn to reprogramme cells without using eggs.

"I think in ten years, people will learn how to convert one cell type to another, without nuclear transfer, without eggs, in a Petri dish," says Jaenisch. "Once we learn all the rules, maybe one day it will be possible."

Erika Check

2003

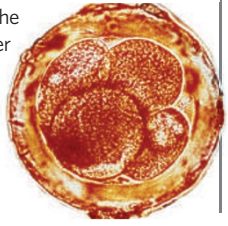
Italian scientists at the Laboratory of Reproductive Technology in Cremona announce Prometea, the first horse (*Equus caballus*) clone created from a skin cell, raising hopes that clones could one day perpetuate the genetic line of castrated geldings.

2003

French and Chinese scientists unveil Ralph the cloned laboratory rat (*Rattus norvegicus*). Rats had been tough to clone because rat eggs divide before the point at which the donor DNA is injected, so the technique relied on using drugs to inhibit division.

2004

Although Seoul National University researcher Woo Suk Hwang's claim to have derived stem-cell lines from cloned human embryos was later discredited, his group can still boast the most experience, and probably the highest number of cloned human embryos, but there is no hard evidence for this.



2005

Hwang's lab announces Snuppy the cloned dog. Although much of the stem-cell research from this lab has been discredited, Snuppy's clonal credentials have been confirmed.

Heidi Ledford

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