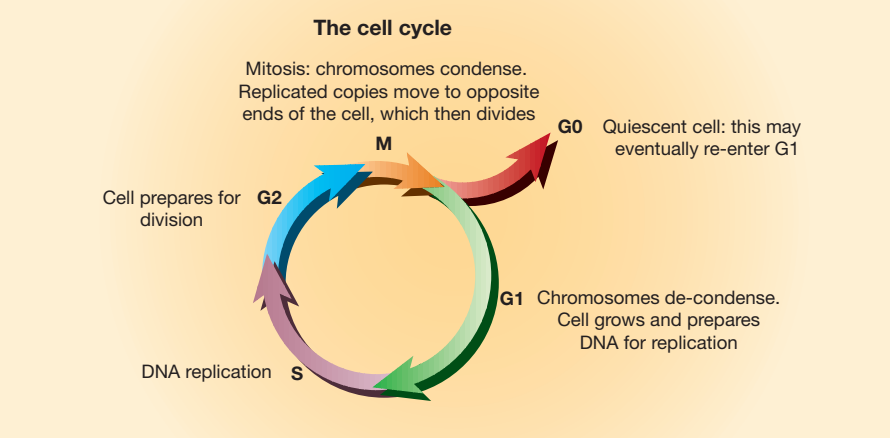
Cloning's owners go to war

The team that created Dolly the sheep captured the headlines, but several groups now have patents on cloning. Peter Aldhous considers how this tangled web of proprietary claims will affect the future of the technology.

To the uninitiated, it seemed like an arcane scientific argument. In September 1998, some 18 months after Dolly the cloned sheep was unveiled to the world, her creators issued a challenge. They disputed one of the conclusions drawn by a group claiming to have repeated the feat of cloning animals, this time in cattle.

Ian Wilmut, of the Roslin Institute near Edinburgh, and Keith Campbell, then with a Roslin spin-off company, PPL Therapeutics, did not question that central claim. But in a letter to *Science*[1], they took issue with their rivals' assertion that the donor cells used to clone the calves had been actively dividing. The cow cloners, from the University of Massachusetts in Amherst, and Advanced Cell Technology (ACT), a company in nearby Worcester, defended their interpretation[2].

This was no ordinary scientific spat. The Roslin researchers' challenge is central to the relative value of the patents that they, and their rivals, have subsequently been awarded. And with others claiming rights to further pieces of intellectual property in cloning, the battle lines are being drawn. Already, one cloning patent infringement suit has come to court, and with more groups muscling in with their own claims, it seems unlikely to be the last. "It's a very tangled web," says Steven Stice, formerly with the Massachusetts team and now at the University of Georgia in Athens.



Taking a breather: many cells opt out of the cell cycle of growth and division to sit in a quiescent state.

The technique used to make Dolly is called nuclear transfer cloning. The idea is to take an egg cell, strip out its chromosomes, and then fuse it with another cell, which donates its nucleus to the egg. If the egg is then activated so that it starts dividing, it can develop into an animal that is a clone of the individual from which the donor cell was taken.

Scientists interested in animal production had been experimenting with nuclear transfer for many years. But they had only succeeded using undifferentiated donor cells from very young embryos. The consensus emerged that, in mammals, it was impossible to produce clones from differentiated cells, specialized for a particular function.

Quiet life

Dolly, cloned from a cell derived from an adult ewe[3], conclusively overturned that dogma. But in truth, the real breakthrough had come in March 1996, when the Roslin team described two lambs, Megan and Morag, cloned from cells that came from fetuses, but were nonetheless differentiated[4].

The Roslin researchers thought the key to such cloning was sending the donor cells into a quiescent state known as G0. Cells that are proliferating go repeatedly through the series of phases in the cell cycle — growing, copying their DNA, and dividing (see above). But many cells cease dividing and sit in the G0 state. In laboratory cultures, cells can be forced into the G0 state by depriving them of nutrient-rich serum. And that is what Campbell, the Roslin team's cell biologist, did. In August 1995, the Roslin researchers filed patents claiming rights to cloning from differentiated, quiescent cells.

At face value, the Massachusetts researchers were way off the pace. In May 1998, they published a paper that repeated the Megan and Morag accomplishment with cows[5]. But in the context of intellectual property, it was significant. The researchers, led by Stice and his colleague James Robl, took their donor fetal cells from an actively dividing culture, missing out

Making cloning pay

AP

PRODUCING ANIMALS OF HIGH GENETIC VALUE
If you have managed to breed a cow that regularly breaks records for its milk yields, why risk losing its combination of high-quality genes in the lottery of sexual reproduction? Cloning offers a means to create herds of carbon-copy animals.

PHARMING HUMAN PROTEINS
Many human diseases — haemophilia, for instance — are caused by defects in the production of proteins. Several companies are now trying to make genetically engineered animals that secrete human proteins in their milk. By combining cloning with a transgenic technology called gene targeting, herds of these 'pharm' animals can be created.

XENOTRANSPLANTATION
Pigs could provide a supply of organs for patients on transplant waiting lists, if the organs can be genetically engineered so that they do not immediately trigger rejection by the human immune system. Again, cloning can be combined with gene targeting to produce these precious animals.

THERAPEUTIC CLONING
Here the goal is to repair the human body with cell and tissue grafts that are perfectly matched to the recipient. Just take a healthy cell from the patient, use it to create a cloned embryo, then — after just a few days — dissect it to remove the embryonic stem cells that, in theory, can be grown in culture to give rise to any of the body's tissues. Working out how to achieve that, however, poses huge scientific challenges.

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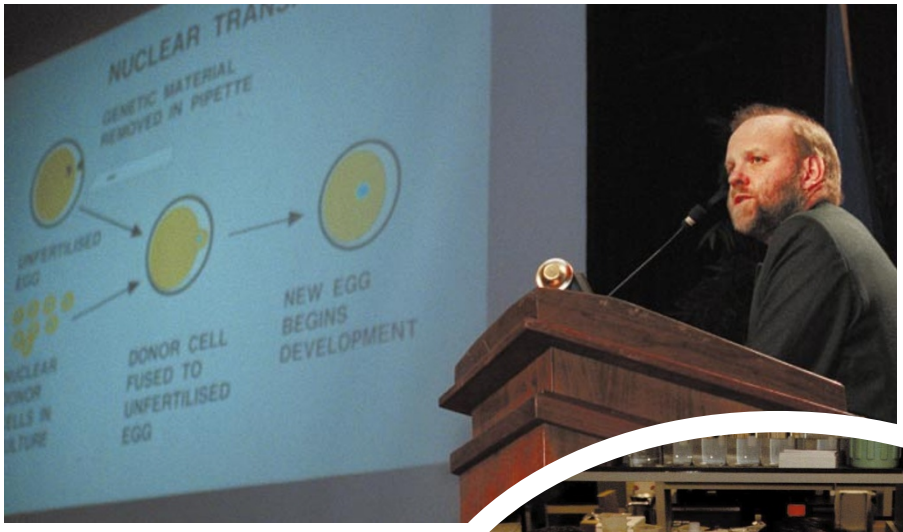

RICK FRIEDMAN

Property dispute: both Wilmut (above) and the team at Advanced Cell Technology (left to right: West, Cibelli and Robl) hope their patents on cloning will dominate the field.

the serum starvation step. They described these cells as "non-quiescent", and argued that they were in a phase called G1, in which cells grow and prepare to copy their DNA. More than a year before, in January 1997, they had applied for a patent. It covered cloning from proliferating differentiated non-human cells — that is, cells in any phase except G0.

As cloning has a range of possible commercial applications (see 'Making cloning pay', opposite), the Roslin team had to respond. The group argued that the Massachusetts researchers could not prove that the individual cells from which they had cloned their calves were not in G0, even though the cells were in a dividing culture — a view that Wilmut still holds. "To my knowledge, there is nothing yet published that clarifies the situation," he said, when questioned by *Nature* on the issue after a lecture in London last month.

Any cell will do?

It is difficult to determine precisely where in the cell cycle an individual cell is. But now that many groups have experimented with cloning from proliferating cell cultures, it is hard to find anyone who thinks that putting donor cells into G0 is the key. "It is probably going to be shown that quiescence is not an essential factor," says Davor Solter of the Max Planck Institute for Immunobiology in Freiburg, Germany. Even Campbell, now at the University of Nottingham, acknowledges the consensus. "I've always been very careful to say that G0 may be beneficial," he says. "It doesn't mean that other stages might not work."

Meanwhile, the disagreement has shaped up into a tussle between ACT and a rival company, Geron of Menlo Park in California. The US patent granted to the University of Massachusetts in August 1999 is exclusively licensed to ACT. Geron won extensive rights to the British patents that were eventually awarded to Roslin in January this year, by acquiring a spin-off, Roslin BioMed, in May 1999.

ACT's chief executive officer, Michael West, knows Geron well — he was one of its founders. "They've got a piece of the pie, and we've got the rest of the pieces," he says. Not surprisingly, Geron sees it differently. David Earp, the company's vice president for intellectual property, argues that it should be possible under US patent law to extend the claims of the Roslin patents to all forms of cloning using adult cells. "Our intellectual property estate eventually will acknowledge the historic breakthrough represented by the cloning of Dolly," he asserts.

Maybe so, but many observers believe ACT has played a clever game. Alan Colman, research director at PPL Therapeutics, describes the broad claims in its patent as "opportunistic", and says he was surprised they were granted. "But it will be quite difficult to get rid of the ACT patent," he adds.

But it is not just the basic patents on cloning from differentiated cells that will determine who makes a commercial success of the technology. To bring many applications to market, nuclear transfer will have to be combined with other technologies. To clone transgenic animals that produce valuable human proteins in their milk, for instance, it must be combined with techniques for creating these animals. For these applications, Roslin has licensed its patents to PPL Therapeutics, which is developing its own transgenic technology. ACT, meanwhile, has linked up with Genzyme, a company based in Cambridge, Massachusetts.

Geron is interested in therapeutic cloning, which aims to grow human tissues to replace those that are damaged or diseased. The company has exclusive access to two other technologies that it sees as crucial. First, it holds licenses to patents on human embryonic stem cells, which would be isolated from cloned embryos a few days after their creation, and from which replacement cells and tissues could be grown. Second, it controls patents on telomerase, an enzyme that rebuilds the caps, or telomeres, on the ends of chromosomes. These caps shorten as cells age, so to ensure that cloned tissue grafts are vibrant and healthy, Geron says, it will be necessary to rejuvenate the donor cells using telomerase.

But true to form, ACT claims it can stake out its own position. The company's existing cloning patent is not relevant here, as it only covers nuclear transfer from a non-human donor cell. But, controversially, ACT revealed in November 1998 that one of its scientists, Jose Cibelli, had fused cells taken from his body with bovine eggs, stripped of their chromosomes, to create embryos which were allowed to grow for several days. Arguably, this would allow ACT to generate embryonic stem cells that would not be covered by Geron's patents, as they could not be regarded as completely human.

Cibelli's experiments are the subject of a patent application from ACT. And in April this year, ACT suggested that the act of cloning itself rejuvenated donor cells in cattle, rebuilding their telomeres to beyond the length seen in newborn calves[6]. "We believe we can make therapeutic cloning work without transgressing any of the Geron patents," says West.

Animal pharm

The idea of using human–cow hybrid cells for therapeutic cloning is viewed with scepticism by many experts — even if it works, the regulatory hurdles would be immense. What is more, Cibelli's work has never been published in a peer-reviewed journal. But biologists are intrigued by ACT's telomere paper[6], and the implication that therapeutic cloning need not depend on telomerase. "It might be it's necessary, but I don't think we have enough information to say," says Colman.

Other companies are adding to the confusion. Most significantly, ACT has found itself on the wrong end of a patent infringement suit from Infigen, a company in DeForest, Wisconsin. Infigen has long been interested in cloning

cows of 'high genetic value', and has recently teamed up with the American Red Cross and Pharming, of Leiden, the Netherlands, to clone animals that produce human proteins in their milk. The company is also working with Imutran of Cambridge, England, and the Swiss-based drugs giant Novartis, on cloning in xenotransplantation — the use of organs from animals in human transplantation.

Staking a claim

Infigen owns a suite of patents on the basic techniques of nuclear transfer, awarded before the Roslin team demonstrated that it is possible to clone mammals from differentiated cells. Last year, it sued ACT for breaching two US patents on cow cloning, one covering a specific culture medium, the other a method for activating bovine eggs after transferring the donor nucleus. Infigen also claimed that Stice, who had once worked for Infigen, stole its trade secrets. That complaint was rejected, but in June 1999, the US District Court in Wisconsin ruled that ACT had indeed infringed Infigen's patents — after which the two companies came to a confidential settlement.

Ominously, it seems that other cow cloners could soon be hearing from Infigen's lawyers. "We're taking steps right now to inform several parties about our patent estate," says Michael Bishop, the company's vice-president for research.

Infigen gained a further US patent in January this year, which is causing raised eyebrows. Again specific to cows, this patent covers cloning from fetal cells that have been developmentally 'reprogrammed' by treating them with specific biochemical growth factors. Given that the Roslin team and others have shown that nuclear transfer itself can reprogramme differentiated cells, most researchers cannot understand the relevance of the extra step.

Although the significance of Infigen's new patent remains unclear, everyone in the field is watching for the emergence of other patents that could alter the intellectual property landscape. "In my mind, there's no overarching patent out there," says Stice. "We are still all trying to find one technique that is efficient." Indeed, most cloning groups only get one or two live births for every hundred nuclear transfer procedures they perform.

Many observers are keeping a close watch on PPL Therapeutics, which in March announced that it had cloned five pigs from adult cells using a novel technique. Details have not yet been published, but Colman claims the method is "significantly different" from anything described previously. That could be important, as pigs have proved hard to clone, and are the animals of choice for xenotransplantation. An Australian company, Stem Cell Sciences of Melbourne, also claims to have developed an alternative method of cloning that similarly remains under wraps.

"The intellectual property situation in this field is very complex at the moment," says Emma O'Donovan, editorial analyst at Derwent Information, a company in London specializing in patent information. "The wording and scope of individual claims will have to be examined very carefully."

If the current confusion is not resolved, the danger is that the situation will restrict the flow of money needed to develop the technology. Investors like the ownership of the key intellectual property to be clear, explains Neal First of the University of Wisconsin in Madison, a cloning specialist who sits on the board of a venture capital company interested in the field.

But perversely, the confusion could have a stimulating effect in the short term. "There are scientists starting little companies without much regard to where the intellectual property lies," says First. "And they are increasing the pool of knowledge." But if the writs begin to fly, some of these scientists may wish they had been more circumspect. ■

Peter Aldhous is *Nature*'s Chief News and Features Editor.

PPL THERAPEUTICS/NEWSMAKERS

Quintuple vision: PPL Therapeutics has devised a new technique that has enabled it to clone five piglets.

Paradise lost in Hawaii

No discussion of cloning's landmark achievements would be complete without mentioning 'team Yana'. Working in the lab of Ryuzo Yanagimachi at the University of Hawaii in Honolulu, scientists led by Teruhiko Wakayama (below) stunned the world in 1998 by cloning scores of mice, some of them clones of clones[7].

Rather than fusing a donor cell with an egg, like other groups working in the field, Wakayama developed a technique in which he removed the donor cell's nucleus and injected it directly into the egg using a piezoelectric device. The university filed for a patent on the method, and granted an exclusive license to a local company called ProBio, headed by Australian businessman, Laith Reynolds.

But the story has since gone sour. ProBio is still waiting for the cloning patent to be granted, but 'team Yana' has broken up. Tony Perry (right), a member of the team, is suing the university over the rights to a transgenic technology, now licensed to ProBio, which was developed by him while he was a European Molecular Biology Organization research fellow in Yanagimachi's lab. Although Wakayama has not sued over the rights to his cloning technique, he is understood to be similarly unhappy. Both scientists have now left for Rockefeller University in New York, but declined to discuss the reasons for their move with *Nature*.

PROBIO AMERICA/NEWSMAKERS

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