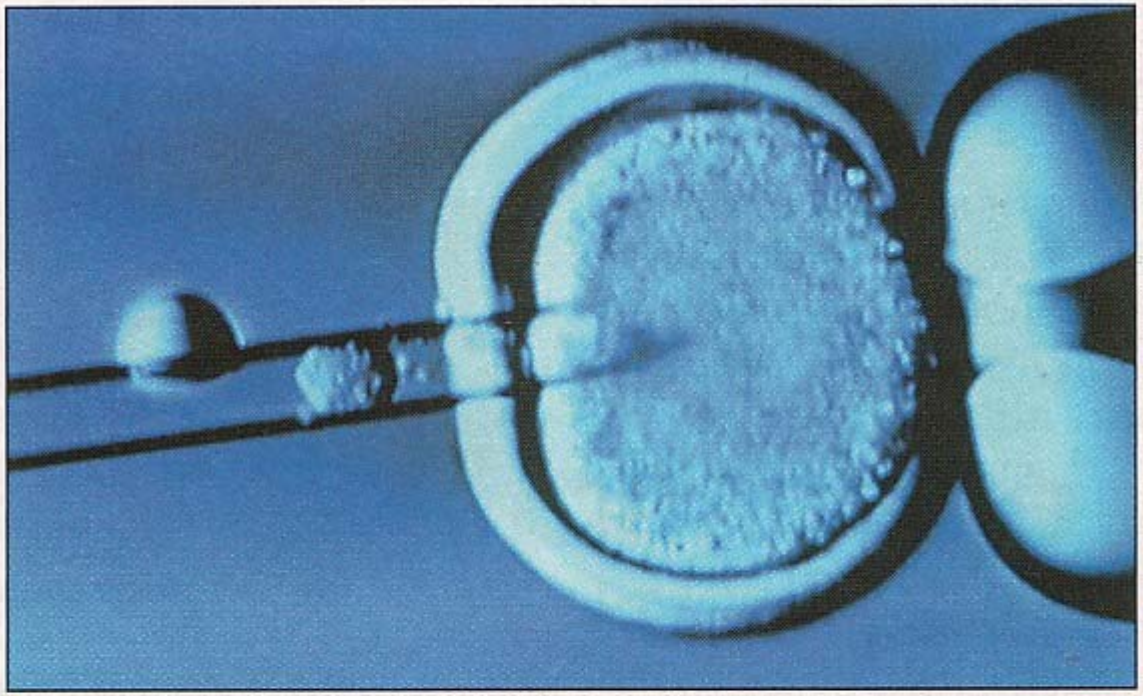
Nisäkkäiden kloonaus

Tuma on se joka kloonataan eli monistetaan (nuclear cloning)

Idea: otetaan halutun luovuttajan (donor) tuma

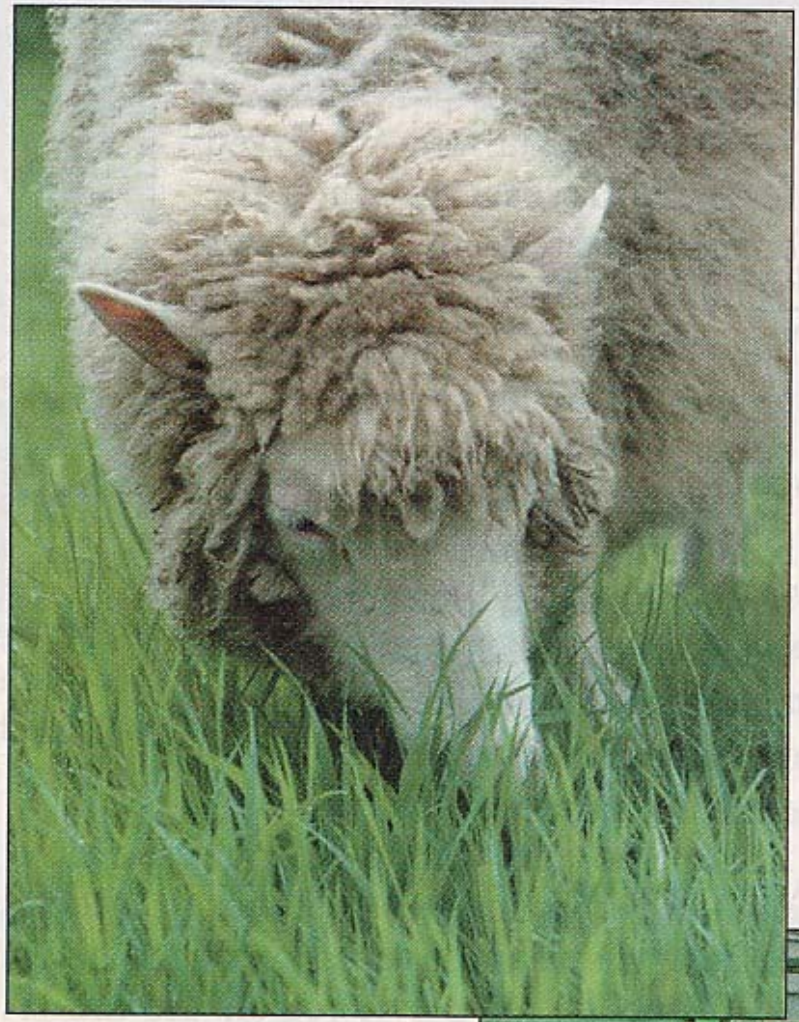
Istutetaan se vastaanottajan (recipient) munasoluun

Siirretään konstrukti kasvamaan luonnonmukaisiin oloihin



Forbidden? Microinjection techniques have enabled scientists to remove and insert nuclear material into ooctyes. If the U.S. Congress has its way, this cloning process will be banned in humans.

Science 10 Aug 2001



Proofs of principle. Dolly (*above*) showed that cloning could be done. Cupid and Diana show that gene targeting is possible.

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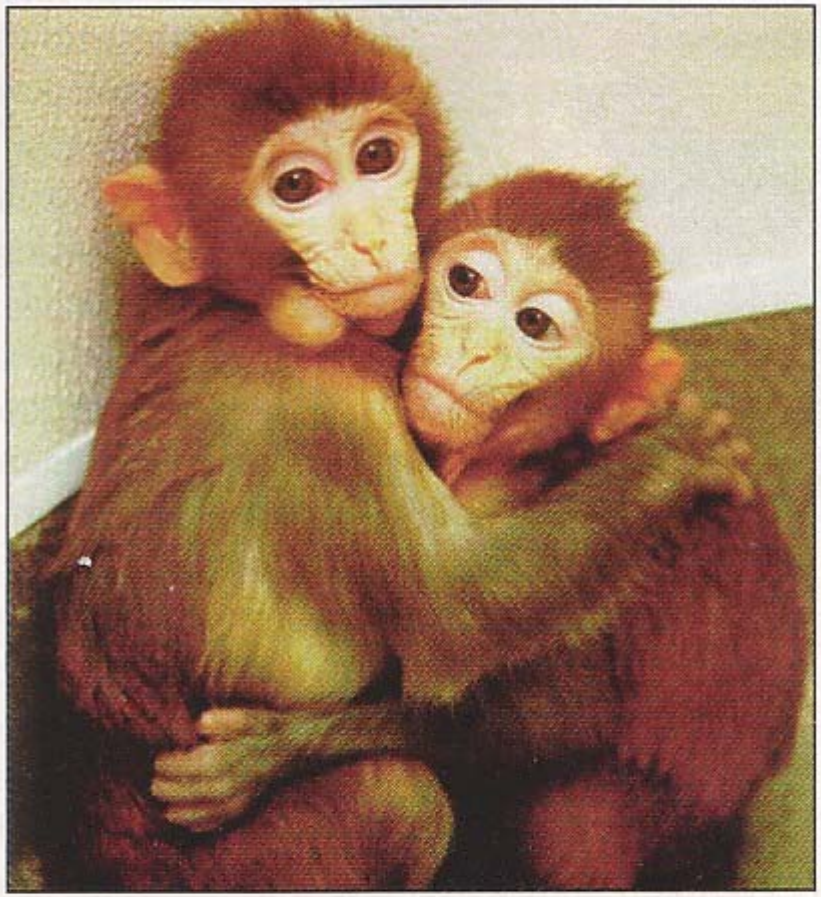


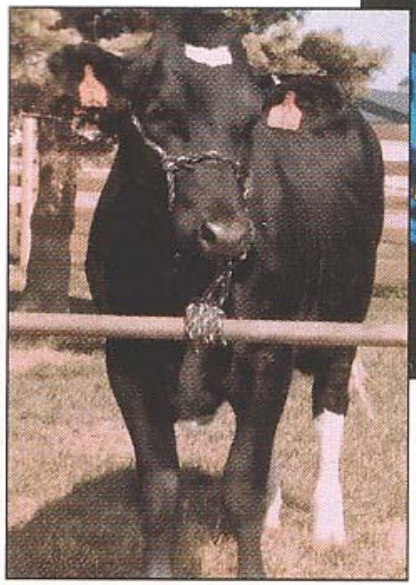
Lonesome twosome. Cloned from embryonic cells, Neti and Ditto are still the only cloned primates, despite years of effort by several groups.

Science 9 June 2000

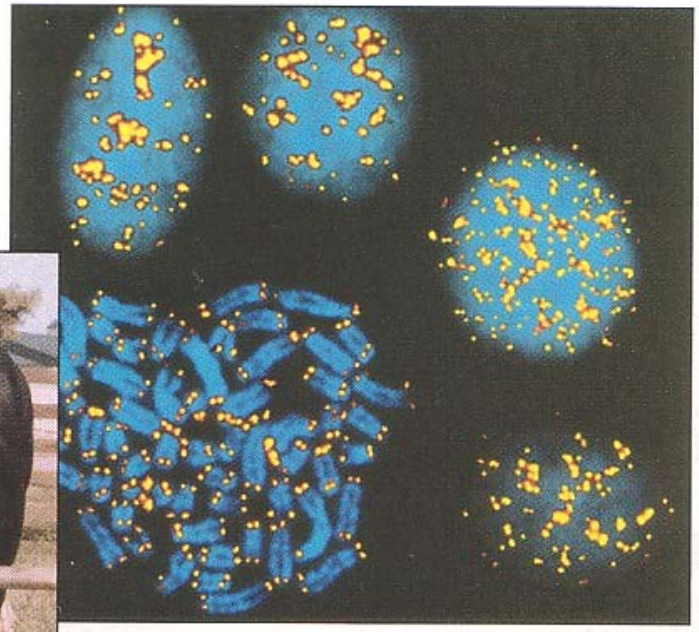
CELL BIOLOGY

In Contrast to Dolly, Cloning Resets Telomere Clock in Cattle

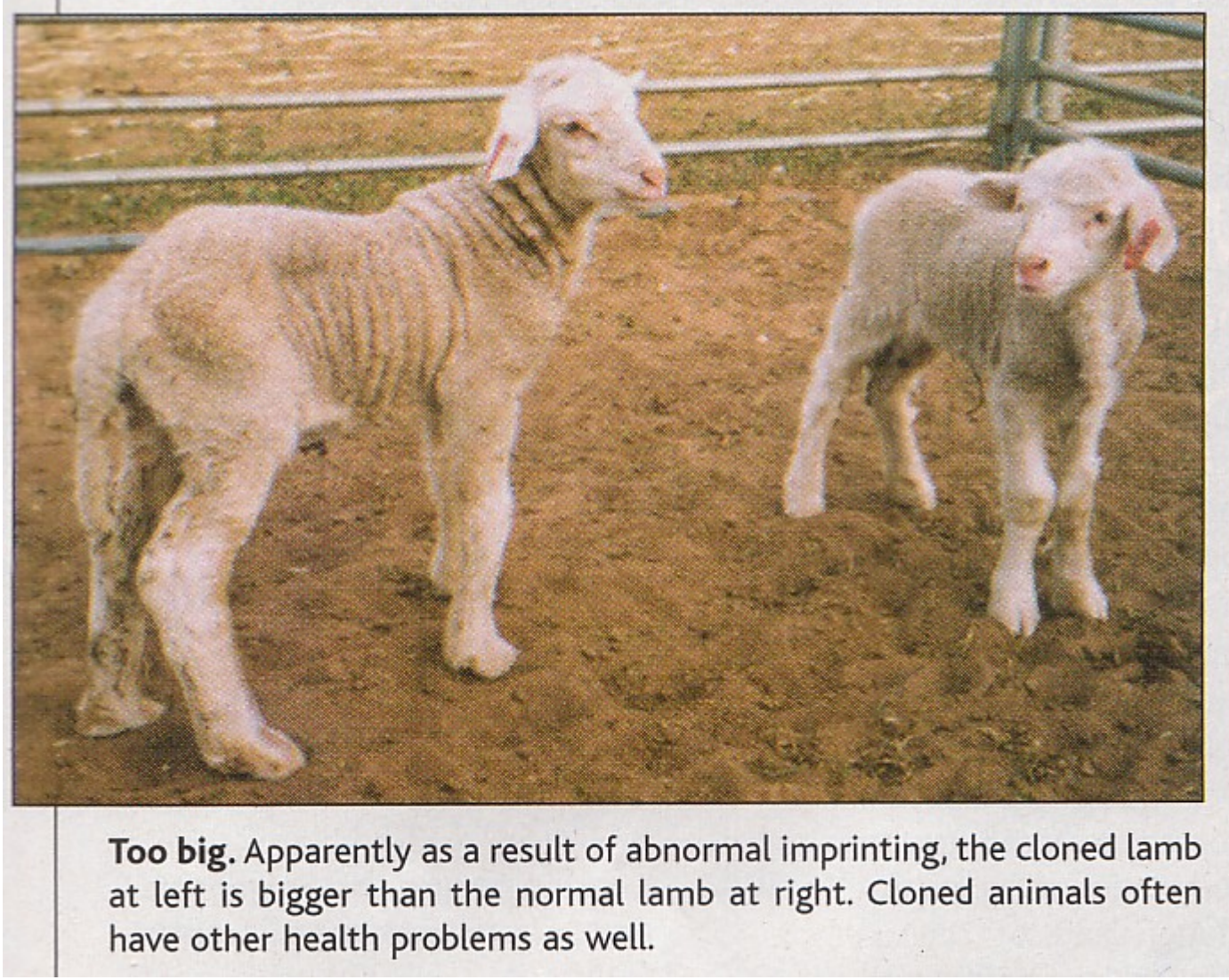
When researchers announced 3 years ago that they had cloned Dolly the sheep, many scientists asked a question that sounds almost metaphysical: Are her cells older than she is? Because Dolly had been cloned from an adult cell, they wondered whether her own cells would show some of the hallmarks of a more mature animal. The answer came in 1998: Dolly's telomeres—the "caps" on the ends of her chromosomes—are shorter than normal. Because telomeres normally shrink with age, this was a disturbing sign that her cellular clock hadn't been reset to zero. Not only did the finding imply that Dolly might age unusually quickly, but it also dampened hopes that the cloning technique might someday be used to produce replacement cells for patients suffering ills such as liver failure or



Cloning is accomplished by transplanting nuclei from somatic cells into eggs whose own nuclei have been removed. When the goal is to produce animal clones, embryos that develop from those cells are implanted



Tying up loose ends. Persephone the clone has longer telomeres than her normal counterparts. The telomeres above glow yellow in cells from a cloned fetal calf.



Too big. Apparently as a result of abnormal imprinting, the cloned lamb at left is bigger than the normal lamb at right. Cloned animals often have other health problems as well.

Ensimmäinen kloonattu nisäkäs oli Dolly-lammas (Wilmut ym. 199Z)

Nämä lampaanpoikaset ovat muita kuin Dolly

Science 10 Aug 2001

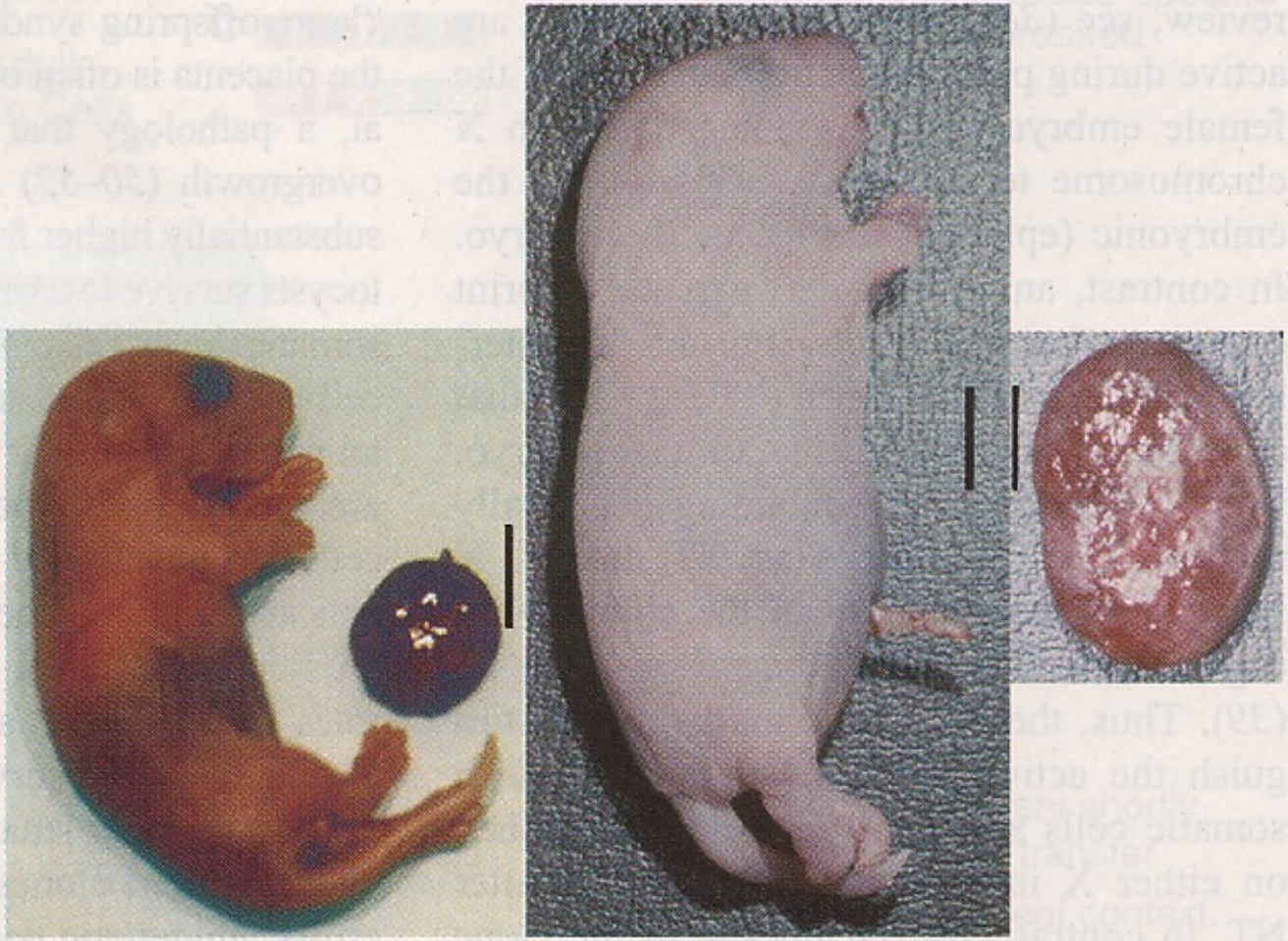


Fig. 2. Photographs of ES cell–derived mice at term. The pup and placenta on the left are derived from tetraploid embryo complementation and appear grossly normal. In contrast, the pup on the right, derived by nuclear transfer of the same ES cell line, shows a dramatic example of the commonly observed overgrowth phenotype seen in cloned mice. Extensive fetal and placental overgrowth are observed accompanied by edema, and this animal did not survive. Scale bars, 1 cm. [from Eggan *et al.* (*55*); copyright 2001 National Academy of Sciences, U.S.A.]

Nuclear Cloning and Epigenetic Reprogramming of the Genome

William M. Rideout III,[1] Kevin Eggan,[1,2] Rudolf Jaenisch[1,2]*

Cloning of mammals by nuclear transfer (NT) results in gestational or neonatal failure with at most a few percent of manipulated embryos resulting in live births. Many of those that survive to term succumb to a variety of abnormalities that are likely due to inappropriate epigenetic reprogramming. Cloned embryos derived from donors, such as embryonic stem cells, that may require little or no reprogramming of early developmental genes develop substantially better beyond implantation than NT clones derived from somatic cells. Although recent experiments have demonstrated normal reprogramming of telomere length and X chromosome inactivation, epigenetic information established during gametogenesis, such as gametic imprints, cannot be restored after nuclear transfer. Survival of cloned animals to birth and beyond, despite substantial transcriptional dysregulation, is consistent with mammalian development being rather tolerant to epigenetic abnormalities, with lethality resulting only beyond a threshold of faulty gene reprogramming encompassing multiple loci.

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Epigenetic reprogram
tion and nuclear transfer
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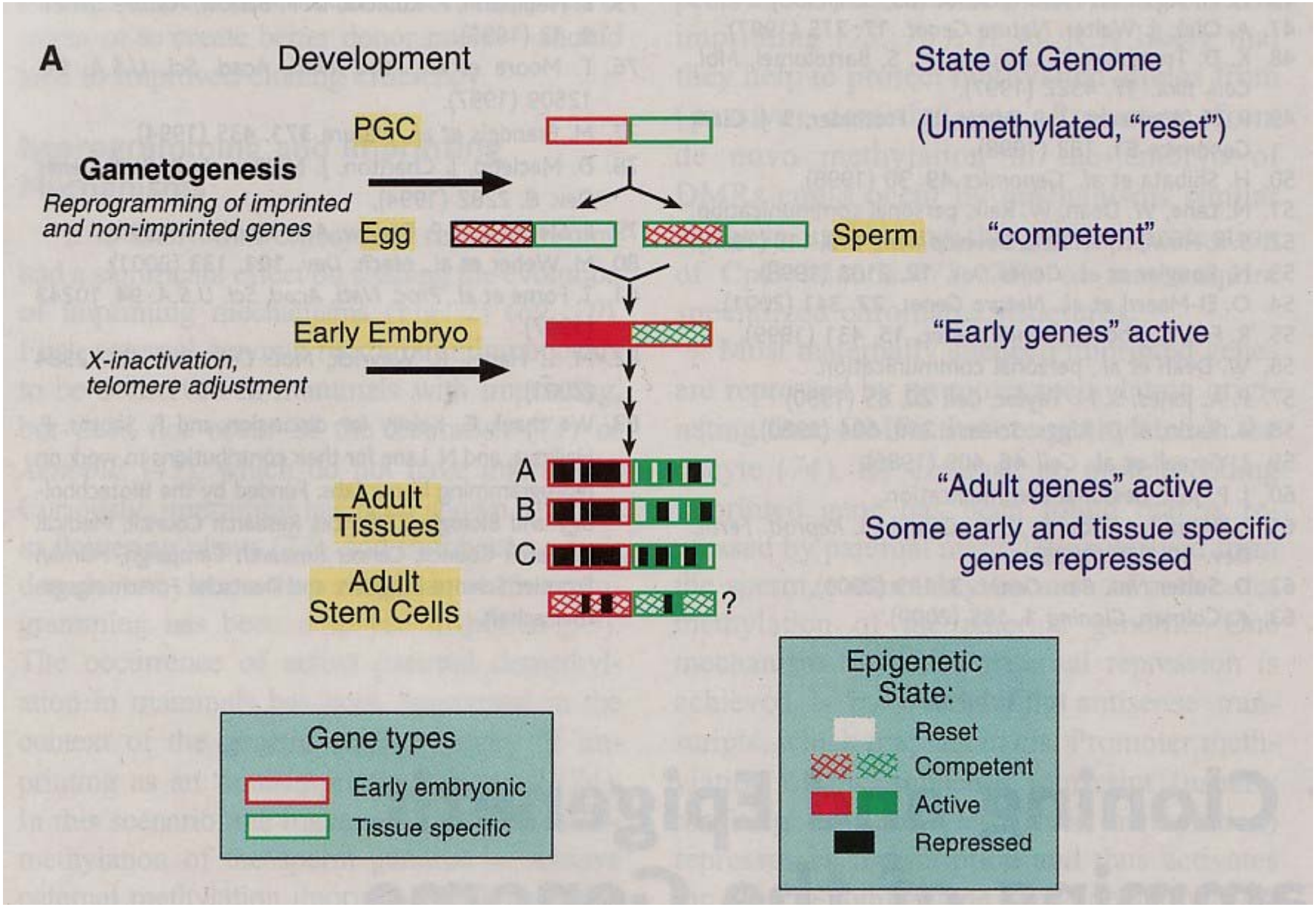
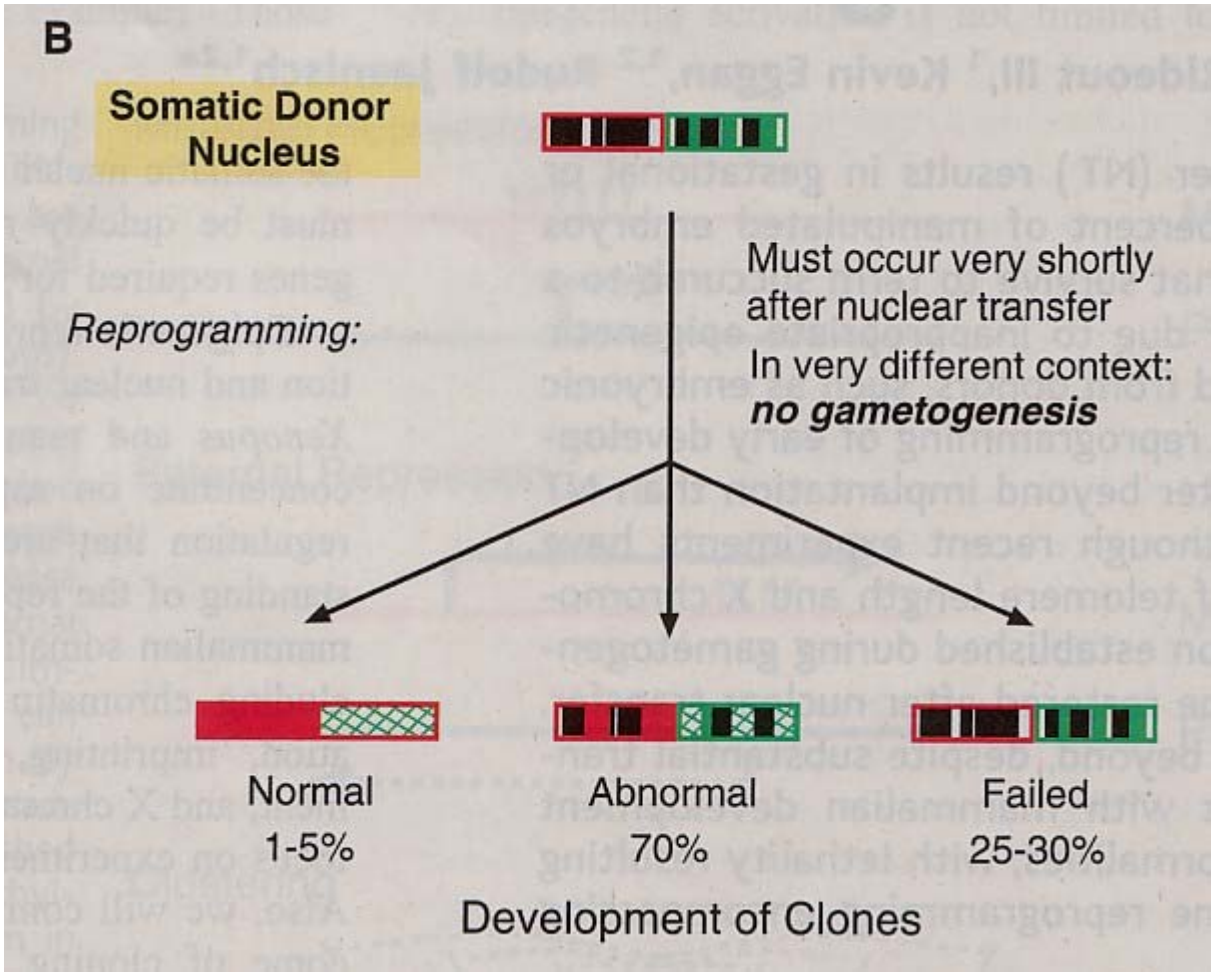
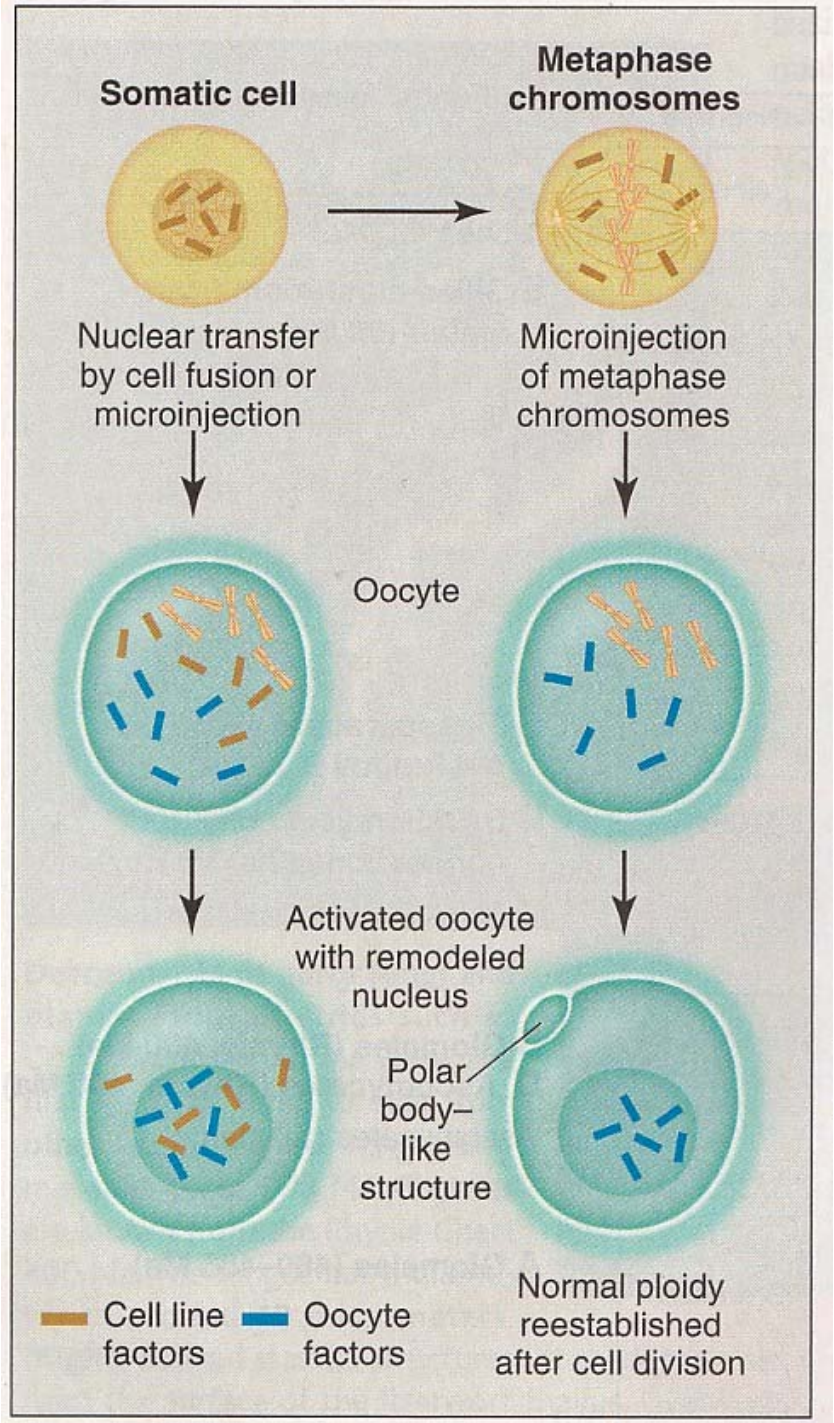


Science 10 Aug 2001



Science 10 Aug 2001



Mitä vähemmän somaattisen solun ainetta (faktoreita) tulee kromosomien mukana, sitä helpommin uskotaan kromosomien ohjelmoituvan uudelleen alusta

Science 15 Sept 2000

Kantasolut (stem cells)

Unipotentit: yhtä tyyppiä

Pluripotentit: monia solutyyppejä

Aikuiset kantasolut

Embryonaaliset kantasolut

Ihmisellä on loppujen lopuksi parisataa erilaista solutyyppiä

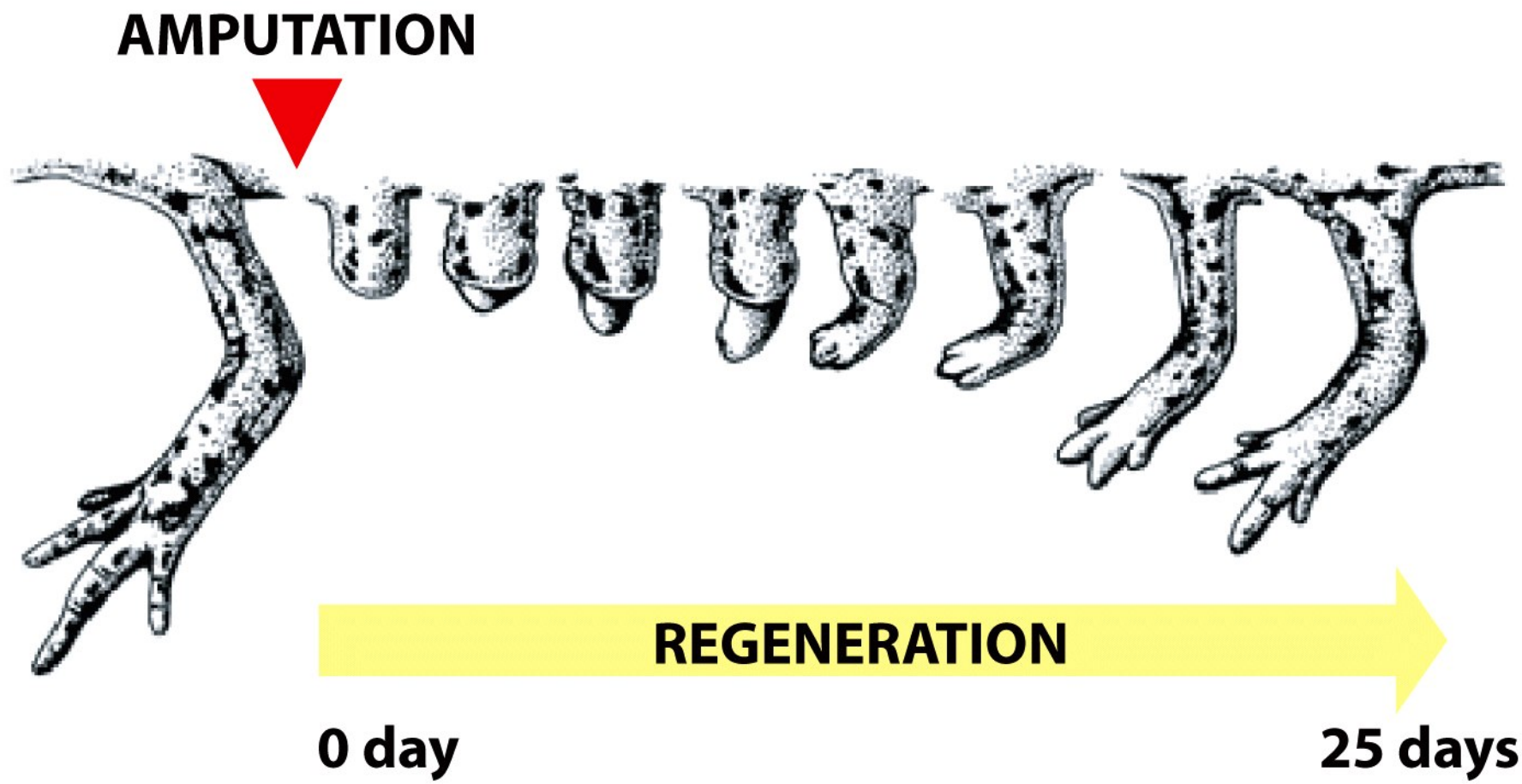
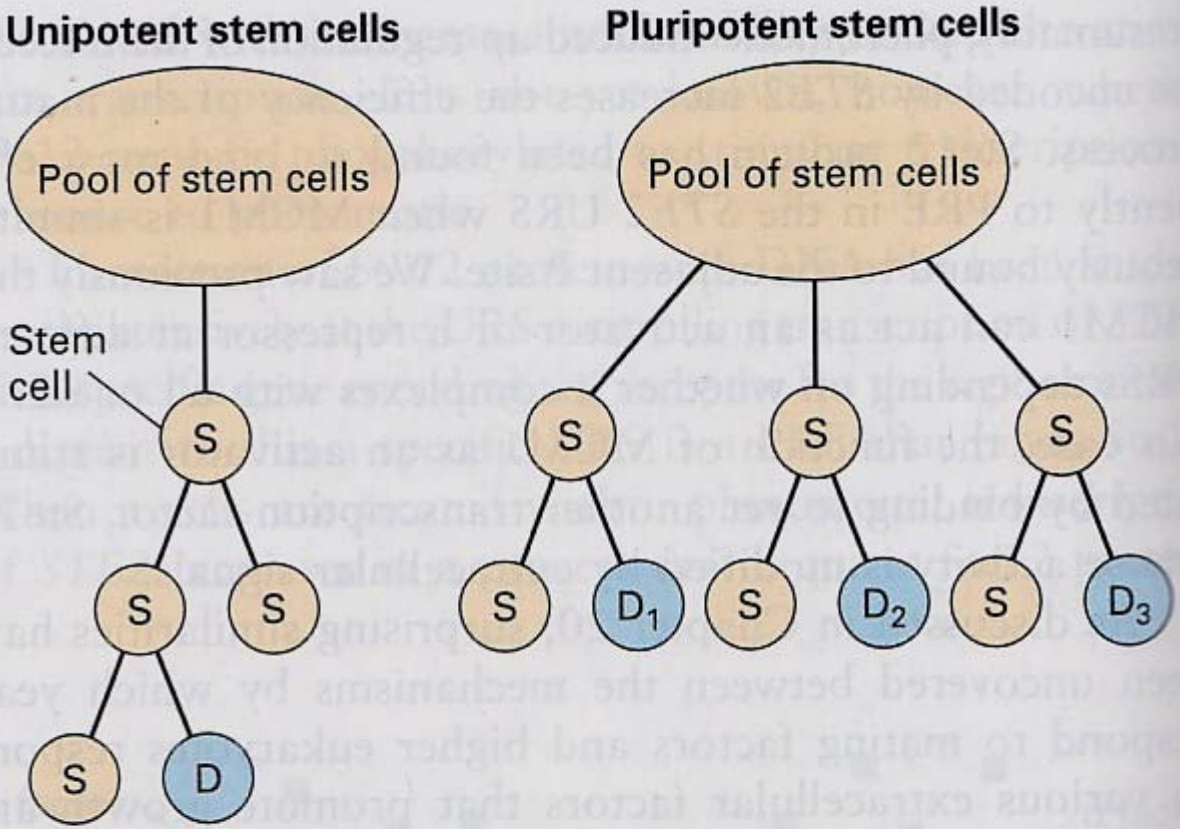
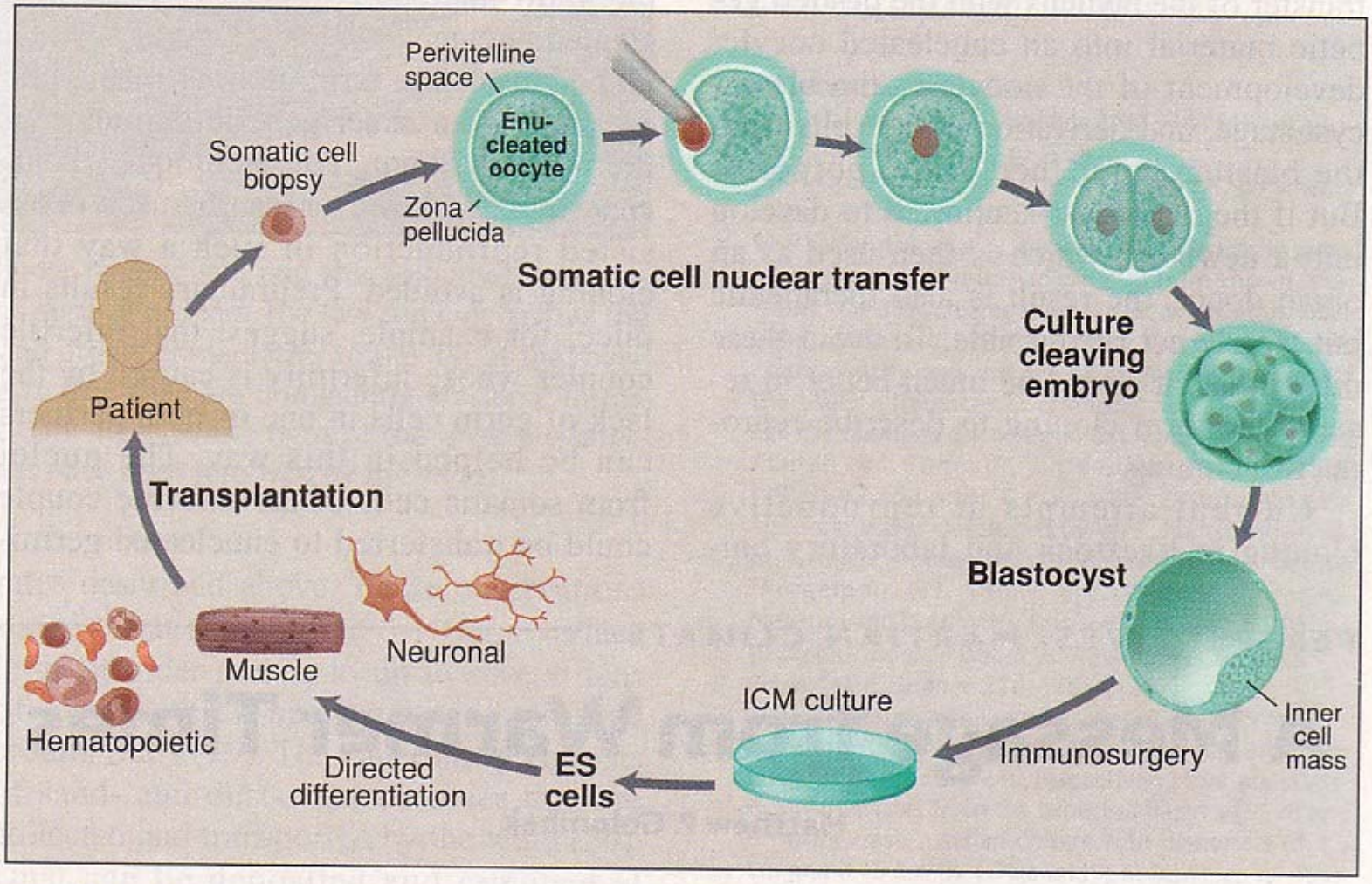


Figure 23-67 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Stem cell engineering: **CELL 1746-**



▲ FIGURE 14-7 The production of differentiated cells (D) from stem cells (S). Unipotent stem cells produce a single type of differentiated cell, whereas pluripotent stem cells may produce two or more types of differentiated cells.



Tissues for transplantation. A cell biopsy is taken from the patient, and the nucleus of the somatic cell is transferred into an enucleated donor oocyte with the nuclear transfer techniques pioneered in mice and sheep. The resulting embryo is allowed to develop until the blastocyst stage. The inner cell mass (ICM) of the blastocyst is then recovered by immunosurgery and cultured, and the embryonic stem (ES) cells are harvested from it. The ES cells are then directed to differentiate into the particular cell type required (for example, dopaminergic neurons to replace those lost in Parkinson's disease, pancreatic islet cells for patients with diabetes, hepatocytes to treat liver cirrhosis) and are transplanted into the patient.

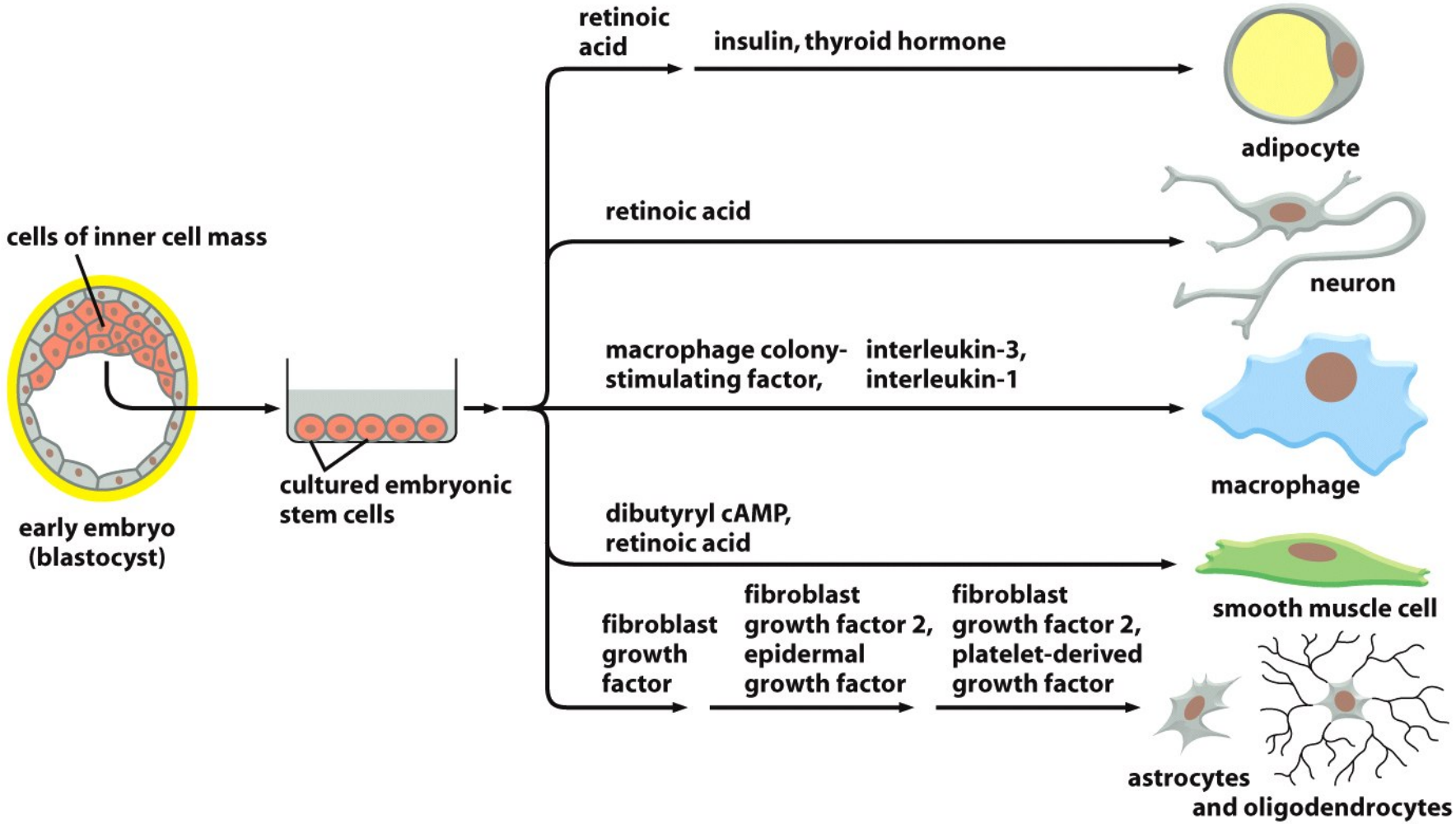


Figure 23-68 Molecular Biology of the Cell 5/e (© Garland Science 2008)

CELL 1481

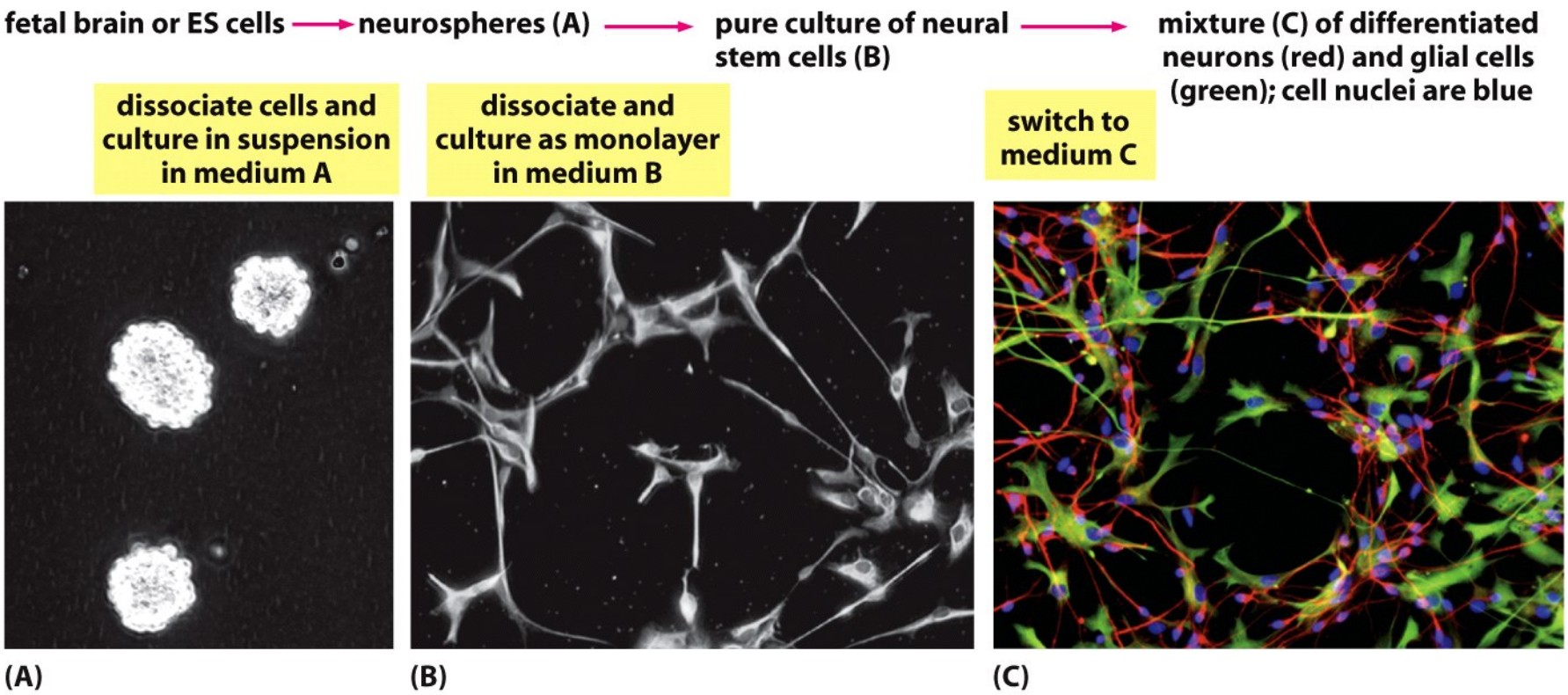
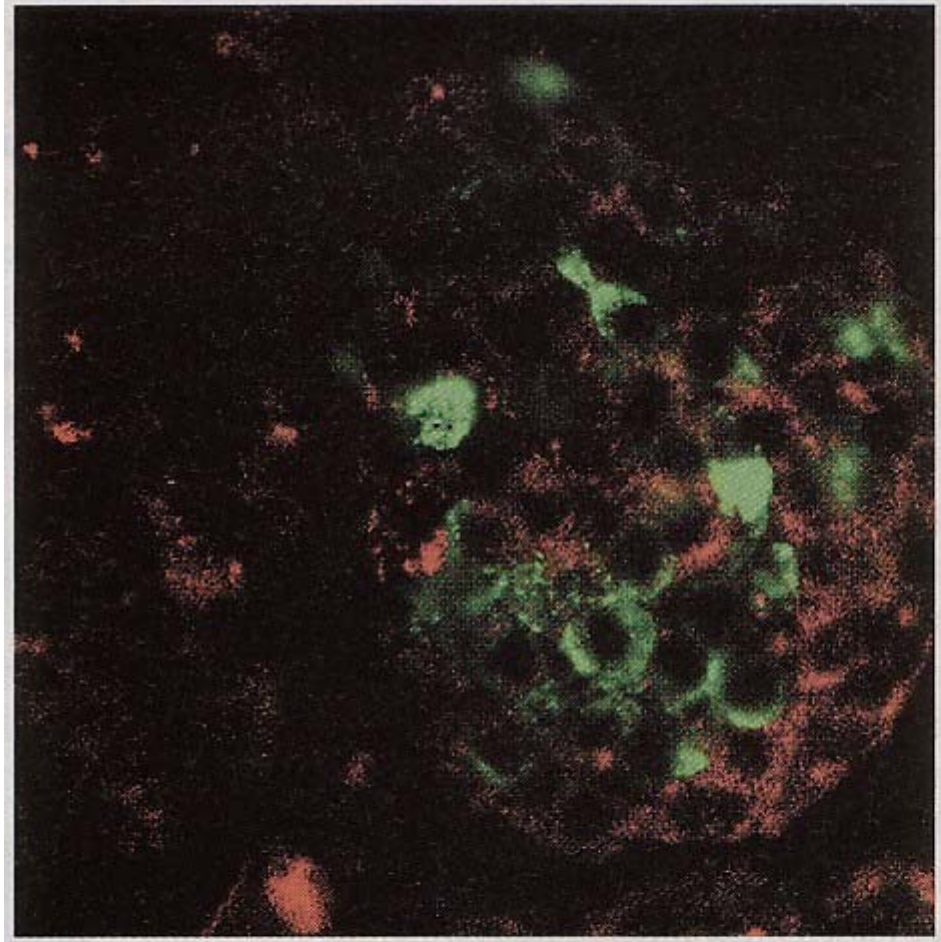


Figure 23-66 Molecular Biology of the Cell 5/e (© Garland Science 2008)

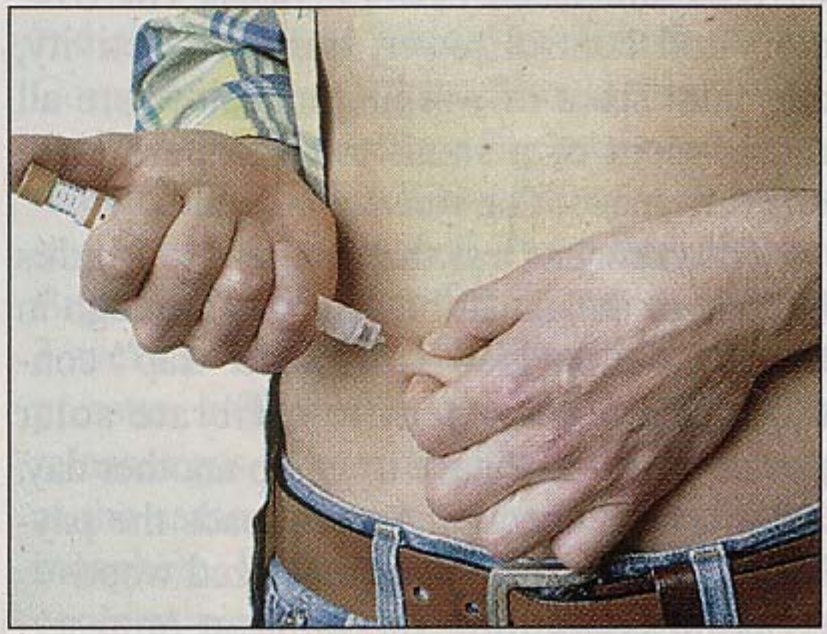
CELL 1479



Future therapy? Someday, cultured cells from islet buds such as this one (red staining indicates insulin and green staining glucagon) may make insulin injections *(right)* obsolete—but not yet.

ly trying to boost cell production levels, but even with the most optimistic scenario, Levine adds, human clinical trials are 5 years away.

Embryonic stem cells offer another promising source of beta cells. Earlier this year, Bernat Soria and his colleagues at the Institute of Bioengineering at Universidad Miguel Hernandez in San Juan, Spain, reported that they had not only produced beta-



Monissa maissa tuli luvalliseksi käyttää tutkimuksiin ym. vain sellaisia embryonaalisia kantasolulinjoja, jotka oli olemassa silloin kun laki laitettiin

Niistä tuli heti arvokkaita kaupallisia tuotteita

Esimerkiksi ruotsalaisilla oli niitä monta rekisterissä,
Suomesta ei yhtään!

Loophole legalizes human cloning

David Adam, London

Britain's cloning regulations have been thrown into chaos by a court ruling that it is not illegal to create a cloned human baby. In a judgement on 15 November, the High Court ruled that existing laws do not cover cloned embryos because they are not formed through fertilization. The government says it will appeal, and is also considering emergency legislation.

Earlier this year the 1990 Human Fertilisation and Embryology Act was amended (see *Nature* 409, 5; 2001) to allow research into using human embryonic stem (ES) cells to grow replacement tissues. This was intended to include cloned embryos, to allow work on 'therapeutic' cloning, which holds the prospect of growing grafts perfectly matched to individual patients. No one has yet applied to the Human Fertilisation and Embryology Authority (HFEA) for a licence to conduct such research, but the law has been praised by stem-cell researchers for appreciating the difference between therapeutic and reproductive cloning.

The new ruling — on a case brought by the ProLife Alliance, which opposes ES-cell research — means that the HFEA no longer has the jurisdiction to award such licences. It effectively leaves human cloning completely unregulated. Severino Antinori, the Italian fertility expert who claims that he will clone human babies, says he now wants to relocate to Britain.

The court's decision will not have an immediate impact on British ES-cell researchers, as they are currently working with cells isolated from embryos created by *in vitro* fertilization. But they say it is important that the cloning regulations are restored. Austin Smith, director of the Centre for Genome Research at the University of Edinburgh, said in a statement that if the court has "identified a loophole that would permit the creation of children by reproductive cloning, it is essential that such a loophole be blocked". ■

Nature 22 Nov 2001

Generation of Fertile Cloned Rats by Regulating Oocyte Activation

Qi Zhou,[1,2] Jean-Paul Renard,[1]* Gaëlle Le Friec,[3] Vincent Brochard,[1] Nathalie Beaujean,[1] Yacine Cherifi,[3] Alexandre Fraichard,[3] Jean Cozzi[3]

The rat is a reference animal model for physiological studies and for the analysis of multigenic human diseases such as hypertension, diabetes, and neurological disorders (*1*). Genetic manipulation in the rat is hampered by the lack of suitable technologies such as embryonic stem cells (ES), which are routinely used to generate targeted mutations in the mouse. Cloning through somatic cell nuclear transfer (SCNT) is a potential alternative approach in species for which ES technologies are unavailable. However, all previous efforts to clone rats have been unsuccessful, with developmental arrest at implantation stage [(*2*) and references therein].

The fine-tuned coordination between nuclear transfer and timing of oocyte activation is critical to the outcome of somatic cloning. This coordination is hampered in the rat because almost all the oocytes spontaneously, although abortively, activate within 60 min of their removal from oviducts (*3*). Such rapid but incomplete activation process is not encountered in other cloned species. To allow embryo reconstruction before the onset of oocyte activation, we initially developed a one-step SCNT procedure for the rapid substitution of the endogenous meiotic metaphase nucleus by an exogenous mitotic one. This latter nucleus was isolated from synchronized cultured fetal CD–Sprague Dawley fibroblasts [12.5 days post coitum (dpc)]. Individual mitotic nuclei were injected into a recipient OFA–Sprague Dawley oocyte, from which the meiotic metaphase nucleus was withdrawn while removing the micropipette from cytoplasm after injection. However, within 30 min after recovery, 70% of oocytes showed clear morphological evidence of spontaneous release from the second meiotic metaphase arrest (oocyte metaphase MII) (Fig. 1A). When activation of cloned embryos (Fig. 1B) was induced and maintained by exposure (2 hours) to a cdc2-specific kinase inhibitor (butyrolactone, 150 μM) (*4*), 201 of 221 reconstructed embryos expelled the polar body and subsequently divided into two-cell embryos. Their transfer into OFA–Sprague Dawley foster mothers (11 recipients, 221 embryos) resulted in nine implantation sites but no fetal development.

Forty percent of oocytes selected for SCNT had been already activated, as evidenced by disjoined sister chromatids moving to opposite poles (Fig. 1C). These observations strongly supported the view that, despite rapid manipulation, most of the oocytes were not suitable for cloning. Because activation is triggered by the inactivation of maturation promoting factor (MPF) activity through a proteasome-mediated cyclin degradation pathway, we used MG132, a protease inhibitor that reversibly blocks the first meiotic metaphase-anaphase transition in the rat (*5*). We found that this drug also reversibly stabilized most oocyte MII metaphases for up to 3 hours [77% (Fig. 1D)].

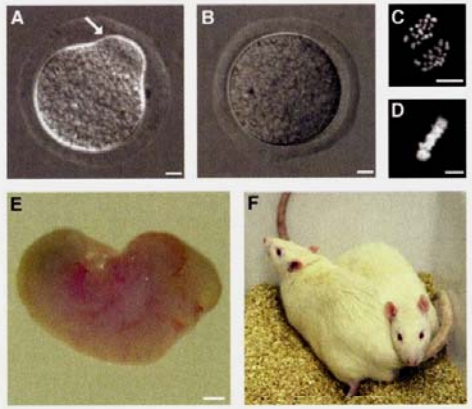


Fig. 1. (**A**) A freshly recovered rat oocyte with a marked cytoplasmic protrusion (arrow) revealing an ongoing activation process. (**B**) A rat oocyte used for micromanipulation. (**C**) DNA status of oocytes after recovery in standard conditions reveals the presence of two separate sets of chromatids, whereas after recovery in MG132 supplemented medium (**D**) a stabilized metaphase plate is revealed. (**E**) Normal cloned fetus at 14.5 dpc and (**F**) two adult cloned male rats obtained using MG132 supplemented medium. Bar: (A) to (D) 10 μm; (E) 1 mm.

We then collected oocytes in the presence of MG132 (5 μM) (*4*) as described. SCNT was performed within 30 min of drug removal. Eight hundred seventy-six embryos were implanted into 12 pseudopregnant foster female rats. At 12.5 dpc, the females were sacrificed, and four females contained 16 fetuses. Thirteen of the fetuses, obtained from three females, were viable with beating hearts (Fig. 1E). In the next series of experiments, we transplanted 129 cloned embryos into two foster mothers and allowed them to go to term. Only one foster mother contained viable fetuses, and this animal delivered three live male pups of fibroblast origin as unambiguously demonstrated by microsatellite marker analysis (*4*). One normal-sized pup (5.9 g) died a few hours after birth. The other two pups grew to sexual maturity (Fig. 1F) and generated normal progeny. We have also obtained normal progenies (in terms of size, weight, and development) from two additional cloned female rats, demonstrating the potential of the technique for the development of fertile rat lines of both sexes [supporting online material (SOM) Text].

Our data highlight the importance of adapting the SCNT procedure to oocyte physiology for successful cloning. Recently, random mutagenesis has been proposed to generate knockout rats (*6*). However, our results pave the way for more extensive genetic modifications such as conditional knock-out and gene replacement, which are required to produce relevant models of human diseases.

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5. L. B. Josefsberg *et al.*, *Biol. Reprod.* **62**, 1270 (2000).
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7. Funding was provided by Institut National de la Recherche Agronomique (INRA) and genOway Company. We thank P. Hardy (DVM, Charles River Laboratories, France) and C. Szpirer (ULB) for their contribution to rat genotype analysis. We also acknowledge P. Adenot for use of the INRA confocal facility and B. Nicolas for technical help during the preparation of this manuscript.

Supporting Online Material

www.sciencemag.org/cgi/content/full/1088313/DC1
Materials and Methods
SOM Text
Fig. S1
Tables S1 to S3
References

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10.1126/science.1088313
Include this information when citing this paper.

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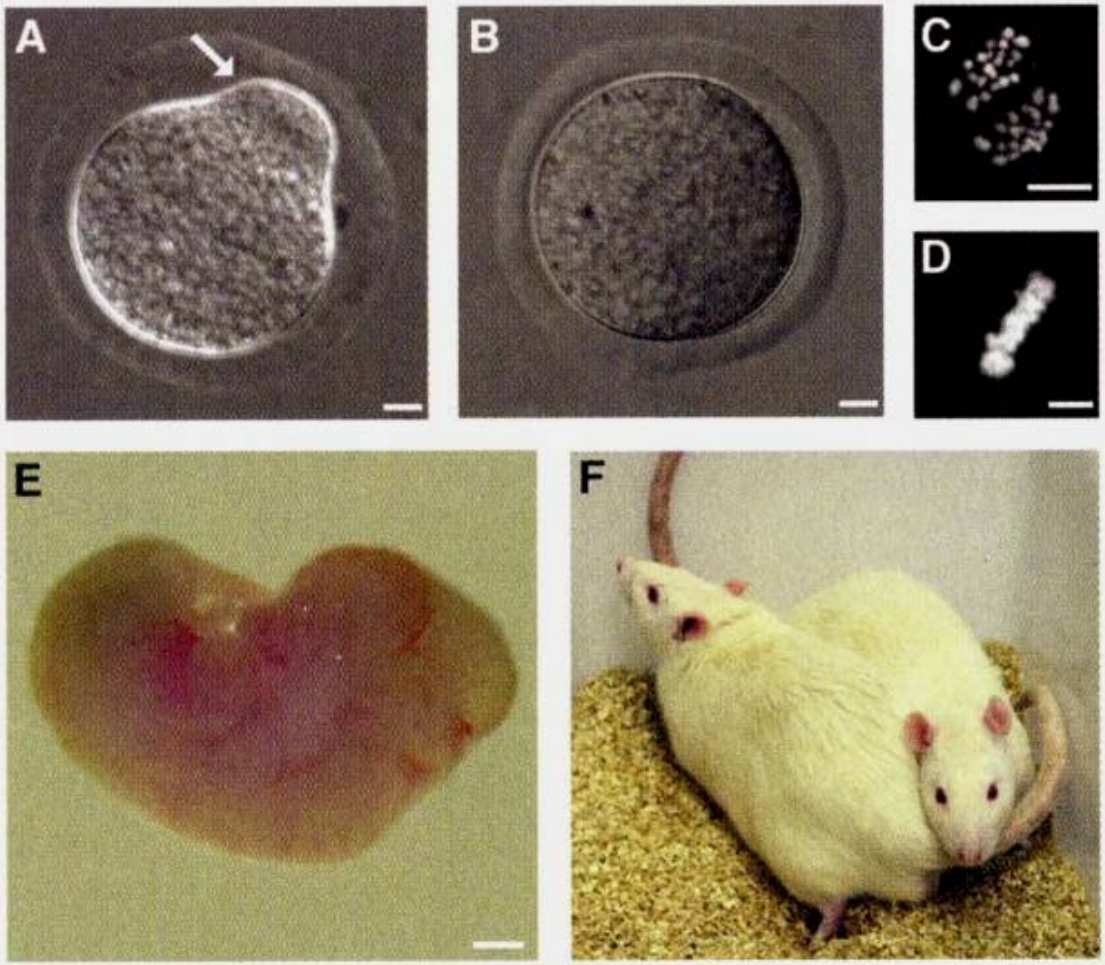

Fig. 1. (**A**) A freshly recovered rat oocyte with a marked cytoplasmic protrusion (arrow) revealing an ongoing activation process. (**B**) A rat oocyte used for micromanipulation. (**C**) DNA status of oocytes after recovery in standard conditions reveals the presence of two separate sets of chromatids, whereas after recovery in MG132 supplemented medium (**D**) a stabilized metaphase plate is revealed. (**E**) Normal cloned fetus at 14.5 dpc and (**F**) two adult cloned male rats obtained using MG132 supplemented medium. Bar: (A) to (D) 10 μm; (E) 1 mm.

ng a human baby, which South aw forbids. "Cloning forces us to out, 'Are we just a mass of cells gical processes?'" says Dr. Robert , a co-director of Columbia ty's Center for Bioethics. "Stem ch on fundamental questions of are, where we come from and e are going." The collection of eggs men is also a delicate issue, as as discovered. The journal *Nature* last year that one of Hwang's stu- d that she and another researcher ated eggs for use in the lab's experiments. Obtaining ova from a ker is ethically controversial be- process is uncomfortable, carries isk for the donors, and a subordi- ght feel pressured to donate. lenied the report at the time, and nt interviewed by *Nature* retract- tory before the article was pub- aying she'd misunderstood the But Gerald Schatten, a biology at the University of Pittsburgh ajor partner in Hwang's World l Hub, which plans to create some 100 human stem-cell provide them to researchers around the globe, pulled

I had already produced many cloned cows and pigs, but when Snuppy was born, it was different . . . I was so happy. He was very healthy.

—Hwang Woo Suk, with Snuppy, at Seoul National University

primarily on $2 million from the government annually—can't explain such resounding success in a country hardly known for its deep scientific roots. Determination is a key, along with the lab's nearly round-the-clock, seven-days-a-week working schedule. "Stem cells do not know Saturday and Sunday," Hwang says.

Encouraged by his success with the notoriously finicky hu-

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Invention of the Year

from the egg and sperm of a mother and father but from a single cell taken from the ear of an adult Afghan hound. That makes Snuppy the first dog created by cloning, the still relatively new—and for some, troubling—branch of biotechnology. Other mammals have been cloned, starting with Dolly the sheep back in 1996 and followed by mice, cows, pigs, rabbits, horses and, most recently, cats. But dogs had remained elusive—until now.

Snuppy is the product of a lab in which the painstaking process of cloning has become routine. Years of exasperating experimentation, countless mishaps and dispiriting failures have produced a technique so finely tuned that it can tackle even the most stubborn cloning challenges, such as dogs. And it suggests that pretty much any mammal can be cloned—given enough expertise.

Snuppy, above right, was created from an ear cell of an Afghan, above left, and carried to term by a surrogate Lab retriever, right

That's the key word. Plenty of labs do mammalian cloning these days, but the group that produced Snuppy is, like the puppy himself, extraordinary. With striking regularity, Hwang Woo Suk and his 45-person team have cranked out one cloning breakthrough after another from his laboratory of veterinary science at Seoul National University in South Korea. Snuppy was just one of the major steps forward for Hwang during this busy year. He also refined his human cell-cloning process to yield the first stem cells from patients with diseases, bringing medicine a step closer to the possibility of curing illnesses from Alzheimer's to diabetes with a patient's own rejection-proof tissues.

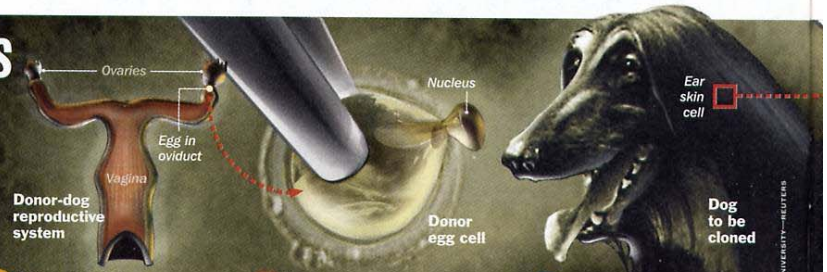
Research on human cells always courts controversy: the technique used to produce stem cells could also be used to clone a human being, although Hwang says he is not in the business of cloning a human baby, which South Korean law forbids. "Cloning forces us to think about, 'Are we just a mass of cells and biological processes?'" says Dr. Robert Klitzman, a co-director of Columbia University's Center for Bioethics. "Stem cells touch on fundamental questions of who we are, where we come from and where we are going." The collection of eggs from women is also a delicate issue, as Hwang has discovered. The journal *Nature* reported last year that one of Hwang's students said that she and another researcher had donated eggs for use in the lab's cloning experiments. Obtaining ova from a lab worker is ethically controversial because the process is uncomfortable, carries a small risk for the donors, and a subordinate might feel pressured to donate. Hwang denied the report at the time, and the student interviewed by *Nature* retracted her story before the article was published, saying she'd misunderstood the reporter. But Gerald Schatten, a biology professor at the University of Pittsburgh and a major partner in Hwang's World Stem Cell Hub, which plans to create some 100 human stem-cell lines and provide them to researchers around the globe, pulled out of the project on Nov. 11 citing concerns over the sourcing of eggs. In response, Hwang said his labs had abided by the South Korean government's ethical guidelines. He also announced that he is conducting an internal investigation into his team's procedures, telling reporters: "I will make an announcement as soon as the investigation is completely finished."

Controversy aside, there's little doubt that Hwang's lab is the trailblazer in the field of both animal- and human-cloning research—certainly far ahead of American labs that dominate other areas of biotechnology. The Bush Administration has banned the use of federal funding for any research involving cloning, including embryonic stem cells, aside from a short list of "grandfathered" cell lines. In contrast, South Korea's President Roh Moo Hyun has given unprecedented political, financial and social support to cloning research. But funding alone—Hwang intentionally keeps his budget lean, relying

FROM TOP: SEOUL NATIONAL UNIVERSITY—GETTY; SEOUL NATIONAL UNIVERSITY—REUTERS

CLONING MAN'S BEST FRIEND

A dog's reproductive cycle makes cloning canines especially tricky. Here's how South Korean scientists finally succeeded in cloning an Afghan after two years of trial and error



1 Donor-dog eggs are **extracted** as they are released into the oviduct. Cell studies and hormone-level readings help pinpoint the event

2 The nucleus of the egg is **squeezed out** through a slit made in the cell's outer membrane, using a delicate technique perfected in the South Korean lab

3 A biopsy of skin is **taken** from the ear of a 3-year-old adult male Afghan, and skin cells are **processed** and **grown** in culture

SEOUL NATIONAL UNIVERSITY—REUTERS

> I had already produced many cloned cows and pigs, but when Snuppy was born, it was different... I was so happy. He was very healthy.
> —Hwang Woo Suk, with Snuppy, at Seoul National University

primarily on $2 million from the government annually—can't explain such resounding success in a country hardly known for its deep scientific roots. Determination is a key, along with the lab's nearly round-the-clock, seven-days-a-week working schedule. "Stem cells do not know Saturday and Sunday," Hwang says.

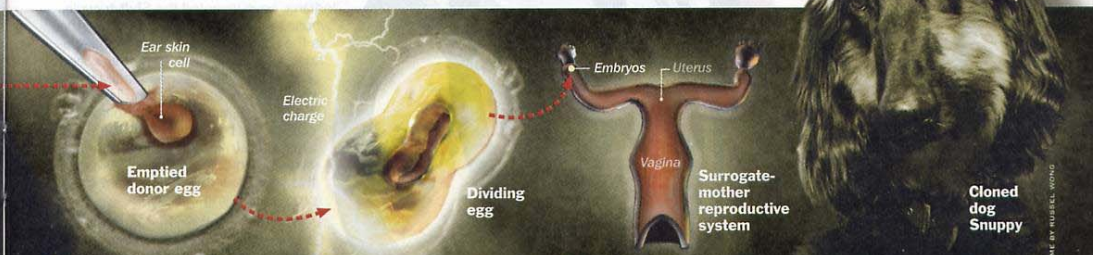
Encouraged by his success with the notoriously finicky human stem-cell lines, Hwang turned his attention to dogs, a challenge even for a lab the caliber of his. Dogs have a limited breeding period; a female's eggs can be harvested for only a few weeks each year, when she is in heat. In addition, the eggs cannot be easily extracted from the ovaries, as they can with pigs and cows. "We failed so many times to get eggs from many egg-donating dogs," says Hwang. "So I studied and surveyed the reproductive cycle and reached a solution. If we try to get the egg not from the ovary but from the oviduct after ovulation, then maybe we would get good-quality eggs."

Hwang and his team began by doing what scientists do best: observe. They meticulously documented the temperature, hormone levels and vaginal cellular changes of potential canine egg donors through an entire ovulation cycle. On some days, they took readings two or three times a day. That way, they could pinpoint when an egg began its journey from the ovary into the oviduct. The next steps were similar to those used in cloning human cells. First, they gently squeezed out the nucleus of each egg and replaced it with an entire ear cell from an adult dog. Then the egg and its new cargo were electrically stimulated and chemically fused in steps that Hwang developed so that the egg would begin dividing and acting like a growing embryo. But the culture medium in which he had been growing his animal clones—even the one that had worked so well for his human stem-cell lines—turned out to be inhospitable to canine embryos. "It took almost two years to get this specific in vitro culture for the dog clones," Hwang says.

After extracting 1,095 eggs from more than 100 donors and transferring five to 12 embryos to each of 123 carefully chosen surrogates, three dogs, including a Labrador retriever belonging to one of Hwang's students, became pregnant. Two fetuses made it to full term and were born by caesarean section last summer. The first, Snuppy, was born to the retriever and was the only one to survive. "I had already produced many cloned cows and pigs, but when Snuppy was born, it was different," says Hwang. "When I pulled out the first cloned dog from the surrogate mother's uterus, I was so happy. He was very healthy." Whether he will remain that way isn't as clear. Having created dozens of cloned animals, Hwang admits that they can face a wide range of genetic abnormalities.

Rather than deterring Hwang, however, the setbacks appear to have fueled his curiosity. "If we study and develop our technique more, I expect that we can find some ways to diminish, or reduce the rate of abnormalities in cloned animals," he says. His approach has introduced some level of control and standardization to the somewhat haphazard process of cloning. Even before Snuppy's birth, Hwang had streamlined his process to yield more canine clones from fewer donor eggs.

Hwang's success on the human side—in creating patient-specific stem-cell lines—is noteworthy for a similar milestone of efficiency: he was able to coax one stem-cell line from just 10 eggs, a remarkable feat that stunned scientists. Given how fast the South Koreans have jumped ahead of the pack in cloning, their lead over rivals in other nations is likely to keep growing. As Hwang sees it, his research team's edge is its ability to remain "continuously hungry" for knowledge. So far, there's no sign that this appetite is anywhere near sated. ■



Cloned dog Snuppy

PHOTOGRAPH FOR TIME BY RUSSELL WONG

4 An entire skin cell is carefully **injected** into the empty donor egg, using the opening created previously

5 An electric charge **fuses** the egg and its new cargo. The resulting cell is then **immersed** in a chemical bath and starts to divide and grow

6 After several days, 10 or more embryos at a time are **transferred** into a surrogate dog, improving the chances that one will grow to term

7 After a normal 61-day pregnancy, Snuppy was **born** by caesarean section in April 2005

TIME Graphic by Lon Tweeten. Text by Alice Park

ng a human baby, which South aw forbids. "Cloning forces us to out, 'Are we just a mass of cells gical processes?'" says Dr. Robert , a co-director of Columbia ty's Center for Bioethics. "Stem ch on fundamental questions of are, where we come from and e are going." The collection of eggs men is also a delicate issue, as as discovered. The journal *Nature* last year that one of Hwang's stu- d that she and another researcher ated eggs for use in the lab's experiments. Obtaining ova from a ker is ethically controversial be- process is uncomfortable, carries isk for the donors, and a subordi- ght feel pressured to donate. lenied the report at the time, and nt interviewed by *Nature* retract- tory before the article was pub- aying she'd misunderstood the But Gerald Schatten, a biology at the University of Pittsburgh ajor partner in Hwang's World l Hub, which plans to create some 100 human stem-cell provide them to researchers around the globe, pulled

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ETHICS

Issues in Oocyte Donation for Stem Cell Research

David Magnus and Mildred K. Cho*

As described by Hwang *et al.* in *Science* (*1*), somatic cell nuclear transfer (SCNT) to create human embryonic stem cell (hESC) lines represents a step toward realizing the promise of stem cell research. They have shown the generalizability and efficiency of the approach in creating 11 cell lines from the nuclei of skin cells of individuals with serious diseases or disabilities and the oocytes of donors. This work raises ethical and policy questions. As hESC research proceeds internationally, these issues must be adequately addressed for public confidence to be maintained. We discuss three areas that particularly deserve attention: (i) ethical oversight of collaborations between scientists working in countries with different standards, (ii) protection of oocyte donors, and (iii) avoidance of unrealistic expectations.

International Oversight

The research described in Hwang *et al.* took place in South Korea. It was conducted with oversight and approval from Korean institutions required by South Korean law. However, one of the researchers is a U.S. scientist. No U.S. Federal funding was used, and the creation of human embryonic stem cells for research under these conditions is not prohibited in either country (*2*, *3*). This scientist obtained Institutional Review Board (IRB) review from his university, in which the IRB determined that the research did not involve human subjects on the basis of the Federal definition of human subjects research. These regulations exempt research from full IRB review if samples cannot be traced back to their donors (*4*). If this had been a clinical trial, his institution in the United States would have been mandated also to provide full IRB oversight for the research. Full IRB review might have been warranted in this case, because at least one of the researchers must be able to ascertain the identity of the donor from the clinician's encoded information if family members were to receive priority for future hESC transplants, in compliance with the Korean Network for Organ Sharing Regulation Code 18-1 [see SOM for (*1*)]. However, until recently, specific guidance to IRBs for review of procurement of oocytes for stem cell research has been minimal.

IRBs now can look to the new U.S. National Research Council—Institute of Medicine (NRC-IOM) report on stem cell research recommending that all such research have IRB approval and additional oversight by a special hESC research ethics oversight committee (*5*). If such oversight is not required by law, but is routine within the United States, should we expect that U.S. researchers working in other countries will voluntarily comply with these requirements? Or should they follow the laws and regulations of the country where the research is taking place unless mandated by U.S. law to do otherwise? This research was conducted before the NRC-IOM recommendations and at this point, it is not clear what the involvement of the U.S. IRB should have been.

Differing ethical standards in international collaborative research are not new, and solutions for reconciling differences have been proposed (*6*, *7*). Therefore, the evolving oversight of hESC research will require that, as new mechanisms are put in place [such as for research funded by the passage of the state's Proposition 71 (*8*) in 2004], U.S. researchers would be wise to seek approval from all relevant bodies and to ensure compatibility with the highest standards. This can help researchers obtain approval of the FDA or other similar bodies of clinical application of their research.

Nonmedical Oocyte Donation

A major challenge facing hESC research will be procurement of oocytes from "nonmedical" donors, meaning those who are donating oocytes neither for reproductive, nor medical purposes. The use of excess embryos and oocytes from in vitro fertilization procedures for research has a clear precedent. It uses a clinical informed consent process for women who are considering using assisted reproductive technology that includes discussion of the risks and benefits with the patient. In addition, there is a research informed consent process, as the patient (now a subject) agrees to allow her gametes to be used for research purposes (*9*, *10*). Agreement will be required on the confidentiality and the use of the material. In other words, for these patients, there is a two-part process—a clinical consent that covers the (not insignificant) risks and benefits of the procedure used to procure the oocytes for reproductive purposes (drugs for hyperstimulation, removal of the follicles, etc.) and then a research consent that focuses on the subject as a tissue donor. If cell lines derived from this material are eventually used in clinical trials, then the consent process for a new clinical trial comes into play.

The clinical consent model does not seem to fit women who agree to donate oocytes entirely for research purposes. These women are not pursuing the procedure for any reproductive or medical benefit to themselves; rather, they are exposing themselves to risk entirely for the benefit of others. If we were to think of them as simply clinical patients, their physician's fiduciary obligations would seem to require counsel against undergoing such a procedure for no benefit (*11*). Between 0.3 and 5% (*12*) or up to 10% (*13*) of women who undergo ovarian stimulation to procure oocytes experience severe ovarian hyperstimulation syndrome, which can cause pain, and occasionally leads to hospitalization, renal failure, potential future infertility, and even death.

Alternatively, these individuals can be viewed purely as research subjects. After all, research often requires individuals to expose themselves to risk for the benefit of others (albeit often with the possibility of direct benefit to themselves). This model may also be inadequate for addressing the status of these women, because the consent process is likely to focus on the post-procurement research risks and benefits. Thus, the risks of the actual procurement process may not be adequately highlighted. There is nothing experimental being tested on these women. The only research aspect of their experience is use of their tissues.

Finally, once current technical limits are overcome, cell lines derived from this research may actually be used in therapy. There may be very little difference for the oocyte donors between donating their

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gametes for research or for clinical purposes—yet the consent processes would seem to require different approaches. All of these factors doubtless contribute to the fact that Hwang and colleagues' discussion of the consent process and their consent forms (*1*) reveal little attention to the risks of the procedure and focus on the research aspects of their contribution.

We may need a new category to deal with this unusual class of participants who expose themselves to substantial risk only for the benefit of others, where the risk is incurred not in the actual research but in the procurement of materials for the research. When the oocytes that are donated are anonymized, current U.S. regulations no longer recognize these donors as research subjects. However, the donors are also not patients. We recommend use of the term "research donors" as distinct from "research subjects" to signify their dissimilar roles. This new category does not apply to donors of sperm used to create hESCs, because they are not exposed to similar risks. It also does not apply to donors of tissue for genetic research projects such as the HapMap (*14*), even though direct benefits do not accrue to those donors, because of the low physical risks involved.

When someone volunteers to donate an organ (such as a kidney or a liver lobe), there is a similar conceptual difficulty (*15*–*17*). These procedures are not now considered research, but it is difficult to see the donors truly as patients in the way that recipients are seen (*18*). Simply taking the best interests of the donor into account, it is hard to justify organ donation.

In dealing with the problem of benefit, the transplant community has moved fairly cautiously, and a great deal of conceptual and procedural work has gone into protecting the individuals who are making a sacrifice for others (*15*–*19*). In general, scrutiny of the motives for undergoing such donation is far greater than would normally be required for an elective procedure, and whole classes of potential live donors are ruled out on principle. For example, altruistic directed donation by live donors (i.e., to strangers) has generally been regarded as problematic, both ethically and practically (*16*, *20*). In general, it has been found that it is much easier to justify donation to close family members and friends.

However, it seems that clinicians and stem cell researchers envision this type of altruistic donation as the primary vehicle for generating hESC lines for research and eventual clinical application. Applying this model to the procurement of oocytes would mean that researchers would have to exercise a great deal of caution and to be rigorous in their assessment of whether someone is an appropriate donor as well as being clear about all of the risks.

Recruiting oocyte donors from families of afflicted patients would follow the pattern of justification typically cited in living organ donation. However, it does raise the question of whether oocyte donors feel coerced by their family situations into donating. Furthermore, there is one significant difference that leads to a final problem—organ donation has fairly clearly established benefits to the recipient; hESC research based on SCNT does not.

Misconception of Therapeutic Use

As the NRC-IOM report highlights, it is necessary that prospective donors recognize the large gap between research and therapy. This is particularly important in frontier areas of research where therapeutic impact in humans is unproven. Because it is likely that oocyte donors will be recruited from individuals with diseases and disabilities or their close family members, researchers must make every effort to communicate to these volunteers that it is extremely unlikely that their contributions will directly benefit themselves or their loved ones. Also, it is nearly certain that the clinical benefits of the research are years or maybe decades away. This is a message that desperate families and patients will not want to hear. Their vulnerability and the risks of oocyte donation make it imperative that prospective donors are adequately counseled and that risks are weighed carefully against a realistic assessment of benefits before allowing research to proceed. Donors who are family members or friends of patients hoping to benefit from downstream stem cell research are more vulnerable than the so-called altruistic donors who are strangers.

The language used to describe the research can reinforce the therapeutic misconception (*21*), misleading donors and subjects into believing that research is therapy. This was recognized as a serious problem in so-called "gene therapy" research (*20*, *22*, *23*) and has led to recommendations that this research should more accurately be described as "gene transfer research." Similarly, it is important not to use the term "therapy" when what is meant is "research" and not to refer to hESC research as "therapeutic cloning." There is currently no such thing as "therapeutic cloning" and this is not "therapeutic cloning research," nor can we say with any certainty that "cell therapy" is in the near future. Similarly, referring to research subjects as "patients" contributes to confusion (*2*). Introducing such terminology increases the likelihood that individuals have been or will be misled into exposing themselves to risk. It is permissible and perhaps even laudatory for women to contribute voluntarily to moving the field forward. But it would be a mistake to allow our language and the enthusiasm of researchers to allow that research to take place through exploitation of vulnerable patients and their friends and family members.

Responsibilities of Journals

Journals have an ethical obligation to publish research such as Hwang *et al.*'s if it is scientifically sound. However, journals must also be satisfied that the research was conducted ethically and must call attention to ethical issues raised by the work that they publish. Research that crosses the boundary into the illegal or clearly ethically unacceptable (e.g., if this research had been conducted without consent) should not be published. Although Hwang *et al.* did not cross those boundaries, their work is in such a novel and controversial area that it is unsurprising that it raises new ethical challenges, and is beset by some ambiguities. Journals are obligated to publish such research and to encourage ethical reflection on how future research should be conducted.

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UK embryo licence draws global attention

Scientists in Britain have been granted permission to perform controversial experiments that will create human embryos using genetic material from three people. Teams carrying out similar research in the United States and China have been forced to shut down their experiments, and say that they hope the UK decision will help push the work forward around the world.

The UK Human Fertilisation and Embryology Authority (HFEA) said on 8 September that it will allow scientists at the University of Newcastle upon Tyne to transfer the nucleus of a fertilized egg into an egg donated by a second woman. The second egg's own nucleus will be removed before the transfer occurs, but it will still contain genetic material in certain structures outside the nucleus. These structures, called mitochondria, generate energy and carry their own genes. So the Newcastle scientists will be creating embryos that contain genes from three people: the biological mother, the biological father and an unrelated egg donor. That makes the experiments highly controversial.

The embryos that will be created in Newcastle will not be transferred into women. But advocates of the technique say that if the experiments work, they could eventually prevent mothers from passing on diseases caused by mitochondrial defects to their children.

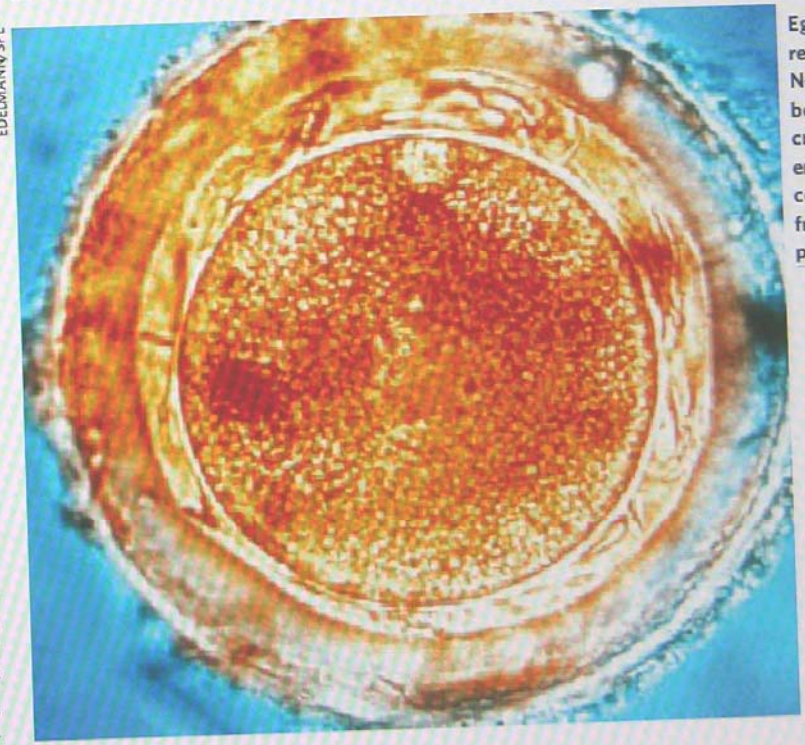
Similar experiments were being done in the United States by James Grifo, a reproductive endocrinologist at New York University Medical Center. But in the late 1990s the US Food and Drug Administration (FDA) halted Grifo's experiments after he had created only a few embryos, because it was worried that the health of the fetuses could not be guaranteed. Grifo's postdoctoral fellow, John Zhang, continued the experiments in China, where altered embryos were transferred into two patients before an international outcry shut those experiments down too. Grifo, who has since been pleading with the FDA to allow him to restart the experiments, told *Nature* that he is encouraged by the HFEA's decision.

"I'm glad that at least one country in the world is pioneering and smart enough to do this, and hopefully it will help patients with mitochondrial diseases," Grifo says.

He predicts that the British move will eventually spur action elsewhere, including in the United States. "Once there's some semblance of success over there, they'll say 'OK, now we can do it.' Traditionally, that's how things go."

More than 30 children have already been born thanks to a related technique that is used to boost fertility. The method does not involve nuclear transfer — instead, scientists transfer some of the cytoplasm that surrounds a cell's nucleus from a donor egg to the fertilized egg. But that method has also been halted in the United States, leaving the Newcastle researchers at the head of the field, for the moment at least. ■

Erika Check



EDELMANN/SPL

Egged on: researchers in Newcastle will be allowed to create an embryo that contains genes from three people.

Simple switch turns cells embryonic

Research reported this week by three different groups shows that normal skin cells can be reprogrammed to an embryonic state in mice[1–3]. The race is now on to apply the surprisingly straightforward procedure to human cells.

If researchers succeed, it will make it relatively easy to produce cells that seem indistinguishable from embryonic stem cells, and that are genetically matched to individual patients. There are limits to how useful and safe these would be for therapeutic use in the near term, but they should quickly prove a boon in the lab.

"It would change the way we see things quite dramatically," says Alan Trounson of Monash University in Victoria, Australia. Trounson wasn't involved in the new work but says he plans to start using the technique "tomorrow". "I can think of a dozen experiments right now — and they're all good ones," he says.

"It's unbelievable, just amazing. It's like Dolly. It's that type of accomplishment."

In theory, embryonic stem cells can propagate themselves indefinitely and are able to become any type of cell in the body. But so far, the only way to obtain embryonic stem cells involves destroying an embryo, and to get a genetic match for a patient would mean, in effect, cloning that person — all of which raise difficult ethical questions.

As well as having potential ethical difficulties, the 'cloning' procedure is technically difficult. It involves obtaining unfertilized eggs, replacing their genetic material with that from an adult cell and then forcing the cell to divide to create an early-stage embryo, from which the stem cells can be harvested. Those barriers may have now been broken down.

"Neither eggs nor embryos are necessary. I've never worked with either," says Shinya Yamanaka of Kyoto University, who has pioneered the new technique.

Last year, Yamanaka introduced a system that uses mouse fibroblasts, a common cell type that can easily be harvested from skin, instead of eggs[4]. Four genes, which code for four specific proteins known as transcription factors, are transferred into the cells using retroviruses. The proteins trigger the expression of other genes that lead the cells to become pluripotent, meaning that they could potentially become any of the body's cells. Yamanaka calls them induced pluripotent stem cells (iPS cells). "It's easy. There's no trick, no magic," says Yamanaka.

The results were met with amazement, along with a good dose of scepticism. Four factors seemed too simple. And although the cells had some characteristics of embryonic cells — they formed colonies, could propagate continuously and could form cancerous growths called teratomas — they lacked others. Introduction of iPS cells into a developing embryo, for example, did not produce a 'chimaera' — a mouse carrying a mix of DNA from both the original embryo and the iPS cells throughout its body. "I was not comfortable with the term 'pluripotent' last year," says Hans Schöler, a stem-cell specialist at the Max Planck Institute for Molecular Biomedicine in Münster who is not involved with any of the three articles.

This week, Yamanaka presents a second generation of iPS cells[1], which pass all these tests. In addition, a group led by Rudolf Jaenisch[2] at the Whitehead Institute for Biomedical Research in Cambridge, Massachusetts, and a collaborative effort[3] between Konrad Hochedlinger of the Harvard Stem Cell Institute and Kathrin Plath of the University of California, Los Angeles, used the same four factors and got strikingly similar results.

"It's a relief as some people questioned our results, especially after the Hwang scandal," says Yamanaka, referring to the irreproducible cloning work of Woo Suk Hwang, which turned out to be fraudulent. Schöler agrees: "Now we can be confident that this is something worth building on."

The improvement over last year's results was simple. The four transcription factors used by Yamanaka reprogramme cells inconsistently and inefficiently, so that less than 0.1% of the million cells in a simple skin biopsy will be fully reprogrammed. The difficulty is isolating those in which reprogramming has been successful. Researchers do this by inserting a gene for antibiotic resistance that is activated only when proteins characteristic of stem cells are expressed. The cells can then be doused with

Nature 07.06.2007

The birth of this chimaeric mouse suggests that the cells used to generate it behave like embryonic stem cells.

NASA/JOHNS HOPKINS UNIV./SOUTH

S. OGDEN

antibiotics, killing off the failures.

The protein Yamanaka used as a marker for stem cells last year was not terribly good at identifying reprogrammed cells. This time, all three groups used two other protein markers — Nanog and Oct4 — to great effect. All three groups were able to produce chimaeric mice using iPS cells isolated in this way; and the mice passed iPS DNA on to their offspring.

Jaenisch also used a special embryo to produce fetuses whose cells were derived entirely from iPS cells. "Only the best embryonic stem cells can do this," he says.

"It's unbelievable, just amazing," says Schöler, who heard Jaenisch present his results at a meeting on 31 May in Bavaria. "For me it's like Dolly [the first cloned mammal]. It's that type of accomplishment."

The method is inviting. Whereas cloning with humans was limited by the number of available eggs and by a tricky technique that takes some six months to master, Yamanaka's method can use the most basic cells and can be accomplished with simple lab techniques.

But applying the method to human cells has yet to be successful. "We are working very hard — day and night," says Yamanaka. It will probably require more transcription factors, he adds.

If it works, researchers could produce iPS cells from patients with conditions such as Parkinson's disease or diabetes and observe the molecular changes in the cells as they develop. This 'disease in a dish' would offer the chance to see how different environmental factors contribute to the condition, and to test the ability of drugs to check disease progression.

But the iPS cells aren't perfect, and could not be used safely to make genetically matched cells for transplant in, for example, spinal-cord injuries. Yamanaka found that one of the factors seems to contribute to cancer in 20% of his chimaeric mice. He thinks this can be fixed, but the retroviruses used may themselves also cause mutations and cancer. "This is really dangerous. We would never transplant these into a patient," says Jaenisch. In his view, research into embryonic stem cells made by cloning remains "absolutely essential".

If the past year is anything to judge by, change will come quickly. "I'm not sure if it will be us, or Jaenisch, or someone else, but I expect some big success with humans in the next year," says Yamanaka. ■

David Cyranoski

Additional reporting by Heidi Ledford

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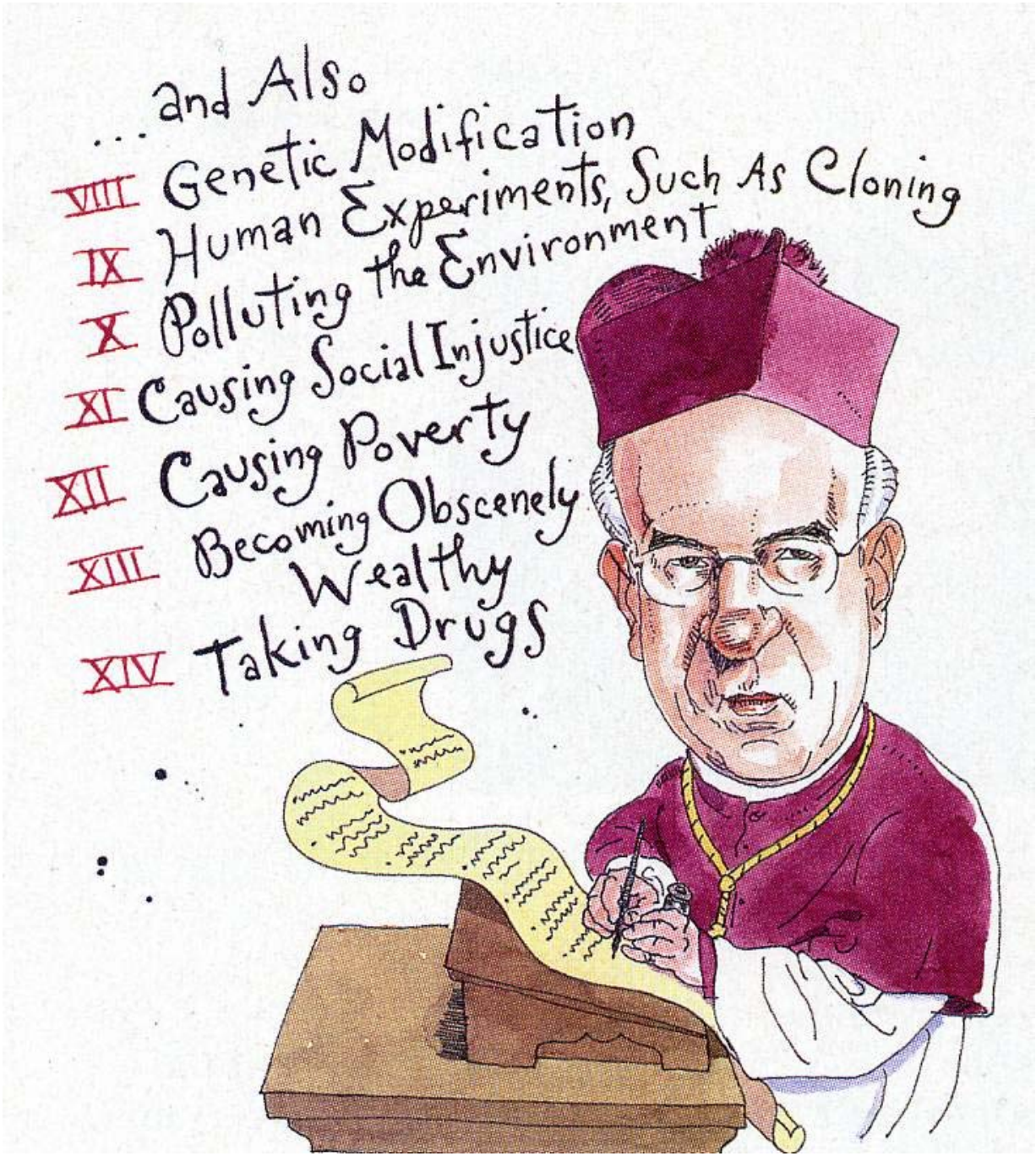
For more on alternative stem-cell work, see page 649; and see www.nature.com/stemcells



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