The people who make policy decisions should damned well know what they are talking about before they make the decisions. There is nobody who is an expert on cloning who would be afraid after seeing “Attack of the Clones.”

Kevin J. Anderson

Evidence for Genomic Equivalence

Possibilities for cellular differentiation mechanism:

1. During development, cells discard genes not needed in their later development.
   
   Corollary: Only germ cells retain all genes.
   
   (A few examples known of chromosomal loss during development: Antibody genes in immune cells.)

2. Differentiated cells retain all genes, but only use some.

Evidence for Genomic Equivalence

Early evidence:

Cytogenetics

- all cells appeared to have same chromosomes

Metaplasia

- differentiated cells ‘de-differentiate,’ generate other differentiated cell types
  
  (seen in regeneration, also some cancers)
Metaplasia in Salamander Lens Regeneration

Normal Unoperated Eye

Metaplasia in Salamander Lens Regeneration

Eye just after lens removal

Lens fully regenerated

Iris cells dedifferentiating to form new lens cells

Evidence for Genomic Equivalence

The ultimate evidence: Animal Cloning

reproducing a complete organism from a single differentiated adult nucleus

First accomplished with frogs:

Briggs & King (1950’s) - work with Rana

Gurdon (1960’s) - work with Xenopus
Figure 4.5(1) Procedure for Transplanting Blastula Nuclei into Activated Enucleated *Rana pipiens* Eggs

**Frog cloning method**

- Animal pole
- Glass needle
- Egg cell membrane
- Vitelline envelope
- Mitotic spindle
- Remove chromosomes and spindle
- Activated matured off
- Isolated mitotic spindle

**Egg Enucleation**

---

Figure 4.5(2) Procedure for Transplanting Blastula Nuclei into Activated Enucleated *Rana pipiens* Eggs

**Nuclear transplantation**

- Somatic cell nucleus
- Extract and lyse donor cell
- Membrane beads
- Donor nucleus inserted into enucleated cell

**Proof of concept:**
Normal, reproductive adult derived from differentiated nucleus

---

Figure 4.6 Percentage of Successful Nuclear Transplants as a Function of the Developmental Age of the Donor Nucleus

Briggs & King actual results - promising but incomplete

- Late blastula
- Early gastrula
- Late gastrula
- Neurula
- Tailbud
- Hatching tadpoles

---

Legend:
- Normal swimming tadpoles (*Rana pipiens*)
- Percent of nuclear transplant embryos that develop normally
- Hours at 18°C
Briggs & King Results

Keywords: Totipotent
      Pluripotent
- Progressive loss of nuclear potency
- Significant pluripotency exists in embryonic nuclei

John Gurdon’s cloning experiments

- used Xenopus vs. Rana
- used serial transplantation of embryonic nuclei
- used donor and host nuclei of different genotypes

Figure 4.7 A Clone of Xenopus laevis Frogs

Gurdon + Xenopus + serial transplantation: ultimate success - cloned frogs
The re-emergence of cloning:

Dolly the cloned sheep, 1997

Figure 4.8(1) Cloned Mammals, Whose Nuclei Came From Adult Somatic Cells

Dolly with offspring
The re-emergence of cloning: Dolly the cloned sheep, 1997

Mammalian cloning, previously thought impossible, achieved by Wilmut et al.

Technical achievement:
Sheep mammary cells in $G_0$ fused with enucleated egg

Reignites long-dormant debate about cloning

Figure 4.8(2) Cloned Mammals, Whose Nuclei Came From Adult Somatic Cells

Figure 4.8(3) Cloned Mammals, Whose Nuclei Came From Adult Somatic Cells

Only 1 in 434 oocytes survived
**Other mammals cloned in quick succession after ‘Dolly’ (1997)**

<table>
<thead>
<tr>
<th>Year</th>
<th>Mammal(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1998</td>
<td>Cow, Mouse</td>
</tr>
<tr>
<td>2000</td>
<td>Goat, Pig</td>
</tr>
<tr>
<td>2002</td>
<td>Cat</td>
</tr>
<tr>
<td>2003</td>
<td>Rat, Mule, Horse</td>
</tr>
<tr>
<td>2004</td>
<td>Deer</td>
</tr>
<tr>
<td>2005</td>
<td>Dog</td>
</tr>
<tr>
<td></td>
<td>Nov 2007 – Rhesus monkey nuclear transfer blastocysts</td>
</tr>
</tbody>
</table>

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**Rhesus monkey nuclear transfer blastocysts**

*Nature* 1 November 2007

**Producing primate embryonic stem cells by somatic cell nuclear transfer**

J Byrne, O Rodaway, I Casper, M Nelson, W Sanger, S Gilmour, D Wolf & S Infante

Derivation of embryonic stem (ES) cells genetically identical to a patient by somatic cell nuclear transfer (SCNT) holds the potential to treat genetic diseases. However, human trials are unlikely until the potential for rejection of the transplant is known. Non-human primates, such as the rhesus macaque, are used to circumvent concerns regarding rejection by the host immune system. However, the concept has only been achieved in the mouse, whereas inefficient reprogramming and poor embryonic development characterise the results obtained in primates. Here, we used a modified SCNT approach to produce rhesus macaque blastocysts from adult skin fibroblasts, and successfully cultured two ES cell lines from these blastocysts. Individual cells were derived from different blastocysts to ensure that the two cell lines were independent. The cultured ES cell lines display a normal karyotype and maintain normal ES cell morphology, represent key stem-cell markers, were transcriptionally similar to control ES cells and differentiated into multiple cell types in vitro and in vivo. Our results represent successful nuclear reprogramming of adult somatic cells into pluripotent ES cells and demonstrate proof-of-concept for therapeutic cloning in primates.

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**Cloned mammals have problems**

*Daily Mail* 16 November 2007
The University of Hawaii medical school said that Cumulina died in her sleep last Friday of natural causes. The mouse was two years, seven months old – about seven months above average. That corresponds to age 95 in human years, the university said in a statement.

Cloning long dead animals?

Production of healthy cloned mice from bodies frozen at −20°C for 16 years

Abstract

Cloning animals by nuclear transfer provides an opportunity to preserve endangered mammalian species. However, it has been suggested that the “resuscitation” of human extinct species such as the woolly mammoth is impractical, as no live cells are available, and the genetic material that remains is morbidly degenerated. Here we report production of cloned mice from bodies kept frozen at −70 °C for up to 18 years without any cryoprotection. All of the cells were harvested after thawing, as used in modified cloning method and examined nuclei from several stages for use in nuclear transfer attempts. Using human nuclear as nuclear donors, we established embryonic stem cell lines from the cloned embryos. Healthy cloned mice were then produced from these nuclear-transferred embryonic stem cells by serial nuclear transfer. Thus, nuclear transfer techniques could be used to “resuscitate” animals or maintain valuable genetic stocks from mice frozen for prolonged periods without cryopreservation.

Just how close is it, really?
a closely related topic:

Embryonic Stem Cells

Stem cells can divide indefinitely, and give rise to differentiated cell types.
Typical Stem Cell Populations

- Hematopoietic stem cells (in bone marrow and circulation)
- Epidermal stem cells
- Intestinal crypt cells
- Germ cells (producing sperm and eggs)

Problems with Adult Stem Cells

- Rare
- Difficult to identify and isolate
- Limited potential to make other cell types (plasticity)
- Limited capacity for self-renewal

If only we were newts.
Stem cells offer the hope of replacing, renewing, regenerating or repairing any cell, tissue or organ in the body.

The fertilized egg (zygote) is totipotent - capable of giving rise to every cell type found in the body, and to all extraembryonic tissues (i.e., the placenta etc.)

Pluripotent Embryonic Stem Cells come from the **Inner Cell Mass** or **Epiblast** of the early embryo
Pluripotent Stem Cells
Capable of giving rise to all body cell types
Unable to generate trophoblast-derived placental tissues
Maintain normal genetic makeup (karyotype)
Capable of indefinite self-renewal
Can remain undifferentiated (without signal)

Using Embryonic Stem Cells
Potential therapeutic uses of stem cells to repair the nervous system
Parkinson’s disease
Huntington’s disease
Spinal cord injury
Stroke
Multiple sclerosis

Dopaminergic nerve cells derived from mouse ES cells
Stem Cell-derived Therapies are Here

Problems with Embryonic Stem Cells
Ethical issues of human embryo manipulation and destruction
Safety concerns with most/all stem cells:
- Immune system rejection of grafts
- Regulation of growth and differentiation
- Transmission of infectious agents
- Long-term safety and efficacy

Solving Technical Problems
Therapeutic cloning (but, new ethical issues)
Genetic manipulation of ES cells to avoid immune system rejection of grafts
Other options: iPS cells

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Induced Pluripotent Stem Cell Lines Derived from Human Somatic Cells

Junying Yu,1,2,3 Hanlin A. Yedoyin,1,2 Kim Seog-Ju Oh,1,2 Jessica Antosiewicz-Bourget,1,2 Jennifer L. Frame,1,2 Shulan Tian,1,2 Jeff Wu,3 Gudrun A. Jenuwein,1 Victor Ruddle,1
Ann Stevens,1 Igor I. Slukins,1 James A. Thomson1,3,4

Somatic cell nuclear transfer allows transecting factors present in the mammalian nucleus to reprogram somatic cell nuclei to an undifferentiated state. We show that four factors (Oct4, Sox2, Nanog, and Lin28) are sufficient to reprogram human somatic cells to pluripotent stem cells that exhibit the essential characteristics of embryonic stem (ES) cells. These induced pluripotent human stem cells have normal karyotypes, express telomerase activity, express cell surface markers and genes that characterize human ES cells, and maintain the developmental potential to differentiate into advanced derivatives of all three primary germ layers. Such induced pluripotent human cells then should be useful in the production of new disease models and in drug development, as well as for applications in transplantation medicine, since technical limitations (for example, mutation through viral integration) are eliminated.

iPSC-derived tissues in teratomas

The coming years will show the promise and controversies of human embryonic and adult stem cells.

The Promise of Stem Cell Research

Drug Development and Toxicity Tests

Experiments to Study Development and Gene Control

Cultured Pluripotent Stem Cells

Tissues/Cells for Therapy

Bone Marrow

Nerve Cells

Heart/Muscle Cells

Pancreatic Islet Cells
Scientific Uses of Embryonic Stem Cells

Creation of Transgenic Organisms

[Transgenic: having experimentally altered genetic material by transfer of DNA from an external source.]

Method:
- ES cells isolated, genetically altered in vitro
- ES cells re-inserted into embryo to create chimera.
- Chimera reproduces to create pure transgenic

Figure 4.20(1) Production of Transgenic Mice

Figure 4.20(2) Production of Transgenic Mice

ES cells can integrate into ICM of blastocyst and contribute to the embryo.
Figure 4.20(3) Production of Transgenic Mice

Chimera has cells of two different genotypes

If ES cells contribute to germline, some gametes are transgenic

Figure 4.20(4) Production of Transgenic Mice

Heterozygous transgenic mice

Cross heterozygous progeny

Homozygous wild-type

Heterozygous transgenic

Homzygous transgenic

The Nobel Prize in Physiology or Medicine 2007

"For their discoveries of principles for introducing specific gene modifications in mice by the use of embryonic stem cells"
Gene Targeting and the use of Embryonic Stem Cells in Mice
(from the Nobel website)

Figure 4.10(1) Cloning of Transgenic Mammals to Produce Protein Pharmaceuticals

Figure 4.10(2) Cloning of Transgenic Mammals to Produce Protein Pharmaceuticals