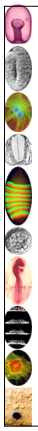


Anderson - Cloning Quote

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

Early evidence:

Cytogenetics

- all cells appeared to have same chromososmes

Metaplasia

- differentiated cells 'de-differentiate,' generate other differentiated cell types
(seen in regeneration, also some cancers)

Evidence for Genomic Equivalence

Possibilities for cellular differentiation mechanism:

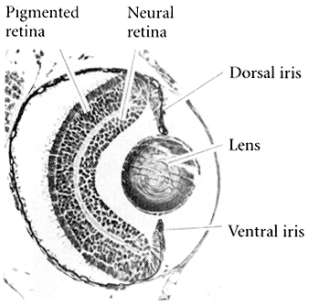
1. During development, cells discard genes not needed in their later development.

   Corollary: Only germ cells retain all genes.

   (A few examples known of chromosomal loss during development: Antibody genes in immune cells.)

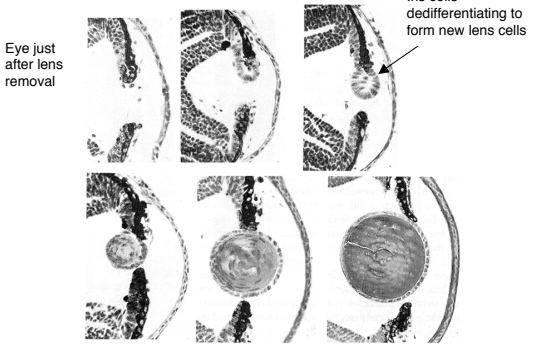
2. Differentiated cells retain all genes, but only use some.

Metaplasia *in Salamander Lens Regeneration*



Normal Unoperated Eye

Metaplasia *in Salamander Lens Regeneration*



Evidence for Genomic Equivalence

The ultimate evidence: ***Animal Cloning***

reproducing a complete organism from a single differentiated adult nucleus

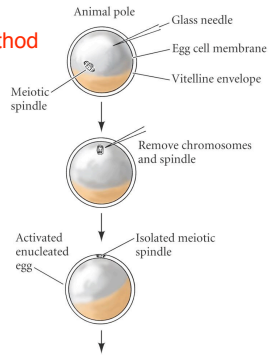
First accomplished with frogs:

Briggs & King (1950's) - work with *Rana*

Gurdon (1960's) - work with *Xenopus*

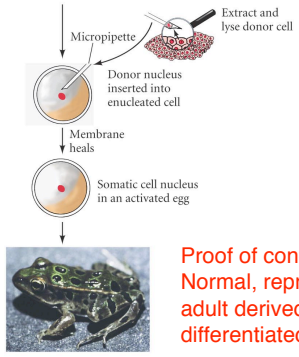
Figure 4.5(1) ***Procedure for Transplanting Blastula Nuclei into Activated Enucleated* Rana pipiens *Eggs***

Frog cloning method



Egg Enucleation

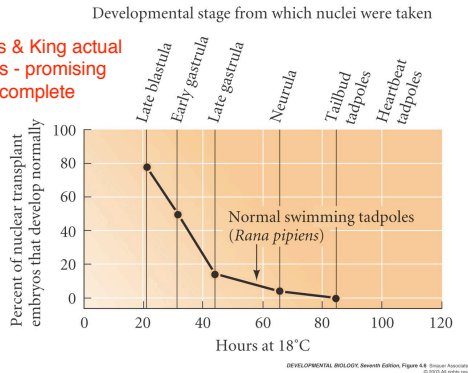
Figure 4.5(2) ***Procedure for Transplanting Blastula Nuclei into Activated Enucleated* Rana pipiens *Eggs***



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



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***

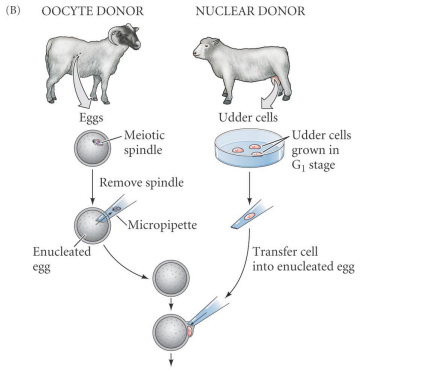
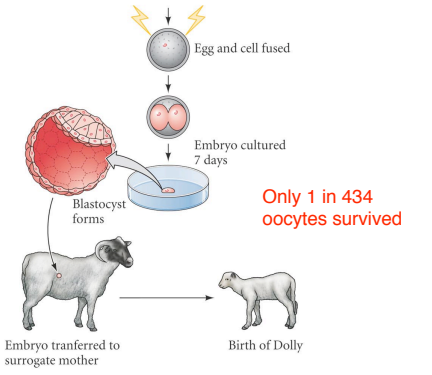


Figure 4.8(3) ***Cloned Mammals, Whose Nuclei Came From Adult Somatic Cells***

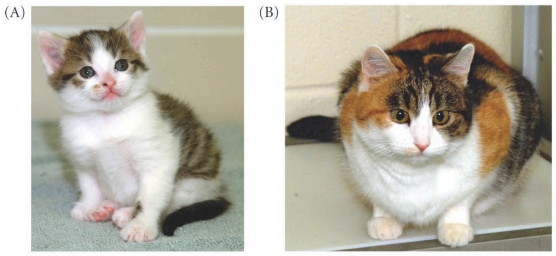


Other mammals cloned in quick succession after 'Dolly' (1997)

1998 - Cow, Mouse
2000 - Goat, Pig
2002 - Cat
2003 - Rat, Mule, Horse
2004 - Deer
2005 - Dog

Not yet: any Primates

Figure 4.9 ***The Kitten "CC" Is a Clone Produced Using Somatic Nuclear Transfer from "Rainbow" (B)***



The ultimate argument against cloning -- cats! (just kidding)

DEVELOPMENTAL BIOLOGY, Seventh Edition, Figure 4.9 Sinauer Associates, Inc. ©2003 All rights reserved.

Cloned mammals have problems

Or not…

First Cloned Mouse Dies Of Old Age

Cumulina Was Two Years, Seven Months Old

HONOLULU May 10, 2000

E-MAIL STORY | PRINT STORY | SPHERE | SHARE | TEXT SIZE: A A A

One of these mice may be Cumulina. Who can tell? **(AP)**

(CBS) Cumulina, the world's first cloned mouse, has died of old age.

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.

The mouse made headlines when the results of the distinctive cloning technique of Dr. Ryuzo Yanagimachi's team were reported in the journal *Nature* in July 1998.

The scientists turned out more than 50 carbon-copy mice using what was believed to be a more reliable cloning technique than the one used to create Dolly the sheep. Cumulina was the first of the 50, born October 3, 1997.

The nucleus of a cell from one mouse was injected through a tiny needle into an egg donated by a second mouse. The egg's original genetic package was removed. The donor nucleus came from cumulus cells, which surround the developing eggs in the ovaries of female mice.

The technique gave Cumulina her name.

Cumulina raised two litters before **"retiring."** About eight months ago, Cumulina developed a skin tumor, common in aging mice, and the tumor was removed. She had otherwise been healthy and active until several days before her death, the university said.

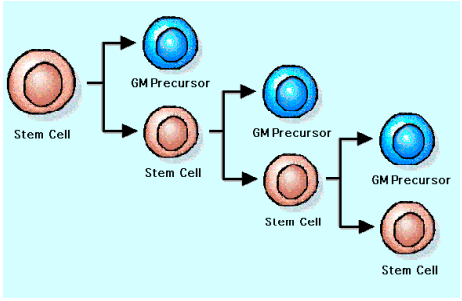
Cumulina's remains will be preserved and mounted in a new permanent exhibit in the new Institute for Biogenesis Research, which is scheduled to be dedicated in the fall, Yanagimachi said.

Just how close is it, really?

a related topic:

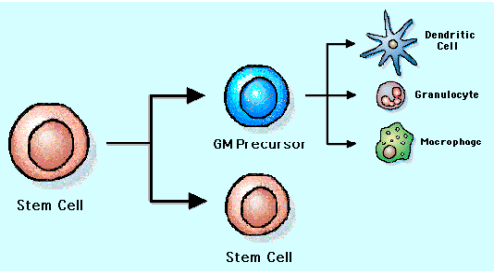
Embryonic Stem Cells

Stem cells



Stem cells can divide indefinitely...

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

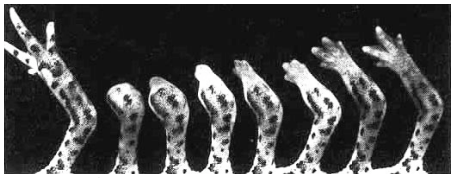
Rare

Difficult to identify and isolate

Limited potential to make other cell types (plasticity)

Limited capacity for self-renewal

If only we were newts.



Before Amputation

0 7 21 25 32 42 72

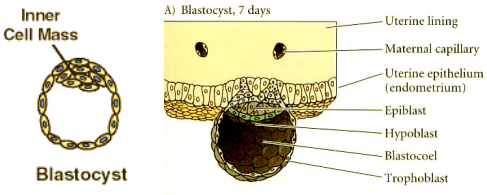
Days Post-Amputation

Stem cells offer the hope of replacing, renewing, regenerating or repairing any cell, tissue or organ in the body.

The Ultimate Stem Cell

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.)

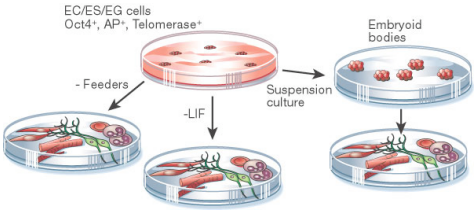
Pluripotent Embryonic Stem Cells come from the **Inner Cell Mass** or **Epiblast** of the early embryo



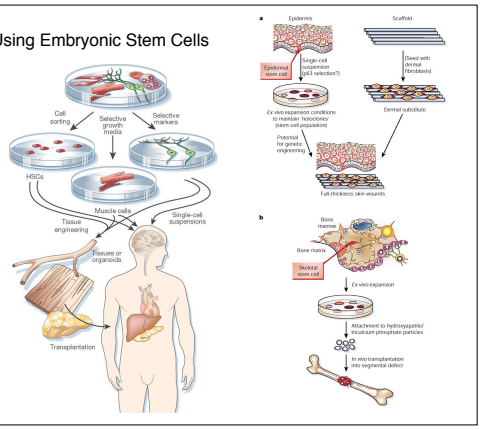
Pluripotent Stem Cells

- 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



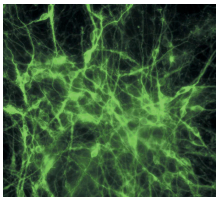
Using Embryonic Stem Cells



Using Embryonic Stem Cells

Potential therapeutic uses of stem cells to repair the nervous system

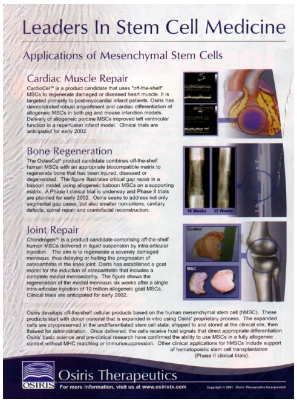
- Parkinson's disease
- Huntington's disease
- Spinal cord injury
- Stroke
- Multiple sclerosis



Dopaminergic nerve cells derived from mouse ES cells

Stem Cell-derived Therapies are Here

OSIRIS



Problems with Embryonic Stem Cells

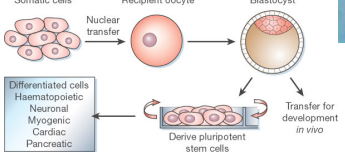
Ethical issues of human embryo manipulation and destruction

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: iPSCs

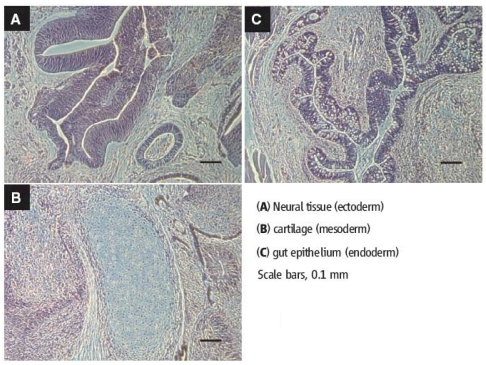
www.sciencemag.org SCIENCE VOL 318 21 DECEMBER 2007

Induced Pluripotent Stem Cell Lines Derived from Human Somatic Cells

Junying Yu,[1,2]* Maxim A. Vodyanik,[2] Kim Smuga-Otto,[1,2] Jessica Antosiewicz-Bourget,[1,2] Jennifer L. Frane,[1] Shulan Tian,[3] Jeff Nie,[3] Gudrun A. Jonsdottir,[3] Victor Ruotti,[3] Ron Stewart,[3] Igor I. Slukvin,[2,4] James A. Thomson[1,2,5]*

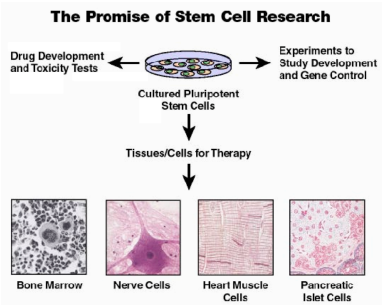
Somatic cell nuclear transfer allows trans-acting factors present in the mammalian oocyte to reprogram somatic cell nuclei to an undifferentiated state. We show that four factors (*OCT4*, *SOX2*, *NANOG*, and *LIN28*) are sufficient to reprogram human somatic cells to pluripotent stem cells that exhibit 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 cell lines should be useful in the production of new disease models and in drug development, as well as for applications in transplantation medicine, once technical limitations (for example, mutation through viral integration) are eliminated.

iPSC-derived tissues in teratomas



(**A**) Neural tissue (ectoderm)
(**B**) cartilage (mesoderm)
(**C**) gut epithelium (endoderm)
Scale bars, 0.1 mm

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



Scientific Uses of Embryonic Stem Cells

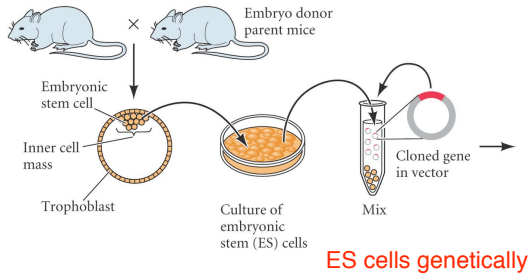
Creation of **Transgenic** Organisms

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

Method:

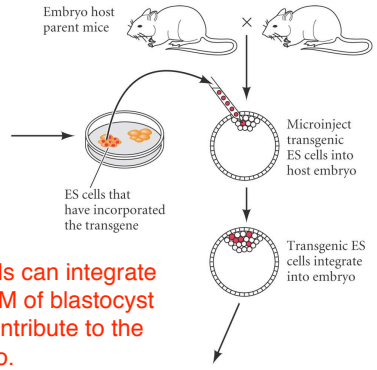
- ES cells isolated, genetically altered *in vitro*
- ES cells re-inserted into embryo to create chimera.
- Chimera reproduces to create pure transgenic

Figure 4.20(1) ***Production of Transgenic Mice***



ES cells genetically altered *in vitro*

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***

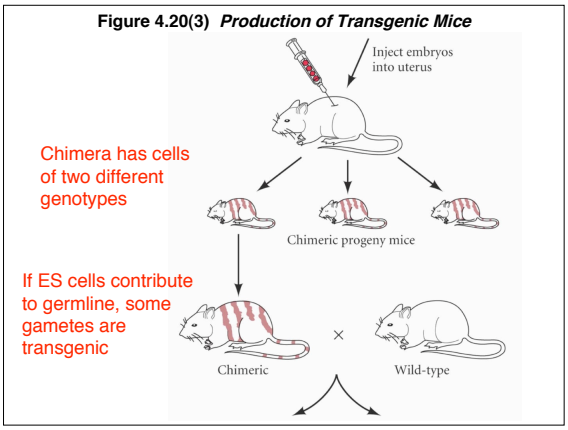
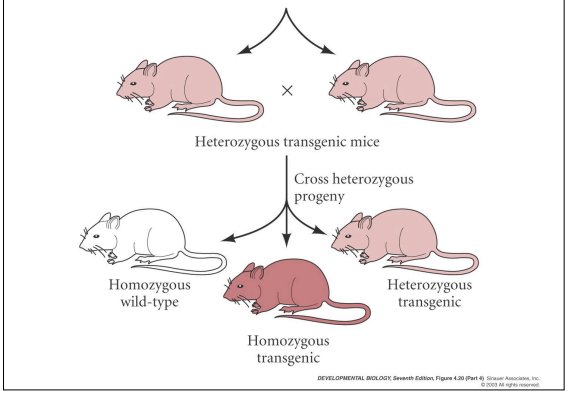


Figure 4.20(4) ***Production of Transgenic Mice***



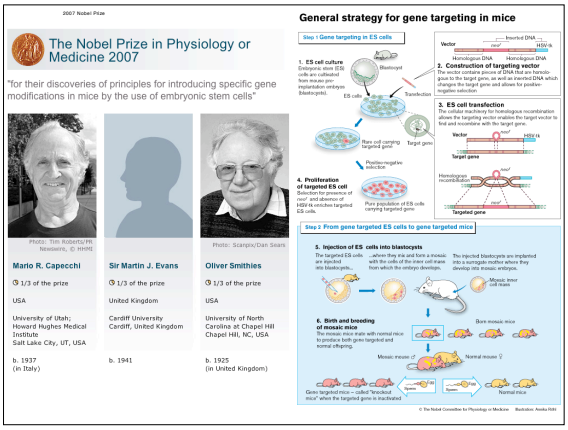


Figure 4.10(1) ***Cloning of Transgenic Mammals to Produce Protein Pharmaceuticals***

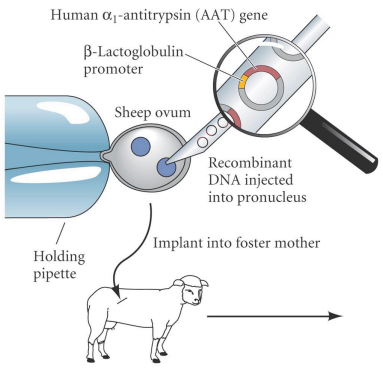


Figure 4.10(2) ***Cloning of Transgenic Mammals to Produce Protein Pharmaceuticals***