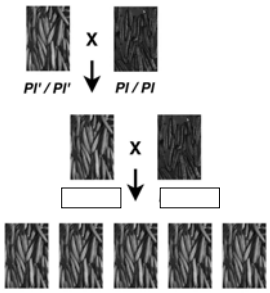
Image courtesy of Prof. Jay Hollick, MCB Department

?!!!



R. Alexander Brink, 1950
Vicky Chandler, Jay Hollick et al

Broadly speaking

An "epigenetic" effect on the genome changes the phenotype without changing the genotype.

→ The power of the environment and of life history

Technically

"A mitotically or meiotically heritable change in gene expression state (or genome functional state) that is not associated with a change in the primary sequence of DNA."

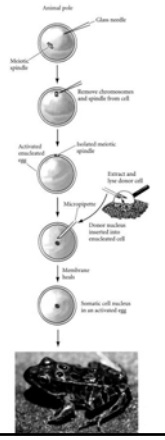
In other words

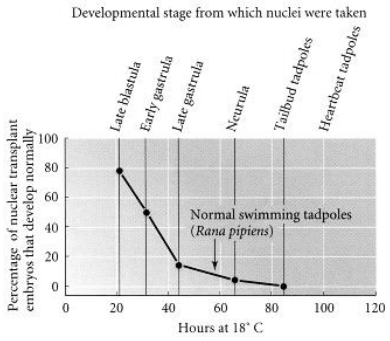
Genetics	Epigenetics
Organism (or a cell) with a phenotype	Organism (or a cell) with a phenotype
↓	↓
Mutation (change in DNA)	Something happens, but **not** a change in the DNA
↓	↓
Different phenotype	Different phenotype

"Cloning":

hello, Dolly,
and
hello again, Dolly

King and Briggs (1956).
Serial transplantation of embryonic nuclei
Cold Spring Harbor Symp. Quant. Biol. 21: 271-289.





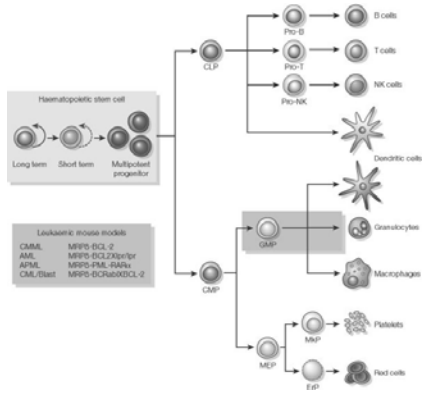
McKinnell, R. G. 1978. *Cloning: Nuclear Transplantation in Amphibia.*
University of Minnesota Press, Minneapolis.

?

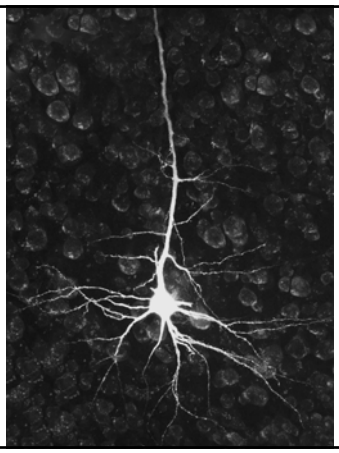
How can one explain the fact that cloning works so much better if one use a cell from an early embryo as the donor of the nucleus?

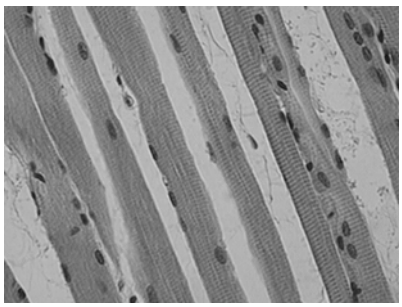
Two explanations

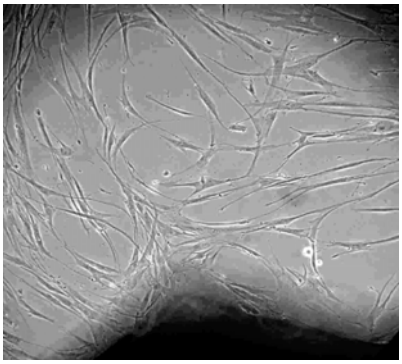
1. Alteration of the actual DNA of the cells as the embryo develops.
2. Something else.



Reya, Clarke, and Weissman. *Nature* 2001.







King and Briggs (1956).
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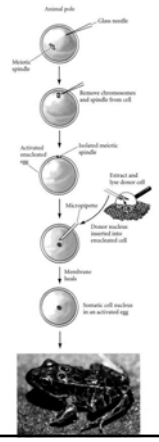
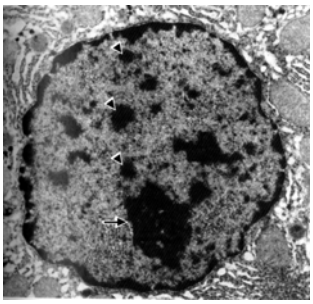
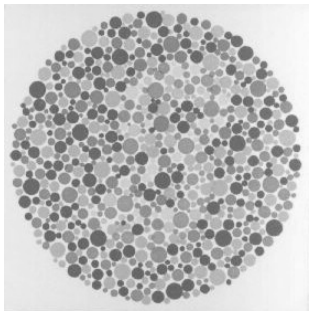






Dolly

Bill Ritchie

Ian Wilmut Dolly

Semantics

1. Reproductive cloning: make new organisms.
2. Therapeutic cloning (aka “somatic cell nuclear transfer”): no organism made.

Extensive abnormalities in cloned animals

- Lung failure
- Liver failure
- Obesity
- Etc etc

Two problems:

1. Cloning is incredibly inefficient.
2. Of the animals that are born, many have severe defects.

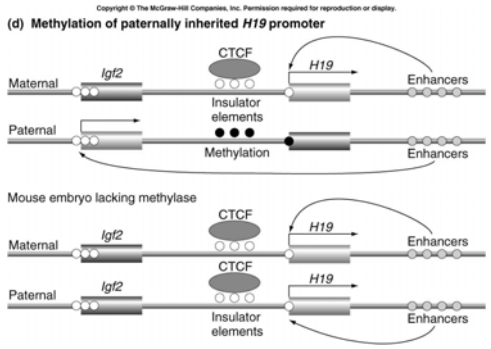
Proof that these abnormalities are entirely epigenetic

Dolly’s lambs, and the offspring of all cloned animals, are normal.

Solter and Surani

Gynogenetic embryos – very small.

Androgenetic embryos – very large.



Ah, terminology

Genes for which you have your Mother's copy turned on:

Maternally expressed

Genes for which you have your Dad's copy turned on:

Paternally expressed

Spontaneous ^{me}CpG deamination (colon cancer)

Should be 4% of all NN – in fact, is 0.8%.

Methylation:

C → 5mC

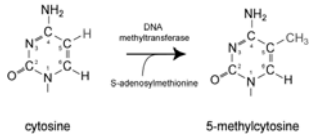
CpG → 5mCpG

5mCpG → TpG → TpA

deamination → MMR

CpG→ UpG → CpG

(no mutation)



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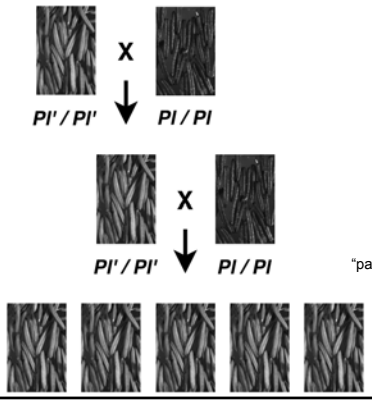


"paramutation"!

The Haig hypothesis

Imprinting evolved as a manifestation of parental conflict over the allocation of maternal resources to the developing fetus: "intrauterine tug of war" over how big the fetus will be.

Paternally expressed genes increase embryo size.

Maternally expressed genes decrease embryo size.

Peromyscus polionotus
(the monogamous mouse)

Vrana et al. Nature Genetics 20: 362 (1998).

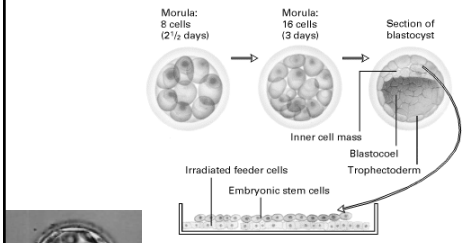
Don't clone humans

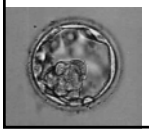
1. Responsibility for child and his/her "developmental abnormalities."
2. Naïve overestimation of role of DNA in shaping the human being.

"Therapeutic cloning"

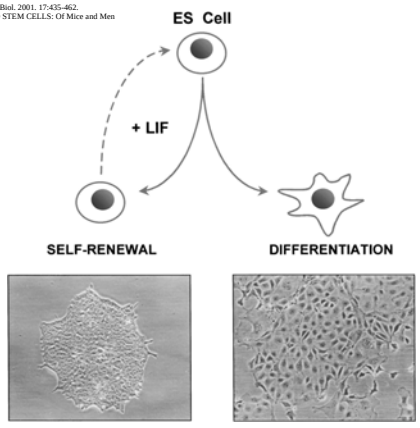
= somatic cell nuclear transfer

Embryonic stem cells





Annu. Rev. Cell Dev. Biol. 2001. 17:435-462.
EMBRYO-DERIVED STEM CELLS: Of Mice and Men
Austin G. Smith



ES cells – status quo

- Limited number of human ES cell lines available for research with federal funds
- Growth on mouse feeders makes them unsuitable for use as therapeutics
- The indications being considered are, among others, cardiovascular and neurological

Why ES cells and not adult stem cells?

For the simple reason that ES cells are *incomparably* easier to grow to large numbers in a dedifferentiated state, and then drive them – in a controlled fashion! – to differentiate into a specific cell type.

Note: in this context, "incomparably" means "the difference between essentially impossible and feasible."

"Dopamine neurons derived from embryonic stem cells function in an animal model of Parkinson's disease" – Ron McKay et al

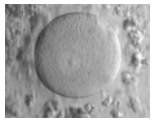
Parkinson's disease is a widespread condition caused by the loss of midbrain neurons that synthesize the neurotransmitter dopamine. Cells derived from the fetal midbrain can modify the course of the disease, but they are an inadequate source of dopamine-synthesizing neurons because their ability to generate these neurons is unstable. In contrast, embryonic stem (ES) cells proliferate extensively and can generate dopamine neurons. If ES cells are to become the basis for cell therapies, we must develop methods of enriching for the cell of interest and demonstrate that these cells show functions that will assist in treating the disease. Here we show that a highly enriched population of midbrain neural stem cells can be derived from mouse ES cells. The dopamine neurons generated by these stem cells show electrophysiological and behavioural properties expected of neurons from the midbrain. Our results encourage the use of ES cells in cell-replacement therapy for Parkinson's disease.

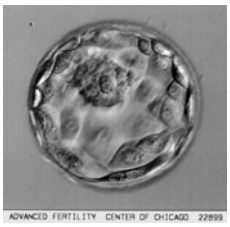
Nature. 2002 Jul 4;418(6893):50-6

The problem

In order to generate ES cells, one has to destroy an early human embryo

- Twenty eight thousand IVF births in the US in 1998
- Six to fourteen embryos per birth – healthy ones frozen, and then discarded (=flushed down a sink)





Reading

Life's Dominion: An Argument about Abortion, Euthanasia, and Individual Freedom

Ronald Dworkin

A way to overcome this entire issue?

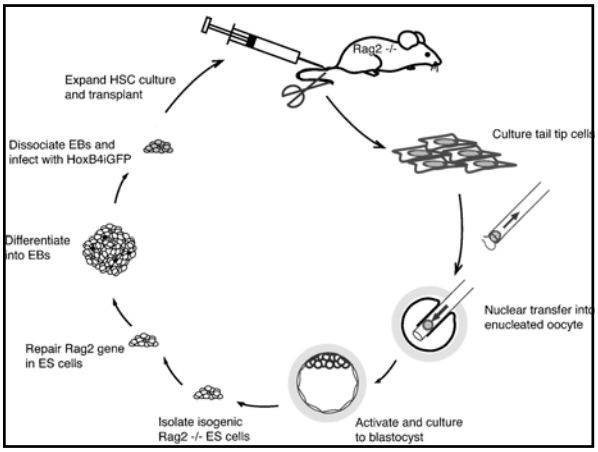
1. Patient with failing organ.
2. Take nucleus from patient's cell.
3. Do somatic cell nuclear transfer to generate ES line from that patient.
4. Transdifferentiate that line *ex vivo* into cell type relevant to disease.
5. Reimplant in patient.

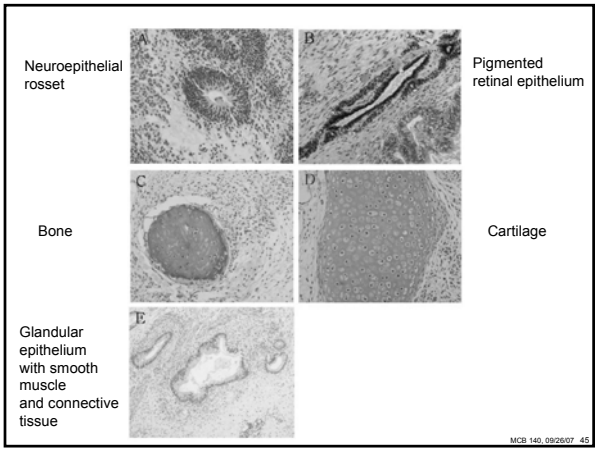
Correction of a Genetic Defect by Nuclear Transplantation and Combined Cell and Gene Therapy

Rideout et al.

Cell (2002)

109, 17-27





What's next with "therapeutic cloning"? ("nuclear transfer")

I don't know.

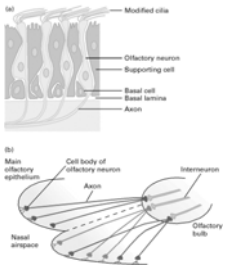
There is likely to be a complex polemic between patient advocacy groups (on the one hand) and groups opposed to somatic cell nuclear transfer on various grounds.

Mice cloned from olfactory sensory neurons

Eggan et al.
(Rudolf Jaenisch and Richard Axel)
Nature (2004)
428(6978):44 9

Natalie Angier
Unnatural Obsessions

"The adjective that scientists use to describe a well-wrought experiment is "elegant" – which means not only that it worked, but it worked in style."



Sense of smell

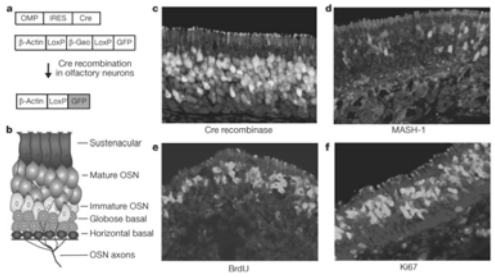
"One particularly clear example of neuronal diversity is provided by the olfactory sensory epithelium. In the mouse, each of the 2,000,000 cells in the olfactory epithelium expresses only one of about 1,500 odorant receptor genes, such that the functional identity of a neuron is defined by the nature of the receptor it expresses. Thus, the sensory epithelium consists of at least 1,500 neuronal types. The pattern of receptor expression is apparently random within one of four zones in the epithelium, suggesting that the choice of receptor gene may be stochastic. One mechanism to permit the stochastic choice of a single receptor could involve DNA rearrangements."

Eggan et al. Nature. 2004 Mar 4;428(6978):44-9

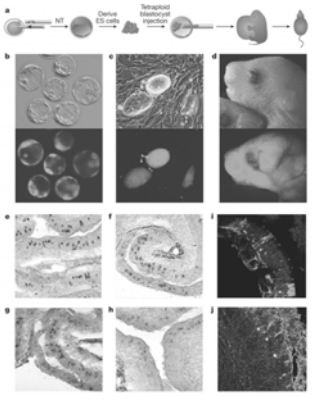
Allelic inactivation regulates olfactory receptor gene expression

We suggest a model in which a hierarchy of controls is exerted on the family of odorant receptor genes to assure that a sensory neuron expresses a single receptor from a family of 1000 genes. We propose that a cis regulatory element directs the stochastic expression of only one gene from a large array of linked receptor genes. Moreover, only one allelic array encoding multiple receptor genes is active in an individual neuron. We demonstrate that in a neuron expressing a given receptor, expression derives exclusively from one allele.

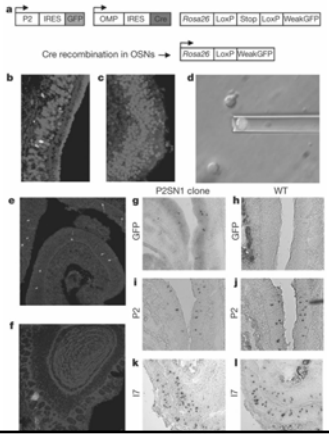
Chess et al. Cell. 1994 Sep 9;78(5):823-34



Eggan et al. Nature. 2004 Mar 4;428(6978):44-9



Eggan et al. Nature. 2004 Mar 4;428(6978):44 9



Question → answer

"The regulation of gene expression by DNA rearrangement is rare, but this mechanism has nonetheless been suggested to explain the diversity inherent in complex nervous systems."

Well, we now know that it is NOT how neuronal diversity in olfactory epithelium is created. The difference between the individual neurons expressing different receptors is not at the level of DNA – it's epigenetic.

Eggan et al. Nature. 2004 Mar 4;428(6978):44-9