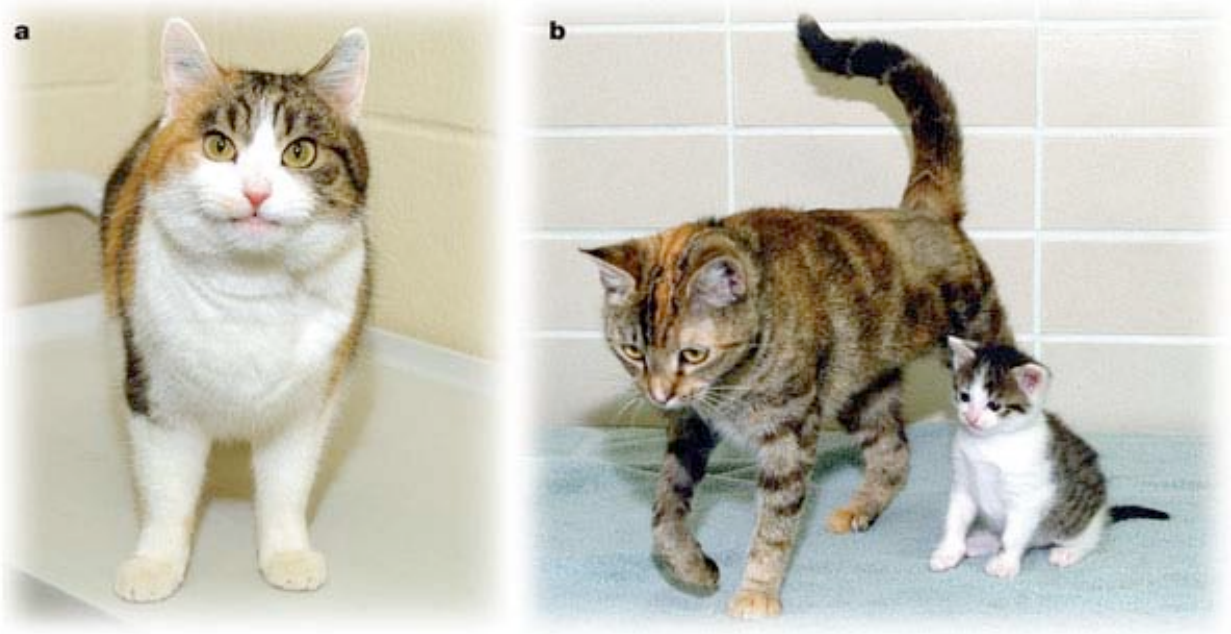
3/9/04 Cloning/Stem Cells

Meet (left to right):
Rainbow, Allie and cc (carbon copy) who is a *genetic clone* of Rainbow. Allie is cc's surrogate mom



February 14, 2002

First Cloned Cat Is Born, Scientists Report

By REUTERS

WASHINGTON (Reuters) - A 2-month-old kitten called "Cc:" is the first successful product of a program aimed at letting people clone their pets, scientists said on Thursday.

(Reuters)
The world's first-ever cloned cat, "Cc," is seen at seven weeks old with Allie, her surrogate mother.

The kitten joins a growing list of animals that have been cloned from adult cells, starting with Dolly the sheep and now including pigs, goats, cattle, mice and an oxlike creature called a gaur.

"She is as cute as a button," said a spokeswoman for Texas A&M, where the work was done using a grant from philanthropist John Sperling's Apollo Group Inc..

"The kitten was vigorous at birth and appears to be completely normal," Mark Westhusin and colleagues write in their report in a letter published in the science journal Nature.

The kitten is a calico-and-white shorthair that looks similar to, but not exactly like, her genetic mother. The kitten looks very different from the tabby that gave birth to her.

The scientists said her coat coloring was unique because not only genetics contribute to an animal's markings, but also conditions in the womb.

To see entire article:
http://fire.biol.wwu.edu/trent/trent/2.14.02NYTCAT.pdf
Note how many attempts it took to generate one viable kitty

February 15, 2002

What Is Warm and Fuzzy Forever? With Cloning, Kitty

By GINA KOLATA

Scientists in Texas have cloned a cat, opening the door to what some experts say will be the first large- scale commercial use of cloning Ñ to reproduce beloved pets.

The effort was supported by a company, Genetic Savings and Clone, of College Station, Tex., and Sausalito, Calif., which wants to offer cloning to dog and cat owners. It is investing $3.7 million in the project.

The study will be published in the Feb. 21 issue of Nature, a British science journal, but Nature released the paper yesterday because the result, although not the details of the study, had become public. News of the company's success was first reported yesterday in The Wall Street Journal.

It was, some said, long expected.

"The commercial future of cloning is absolutely in animals," said Dr. Arthur Caplan, an ethicist at the University of

College of Veterinary Medicine, Texas A&M University
Ushering in pet cloning are donor, Rainbow, top, and offspring, cc.

Cloning experts say that if their experience is any guide, Genetic Savings and Clone will not lack for interested pet owners.
Dr. George Seidel, a cloning researcher at Colorado State University, said he had heard from many interested pet owners, though he could not help them.
He said he remembered one woman who called him about her cat, named Stinky. "Stinky had died,and she had put it in her freezer three weeks earlier," Dr. Seidel recalled. "She asked me what we could do. I said, `I don't think you've got any hope there. Take Stinky out of the freezer and bury it.' "
Read entire article:
http://fire.biol.wwu.edu/trent/trent/2.15.02NYTCAT.pdf

Clone: population of cells or organisms produced by repeated asexual reproduction

[Cloning a gene: to produce many copies of a gene by repeated cycles of replication]

But Cloning a mammal?

Why?
How?
How come it is so hard to do?

Why Clone animals?

To address a scientific question

To generate genetic clones of animals for use in basic and applied scientific research

To generate "personalized" embryonic clones of human embryos from which stem cells can be derived

To generate genetic clones of animals (pets) for commercial purposes… to make money

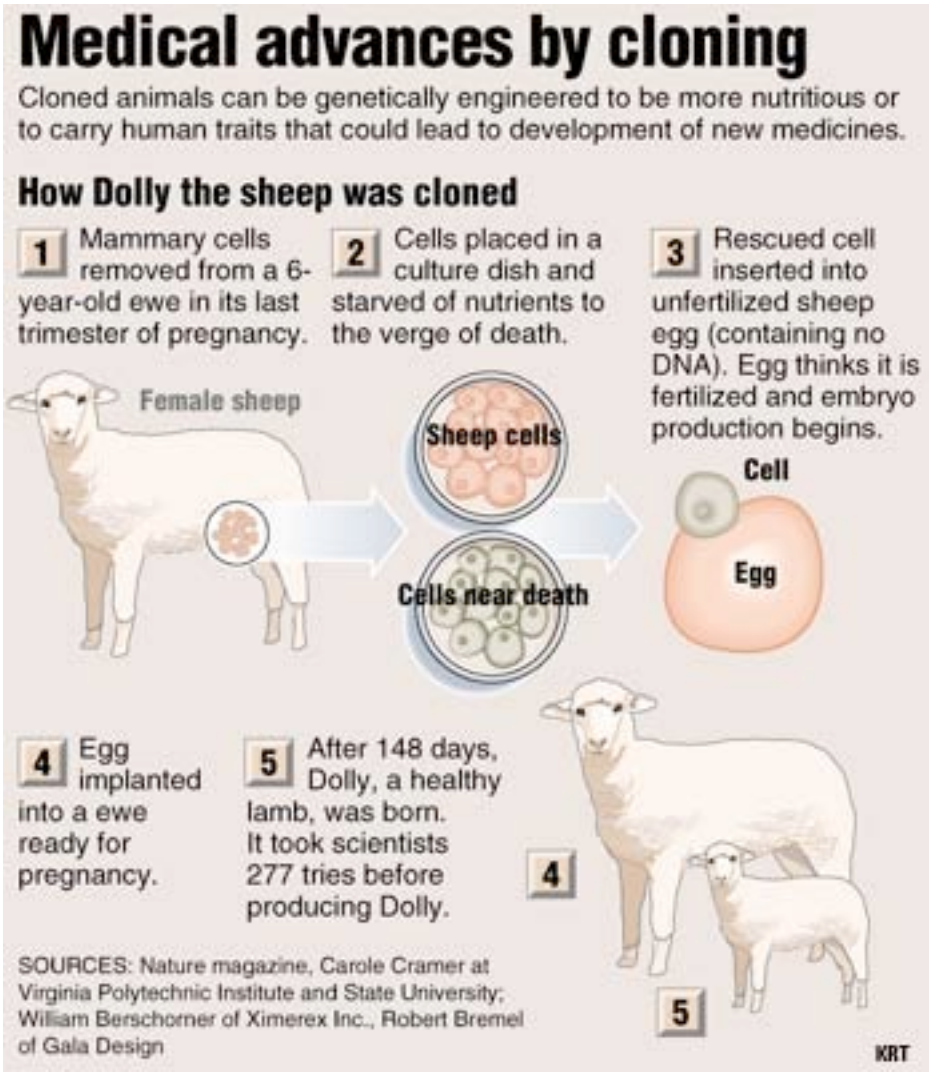
To address a scientific question

Original cloning experiment were performed in amphibians (decades ago) to address the following question:

Do the nuclei of differentiated (specialized) adult cells (such as an intestinal cell or a nerve cell) still contain a complete genome capable of directing the development of an entire organism?

Experiments demonstrated that the nucleus of an intestinal cell could direct the development of viable frogs -- indicating that adult cells retain a complete genetic complement

Figure 2 Cloned frogs. These 19 identical male albino frogs were prepared by nuclear transplantation into unfertilized eggs of the dark green female frog33. (Male frogs are about half the size of females.)

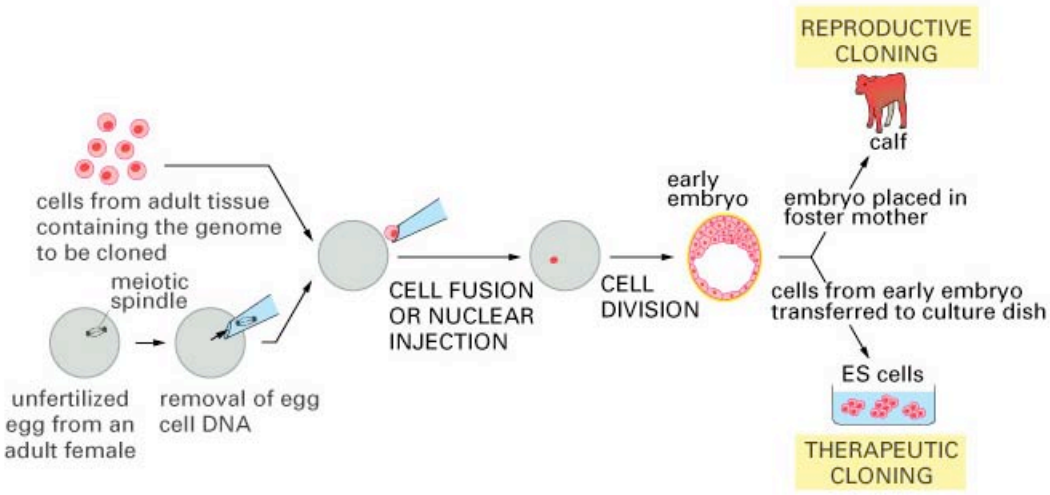


****

****

Since the birth of Dolly, the sheep, successful cloning experiments have been reported in mice, cattle, goats, pigs and cats. Dogs have not been successsfully cloned. But mules have!

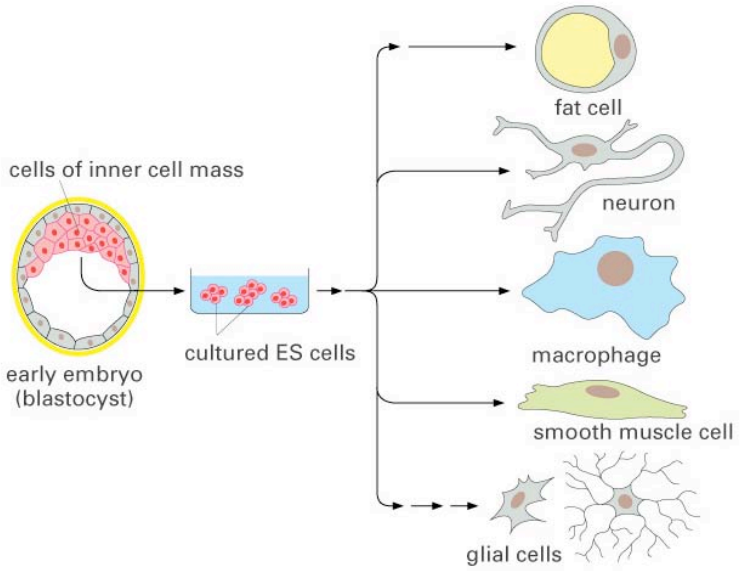
Reproductive cloning vs. Therapeutic Cloning



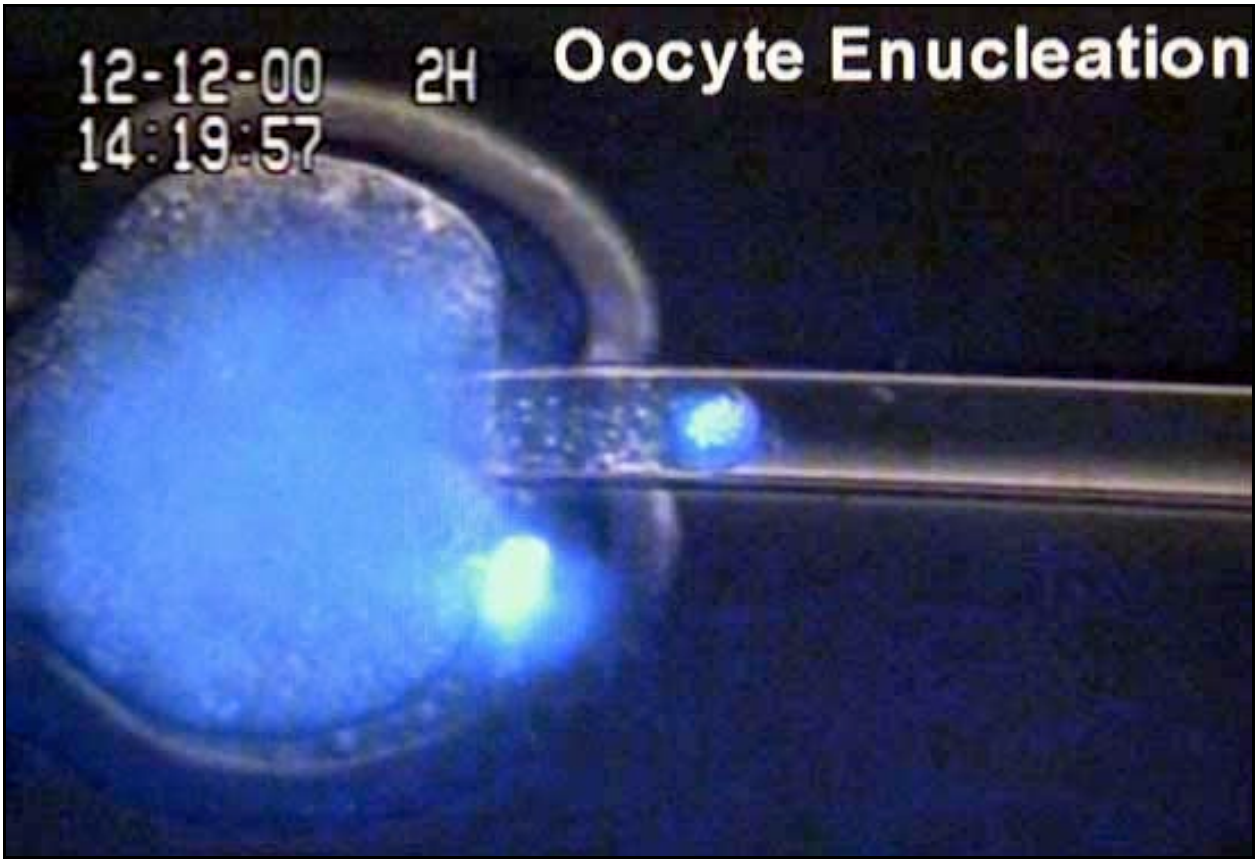
Nuclear-transplantation techniques in mammals. The genetic material is removed from the recipient cell (an egg), then replaced by a nucleus from a donor cell. The resulting embryo is then transferred to a surrogate mother. The clones are (almost) genetically identical to the donor.

Many scientists agree that the human embryonic stem cells could serve as a universal repair kit for patching up impaired body tissues. Other scientists argure that stem cells are "over-hyped"

Therapuetic cloning generates "genetically personalized" stem cell populations



Many differentiated (specialized) cells, such as red blood cells, skin & gut cells, are themselves unable to divide. The integrity of our body depends on stem cells, that can proliferate (divide) indefinately and produce differentiated cell progeny. Stem cells allow for the continued renewal of normal tissue, as well as the repair of tissue lost through injury.



DNA is removed from a mammalian egg using suction through a pipette.
DNA is stained with a fluorescent dye

February 2004
Researchers in South Korea reported that they produced cloned human embryos by nuclear transfer:

- Nuclear DNA from the cumulus cells of 16 women was injected into 242 enucleated eggs. [Cumulus cells are a specialized type of cell that helps to nourish developing eggs.]
- 30 embryos were obtained
- The embryos were allowed to divide in culture for 5-6 days; then the inner cell mass (embryonic stem cells) of each embryo was harvested
- ONE viable stem cell culture (propagated in the lab for over a year) was generated from only one of the embryos
- This stem cell line was induced to form muscle, bone, cartilage and connective tissue in mice

The Korean group did not try to produce a baby. Therapeutic stem cell cultures was the goal.

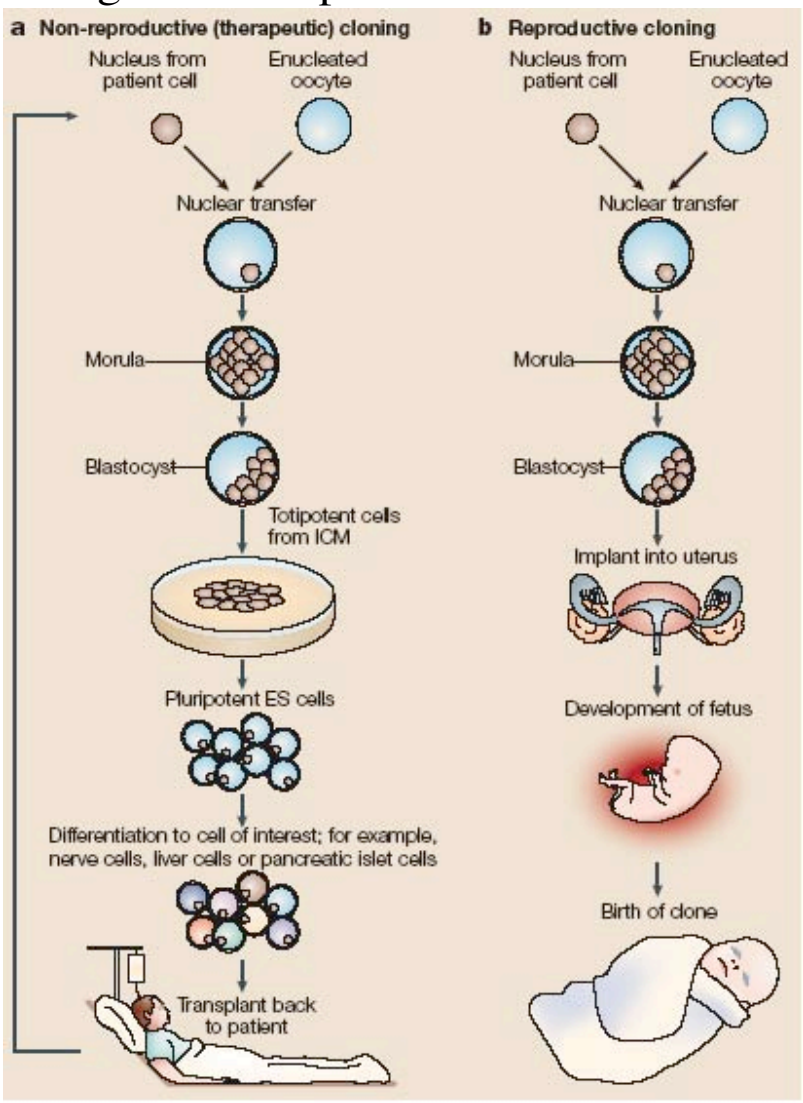
An interesting aspect of the South Korean research:

- DNA from adult male cells failed to produce embryos
- DNA from females cells genetically unrelated to the donor eggs failed to produce embryos

Does the (rare) successful generation of a stable embryonic stem cell line mean that this embryo could have produced a viable baby?

Many biologist say NO and distinguish cloned embryos from the blastocytes that form during normal reproduction.

So what's the difference in generating a pregnancy and an embryonic stem cell line that can generate many different types of tissues?



Enough experience has accumulated to assess the risks of cloning:

- animal cloning is inefficient (its took 276 unsuccessful attempts before the sheep Dolly was produced) and is likely to remain so for the foreseeable future
- cloning results in gestational and neonatal developmental failures
- at BEST, a few percent of the nuclear transfer embryos survive to birth and, of those, many die within the perinatal period
- newborn clones often display respiratory distress and circulatory problems and even apparently healthy survivors may suffer from immune dysfunction, or kidney or brain malfunction
- and Dolly (the sheep) developed premature arthritis

Many of the cloning failutes and many of the fetal abnormalities and abnormalities in those few clones that are born alive probably result from failures in **genomic reprogramming**.

Cloning and Genomic Programming

From skin and muscle to nerve and blood, our bodies are composed of more than 200 distinct types of cells.

During develoment of a multicellular organism from a single fertilized eggs, cell lineages become

- progressively more restricted with respect to the types of cells that they can form and
- progressively more commited to forming a particular type of cell.

lineage: the natural progression from an immature cell type to one or more differentiated cell types

committed: used to describe cells that whose fate is already determined along a particular path of differentiation

differentiation: the development of specialized cell types from the single fertilized egg

Differentiation events are associated with the genomic programming events that control (in part) expression of the 30,000 -40,000 genes in the human genome

Differentiated cell types exhibit different patterns of gene expression (transcription/translation):

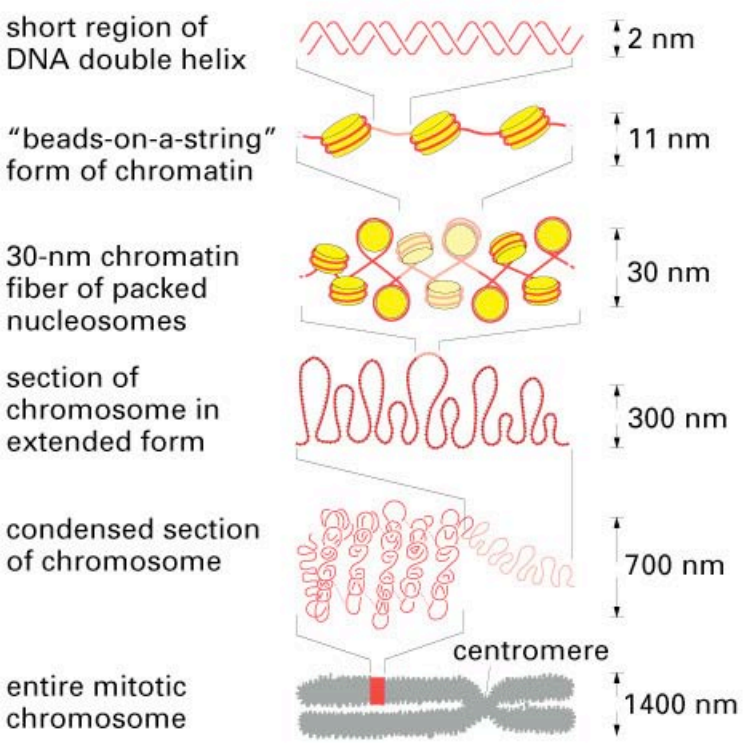
- during cell differentiation, expression of cell-specific genes is activated: which genes are turned “on” or “off” will be specific to each cell type
- during differentiation, the cell’s genome is programmed in a way that limits its capacity to express embryonic genes as well as some types of adult cell-specific genes
- differentiated cell types tend to have a limited capacity to divide

Gene/genomic Reprogramming:
In summary, normal develoment depends on a precise sequence of changes in the pattern of *gene expression (or transcription and translation)*:

- Some genes are expressed in only embryonic cells; other genes are expressed only in adult cells
- Other genes are expressed all the time in all cells (housekeeping genes)

Epigentic programming: during development some genes are more or less "permanently" turned off (and never transcribed again):

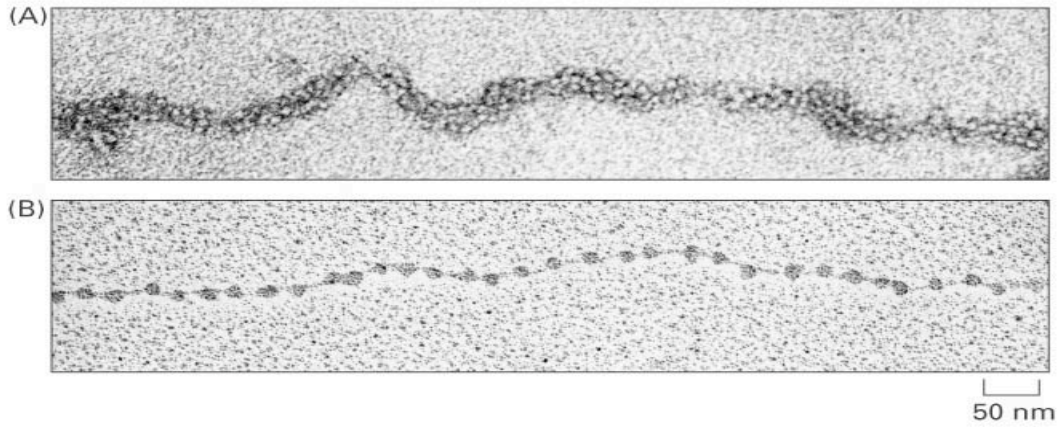
- they are chemically modified (methylation of purine/pyrimidine bases) and complexed with specific proteins (usually histones) that prevent access by the transcription machinery
- These epigenetic modifications are important in tissue-specific expression of genes



NET RESULT: EACH DNA MOLECULE HAS BEEN PACKAGED INTO A MITOTIC CHROMOSOME THAT IS 10,000-FOLD SHORTER THAN ITS EXTENDED LENGTH

Figure 5-24 Essential Cell Biology, 2/e. (© 2004 Garland Science)

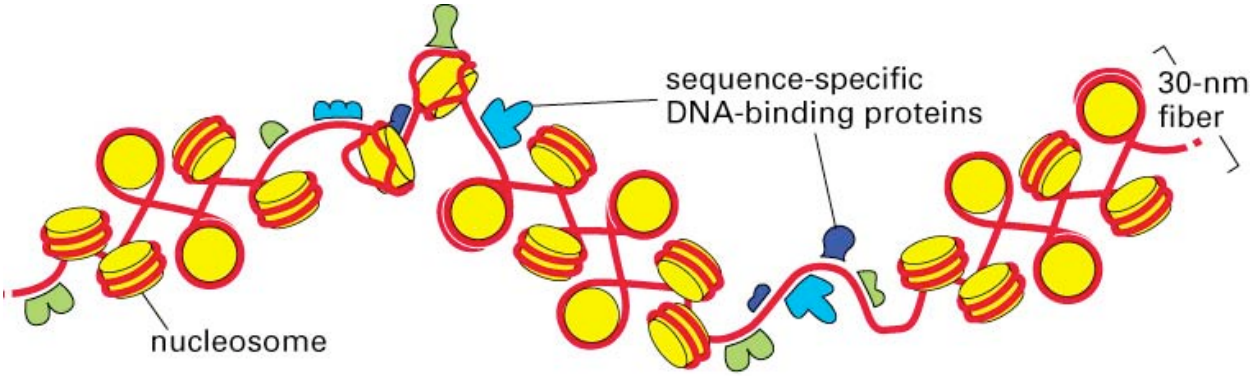
Yellow = histone proteins



a) Chromatin isolated from *interphase* nucleus appears in the electron microscope as a thread 30 nm thick.
b) Chromatin experimentally decondensed to show the nucleosomes

Epigentic programming: during development some genes are more or less "permanently" turned off (and never transcribed again):

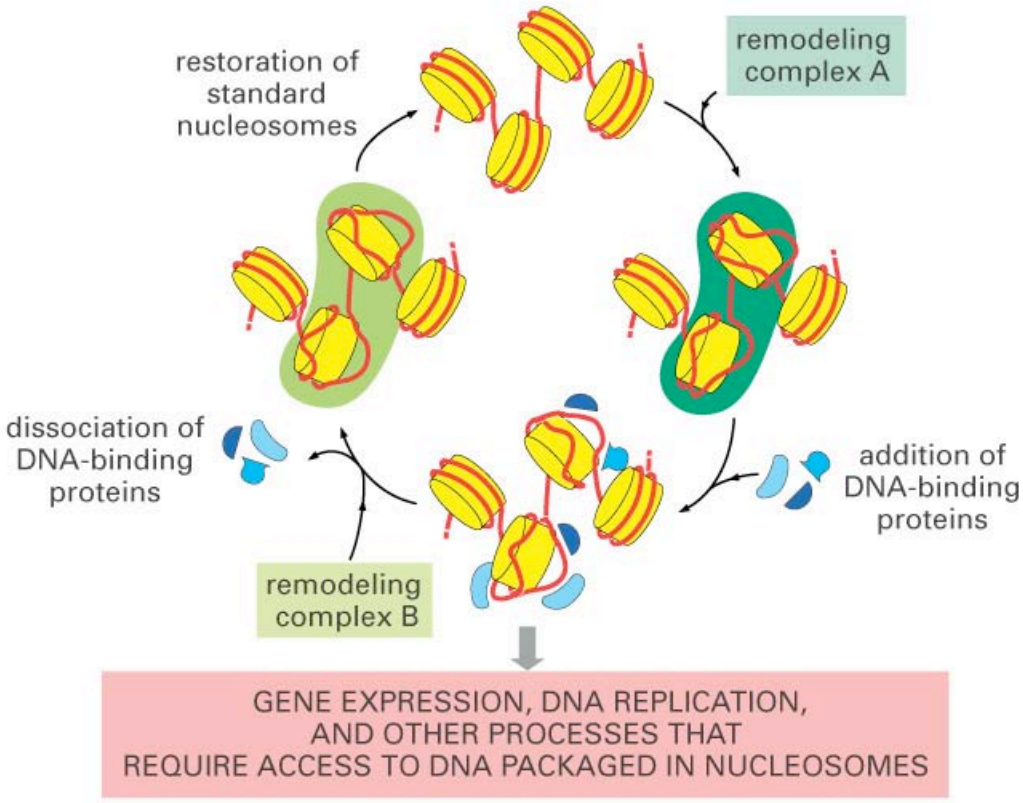
- they are chemically modified (methylation of purine/pyrimidine bases) and complexed with specific proteins (usually histones) that prevent access by the transcription machinery



Interphase chromatin. Red = DNA double helix. Yellow = histone proteins
Note the interruptions in nucleosome structure making some stretches of the 30nm fiber are more accessible to the transcriptional machinery than others. Highly transcribed genes would be in the stretches of DNA that have sequence-specific proteins bound.

Natural genomic reprogramming during egg and sperm formation and unnatural reprogramming during cloning experiments

Natural Reprogramming in normal development renders the egg and sperm genome competent to express embryonic genes (and takes place in the gonads over months/years). In normal reproduction, the sperm and egg genomes are competent to express the genes that need to be activated in early development (designated early genes or embryonic genes)



Chromatin remodeling complexes alter nucleosome structure. The DNA-binding proteins could be involved in transcription, DNA replication or DNA repair.

For a cloning experiment to be successful, the adult nucleus must be reprogrammed so that it can express embryonic genes

Unnatural reprogramming:

- During nuclear cloning, the reprogramming of the somatic nucleus must occur within minutes or, at most, hours between the time that nuclear transfer is completed and the onset of cell division in the activated egg
- Cloning of a somatic nucleus may lead to three outcomes:

1. no reprogramming (no activation of embryonic genes and early death--"failure")
2. partial reprogramming (some embryonic genes are activated--"abnormal" development)
3. complete reprogramming (faithful activation of embryonic genes--"normal" development).

Science 293: 1093 August 10, 2001

Black = genes turned off **red**= early embryonic genes **green** = tissue specific genes PGC = primordial germ cell

Repressed = transcription of gene repressed (turned off)

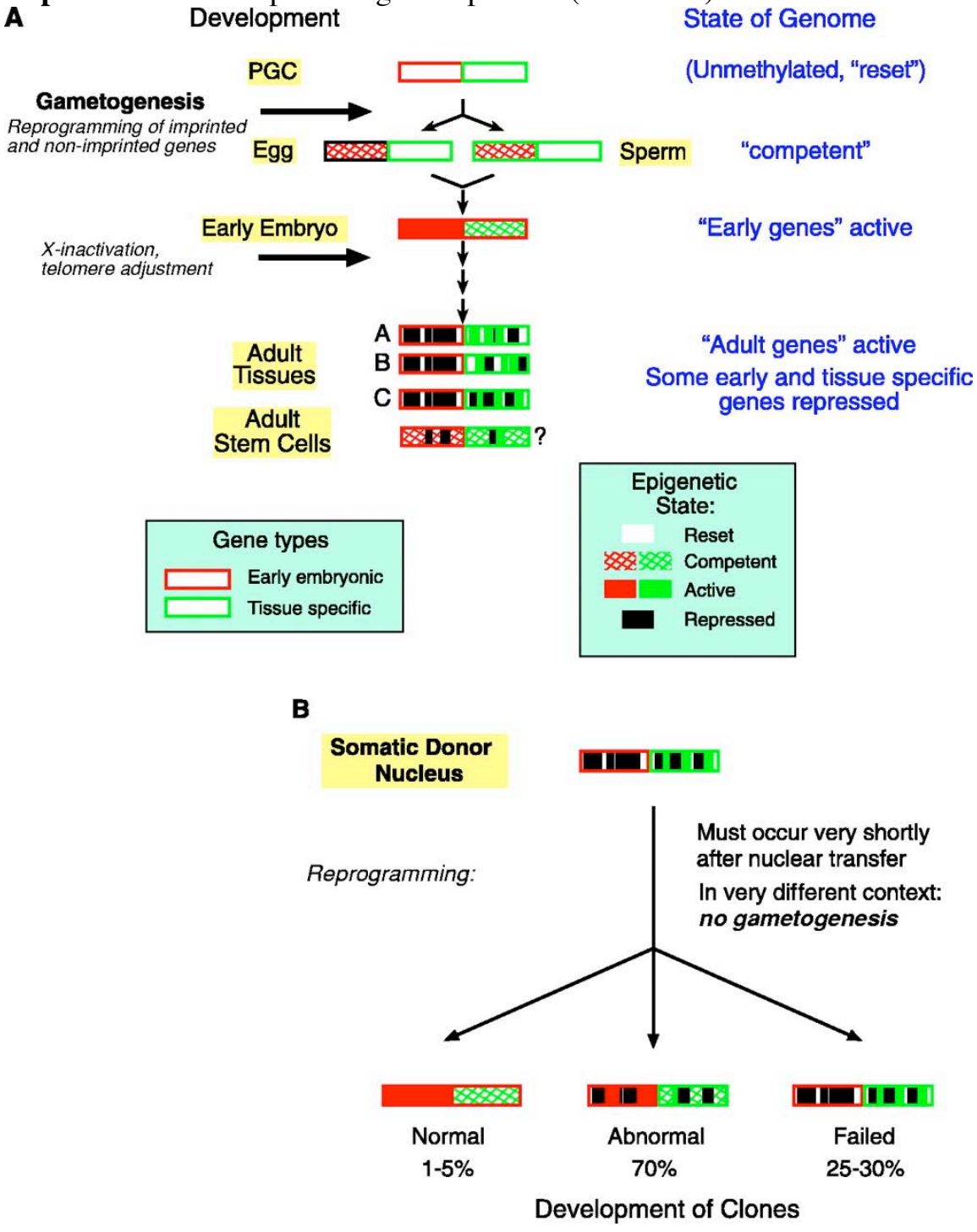
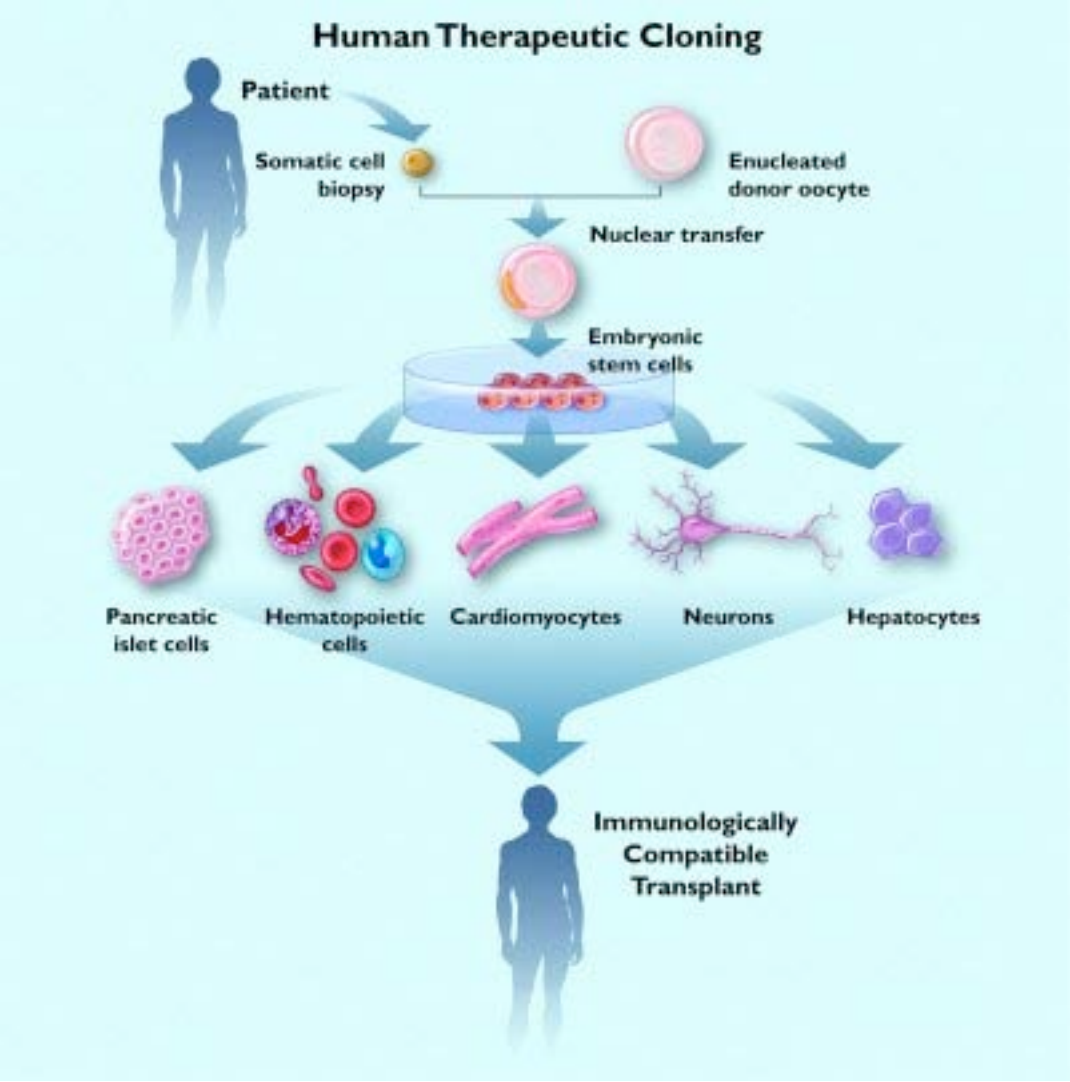


Figure 1. Reprogramming in normal development and nuclear cloning. (A) The genome of primordial germ cells is hypomethylated ("reset," white boxes). Reprogramming and establishment of parent-specific epigenetic marks occur over the course of gametogenesis so that the genome of sperm and egg is competent to express the genes that need to be activated in early embryonic (red hatched box) and later (green hatched box) development. During cleavage and early postimplantation development, "embryonic" genes, such as Oct 3/4, become activated (solid red box) and are repressed at later stages (black boxes) when tissue-specific genes (green boxes) are activated in adult tissues (labeled A, B, and C). Adult stem cells are thought to be less differentiated and may be more effective NT donors because they may require less reprogramming (see text). Epigeneticreprogramming of imprinted and nonimprinted genes occurs during gametogenesis in contrast to X inactivation and the readjustment of telomere length, which take place postzygotically. (B) Reprogramming of a somatic nucleus after nuclear transfer may result in (i) no activation of "embryonic" genes and early lethality, (ii) faulty activation of embryonic genes and an abnormal phenotype, or (iii) faithful activation of "embryonic" and "adult" genes and normal development of the clone. The latter outcome is the exception, and the percentage in each category is estimated from data on cumulus cell NT animals



genetic identity between transplant tissue and recipient

STEM CELLS
http://www.sciam.com/specialissues/0600aging/0600may.html

http://www.nih.gov/news/stemcell/scireport.htm

http://www.nih.gov/news/stemcell/primer.htm

A Stem cell:

- has the ability to divide for an indefinite period of time -- often throughout the lifetime of the organisms
- and, given the right signals, can give rise (differentiate) into many different cell types that make up the organism

A totipotent stem cell can give rise to all cell types:
the fertilized egg is said to be totipotent because it can give rise to the >200 different cell types found in an adult human as well as the extraembryonic tissues, placenta and umbilical cord

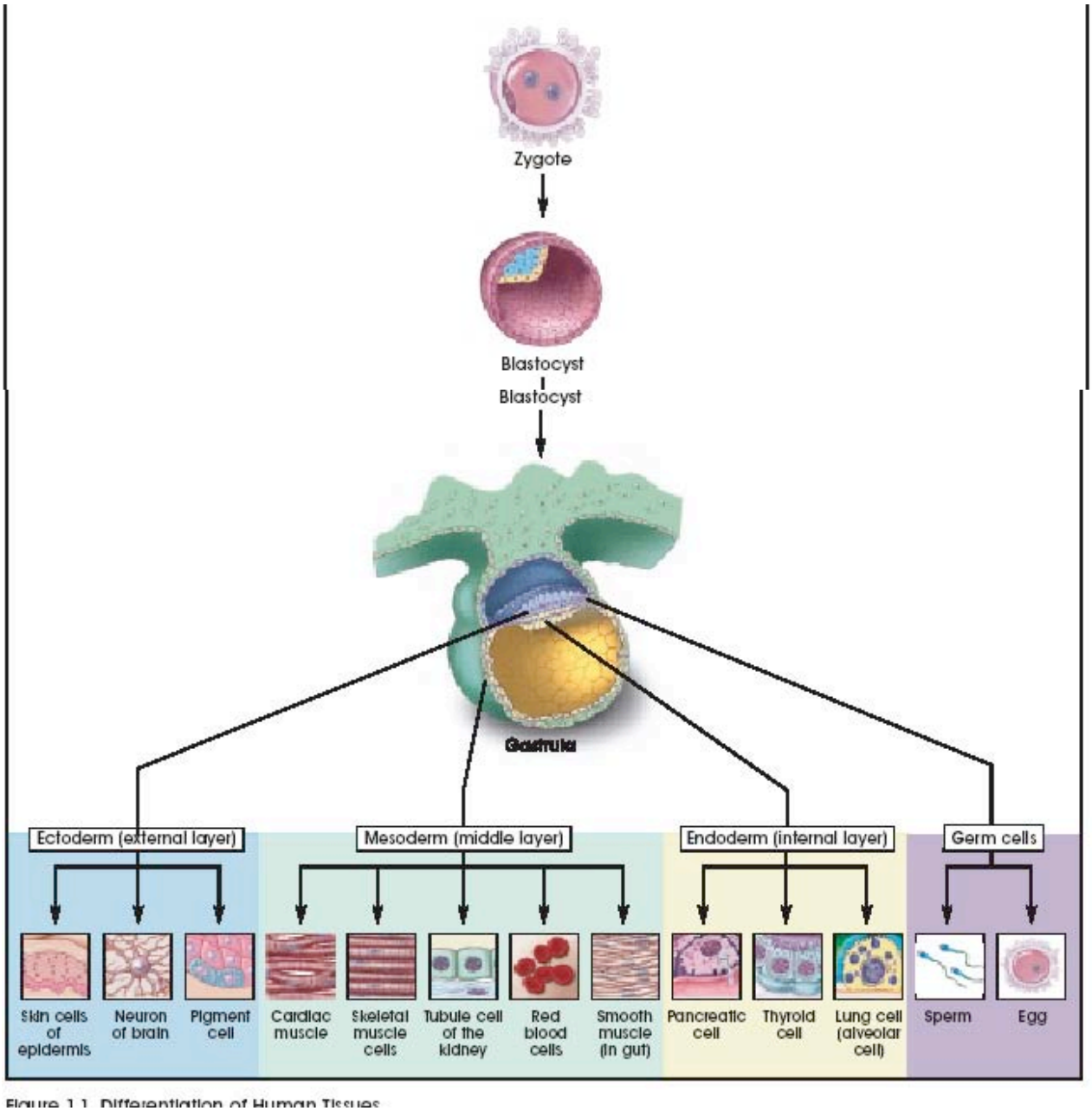
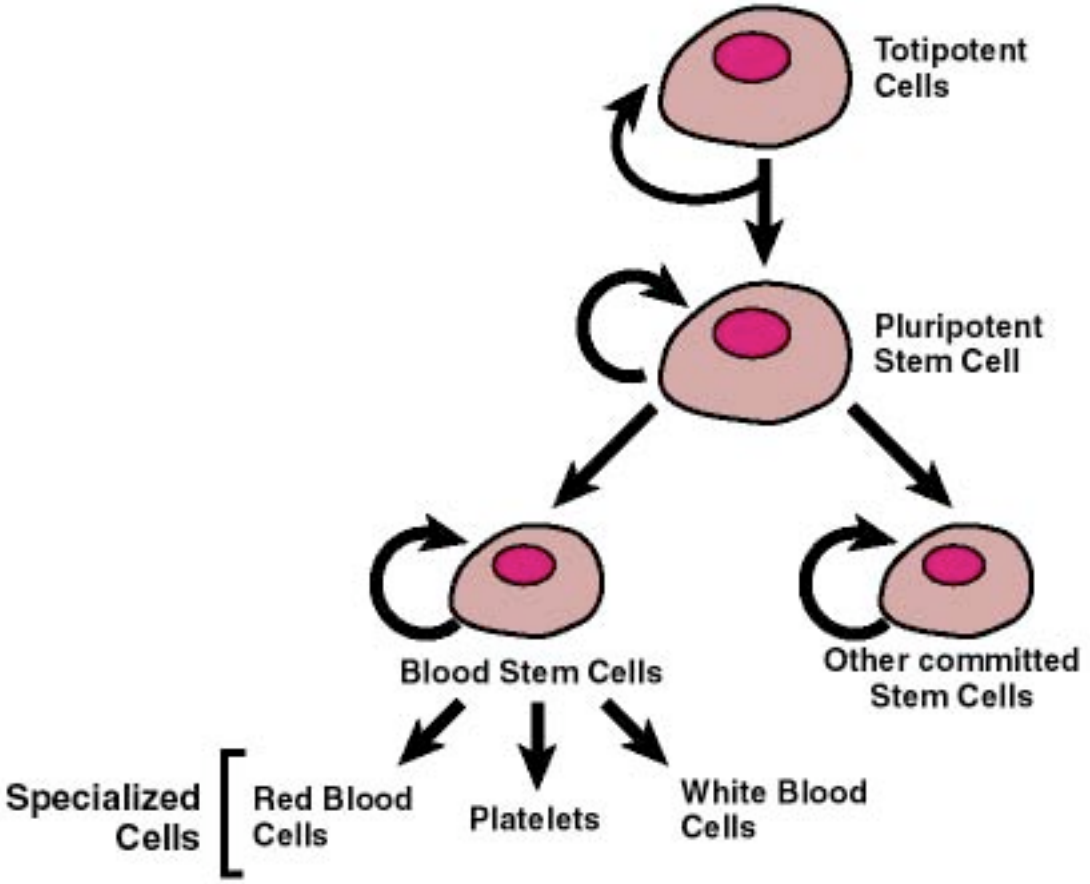


Figure 1.1 Differentiation of Human Tissues

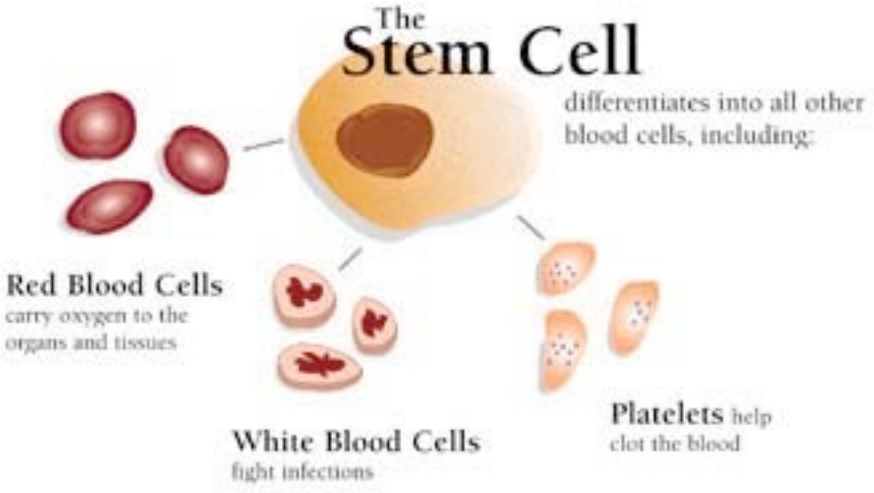
Pluripotent stem cells: able to give rise to all cells found in the embryo and adult animal

Embryonic stem cells from the inner cell mass of the blastocyst are pluripotent



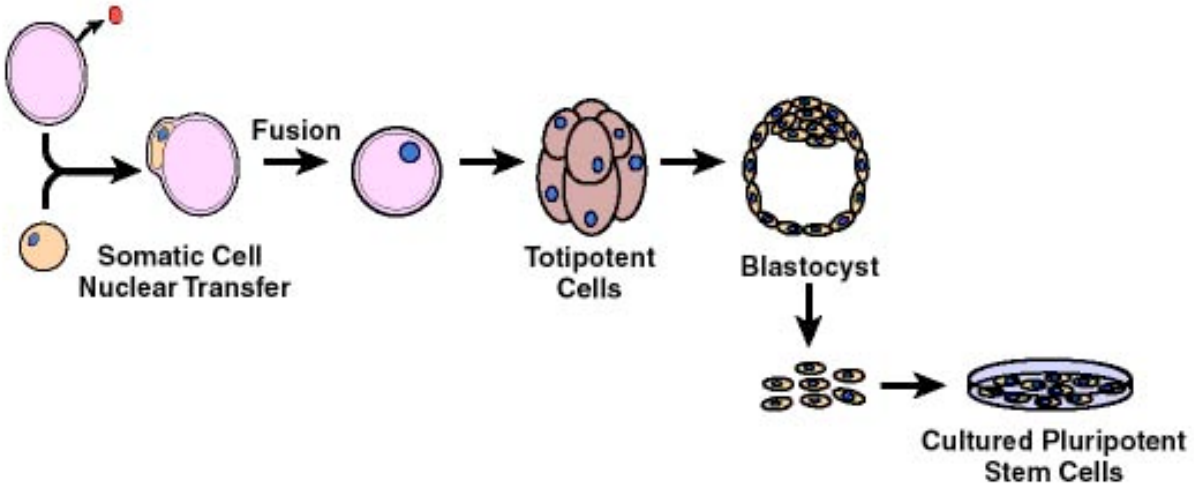
Adult stem cell:

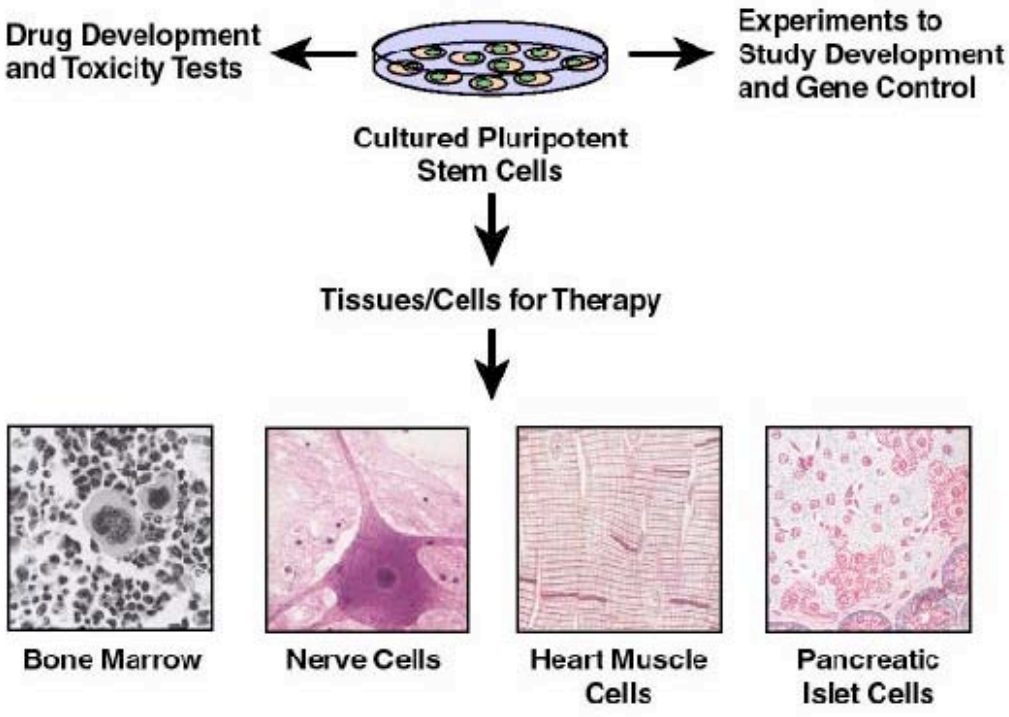
- an undifferentiated (unspecialized) cell that is found in differentiated tissue
- it can renew itself and yield all of the specialized cell types of the tissue from which it originated
- unlike embryonic stem cells, there is no evidence for adult stem cells that are capable of forming all of the cells of the body (under natural conditions)



An adult bone marrow stem cell is multipotent. It divides to produce more multipotent stem cells and committed progenitor cells that which are limited in the number of times they can divide before differentiating to form mature blood cells.

The Promise of embryonic stem cell research





The promise of stem cells:

- Stem cells may greatly extend the numbers and range of patients who could benefit from transplants
- may provide cell replacement therapy to treat debilitating diseases such as diabetes and neurodegenerative disease such as Parkinson's

Why not use adult stem cells?

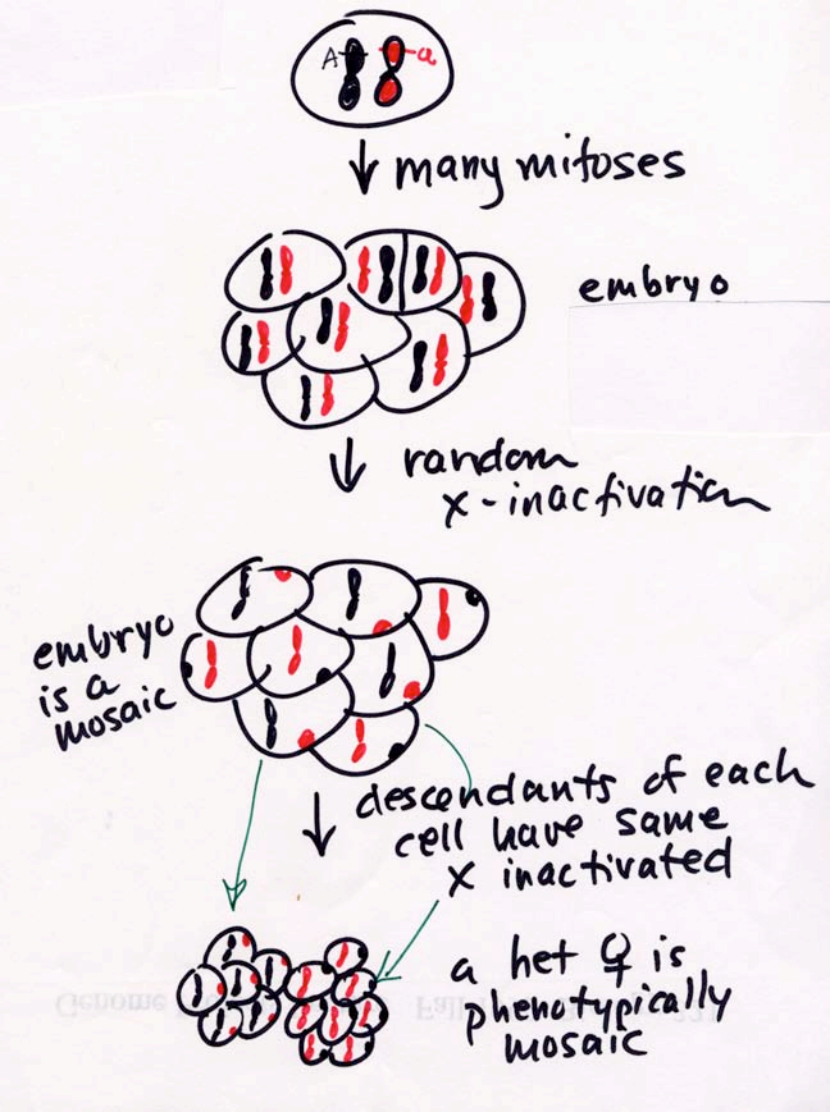
- not clear at this point in time that adult stem cells will prove to be as verstile as embryonic stem cells
- stem cells from adults have not been isolated for all tissues of the body
- adult stem cells are often present in only minute quantities, are difficult to isolate and purify and their numbers may decrease with age

Why doesn't cc's coat look exactly like her genetic mothers?

College of Veterinary Medicine, Texas A&M University

Ushering in pet cloning are donor, Rainbow, top, and offspring, cc.

TORTOISESHELL AND CALICO CATS: females who are heterozygous for an X-linked pigment gene: one allele results in orange pigment, the other allele in black pigment . A calico cat also has a dominant autosomal allele that causes white-spotting.



X-chromosome inactivation

- **In any given cell of a mammalian female, only one of the X chromosomes is active.**
- **Early during the development of a female, one X chromosome in each embryonic cell becomes highly condensed and irreversibly inactivated. This inactivation produces the Barr bodies seen in female cells.**
- **The inactivation is random with respect to paternal or maternal X chromosomes. So on the average, about half of the cells of the embryo will shut down the paternal X chromosome and half will shut down the maternal X chromosome. The female body will be a mosaic of cells that have an active paternal X and cells that have an active maternal X chromosome.**