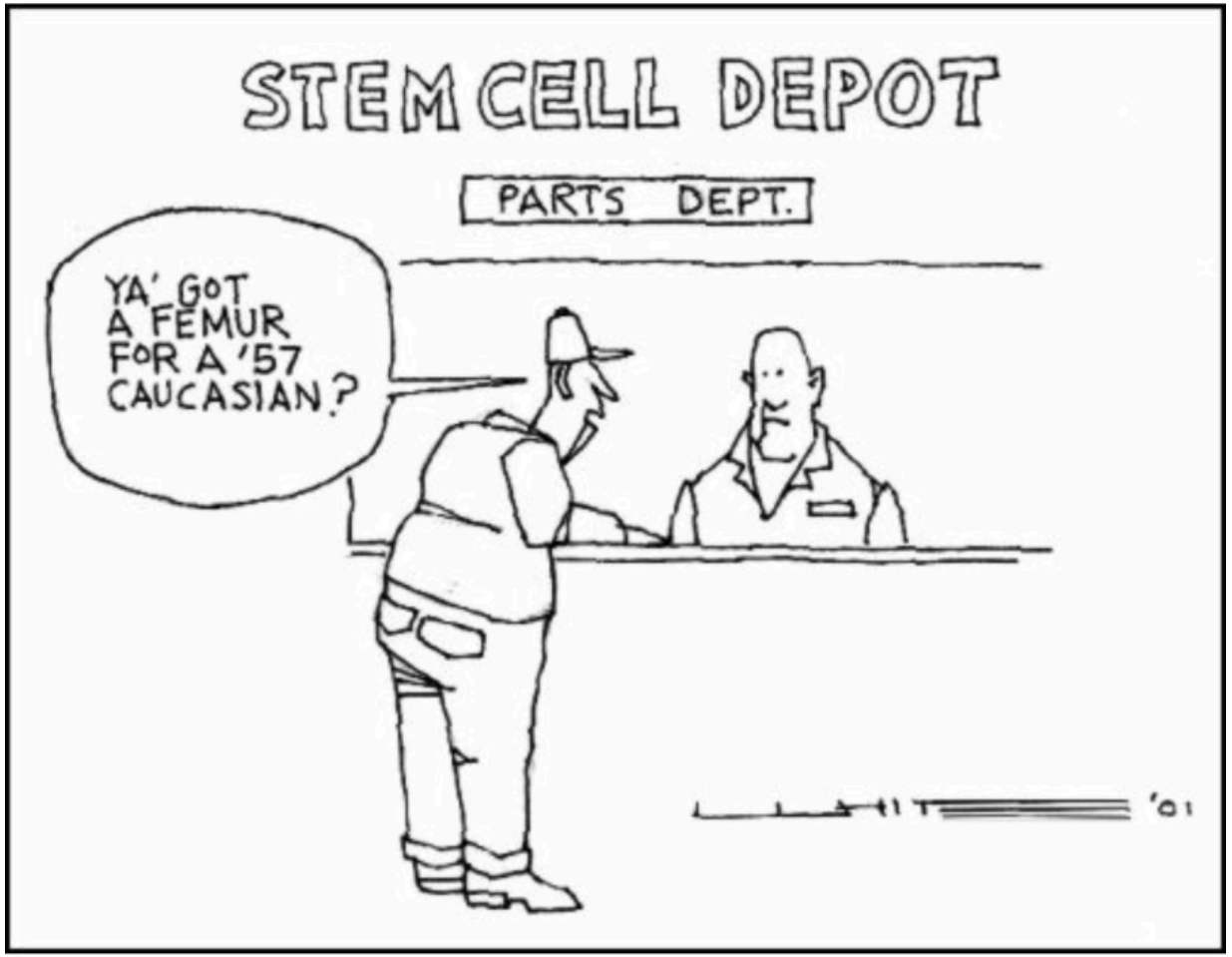
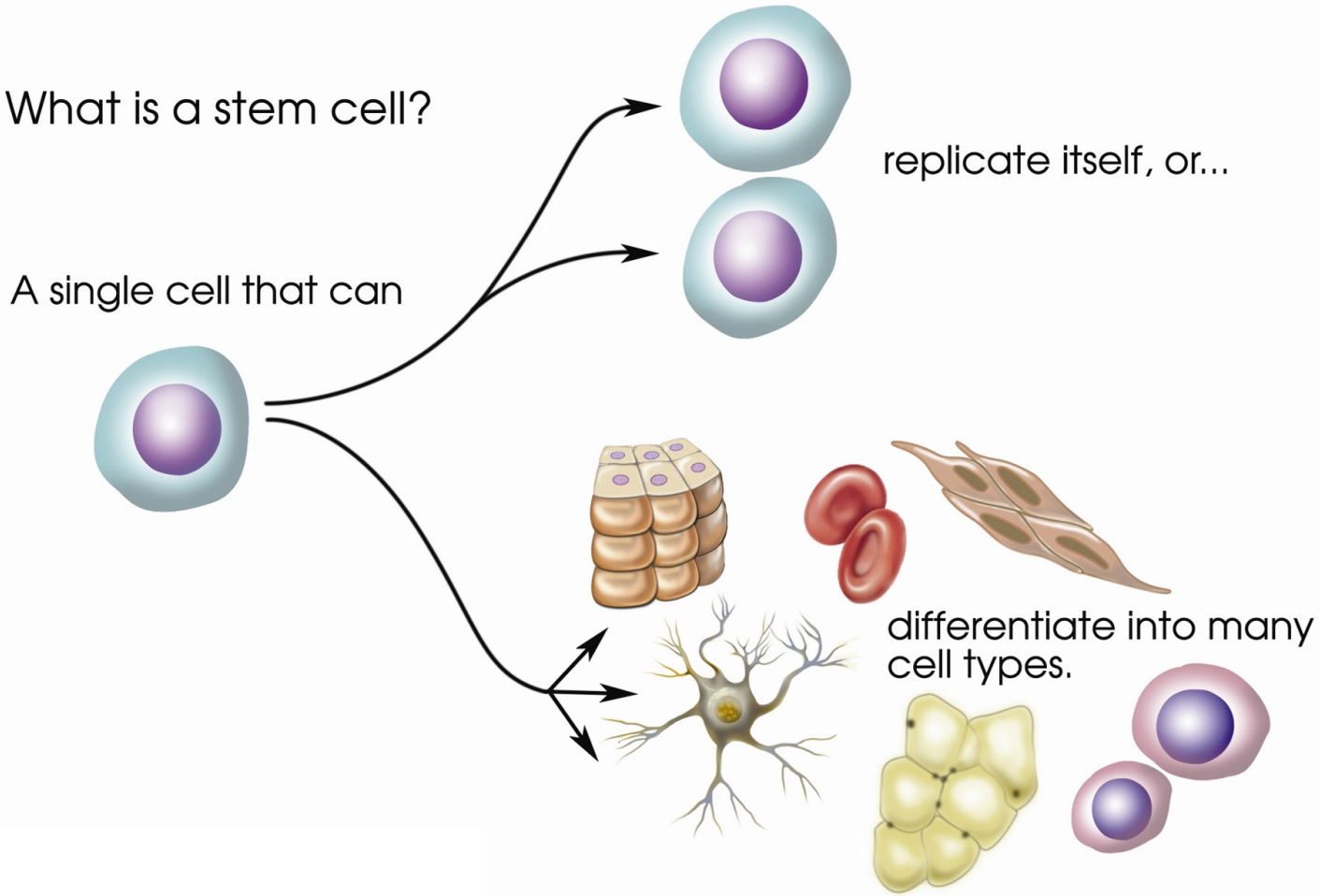


Introduction to Stem Cells

HC70A Winter 2016

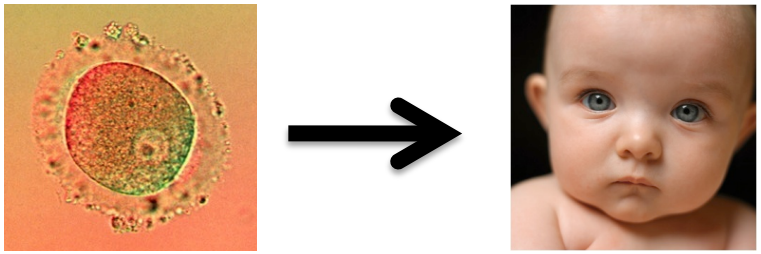
Dr. Pei Yun Lee



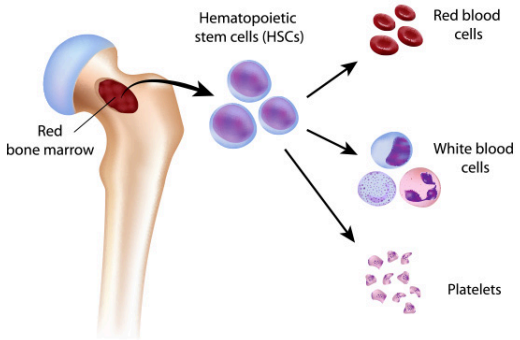
Stem cells: unspecialized cells that can **differentiate and self renew**

e.g. hematopoietic stem cell, embryonic stem cells, induced pluripotent stem cells

What are the Normal Uses of Stem Cells?



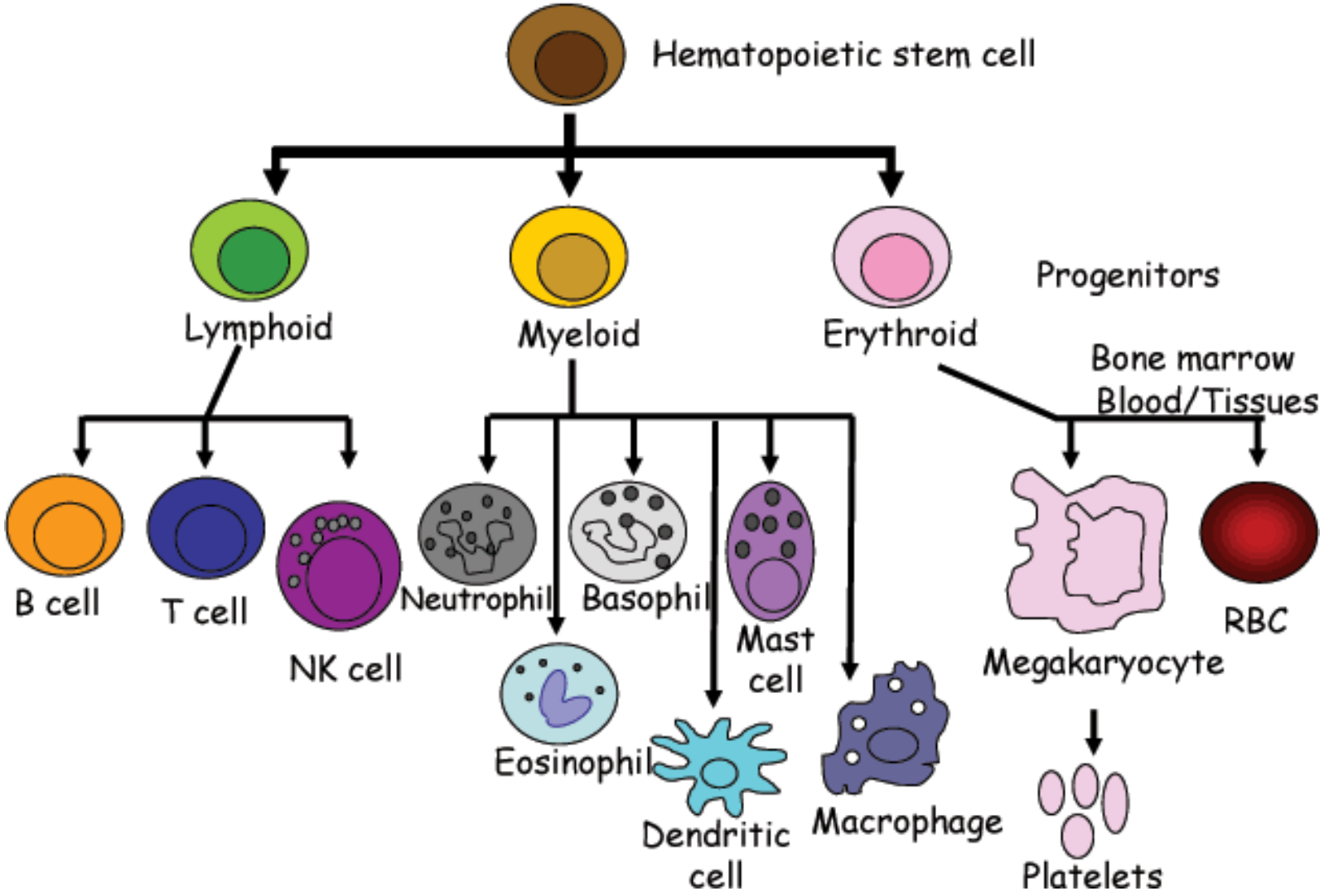
Development



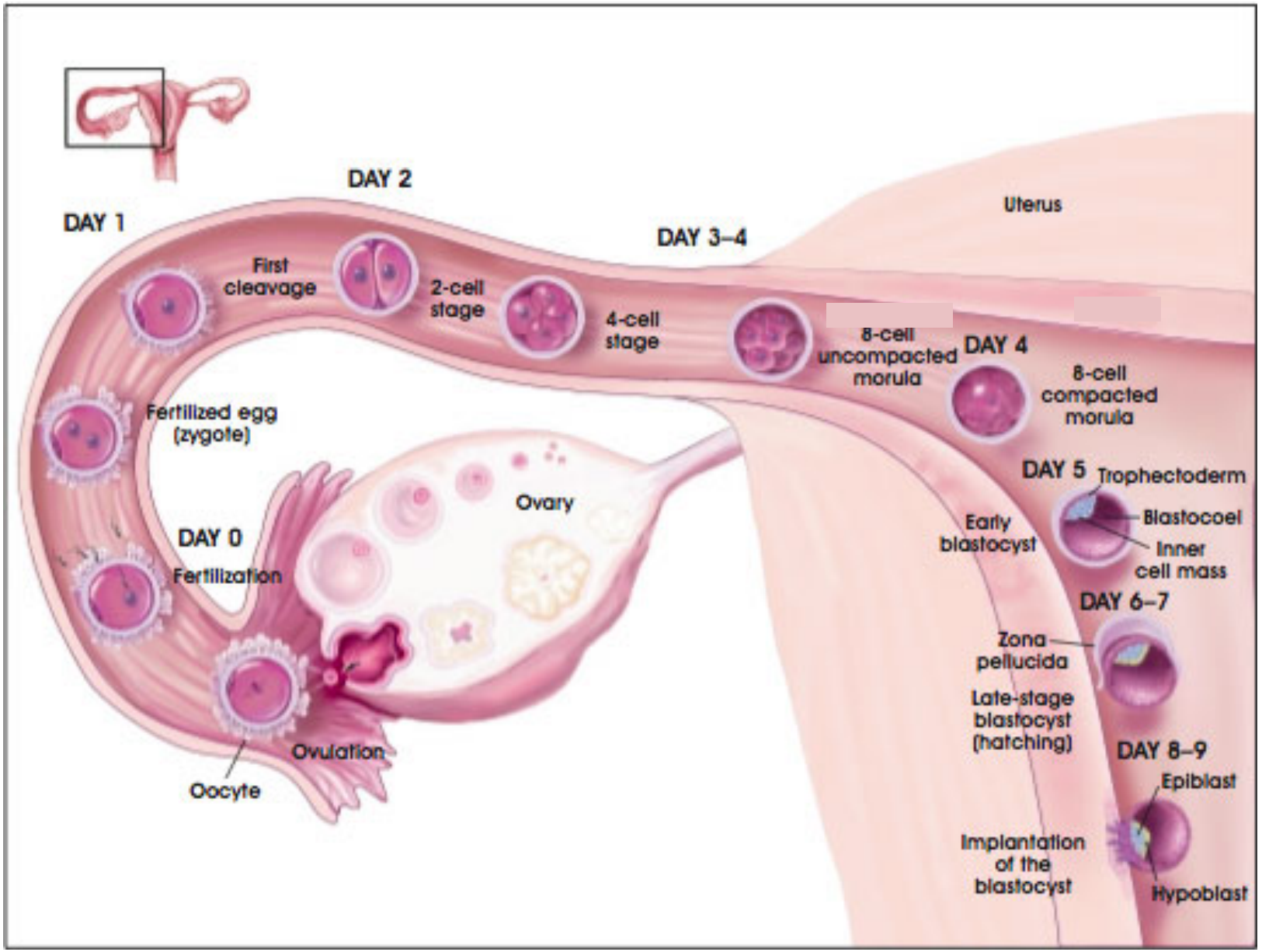
Tissue homeostasis

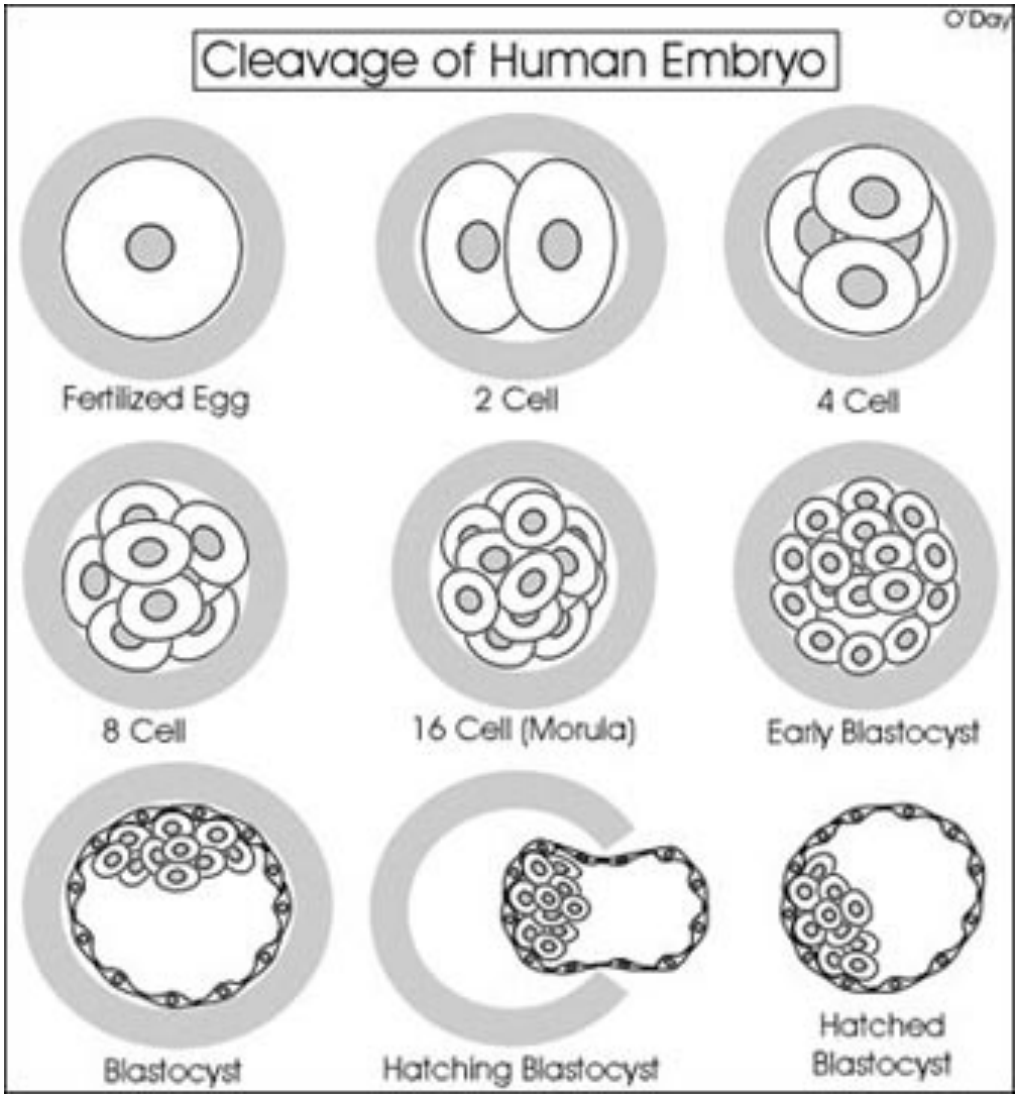
Tissue repair following injury

Immune cell development: Hematopoiesis



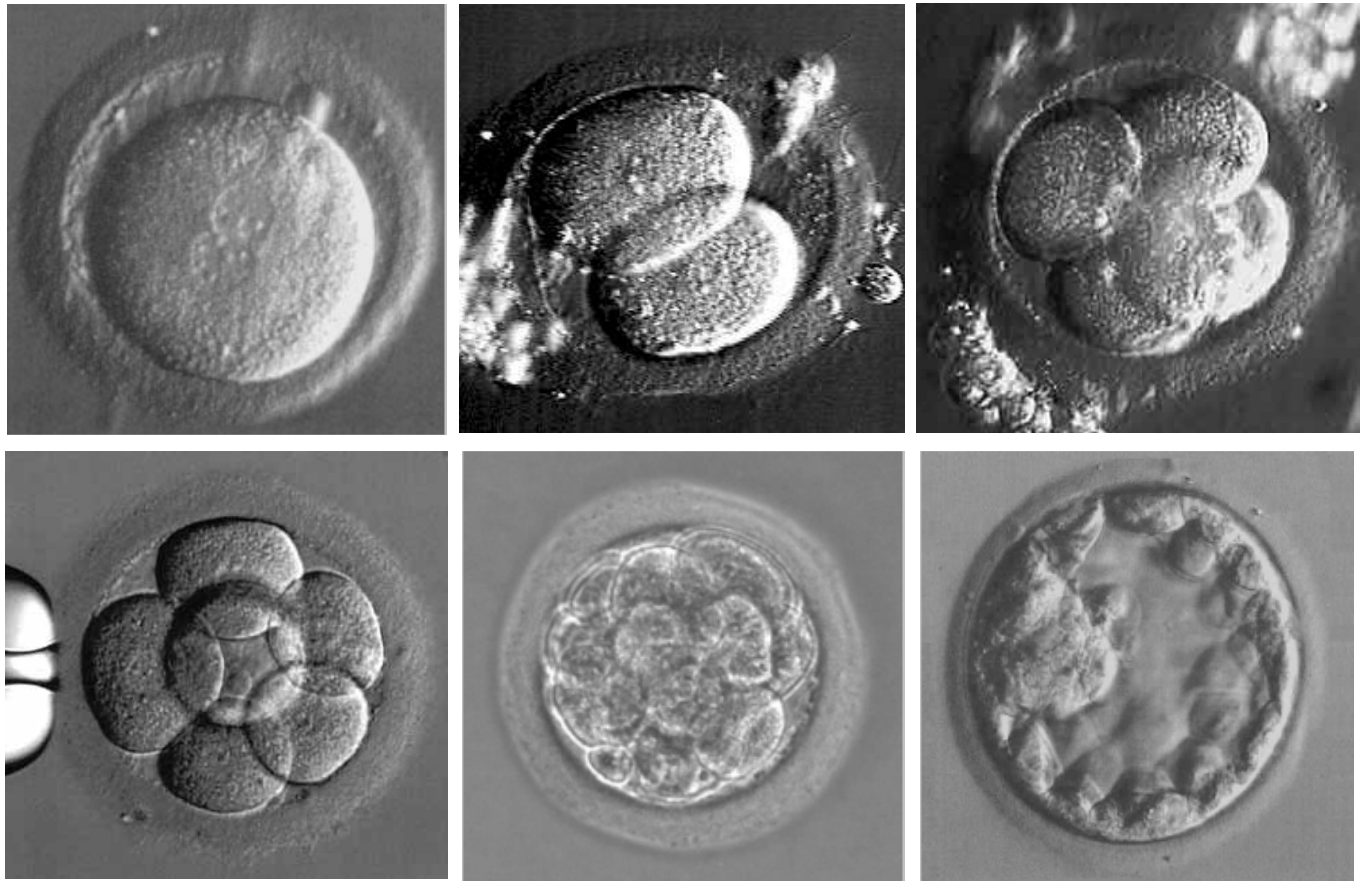
Early Embryonic Development



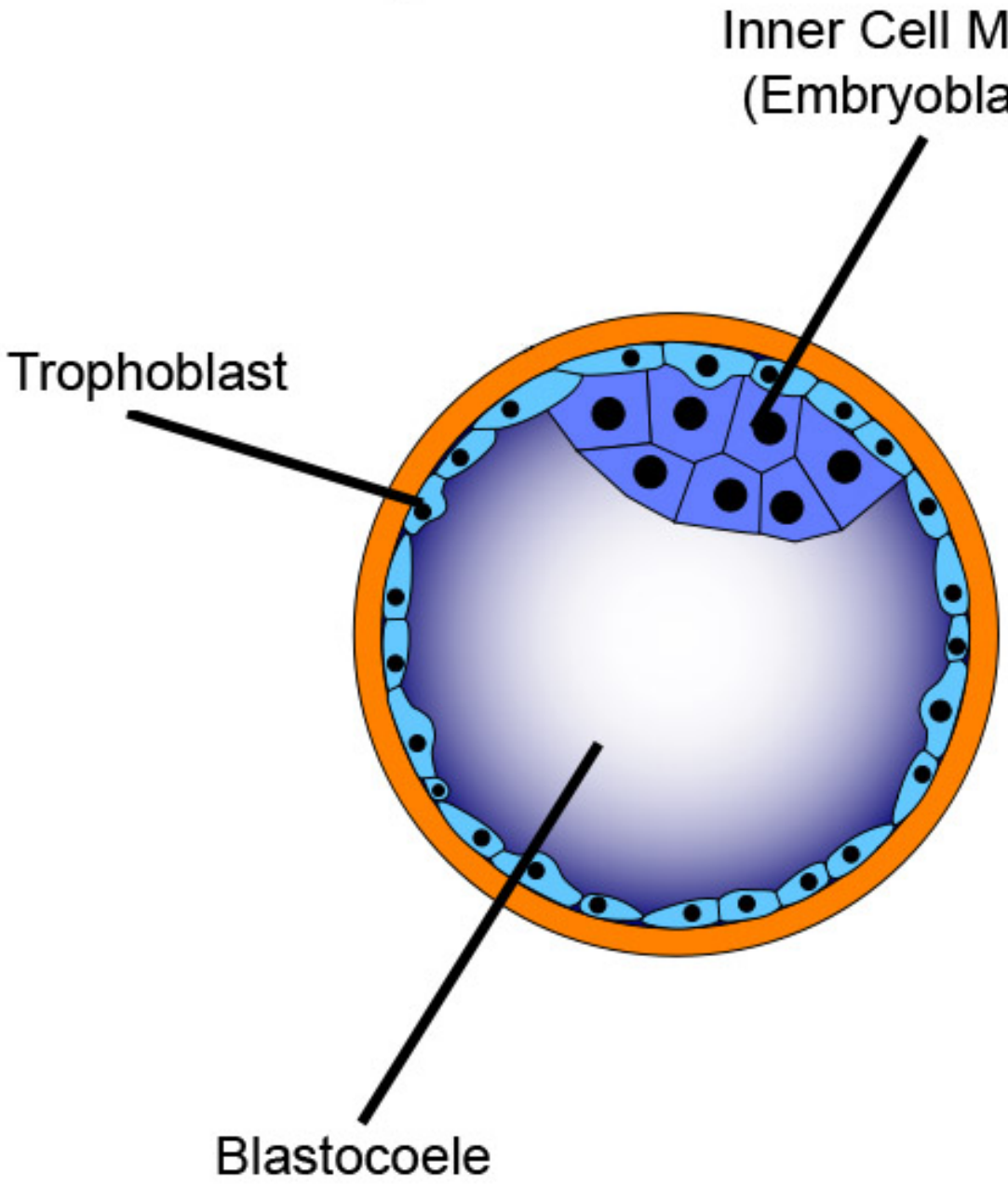


Cleavage: cell division without growth

Cells up to the morula stage are totipotent



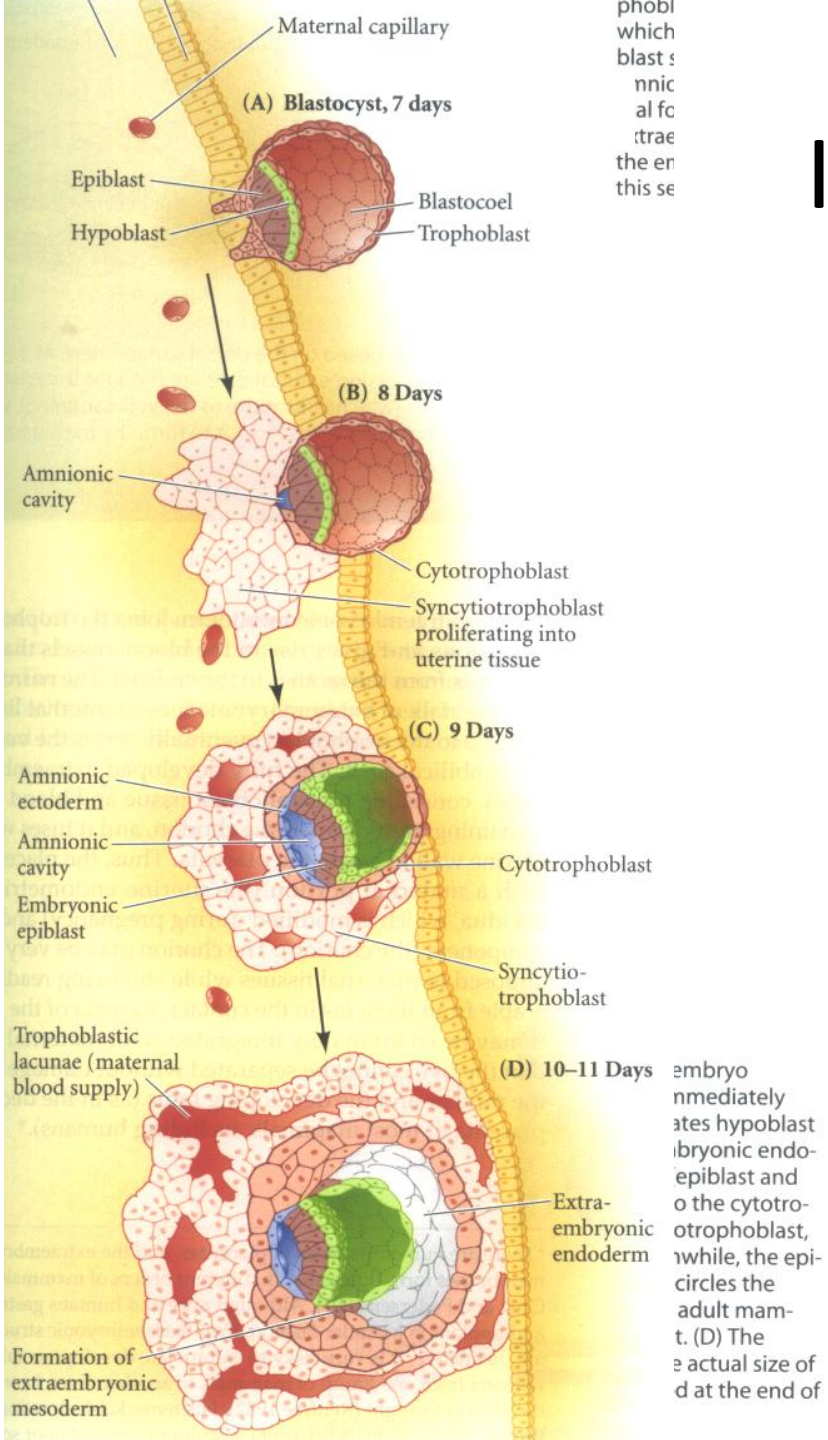
The Blastocyst



Trophoblast: forms placenta and other extraembryonic structures

Inner cell mass: develops into the fetus, pluripotent

Implantation of the Blastocyst



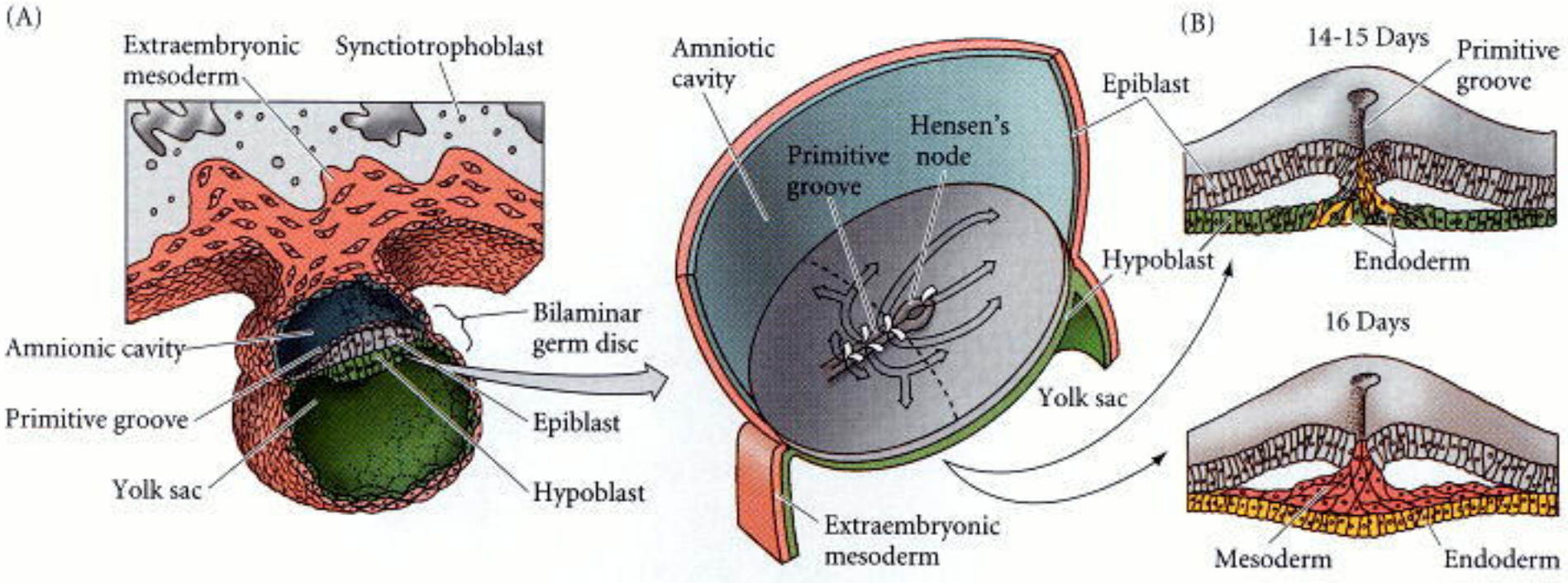
6-12 days after ovulation

Allows embryo to obtain oxygen and nutrients for it to grow

Steps:

1. Hatching
2. Apposition (loose association)
3. Adhesion—penetrates endometrium
4. Invasion—secretion of human choronic gonadotropin (HCG)—positive pregnancy test!

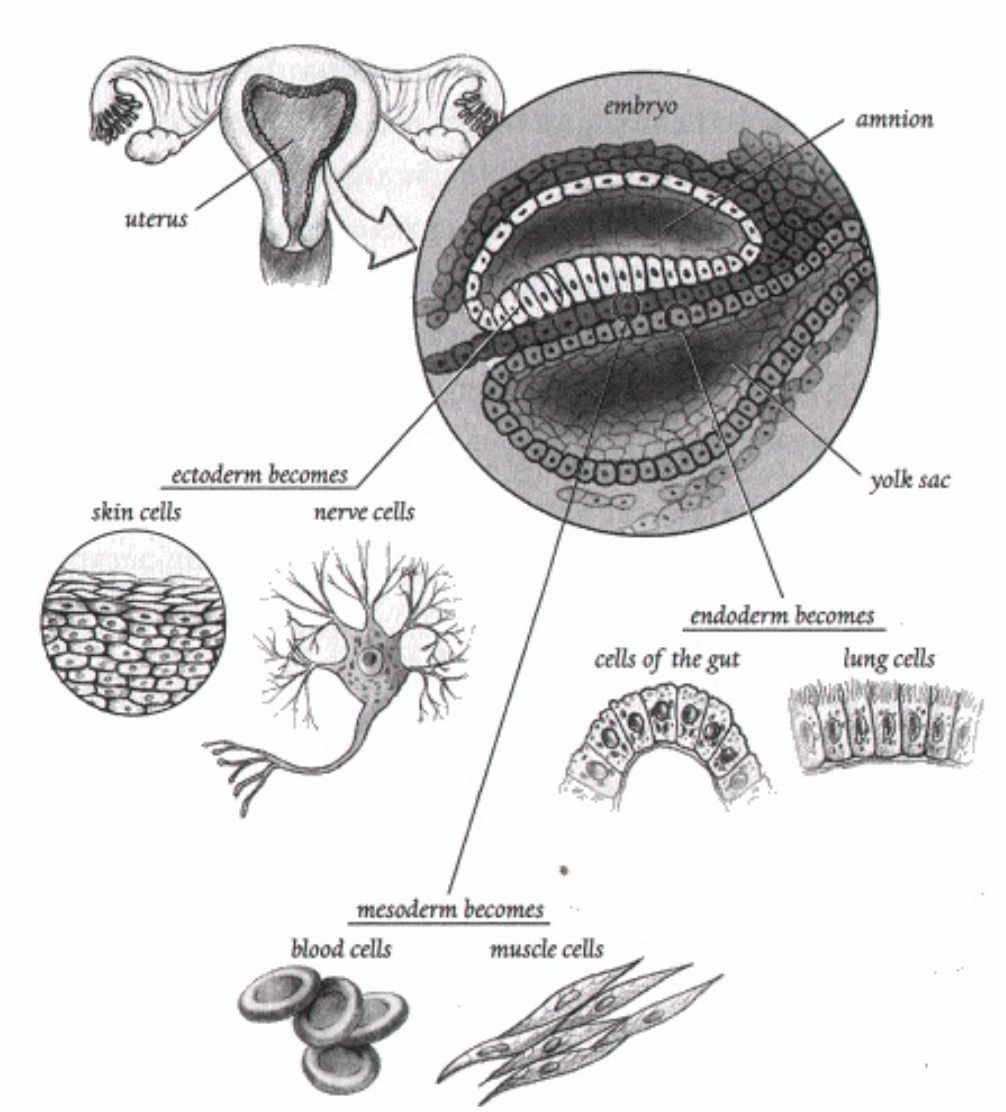
Gastrulation



Inner cell mass organized into disc composed of epiblast and hypoblast
Hypoblast—extra embryonic endoderm
Epiblast—fetus

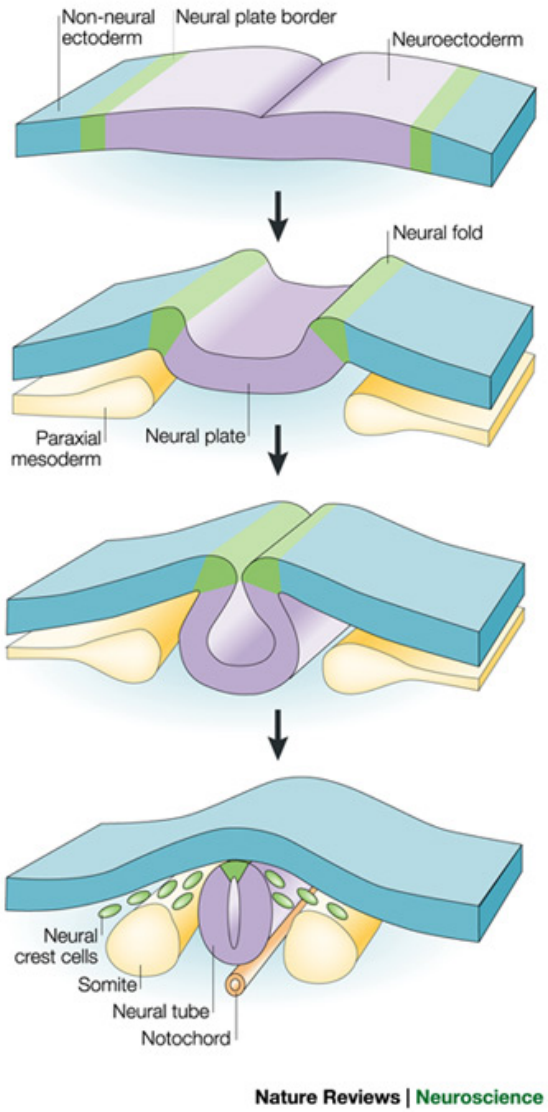
Movement and reorganization of cells in the embryo, begins at anus and moves anteriorly
Resulting embryo has 3 germ layers: endoderm, mesoderm, ectoderm

Cells of different germ layers develop into different parts of the body



Endoderm: Internal layer
Mesoderm: Middle layer
Ectoderm: External layer

Organogenesis--Neurulation



Organogenesis: formation of organs
Neurulation: formation of neural tube, which develops into the brain and spinal cord

Organogenesis takes place from 3-8 weeks after fertilization

Major events in organogenesis

Week 4:

- Primitive heart starts to beat
- Eyes, ears, liver, blood and arms beginning to form

Weeks 5 and 6:

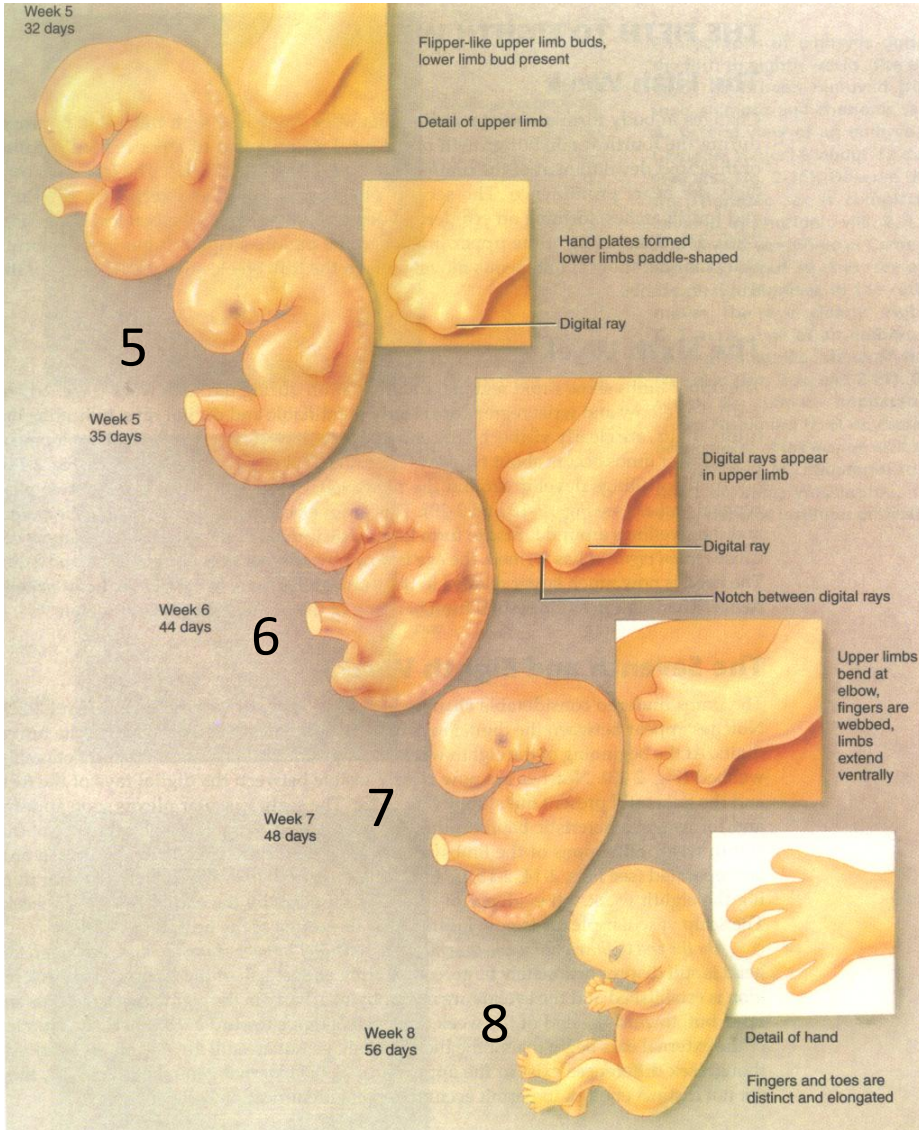
- Muscle, liver, stomach, pancreas, lung, kidneys start to develop

Week 7:

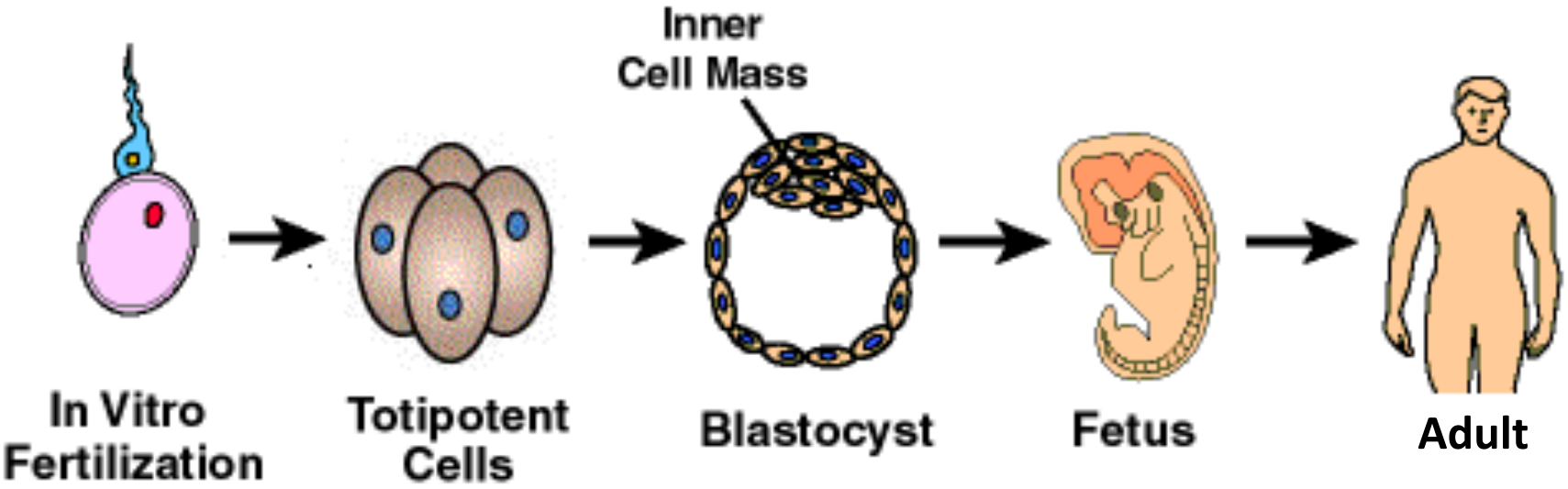
- Sex development begins
- Fingers appear

Week 8:

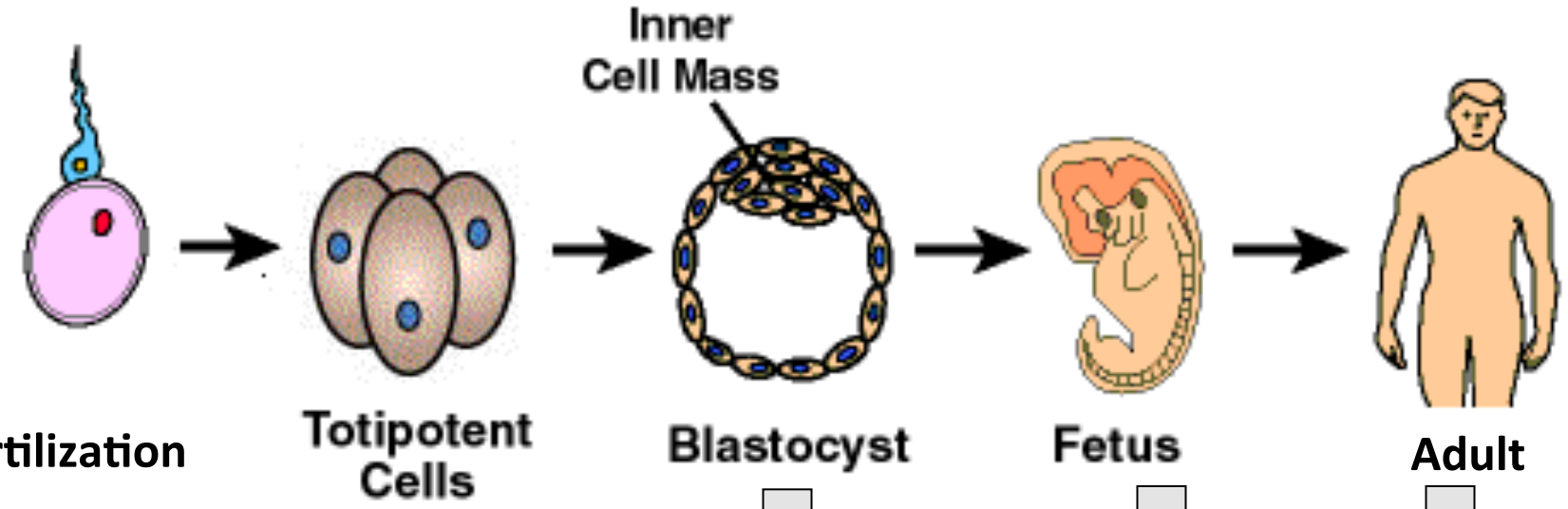
- Major organ development is complete
- Distinctly human, head still large, tail small
- At the end of week 8 embryonic development is complete and embryo is called a fetus
- Probability of miscarriage decreases
- Approximately 2.5 cm



Where can stem cells be found?

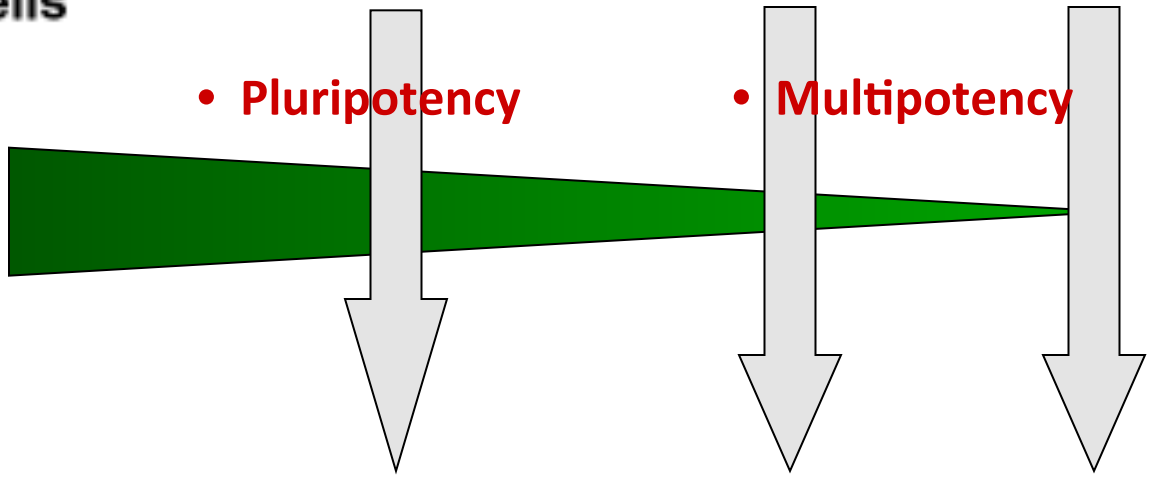


Progressive Restriction of Developmental Potential



- **Totipotency**
- **Pluripotency**
- **Multipotency**

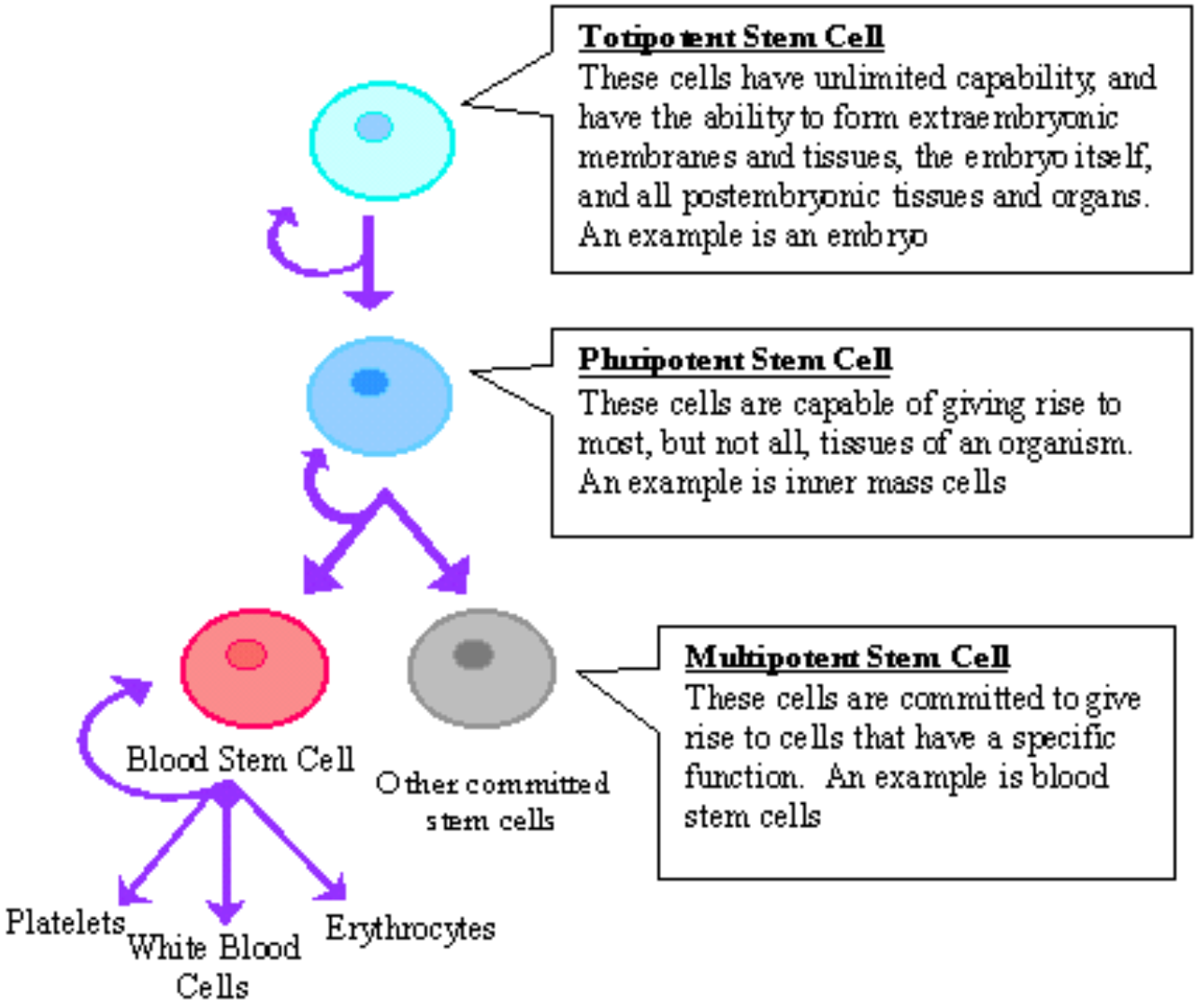
Differentiation potential



Embryonic Stem Cell

Fetal & Adult Stem Cell

Stem cells have different developmental potentials

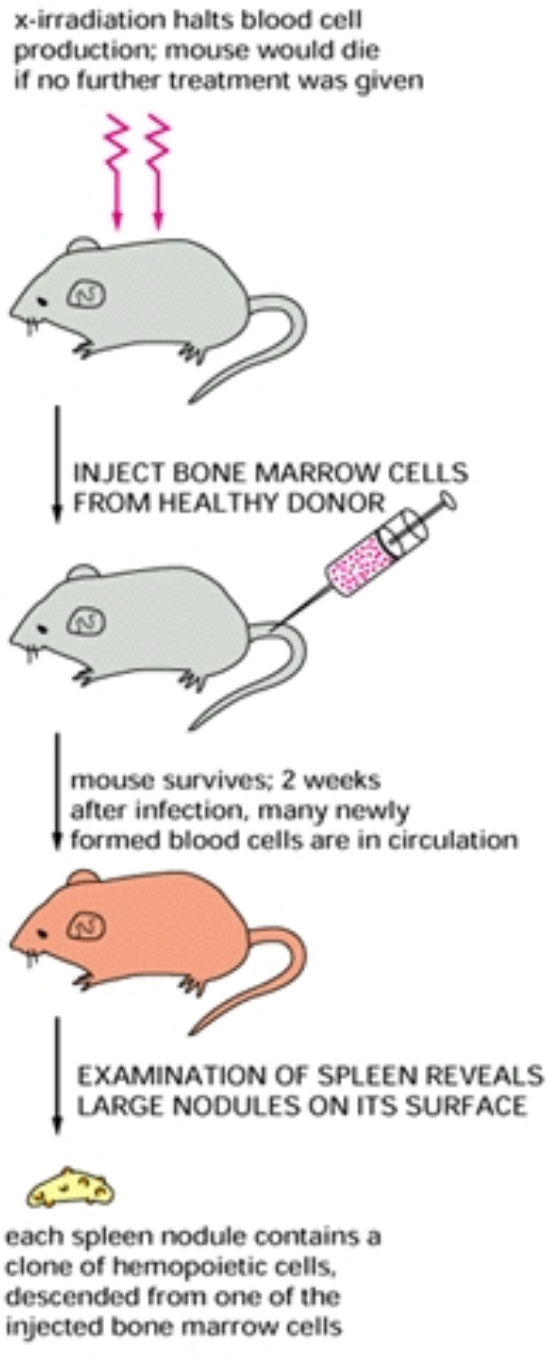


Discovery of stem cells

Alexander Maximow 1909

Unitarian theory of hematopoiesis (blood formation)

-all blood cells descended from a common precursor “stem” cell



McCulloch and Till were the first to demonstrate the existence of multipotent stem cells!

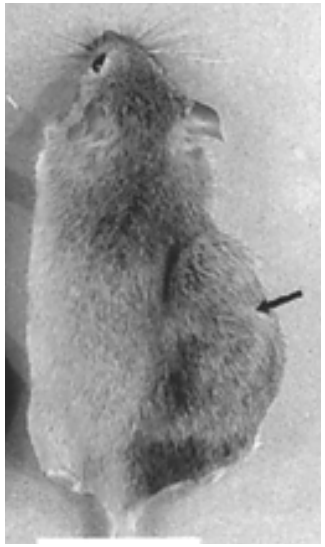
Analysis of the cells in the spleen demonstrated that the cells in each nodule were clones

1961

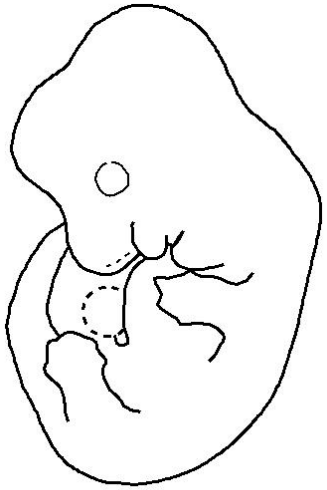
Discovery of pluripotent stem cells

Leroy Stevens

Mouse lines are inbred and therefore genetically identical!

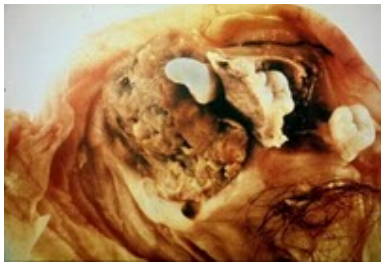


Strain 129!



Isolate cells from mouse embryos

Transplant into adult mouse testes

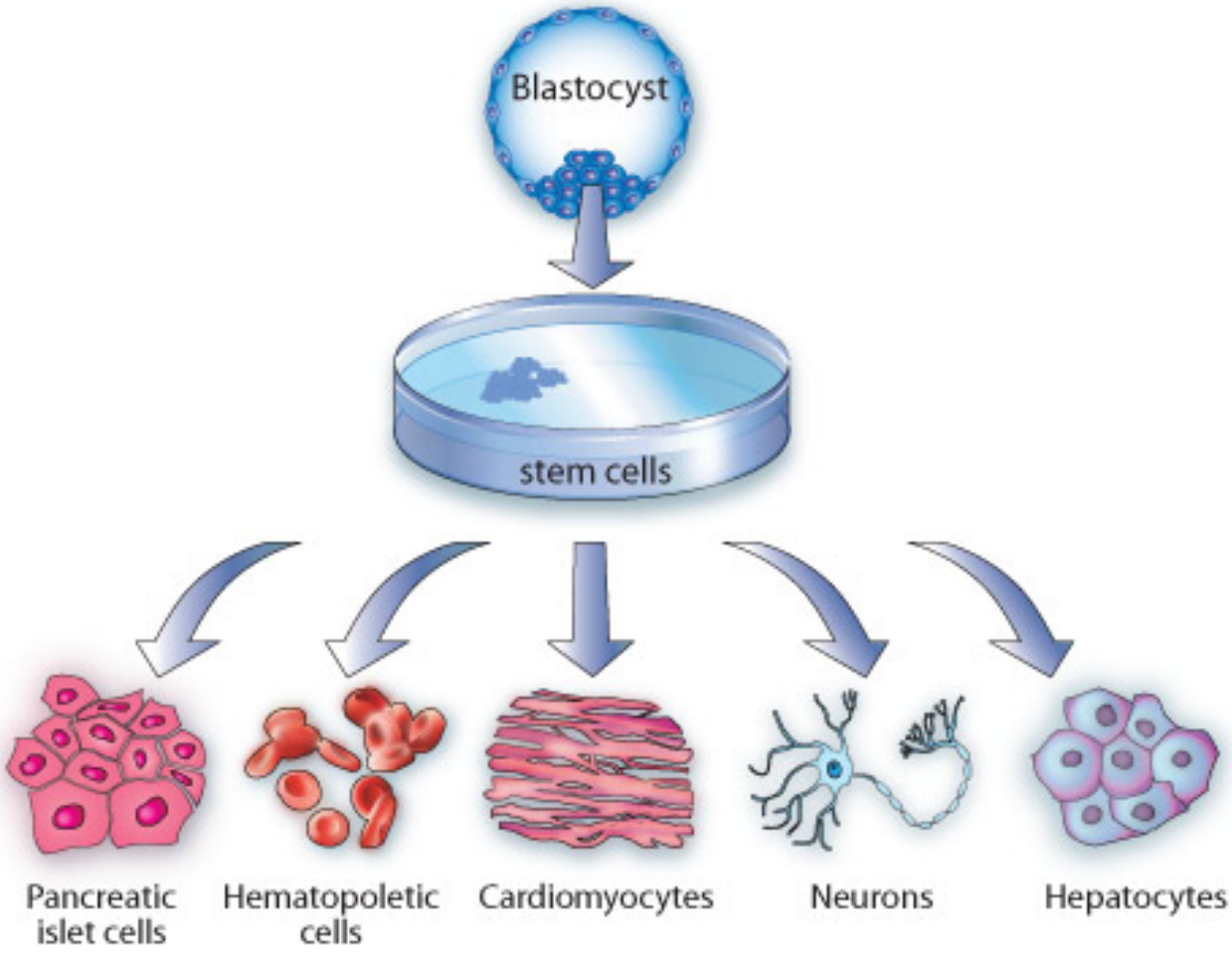


Teratomas!

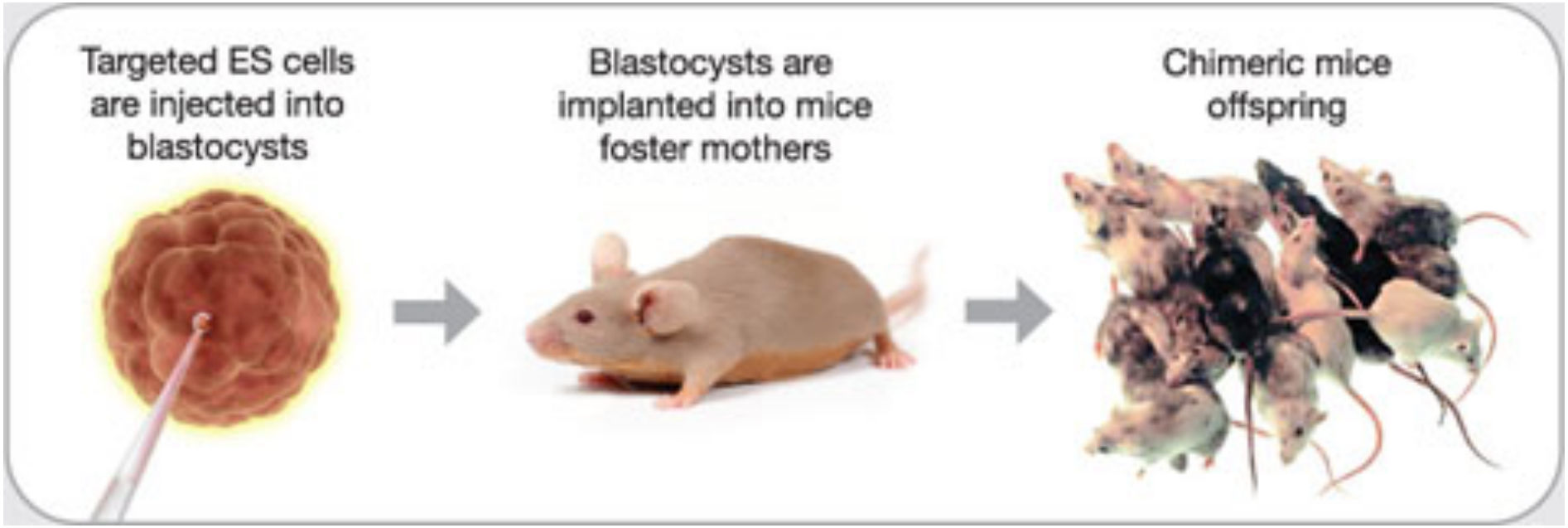
Tumors develop from pluripotent stem cells in the mouse gonad

Pluri=several

Pluripotent stem cells have the potential to develop into any cell in the human body!

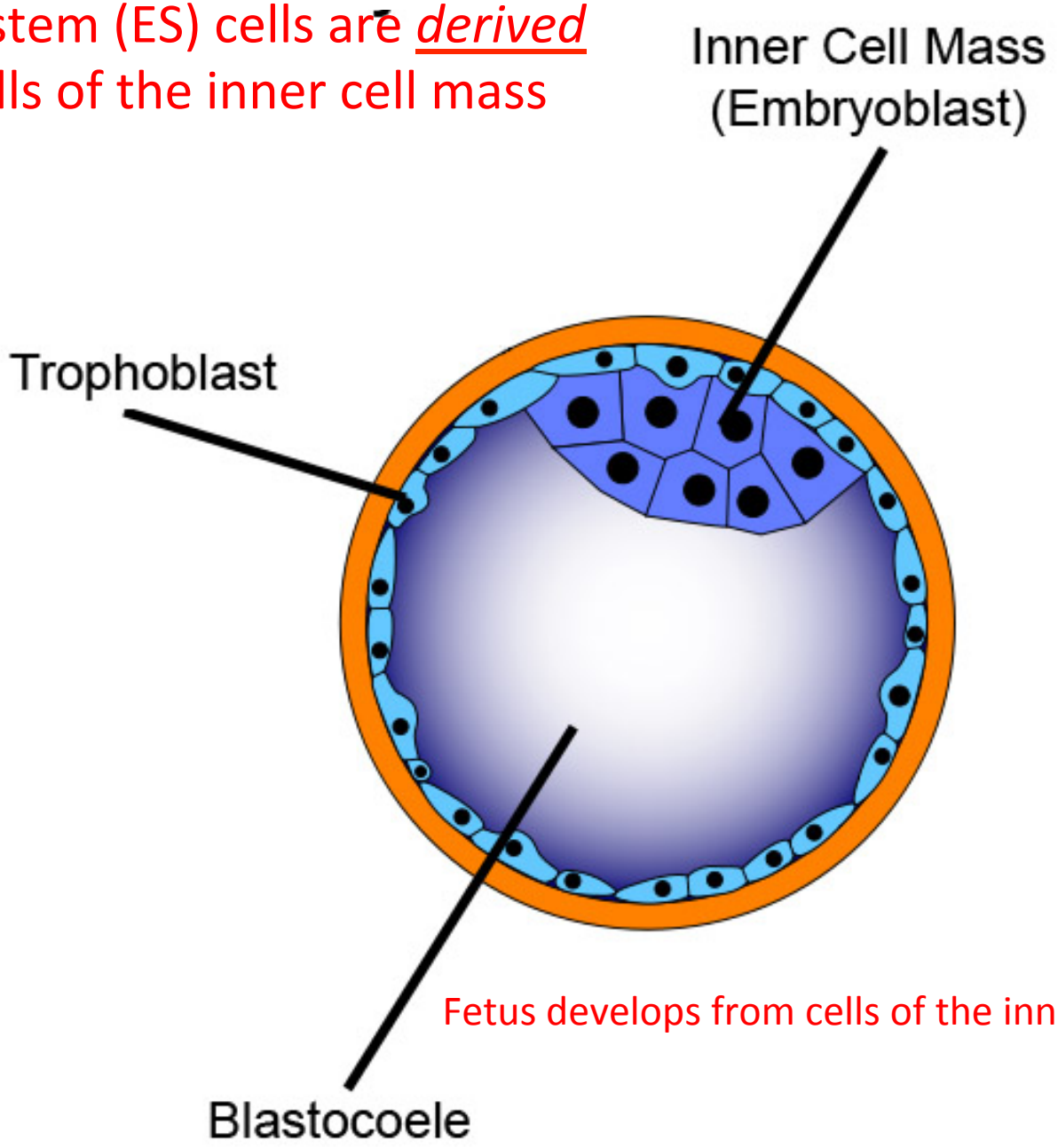


ES cells are pluripotent stem cells!


Targeted ES cells are injected into blastocysts
Blastocysts are implanted into mice foster mothers
Chimeric mice offspring

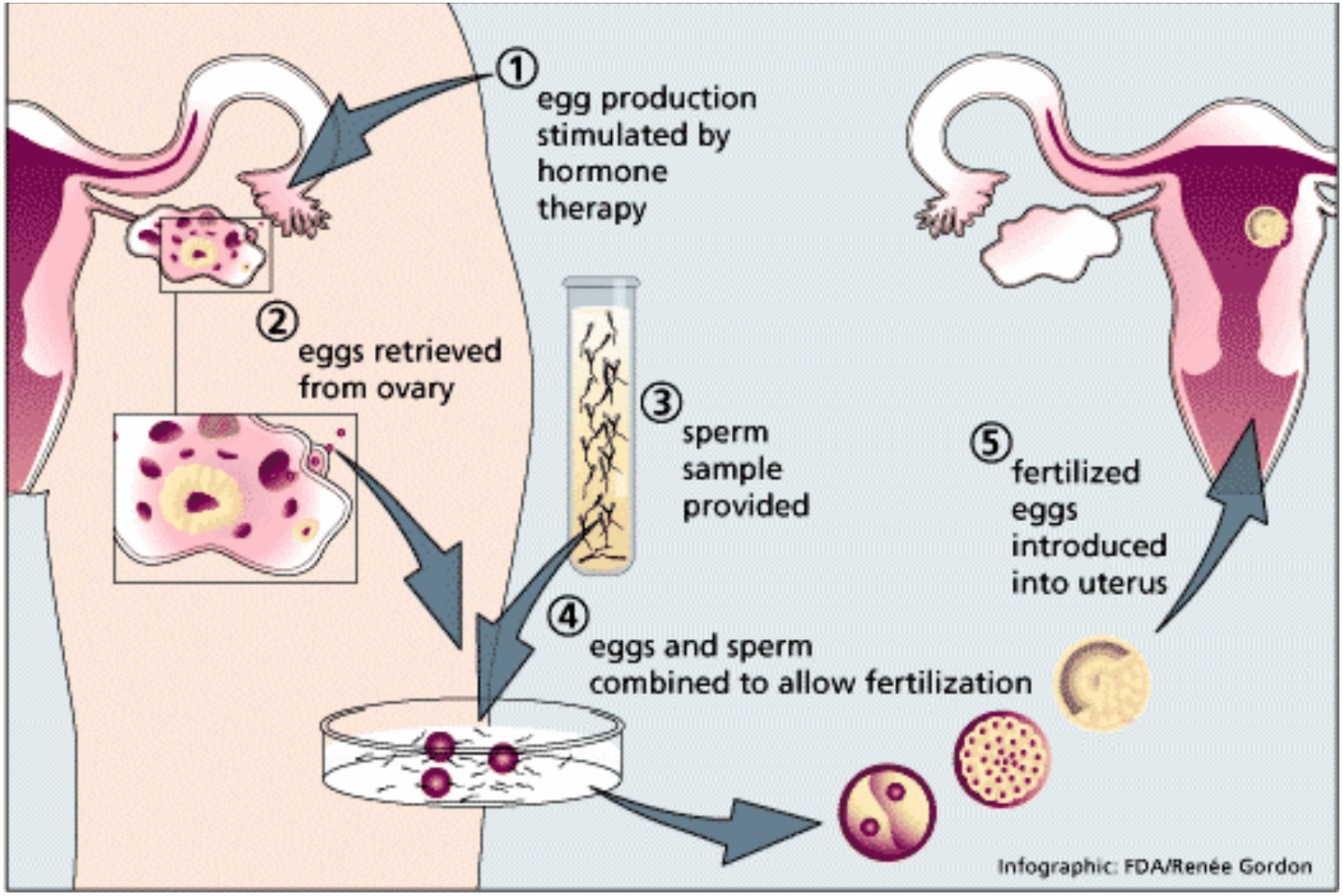
How are ES cells derived?

Embryonic stem (ES) cells are *derived* from the cells of the inner cell mass (ICM)



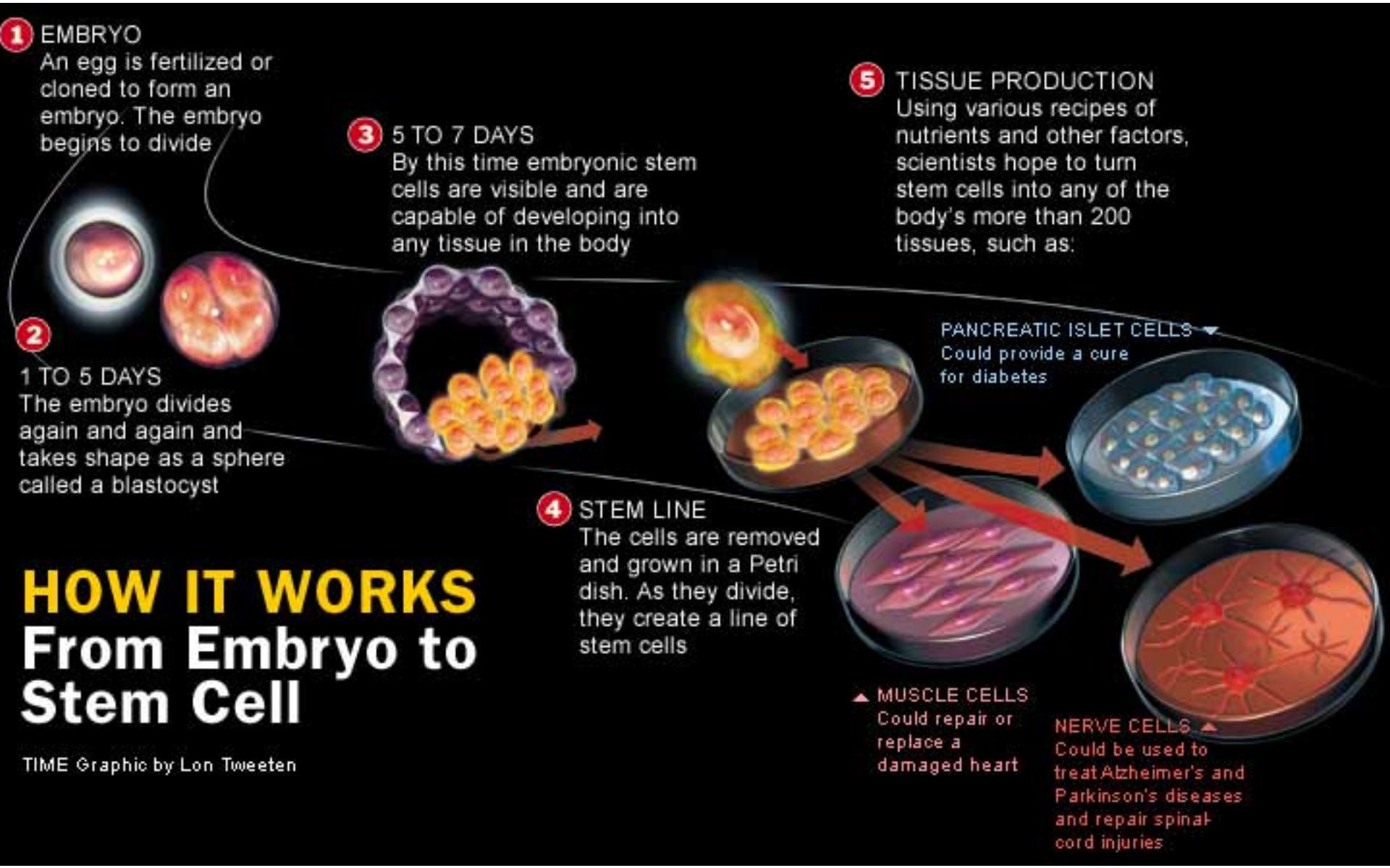
Fetus develops from cells of the inner cell mass

Embryos for ES cell research are obtained through *in vitro* fertilization

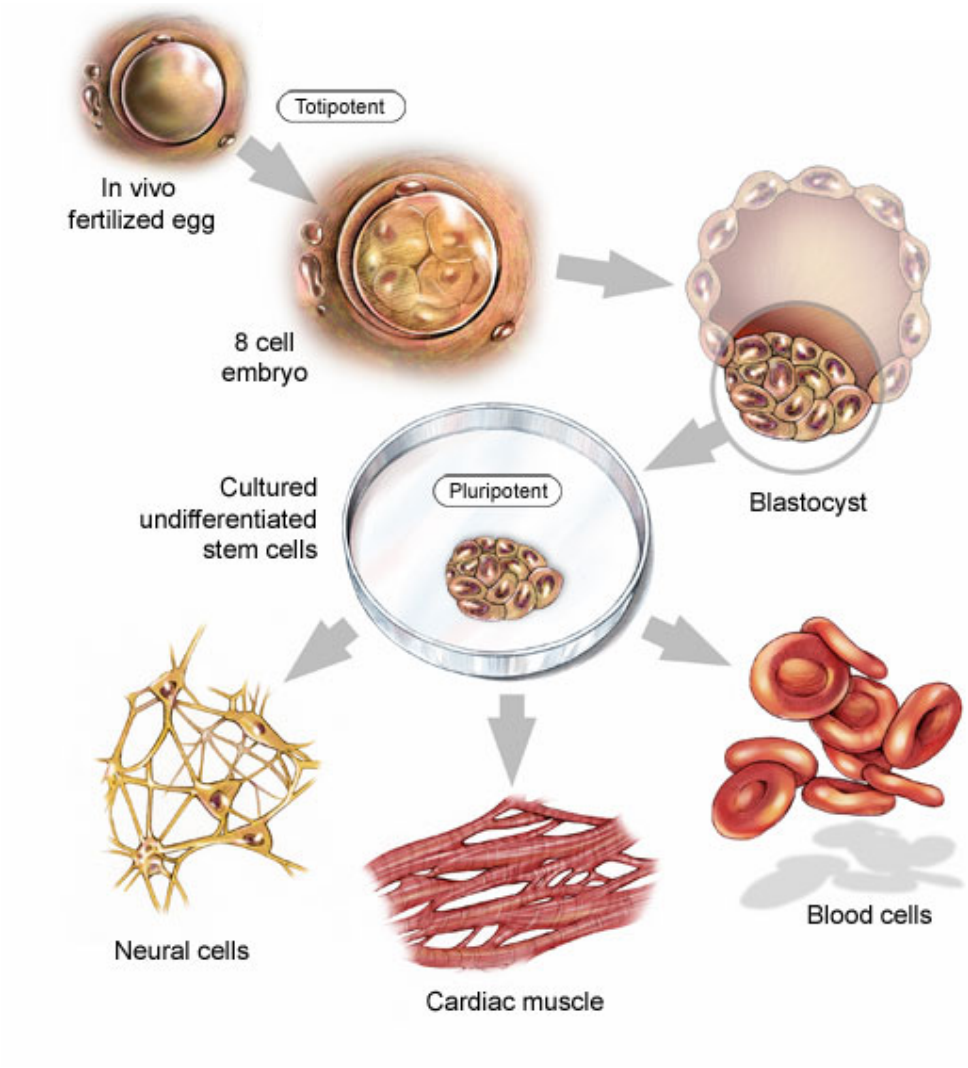


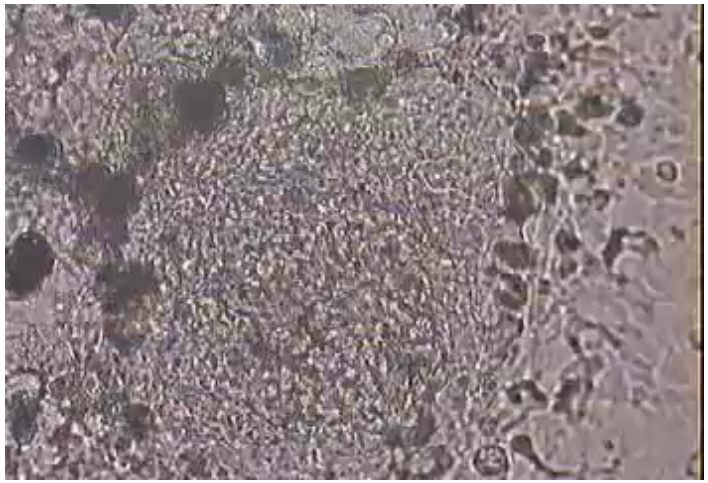
Accounts for 99% of ART procedures

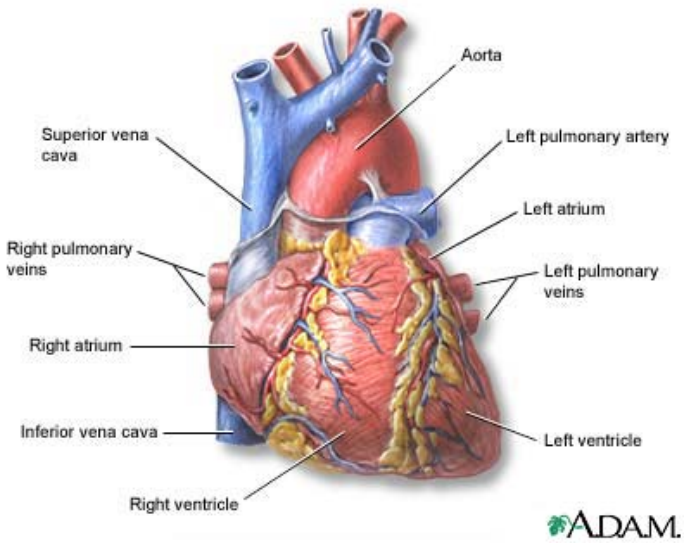
Estimated 3 million worldwide (2% of all US births)


1 EMBRYO
An egg is fertilized or cloned to form an embryo. The embryo begins to divide
2 1 TO 5 DAYS
The embryo divides again and again and takes shape as a sphere called a blastocyst
3 5 TO 7 DAYS
By this time embryonic stem cells are visible and are capable of developing into any tissue in the body
4 STEM LINE
The cells are removed and grown in a Petri dish. As they divide, they create a line of stem cells
5 TISSUE PRODUCTION
Using various recipes of nutrients and other factors, scientists hope to turn stem cells into any of the body's more than 200 tissues, such as:
PANCREATIC ISLET CELLS
Could provide a cure for diabetes
MUSCLE CELLS
Could repair or replace a damaged heart
NERVE CELLS
Could be used to treat Alzheimer's and Parkinson's diseases and repair spinal-cord injuries
HOW IT WORKS
From Embryo to Stem Cell
TIME Graphic by Lon Tweeten

Beating heart muscle from ES cells!

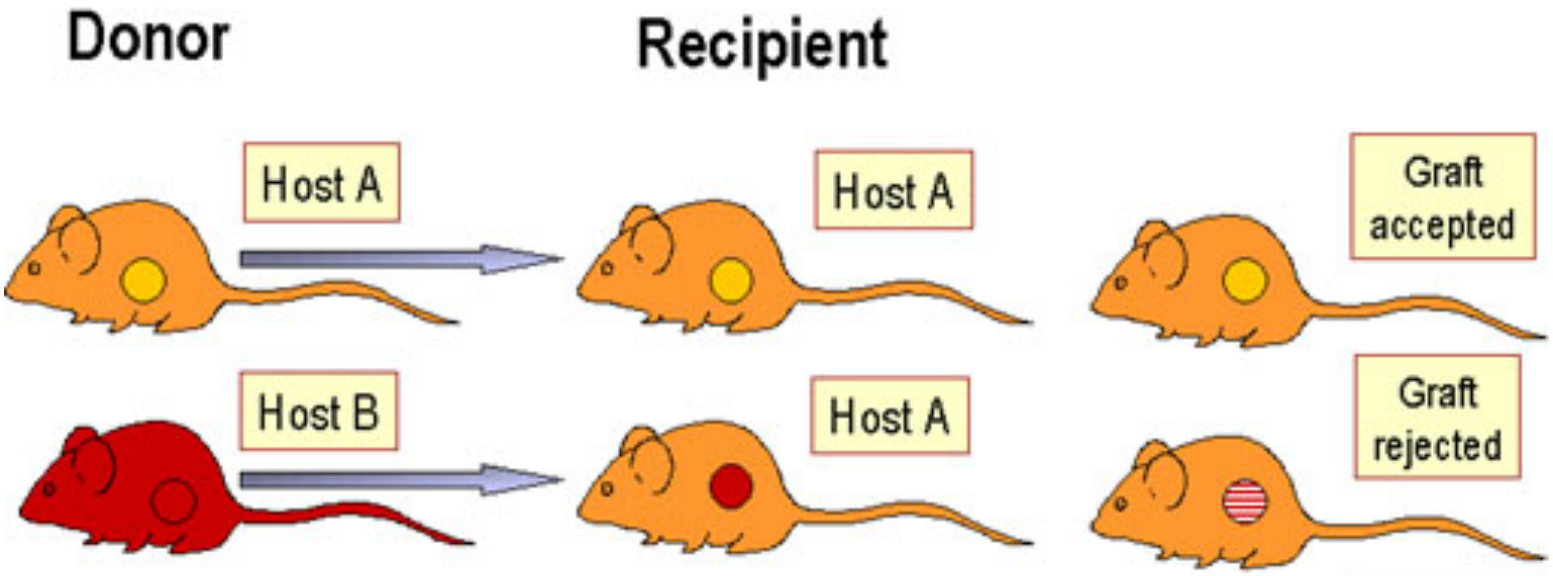






Why do we need more ES cell lines? Aren't the ones we have sufficient? And how does cloning fit into the picture?

Our bodies can recognize self vs non-self



What confers this ability?

Human MHC genes are highly polymorphic

Table 17.3

MHC Class II Alleles

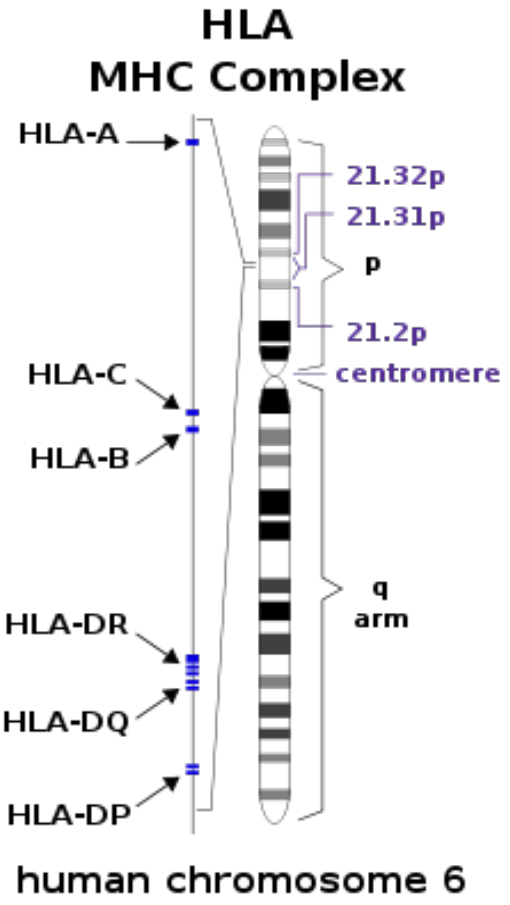
	Locus	Number of Alleles
	HLA-DRA	3
*	HLA-DRB	542
	HLA-DQA	34
	HLA-DQB	73
	HLA-DPA	23
	HLA-DPB	125
	HLA-DMA	4
	HLA-DMB	7
	HLA-DOA	12
	HLA-DOB	9

MHC Class I Alleles

	Locus	Number of Alleles
*	HLA-A	479
*	HLA-B	805
	HLA-C	257
	HLA-E	9
	HLA-F	20
	HLA-G	7

Note: Several other class I alleles are not listed.

Table 17-3 Cell and Molecular Biology, 5/e (© 2008 John Wiley & Sons)



What is the probability that a full sibling will be a genetic match?

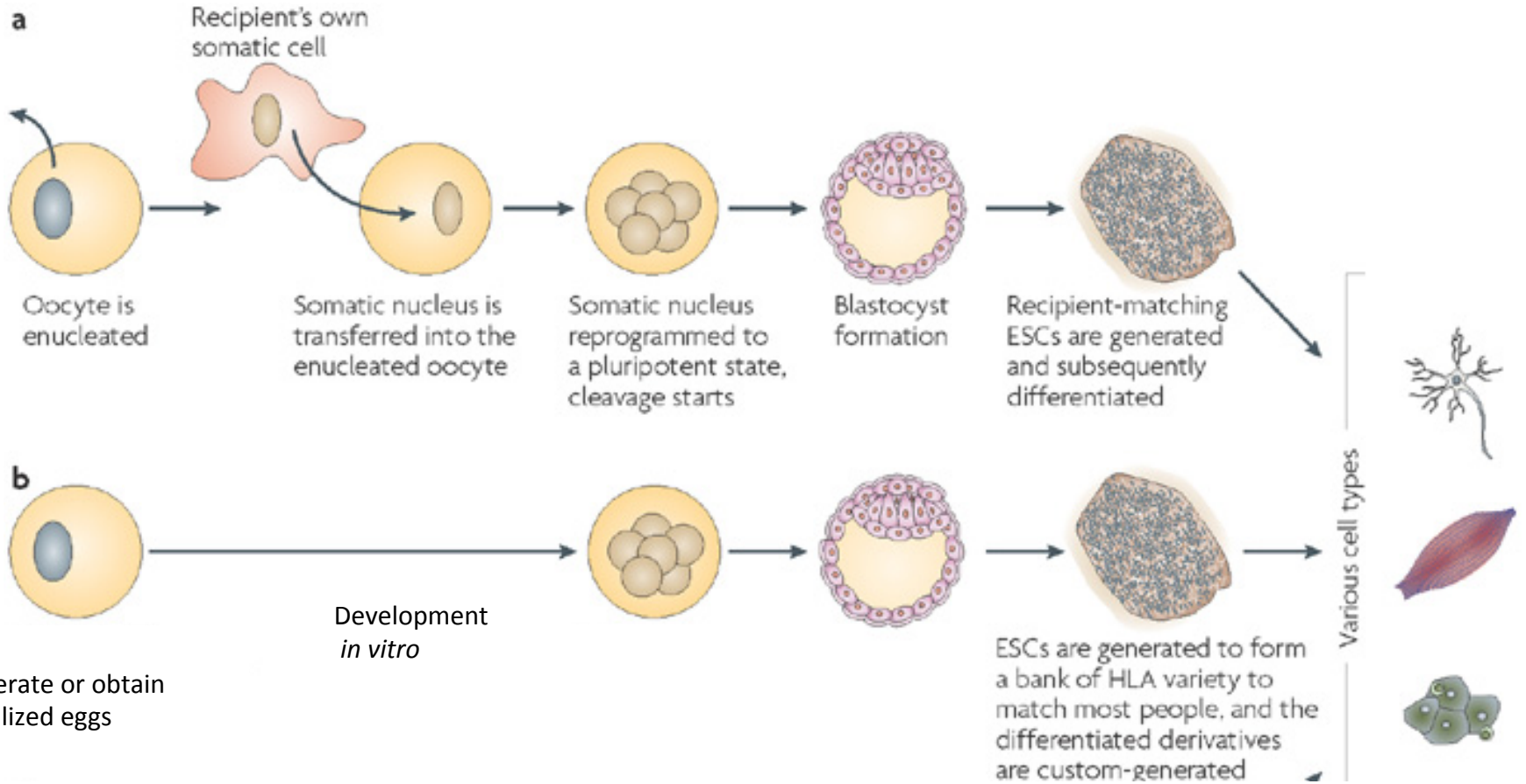
Probability of finding bone marrow match in the general population

	Caucasian	African-American	Asian	Hispanic
Caucasian	1/8,000			
African-American	1/133,000	1/127,000		
Asian	1/270,000	1/2,000,000	1/37,000	
Hispanic	1/45,000	1/370,000	1/370,000	1/39,000

Probability of finding a match is greatest within own ethnic group

Mixed-race individuals face greater challenges

SCNT vs. ES cell bank



Generate or obtain fertilized eggs

Solutions to the Tissue Rejection Problem

- Have a bank of ES cells of different HLA haplotypes representative of different populations
  - similar to bone marrow registry where chances of a match is related to what is available in the bank
- Obtain ES cell lines for each individual through somatic cell nuclear transfer (SCNT) or cloning
  - Personalized medicine!

How do you “clone” a human embryo?

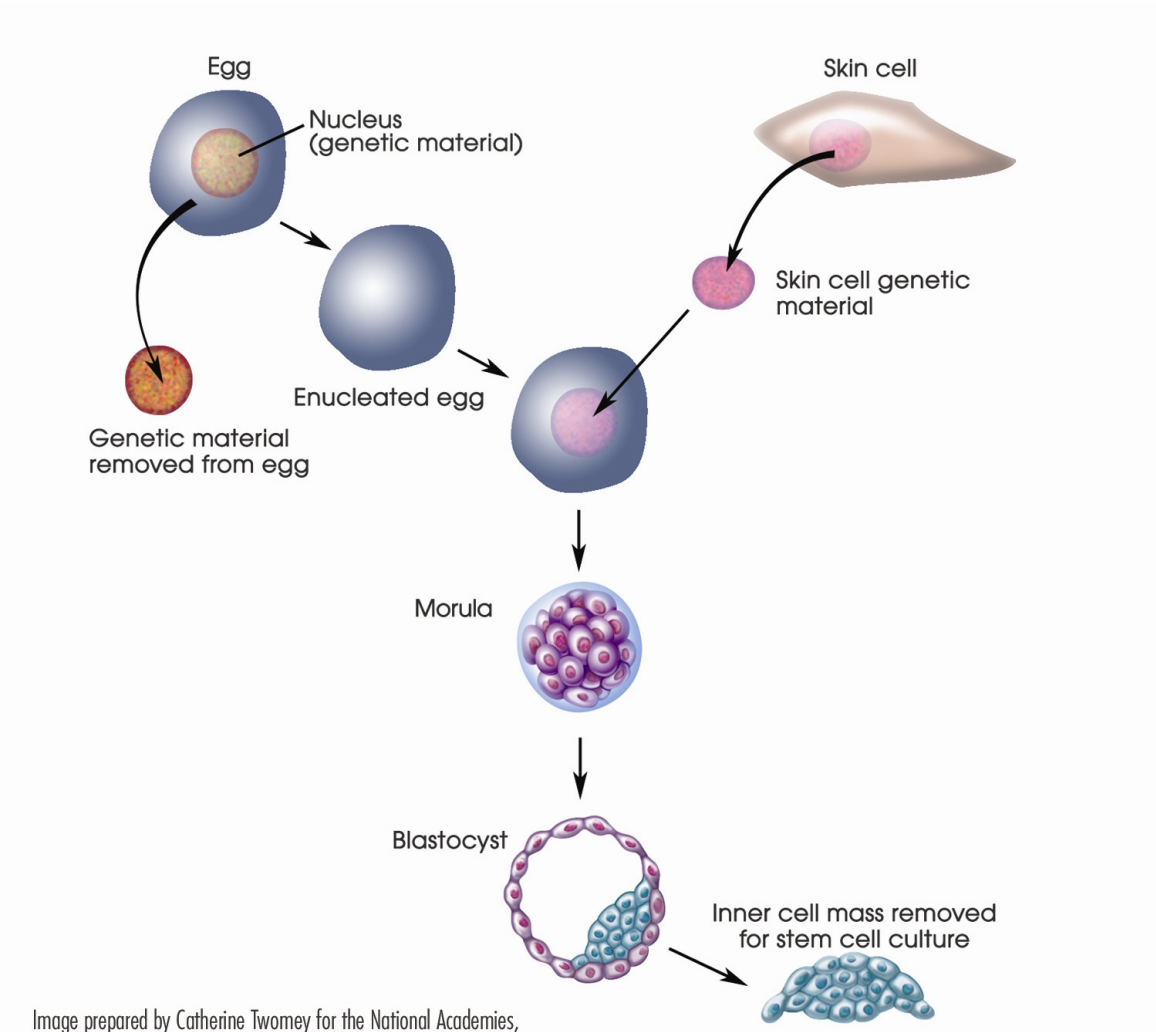
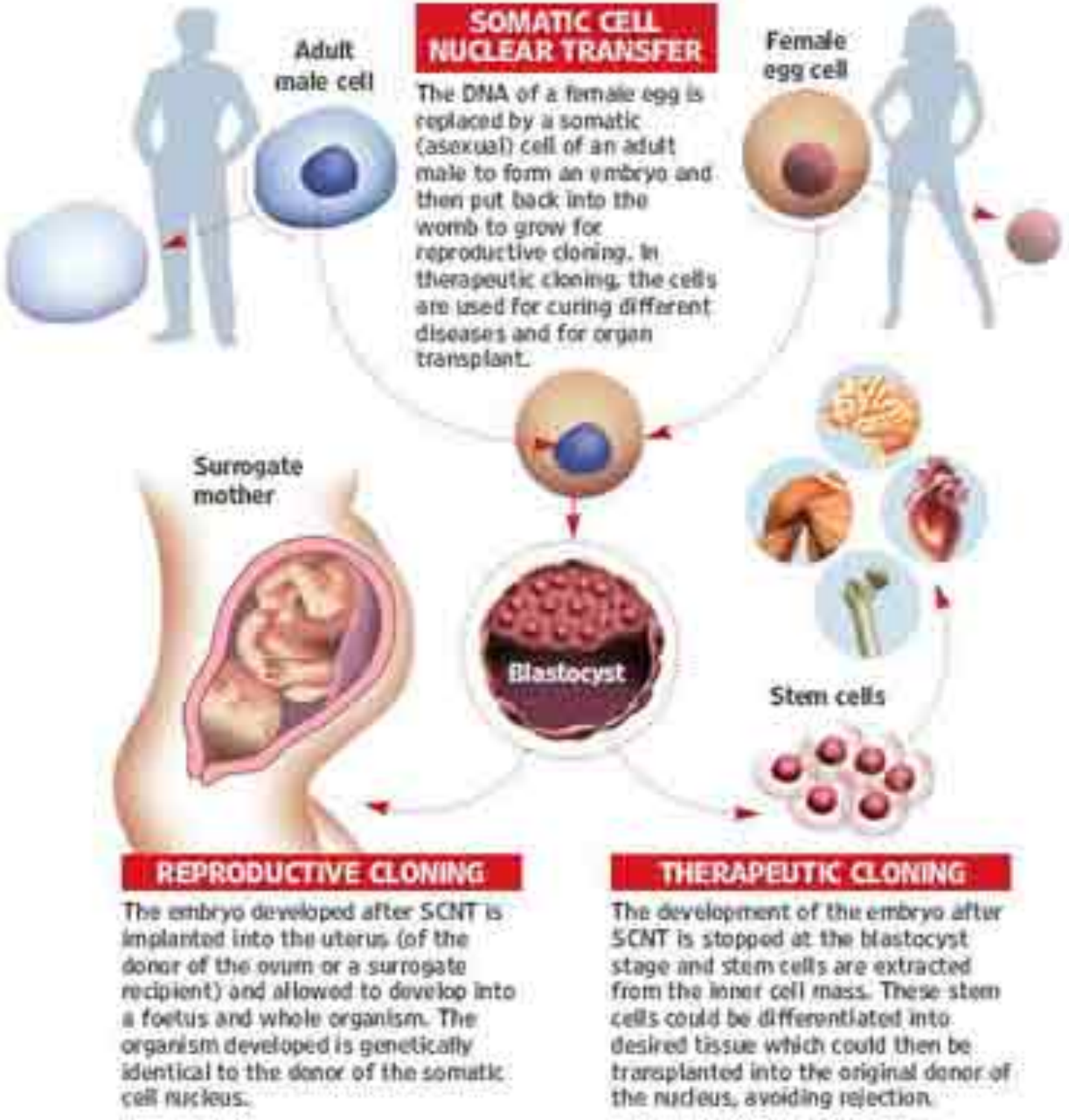


Image prepared by Catherine Twomey for the National Academies,
Understanding Stem Cells: An Overview of the Science and Issues
from the National Academies, http://www.nationalacademies.org/stemcells.
Academic noncommercial use is permitted.

Reproductive vs. Therapeutic Cloning



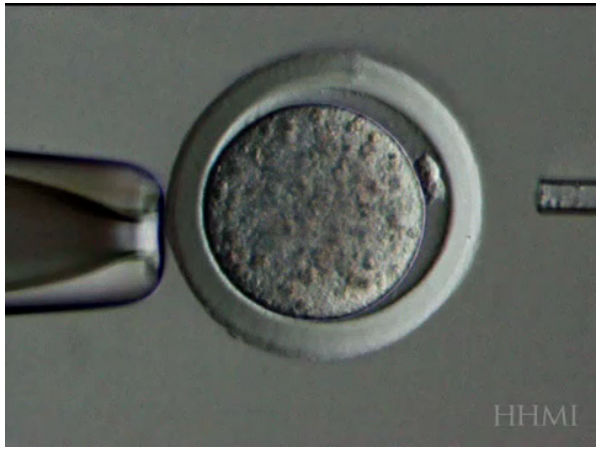
Human Cloning Laws

Reproductive Cloning Ban	Reproductive and Therapeutic Cloning Ban	Ban on State Funding for Cloning Related Research	State Funding Allowed for ES Cell Research (IVF and Cloning)
California, Connecticut, Illinois, Iowa, Maryland, Massachusetts, Missouri, Montana, New Jersey	Arizona, Arkansas, Indiana, Michigan, North Dakota, Oklahoma, South Dakota, Virginia	Arizona, Indiana, Louisiana, Michigan	California, Illinois, Missouri, Maryland, New York

There is no federal law banning reproductive cloning

5/2011

Using Cloned ES Cells to Treat Disease



No need for anti-rejection medication!

What are some challenges to using SCNT to generate pluripotent stem cells?

Why is there controversy surrounding human ES cell research?

Moral Status

- Have protection afforded by moral norms
  - Owe obligation to such individuals

- Who should have moral status?
- What criteria should be used to determine whether a person has moral status?

When do you think personhood begins?

A. Fertilization
B. Implantation in uterus
C. End of embryogenesis (has human body plan)
D. Viability outside uterus
E. Birth

What criteria should we use to define personhood?

Roe v. Wade (1973)

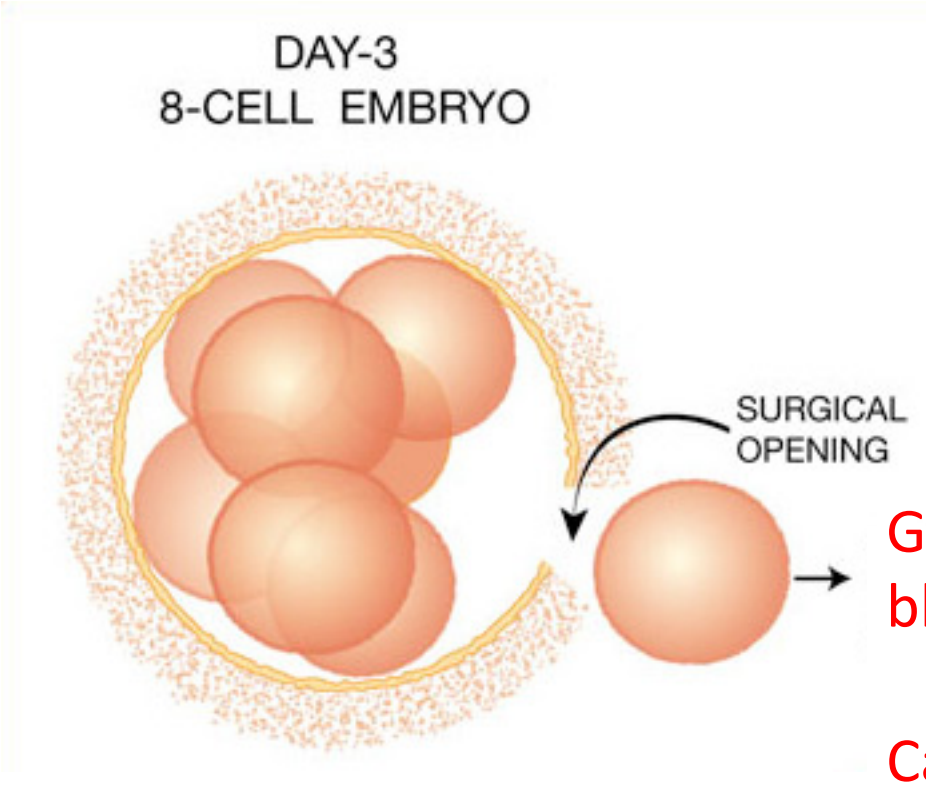
- Right to privacy extends to a woman's right to have an abortion
- Right must be balanced against the protection of prenatal life and mother's health

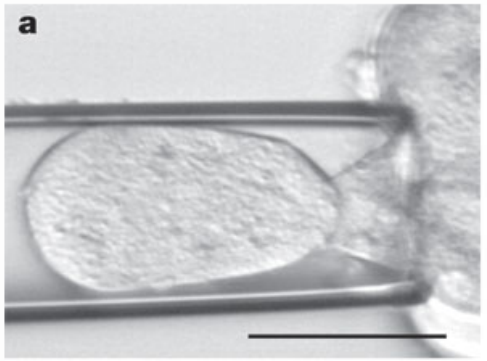
What does Roe v. Wade say about moral status and when personhood begins

- Original opinion:
  - The state could not restrict abortion in the first trimester
  - In second trimester, the state can issue regulations "that are reasonably related to maternal health"
  - In third trimester, the state and regulate or prohibit abortion except "where it is necessary, in appropriate medical judgment, for the preservation of the life or health of the mother"
- Planned Parenthood v. Casey (1992)
  - Supreme court rejected rigid trimester formula, rather it asserted ***viability as the point where the protection of the life of the fetus outweighs the rights of the woman*** and abortion can be banned "except where it is necessary, in appropriate medical judgment, for the preservation of the life or health of the mother"

Can we generate pluripotent stem cells without destroying an embryo?

ES cells can be derived from a single blastomere





Grow biopsied blastomere in culture

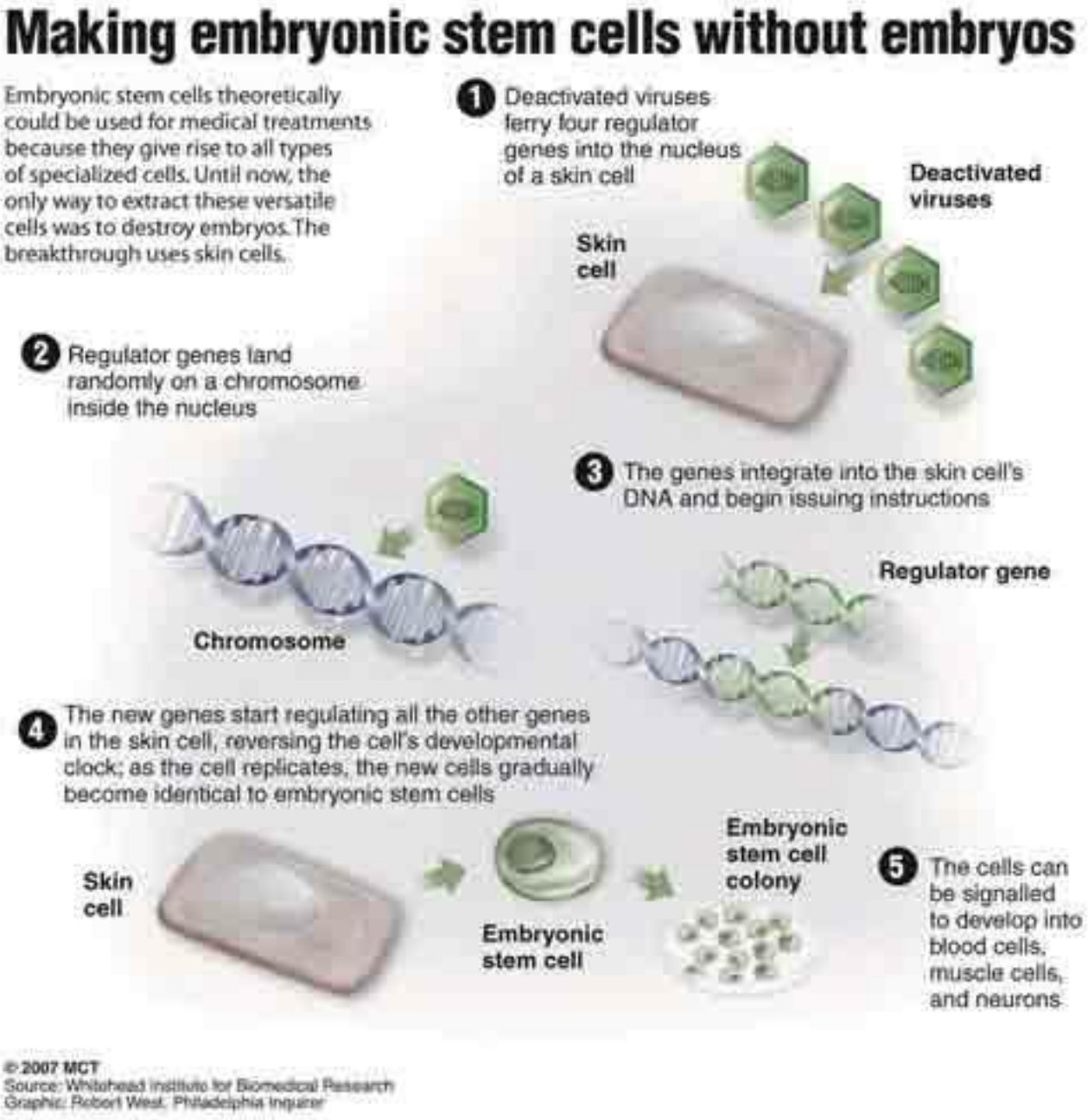
Can develop into ES cells and trophoectoderm

Uses same technique as that for PGD

No babies have been born from biopsied embryos

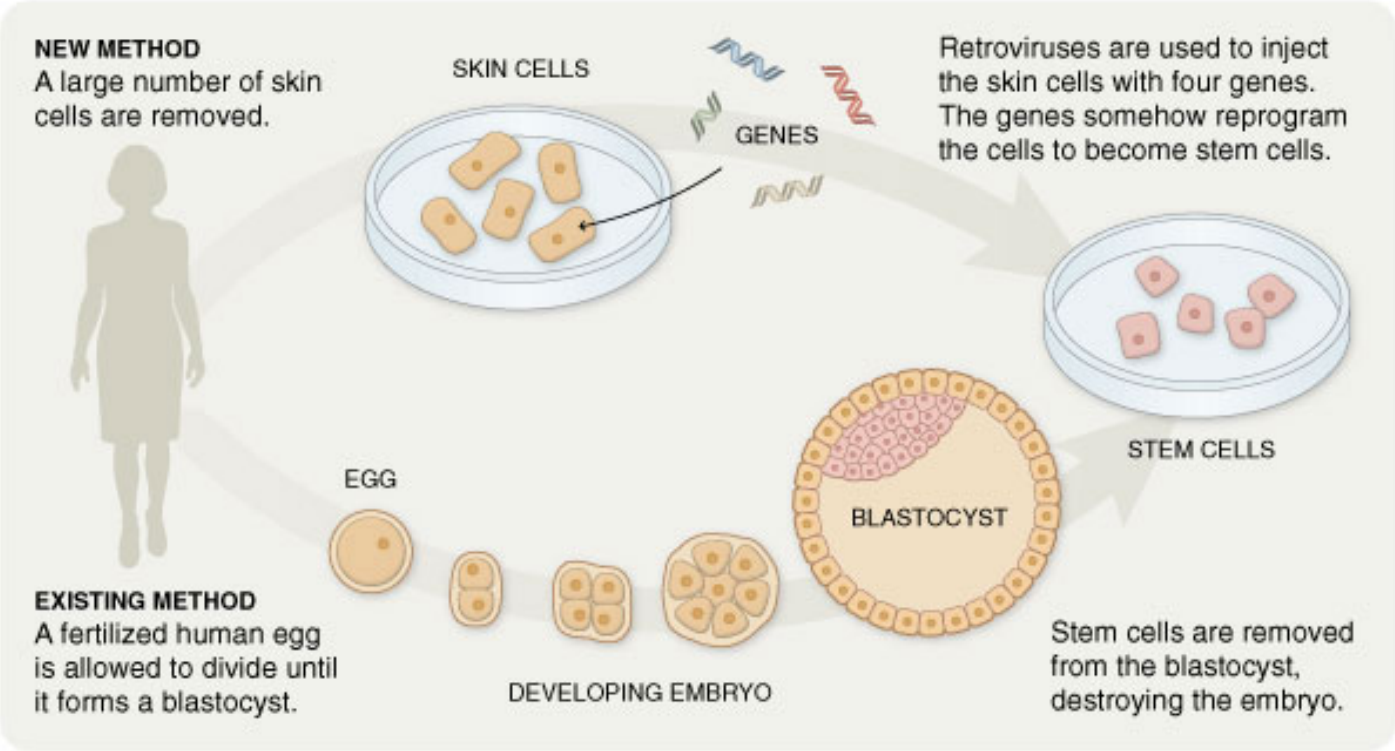
Deactivated viruses have viral genes replaced with targeted human gene

Human gene(s) are inserted into the chromosome of the infected cell and directs the expression of the inserted gene



Reprogramming Human Skin Cells

Researchers have developed a technique for creating stem cells without the controversial use of human eggs or embryos. If the method can be perfected, it could quell the ethical debate troubling the field.



TIMELINE

1995 | 1996 | 1997 | 1998 | 1999 | 2000 | 2001 | 2002 | 2003 | 2004 | 2005 | 2006 | 2007

July 1995
Congress bans federal financing of research on human embryos.

July 1996
Dolly is born. The lamb is the first clone of an adult mammal.

Nov. 1998
First isolation and cultivation of embryonic stem cells. The cells are derived from fertilized human eggs.

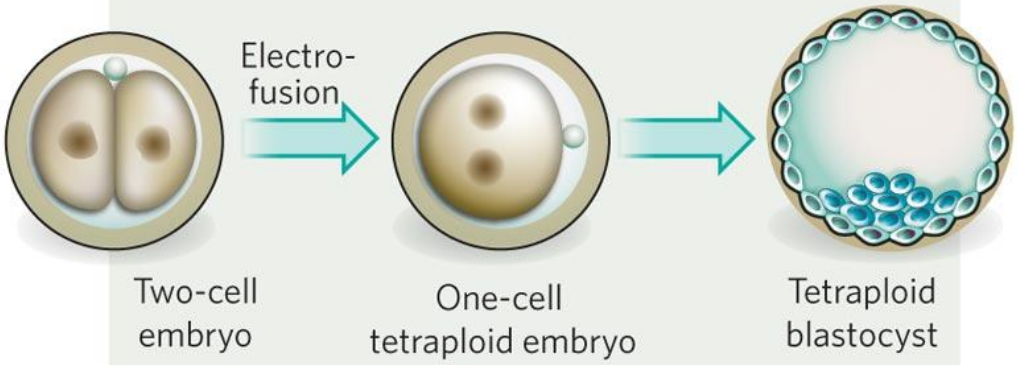
Aug. 2001
President Bush announces that federal money will pay for research on existing stem cell lines, but not new lines.

Nov. 2004
California voters approve a measure to spend $3 billion over 10 years on embryonic stem cell research.

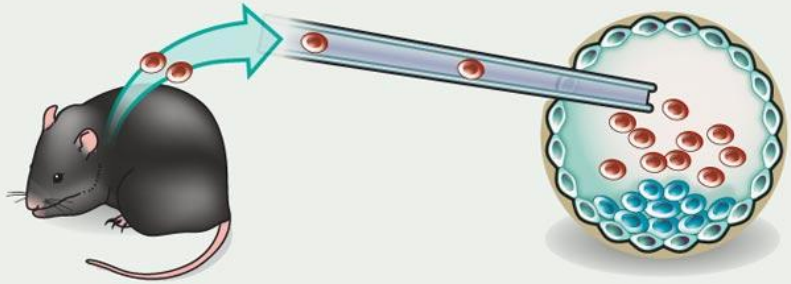
Nov. 2007
New Jersey voters reject a measure to borrow $450 million for stem cell research.

MAKING AN iPS-CELL MOUSE

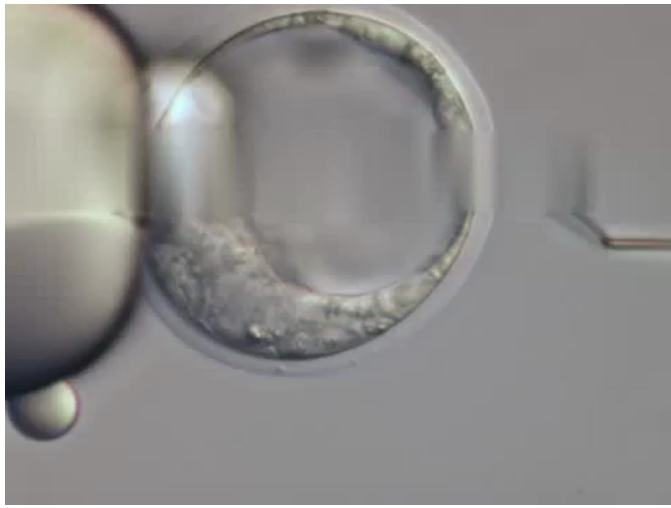
Two-cell embryo is fused to generate a tetraploid blastocyst



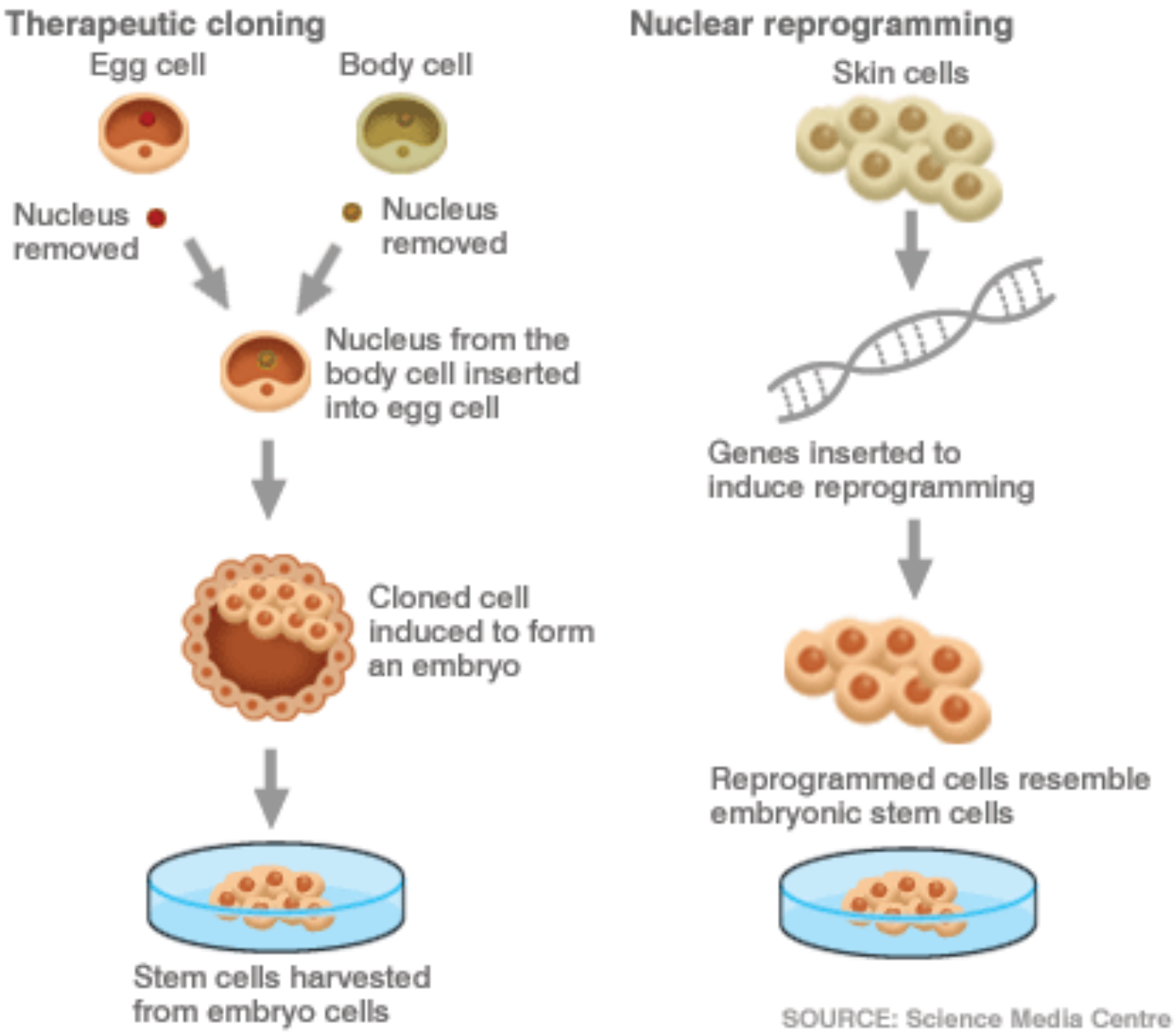
iPS cells are injected into the tetraploid blastocyst, which then steer development



Developing embryo is implanted in surrogate mother



SCNT vs. IPS cells



Use iPS cells to study diseases!

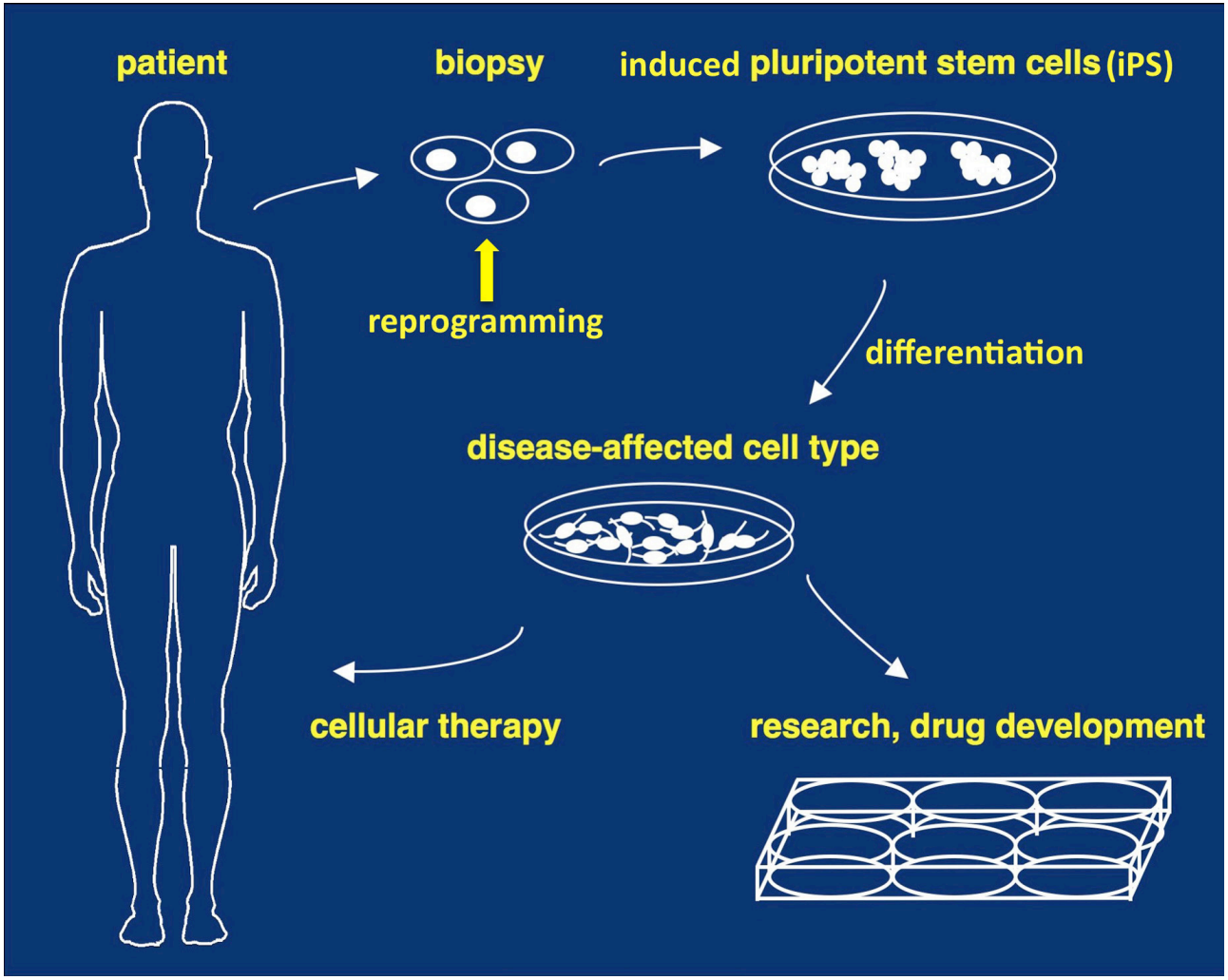


Table 1. iPS Cells Derived from Somatic Cells of Patients with Genetic Disease

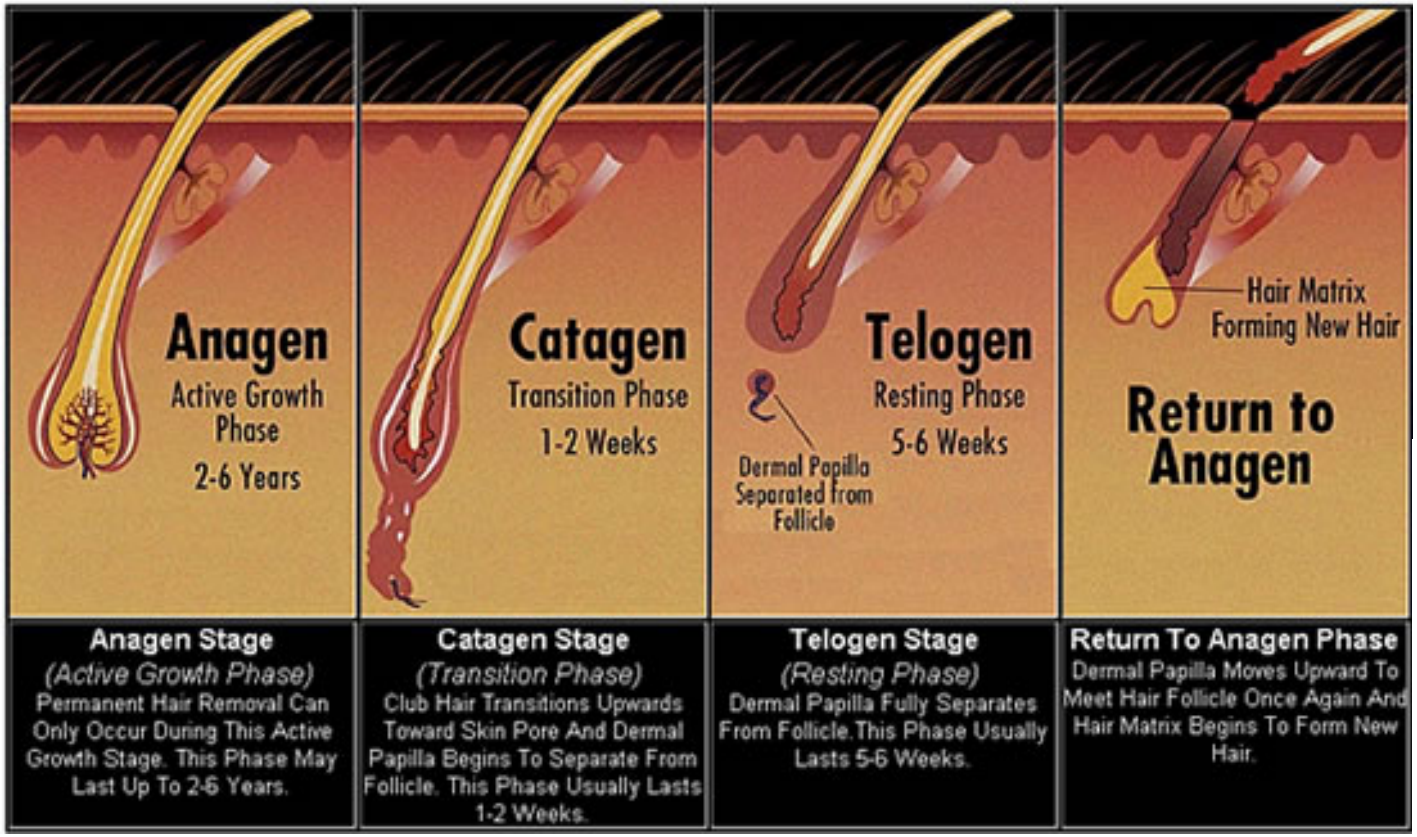
Name	Disease	Molecular Defect	Donor Cell	Age	Sex
ADA	ADA-SCID	GGG >AGG, exon 7 and Del(GAAGA) exon 10, *ADA* gene	Fibroblast	3 M	Male
GD	Gaucher disease type III	AAC > AGC, exon 9, G-insertion, nucleotide 84 of cDNA, *GBA* gene	Fibroblast	20 Y	Male
DMD	Duchenne muscular dystrophy	Deletion of exon 45–52, *dystrophin* gene	Fibroblast	6 Y	Male
BMD	Becker muscular dystrophy	Unidentified mutation in *dystrophin*	Fibroblast	38 Y	Male
DS1, DS2	Down syndrome	Trisomy 21	Fibroblast	1 Y, 1 M	Male
PD	Parkinson disease	Multifactorial	Fibroblast	57 Y	Male
JDM	Juvenile diabetes mellitus	Multifactorial	Fibroblast	42 Y	Female
SBDS	Swachman-Bodian-Diamond syndrome	IV2 + 2T > C and IV3 − 1G > A, *SBDS* gene	Bone marrow mesenchymal cells	4 M	Male
HD	Huntington disease	72 CAG repeats, *huntingtin* gene	Fibroblast	20 Y	Female
LNSc	Lesch-Nyhan syndrome (carrier)	Heterozygosity of *HPRT1*	Fibroblast	34 Y	Female

What about “adult” stem cells?

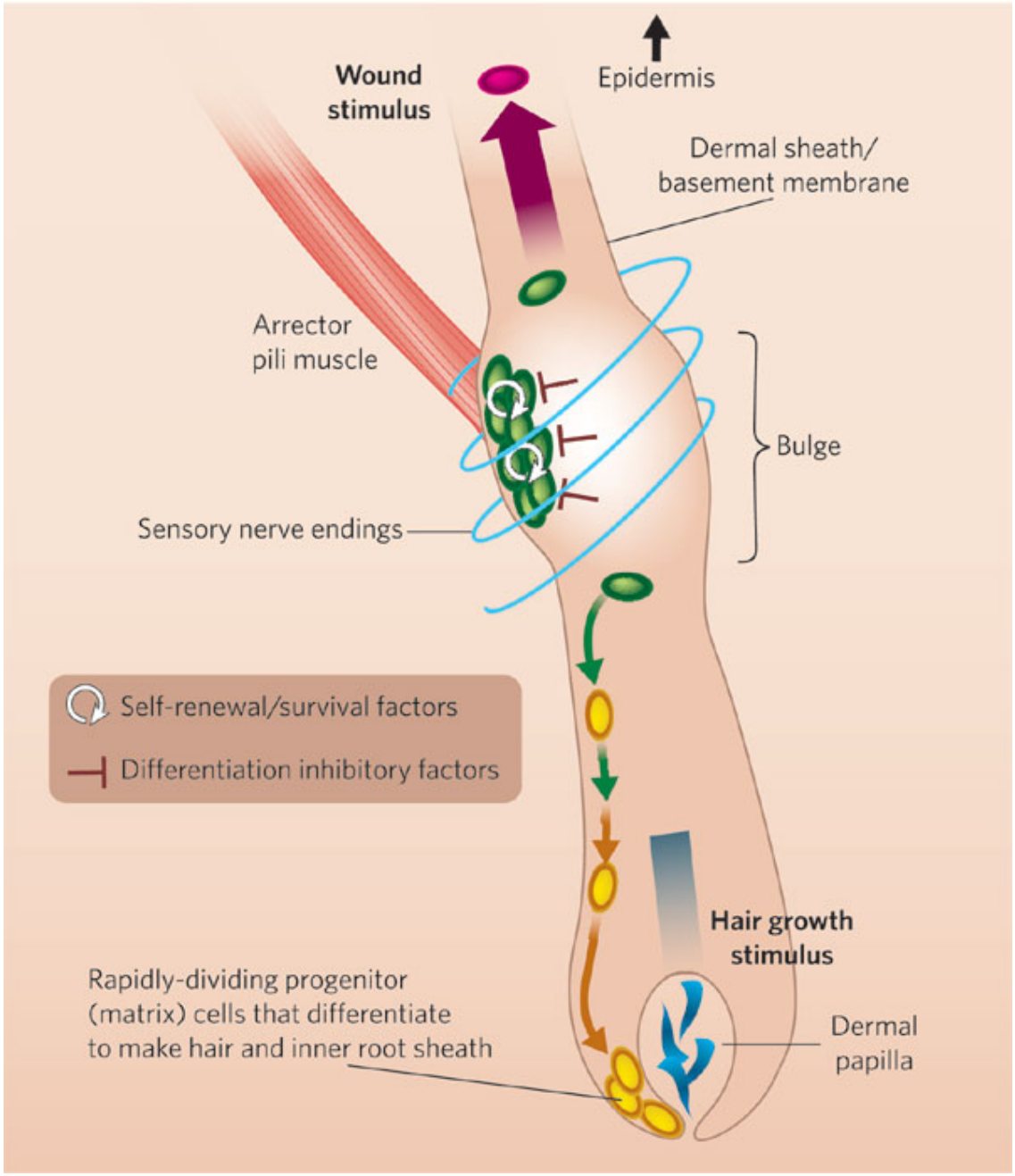
Adult stem cells are…

- Also present in children
- Have limited differentiation potential, usually restricted to a few cell types

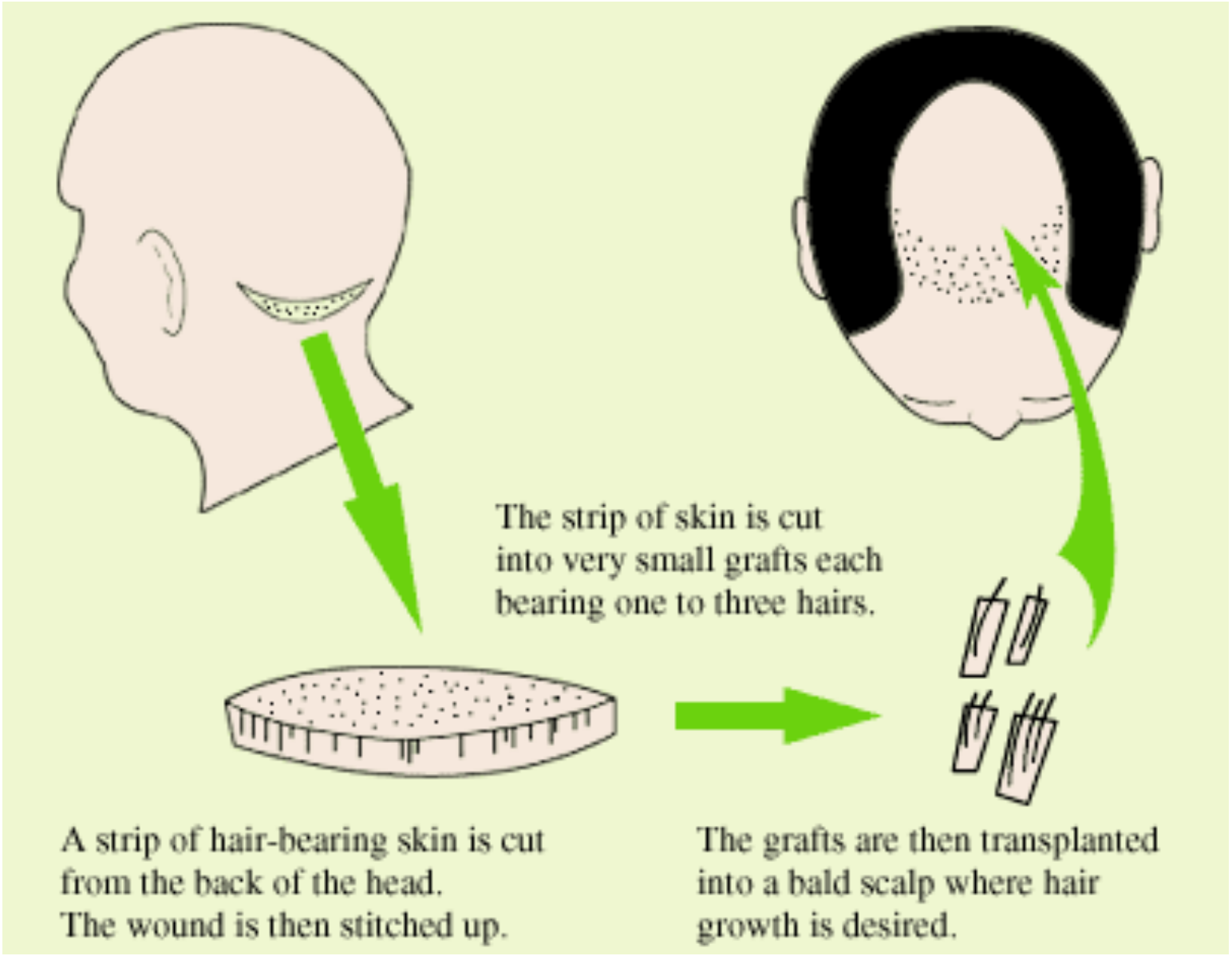
Stem Cells in the Hair Follicle



Sorry Rapunzel!


Wound stimulus
Epidermis
Dermal sheath/ basement membrane
Arrector pili muscle
Bulge
Sensory nerve endings
Self-renewal/survival factors
Differentiation inhibitory factors
Hair growth stimulus
Rapidly-dividing progenitor (matrix) cells that differentiate to make hair and inner root sheath
Dermal papilla

Hair Transplantation is Stem Cell Therapy!



Limitations of multipotent stem cells

- Limited developmental potential
- Difficult to find and isolate
- Difficult to grow in culture

What is the current stem cell policy in the United States?

Policy under Barack Obama

March 9, 2009

Executive order 13505

- The Secretary of Health and Human Services (Secretary), through the Director of NIH, may support and conduct responsible, scientifically worthy human stem cell research, including human embryonic stem cell research, to the extent permitted by law.
- The Presidential statement of August 9, 2001, limiting Federal funding for research involving human embryonic stem cells, shall have no further effect as a statement of governmental policy.
- Executive Order 13435 of June 20, 2007, which supplements the August 9, 2001, statement on human embryonic stem cell research, is revoked.

NIH Guidelines (July 2009)

ES cell research eligible for NIH funding if:

1. that were created using in vitro fertilization for reproductive purposes and were no longer needed for this purpose
2. that were donated by individuals who sought reproductive treatment (hereafter referred to as "donor(s)") and who gave voluntary written consent for the human embryos to be used for research purposes
3. No payments, cash or in kind, were offered for the donated embryos.
4. Decisions related to the creation of human embryos for reproductive purposes should have been made free from the influence of researchers proposing to derive or utilize hESCs in research.

Research NOT eligible for NIH funding:

1. NIH funding of the derivation of stem cells from human embryos is prohibited by the annual appropriations ban on funding of human embryo research (Section 509, Omnibus Appropriations Act, 2009, Pub. L. 111-8, 3/11/09), otherwise known as the Dickey Amendment.
2. Research using hESCs derived from other sources, including somatic cell nuclear transfer, parthenogenesis, and/or IVF embryos created for research purposes, is not eligible for NIH funding.

Dickey-Wicker Amendment (1995)

Prohibits Dept HHS appropriations for:

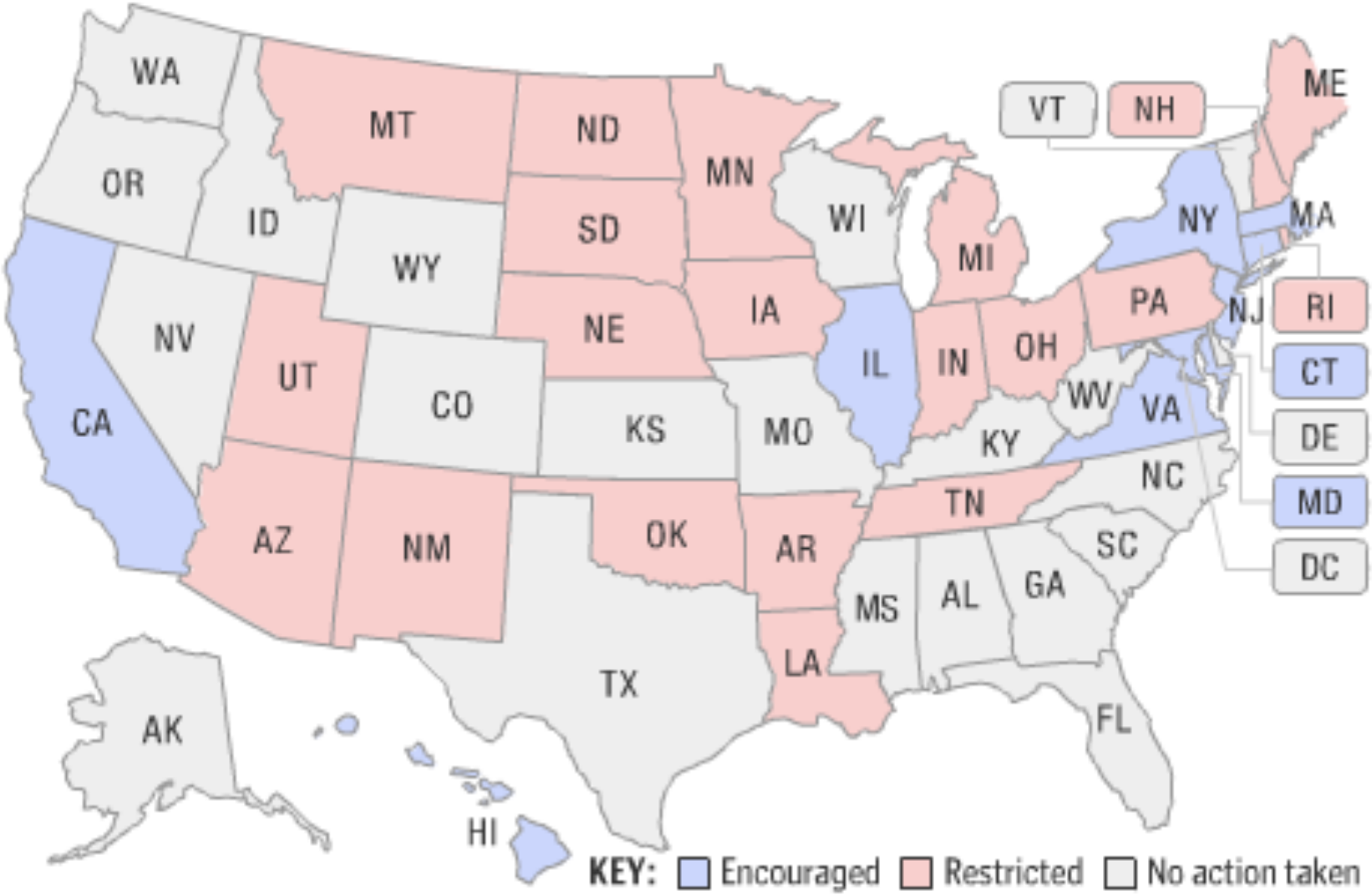
(1) the creation of a human embryo or embryos for research purposes; or

(2) research in which a human embryo or embryos are destroyed, discarded, or knowingly subjected to risk of injury or death greater than that allowed for research on fetuses in utero

Sherley v. Sebelius

- 8/19/2009—complaint filed challenging the legality of NIH guidelines
- 8/23/2010—preliminary injunction from DC District court blocking implementation of NIH's 2009 guidelines, saying that it violates the Dickey-Wicker amendment
- 9/9/2010—preliminary injunction lifted pending decision from US court of appeals
- 4/29/2011—injunction vacated by US court of appeals. Using the Chevron doctrine, the court concluded that the Dickey-Wicker amendment is ambiguous and that NIH has acted reasonably in concluding that public funds could be used for human embryonic stem cell research. Also, the panel that the government would be harmed by the injunction more than the plaintiffs by not having one.
- 1/7/13—US Supreme court refuses to hear appeal

Stem cell research policy by state:



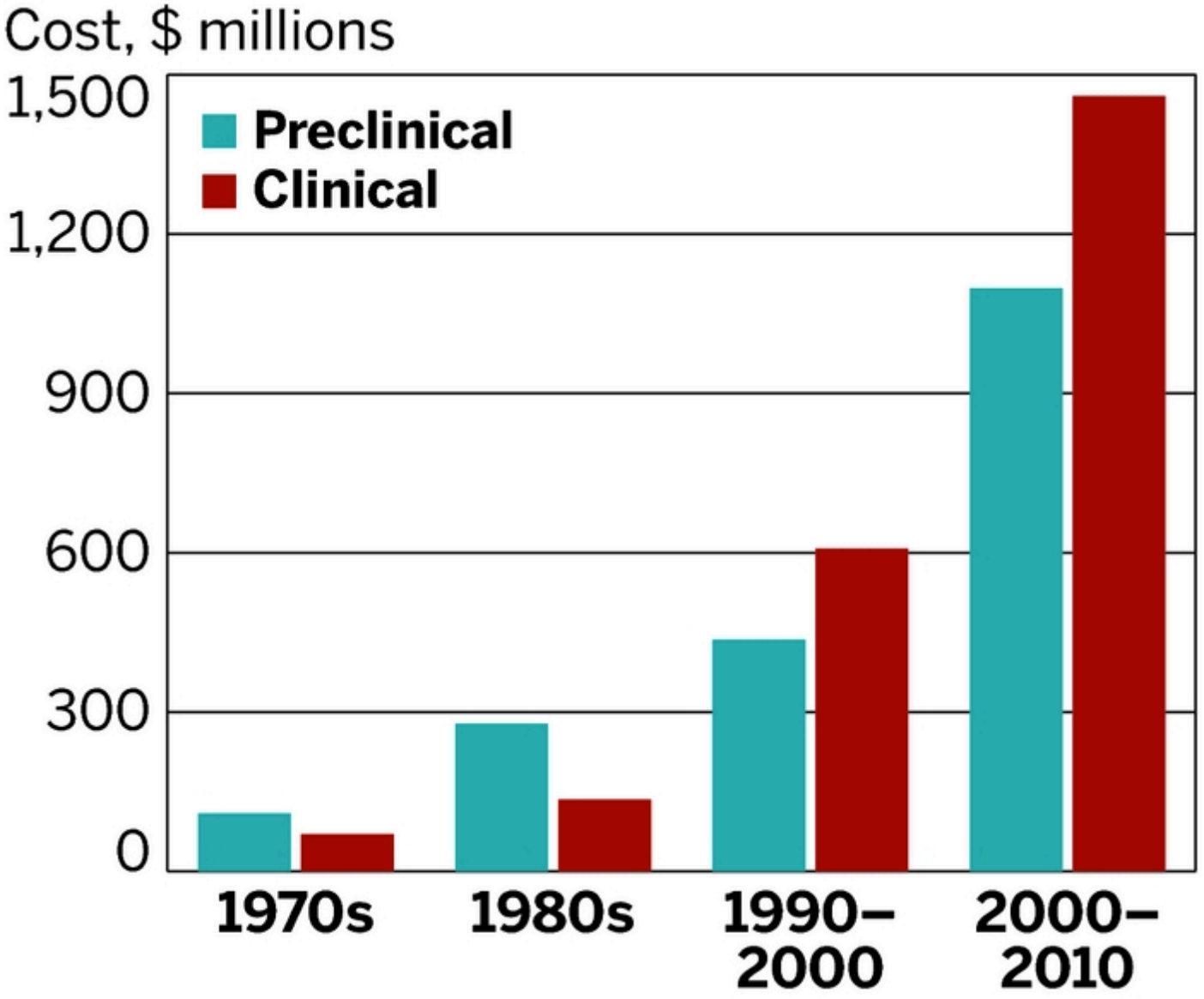
California Institute for Regenerative Medicine (CIRM)

- Created in 2004 through the passage of prop 71 (59% of vote)
  - Allocates grant money for research purposes
  - Sets appropriate regulatory standards
- Prop 71:
  - Makes conducting stem cell research a constitutional right
  - Uses general obligation bonds to fund scientific research (normally for brick and mortar projects)
  - Takes on typical federal government role of funding scientific research
  - Represents a unique example where the public decided to fund scientific research
- Issues 3 billion in grants funded by bonds over 10 years
  - Money can be used for all stem cell research, with priority for human embryonic stem cell research
- First research grants were awarded in 2007
- Funding through prop 71 expected to end by 2017

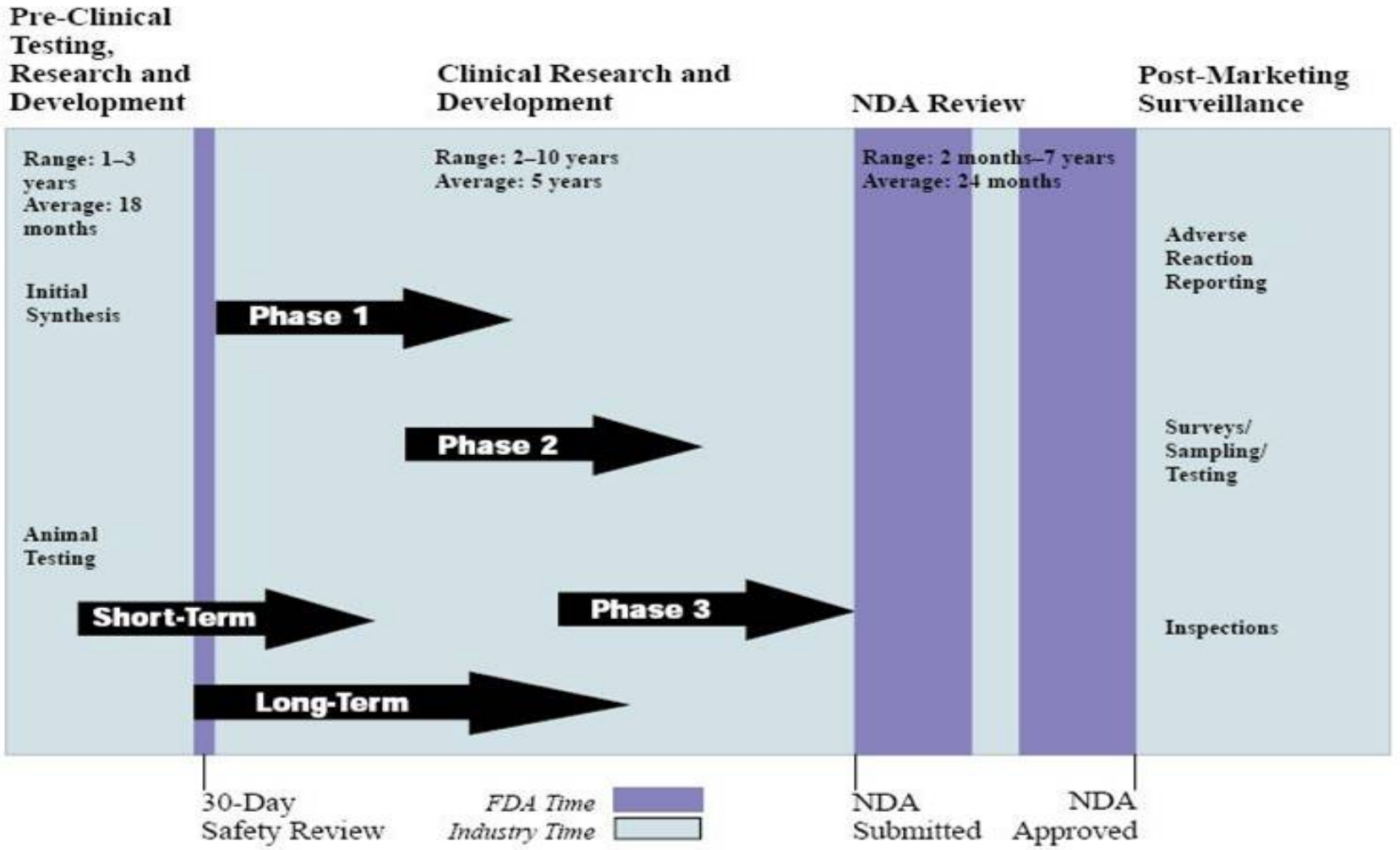
The Promise of Regenerative Medicine

"The cure to your disease came from stem cell research. Sign here if you wish to refuse treatment for moral, religious or ideological reasons."

Cost of Developing a New Drug



New Drug Development Timeline



Clinical Trials

- Phase I: Safety
  - Usually includes healthy (paid) volunteers
- Phase II: Efficacy
  - Patients are involved
  - Usually where drug fails
- Phase III---Randomized controlled trial
  - Involves larger numbers of patients
  - Compares efficacy of drug against current "gold standard" treatment
  - Expensive

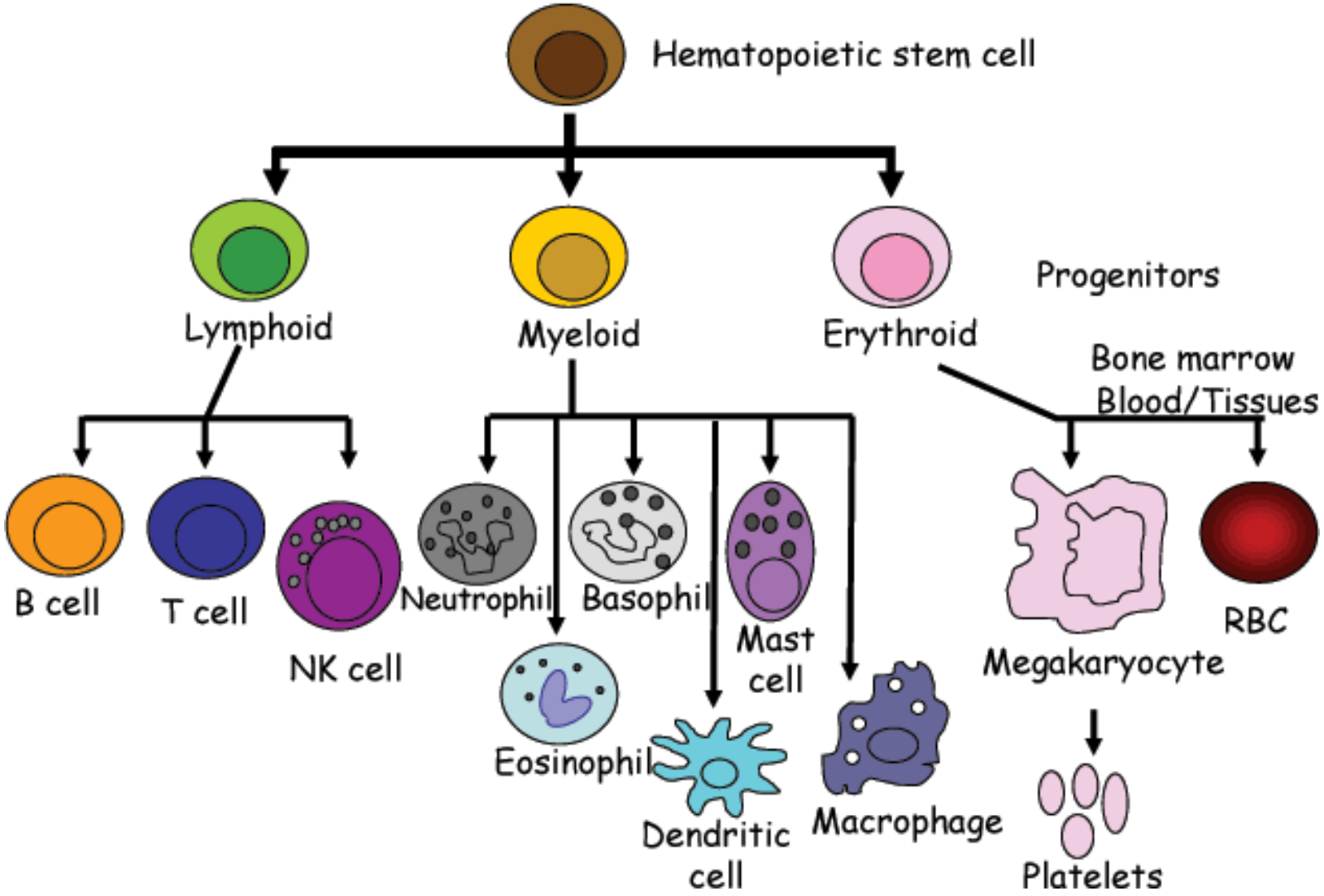
There are currently 2 clinical trials using ES cell derived therapy

Hurdles to using stem cells for disease treatment

- Reproducibly proliferate and generate sufficient tissue
- Reproducibly differentiate into the desired cell type
- Delivery to desired organ
- Survive in the recipient after transplant
- Integrate into the surrounding tissue
- Function properly
- No harm (esp. ESC)

Using Stem Cells to Treat 2 Diseases of the Hematopoietic System—SCID and Sickle Cell Anemia

Immune cell development: Hematopoiesis



Using Stem Cell Therapy to Treat Immune System Disorders

- Certain characteristics of the hematopoietic (blood) system make its diseases good candidates for stem cell therapy
  - Hematopoietic stem cells have been well studied
  - Long history of using the hematopoietic stem cell to treat disease (bone marrow transplant)
  - Easy to isolate, manipulate and replace

- 2 diseases:
  - SCID
  - Sickle cell anemia

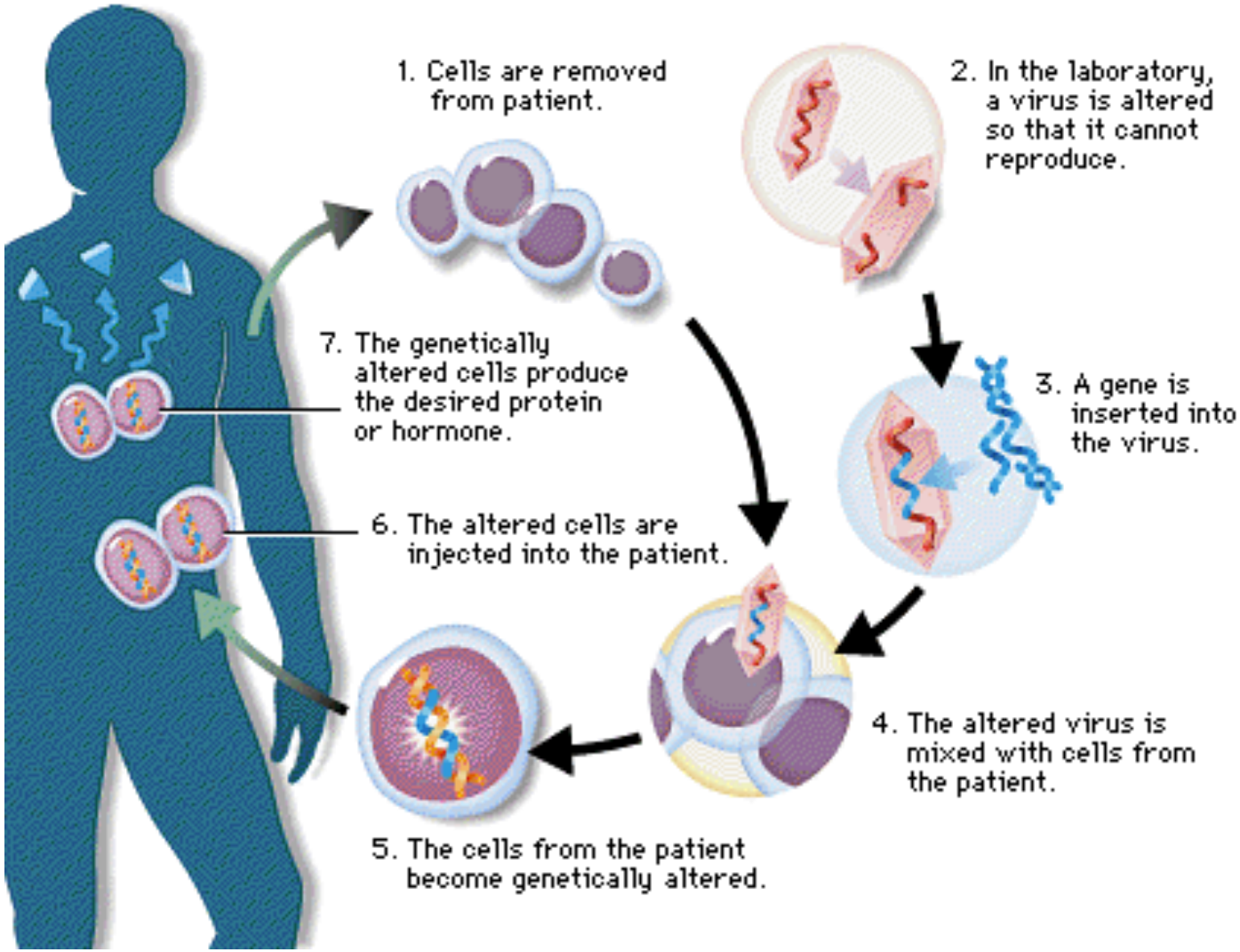
- Both from research done in Donald Kohn's lab here at UCLA!

Severe Combined Immune Deficiency (SCID)

- Bubble boy disease
- Defect leads to lack of functional B or T cells of the immune system
- SCID babies generally die in the first year of life
- Many different mutations can lead to disease, caused by a loss of function of the protein

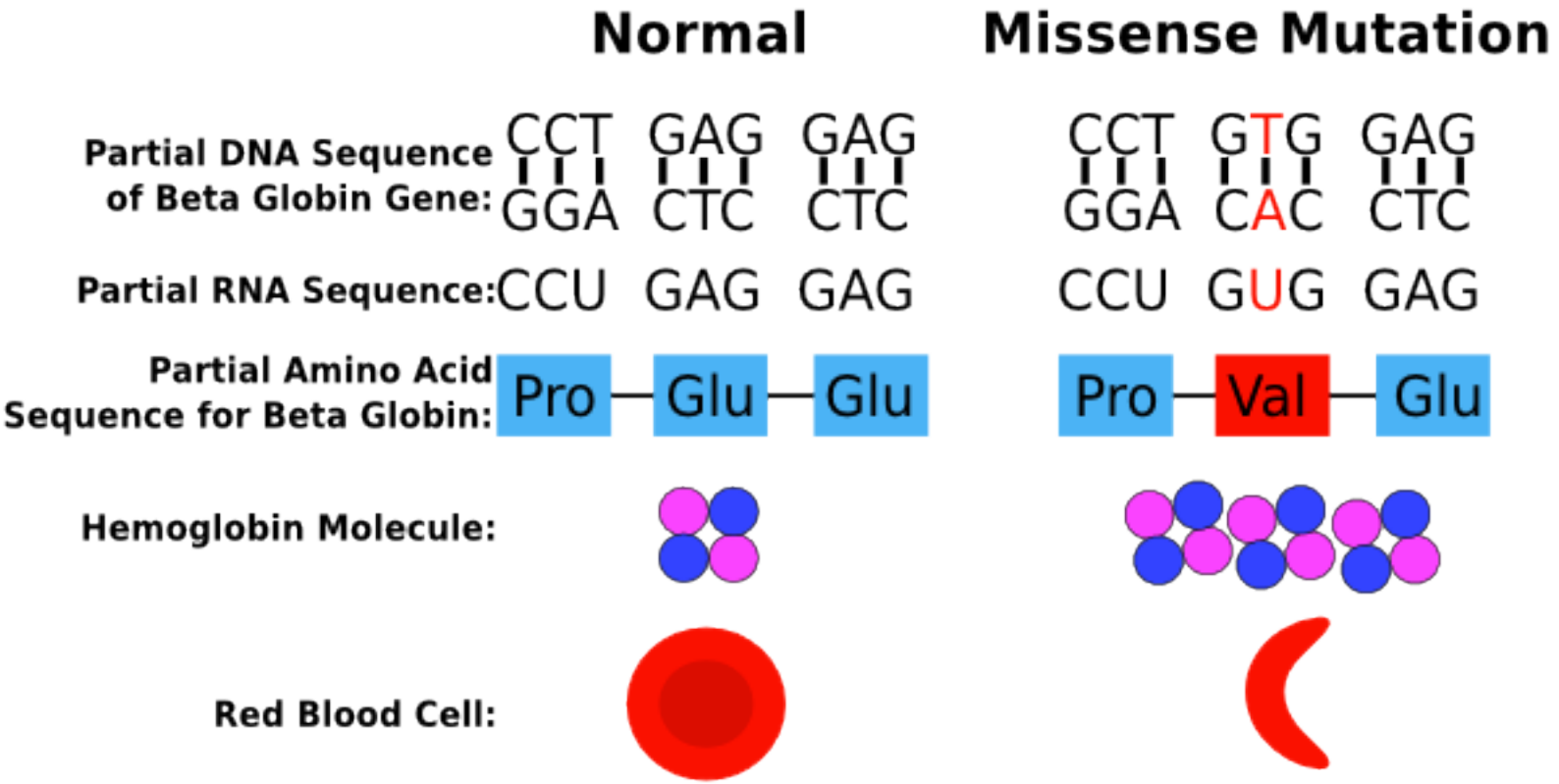
David Vetter—the "bubble boy"
1971-1984

Gene Therapy Using Hematopoietic Stem Cells

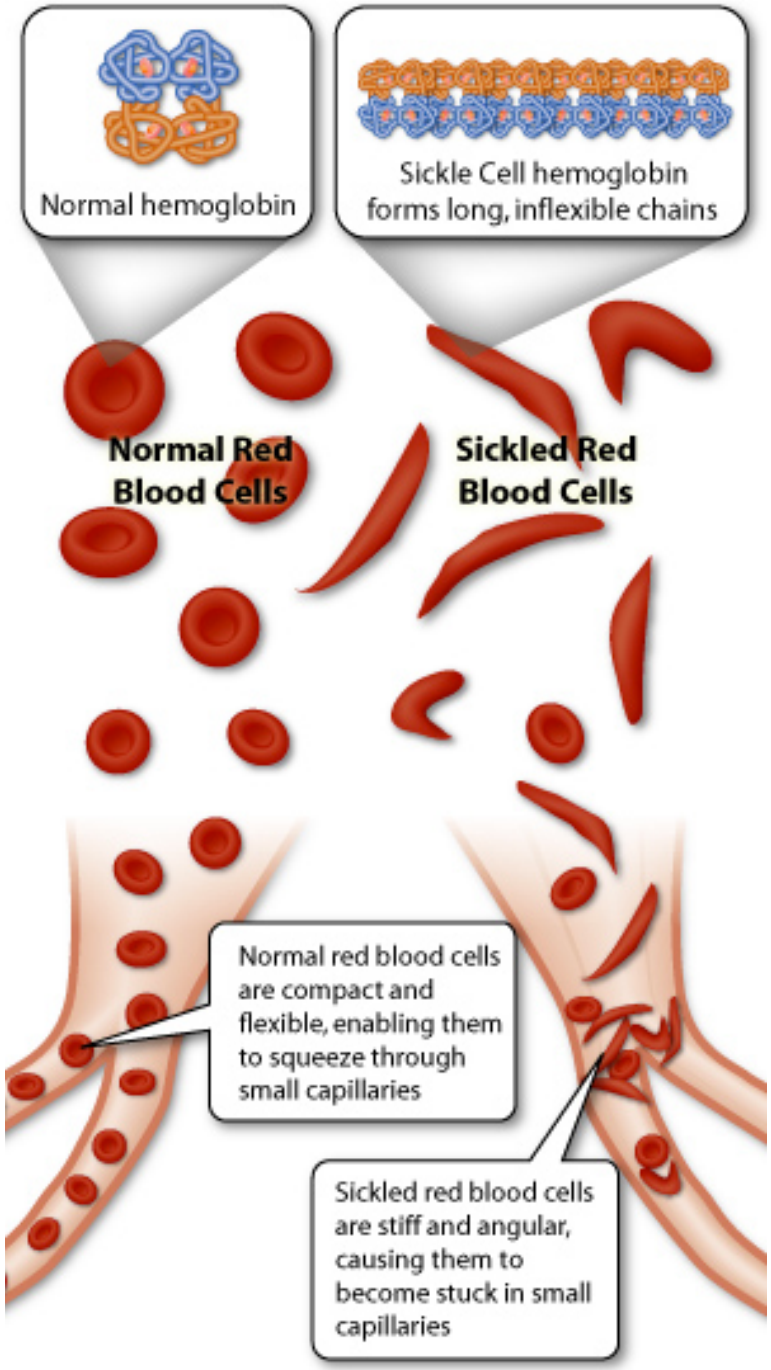


18 children have been cured of the disease using gene therapy carried out at UCLA and NIH

Sickle Cell Anemia

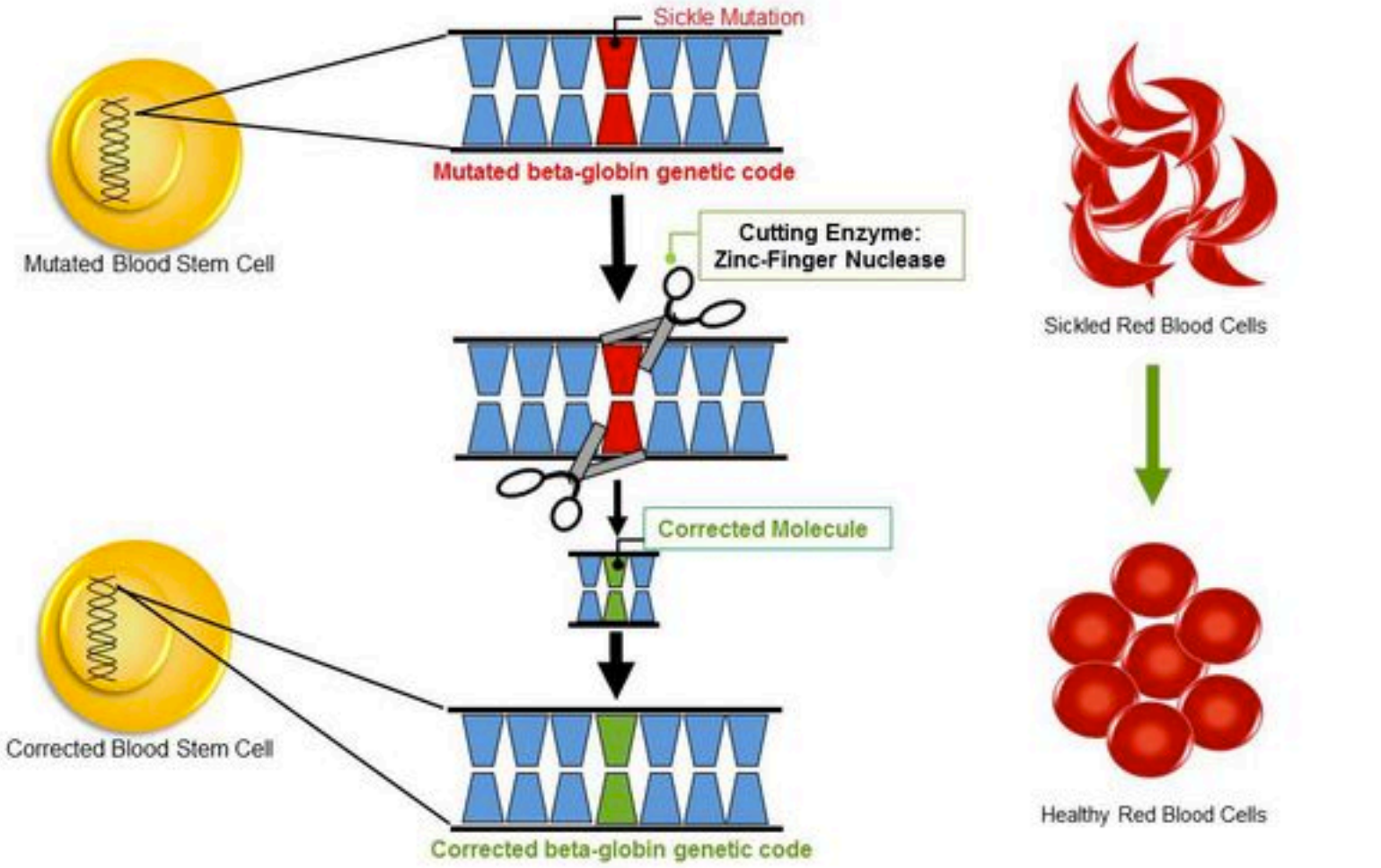


Sickle cell anemia is more difficult to treat because it is not caused by the *lack* of a protein, but the incorrect function of a mutated protein



- Mutation causes the hemoglobin protein to form long stiff rods, leading the sickle shape
- Lifespan of a patient with sickle cell anemia is under 40 years
- Clinical trials currently under way

Correction of Sickle-Cell Disease Mutation in Human Blood Stem Cells



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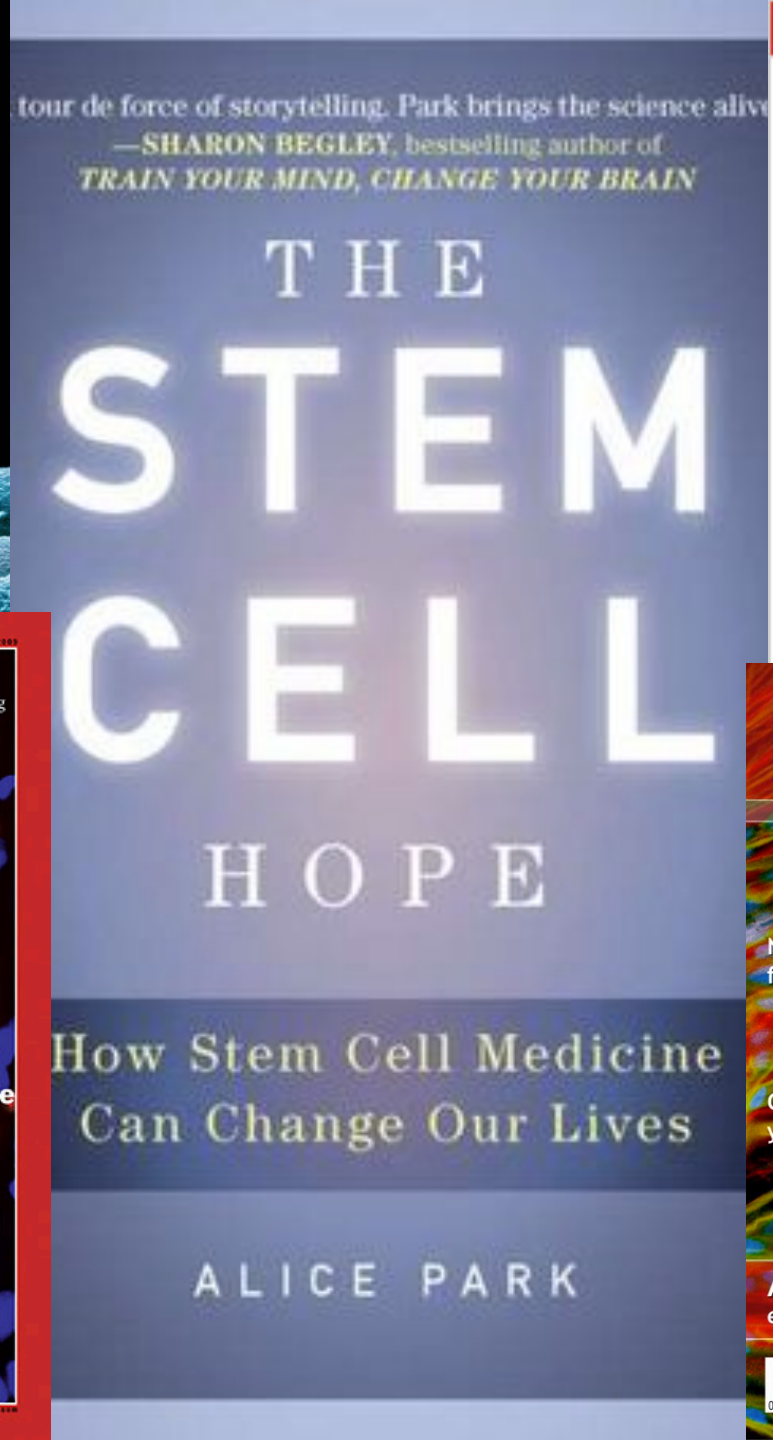
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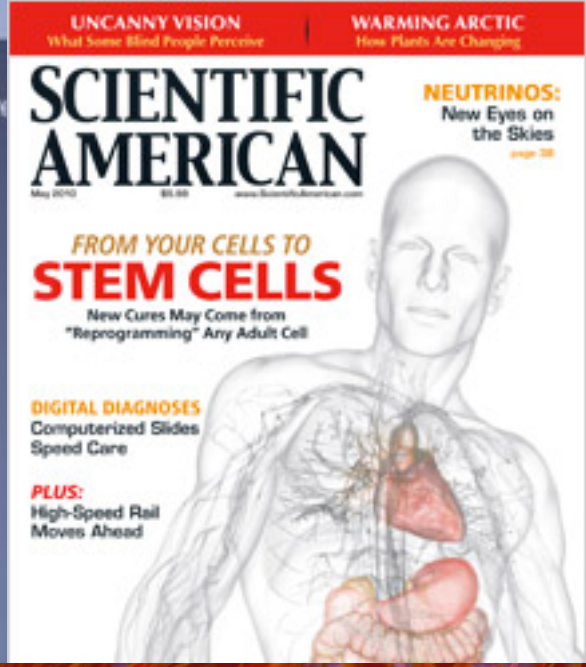
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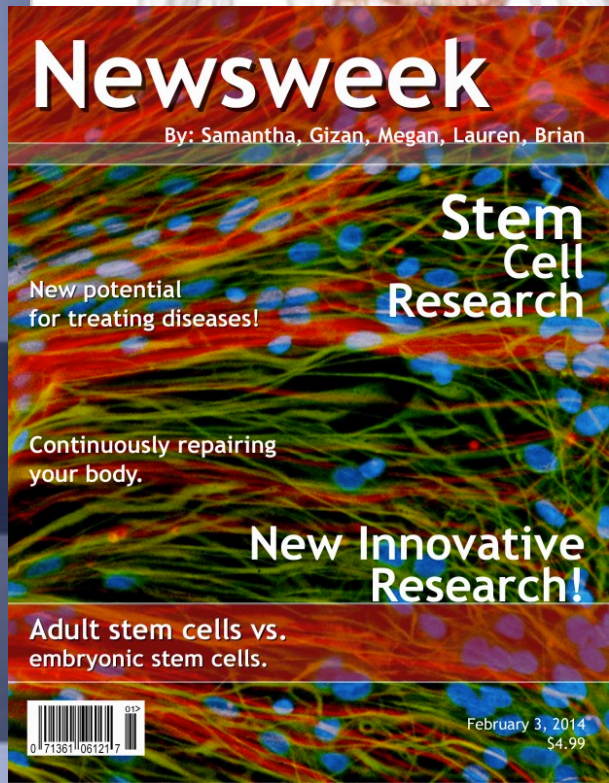
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