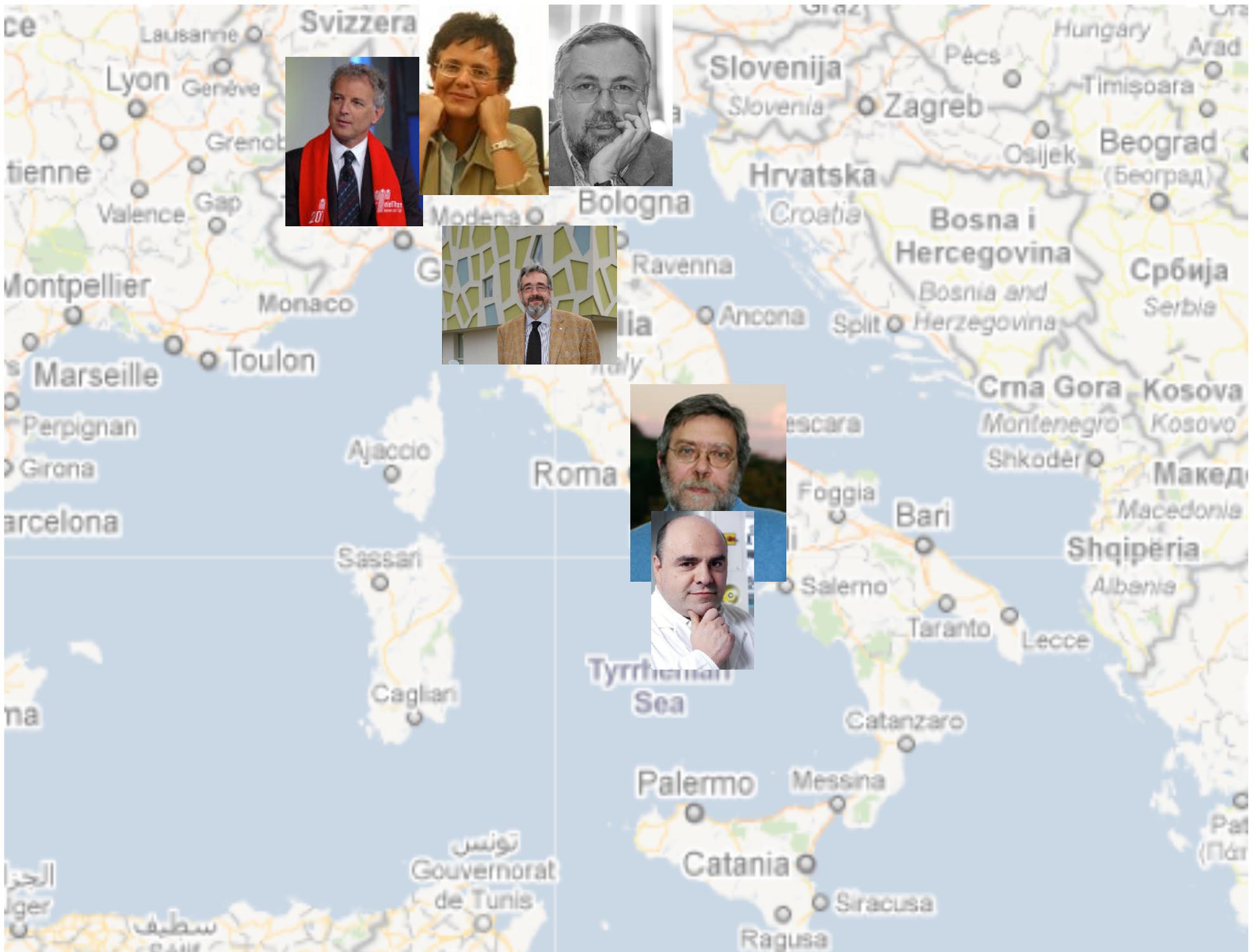


“Somewhere, something incredible is waiting to be known.”

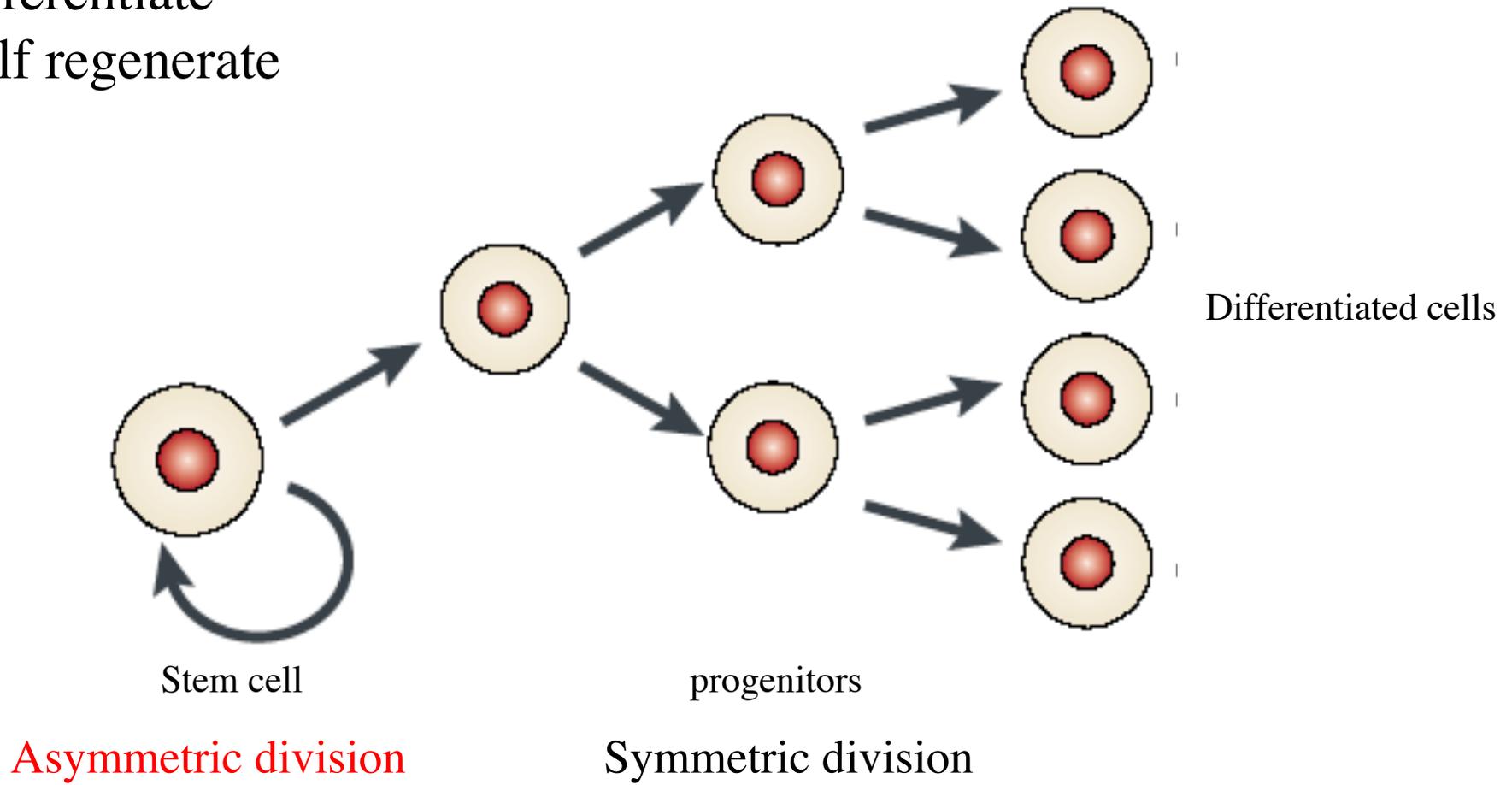
Carl Sagan

Stem cells and gene therapy



Stem cells properties

- 1 Differentiate
- 2. Self regenerate



History

~1900 self regenerating cell

1961 hemopoietic stem cell

1968 skeletal stem cells

1983 mouse ES

1997 Dolly the sheep (nuclear transfer)

1998 human ES

1999 adult stem cell

2006 IPS

Categories

embryonic



pluripotent

ES

EG (germ)

EC (carcinoma)

post-natal



multipotent

unipotent

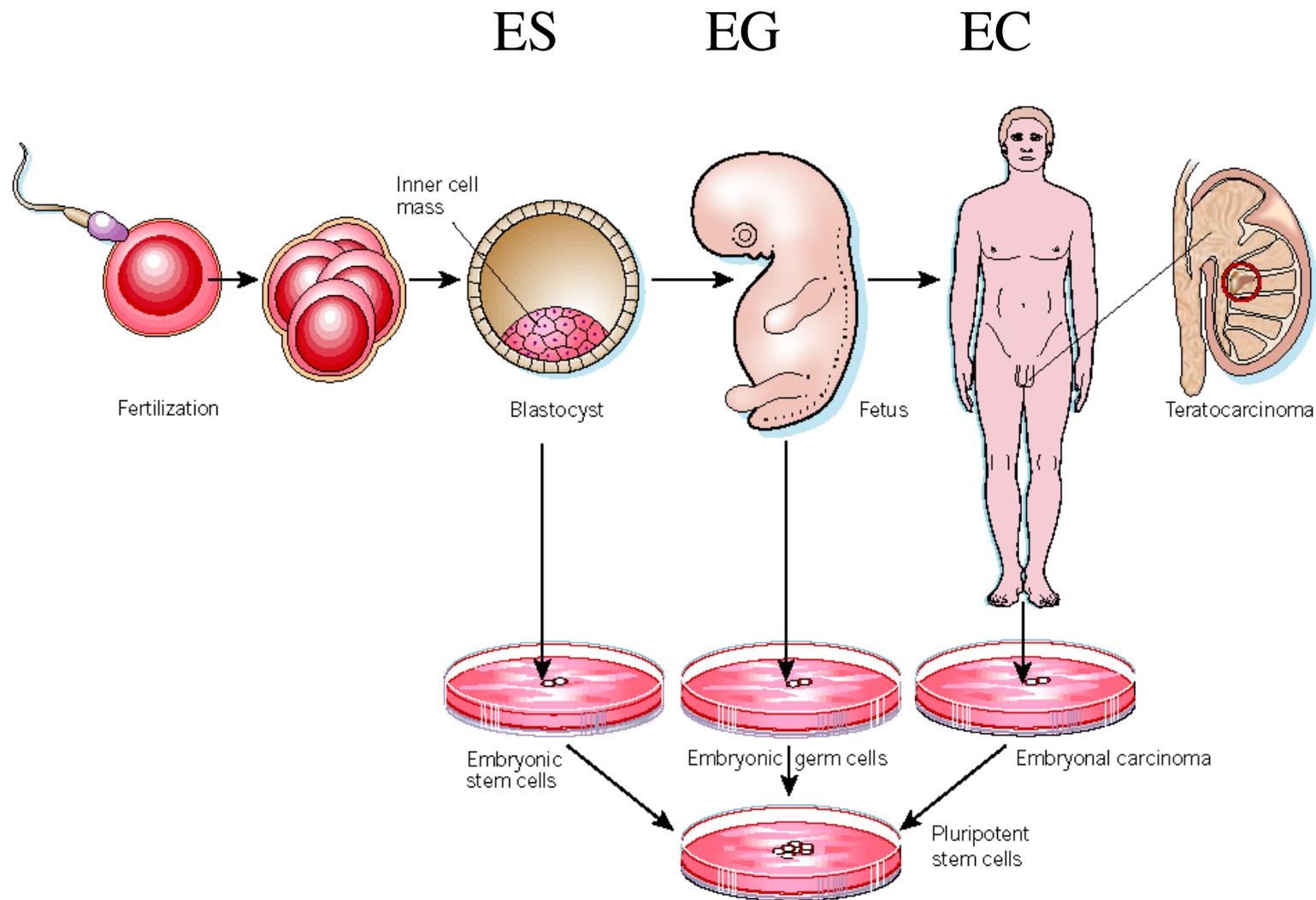
hemopoietic

epithelial

skeletal

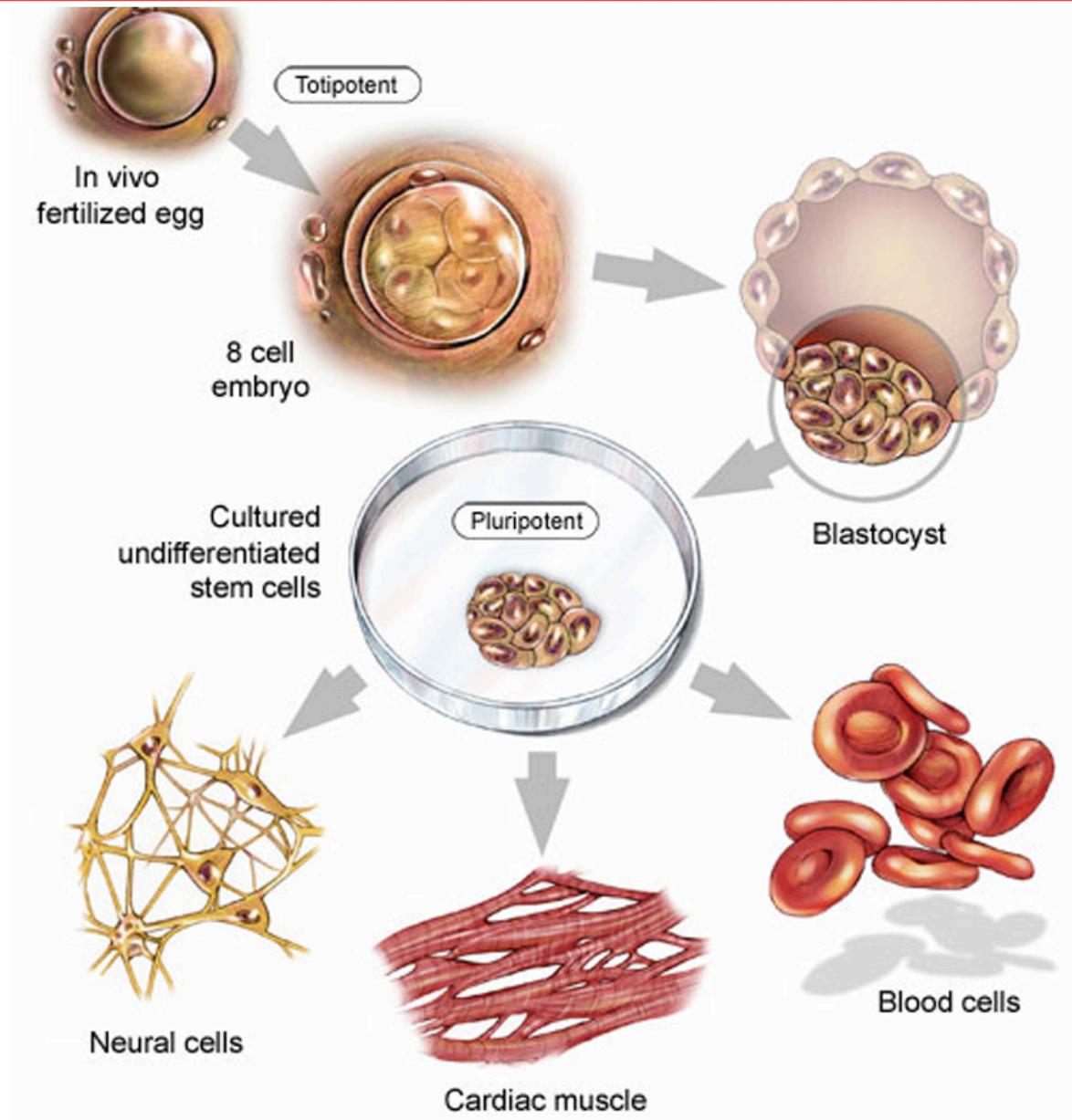
.....

ES origin

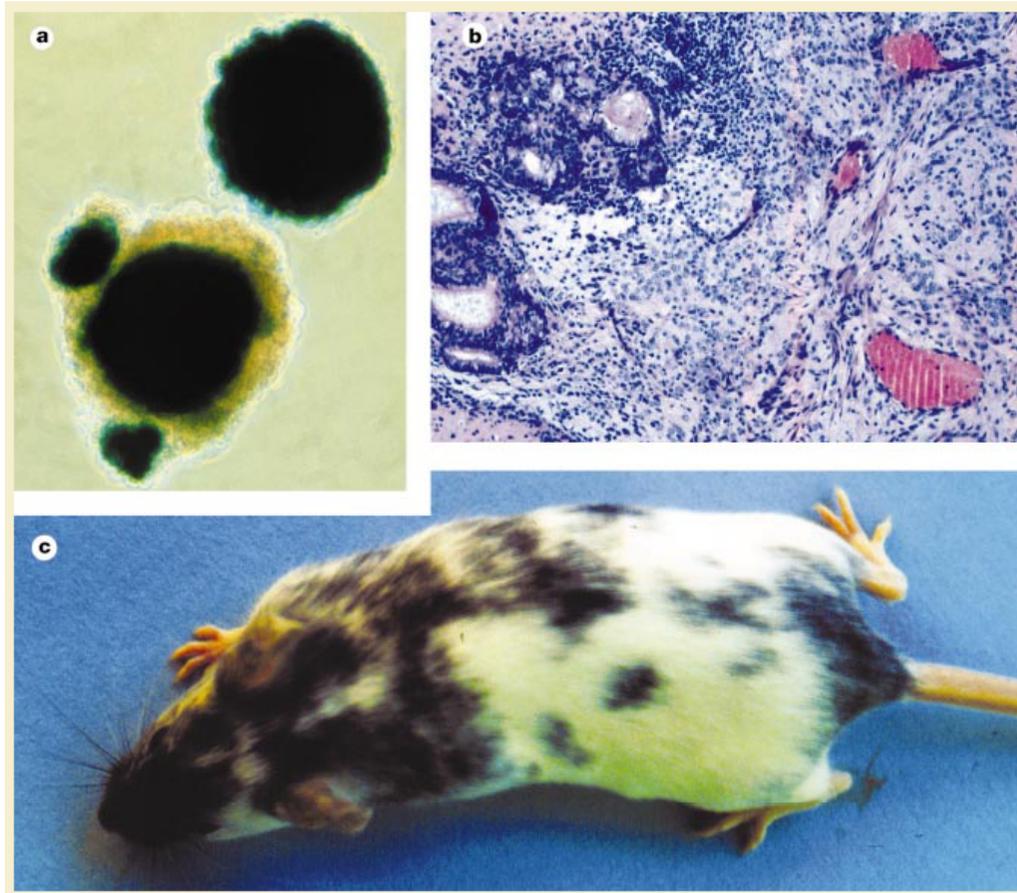


Teratocarcinoma: germ cell tumor

ES: in vitro differentiation



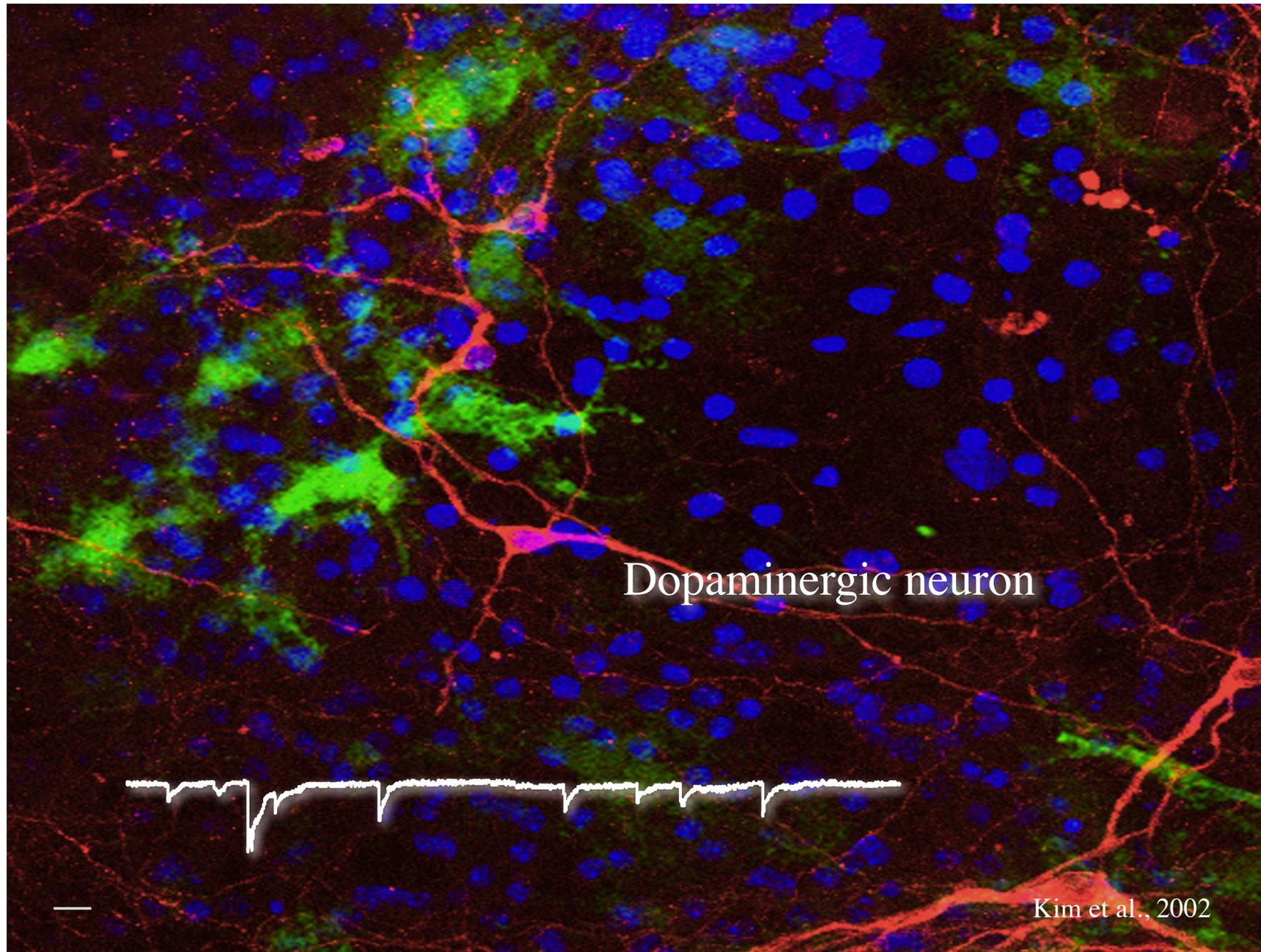
ES: in vivo differentiation



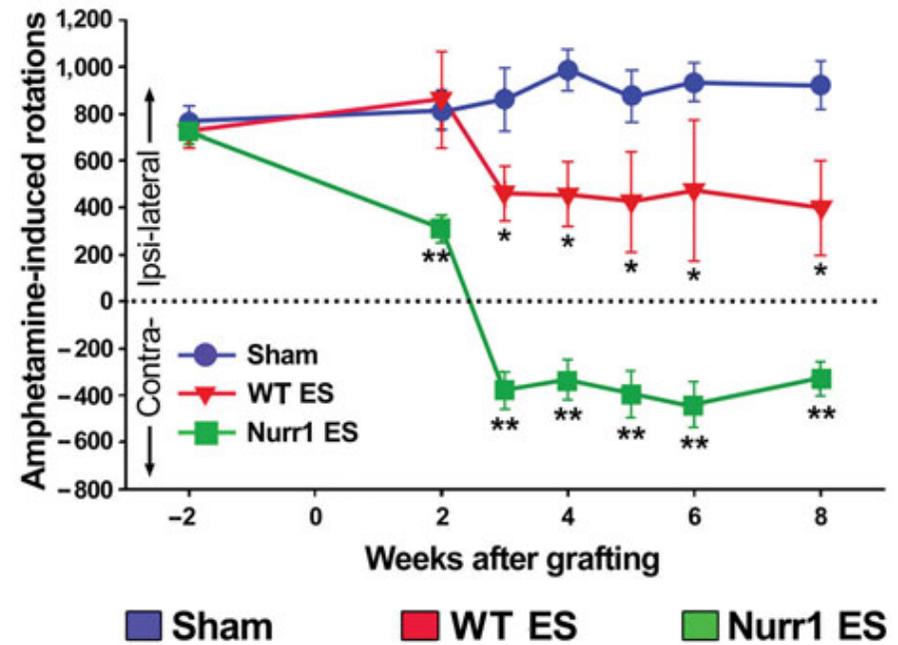
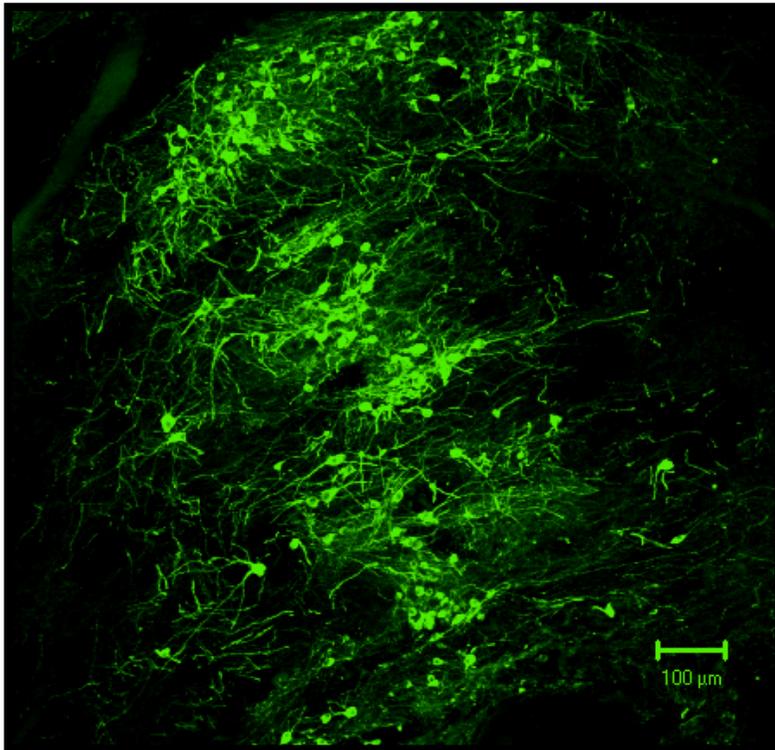
teratoma
(in mouse after
transplantation)

chimera
(implanted in the blastocyst)

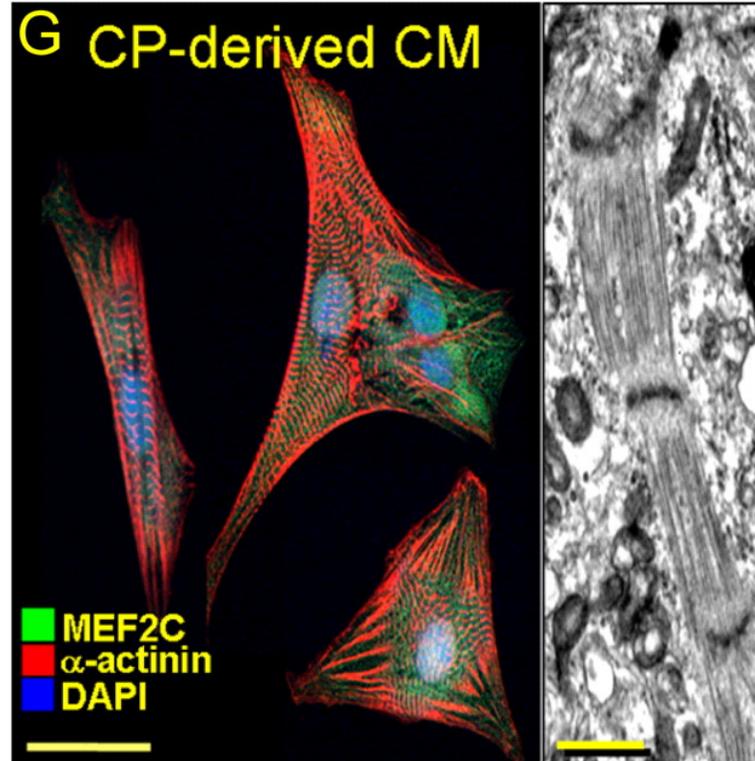
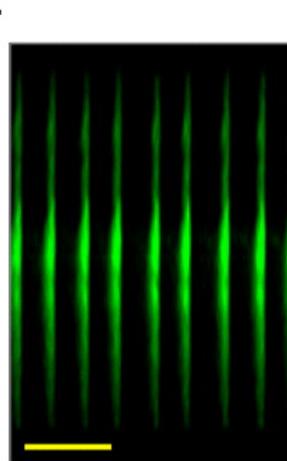
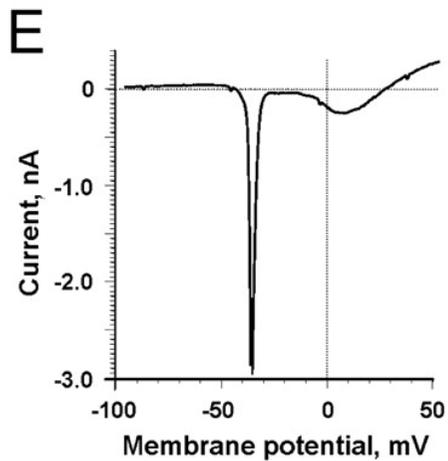
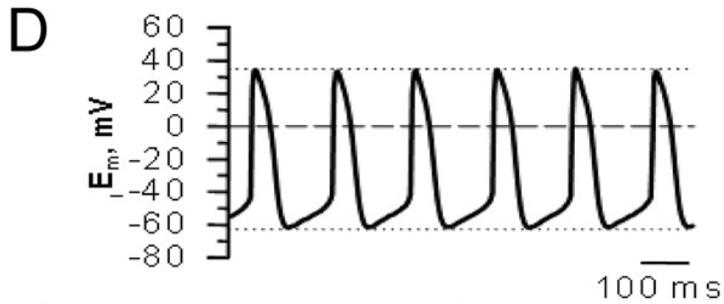
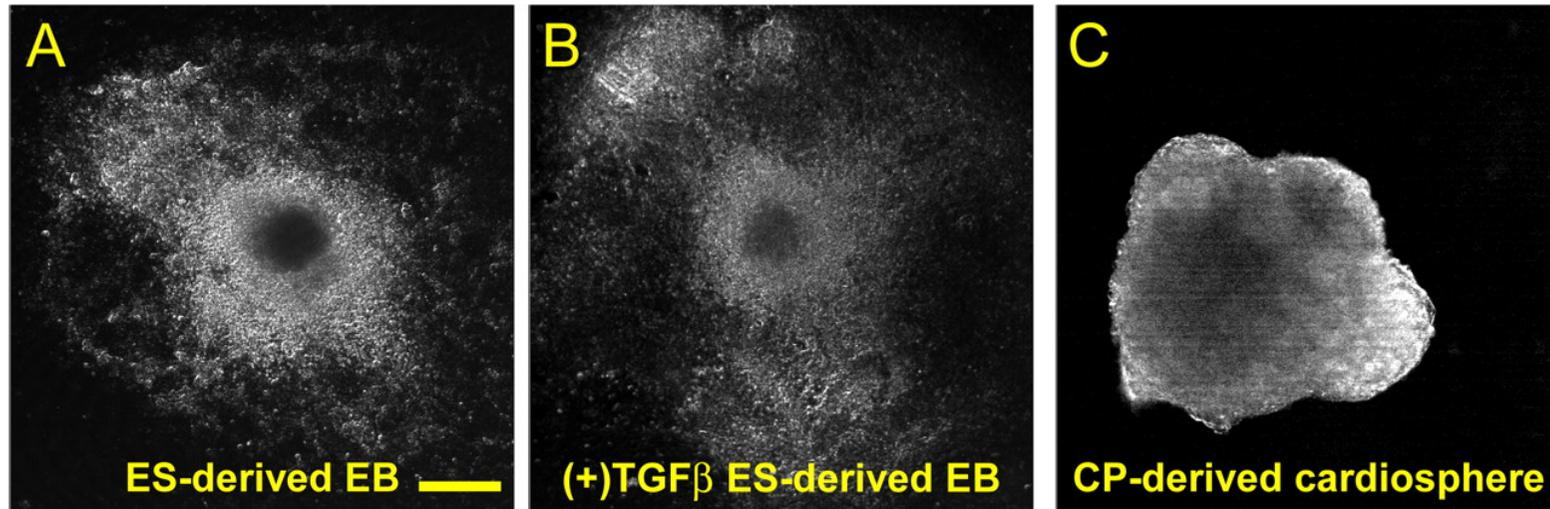
Dopaminergic neurons from mouse ES differentiated in vitro



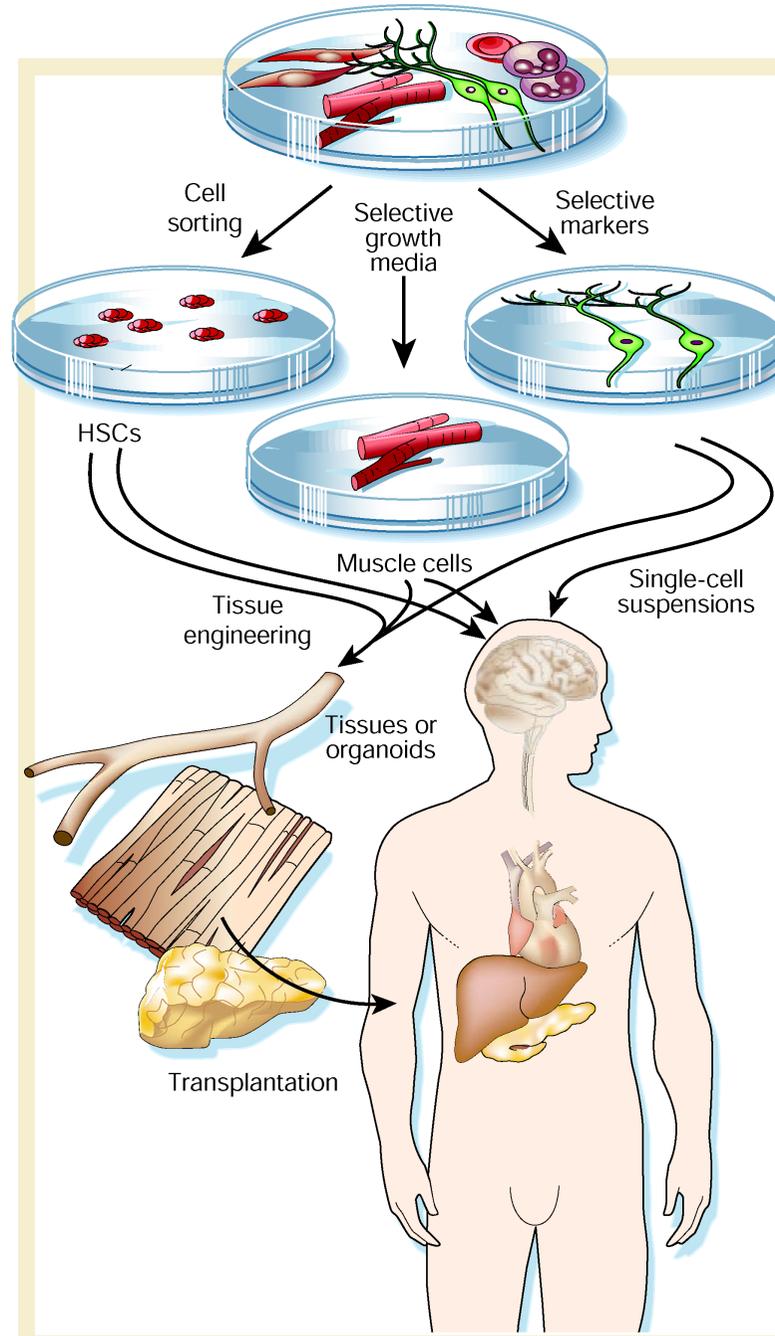
In vivo transplantation



Cardiac cells from mouse ES



ES: in therapy



ES: problems

A) Technical

manipulation

histocompatibility

.....

B) Bioethics

religion

laws

.....

Stem cells from human embryos

(Europe: 100.000 spare frozen embryos from FIVET)

Germany illegal

Great Britain legal

Italy illegal

Israel legal

USA Bush: illegal (cells derived before 9.08.2001)

Obama: legal

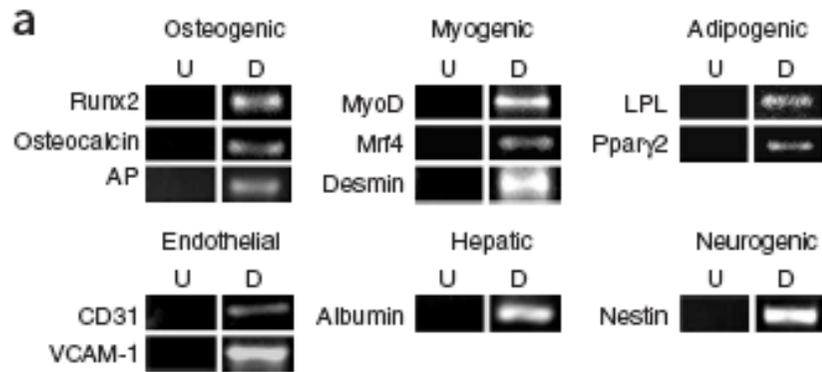
(Executive Order 13505 - Removing Barriers to Responsible Scientific

Research Involving Human Stem Cells - March 9, 2009)

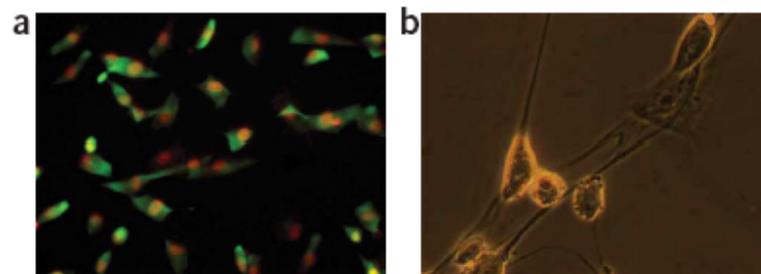
Napolitano Cattaneo 2013



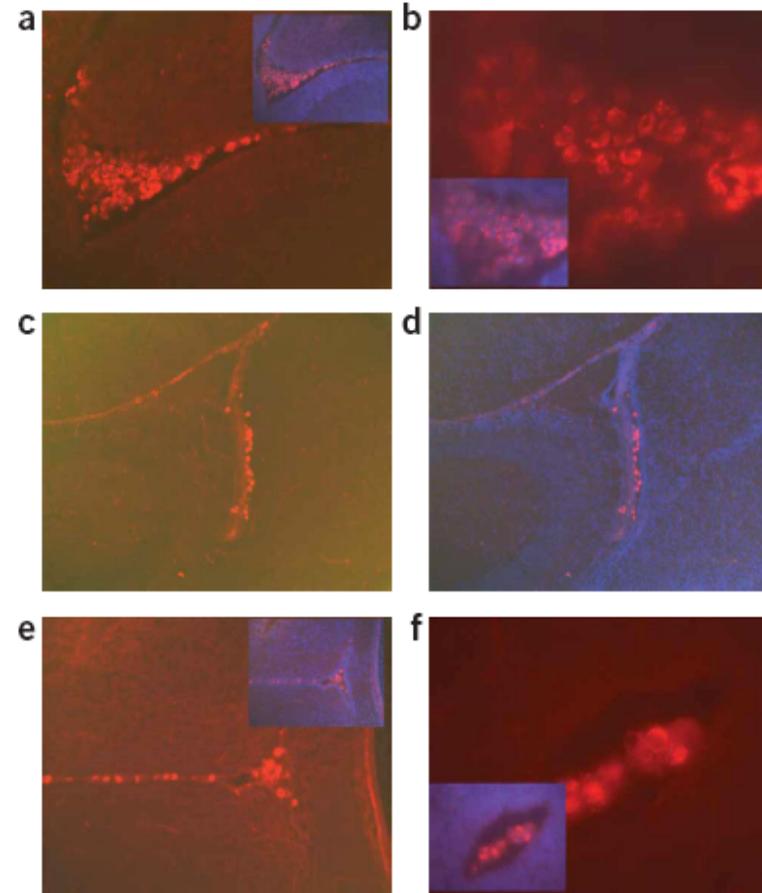
Amniotic fluid stem cells - *life in plastic is fantastic*



RT PCR for mRNA lineage specific

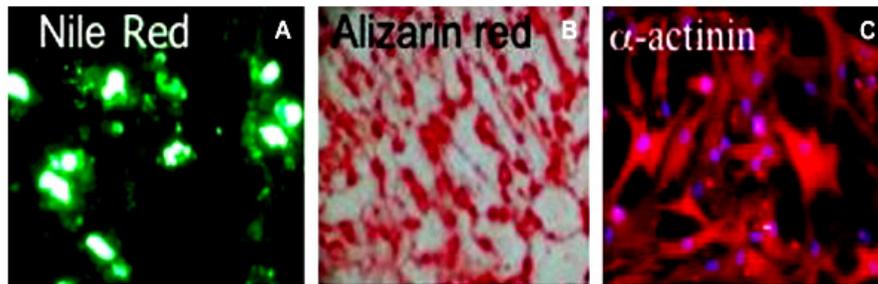


Neuron markers (nestin)



Mouse brain sections after implants of AFC: red hum mitoc

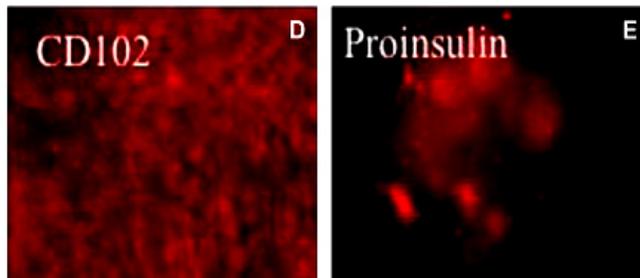
Umbilical cord blood cells (UCB)



Adipocytes

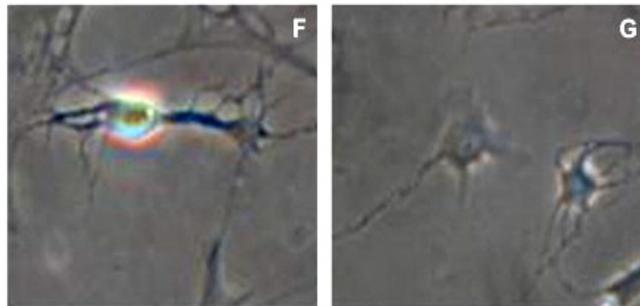
Osteoblasts

Myocytes



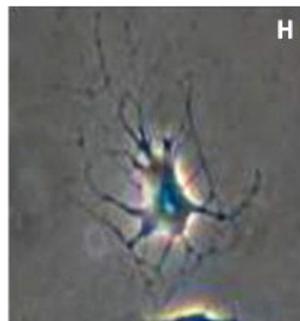
Endothelium

Hepato-pancreatic body



Neurons

Astrocytes



- Hemopoietic cells
- Endothelial progenitors
- Mesenchymal progenitors
- Stem cells pluri/multipotent

Advantages:

- amount
- immunological “youth”
- banking

Post natal stem cells

Limited proliferation

Limited pluripotency

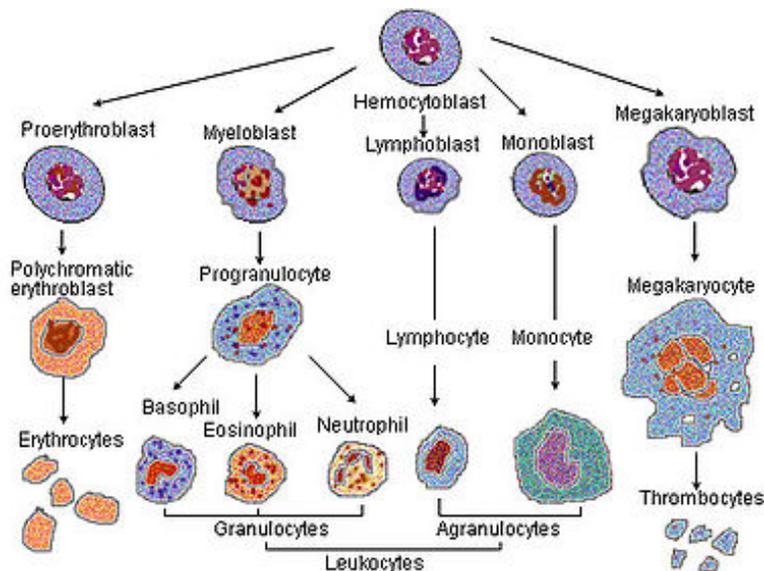
But.....

Best characterized adult stem cells: hematopoietic stem cells (HSC)- (Spangrude 1988)

- Short term hematopoietic stem cells: 2 months
- Long term hematopoietic stem cells: greater than 6 months
- CD34+, enrichable on the basis of membrane markers 10000 -fold, 80% purity (and also marker negative selection)

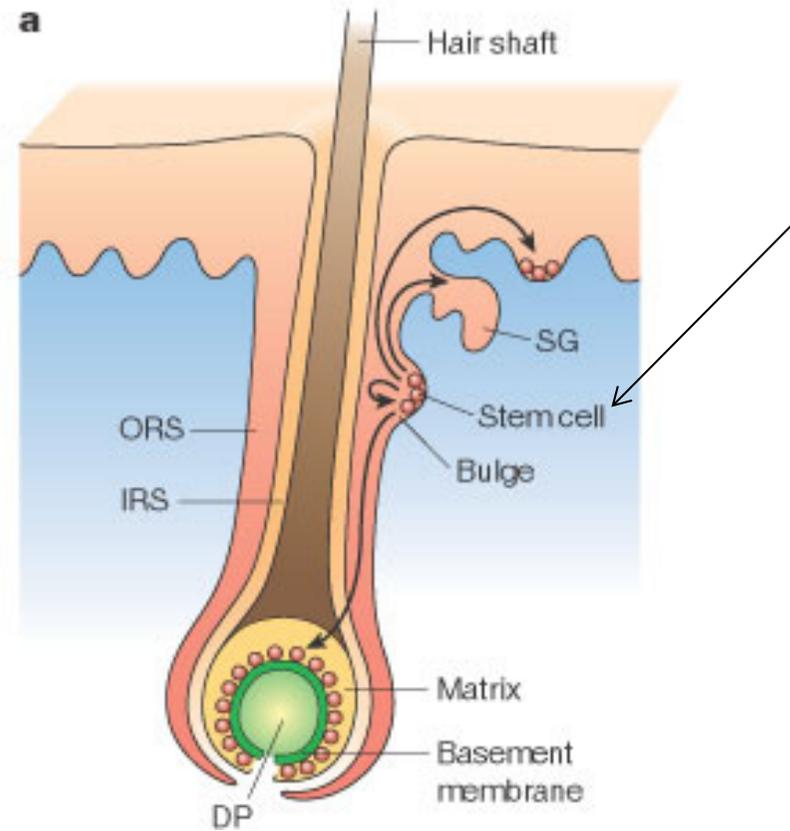
- **Mouse HSC** : CD34^{lo/-}, SCA-1⁺, Thy1.1^{+/lo}, CD38⁺, C-kit⁺, lin⁻
- **Human HSC** : CD34⁺, CD59⁺, Thy1/CD90⁺, CD38^{lo/-}, C-kit/CD117⁺, lin⁻

- Can reconstitute **blood**



Stem cells for skin

- Hair follicles keratinocytes
- expressing keratin K5 and K14



Stem cells for skin



Michele De Luca

Hepatic stem cells

- Liver is an organ capable of extensive regeneration

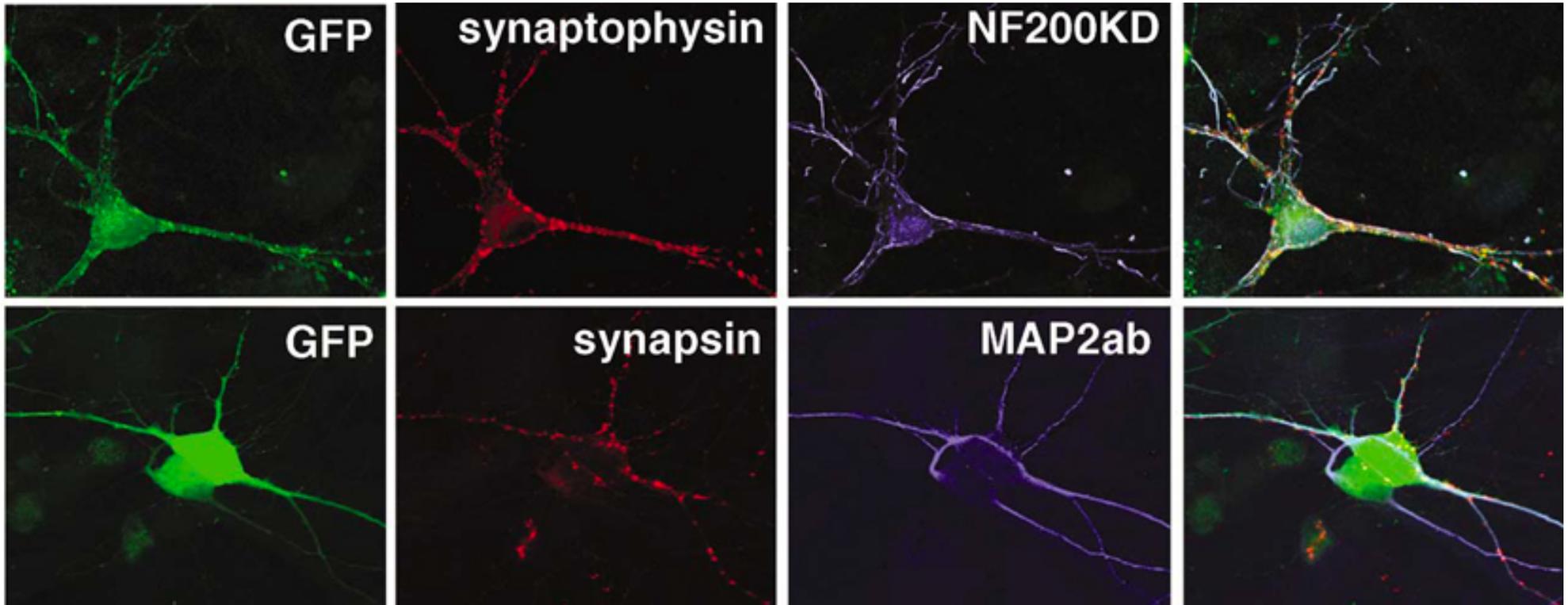
But

- The precise source of stem cells remains unclear (terminal bile ductules ?)

Neural stem cells

- Old studies in rats and songbirds (1969)
- More recent studies in mammals: neuronal progenitors exist, are capable of extensive cell division and self renewal
- Can be obtained by differential sedimentation on a gradient
- Available markers allow only 45 fold enrichment
- Neural progenitors can migrate and home to specific sites of damage or regeneration

Post natal neural cells



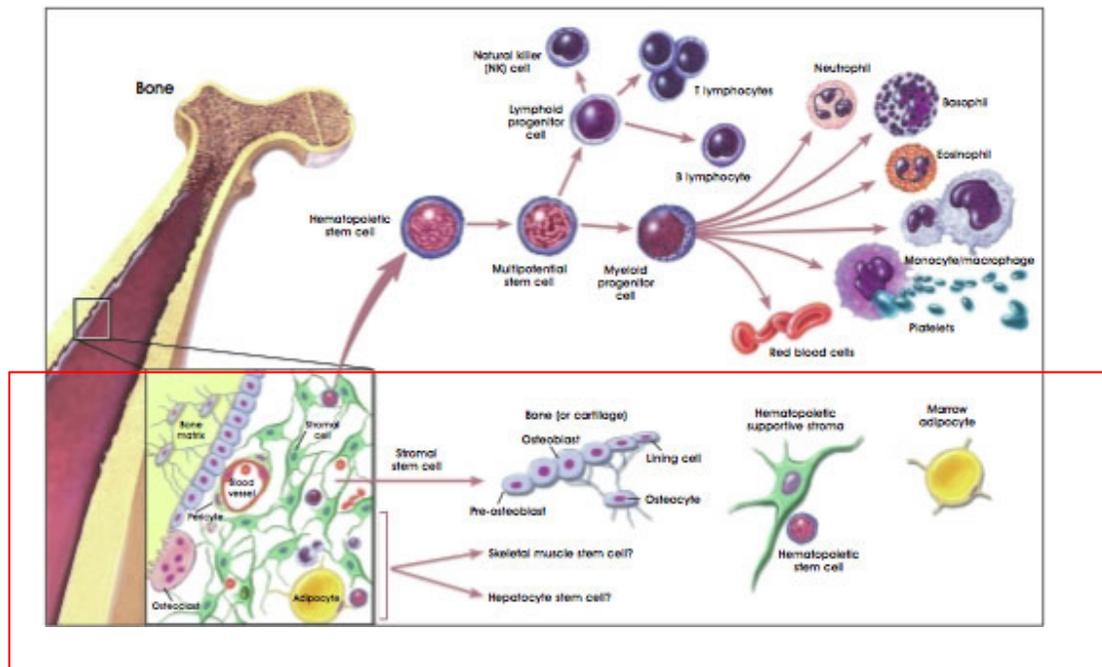
Song et al, 2002

Skeletal muscle stem cells

- Satellite cell: mononucleated cell ensheathed under the basal lamina that surrounds multinucleated muscle fibers (1961)
- Can be activated, induced to proliferate, and contribute to intact skeletal muscle fibers even after extensive tissue doublings
- Heterogeneous, no specific markers
- Are rapidly depleted in muscle of Duchenne patients

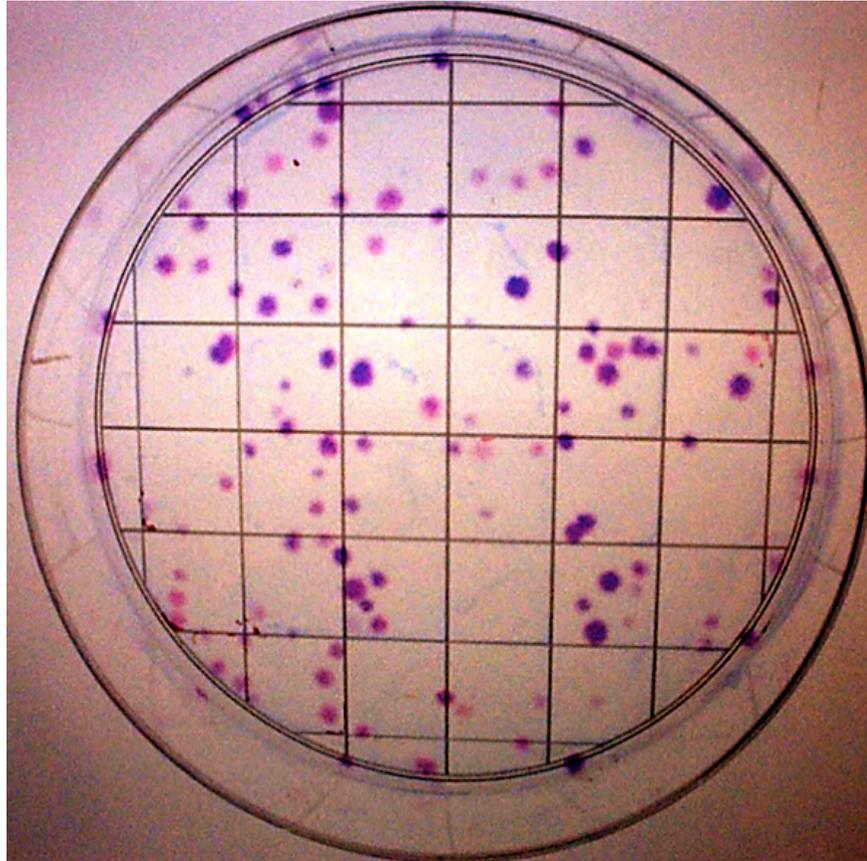
Mesenchymal stem cells

- Bone marrow-derived (non circulating fraction)
- Isolated on the basis of their adhesive properties
- Remarkable plasticity (chondrocytes, osteoblasts, adipocytes, cardiac and skeletal muscle cells, neurons, astrocytes)



MSC properties

Alexander
Friedenstein



In bone marrow (“fibroblasts”)
Can be isolated and amplified ex vivo
transplantable
multipotent

MSC/skeletal stem cells, transplant

