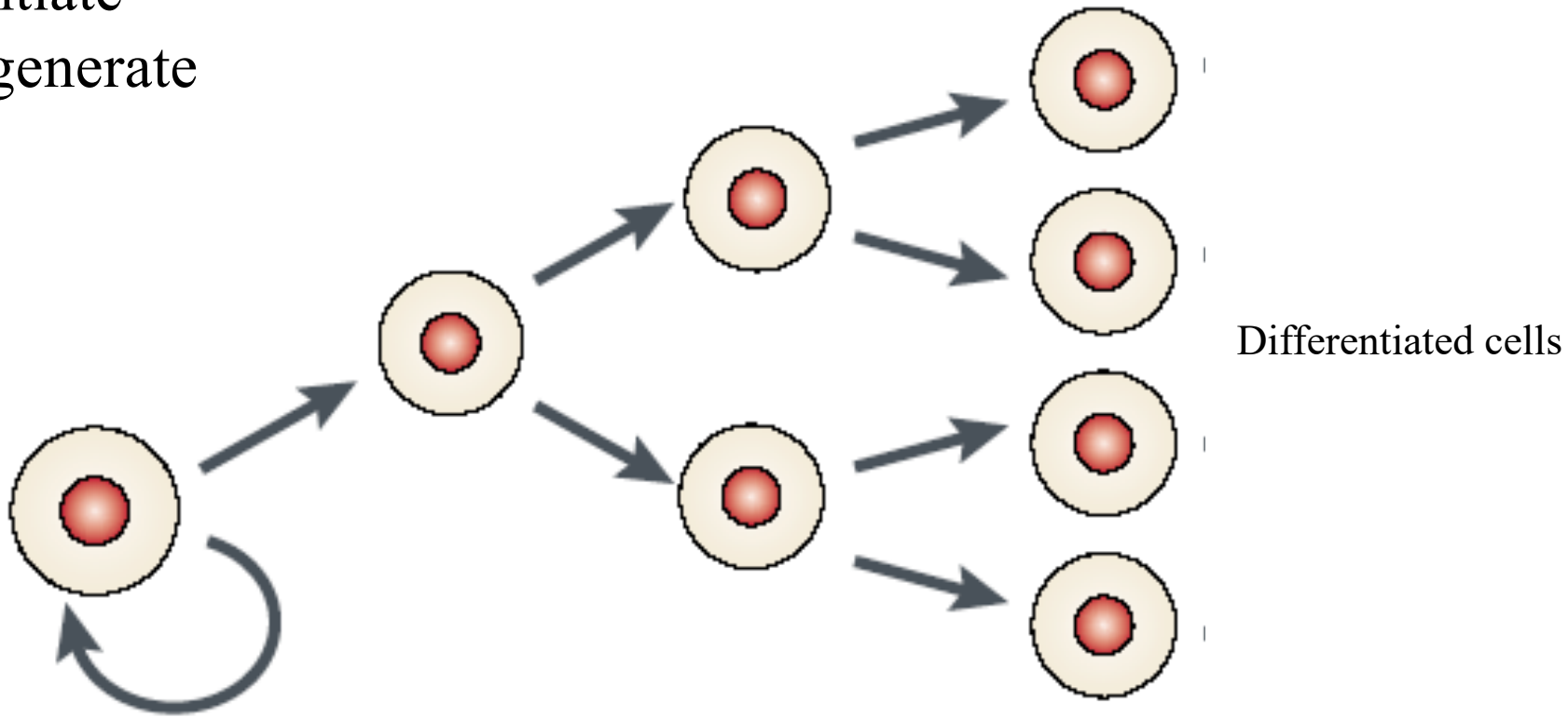
“open up your mind let your fantasies unwind.”

QUESTIONS?


Svizzera
Lausanne
Lyon
Genève
Grenob
tienne
Valence
Gap
Montpellier
Monaco
Marseille
Toulon
Perpignan
Girona
arcelona
Ajaccio
Sassari
Cagliari
Modena
Bologna
Ravenna
Ancona
Roma
Foggia
Bari
Salerno
Taranto
Lecce
Tyrrhenian Sea
Catanzaro
Palermo
Messina
Catania
Siracusa
Ragusa
Gouvernorat de Tunis
Slovenija
Slovenia
Zagreb
Hrvatska
Croatia
Hungary
Pécs
Timişoara
Arad
Osijek
Beograd
(Београд)
Bosna i Hercegovina
Bosnia and Herzegovina
Split
Србија
Serbia
Crna Gora
Montenegro
Kosova
Kosovo
Shkodër
Shqipëria
Albania
Macedonia

Stem cells properties

1 Differentiate
2. Self regenerate



Asymmetric division

Symmetric division

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

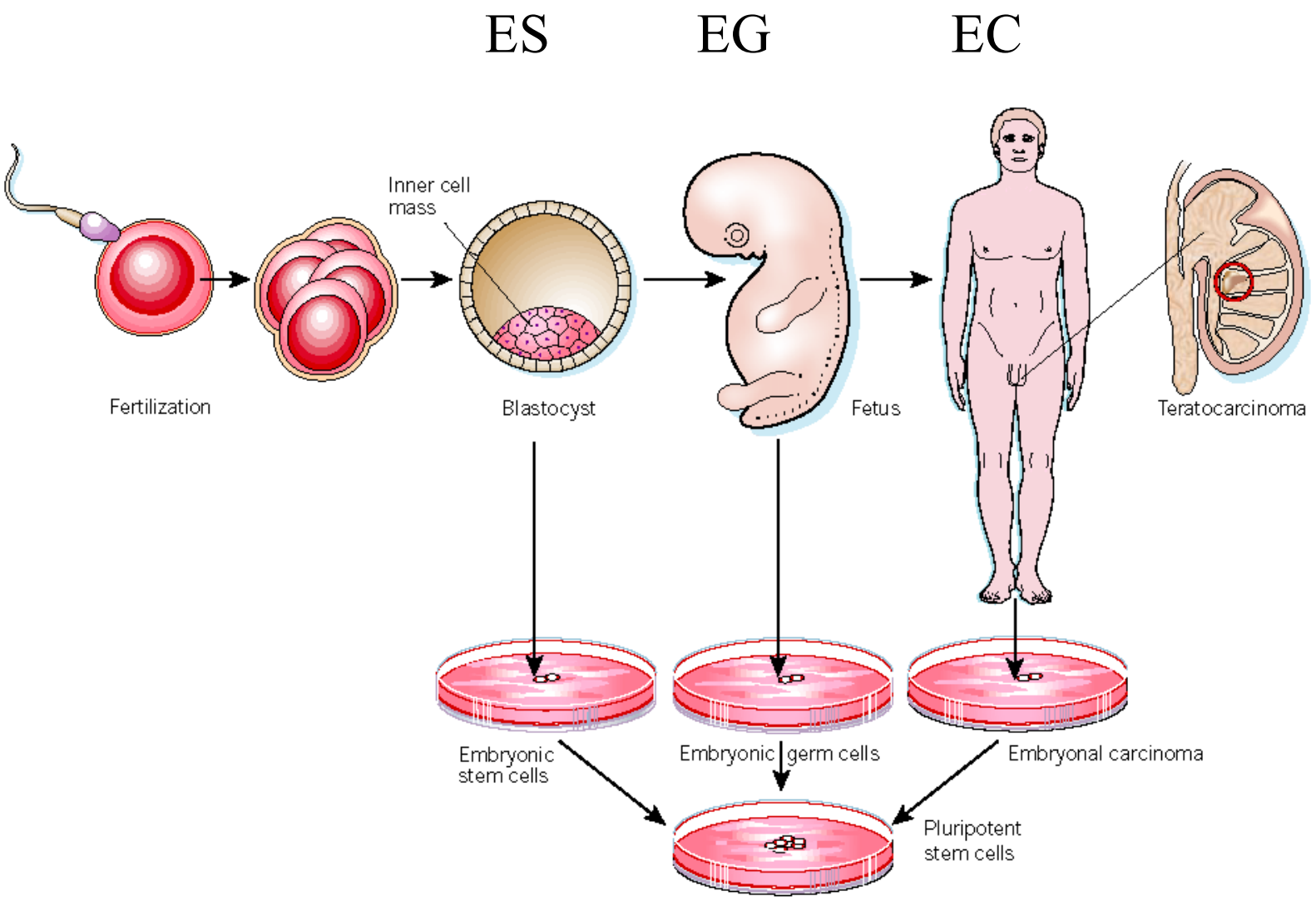
1999 adult stem cell

2006 IPS

Categories

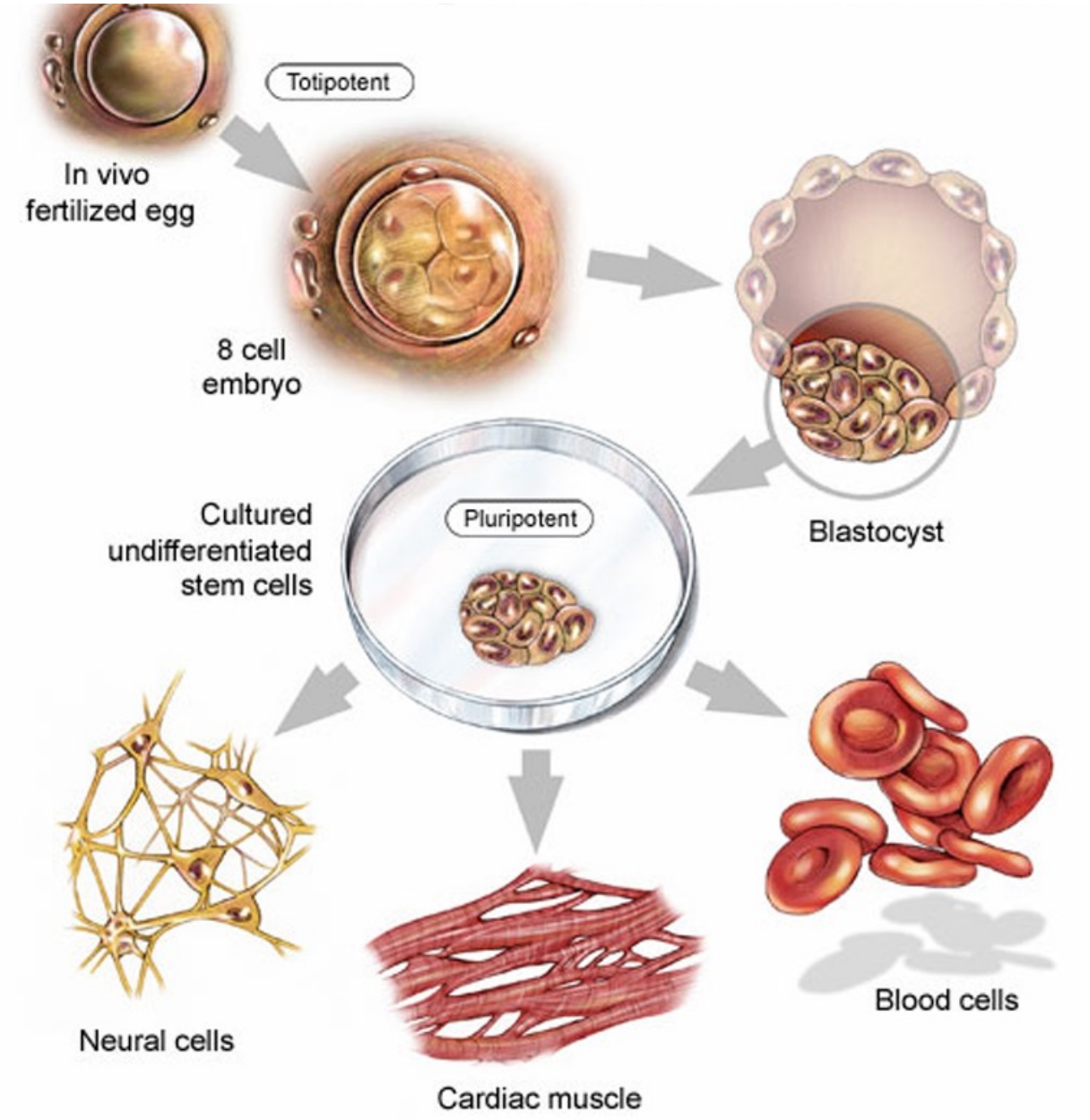
embryonic	ES
↓	EG (germ)
pluripotent	EC (carcinoma)
post-natal	hemopoietic
↓	epithelial
multipotent	skeletal
unipotent	……

ES origin

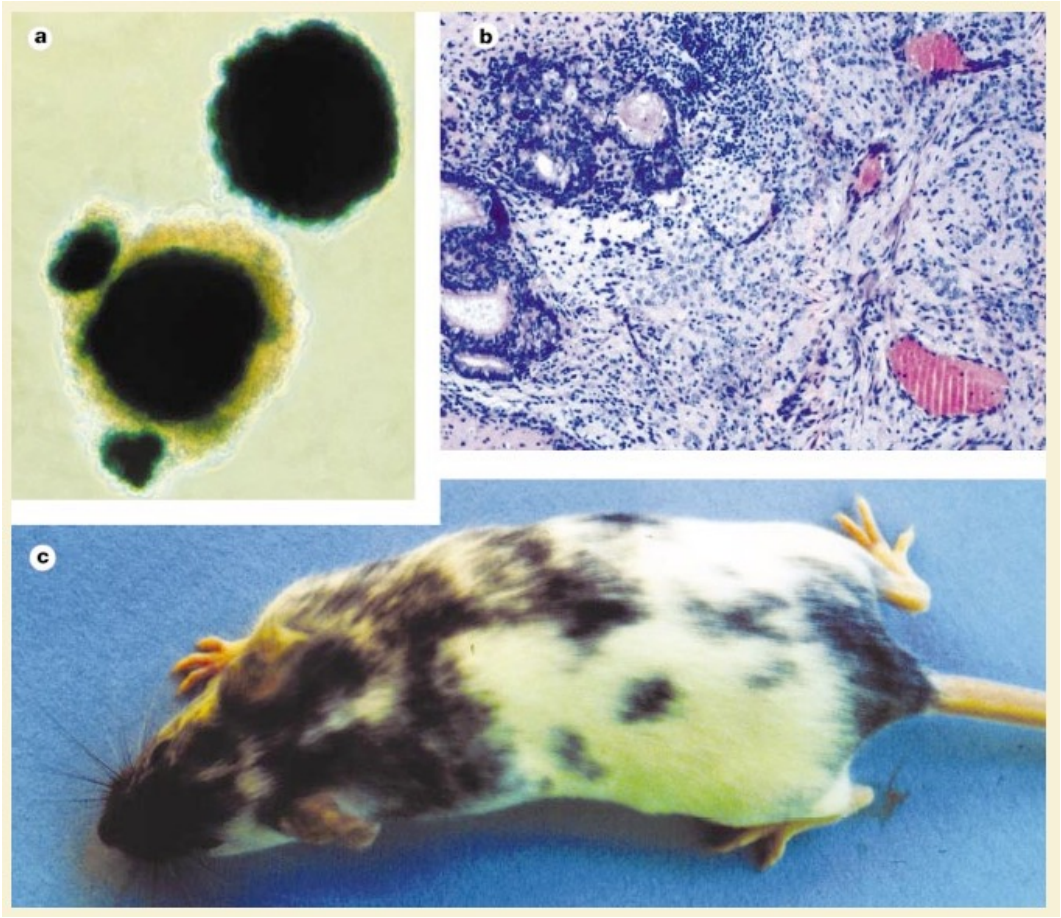


Teratocarcinoma: germ cell tumor

ES: in vitro differentiation



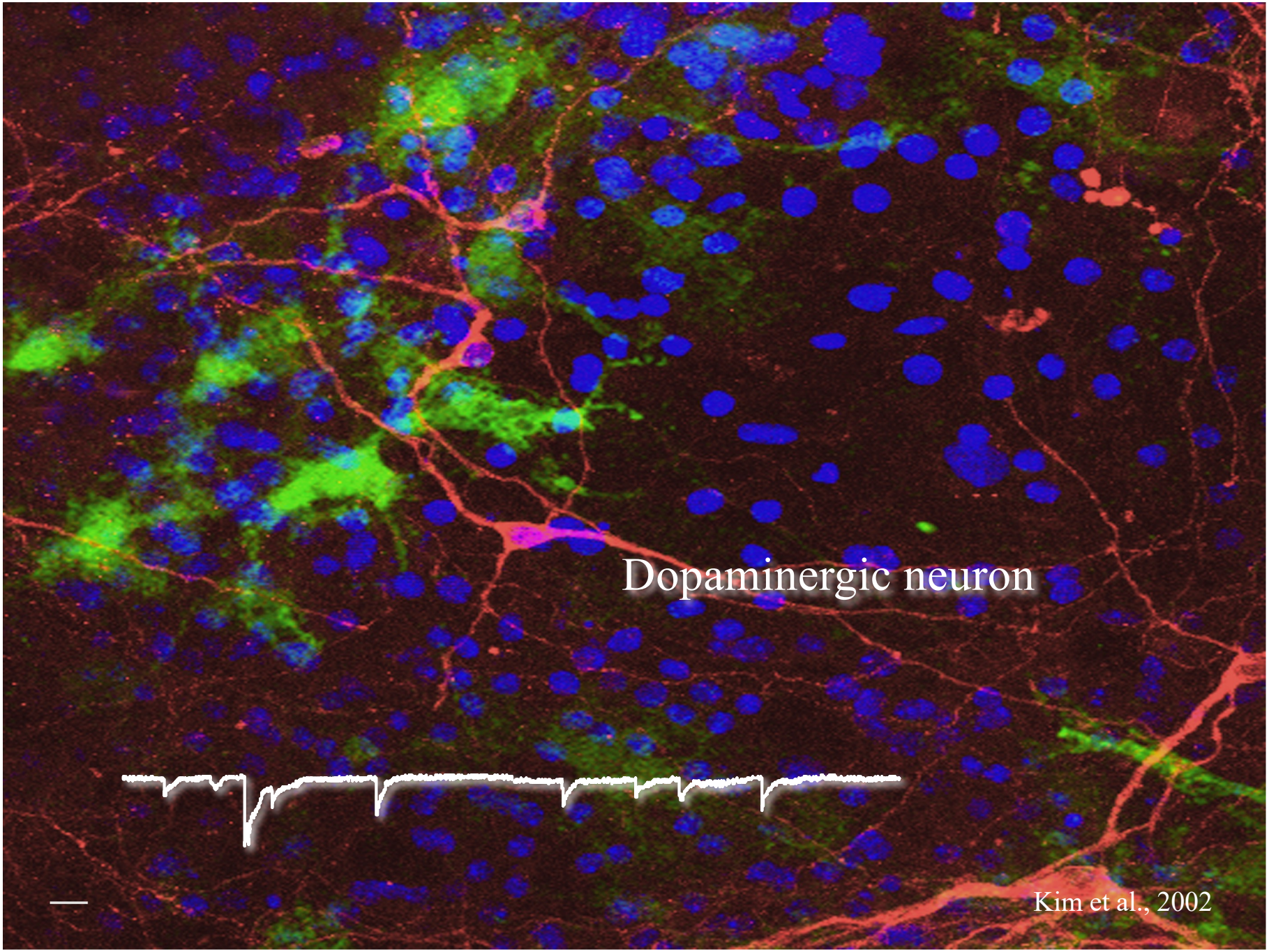
ES: in vivo differentiation



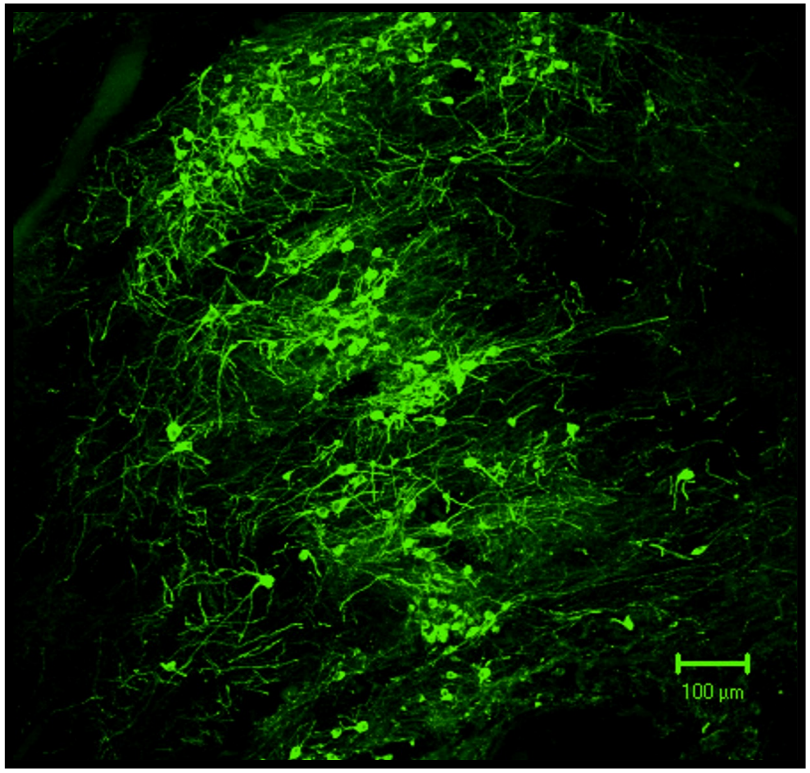
teratoma
(in mouse after transplantation)

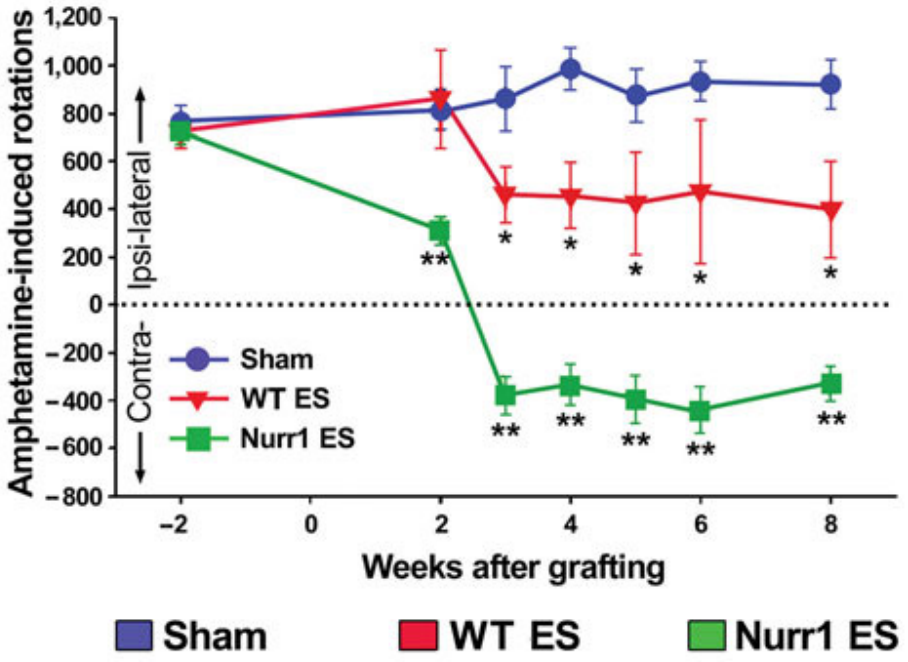
chimera
(implanted in the blastocyst)

Dopaminergic neurons from mouse ES differentiated in vitro



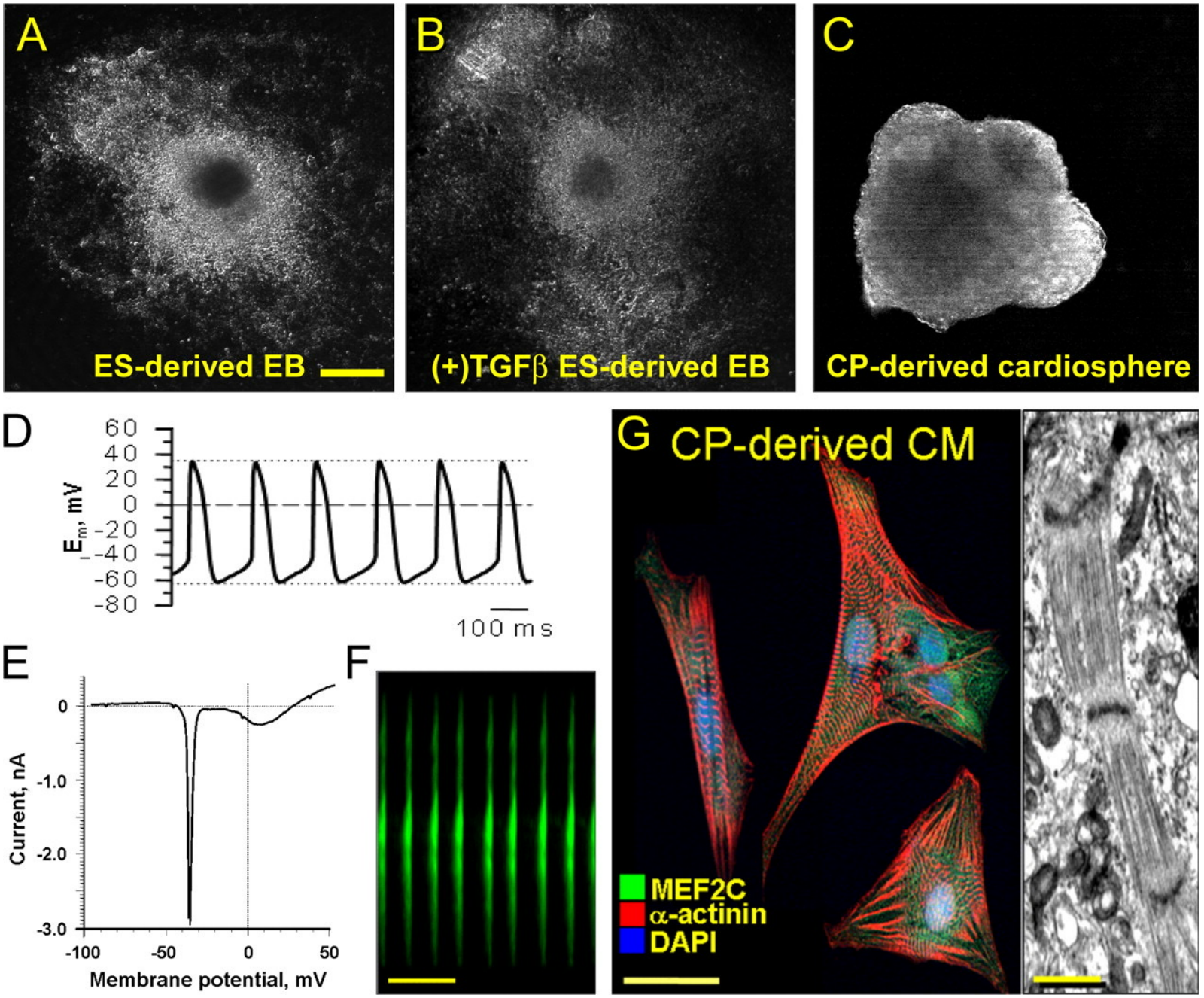
In vivo transplantation



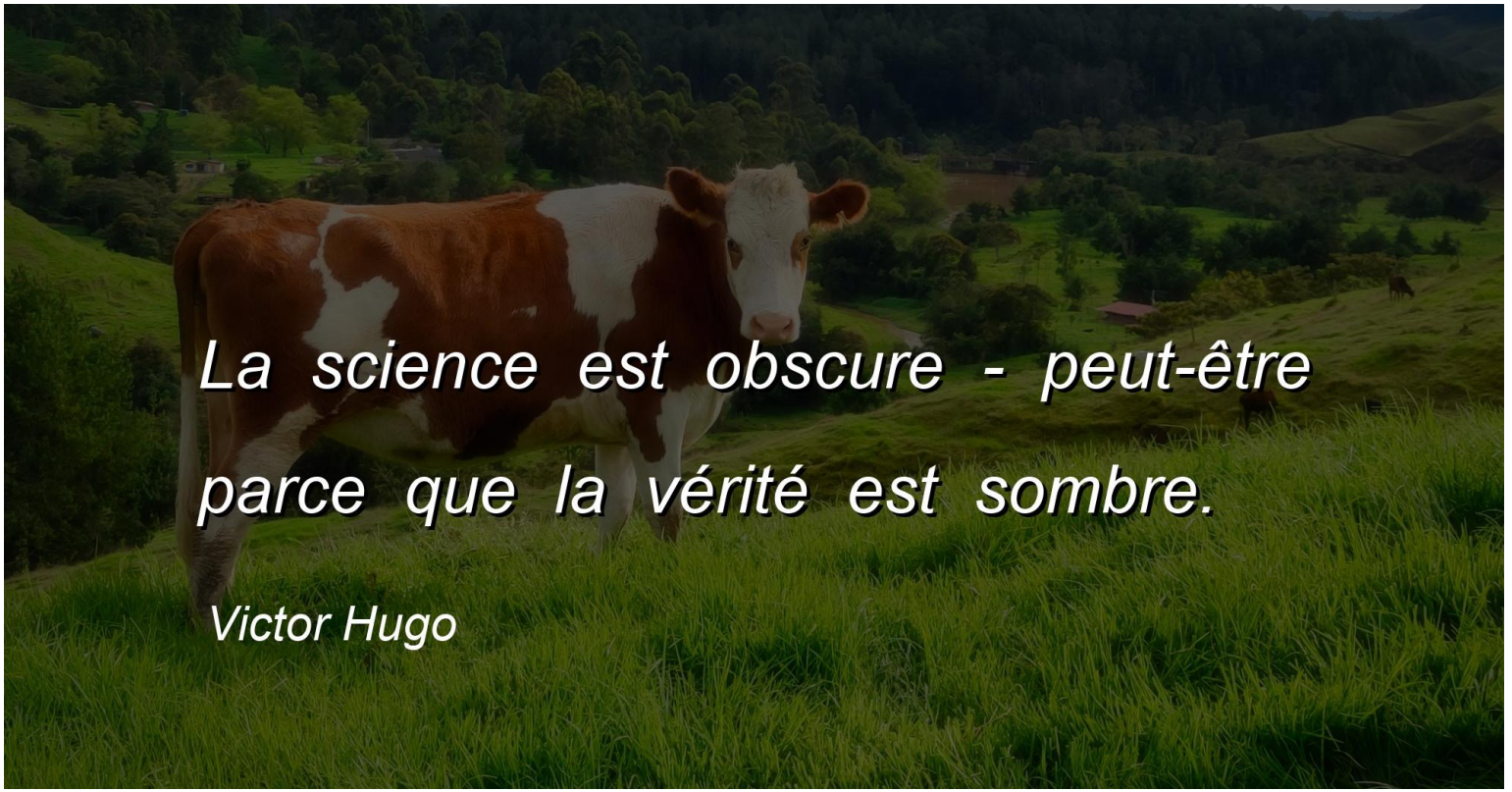


Kim et al., 2002 Nature ES murine

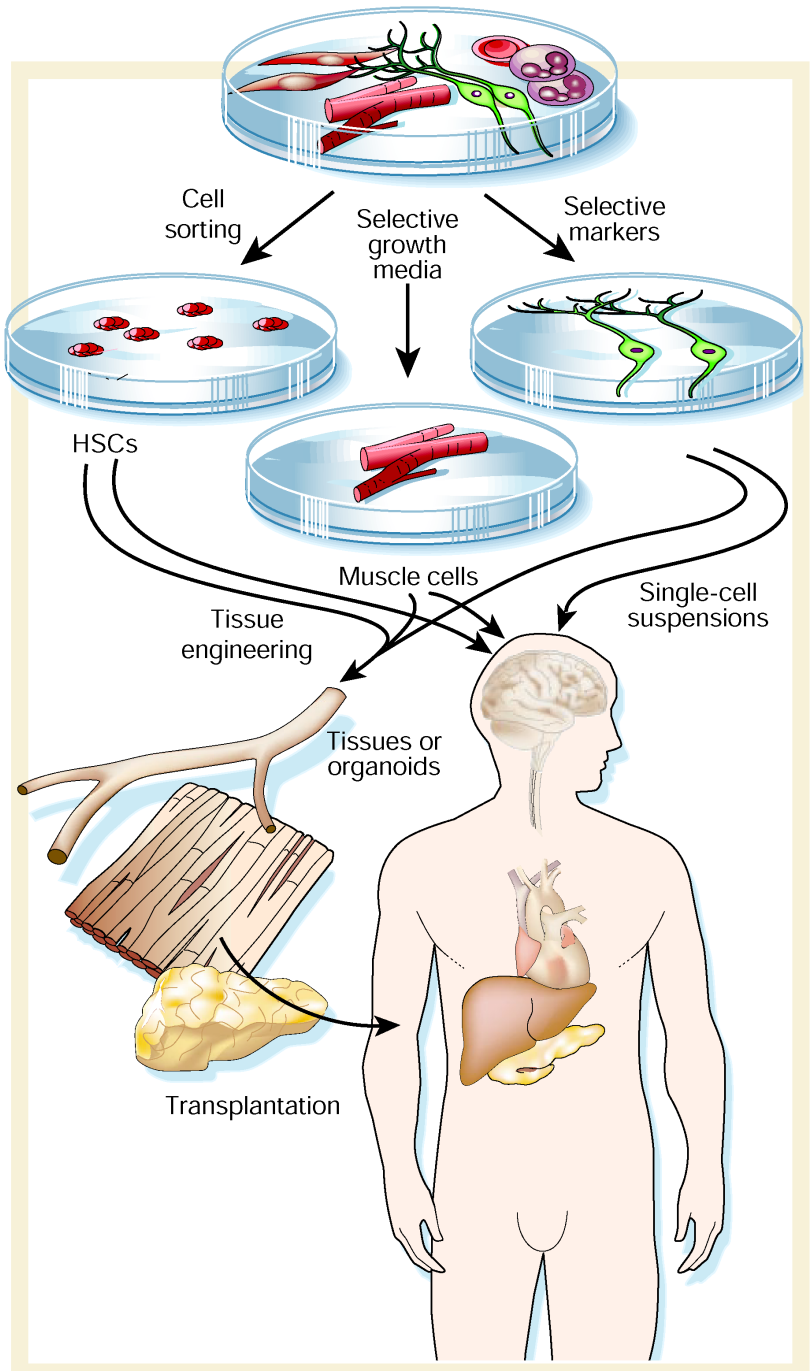
Cardiac cells from mouse ES



Behfar et al, 2007


La science est obscure - peut-être parce que la vérité est sombre.
Victor Hugo

ES: in therapy



ES: problems

A) Technical

manipulation

histocompatibility

……..

B) Bioethics

religion

laws

……..

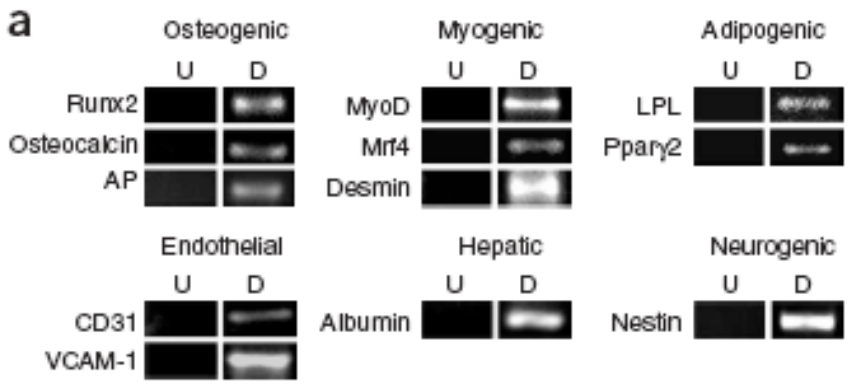
Stem cells from human embryos

(Europe: 100.000 spare frozen embryos from FIVET)

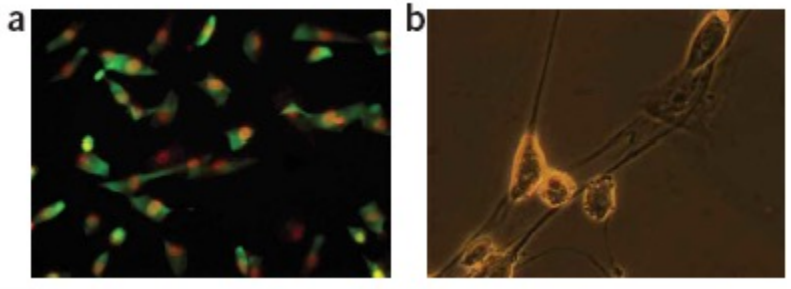
Germany	illegal
Great Britain	legal
Italy	illegal
Israel	legal
USA	Bush: illegal (cells derived before 9.08.2001)
	Obama: legal
	(Executive Order 13505 - Removing Barriers to Responsible Scientific Research Involving Human Stem Cells - March 9, 2009)

Napolitano Cattaneo 2013

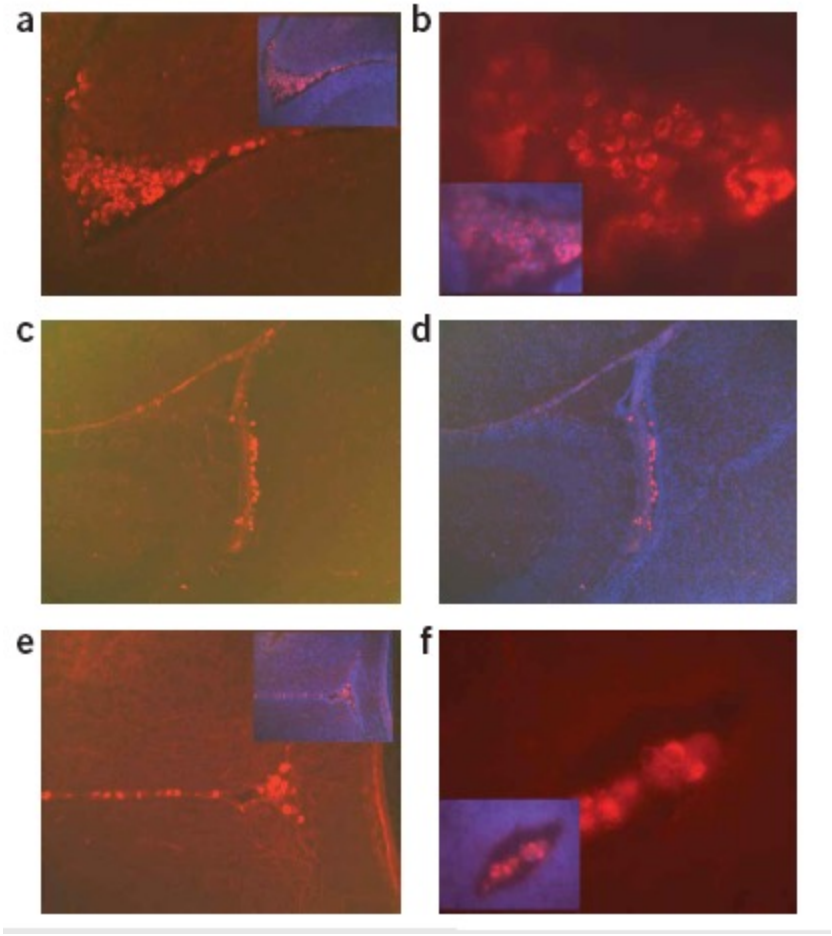
Amniotic fluid stem cells - *life in plastic is fantastic*



RT PCR for mRNA lineage specific



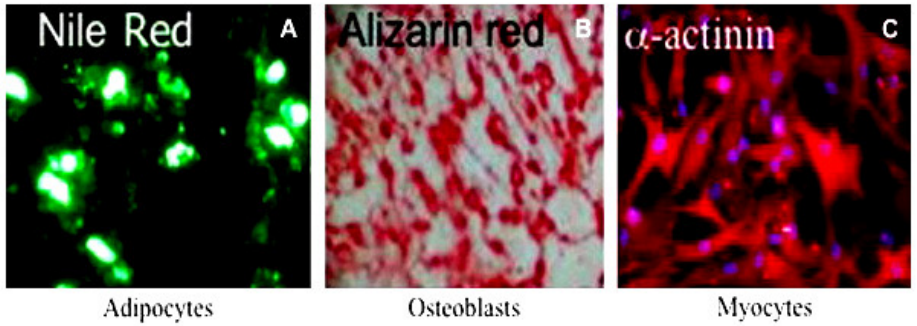
Neuron markers (nestin)



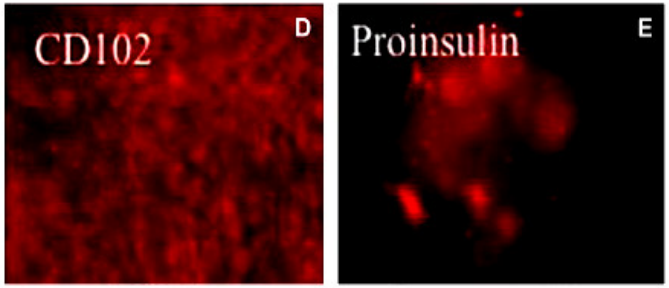
Mouse brain sections after implants of AFC: red hum mitoc

Coppi et al NBT 2007

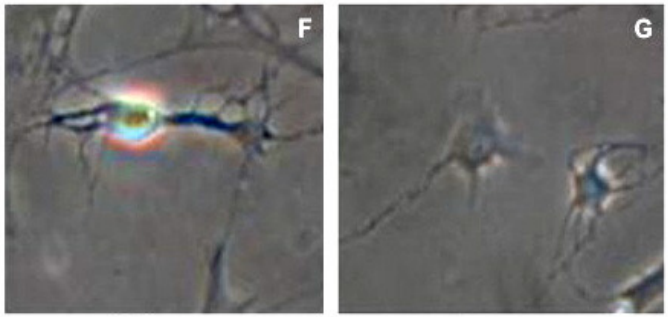
Umbilical cord blood cells (UCB)



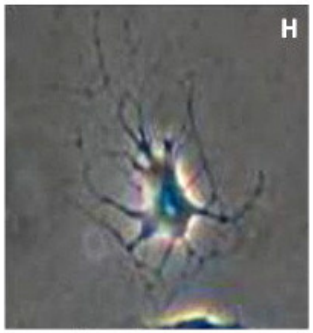
Adipocytes Osteoblasts Myocytes



Endothelium Hepato-pancreatic body



Neurons Astrocytes



- Hemopoietic cells

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

Advantages:

•amount
•immunological “youth”
•banking

Van de Ven et al 2007

Post natal stem cells

Limited proliferation

Limited pluripotency

But……

How to isolate stem cells – localization of post natal stem cells

In regenerating tissues (blood and skin)

In low regenerating tissues (bone)

In non regenerating tissues (teeth, CNS)

How to isolate stem cells

- Antigen markers
- Efflux of the DNA binding dye Hoechst 33342

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

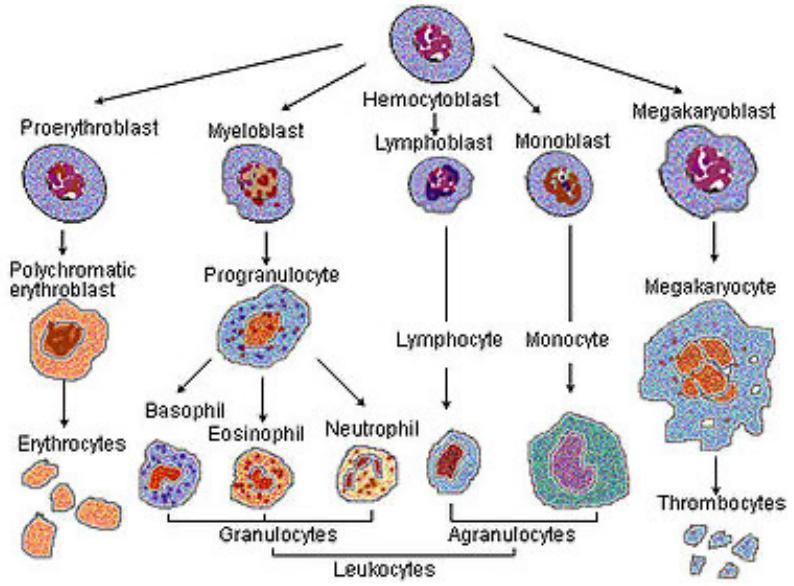
•Short term hematopoietic stem cells: 2 months

•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)

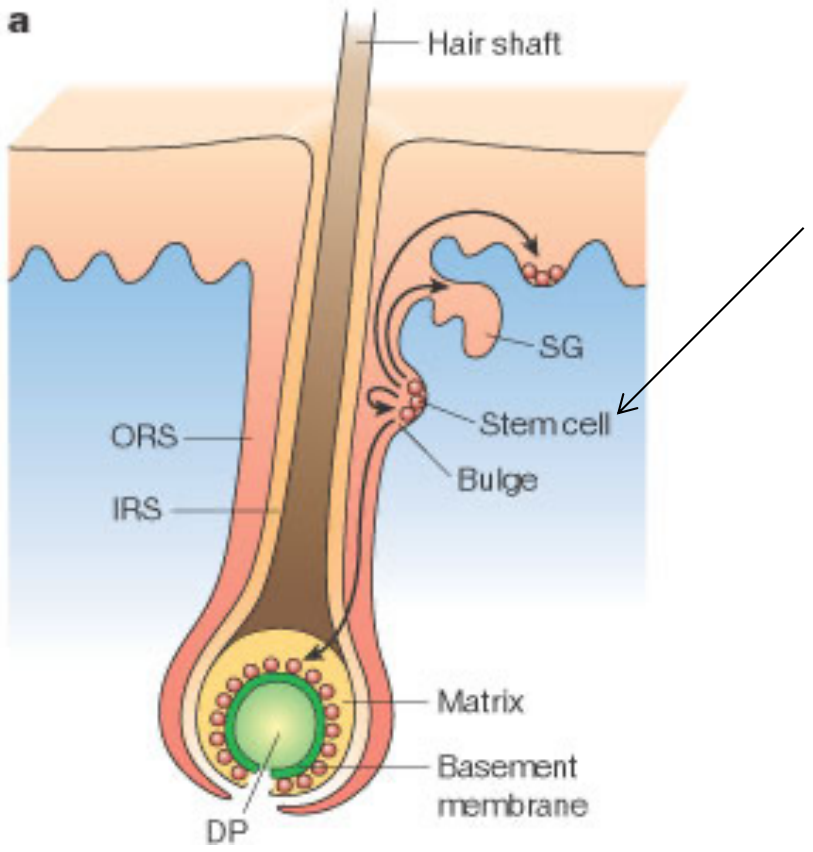
- **Mouse HSC :** $CD34^{lo/-}$, $SCA\text{-}1^{+}$, $Thy1.1^{+/lo}$, $CD38^{+}$, $C\text{-}kit^{+}$, lin^{-}
- **Human HSC :** $CD34^{+}$, $CD59^{+}$, $Thy1/CD90^{+}$, $CD38^{lo/-}$, $C\text{-}kit/CD117^{+}$, lin^{-}

•Can reconstitute **blood**



Stem cells for skin

- Hair follicles keratinocytes
- expressing keratin K5 and K14



Stem cells for skin

Michele De Luca