Un esperimento è una domanda che la scienza pone alla natura, ed una misurazione è la registrazione della risposta della Natura.
(Max Planck)

Hepatic stem cells

- Liver is an organ capable of extensive regeneration

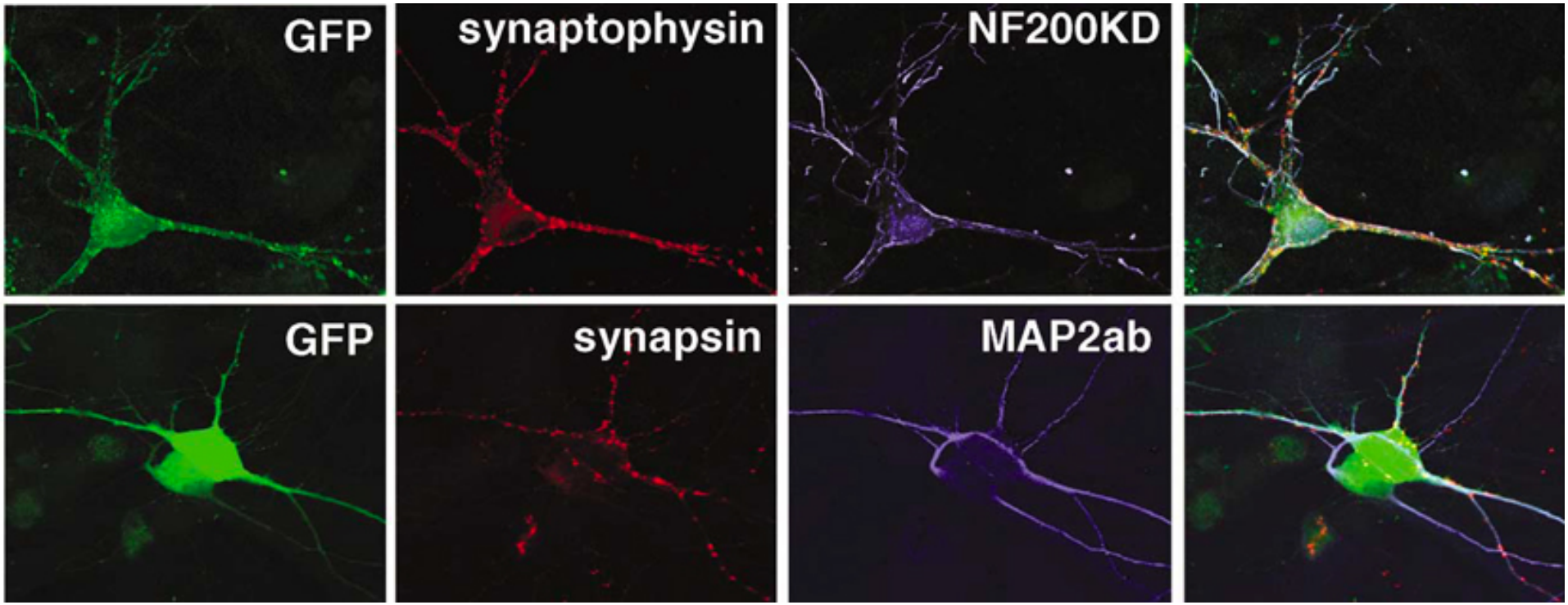
But

- 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

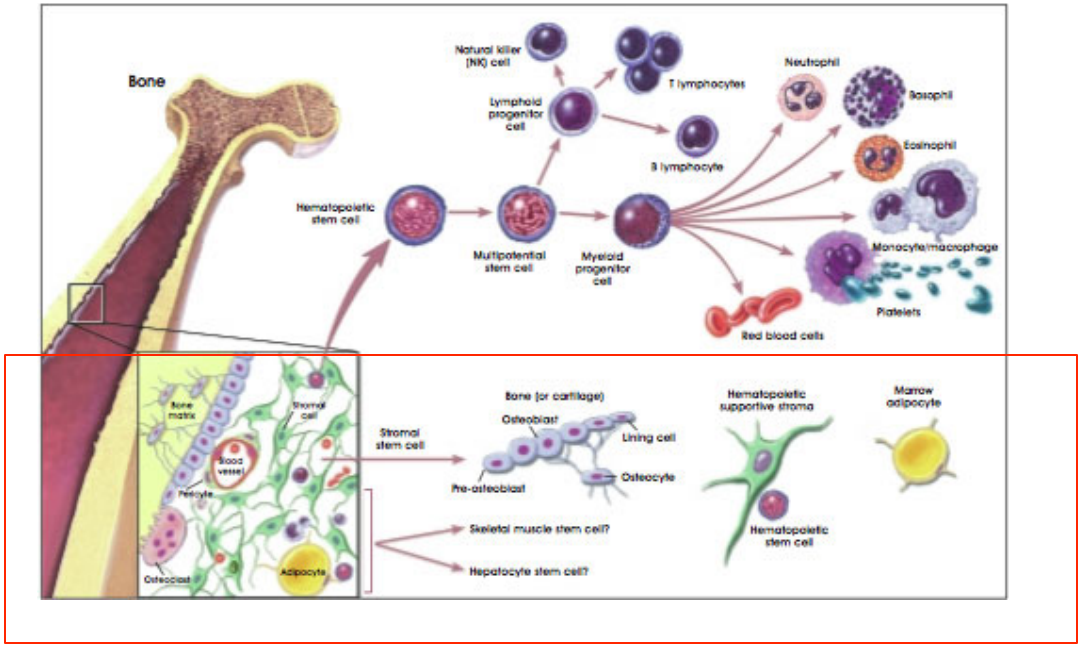
- Heterogeneous, no specific markers

- Are rapidly depleted in muscle of Duchenne patients

Mesenchymal stem cells

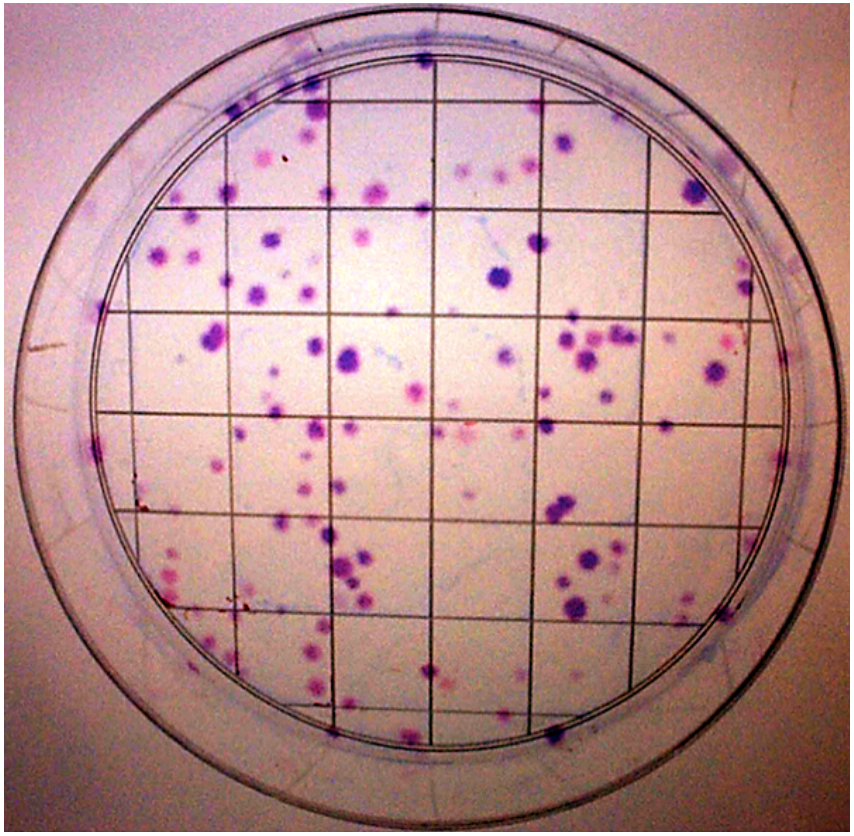
- Bone marrow-derived (non circulating fraction)
- Isolated on the basis of their adhesive properties

- Remarkable plasticity (condrocytes, osteoblasts, adipocytes, cardiac and skeletal muscle cells, neurons, astrocytes)



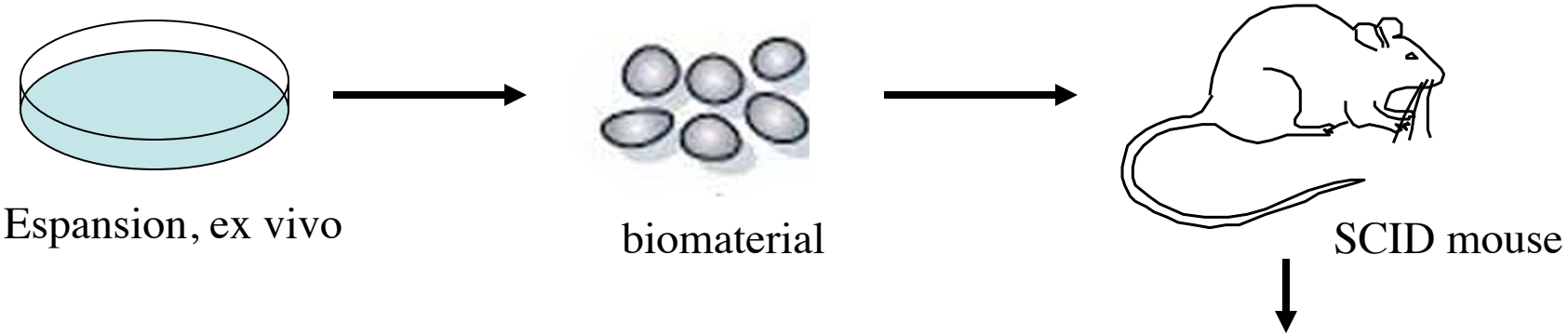
MSC properties

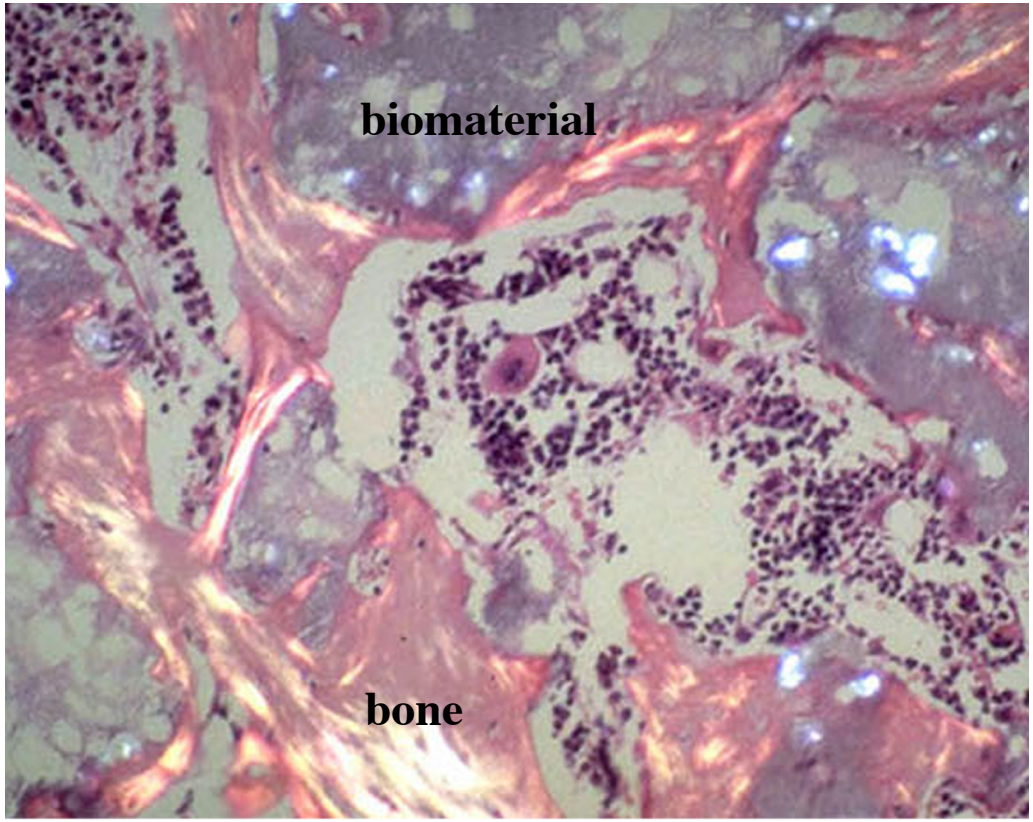
Alexander
Friedenstein

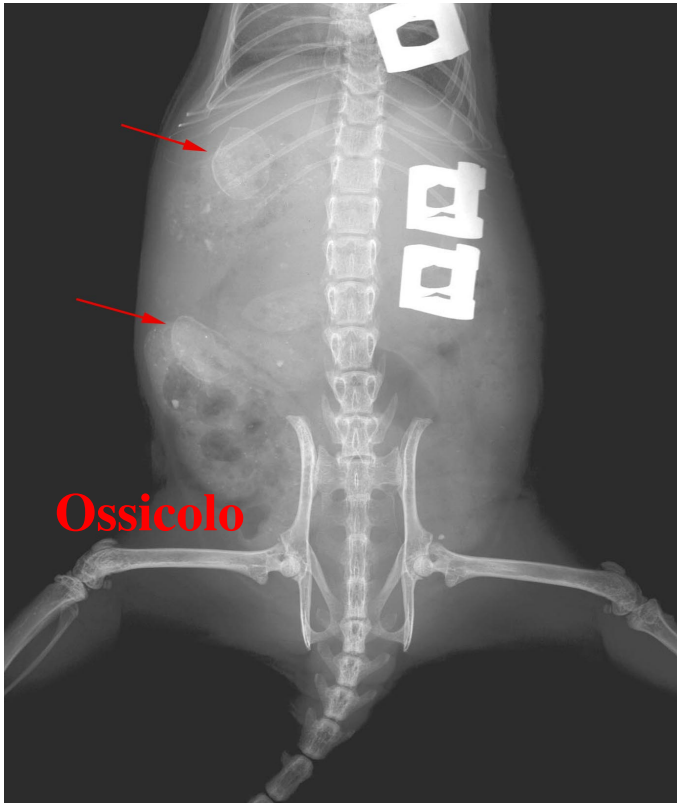


In bone marrow (“fibroblasts”)
Can be isolated and amplified ex vivo
transplantable
multipotent

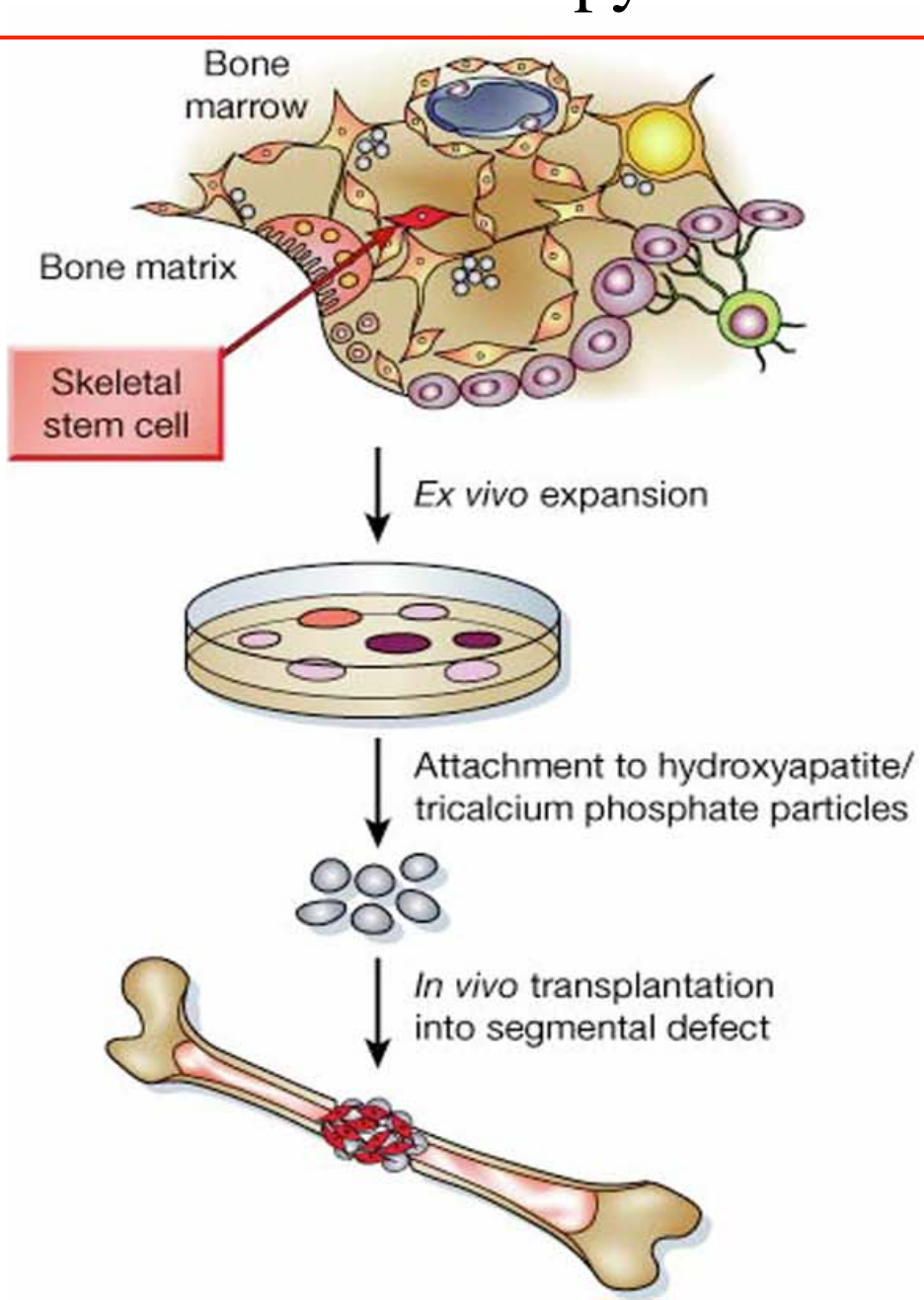
MSC/skeletal stem cells, transplant



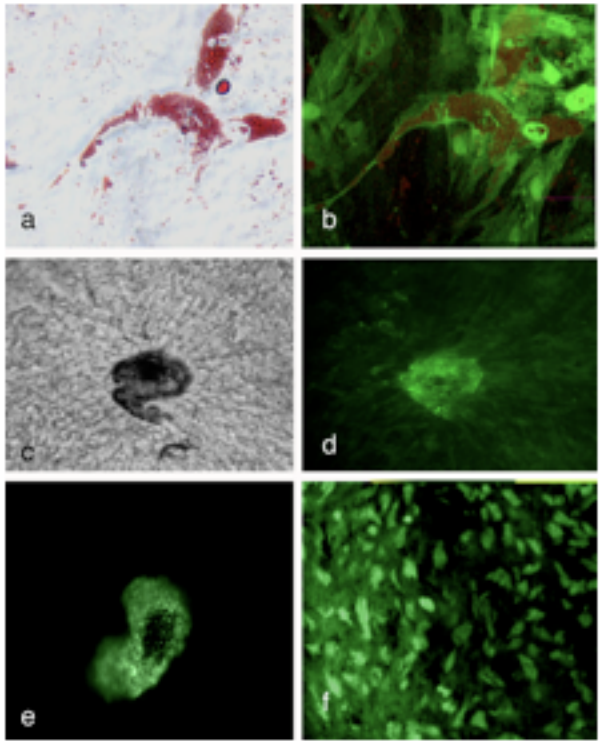


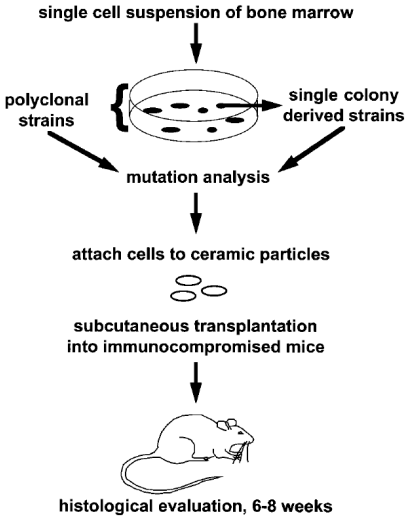


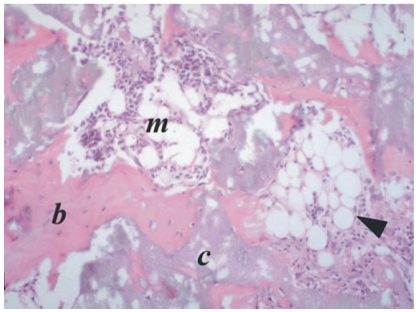
MSC in therapy



Mesenchymal stem cells pluripotency







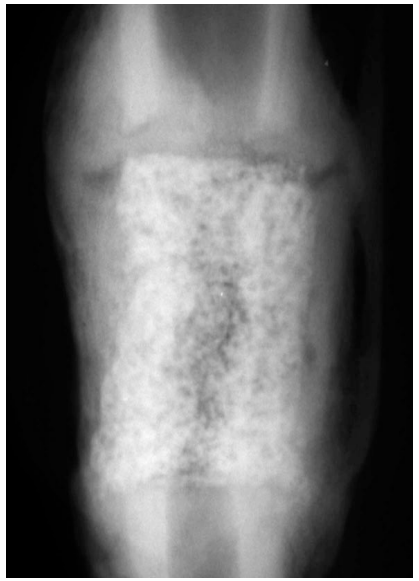
Spaces separating newly formed bony structures are occupied by hematopoietic marrow (m), in which all hematopoietic lines are detected (meg, megakaryocyte). Adipocytes are readily recognizable in the ectopic marrow (arrowheads).

www.bianco-lab.it

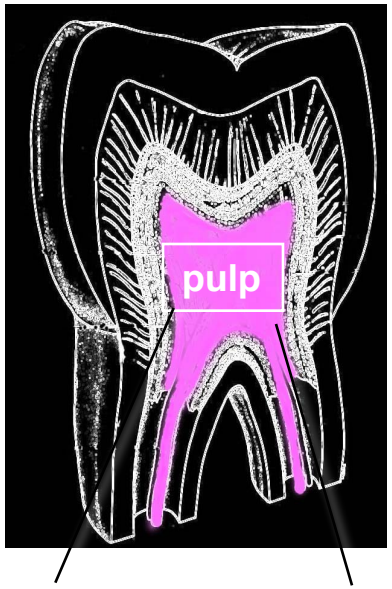
Animal models

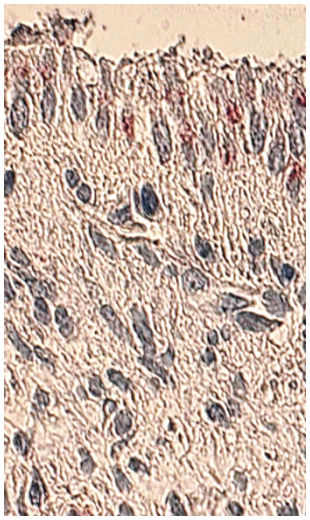
Preclinical models

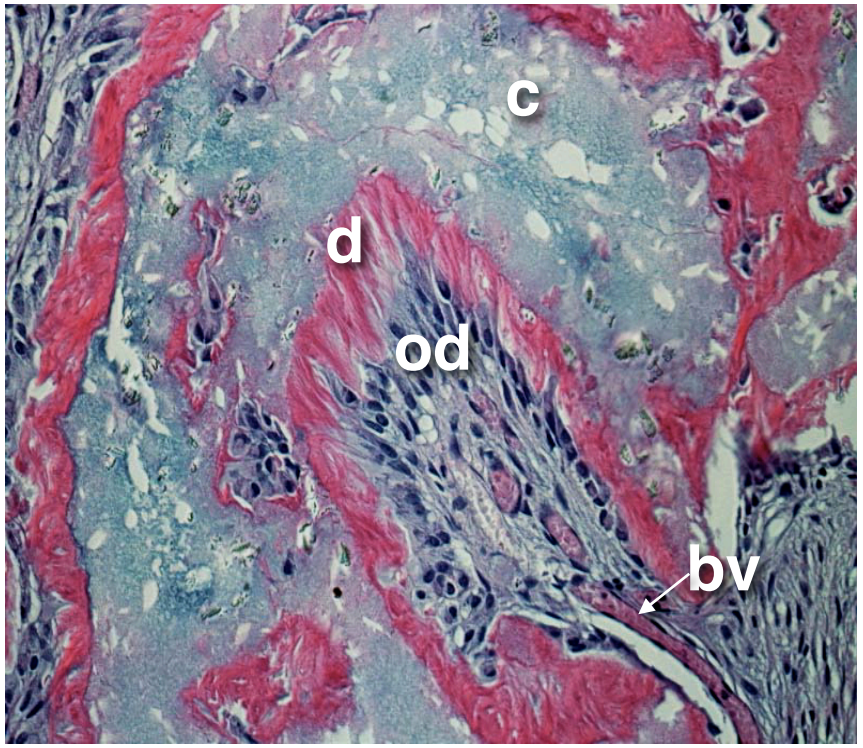
Mice
Dogs
Sheep
….

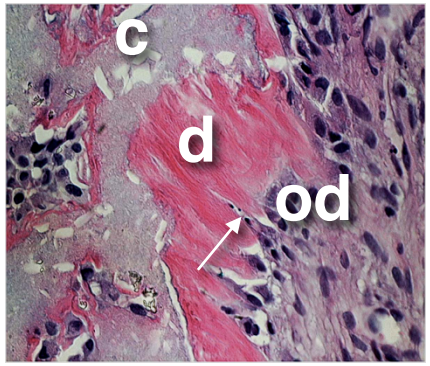


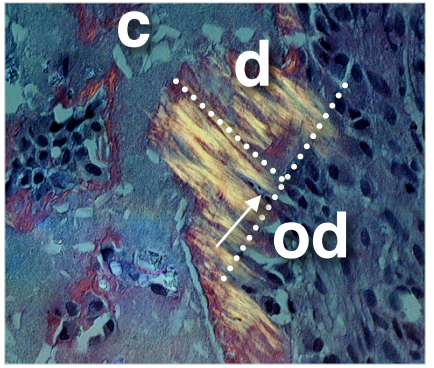
Post natal dental stem cells











Gronthos et al., 2000

MSC in therapy: problems

purification	CD146
espansion ex vivo	gmp/glp
	culture medium quality
biomaterial	
Way of injection	--

Adult cell plasticity: an old concept - truth or not??

- Cloning experiments in amphibia (1962)
- Cloning experiments in sheep (1997)

provide evidence that the differentiated state in adult is not irreversible.

Adult genes in enucleated cell; fusions..

But more in detail? And without fusions or egg inductions?

Original idea on adult stem cells: **self renewal and differentiation potential**

hematopoietc stem cell

blood

Satellite cell

muscle

Liver stem cell

liver

Skin stem cell

epithelium

Plasticity of adult stem cells: self renewal, differentiation and transdifferentiation

Bone marrow derived cells:

Blood
Muscle
Brain
Liver
Heart
Vascular endothelium

Muscle cell

blood

CNS cell

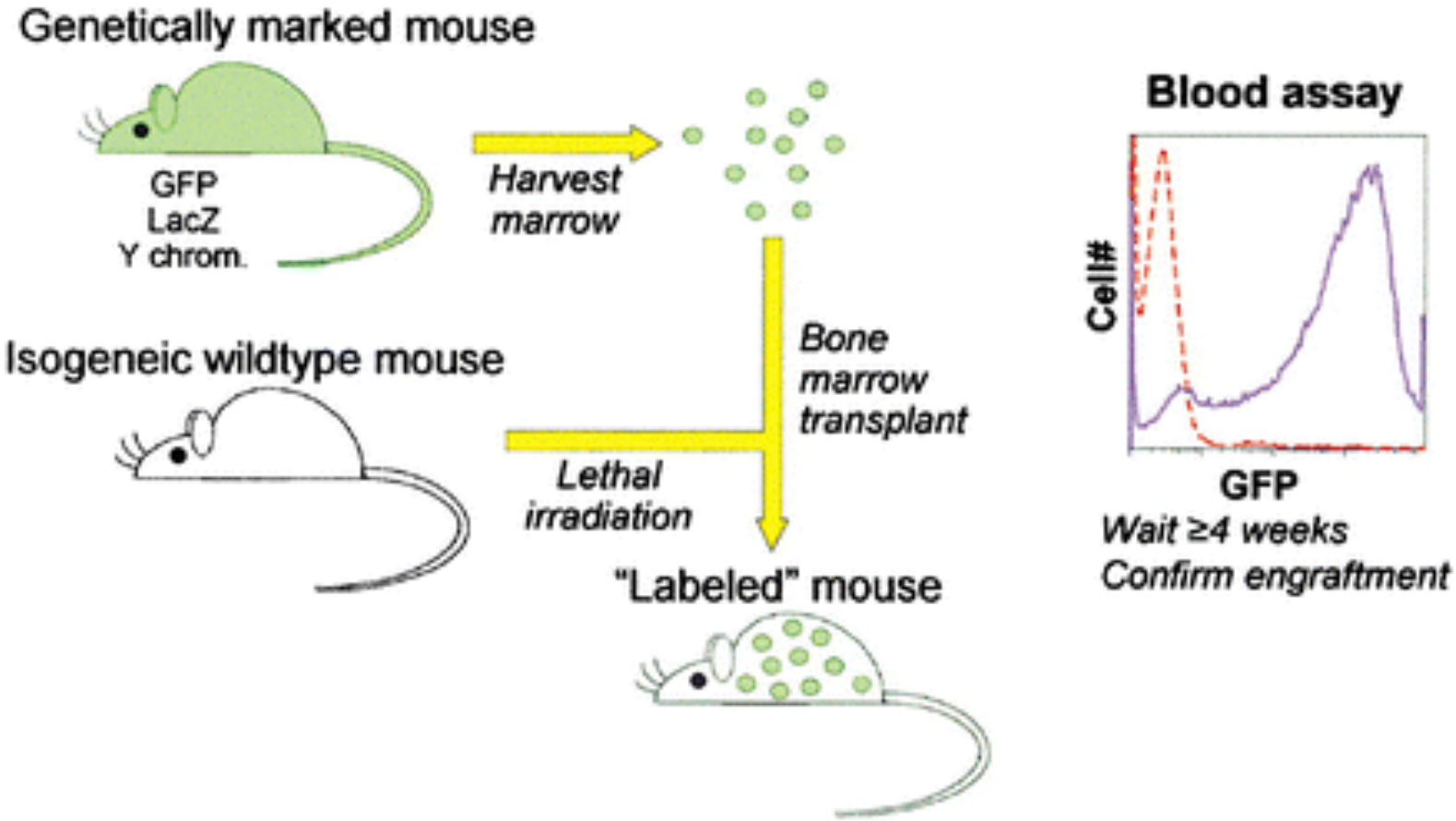
Blood
muscle

BM stromal cells (tissue injury…)

Adipocytes
Muscle
bone

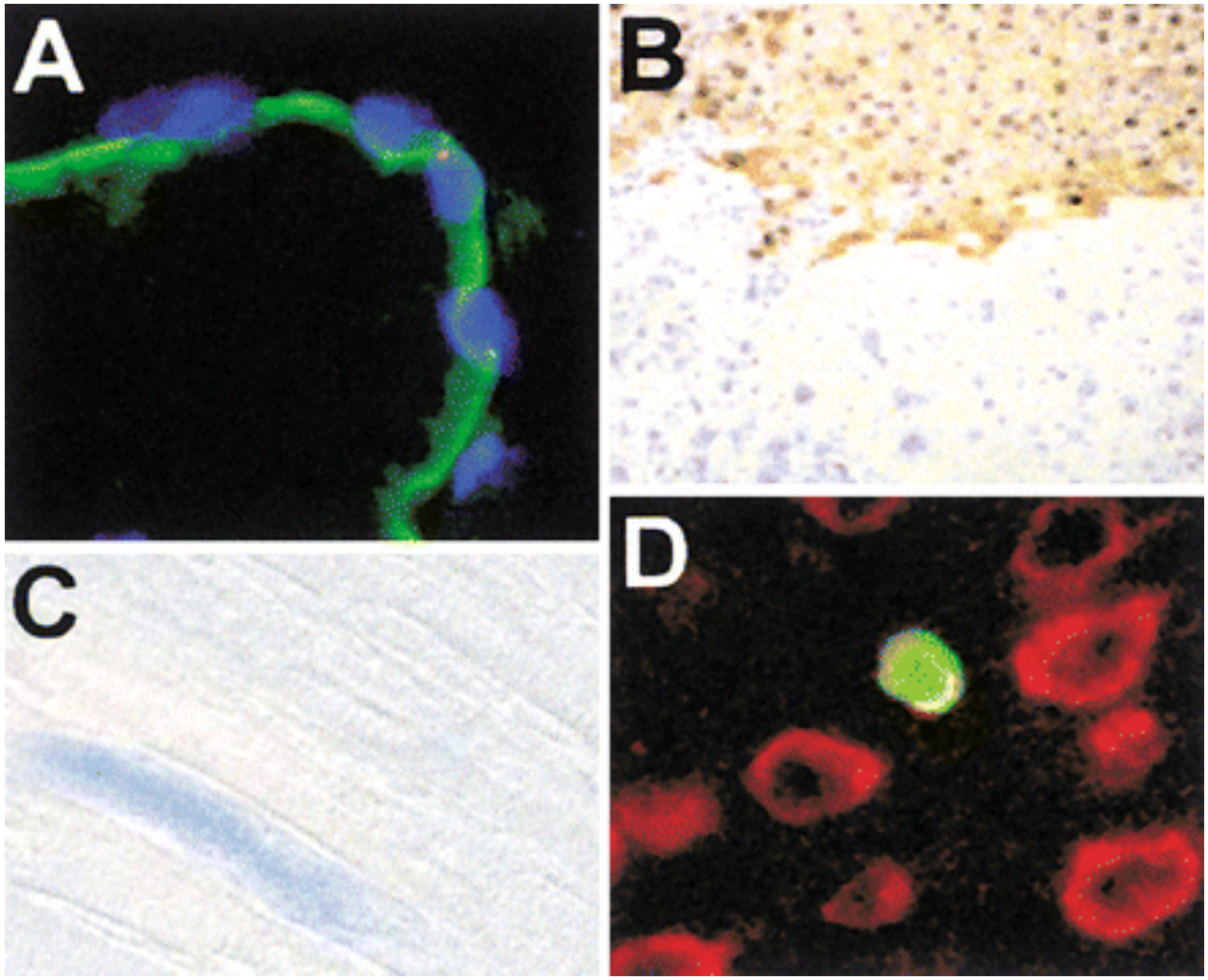
General strategy for identifying cell fate transitions using BM-derived cells - same tissue

(transgenic mouse)



Derivation of diverse tissue-specific cell types from BM-derived stem cells - different tissue

Dystrophin (green) and Y chromosome (blue) in BM-transplanted female mdx mice



FAH staining hepatocytes in FAH-/- BM-transplanted mice 30-50% of liver mass **7 months** posttransplant

Beta gal positive myocardium in a murine model of infarctum BM-transplanted (SP fraction intravascular delivery)

Neurons (red) GFP positive (green) in the cortex of a mouse intravascularly delivered with GFP +BM.

Criteria for trans-differentiation

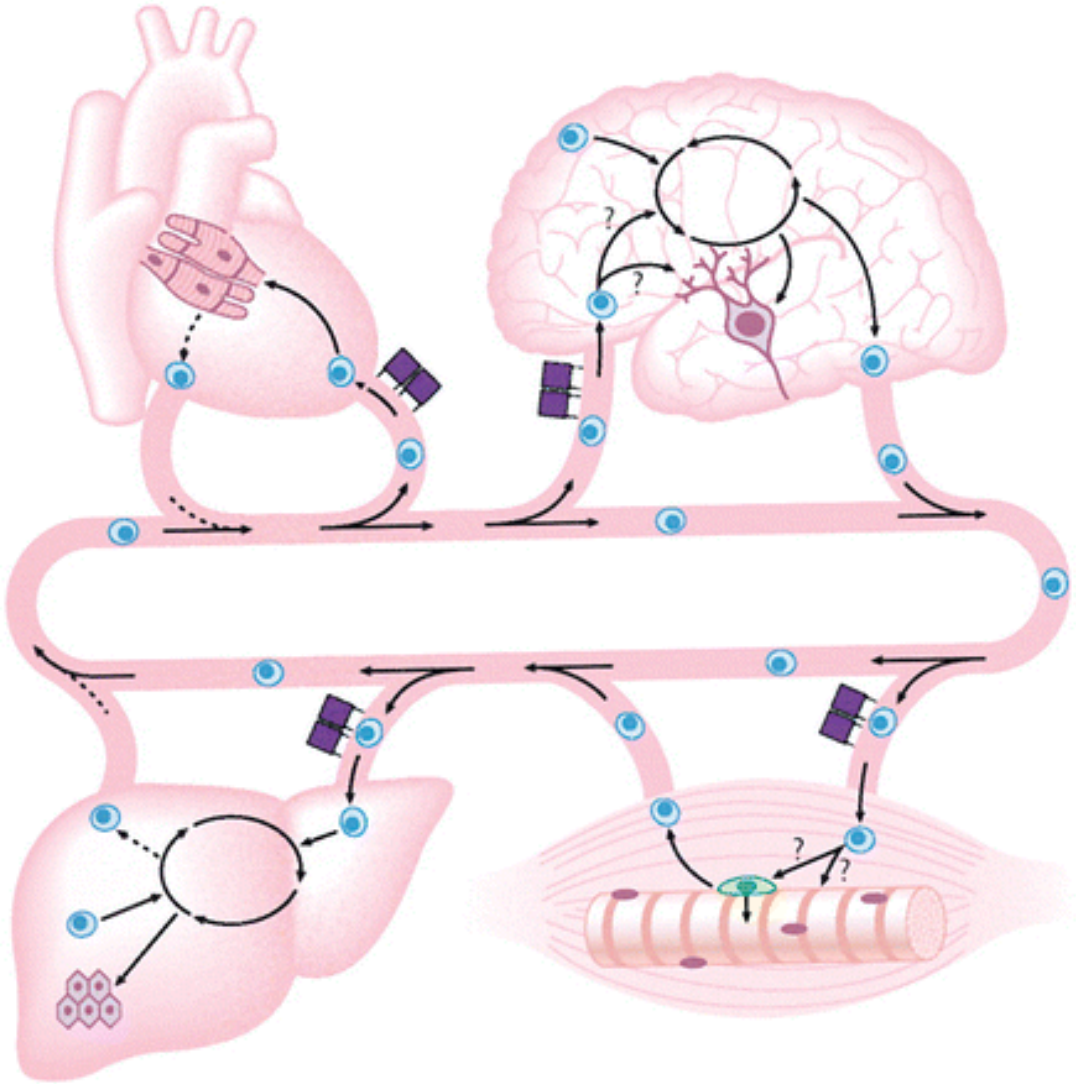
- New specific gene expression, in vitro and in vivo
- Marker of the stem cell (Y, GFP, lacZ..)
- Colocalization (confocal)
- Integration in the tissue
- Functional assay

Stem cells

Entity or function?

HM Blau Cell - 2001

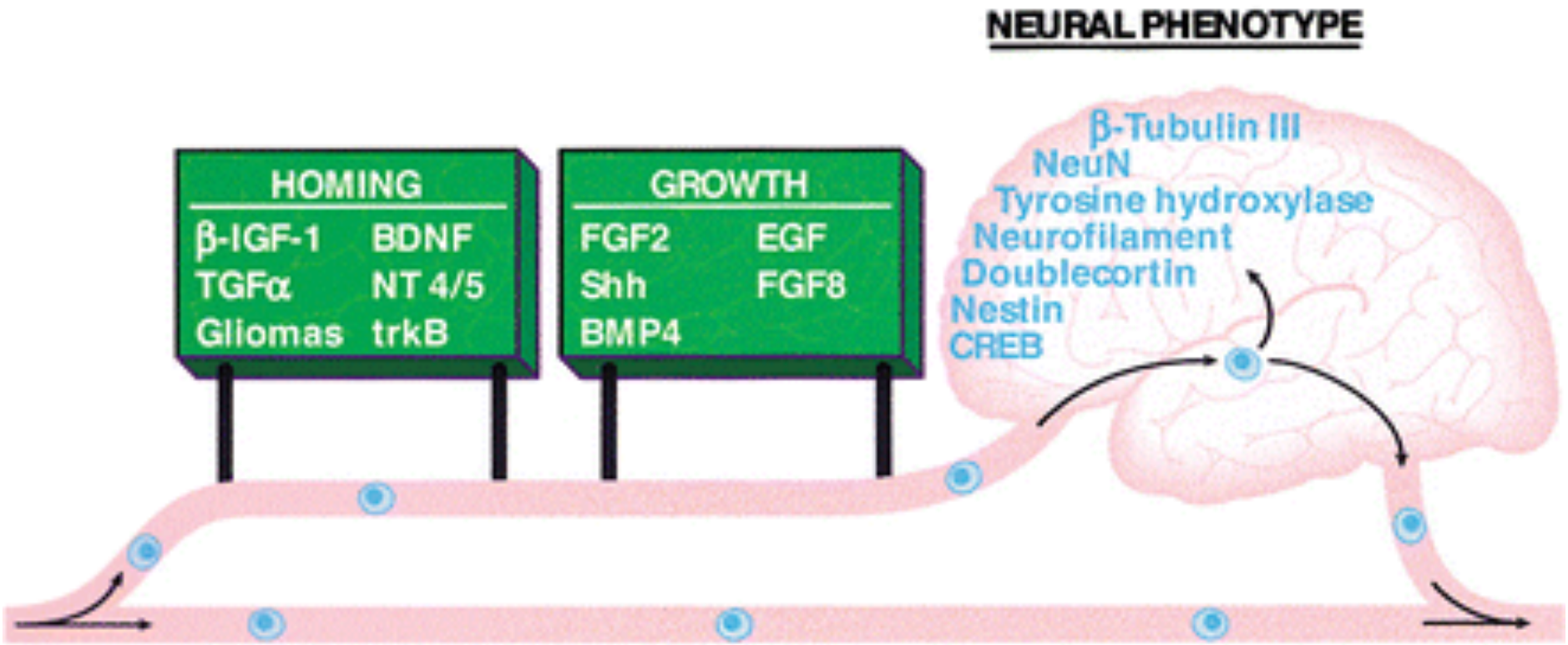
Circulation: the highway of stem cells



•contact with surrounding cells,
•Extra-cellular matrix,
•local milieu,
•growth and differentiation factors

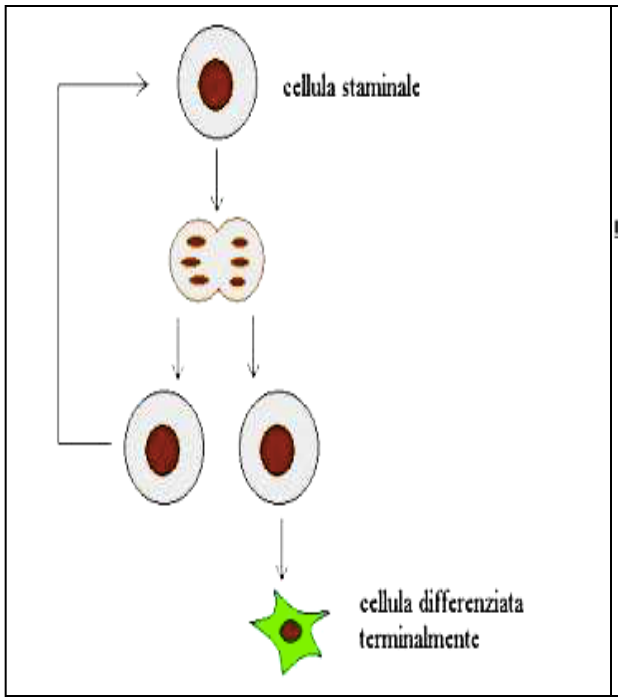
play a key role in determining stem cell function

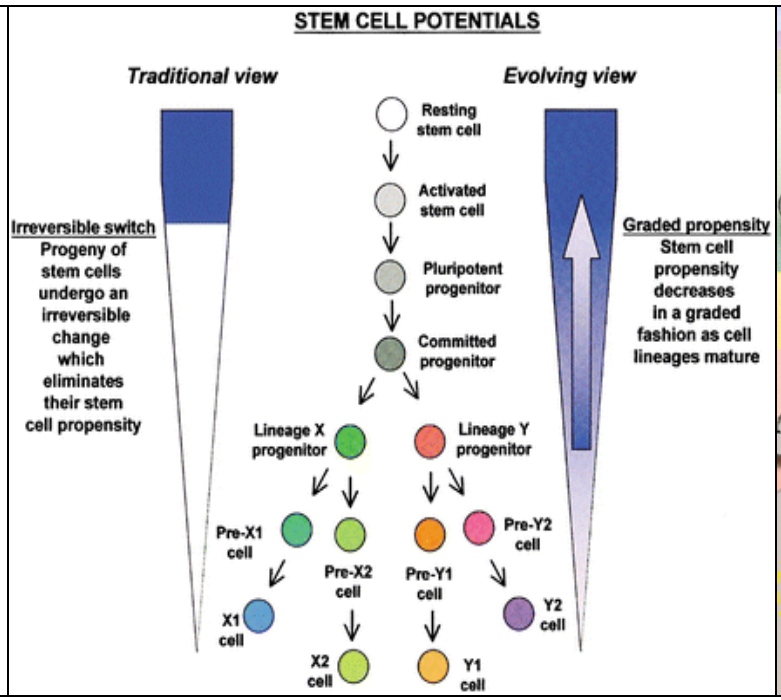
Factors that control trans-differentiation

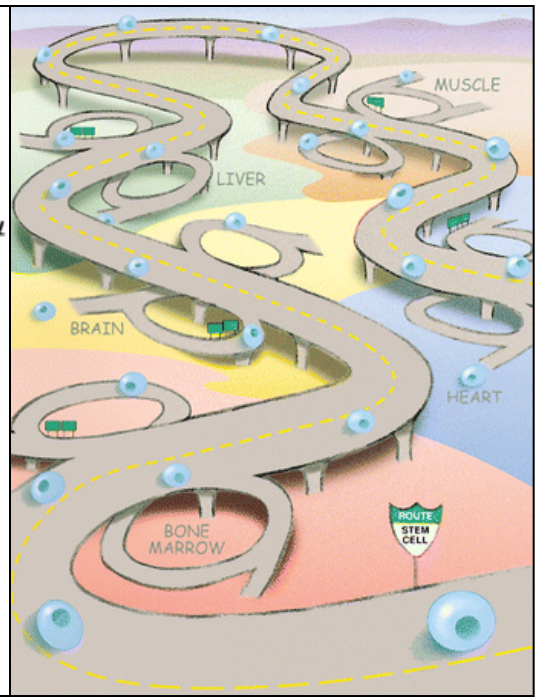


General concepts

1. 2. 3.





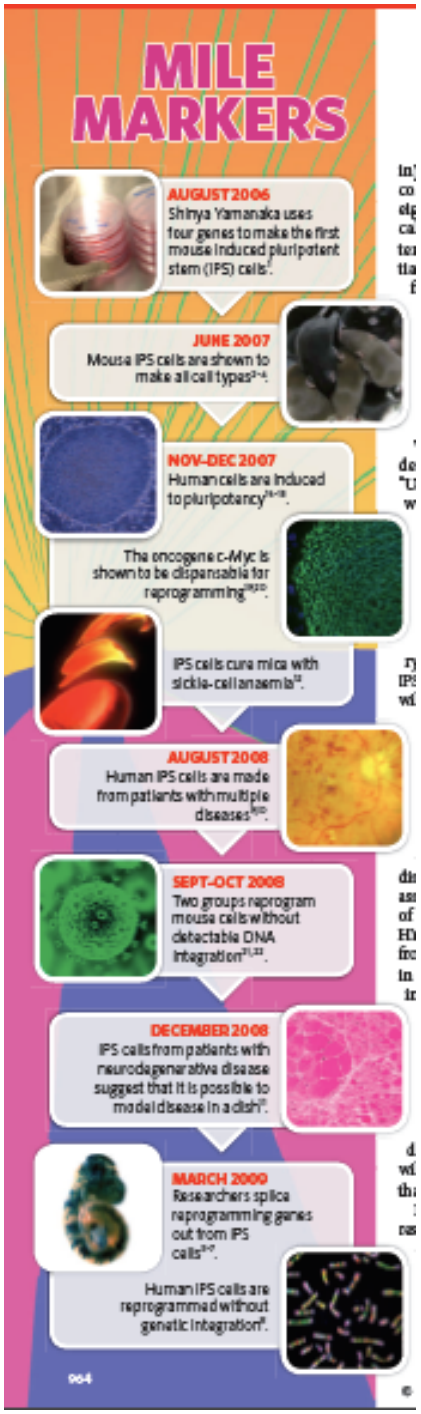


HM Blau Cell - 2001

QUESTIONS
REFERENCES

IPS-ES like

Baker Nature 2009



IPS mile markers

Baker Nature 2009

iPS history

Mouse

Generation of pluripotent stem cells from adult mouse liver and stomach cells. Science 2008; 321: 699

Man

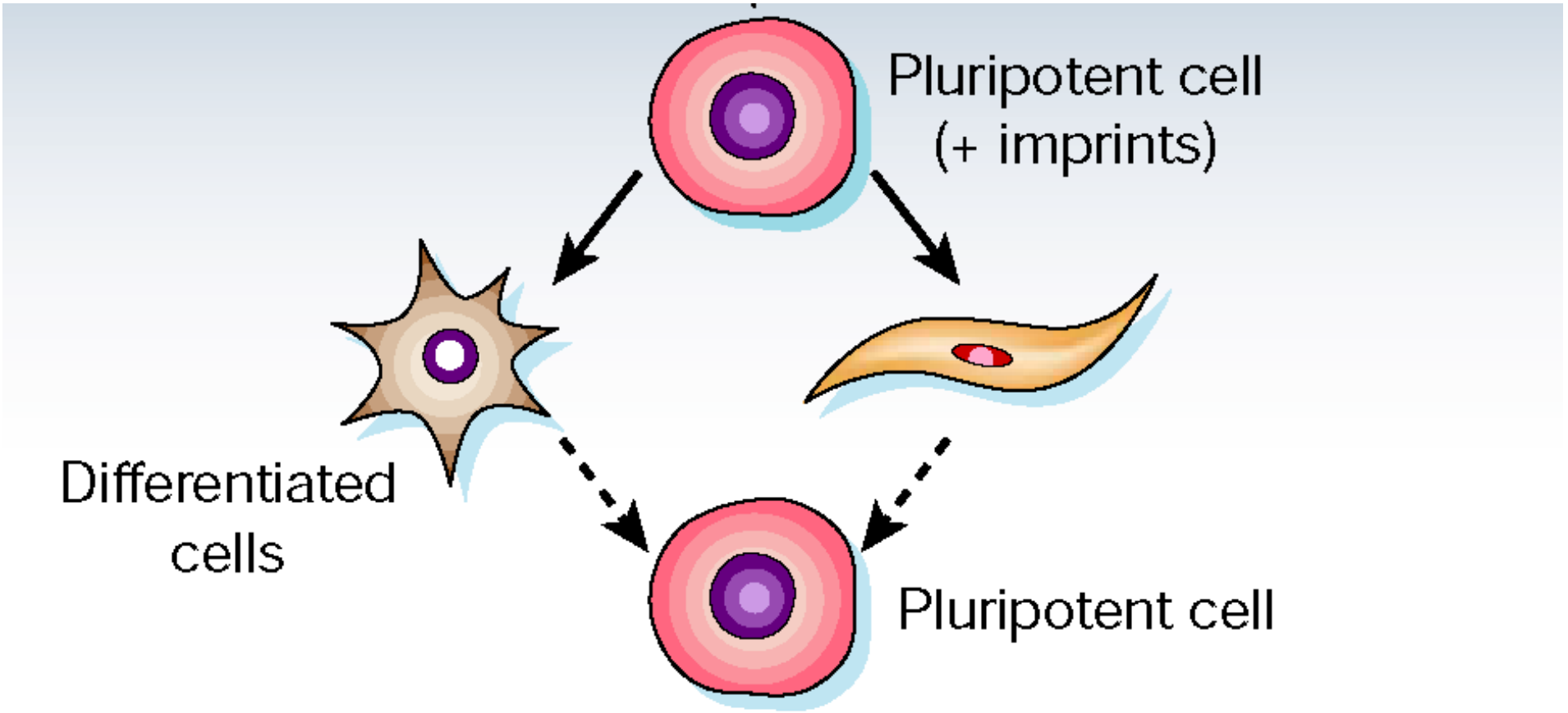
Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell 2007; 131: 861

Reprogramming of human somatic cells to pluripotency with defined factors. Nature 2008; 451: 141

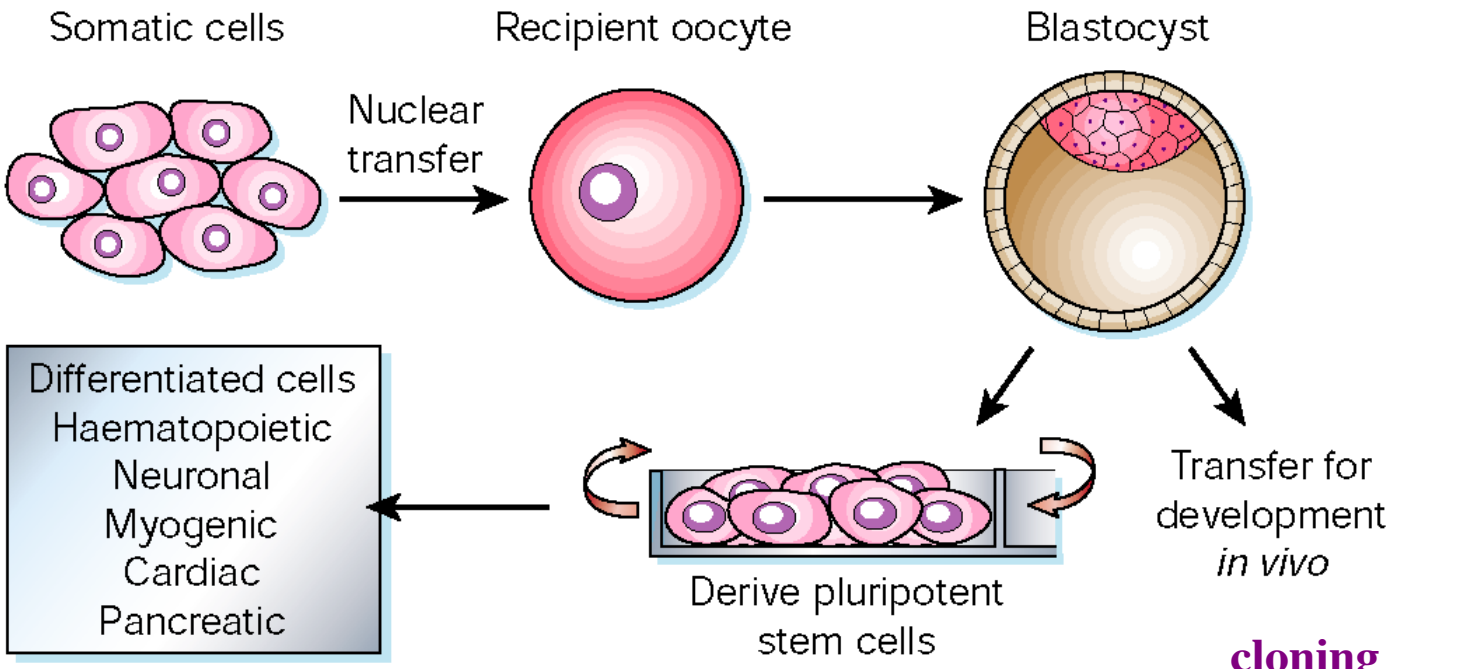
Functional cardiomyocytes derived from human induced pluripotent stem cells. Circ Res 2009; 104: e30

Disease-specific induced pluripotent stem cells. Cell 2008; 134: 877

Epigenetics reversibility



Somatic cell nuclear transfer (SCNT)



cloning terapeutico

cloning riproduttivo

Dolly 1997-2003

Somatic cell nuclear transfer (SCNT)

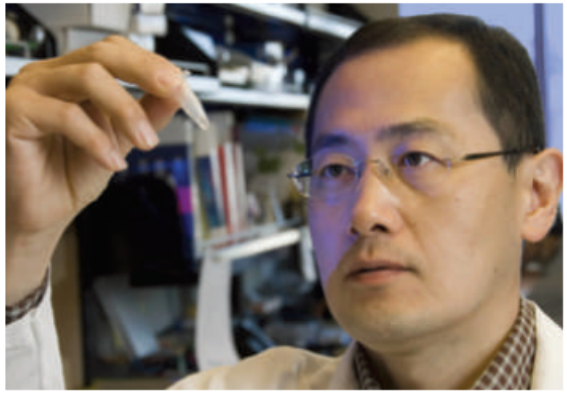
Advantages	no ethics
	histocompatibility
Disadvantages	egg cells
	cost

IPS

Adult stem cells: multipotent and self-regenerating

Embryonic stem cells: pluripotent not self-regenerating

Embryonic stem like cells
Oct4 : transcription factor
Nanog: transcription factor
Sox2: transcription factor
c-Myc: transcription factor /proto-oncogene
Klf-4: transcription factor



Shinya Yamanaka made mouse iPS cells in 2006.

IPS

donor

Germ cells

transplant

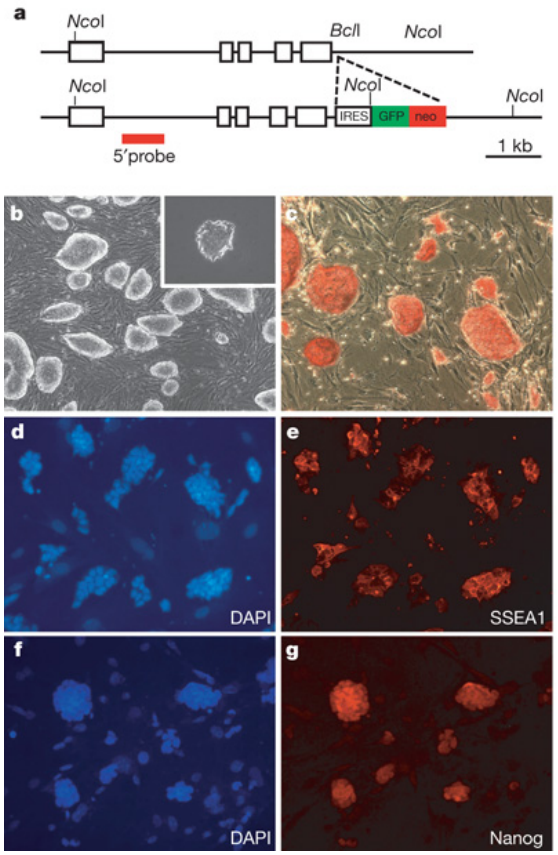
….

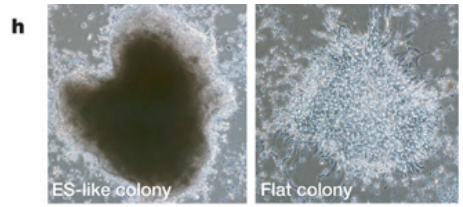
Alessandro Rosa, Erasmus Seminar

Selection of **mouse** fibroblasts for Oct4 or Nanog activation

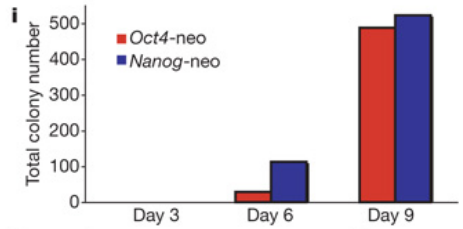
Homologous recombination in MEF to obtain Oct4-neo or Nanog-neo. Neo selection kills the cells because in differentiated cells these genes are silenced.
Then addition of retro-Oct4, Sox2, c-Myc, or Klf4

Colony derived cell line -iPS

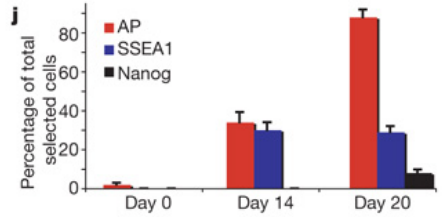




Colony G418 res



Time is needed for colony formation



Different ES markers at different times

k

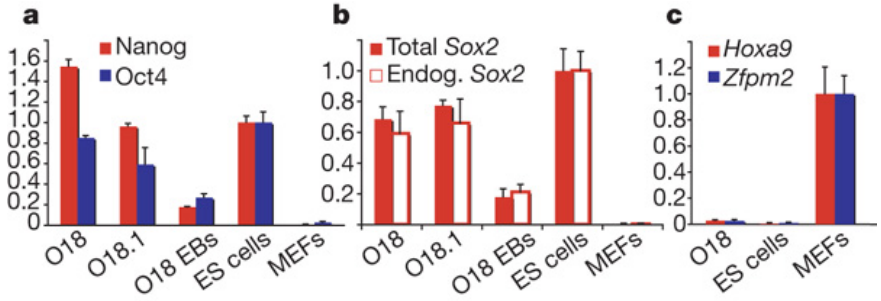
	Total col./ 100,000 cells	ES-like col./ total col. (%)	iPS line/ ES-like col. (%)	Estimated efficiency (%)
Oct4	156 ± 31	24.0 ± 7	22	0.080
Nanog	947 ± 187	11.5 ± 4	5	0.050

Oct4 less colonies more iPS
Nanog easier to activate oct4 more important for the pluripotent state

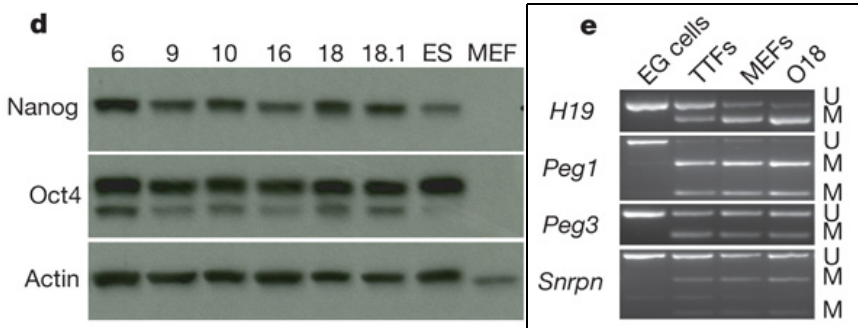
Wernig et al Nature 2007

Expression and DNA methylation

Measurement of markers of ES or MEF or embryoid bodies byrtQPCR

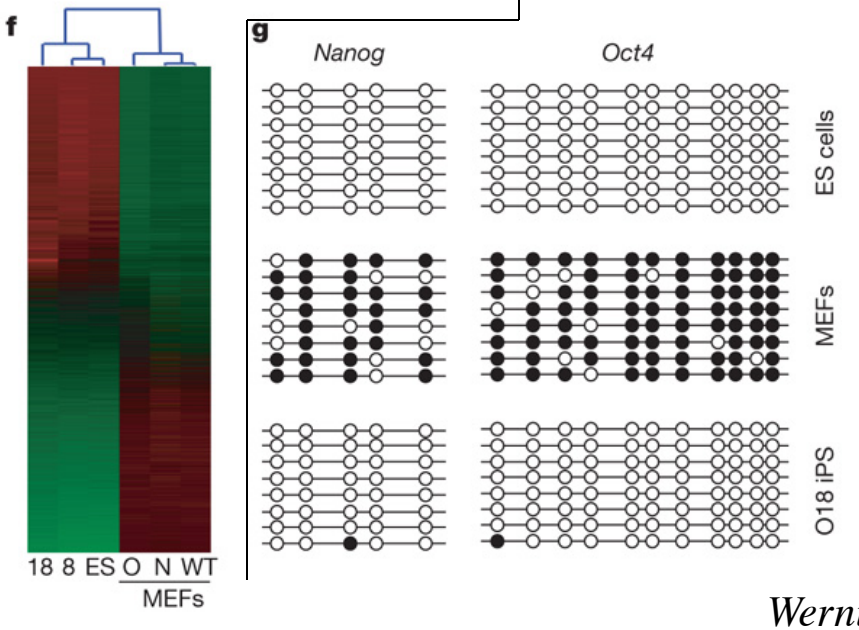


Measurement of markers of ES or MEF by western on Ips and controls



Measurement of promoter methylation (in germ cells mprinting is erased)

Measurement of markers of ES or MEF by gene chip on Ips and controls



Measurement of promoter methylation

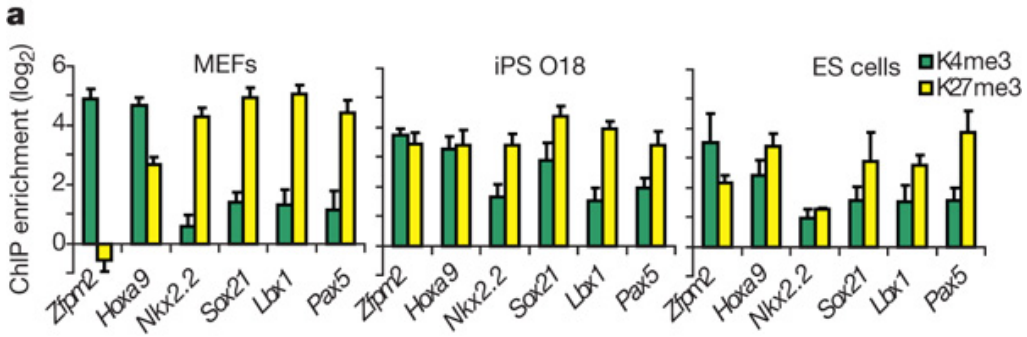
COBRA

Wernig et al Nature 2007

Chromatin modifications

Histone H3 lysine4 and 27 are active or repressive marks. Down stream targets of oct4, nanog, sox2

ChIP and Q PCR to measure H3 methylation state in association with specific genes

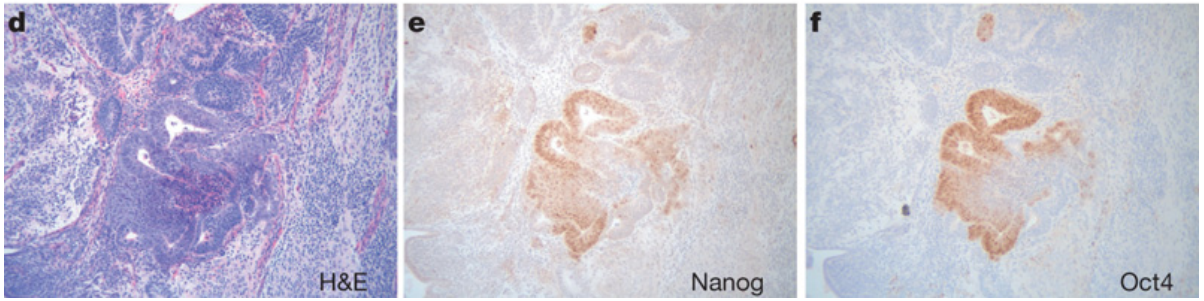


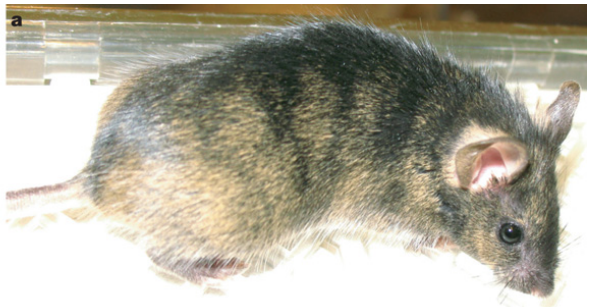
ES = Ips # MEF

Wernig et al Nature 2007

Developmental potential

Teratoma from Ips- three germ layers

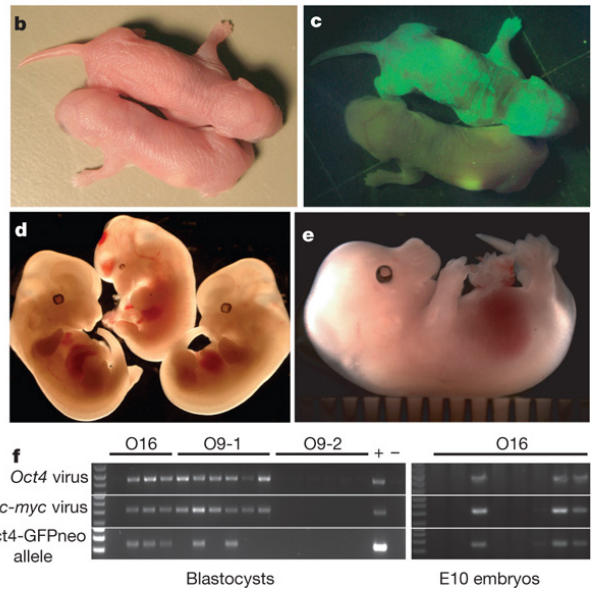




Ips injected in 2N or 4N blastocysts

for chimeras

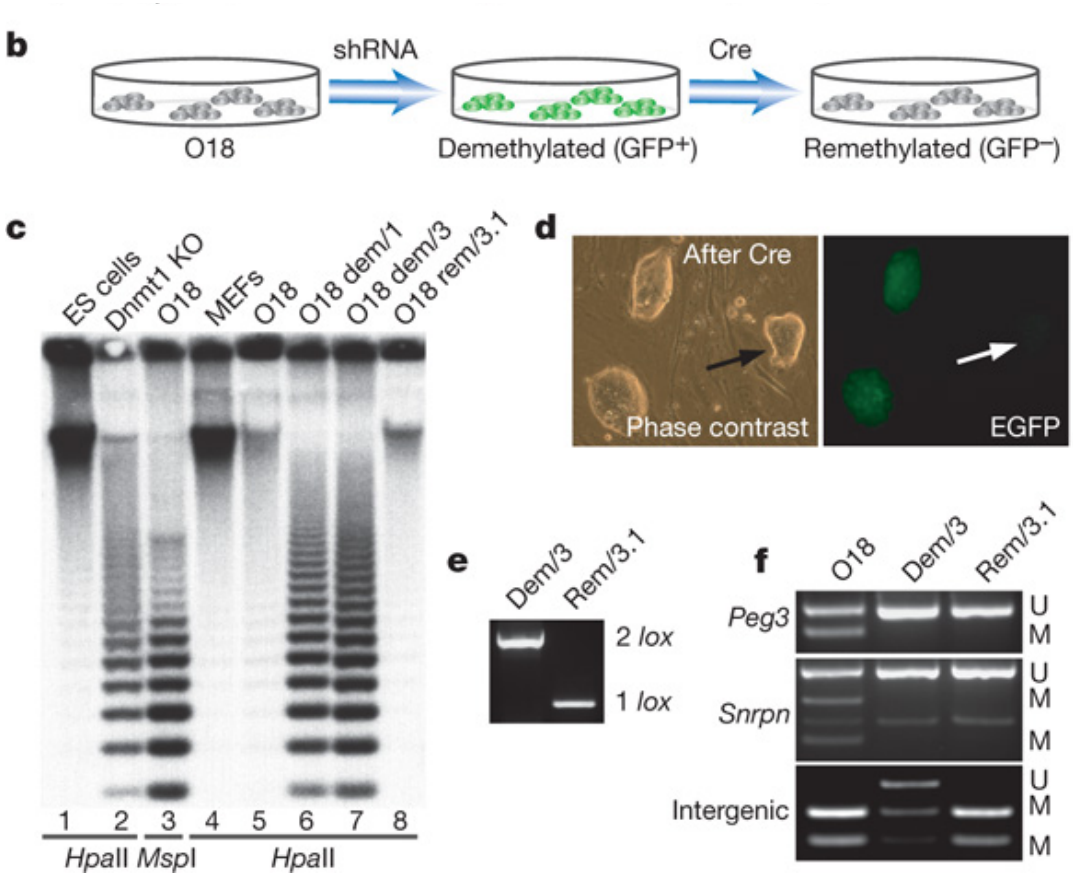
F0 and F1



Wernig et al Nature 2007

Ips tolerate genomic demethylation

LV-siDnmt1/GFP/loxP



Southern with methylation sensitive enzyme (HpaII) And methylation insensitive (MspI)

Morphology

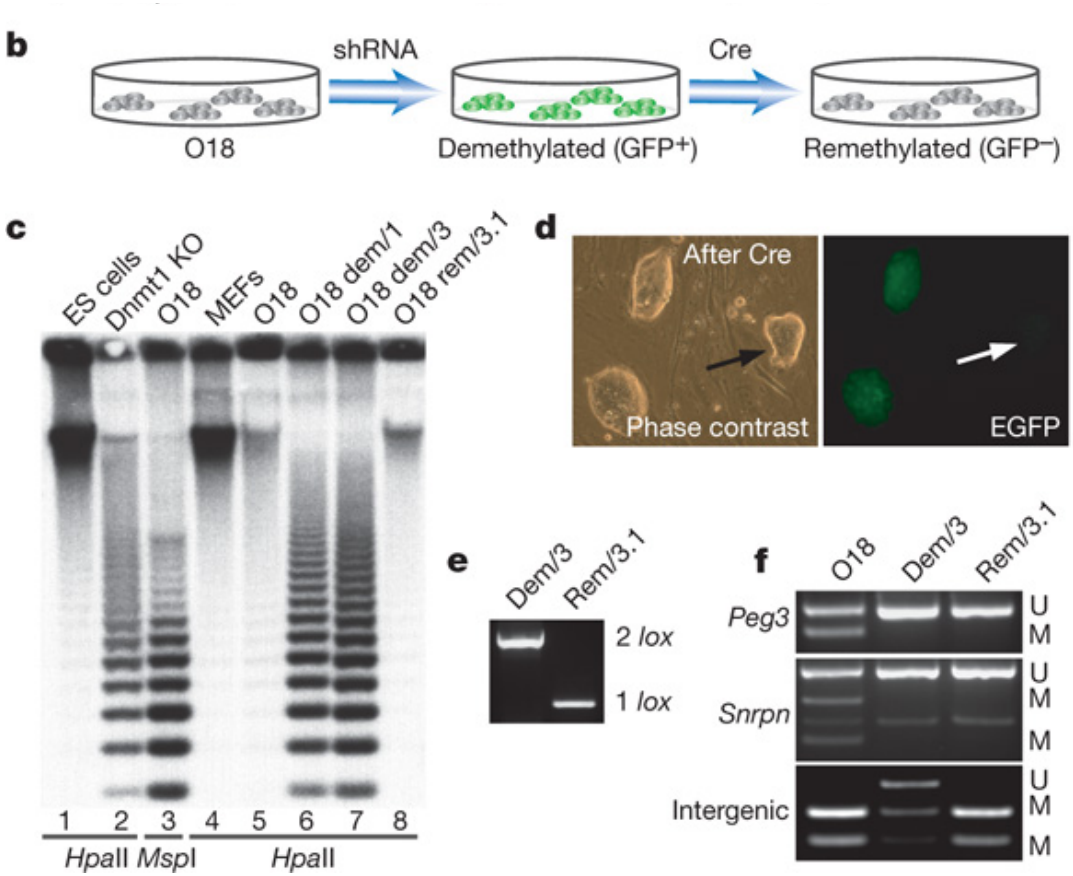
Ips tolerate genomic demethylation (a unique property of ES cells)

No de novo methylation of imprinted genes

Wernig et al Nature 2007

Ips tolerate genomic demethylation

LV-siDnmt1/GFP/loxP



Southern with methylation sensitive enzyme (HpaII) And methylation insensitive (MspI)

Morphology

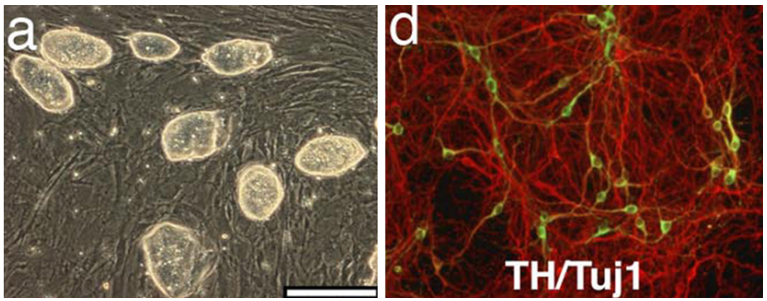
Ips tolerate genomic demethylation (a unique property of ES cells)

No de novo methylation of imprinted genes

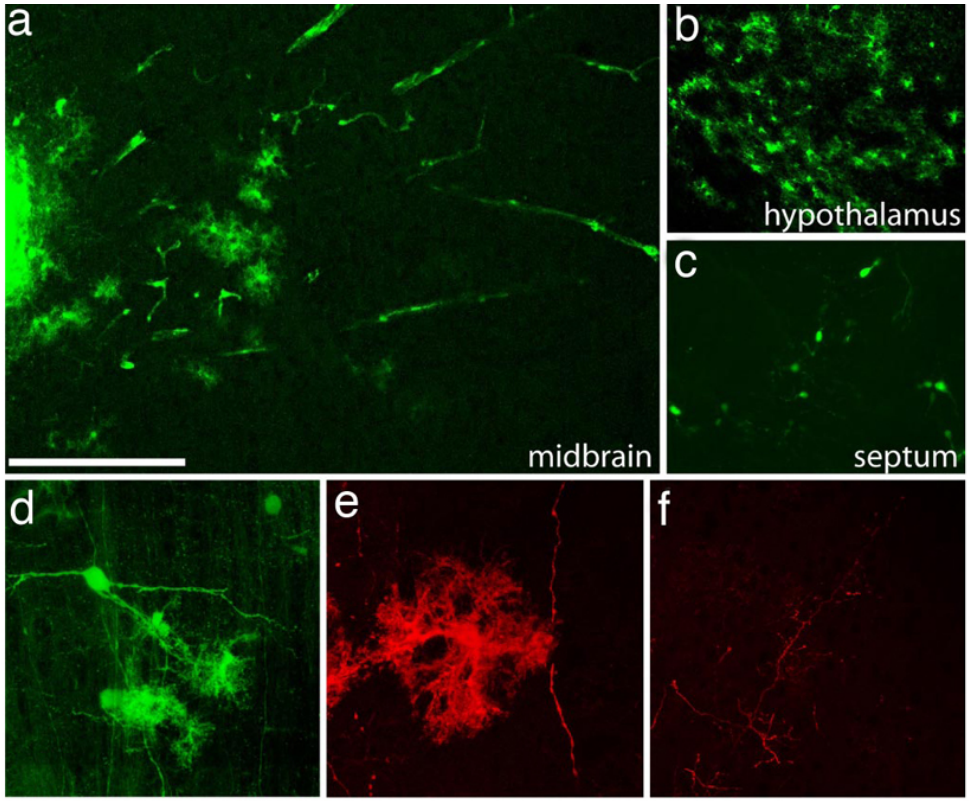
Wernig et al Nature 2007

into neurons

in vitro



in vivo



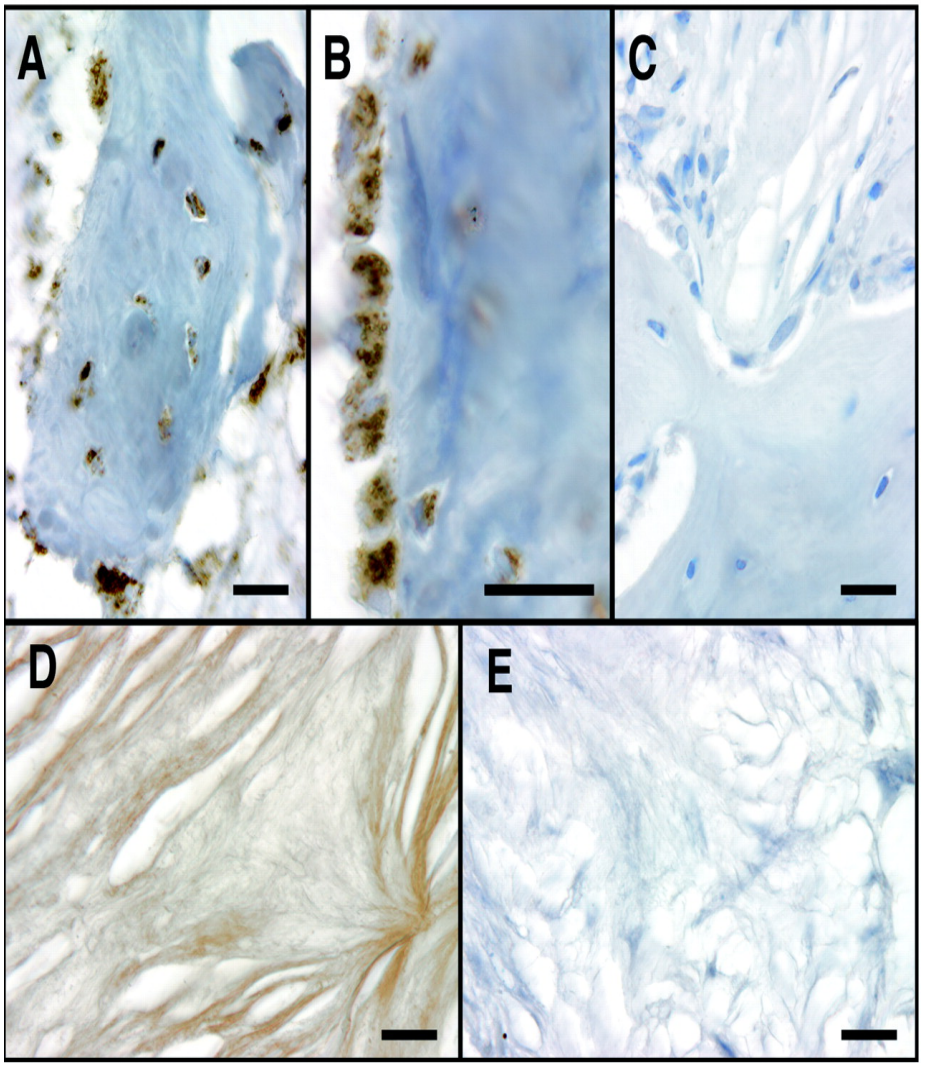
Wernig et al, 2008

QUESTIONS?

In vivo bone formation by targeted MSC (clones and polyclonal). Demonstration of human bone

A, Band C AAV MSC

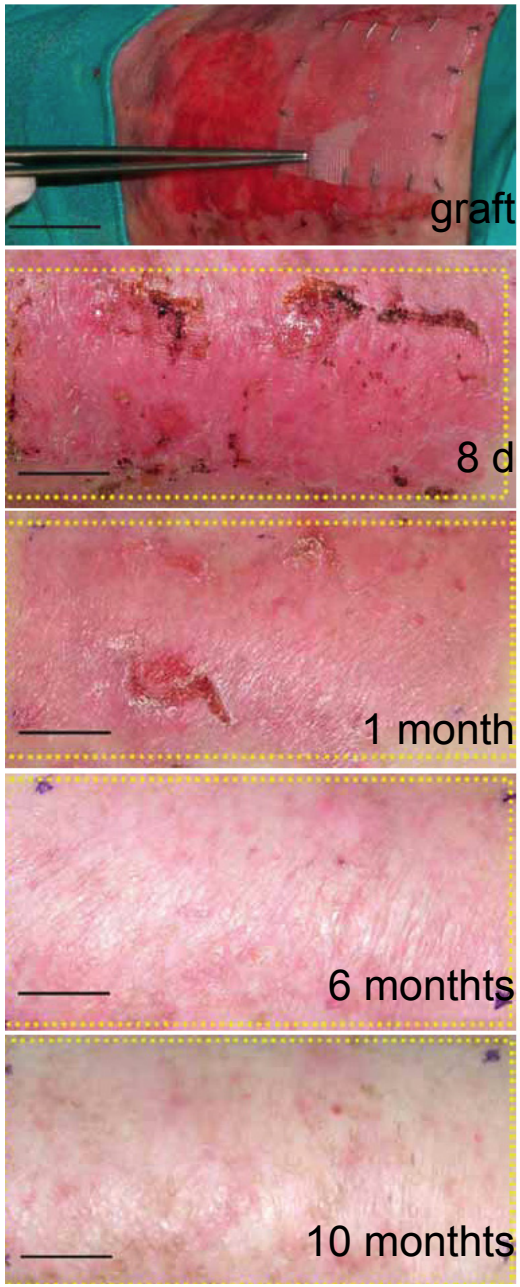
C and murine MSC

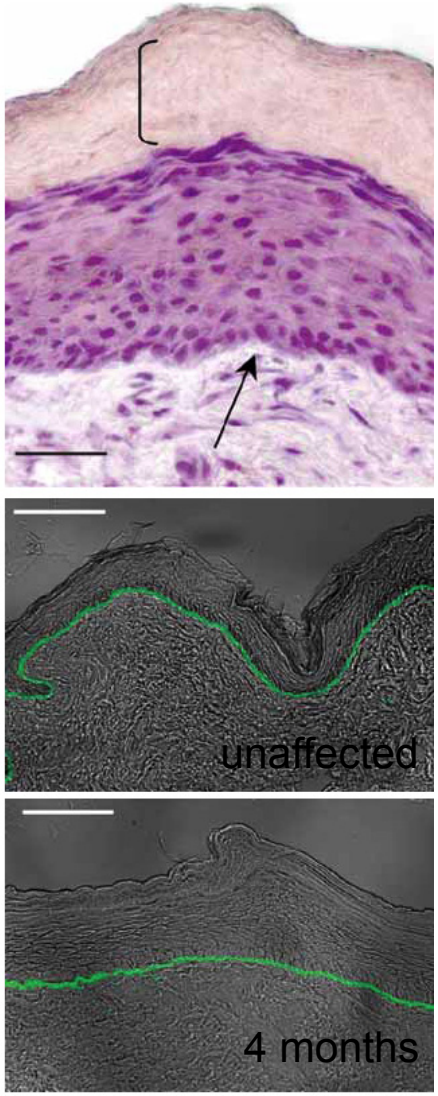


Hum mit stained

Hum collagen

Post natal epithelial cells





"Correction of junctional epidermolysis bullosa by transplantation of genetically modified epidermal stem cells"

Mavilio et al, 2006

QUESTIONS and biblio?

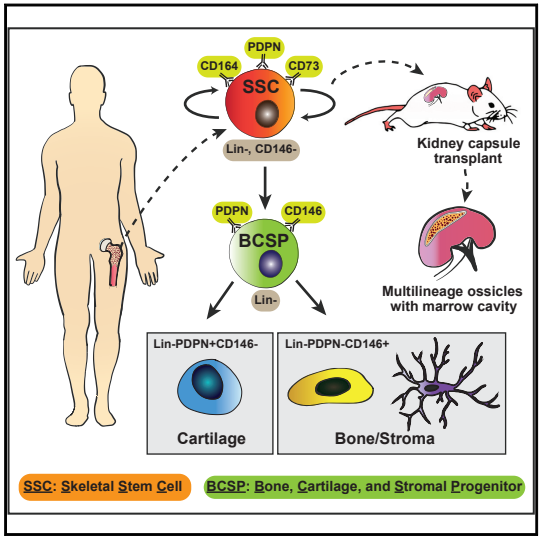
Test facoltativo

What would you do next

Cell

Identification of the Human Skeletal Stem Cell

Graphical Abstract



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In Brief

Identification of a human skeletal stem cell reveals conserved and species-specific pathways in skeletal development, and response to injury and will guide future regenerative approaches.

Highlights

- PDPN^{+}CD146^{-}CD73^{+}CD164^{+} marks a self-renewing, multipotent human skeletal stem cell
- hSSCs can be isolated from fetal, adult, BMP2-treated human adipose stroma, and iPSCs
- hSSCs undergo local expansion in response to acute skeletal injury
- Comparison of mouse and human SSCs reveals evolutionary differences in skeletogenesis

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