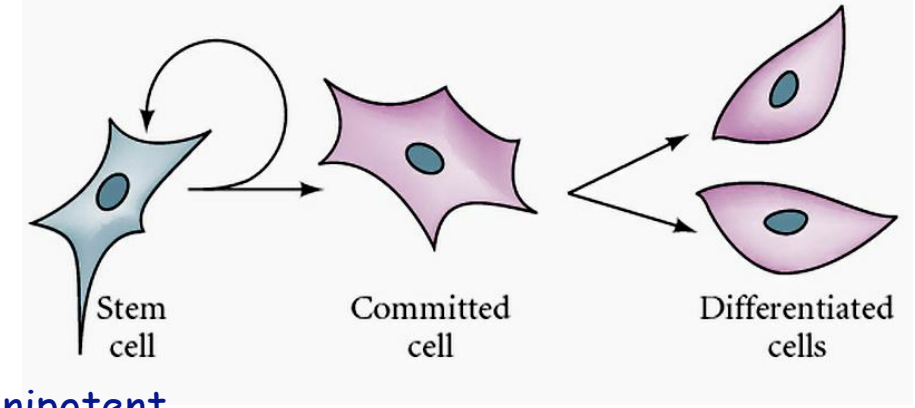
Stem cells

Learning Goals:

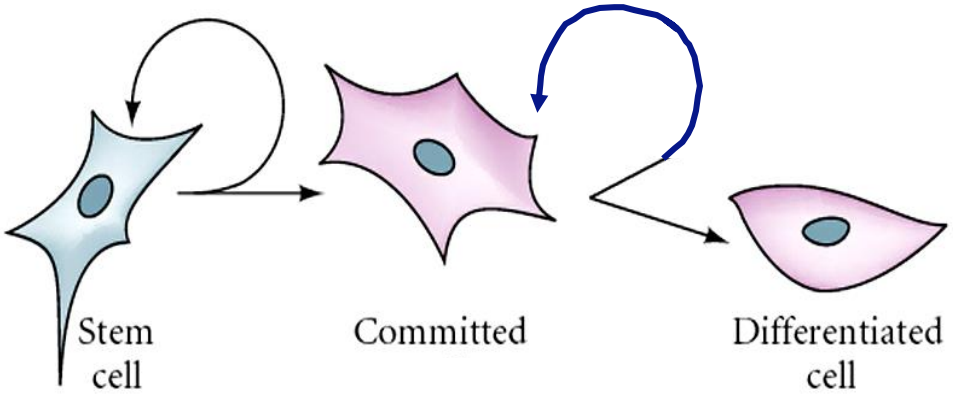
- Define what a stem cell is and describe its general properties, using hematopoietic stem cells as an example.
- Describe to a non-scientist the current progress of human stem cell research.
- Appreciate induced pluripotent stem cells and its implication for regenerative medicine.

Fundamental properties of Stem Cells



unipotent
e.g. epidermal stem cell

e.g. keratinocytes



Pluripotent or multipotent

Progenitor

First documentation of Stem Cells

CYTOLOGICAL DEMONSTRATION OF THE CLONAL NATURE OF SPLEEN COLONIES DERIVED FROM TRANS-PLANTED MOUSE MARROW CELLS

By Dr. A. J. BECKER, E. A. McCULLOCH and J. E. TILL

Department of Medical Biophysics, University of Toronto and

Ontario Cancer Institute, Toronto

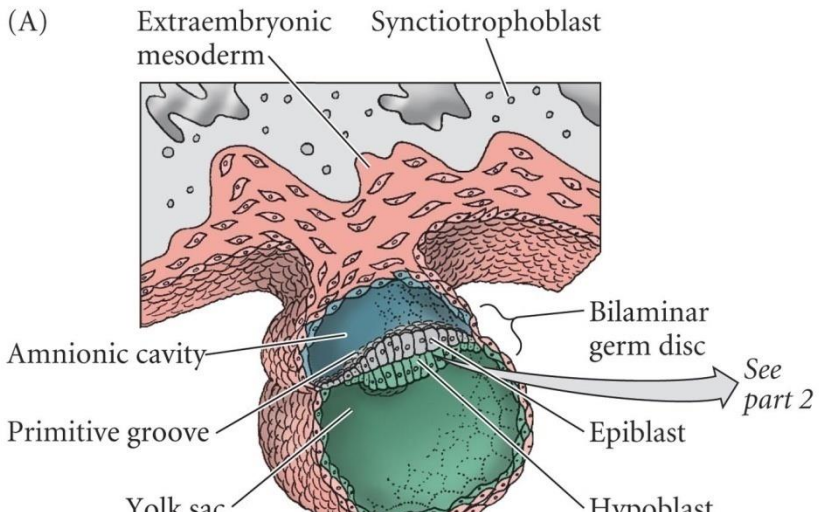
Nature 1963

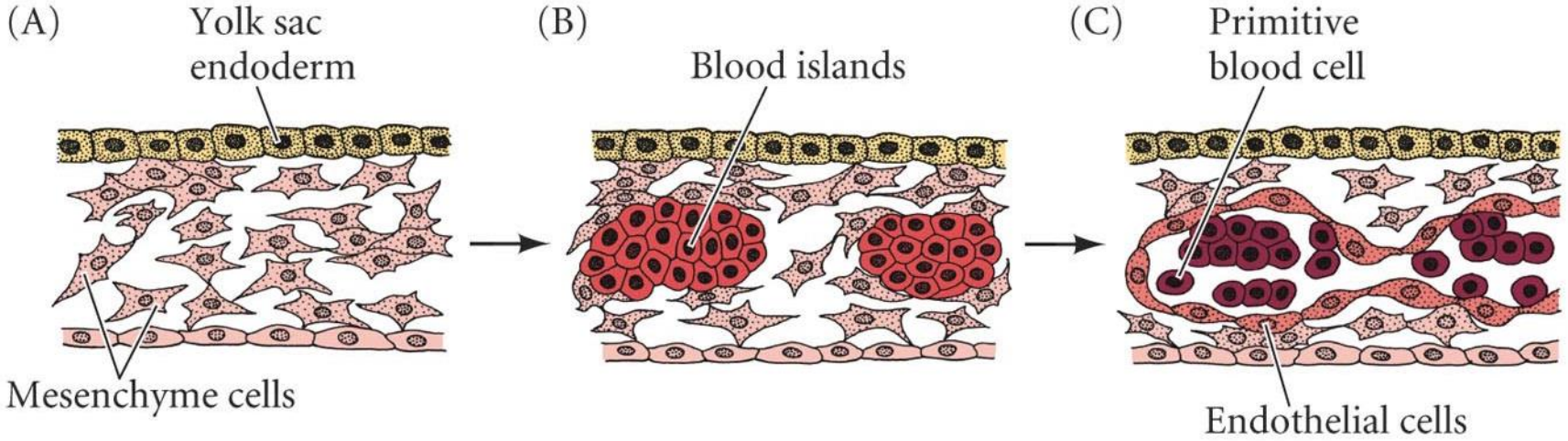
IN normal mouse hæmatopoietic tissue, there is a class of cells which, on being transplanted into heavily irradiated mice, can proliferate and form macroscopic colonies. In the spleen, the colonies formed in this manner are discrete and easy to count[1,2]. Microscopically, each colony appears as a cluster of hæmatopoiotic cells, many of which are dividing; and often, within a given colony, the cells which are observed indicate that differentiation is occurring along three lines, into cells of the erythrocytic, granulocytic and megakaryocytic series, respectively[1].

cent of the colonies. Nevertheless, if the direct cytological evidence is considered together with the indirect support provided by the dilution and radiation-survival data, the general view that spleen colonies are clones is a most reasonable conclusion. The spleen colony procedure may, therefore, be regarded as an *in vivo* single-cell technique, analogous to the

Hematopoietic Stem Cells

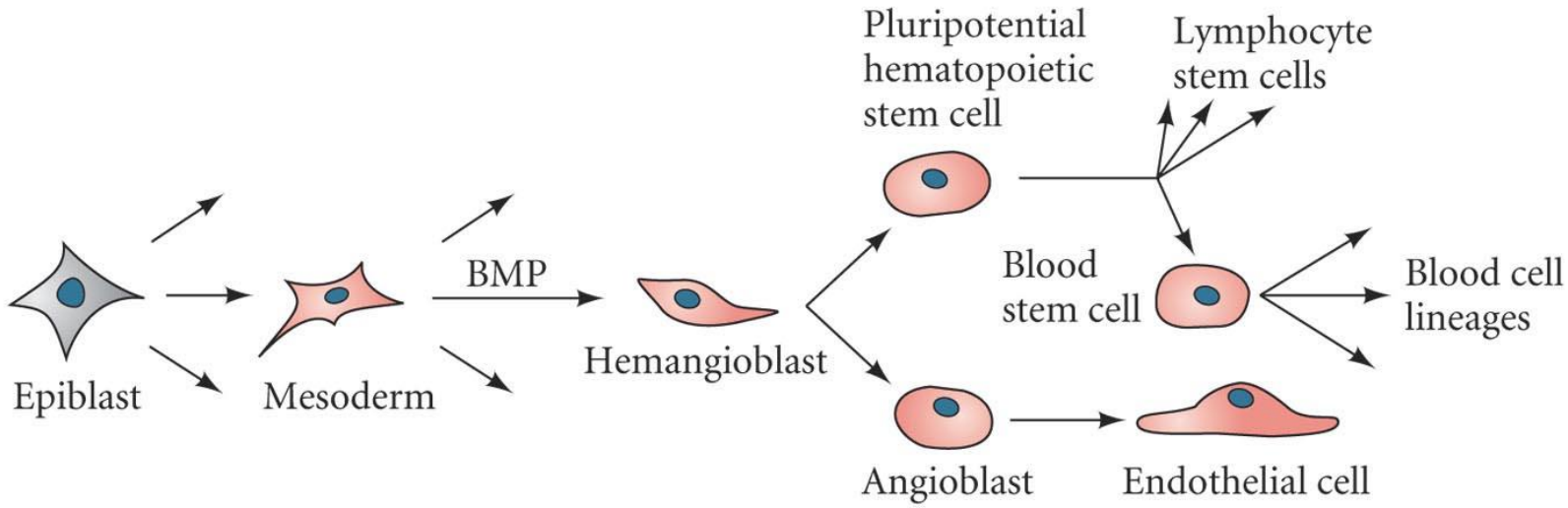
HSCs first arise in the blood islands that form in the yolk sac



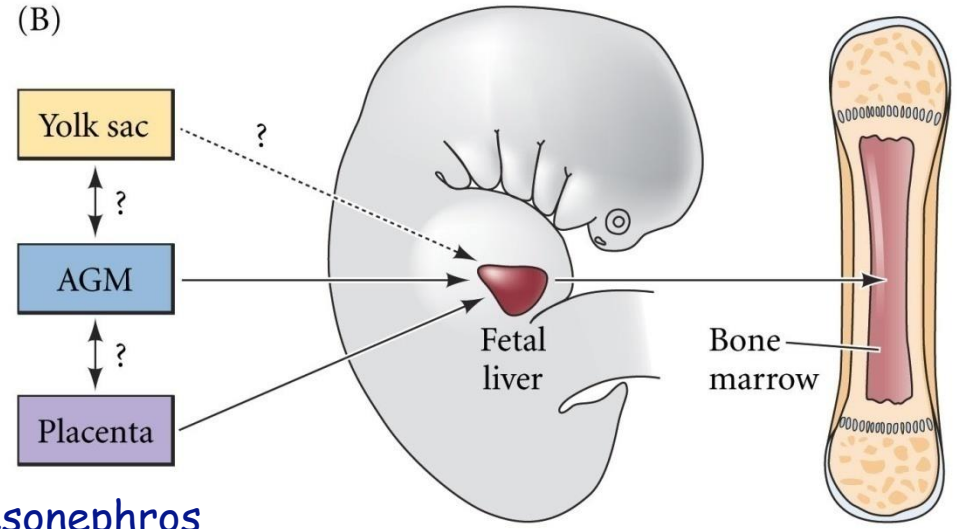


Blood island formation in the wall of the yolk sac

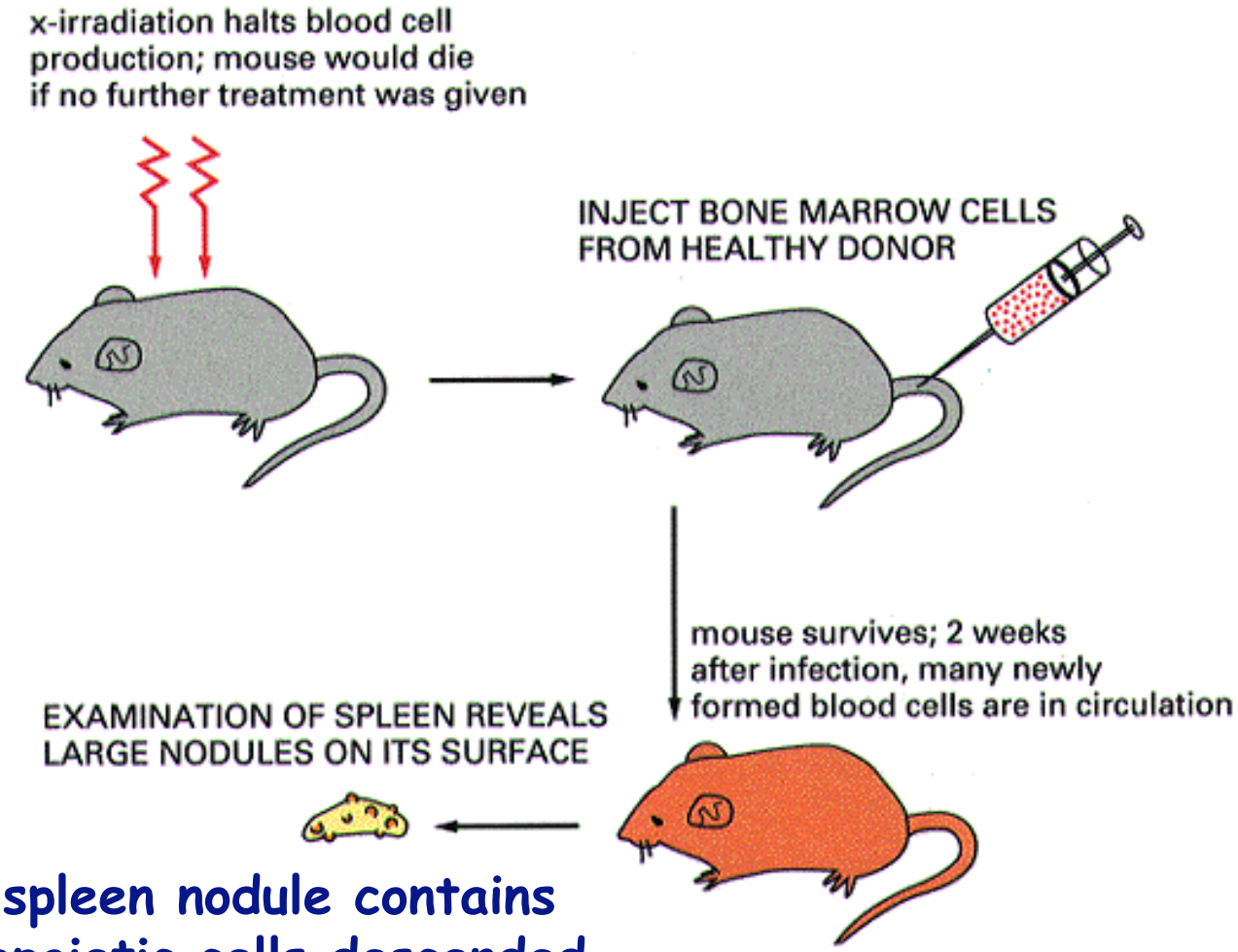
Common origins of HSCs and angioblasts



Source of blood cells to adult bone marrow



AGM= aorta-gonad-mesonephros

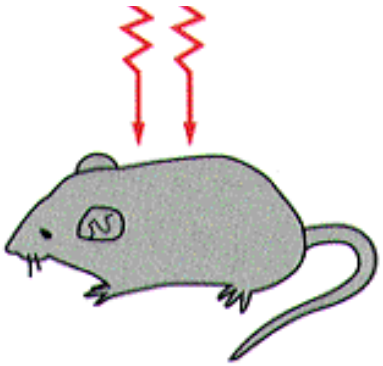


Each spleen nodule contains hematopoietic cells descended from injected bone marrow cells:
Bone marrow cells are stem cells that can make all blood cells

Early studies of the origin of blood cells

How are the different blood types determined?

Do they all derive from a single stem cell?



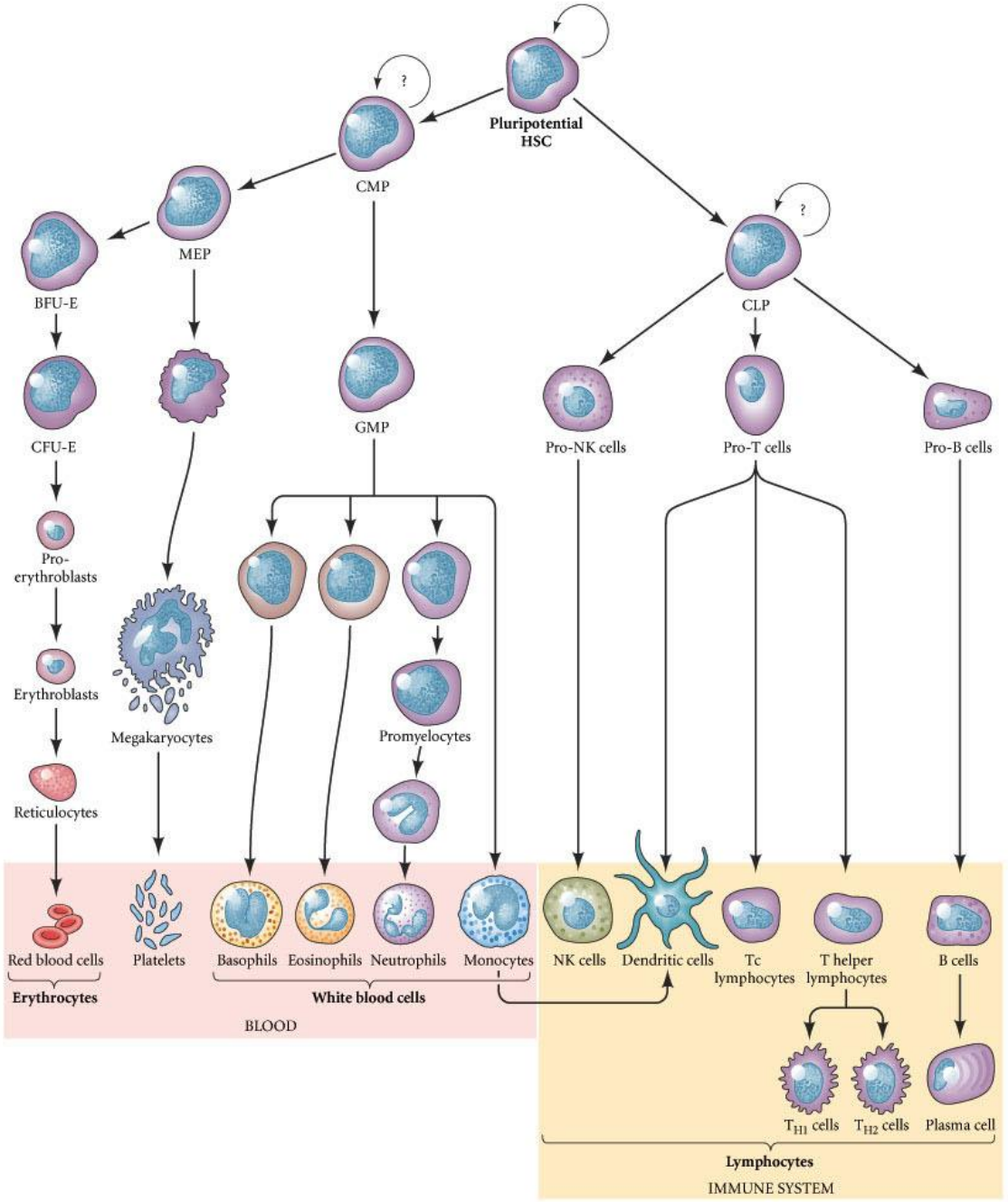
Irradiate an adult mouse (high does X-irradiation). After this adult mouse has been irradiated, it will die very soon unless treated because it

a) has lost all immune system function.
b) can no longer make red blood cells.
c) has suffered major damage to several tissues.
d) has too much cell death in many tissues.

The myeloid and lymphoid cell lineages

There is a common precursor for myeloid and lymphoid cell lineages (it's very rare)

The myeloid and lymphoid stem cells arise from the common HSC



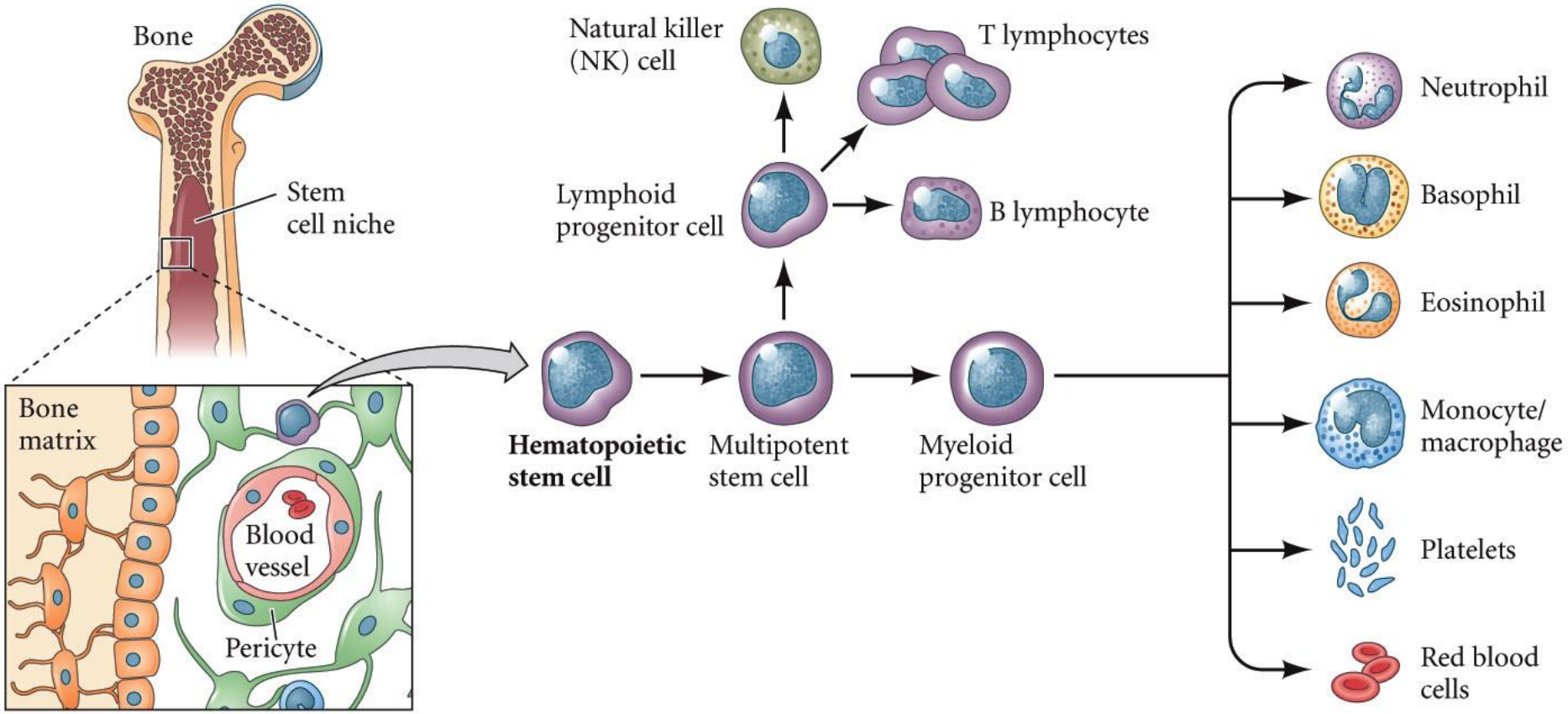
Discussion

- Given the hematopoietic lineages, which cell(s) do you want to isolate for bone marrow transplantation?
- How do you specifically isolate one cell type from the mixed population?

How to isolate HSCs?

- The method should be applicable to live cells.
- The method should enrich the stem cells.
- The method should not require genetic modification.
- You have to identify cell surface markers that are specific to HSCs!

The environment of a stem cell (its "niche") impacts division and differentiation

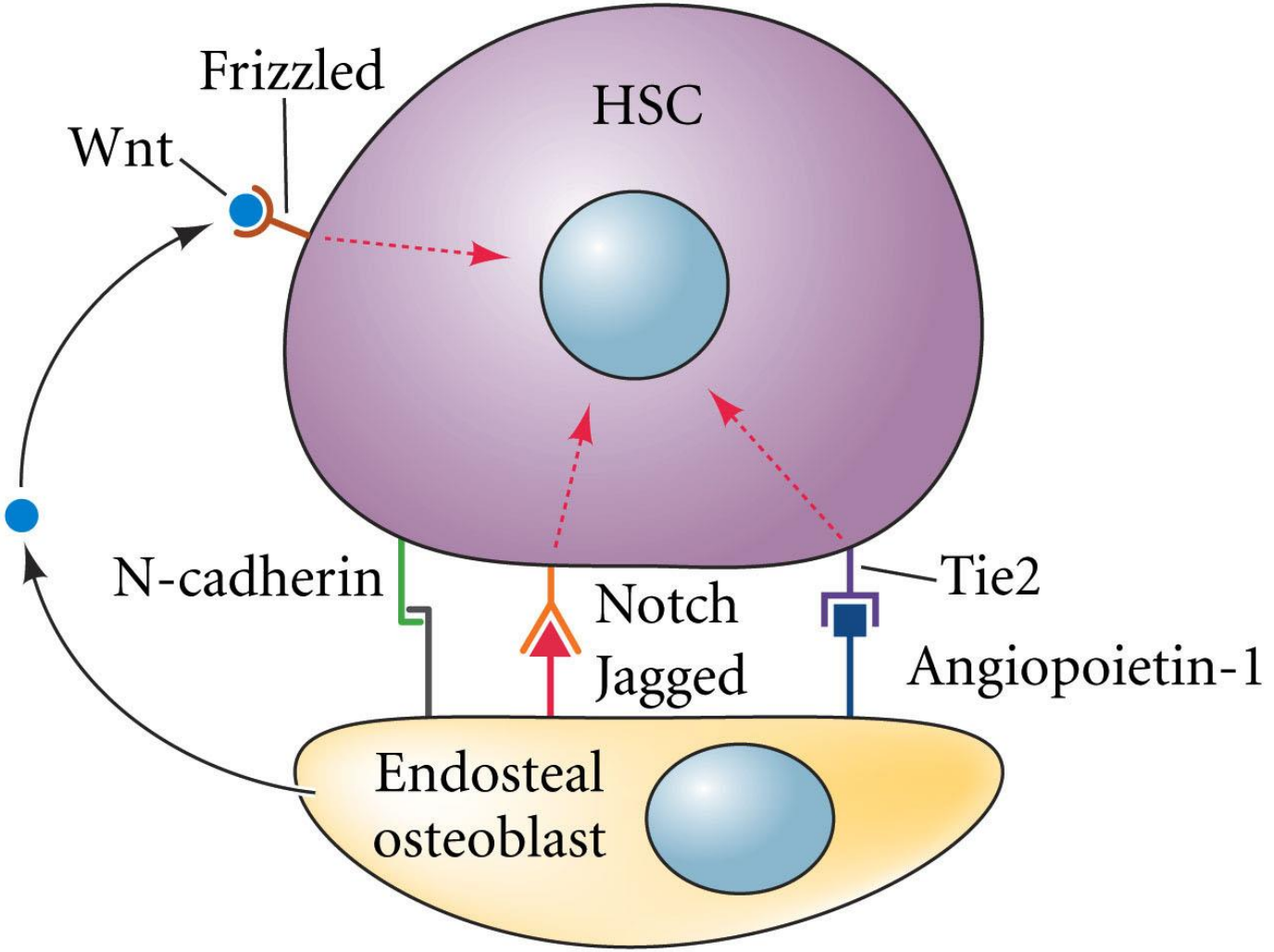


The niche includes signals from surrounding cells

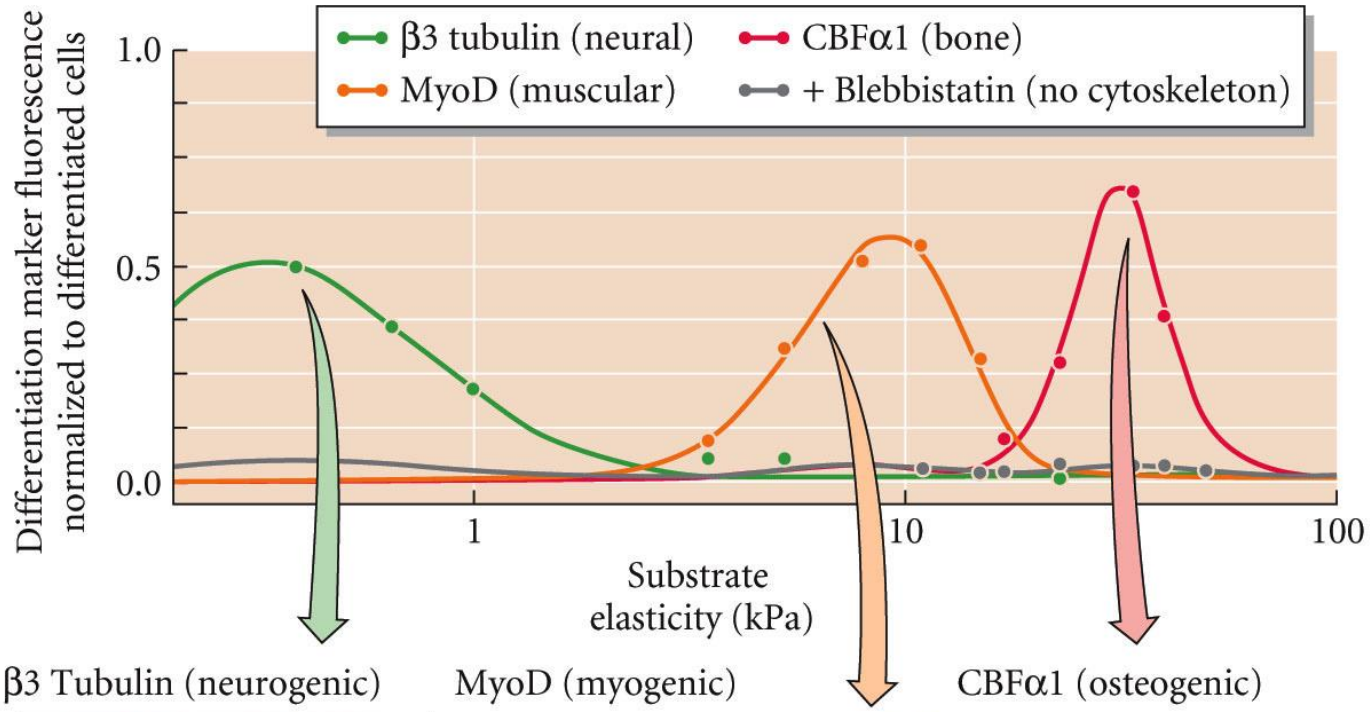
Osteoblasts of the bone provide a "niche" for the HSC to continue dividing

Wnt
Jagged
Angiopoietin

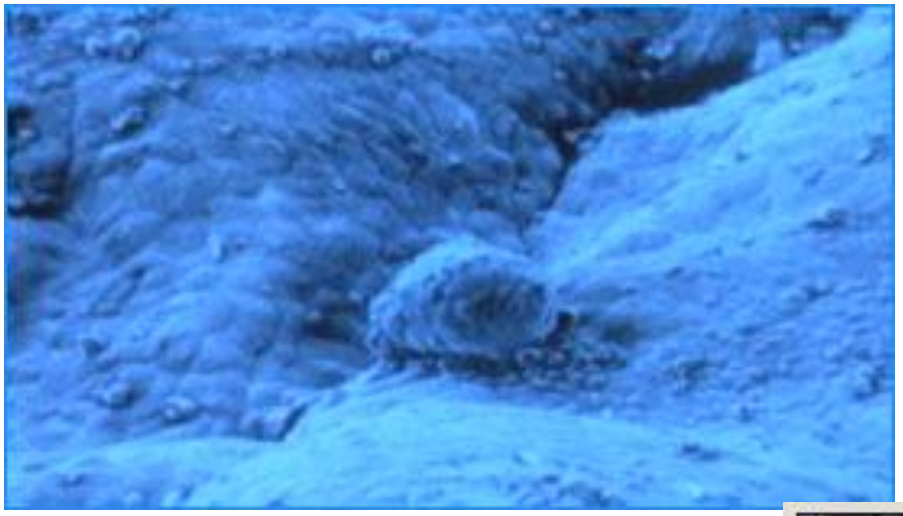
Cytokines (growth factors, interleukins) provide the stimulus for differentiation of different cell types from the HSCs



And from surrounding extracellular matrix components Mesenchymal stem cells (umbilical cord, muscle fat): Differentiation influenced by elasticity of collagen matrix

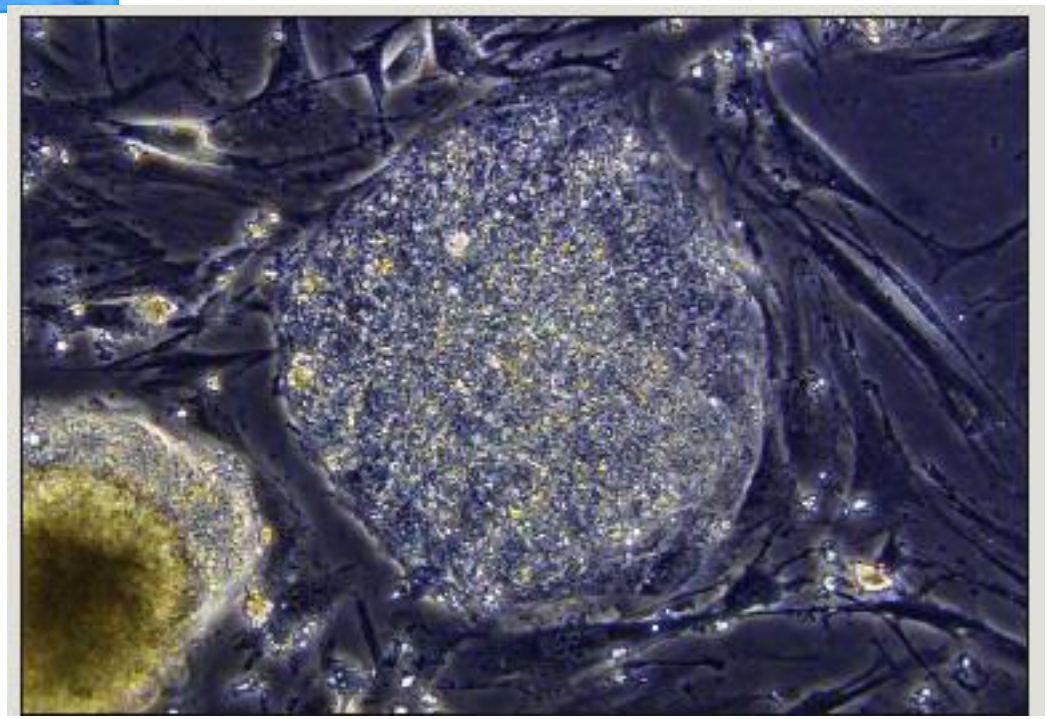


Stem cell research and biomedical applications



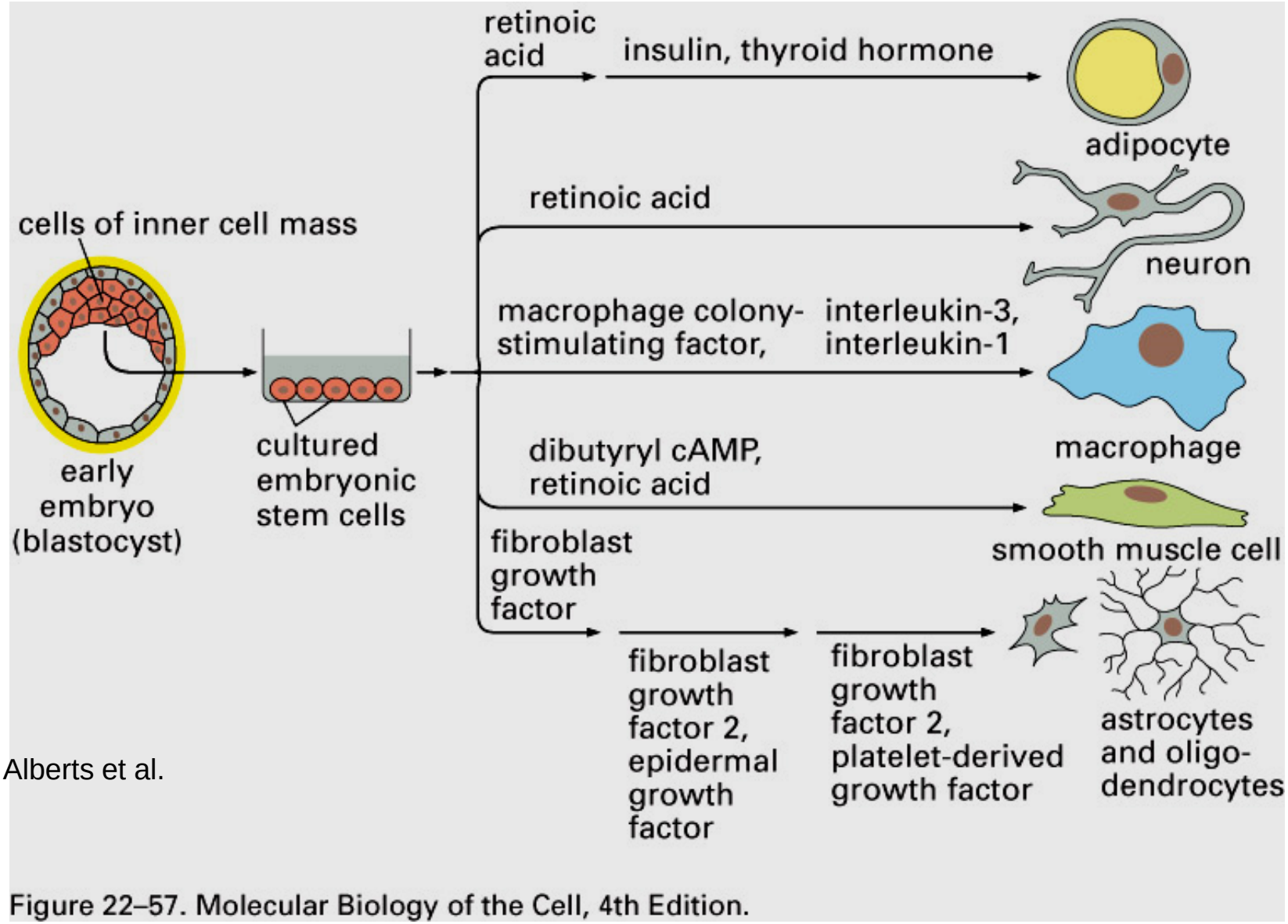
← Human blastocyst on uterine wall

A colony of human ES cells resting on elongated "feeder" cells (irradiated fibroblasts) ↓



Essentially all the technology developed for mouse embryos and ES cells can also be used on human embryos and ES cells.

Differential programming of ES cells with growth factors in vitro



Alberts et al.

Figure 22–57. Molecular Biology of the Cell, 4th Edition.

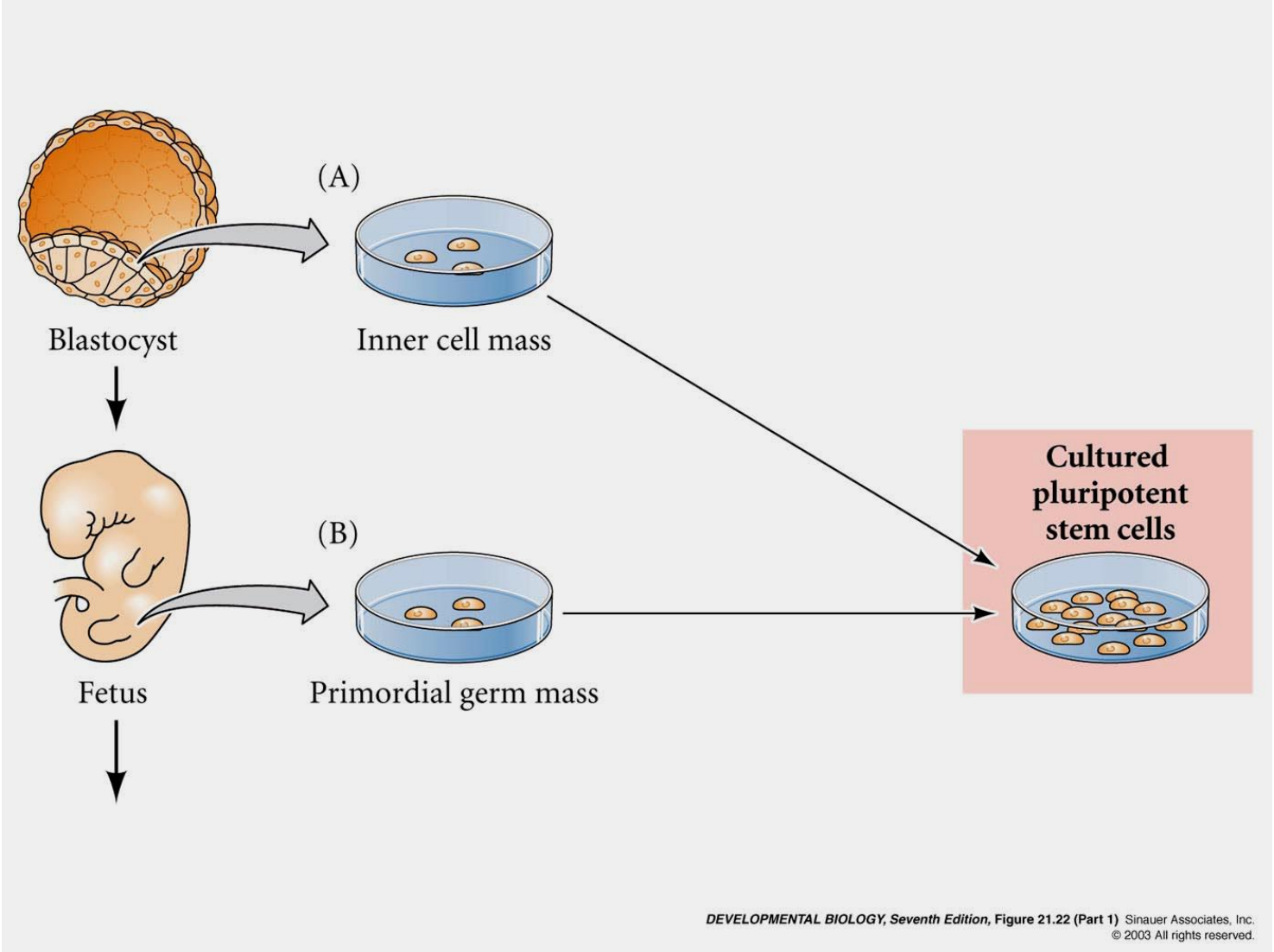
The Three major problems of human tissue engineering with stem cells

Finding an appropriate source of pluripotent stem cells (limited number of embryonic stem cell lines; restrictions on creating new lines with federal funding) with matching MHC loci to avoid rejection

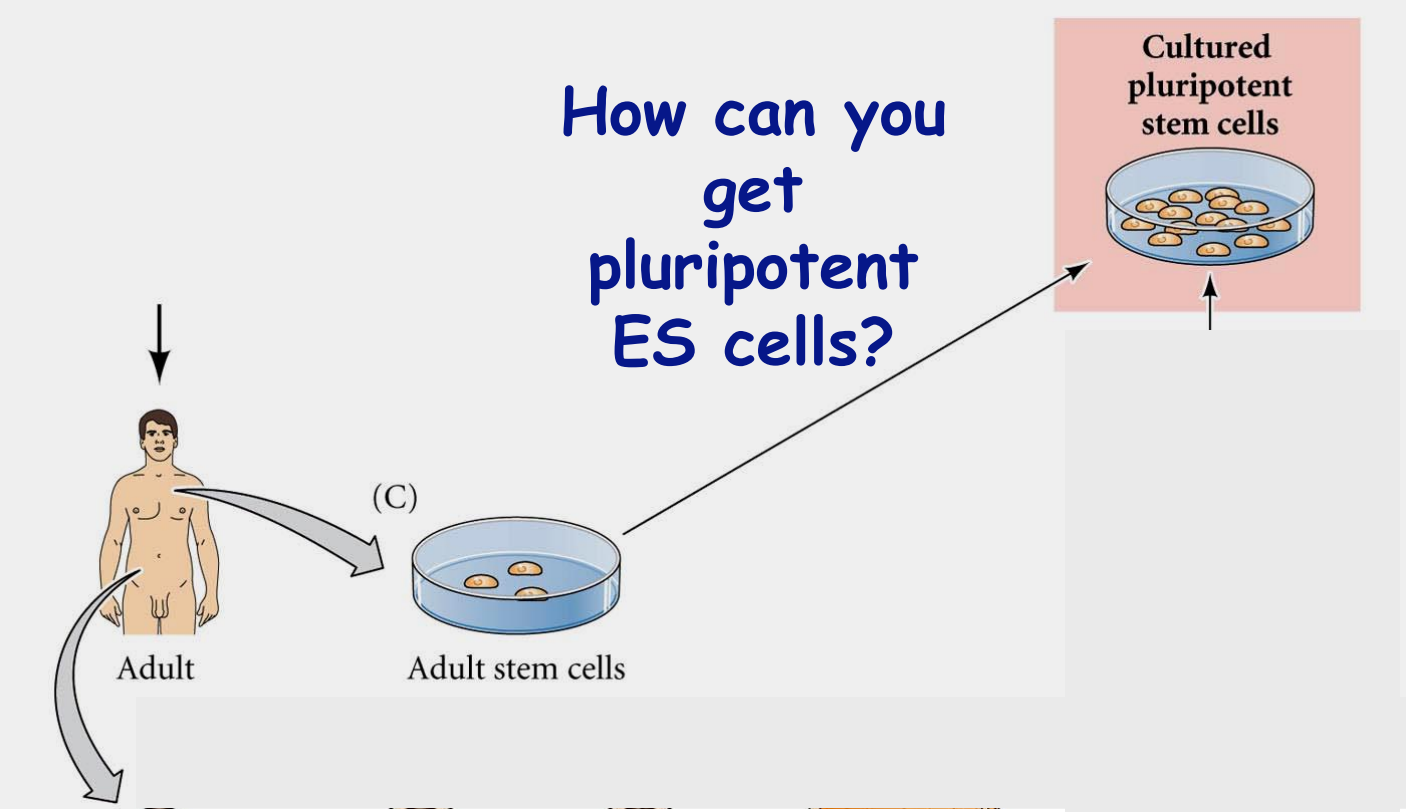
How to differentiate ESCs to the desired cell types?

Safety.

Realistically, we're going to need abundant stem cells to do this kind of treatment: the more pluripotent, the better



What about cells from an adult?



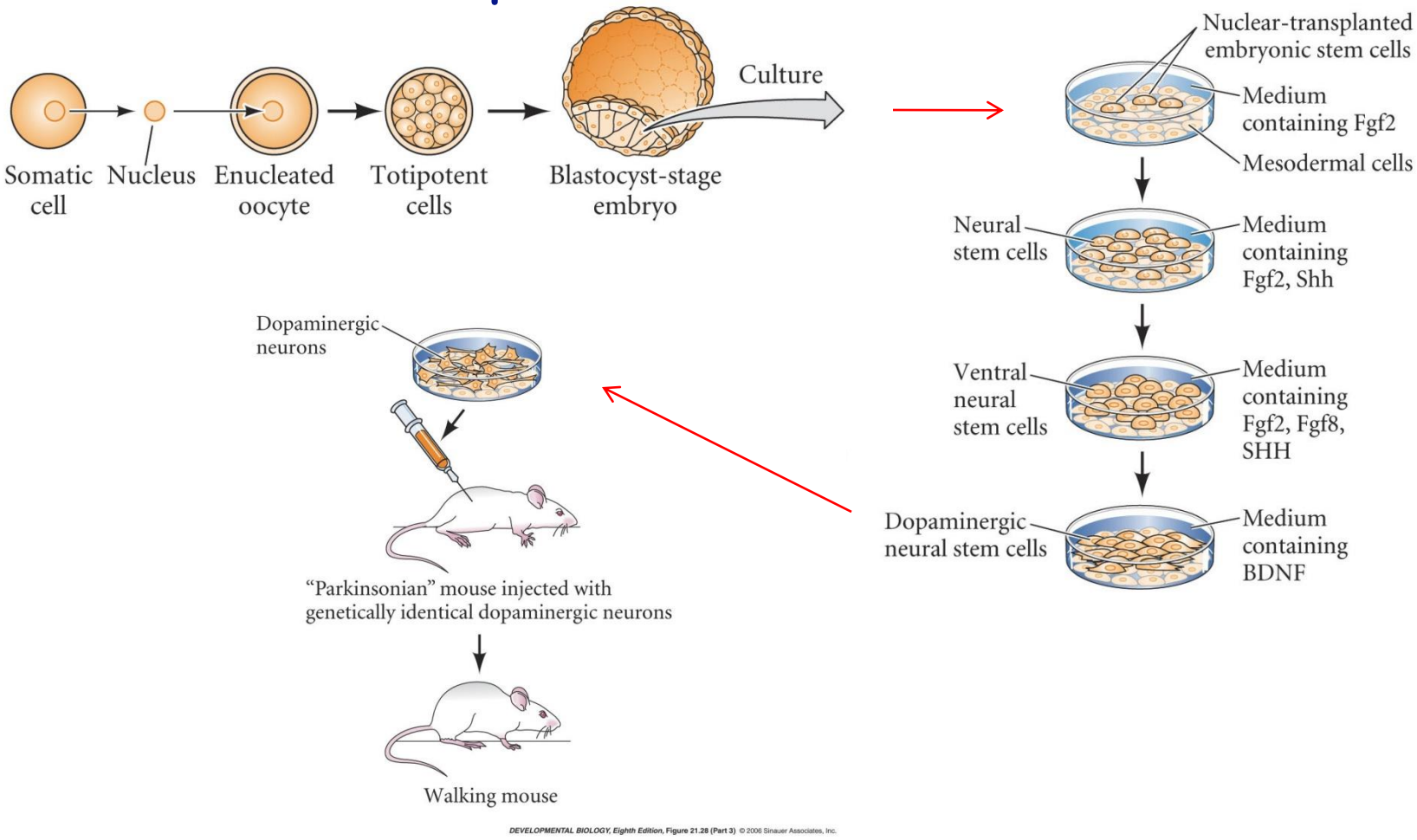
Therapeutic cloning

This differs from reproductive cloning in that the embryo created is not brought to term

(p.s. reproductive cloning is illegal for humans)

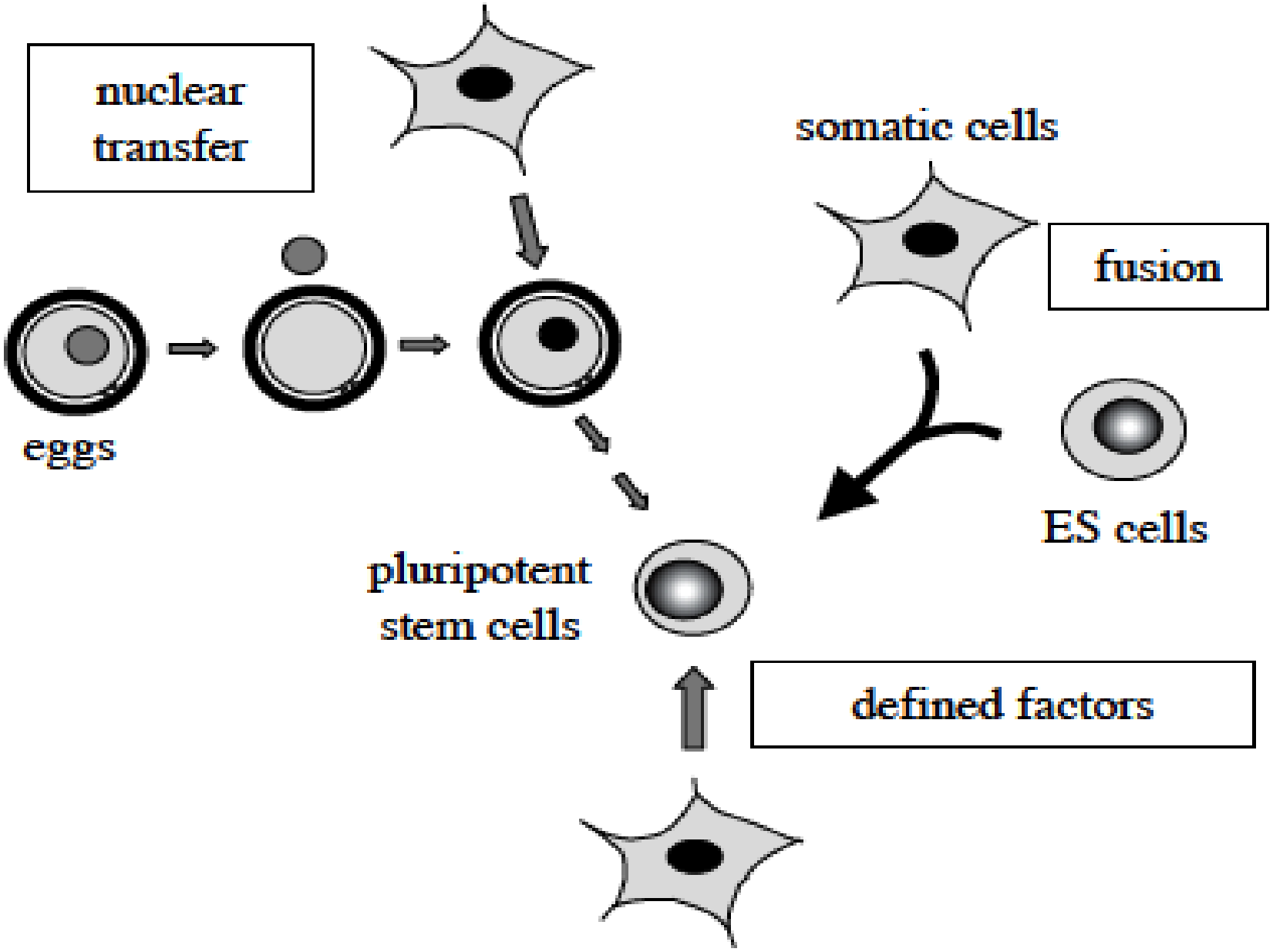
When you have human ES cells that you can use to produce hematopoietic stem cells, which can be injected back into the patient to rescue his production of normal blood cells

This technique has been used with success in mice



These techniques still require the use of an oocyte and cloning… which have their own ethical issues and the efficiency is low (<1%).

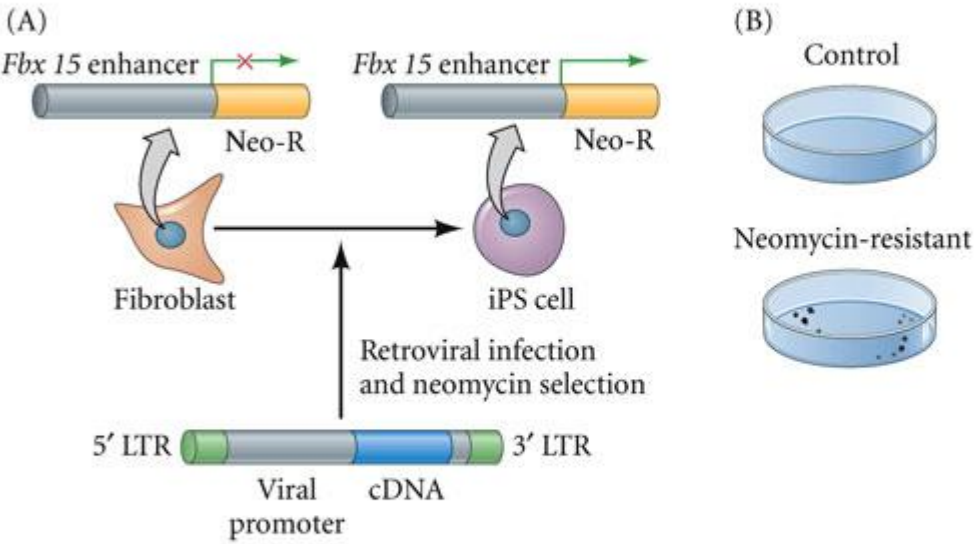
We know that somatic nuclei can be re-programmed by factors in oocyte cytoplasm to produce ES cells.


nuclear transfer
somatic cells
fusion
eggs
ES cells
pluripotent stem cells
defined factors

- April 25, 1953->1962
- (double helix structure of DNA)

- August 25, 2006 -> 2012
- (induced pluripotent stem cells)

Induced Pluripotent Stem Cells
2006



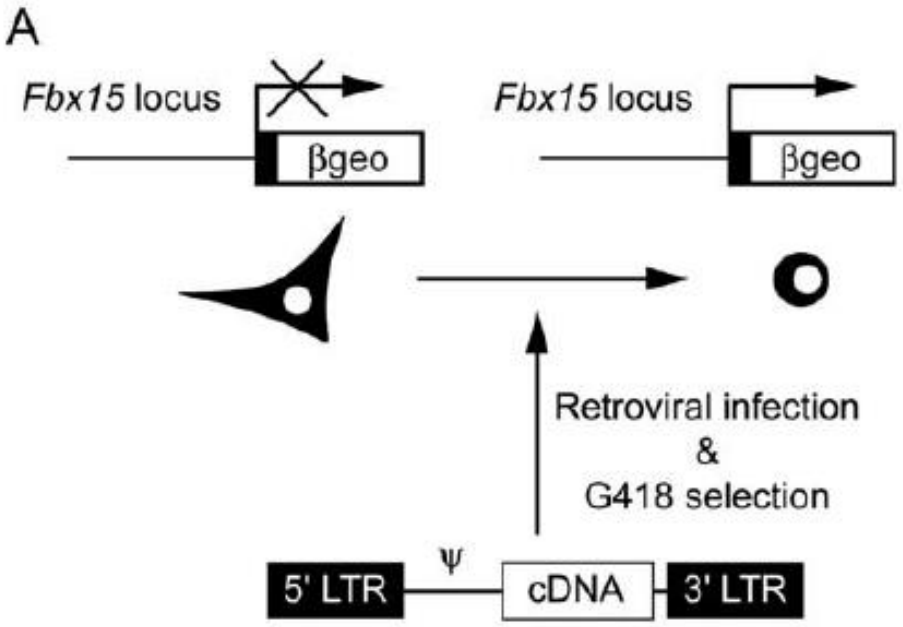
1. Start with mouse fibroblast cells carrying a *neoR* transgene under control of the Fbx15 enhancer promoter, which is activated in ES cells but not in normal fibroblasts
2. Transform cells with viral vectors expressing subsets of the 24 Tx factors characteristic of ES cells.
3. Cells that survive (are neo resistant) do so because Fbx15 has been activated
4. Four genes were required: Oct4, Sox2, Klf4 and C-Myc

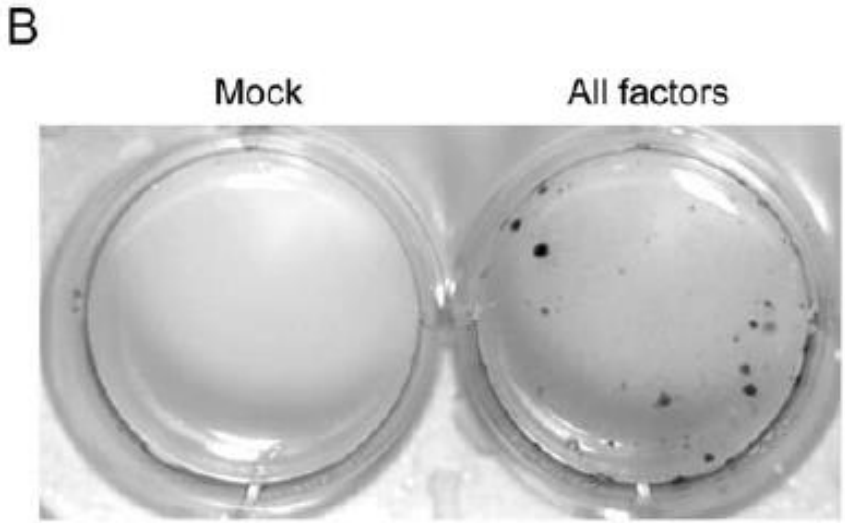
Umbilical cord cells can become iPS cells with only Oct and Sox
Neural stem cells with only Oct

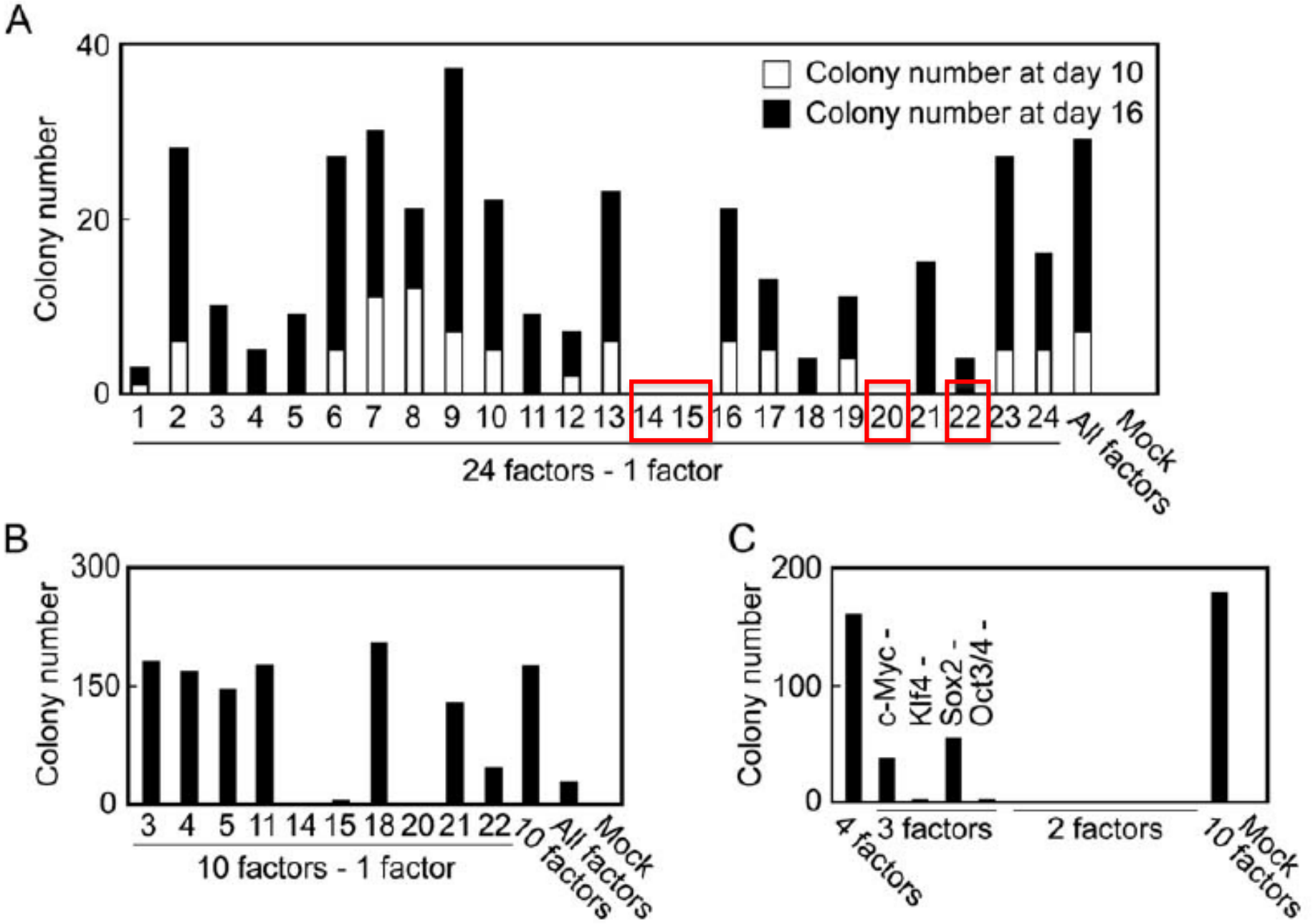
Cell

Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors

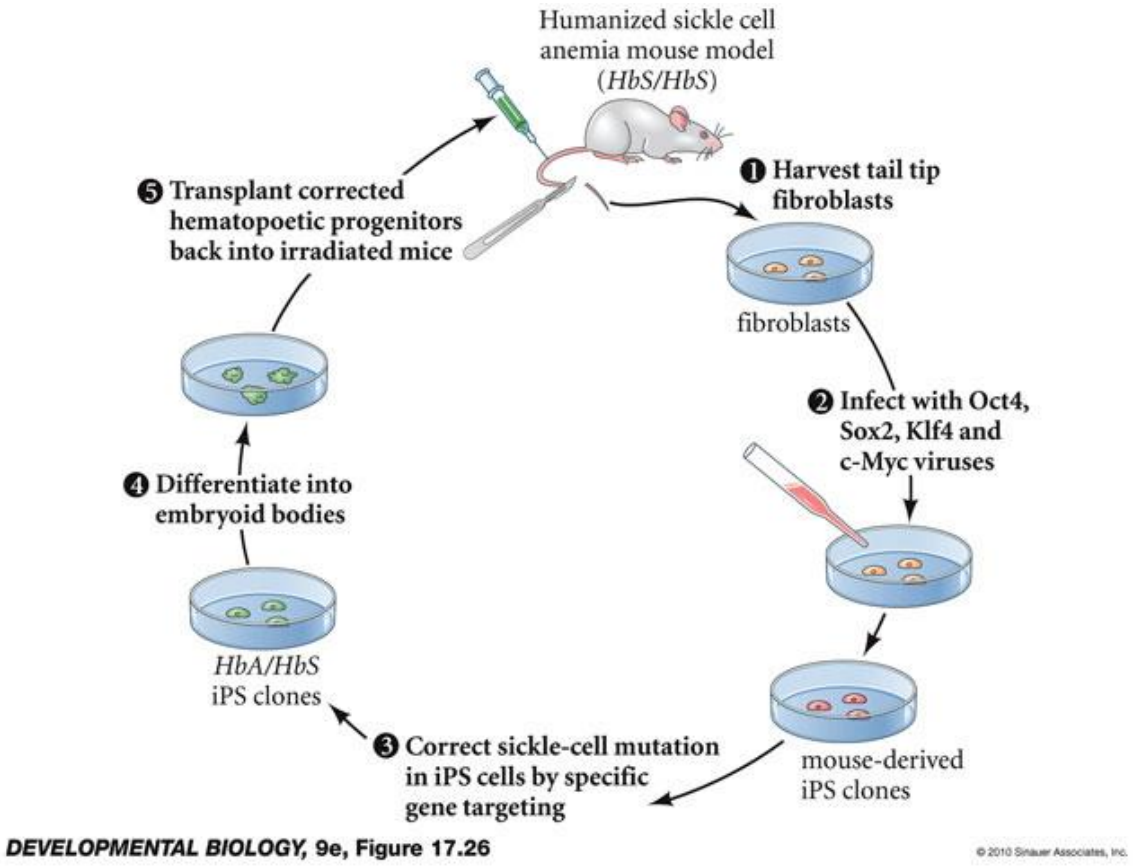
Kazutoshi Takahashi[1] and Shinya Yamanaka[1,2,*]






A
Colony number
40
20
0
Colony number at day 10
Colony number at day 16
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
All factors
Mock
24 factors - 1 factor
B
Colony number
300
150
0
3 4 5 11 14 15 18 20 21 22
10 factors - 1 factor
10 factors
All factors
Mock
C
Colony number
200
100
0
c-Myc -
Klf4 -
Sox2 -
Oct3/4 -
4 factors
3 factors
2 factors
10 factors
Mock

Success with iPS cells in animal model



DEVELOPMENTAL BIOLOGY, 9e, Figure 17.26

Pluripotent Stem Cells Induced from Mouse Somatic Cells by Small-Molecule Compounds
Pingping Hou *et al.*
Science **341**, 651 (2013);
DOI: 10.1126/science.1239278

LETTER

doi:10.1038/nature09915

Modelling schizophrenia using human induced pluripotent stem cells

Kristen J. Brennand[1], Anthony Simone[1]*, Jessica Jou[1]*, Chelsea Gelboin-Burkhart[1]*, Ngoc Tran[1]*, Sarah Sangar[1], Yan Li[1], Yangling Mu[1], Gong Chen[2], Diana Yu[1], Shane McCarthy[3], Jonathan Sebat[4] & Fred H. Gage[1]

Discussion

- So when ES factors are identified, it is possible to reprogram somatic cells directly and convert them to pluripotent stem cells without cloning.
- What does this imply about any cell types?

Direct Reprogramming of Fibroblasts into Functional Cardiomyocytes by Defined Factors

Masaki Ieda,[1,2,3,6,*] Ji-Dong Fu,[1,2,3] Paul Delgado-Olguin,[1,2,4] Vasanth Vedantham,[1,5] Yohei Hayashi,[1] Benoit G. Bruneau,[1,2,4] and Deepak Srivastava[1,2,3,*]