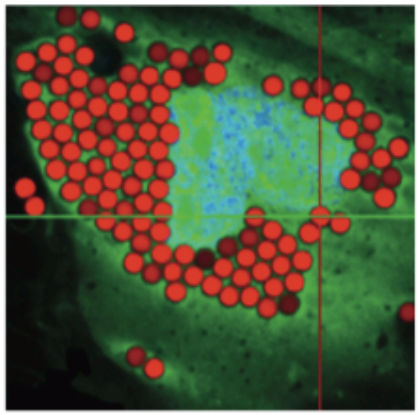
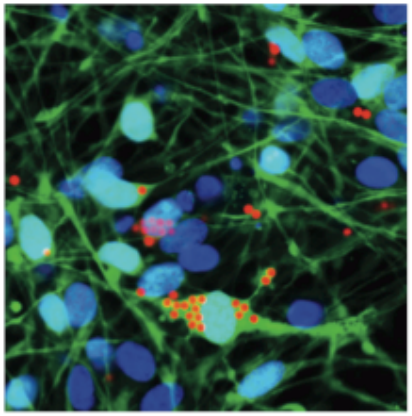
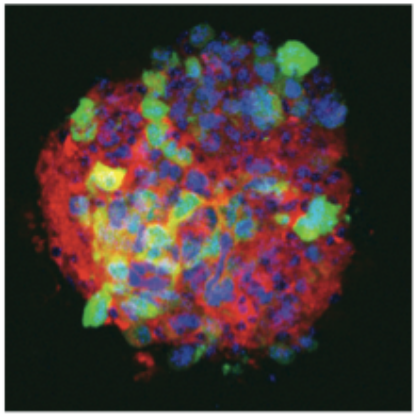
Principles of Stem Cell Biology

A one-day lecture course on what stem cells are, how they behave, how they are regulated, and how they can be used clinically.

NORWEGIAN CENTER FOR
STEM CELL RESEARCH

"Principles of Stem Cell Biology" is offered by the Norwegian Center for Stem Cell Research (www.stemcellnorway.org)

STEM CELLS - BASIC CONCEPTS

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http://stemcells.nih.gov/info/basics/
http://www.stemcellresearchfoundation.org
http://www.stemcellnorway.org

WHAT IS A STEM CELL?

A cell that can undergo self-renewing (expanding) proliferation and give rise to specialized differentiated cells

3 CONCEPTUAL CATEGORIES

Embryonic

Somatic

Tumor

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Embryonic
Found in blastocyst stage embryos, can generate all tissues of the body

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Tumor

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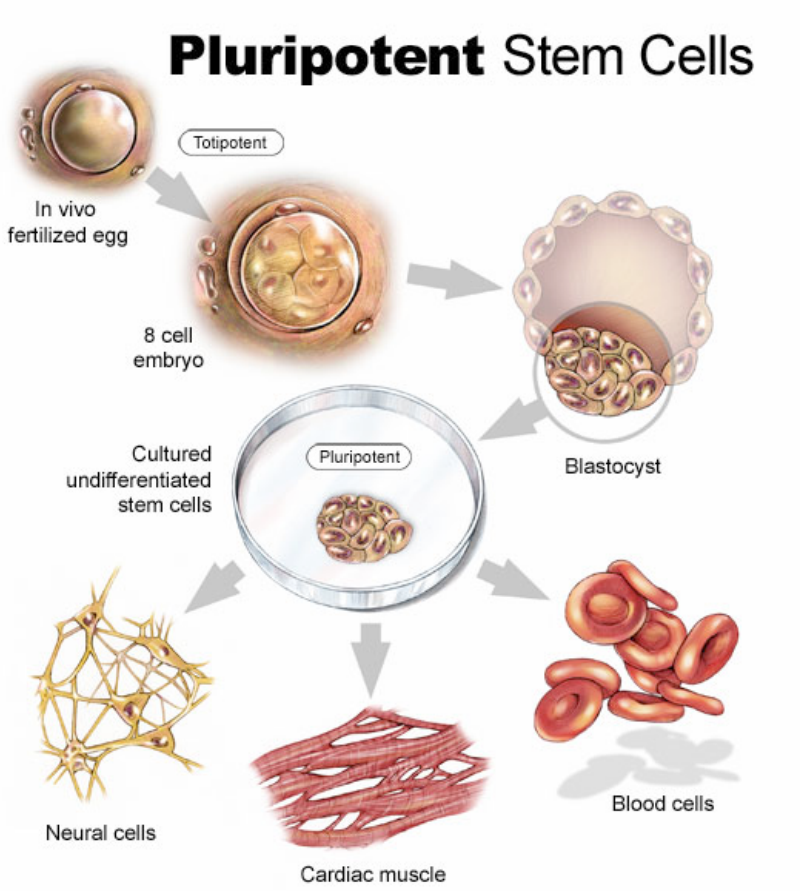
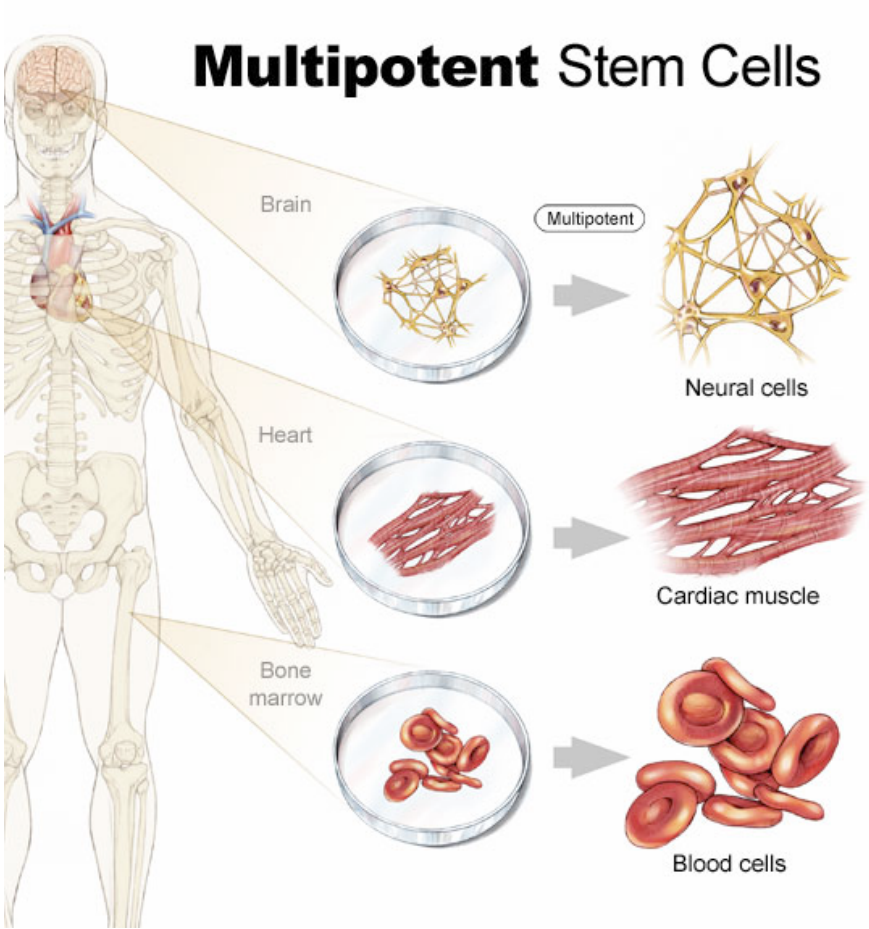
Found in fully-formed organs, can generate multiple cell types characteristic of organ of origin.

Tumor
Found in tumors, can reconstitute new tumors of same type, presumed source of metastases, controversial

THE CONCEPT OF STEM CELL POTENCY

Totipotent (entire body)	fertilized egg first few blastomeres
Pluripotent (most - all cell types)	embryonic stem cells embryonic germ cells embryonal carcinoma cells
Multipotent (several cell types)	somatic stem cells


Pluripotent Stem Cells
Totipotent
In vivo
fertilized egg
8 cell
embryo
Blastocyst
Cultured
undifferentiated
stem cells
Pluripotent
Neural cells
Cardiac muscle
Blood cells


Multipotent Stem Cells
Brain
Multipotent
Neural cells
Heart
Cardiac muscle
Bone
marrow
Blood cells

3 CONCEPTUAL CATEGORIES

Embryonic
Found in blastocyst stage embryos, can generate all tissues of the body

Somatic
Found in fully-formed organs, can generate multiple cell types characteristic of organ of origin.

Tumor
Found in tumors, can reconstitute new tumors of same type, presumed source of metastases, controversial

HISTORICAL PERSPECTIVE

Fertilized egg + first few blastomeres are totipotent
Separated blastomere experiments of Driesch 1892

Embryonic stem cells first isolated from mouse blastocysts by Martin and Evans & Kaufman 1981
“inner cell mass”
established as expandable cell lines, are pluripotent
allowed for the generation of transgenic mice

Embryonic stem cells first isolated from human blastocysts by Thomson et al, Gearhart et al 1998
Established as expandable cell lines (first USA, now many countries including Sweden)
Requires use of human blastocysts, obtained in connection with *in vitro* fertilization for couples with fertility problems

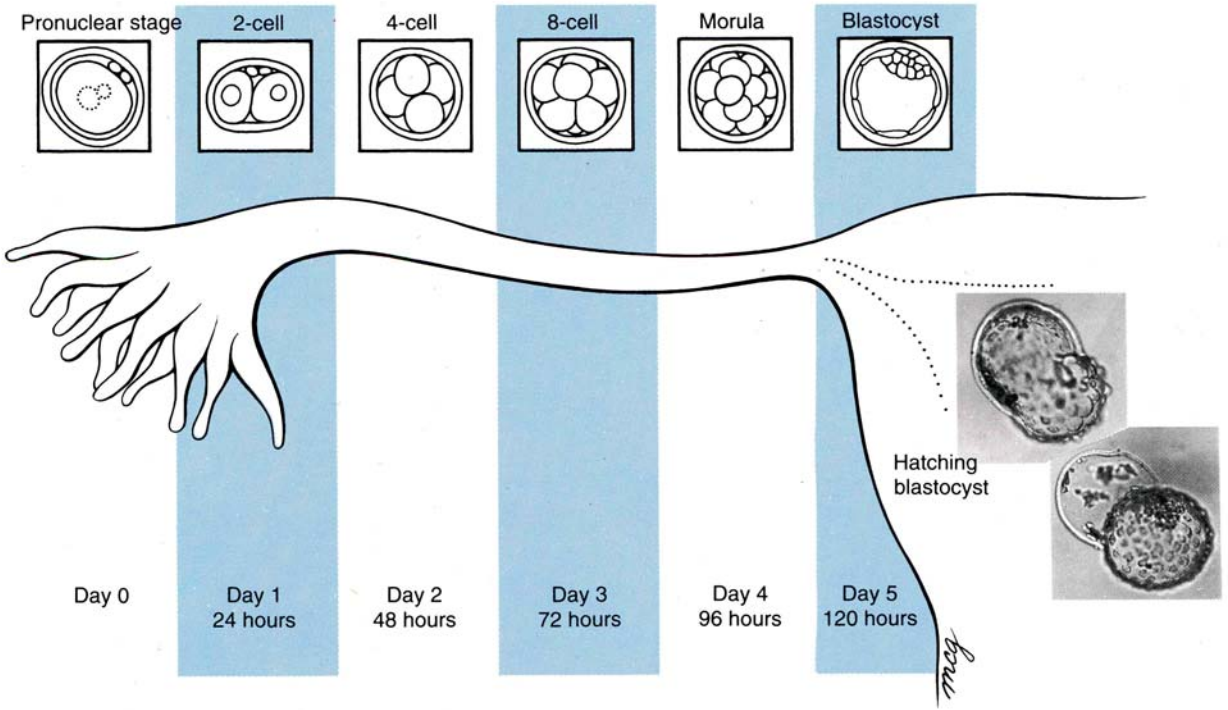


Fig. 1-11. Cleavage and transport down the oviduct. Fertilization occurs in the ampulla of the oviduct. During the first five days, the zygote undergoes cleavage as it travels down the oviduct and enters the uterus. On day 5, the blastocyst hatches from the zona pellucida and is then able to implant in the uterine endometrium. (From Boatman DE. 1987. In vitro growth of non-human primate pre- and peri-implantation embryos. p 273. In Bavister BD (ed): The Mammalian Preimplantation Embryo. Plenum, NY, with permission. Photos courtesy of Drs. Barry Bavister and D.E. Boatman.)

fra Larsen, 1. utg.

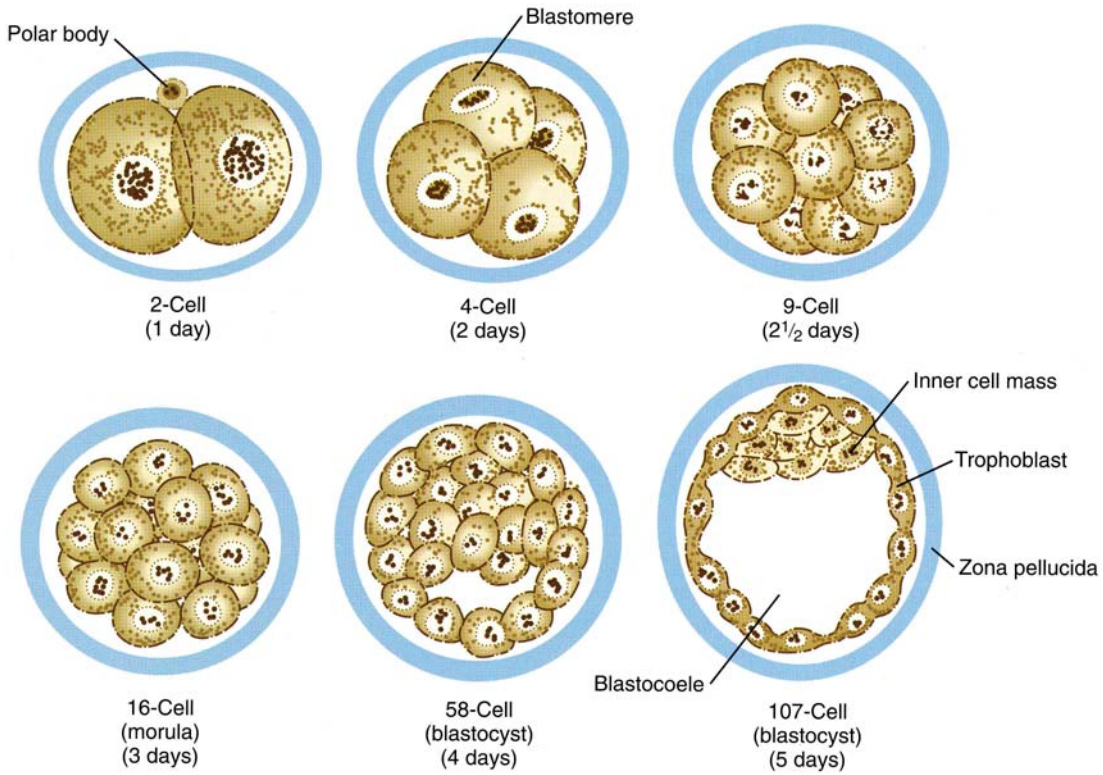


FIGURE 3-1 Drawings of early cleavage stages in human embryos. The drawings of the 58- and 107-cell stages represent sections made through the embryos.

fra Carlson, 3. utg.

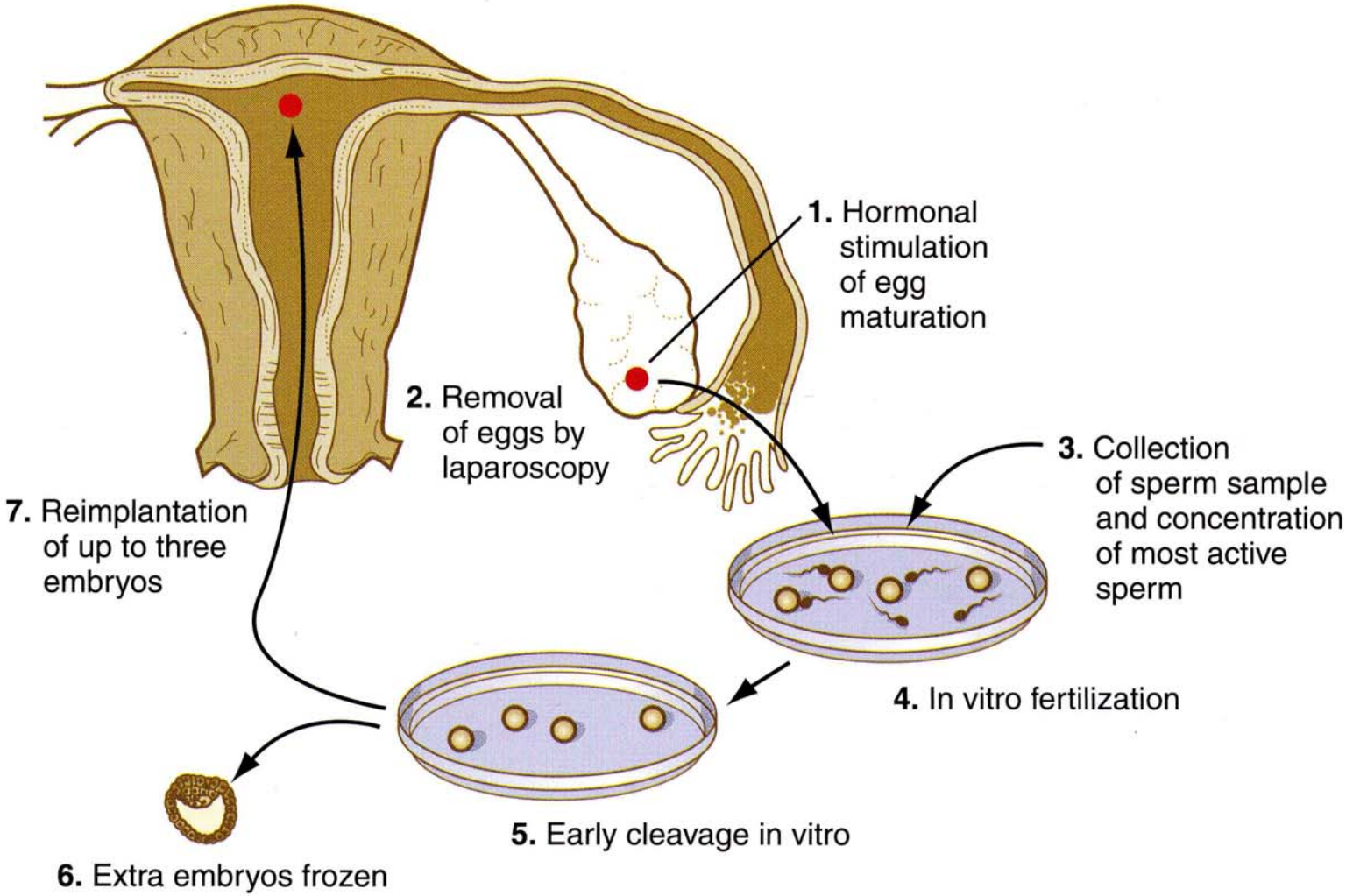


FIGURE 2-7 Schematic representation of a typical in vitro fertilization and embryo transfer procedure in humans.

In vitro fertilization – typical procedure

fra Carlson, 3. utg.

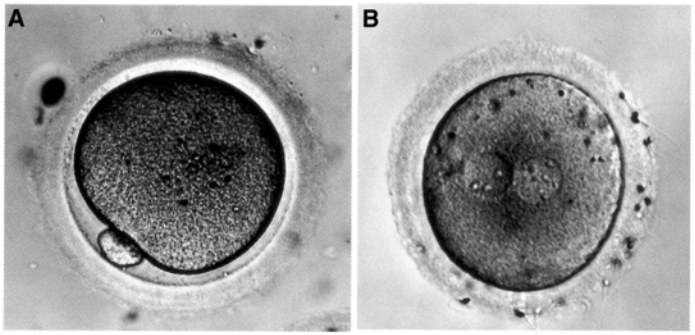


FIGURE 2-9 **A,** Photomicrograph of a mature human oocyte arrested at metaphase II. This oocyte will be fertilized in vitro. **B,** Photomicrograph of a human oocyte newly fertilized in vitro. Two pronuclei are visible. (From Veeck LL: *Atlas of the human oocyte and early conceptus*, vol 2, Baltimore, 1991, Williams & Wilkins.)

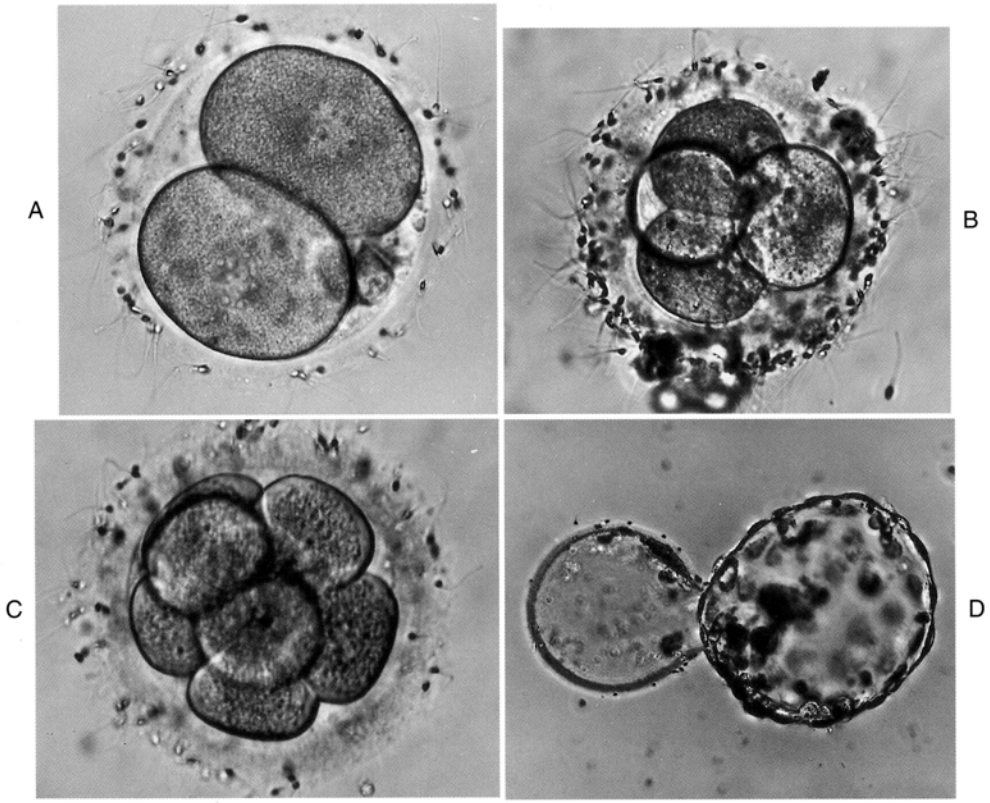


FIGURE 3-2 Photomicrographs of cleavage stages of human eggs fertilized in vitro. **A,** Two blastomeres 39 hours after fertilization. A polar body is seen to the right of the boundary between the blastomeres. **B,** Four blastomeres 42 hours after fertilization. **C,** Eight blastomeres 49 hours after fertilization. **D,** Hatching blastocyst 123 hours after fertilization. The empty zona pellucida is on the left. In **A** to **C,** numerous spermatozoa can be seen clinging to the zona pellucida. (From Veeck LL: *Atlas of the human oocyte and early conceptus*, vol 2, Baltimore, 1991, Williams & Wilkins.)

fra Carlson, 3. utg.

Embryonic Stem Cell Lines Derived from Human Blastocysts

James A. Thomson,* Joseph Itskovitz-Eldor, Sander S. Shapiro, Michelle A. Waknitz, Jennifer J. Swiergiel, Vivienne S. Marshall, Jeffrey M. Jones

Human blastocyst-derived, pluripotent cell lines are described that have normal karyotypes, express high levels of telomerase activity, and express cell surface markers that characterize primate embryonic stem cells but do not characterize other early lineages. After undifferentiated proliferation in vitro for 4 to 5 months, these cells still maintained the developmental potential to form trophoblast and derivatives of all three embryonic germ layers, including gut epithelium (endoderm); cartilage, bone, smooth muscle, and striated muscle (mesoderm); and neural epithelium, embryonic ganglia, and stratified squamous epithelium (ectoderm). These cell lines should be useful in human developmental biology, drug discovery, and transplantation medicine.

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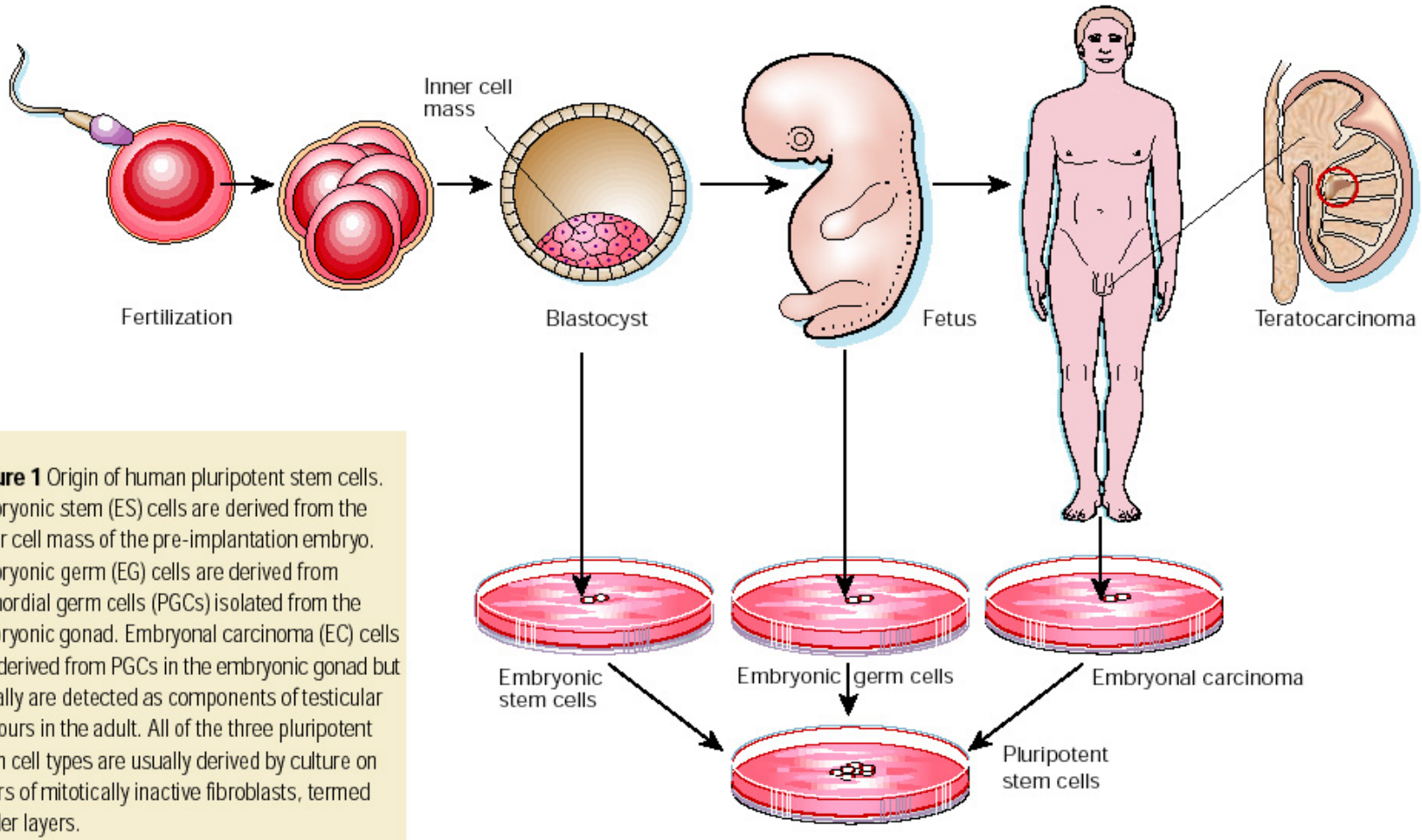

Figure 1 Origin of human pluripotent stem cells. Embryonic stem (ES) cells are derived from the inner cell mass of the pre-implantation embryo. Embryonic germ (EG) cells are derived from primordial germ cells (PGCs) isolated from the embryonic gonad. Embryonal carcinoma (EC) cells are derived from PGCs in the embryonic gonad but usually are detected as components of testicular tumours in the adult. All of the three pluripotent stem cell types are usually derived by culture on layers of mitotically inactive fibroblasts, termed feeder layers.

Embryonic stem cells: example of a potential use

Embryonic stem cells develop into functional dopaminergic neurons after transplantation in a Parkinson rat model

Lars M. Björklund*†‡, Rosario Sánchez-Pernaute*†§, Sangmi Chung*¶, Therese Andersson*¶‖, Iris Yin Ching Chen§, Kevin St. P. McNaught*†, Anna-Liisa Brownell*§, Bruce G. Jenkins§, Claes Wahlestedt‖, Kwang-Soo Kim*¶, and Ole Isacson*†‡**

*Udall Parkinson's Disease Research Center of Excellence, †Neuroregeneration Laboratories, and ¶Molecular Neurobiology Laboratory, McLean Hospital/Harvard Medical School, 115 Mill Street, Belmont, MA 02478; Departments of §Radiology and **Neurology, Massachusetts General Hospital and Program in Neuroscience, Harvard Medical School, Boston, MA 02114; and ‖Karolinska Institute, SE-17177 Stockholm, Sweden

Edited by Gerald D. Fischbach, Columbia University College of Physicians and Surgeons, New York, NY, and approved November 29, 2001 (received for review August 20, 2001)

2344–2349 | PNAS | February 19, 2002 | vol. 99 | no. 4

Efficient production of mesencephalic dopamine neurons by Lmx1a expression in embryonic stem cells

Stina Friling[a,1], Elisabet Andersson[b,1], Lachlan H. Thompson[c], Marie E. Jönsson[c], Josephine B. Hebsgaard[c], Evanthia Nanou[d], Zhanna Alekseenko[b], Ulrika Marklund[b], Susanna Kjellander[a], Nikolaos Volakakis[a], Outi Hovatta[e], Abdeljabbar El Manira[d], Anders Björklund[c], Thomas Perlmann[a,b,2], and Johan Ericson[b,2]

[a]The Ludwig Institute for Cancer Research and [b]Departments of Cell and Molecular Biology, [d]Neuroscience, and [e]Biosciences and Nutrition, Karolinska Institutet, 17177 Stockholm, Sweden; and [c]Wallenberg Neuroscience Center, Lund University, 221 84 Lund, Sweden

Communicated by Thomas M. Jessell, Columbia University College of Physicians and Surgeons, New York, NY, March 13, 2009 (received for review December 10, 2008)

PNAS | May 5, 2009 | vol. 106 | no. 18 | 7613–7618

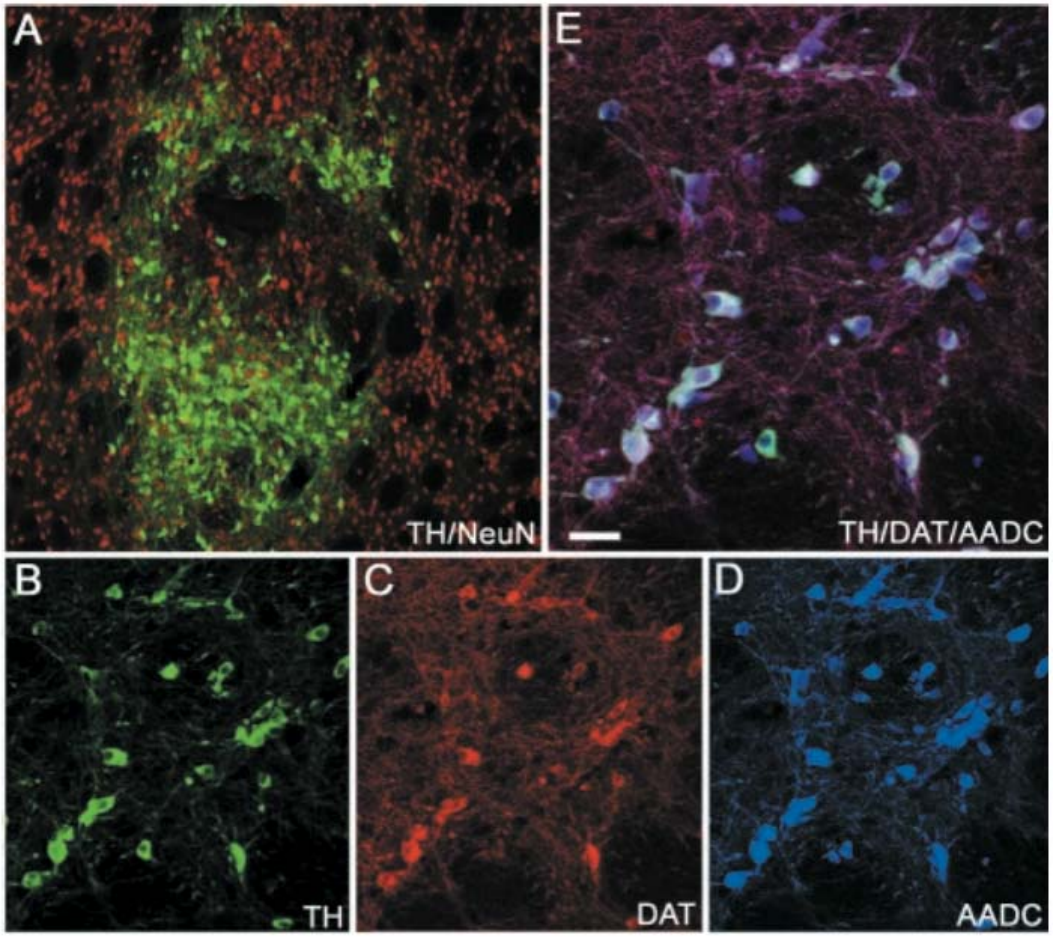

Fig. 1. Immunohistochemical staining of a graft 16 weeks after implantation of a low concentration (1,000–2,000 cells per μl) of D3 ES cells into adult 6-OHDA lesioned striatum. Numerous TH-positive neurons were found within the graft (*A* and *B*, green). All TH-positive profiles coexpressed the neuronal marker NeuN (*A*, red). TH (*B*) also was coexpressed with DAT (*C*, red) and AADC (*D*, blue), demonstrated by white triple labeling (*E*). (Scale bars: *A*, 150 μm; *B–D*, 50 μm; *E*, 25 μm.)

Bjørklund et al (2002) PNAS 99:2344-2349

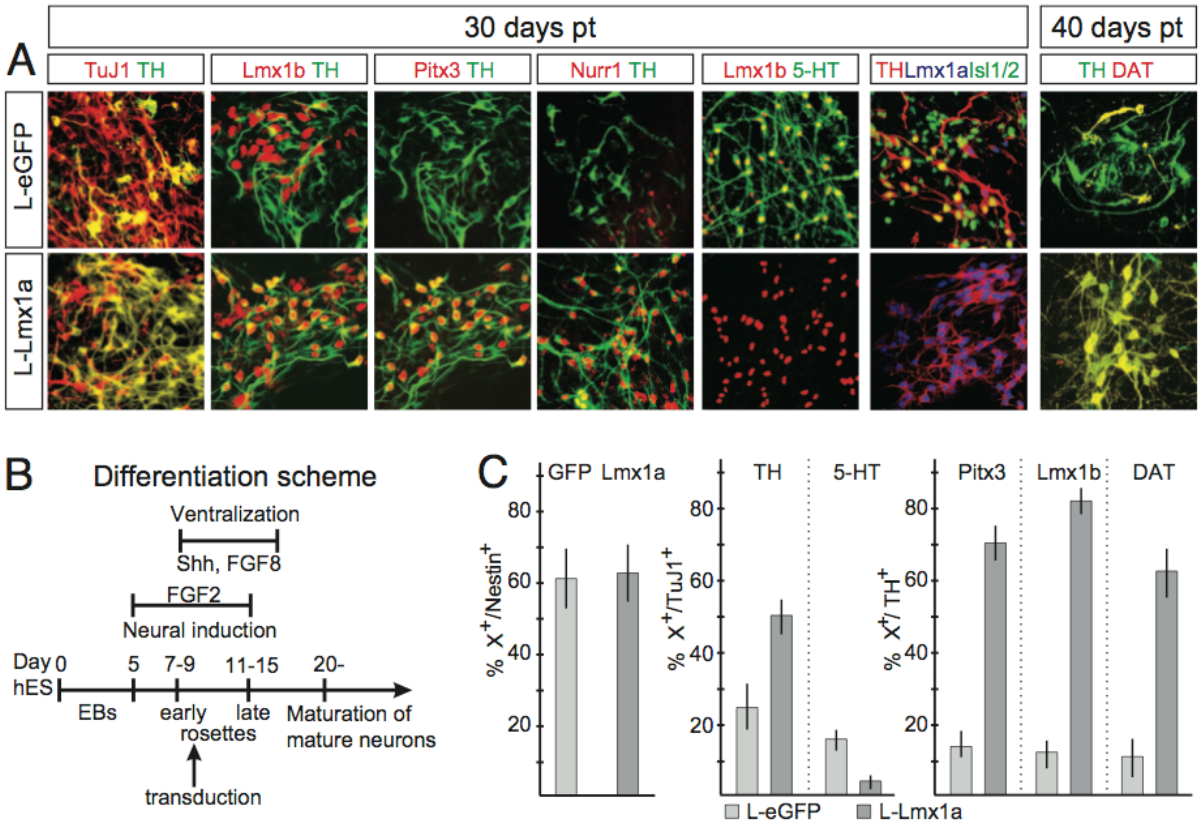

Fig. 5. Lmx1a promotes mesDA^{hES} neurons in differentiating hESCs. hESC-derived neuroepithelial progenitors were infected with lentiviral (*L*) vectors carrying Lmx1a or eGFP and analyzed at day 30 to 40 pt. (*A*) In L-Lmx1a-infected cultures, >50% of TuJ1$^+$ neurons co-expressed TH at d 30 pt, compared with 25% in L-eGFP-infected cultures. Most TH$^+$ neurons co-expressed mesDA markers, e.g., Lmx1b, Pitx3, Nurr1, and DAT, whereas markers for 5-HT neurons were suppressed. Few TH$^+$ neurons derived from L-eGFP-infected cells co-expressed mesDA markers. (*B*) Differentiation scheme. (*C*) Quantification of marker expression. Error bars indicate SD, $n = 4$.

Friling et al (2009) PNAS 106:7613-7618

Embryonic stem cells: example of a potential use

4694 • The Journal of Neuroscience, May 11, 2005 • 25(19):4694 – 4705

Development/Plasticity/Repair

Human Embryonic Stem Cell-Derived Oligodendrocyte Progenitor Cell Transplants Remyelinate and Restore Locomotion after Spinal Cord Injury

Hans S. Keirstead,[1] Gabriel Nistor,[1] Giovanna Bernal,[1] Minodora Totoiu,[1] Frank Cloutier,[1] Kelly Sharp,[1] and Oswald Steward[1,2,3]

Departments of [1]Anatomy and Neurobiology, [2]Neurobiology and Behavior, and [3]Neurosurgery, Reeve-Irvine Research Center, College of Medicine, University of California at Irvine, Irvine, California 92697-4292

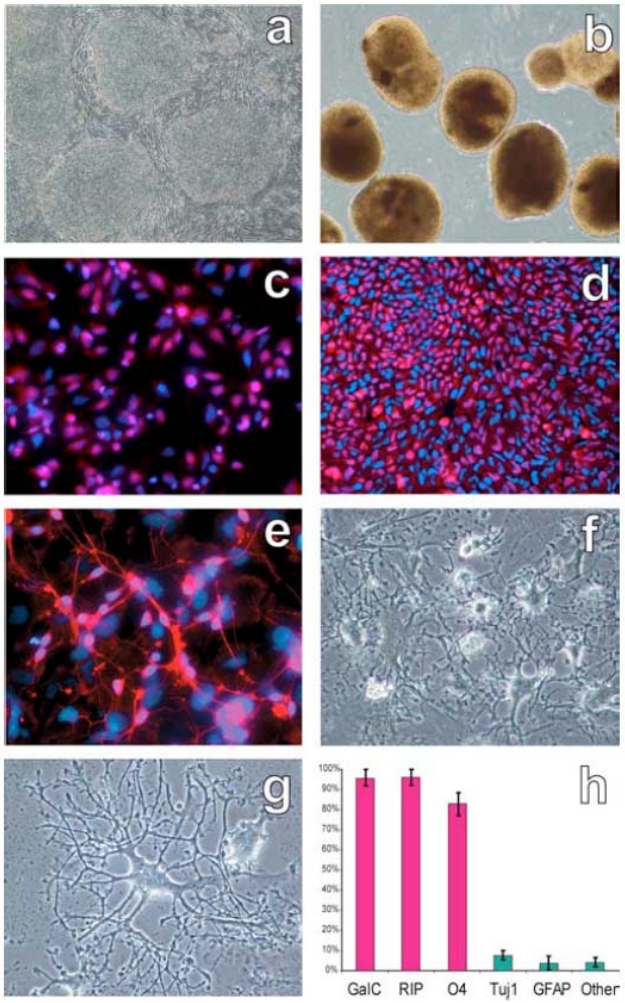

Figure 1. Commitment of hESCs to oligodendrocyte progenitors. ***a***, Undifferentiated hESCs readily expand in colonies, separated by stromal cells. ***b***, Yellow spheres appeared within 5 d of exposure to RA and grew rapidly in the presence of GRM, evidenced by an increase in their size and proportion relative to other culture components. ***c***, A total of 83 ± 7% of cells expressed the transcription factor Olig1 (red) associated with oligodendrocyte and motoneuron specification. ***d***, A total of 72 ± 12% of cells expressed the DNA binding protein SOX10 (red) expressed within oligodendrocyte precursors. ***e***, More than 95% of cells labeled with the mature oligodendroglial marker RIP (red). Cells that did not label with oligodendroglial markers were primarily GFAP positive or Tuj1 positive. ***f***, ***g***, Plated cells adopted a typical oligodendroglial morphology characterized by multiple branches. ***h***, Quantitation of immunolabeling. Error bars illustrate SD. Magnification: ***a***, 50×; ***c***, ***d***, 100×; ***e***, ***f***, 200×; ***b***, ***g***, 400×.

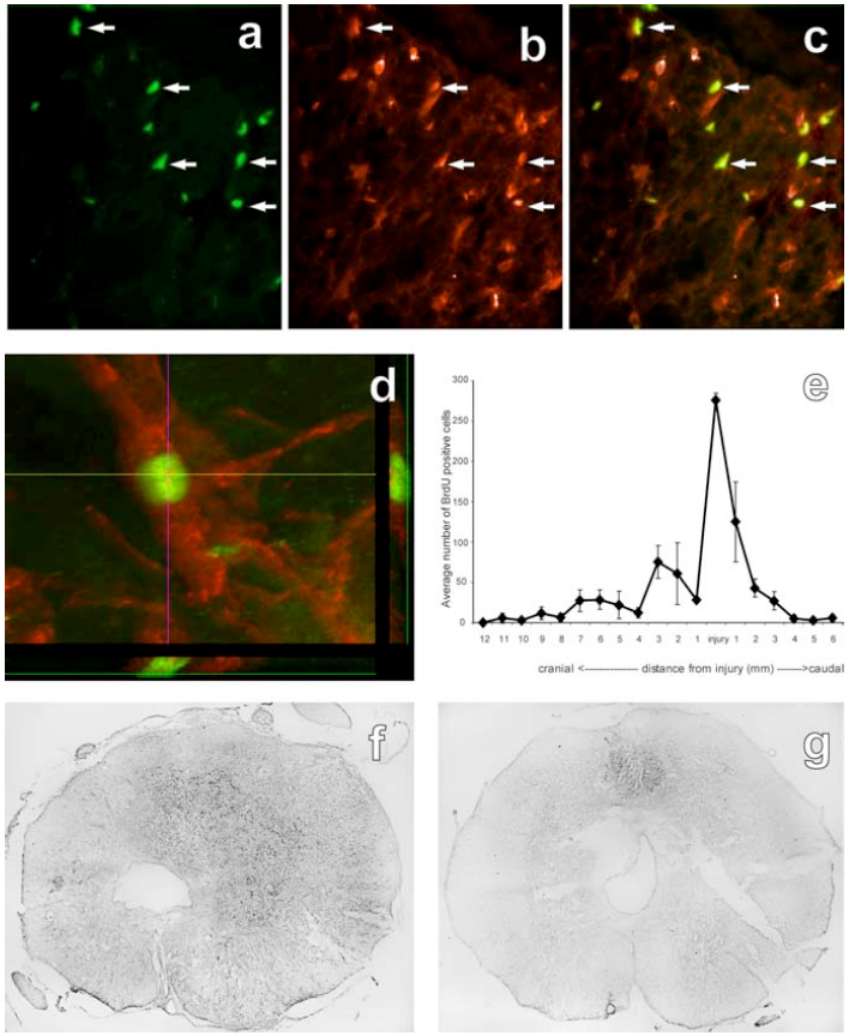

Figure 2. Acute transplantation of hESC-derived OPCs resulted in cell survival, limited redistribution from the site of implantation, and differentiation to mature oligodendrocytes. ***a***, Anti-human nuclei-positive OPCs (arrows) double labeled with the mature oligodendrocyte marker APC-CC1 (arrows; ***b***); a composite is shown in ***c***. ***d***, Anti-human nuclei-positive, APC-CC1-positive double labeling was confirmed using 3-D reconstruction of confocally scanned thin-plane images. ***e***, Distribution of total numbers of BrdU-prelabeled cells within spinal cord transverse sections 2 months after transplantation at 7 d after SCI. Error bars illustrate SD. ***f***, Anti-human nuclei-immunostained transverse section 1 mm caudal to the site of implantation showing transplanted cells (black dots) located primarily within the gray matter but also redistributed throughout the white matter. ***g***, Anti-human nuclei-immunostained transverse section 6 mm cranial to the site of implantation, showing transplanted cells (black dots) located primarily within the dorsal column. Magnification: ***a***–***c***, 400×; ***d***, 2000×; ***f***, ***g***, 20×.

Keirstead et al (2005) J Neurosci 25:4694-4705

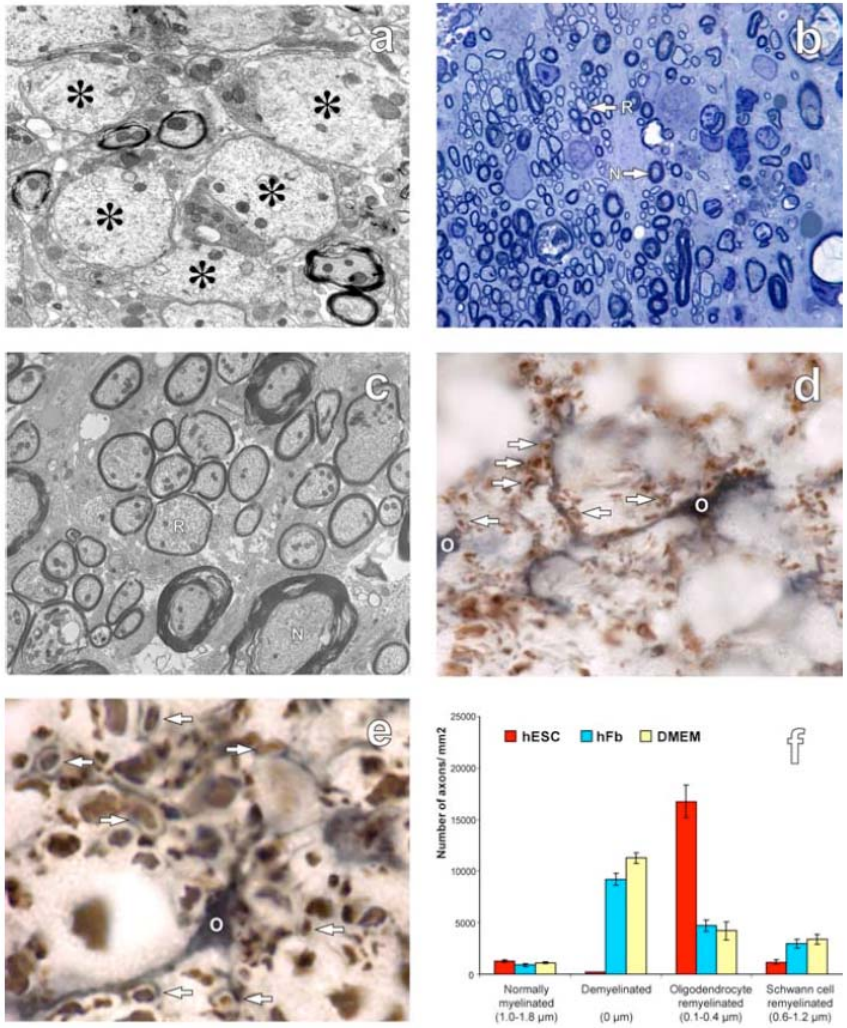

Figure 4. Acute transplantation of hESC-derived OPCs resulted in a significant increase in the density of oligodendrocyte remyelination compared with controls. ***a***, Electron micrograph of the transplant environment at 7 d after injury, illustrating demyelinated axons (*) in an extracellular environment free of astrogliosis. ***b***, Toluidine blue-stained transverse section and electron micrograph (***c***), illustrating robust oligodendrocyte remyelination (R; with characteristically thin myelin sheaths) among few normally myelinated axons (N). ***d***, ***e***, Anti-GFP and anti-neurofilament double immunostains illustrating highly branched GFP-positive OPCs (O) extending processes that ensheath nearby neurofilament-positive axons (arrows), confirming that remyelination was performed by eGFP-labeled transplanted cells. ***f***, Quantification of normally myelinated, demyelinated, and oligodendrocyte or Schwann cell-remyelinated axons in hESC-derived OPC-transplanted, hFb-transplanted, and DMEM-injected animals. Error bars illustrate SD. The myelin sheath thickness for each class of axons is indicated in brackets. Magnification: ***a***, 6000×; ***b***, 400×; ***c***, 3000×; ***d***, 600×; ***e***, 1000×.

Keirstead et al (2005) J Neurosci 25:4694-4705

3 CONCEPTUAL CATEGORIES

Embryonic
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Tumor
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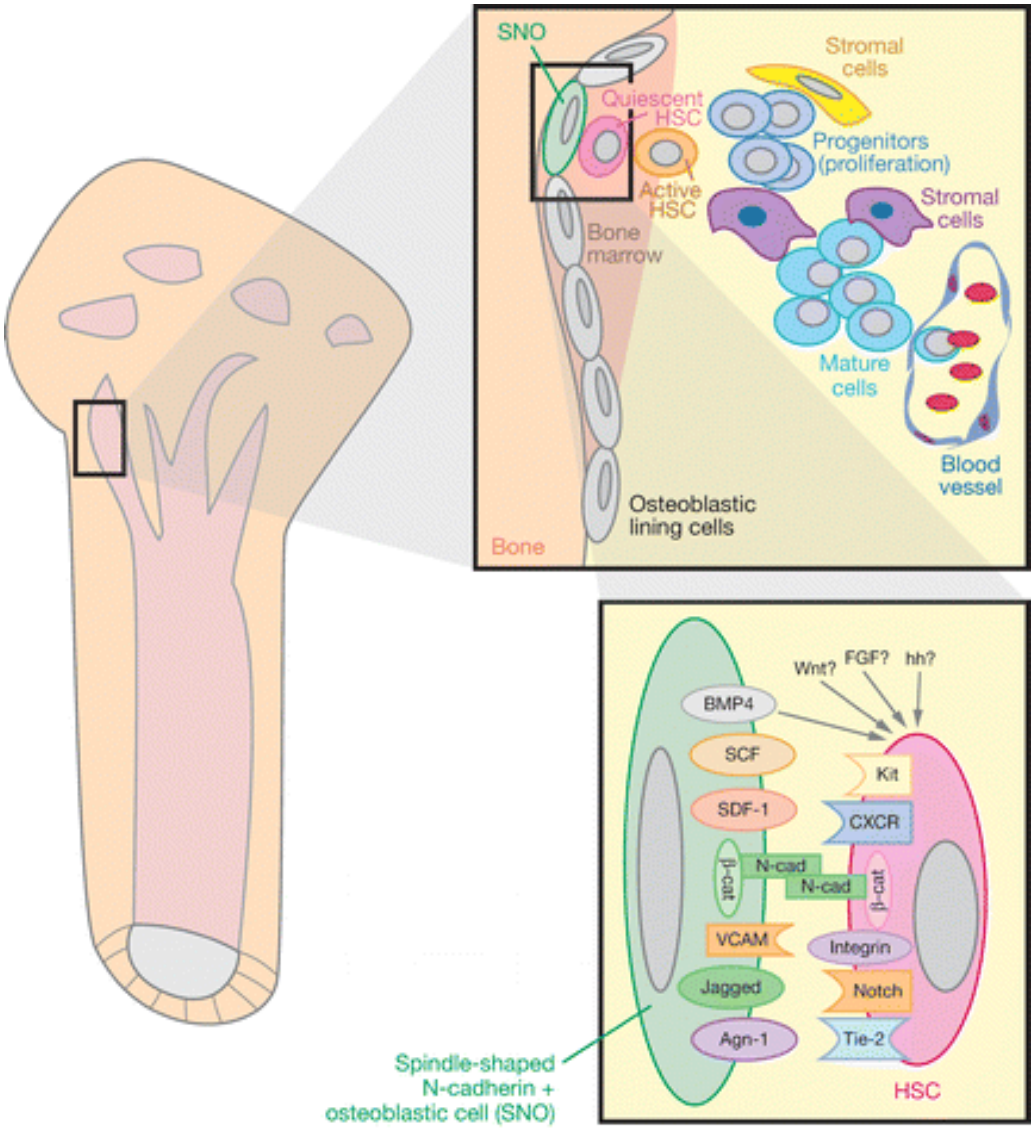
HISTORICAL PERSPECTIVE

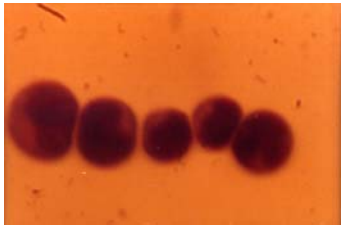
Previously known to exist in organs with obvious self-renewal
(bone marrow, skin, intestinal epithelium), and in organs with some capacity to regenerate after cell loss (liver, muscle)

Previously believed NOT to exist in organs with no obvious self-renewal
(like brain)

More recently demonstrated in precisely such organs (like brain)



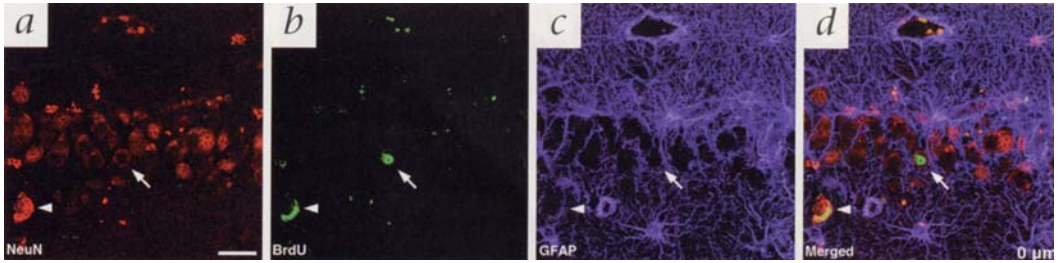
Li L, Xie T. 2005.
Annu. Rev. Cell Dev. Biol. 21:605–31



hHSCs in vitro

from Torstein Egeland, IMMI, RH

Johansson CB, Svensson M, Wallstedt L, Janson AM, Frisen J. Neural stem cells in the adult human brain. Exp Cell Res 1999; 253:733-736.



NATURE | VOL 412 | 16 AUGUST 2001 | www.nature.com

Purification of a pluripotent neural stem cell from the adult mouse brain

Rodney L. Rietze*, Helen Valcanis†, Gordon F. Brooker*, Tim Thomas*, Anne K. Voss* & Perry F. Bartlett*

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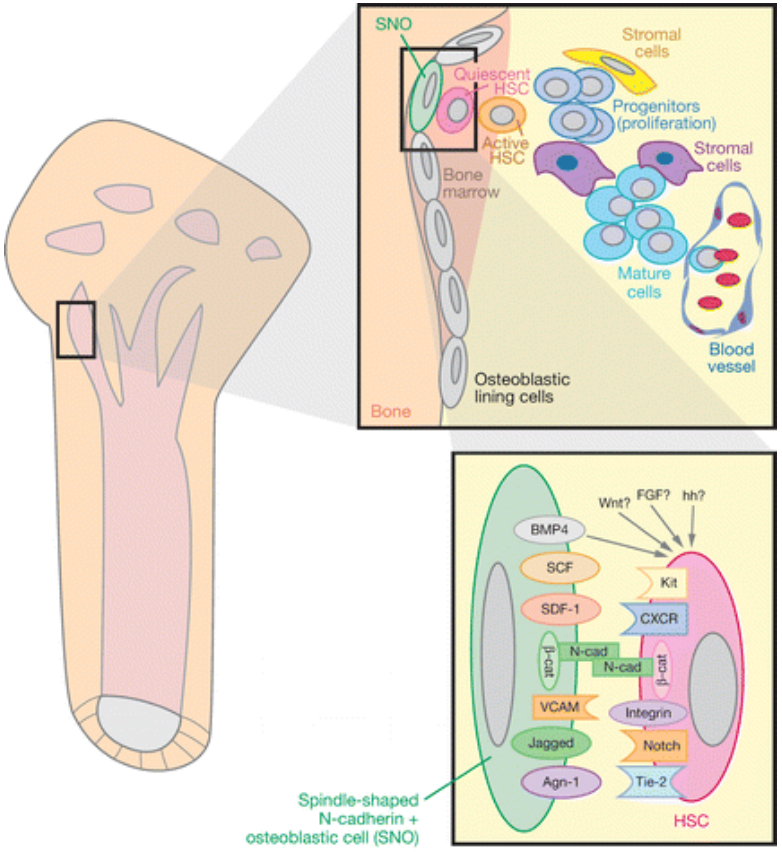
CONCEPT OF THE STEM CELL “NICHE”



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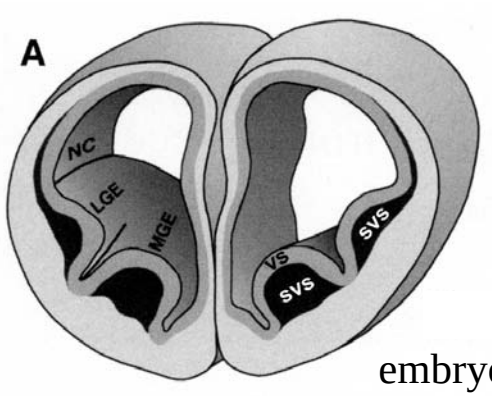
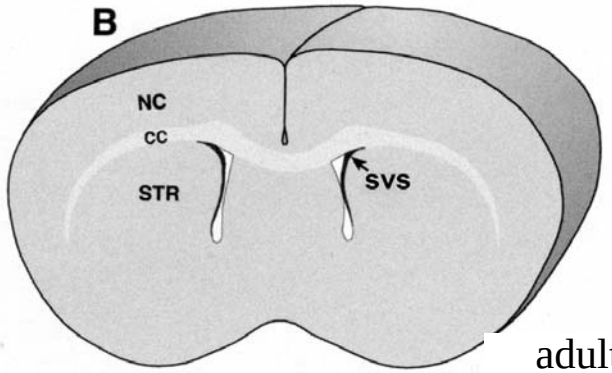
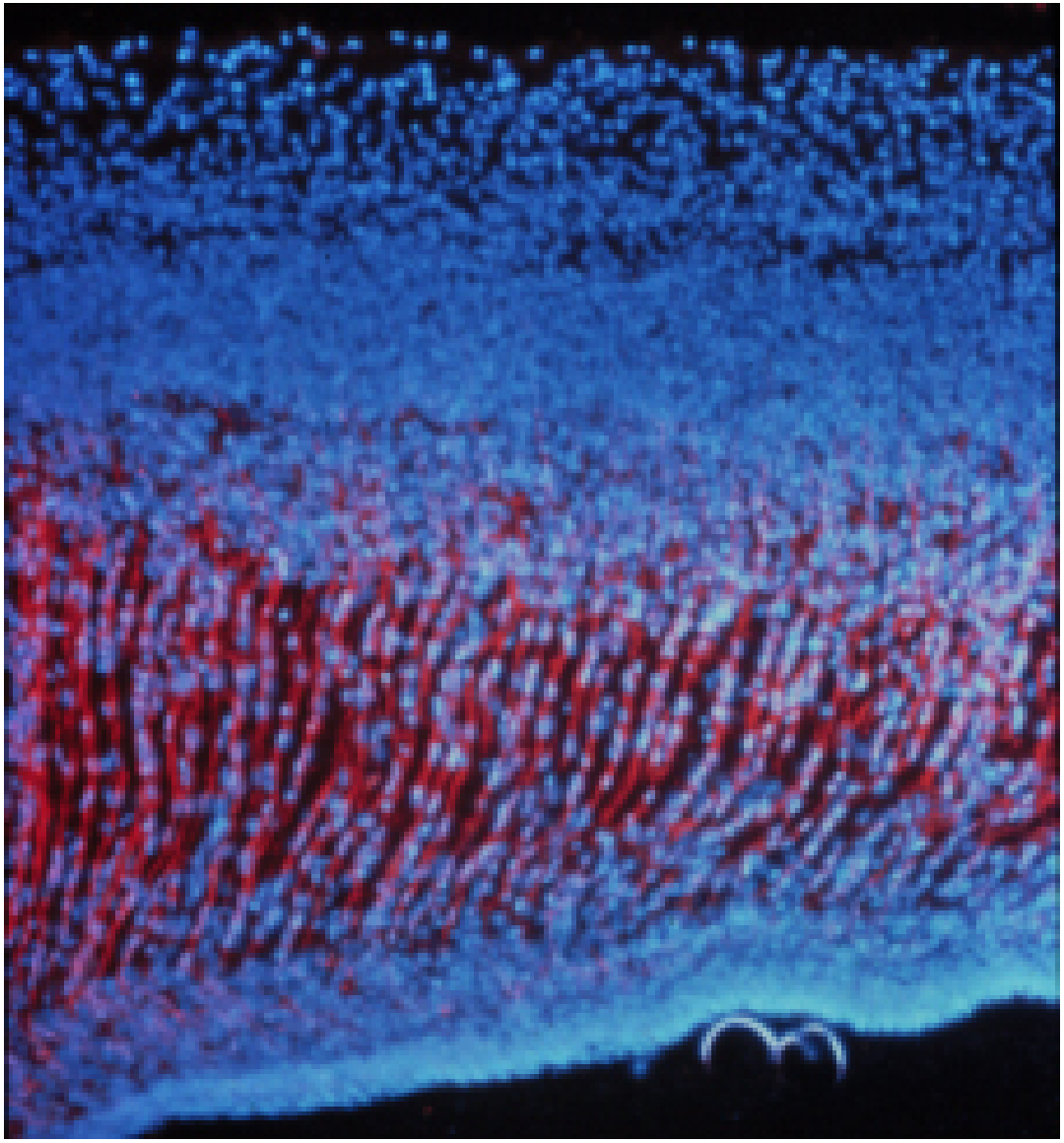


embryo

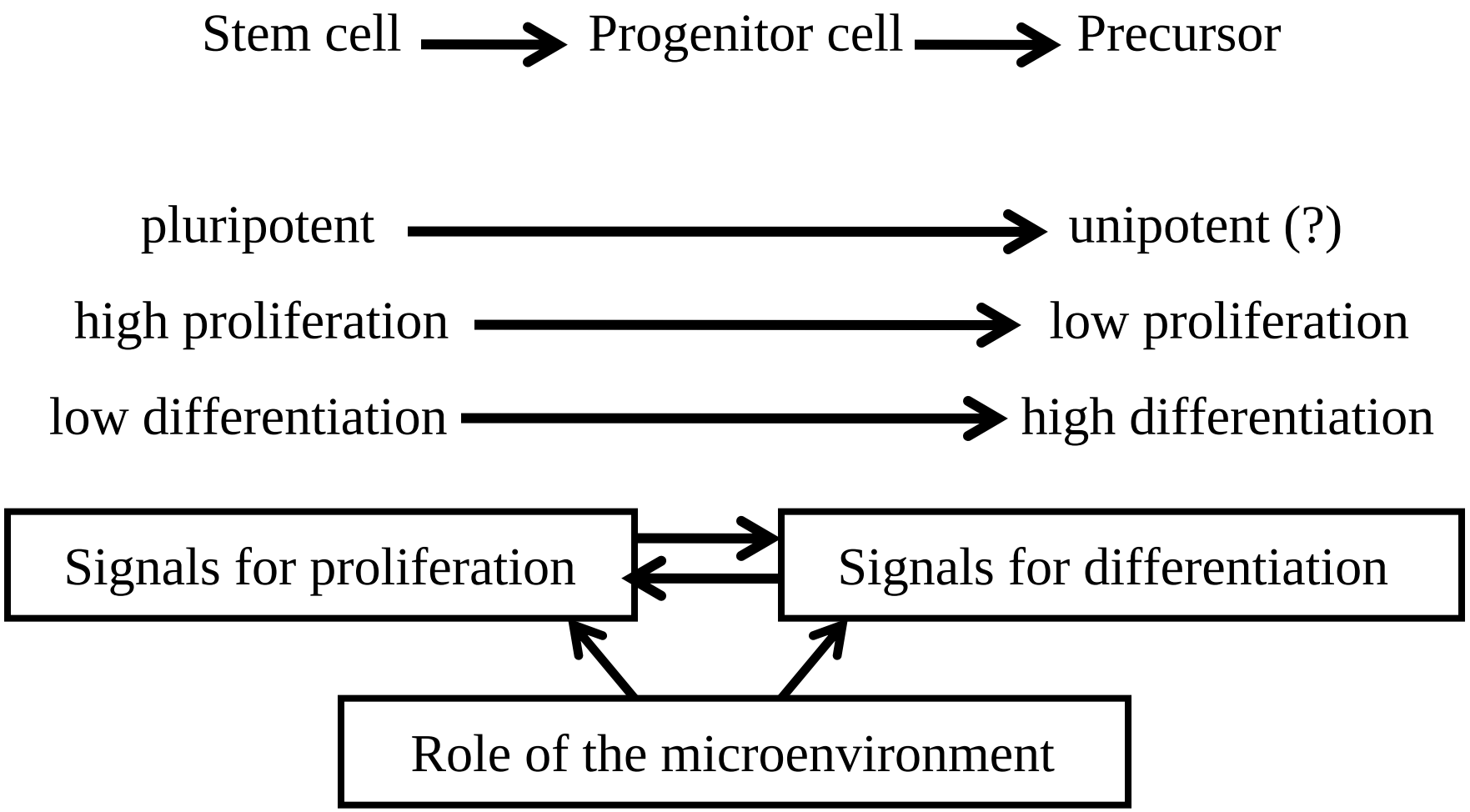


adult

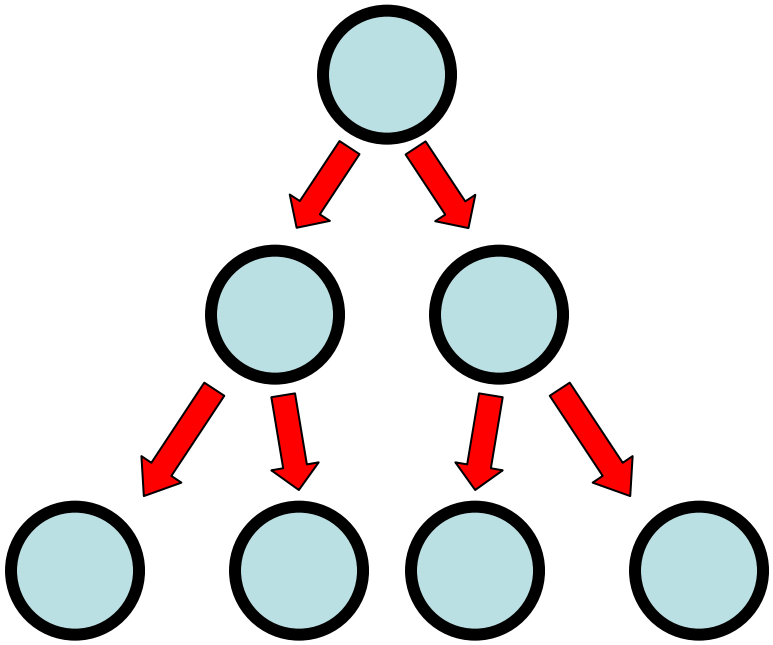
Somatic stem cells: Remnants of embryogenesis?



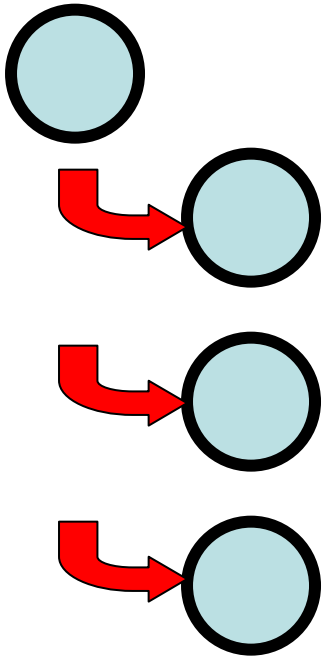
“Stages” of development: proliferation versus differentiation



Proliferative kinetics: relationship to expansion *in vitro* *(and to evolution!)*



number of cells = 2^n



number of cells = n + 1

AN IMPORTANT QUESTION REGARDING SOMATIC STEM CELLS

What is the differentiation potential of somatic stem cells?

Organ-restricted (multipotent), or broader (pluripotent)?

Much circumstantial evidence. Requirement for definitive studies proving full differentiation to specific cell types *in vivo*.

Somatic stem cells: examples of specific uses

Hematopoietic stem cells have been used for years in the treatment of bone marrow and blood disorders such as leukemia, aplastic Anemia

Skin transplants are de facto stem cell treatments

More recent advances in regenerative medicine:
Liver, connective tissue, etc……
(homotypic, as for bone marrow transplants)

In the future: Tissues derived from heterotypic stem cell sources?
(for example, nerve cells from hematopoietic stem cells or from fat stem cells)

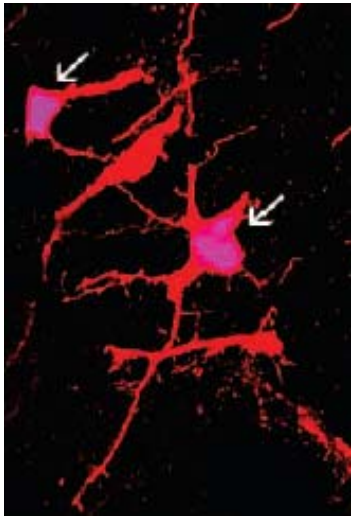
PNAS

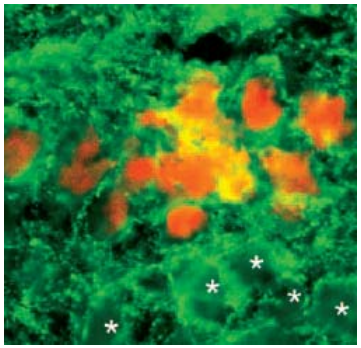
Adult human hematopoietic stem cells produce neurons efficiently in the regenerating chicken embryo spinal cord

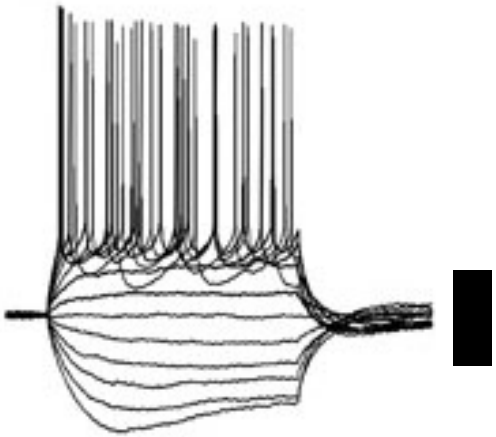
Olafur E. Sigurjonsson*, Marie-Claude Perreault†, Torstein Egeland*, and Joel C. Glover†‡

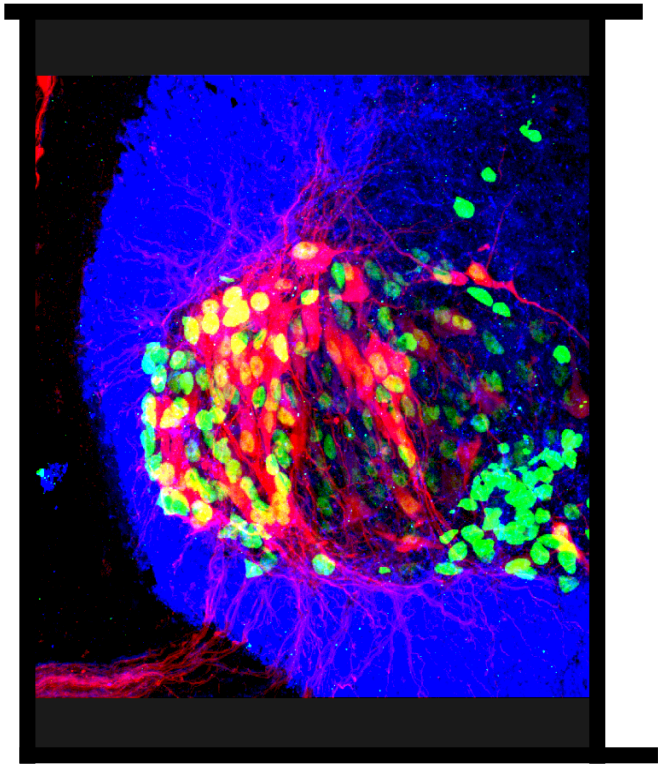
*Institute of Immunology, Rikshospitalet University Hospital and University of Oslo Rikshospitalet, 0027 Oslo, Norway; and †Department of Physiology, Institute of Basic Medical Science, University of Oslo, 0319 Oslo, Norway

Communicated by Joshua R. Sanes, Harvard University, Cambridge, MA, February 7, 2005 (received for review August 31, 2004)









Somatic stem cells: examples of specific uses

Make pluripotent stem cells!

Induced pluripotent stem cells (iPS cells): Pluripotent stem cells derived from somatic cells that have been reprogrammed to revert to a pluripotent state as in embryonic stem cells

Cell *126*, 663–676, August 25, 2006

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

Kazutoshi Takahashi[1] **and Shinya Yamanaka**[1,2,*]
[1] Department of Stem Cell Biology, Institute for Frontier Medical Sciences, Kyoto University, Kyoto 606-8507, Japan
[2] CREST, Japan Science and Technology Agency, Kawaguchi 332-0012, Japan
*Contact: yamanaka@frontier.kyoto-u.ac.jp
DOI 10.1016/j.cell.2006.07.024

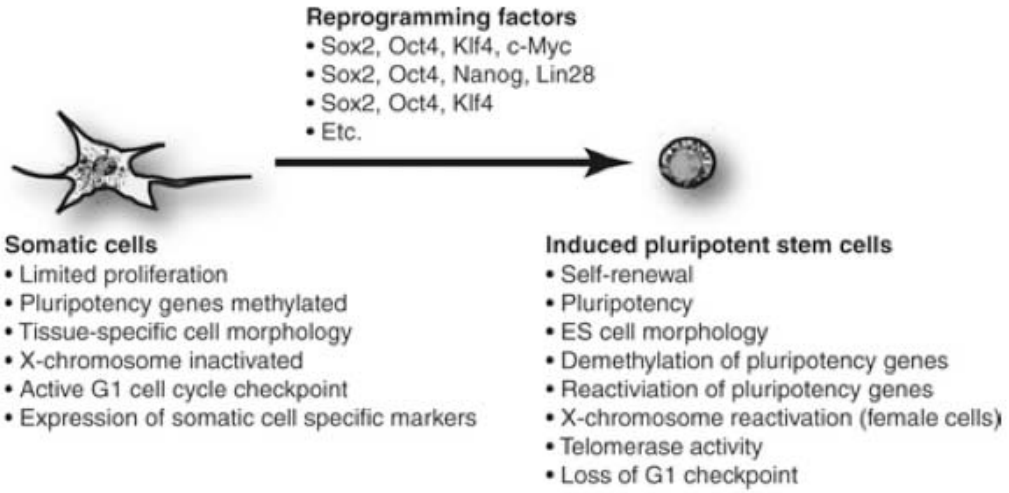


Figure 1 Reprogramming of somatic cells to induced pluripotent stem (iPS) cells. Examples of reprogramming factors are provided along with the characteristics of a typical starting somatic cell and those of an iPS cell

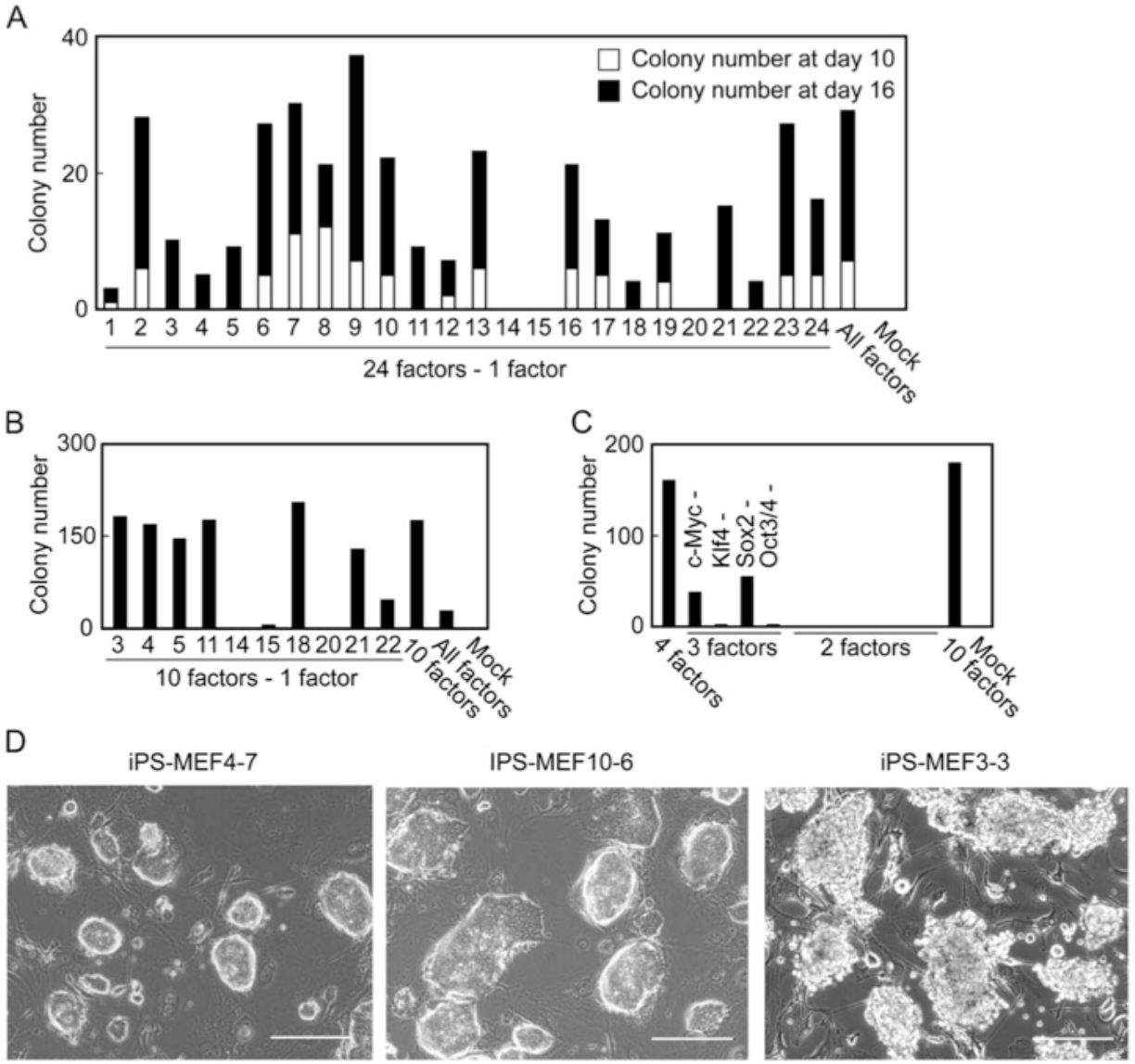


Figure 2. Narrowing down the Candidate Factors

Takahashi & Yamanaka (2006) Cell 126:663-676

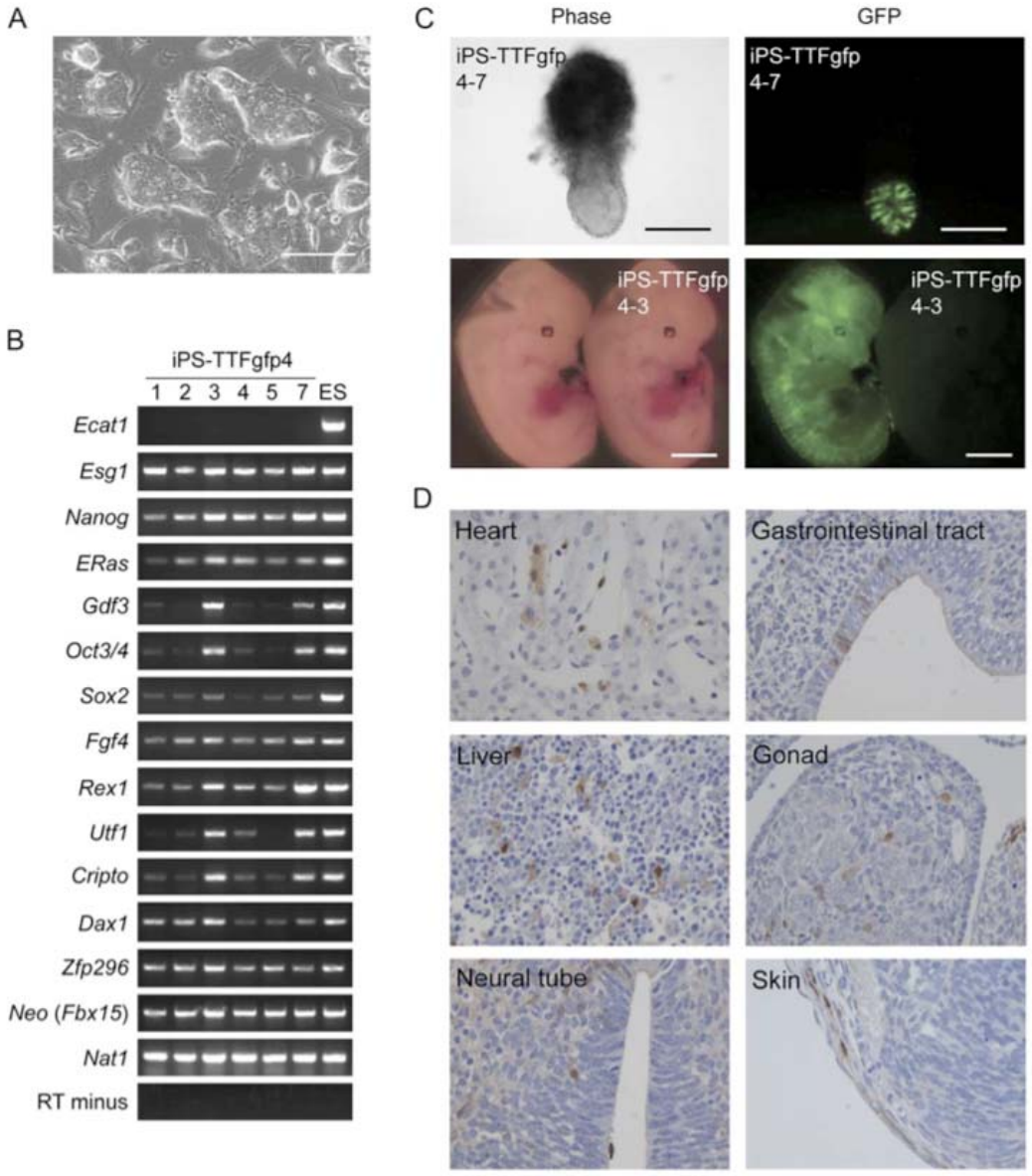


Figure 6. Characterization of iPS Cells Derived from Adult Mouse Tail-Tip Fibroblasts

Takahashi & Yamanaka (2006) Cell 126:663-676

Nature **448**, 318-324 (19 July 2007) | doi:10.1038/nature05944; Rece
22 May 2007; Published online 6 June 2007

In vitro reprogramming of fibroblasts into a pluripotent ES-cell-like state

Marius Wernig[1,6], Alexander Meissner[1,6], Ruth Foreman[1,2,6], Tobias Brambrink[1,6], Manching Ku[3,6], Konrad Hochedlinger[1,7], Bradley E. Bernstein[3,4,5] & Rudolf Jaenisch[1,2]

Nature **454**, 646-650 (31 July 2008) | doi:10.1038/nature07061; Receiv
May 2008; Published online 29 June 2008

Pluripotent stem cells induced from adult neural stem cells by reprogramming with two factors

Jeong Beom Kim[1,3], Holm Zaehres[1,3], Guangming Wu[1], Luca Gentile[1], Kinarm Ko[1], Vittorio Sebastiano[1], Marcos J. Araúzo-Bravo[1], David Ruau[2], Dong Wook Han[1], Martin Zenke[2] & Hans R. Schöler[1]

PNAS

Neurons derived from reprogrammed fibroblasts functionally integrate into the fetal brain and improve symptoms of rats with Parkinson's disease

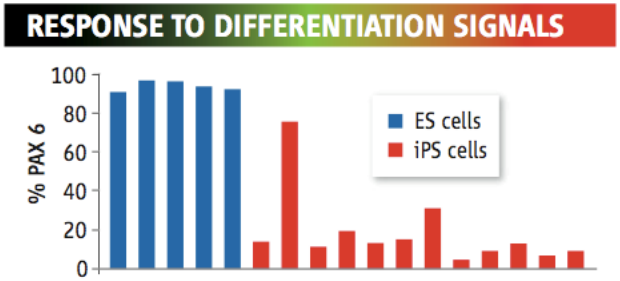
Marius Wernig*, Jian-Ping Zhao†, Jan Pruszak‡, Eva Hedlund‡, Dongdong Fu*, Frank Soldner*, Vania Broccoli§, Martha Constantine-Paton†, Ole Isacson‡, and Rudolf Jaenisch*¶‖

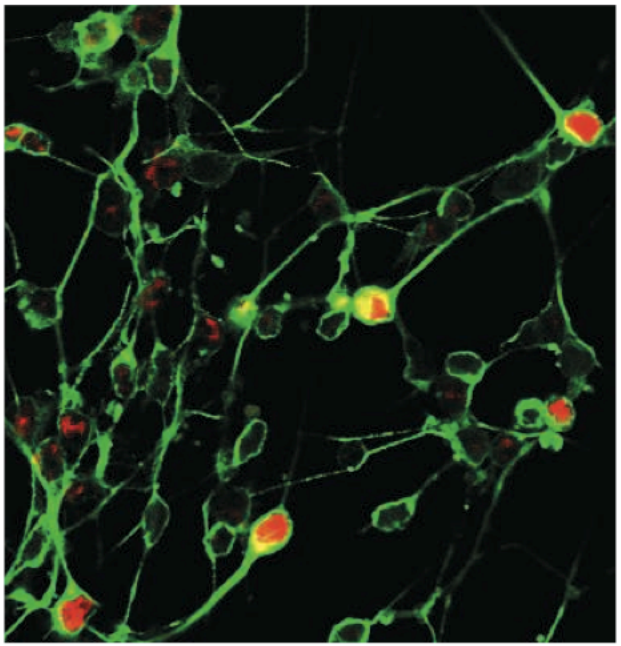
*The Whitehead Institute for Biomedical Research, Cambridge, MA 02142; †The McGovern Institute for Brain Research and ¶Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139; ‡Udall Parkinson's Disease Research Center of Excellence and Neuroregeneration Laboratories, McLean Hospital/Harvard University, Belmont, MA 02478; and §San Raffaele Scientific Institute, 20132 Milan, Italy

STEM CELLS

Reprogrammed Cells Come Up Short, for Now

www.sciencemag.org SCIENCE VOL 327 5 MARCH 2010





Work in progress. iPS cells can differentiate into functional neurons (*above*), but analysis of PAX6 gene expression shows they are less responsive than human ES cells to neuron-making cues (chart).

STEM CELLS

Embryonic Stem Cells/Induced Pluripotent Stem Cells

Hemangioblastic Derivatives from Human Induced Pluripotent Stem Cells Exhibit Limited Expansion and Early Senescence[†‡§]

Qiang Feng [1], Shi-Jiang Lu [1 *¶], Irina Klimanskaya [2], Ignatius Gomes [3], Dohoon Kim [4], Young Chung [1], George R. Honig [3], Kwang-Soo Kim [1 4], Robert Lanza [1 2 *‖]

Embryonic
Advantages: Clearly pluripotent, easy to expand and differentiate, platform for many model systems for studying normal and disease mechanisms

Disadvantages: Not autologous, may cause tumors, derived from embryos

Somatic
Advantages: Autologous, already programmed towards specific cell types, lower risk of tumorigenesis

Disadvantages: Restricted potential, some are hard to get, still carry genetic disease burden

Induced pluripotent
Advantages: Autologous, greater potential, platform for in vitro disease models

Disadvantages: Harder to generate and expand, require genetic/epigenetic “harassment”, may enter senescence sooner

The main message:

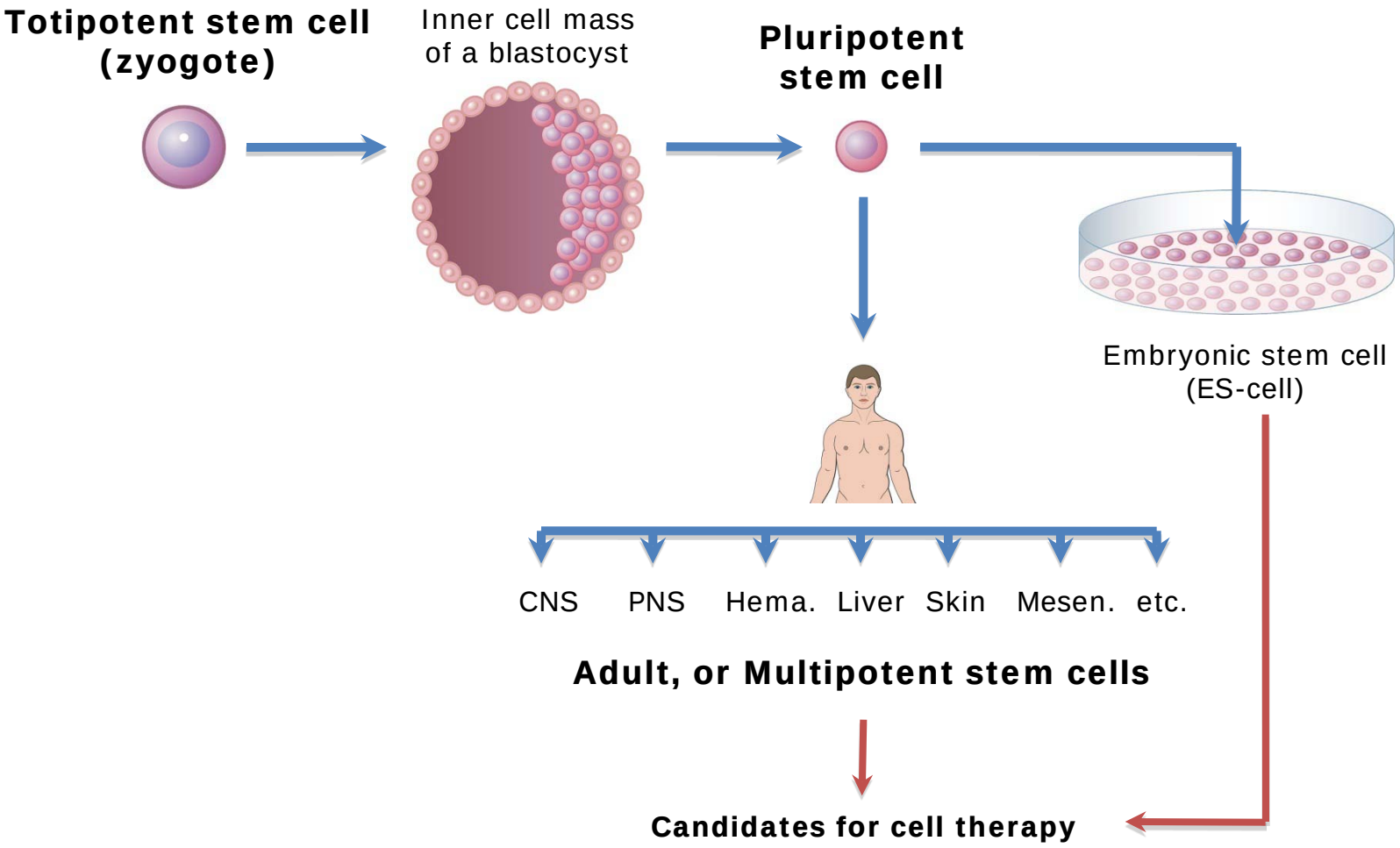
STEM CELL BIOLOGY STILL PRESENTS MANY CHALLENGES

What is needed is continued, integrated research into embryonic, somatic, and induced pluripotent stem cells

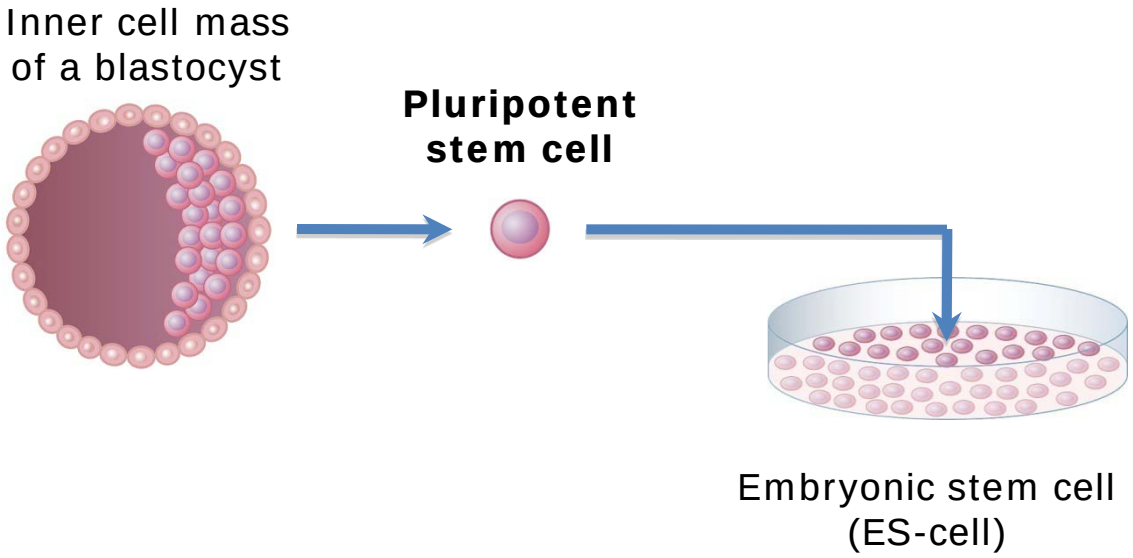
Current clinical applications of stem cells in Norway

Jan E. Brinchmann, MD, PhD
Group leader
Norwegian Center for Stem Cell Research
Oslo University Hospital Rikshospitalet
and University of Oslo

The stem cell hierarchy

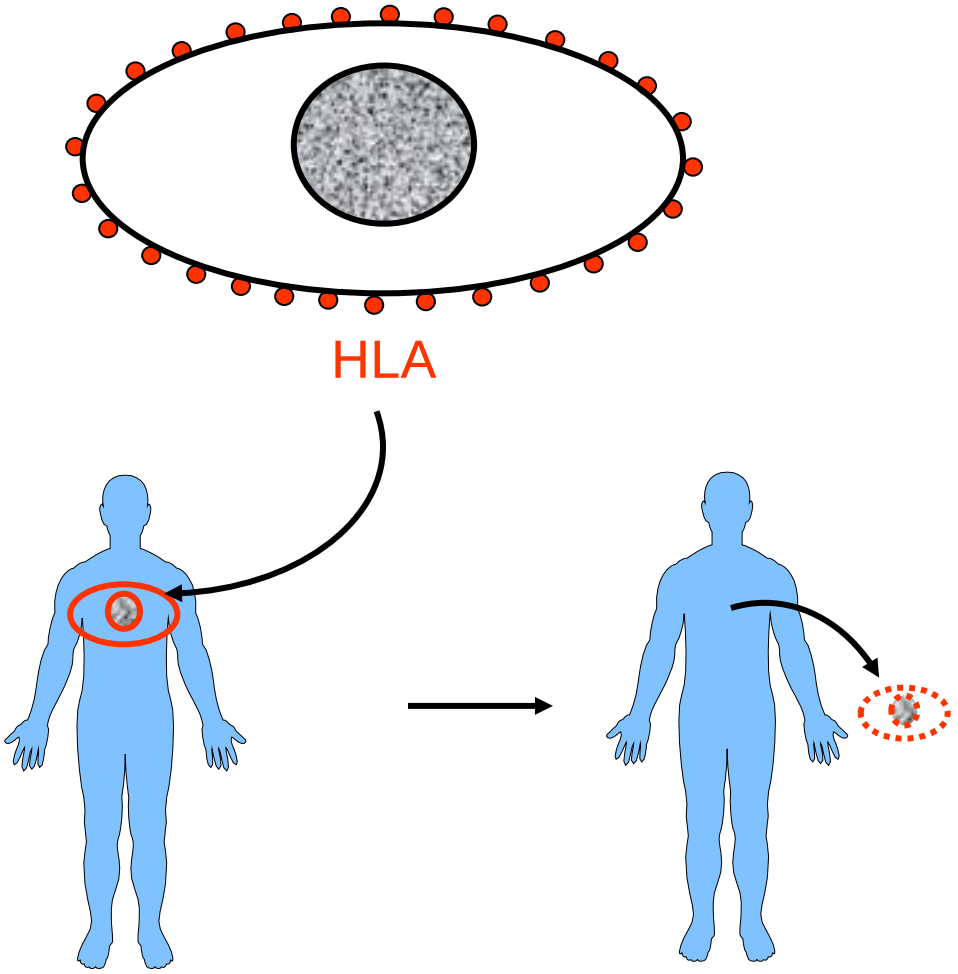


Embryonic stem cells



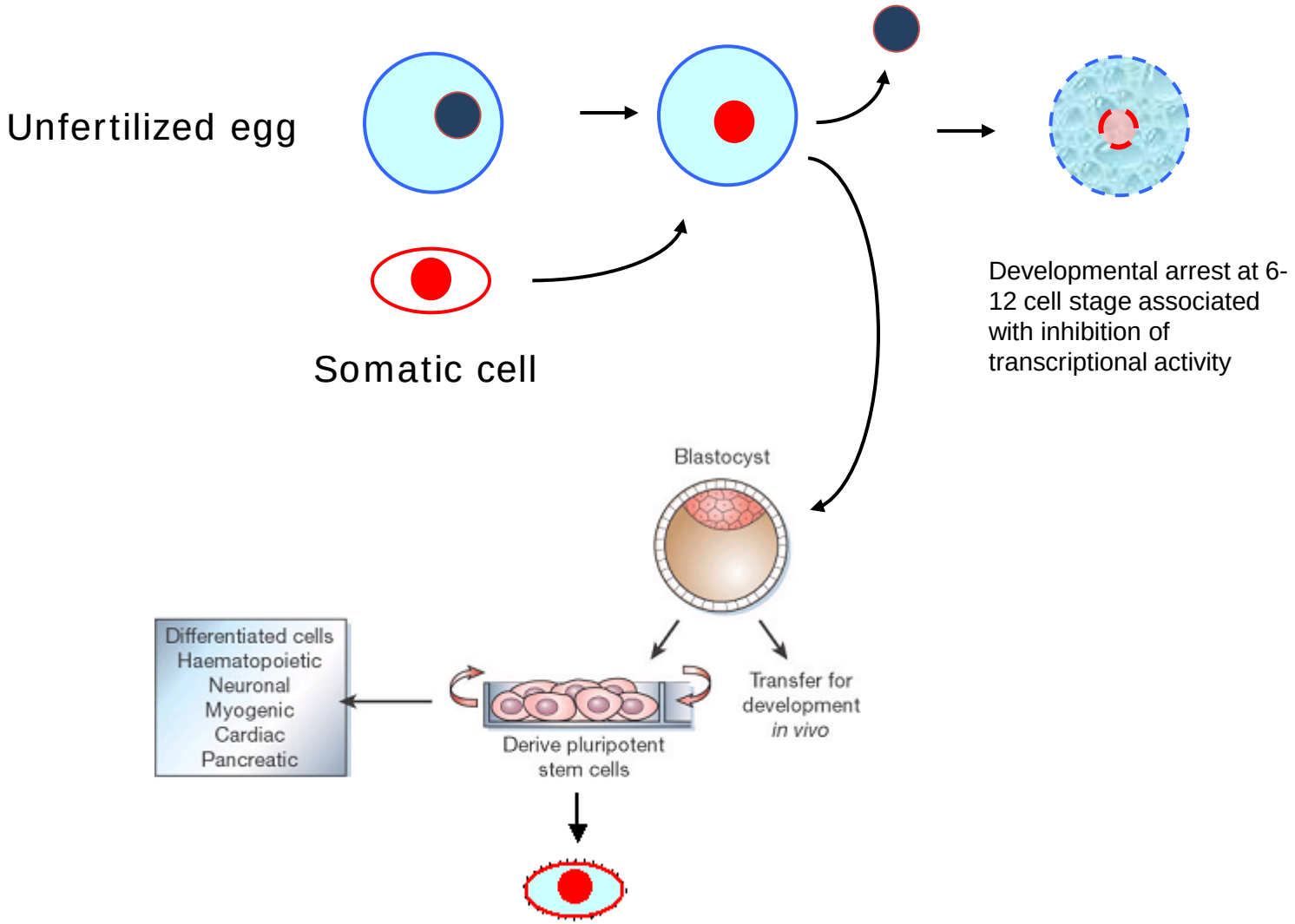
- Proliferates indefinitely
- Always pluripotent (teratoma assay)
- Can differentiate to cells typical of all three germ layers (ectoderm, mesoderm, endoderm)
- But: we can not yet fully control the differentiation
- Teratogenesis
- Always allogeneic

Cells from different people are different



Can stem cells from one individual still be used to treat another individual?

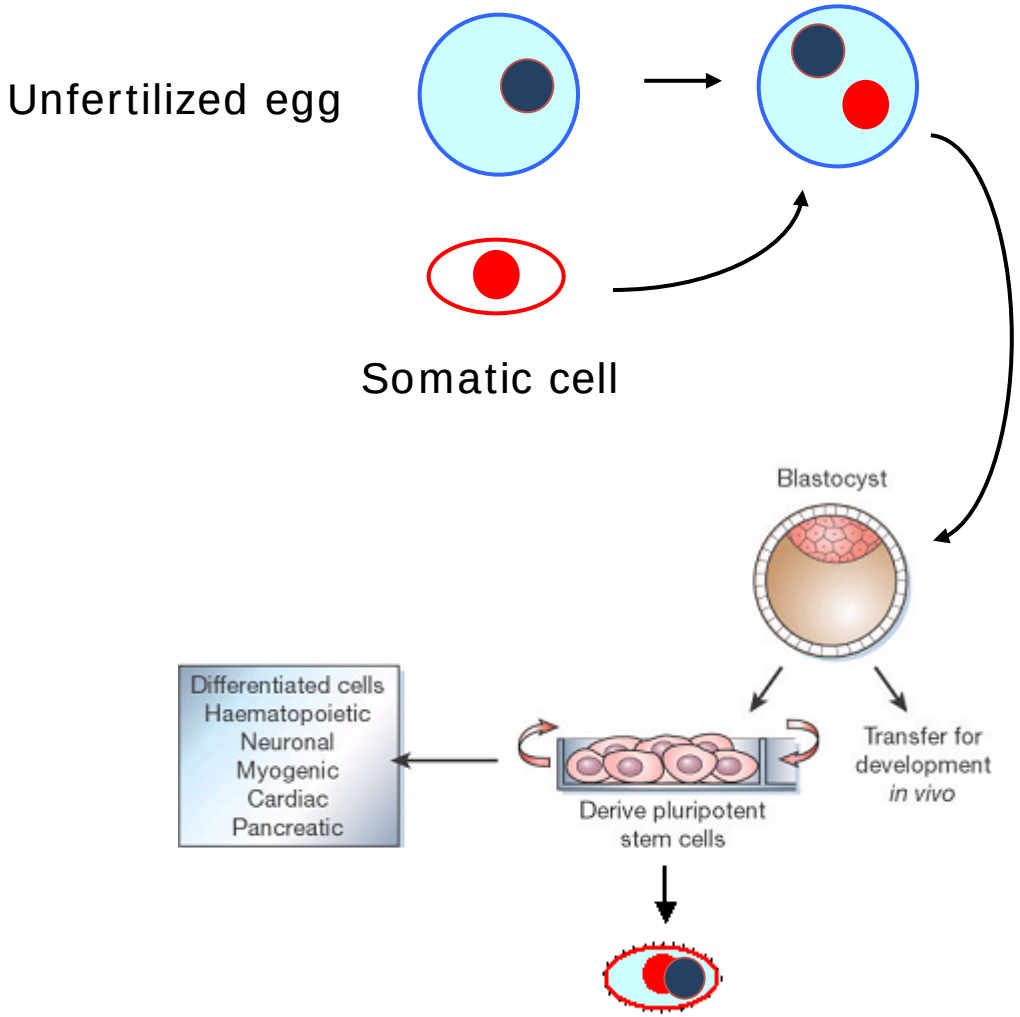
Somatic cell nuclear transfer



Pluripotent stamcelle med pasientens HLA

Human oocytes reprogram somatic cells to a pluripotent state

Scott Noggle, Ho-Lim Fung, Athurva Gore, Hector Martinez, Kathleen Crumm Satriani, Robert Prosser, Kiboong Oum, Daniel Paull, Sarah Druckenmiller, Matthew Freeby, Ellen Greenberg, Kun Zhang, Robin Goland, Mark V. Sauer Rudolph L. Leibel & Dieter Egli



Pluripotent stamcelle med pasientens HLA

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

Kazutoshi Takahashi[1] and Shinya Yamanaka[1,2,*]
[1] Department of Stem Cell Biology, Institute for Frontier Medical Sciences, Kyoto University, Kyoto 606-8507, Japan
[2] CREST, Japan Science and Technology Agency, Kawaguchi 332-0012, Japan
*Contact: yamanaka@frontier.kyoto-u.ac.jp
DOI 10.1016/j.cell.2006.07.024

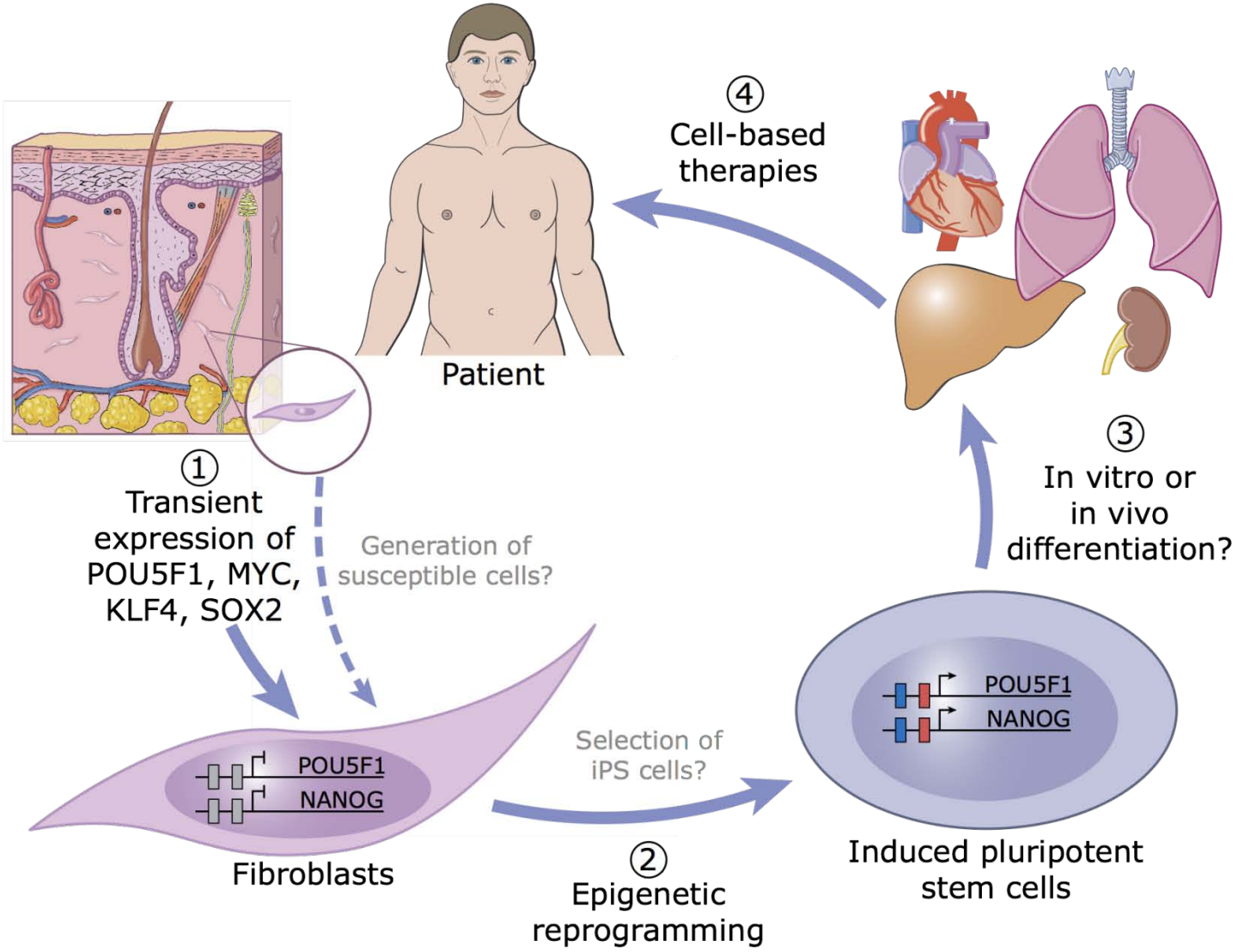
Background:

Reprogramming of differentiated cells has been shown to be possible:

- Somatic cell nuclear transfer (Wilmut et al., 1997)
- cell fusion with embryonic stem cells (Cowan et al., 2005; Tada et al., 2001)

Is it possible to induce pluripotency in end differentiated cells by introducing a limited number of genes?

Induced pluripotent stem cells



Unsolved issues for the clinical use of hIPCs

Gene transduction invoves random insertion of transgene. This may lead to cancer.

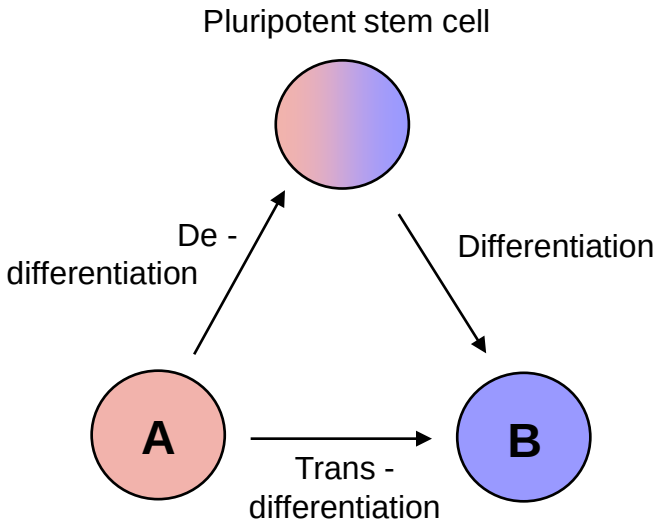
Other reprogramming strategies: microRNAs, synthetic mRNAs, transient gene transfection, protein transfection

Clinical use requires full control of differentiation strategy

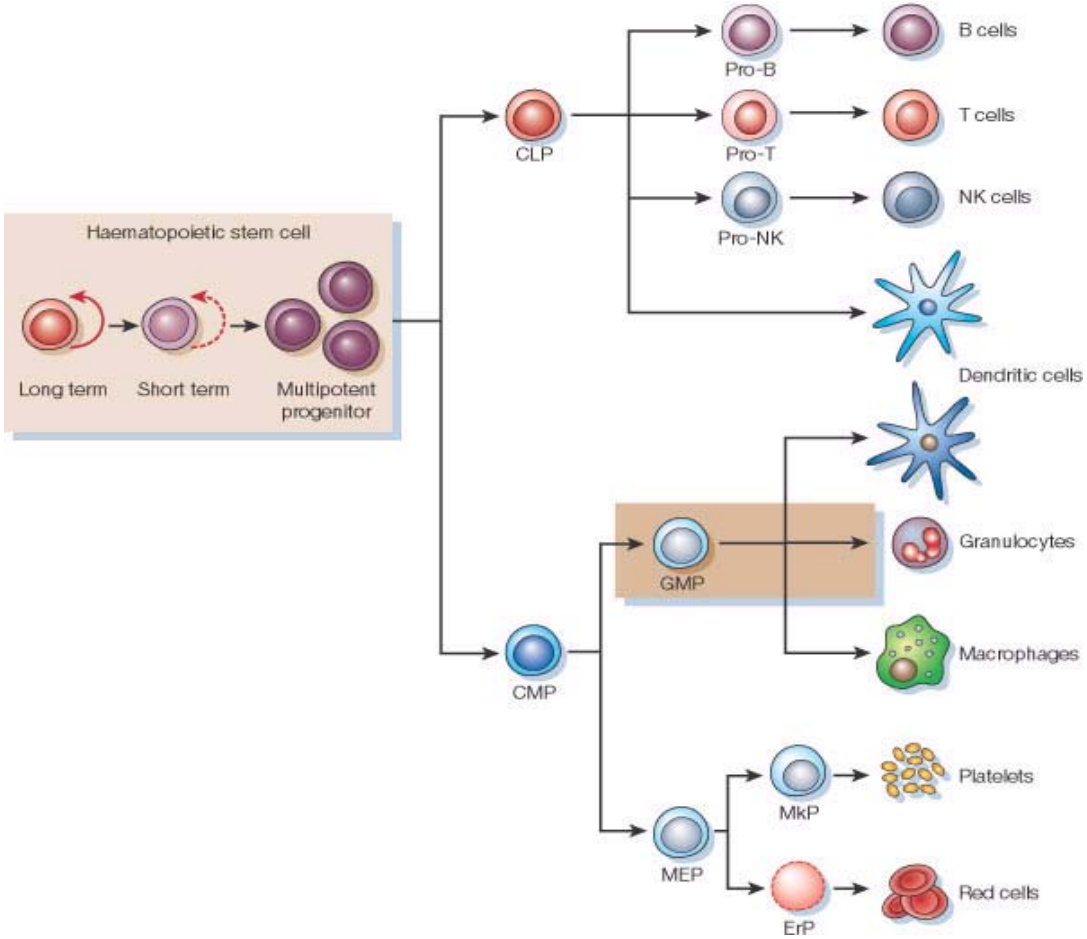
Are iPSC truly pluripotent? Memory of mother cell

Unsolved issues for the clinical use of hIPCs (cont)

Do the cells need to be reprogrammed to pluripotency, or is transdifferentiation possible?



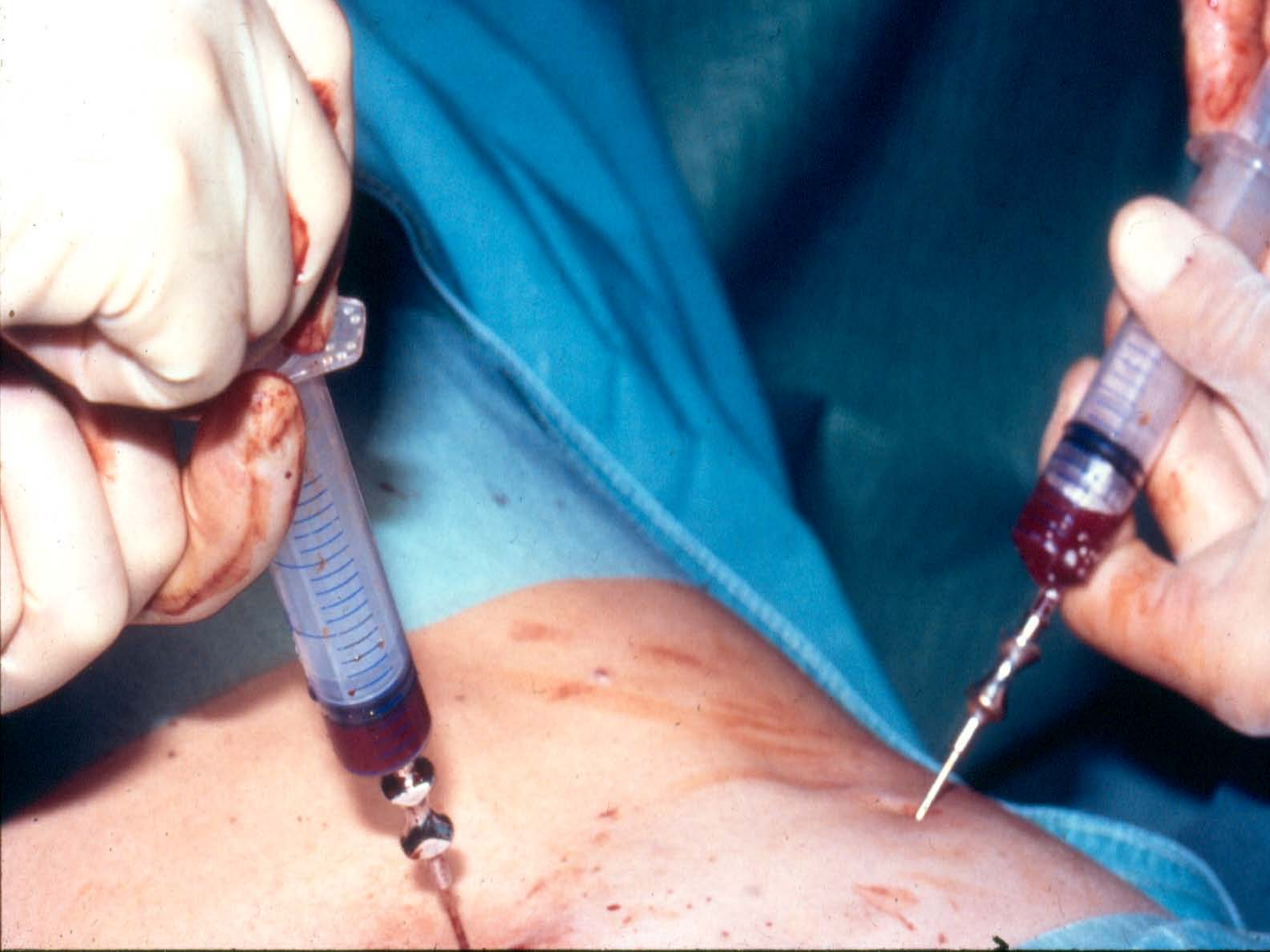
Hematopoietic stem cell transplantation has been used in the clinic for more than 40 years



Hematopoietic stem cell transplantations

- Autologous: From the patient herself
- Allogeneic: From another individual
  - » Family (including umbilical cord blood)
  - » Bone marrow donor registries
  - » Umbilical cord biobanks
  - » **For all these: HLA compatibility very important**

Lorentz Brinch, Department of Blood Diseases, OUS



Organization of stem cell transplants in Norway:

Autologous (høydosebehandling med autolog stamcellestøtte: HMAS)

- All University hospitals in Norway
- Oslo Universitetssykehus:
  - Ullevål: Lymphomas and multiple myelomas
  - Rikshospitalet: Multiple myelomas, solid tumors (children)
  - Radiumhospitalet: Lymphomas, some solid tumors

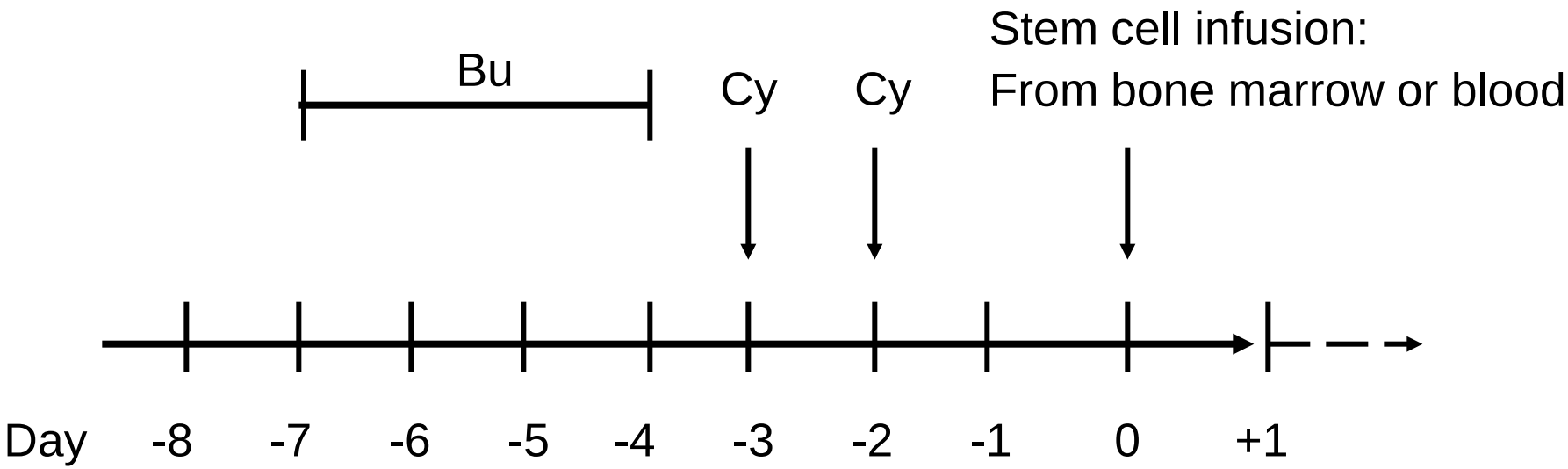
Lorentz Brinch, Department of Blood Diseases, OUS

High dose chemotherapy followed by autologous bone marrow transplantation is an option for patients with lymphomas

Histology	1.line	First chemosensitive relapse	Later chemosensitive relapse	
Hodgkins lymphoma	Not recommended	Clinical option	Clinical option	
T/B lympho-blastic lymphoma	Clinical option	Not recommended	Not recommended	
Aggressive B cell NHL	Not recommended	Clinical option	Clinical option	
Transforme d NHL	Not recommended	Clinical option	Clinical option	
Follicular NHL	Not recommended	Not recommended	Clinical option	
Mantle cell NHL	Clinical option	Not recommended	Not recommended	
Aggressive T cell NHL	ACT-1 randomised study Clinical option	Clinical option		

Arne Kolstad,
Norwegian
Radium Hospital
OUS

Allogeneic stem cell transplantation: bone marrow depletion



Bu: Busulfan : 16 mg/kg in total
Cy: Cyclofosfamid : 120 mg/kg in total

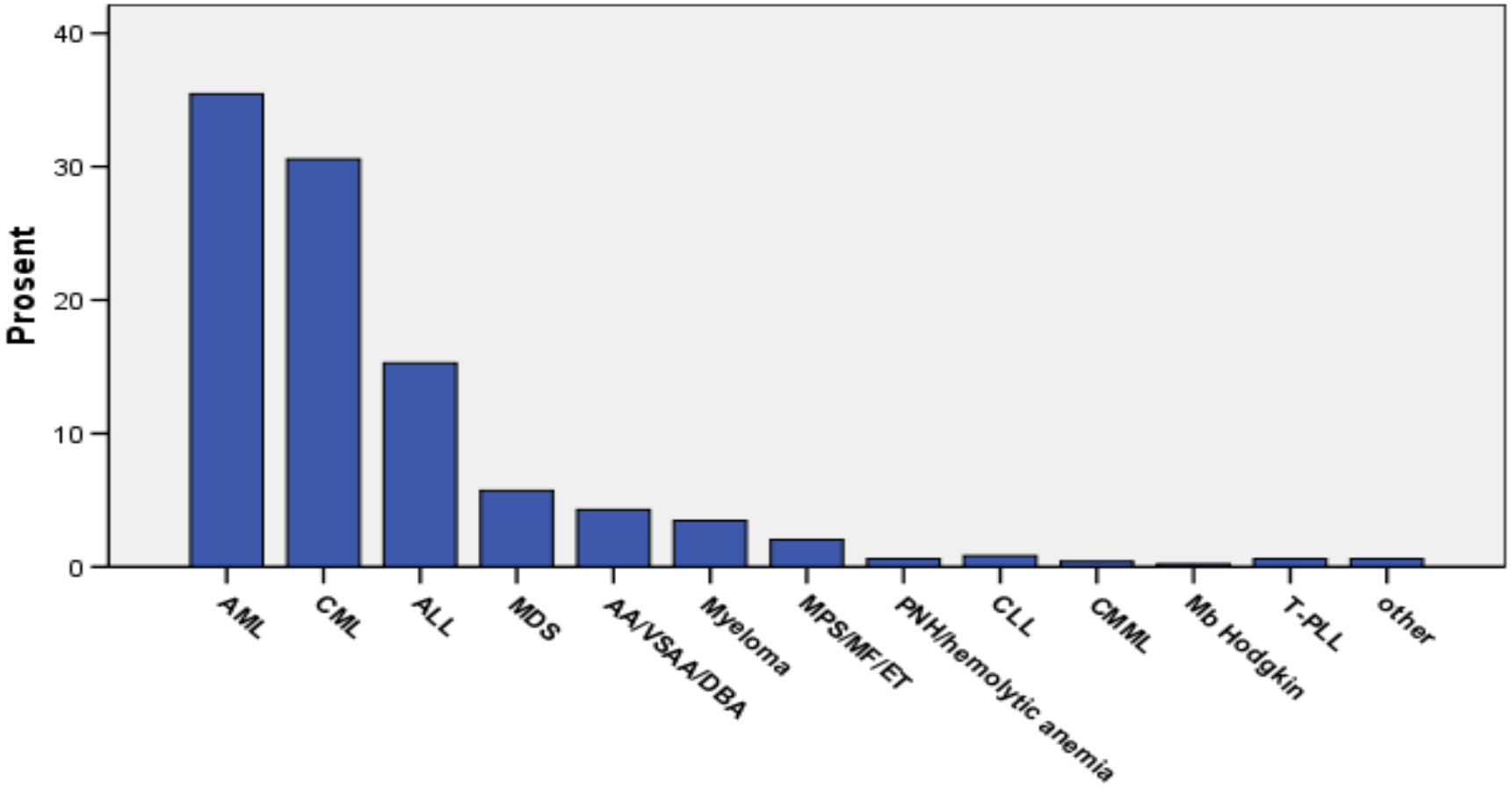
Lorentz Brinch, Department of Blood Diseases, OUS

Difference between autologous and allogeneic HSC transplantation

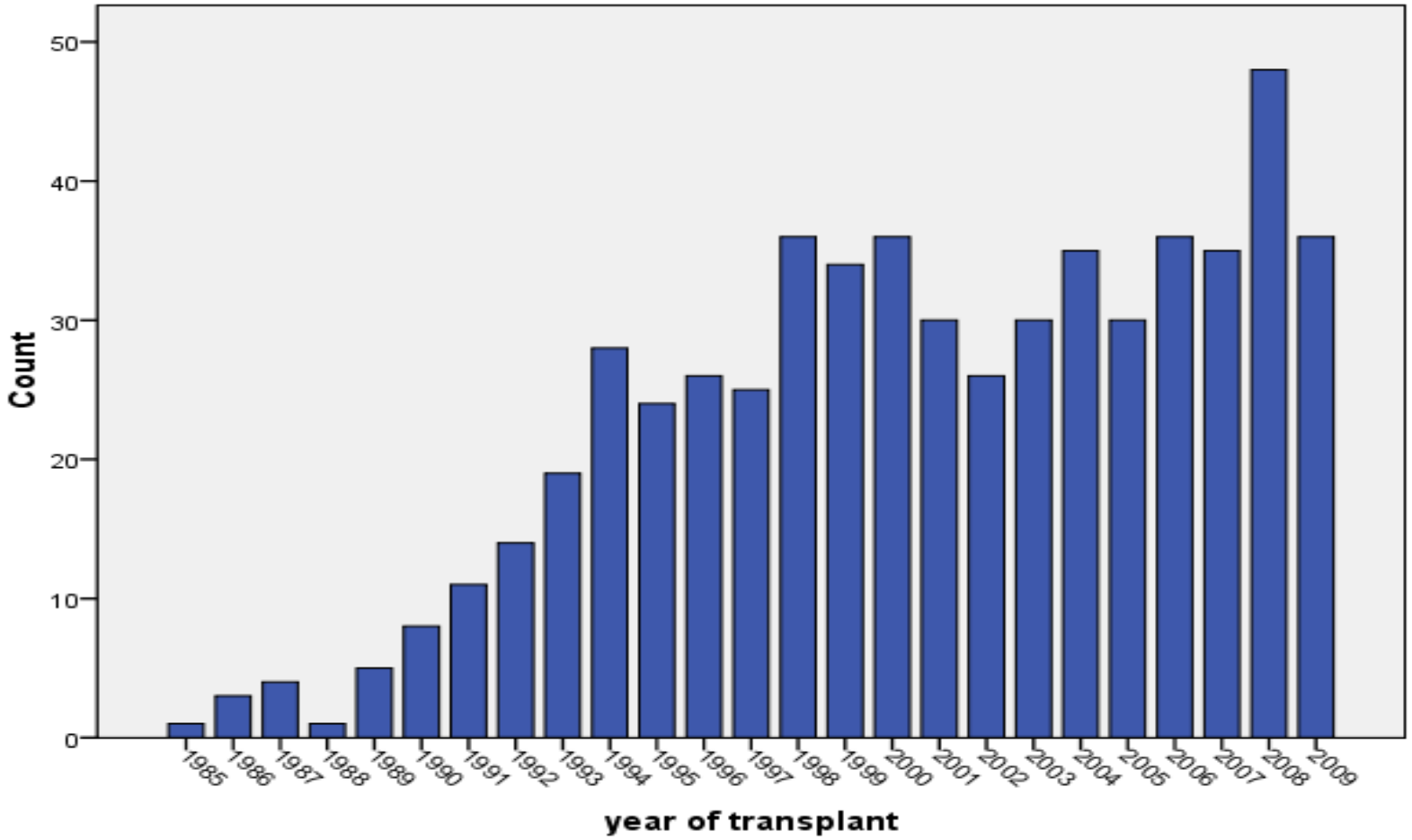
	Autologous	Allogeneic
Healthy stem cells	±	+
HLA compatibility	Yes	Very important
Transplant rejection	-	+
Need for treatment against rejections	-	+
Transplant versus malignancy effect	-	+

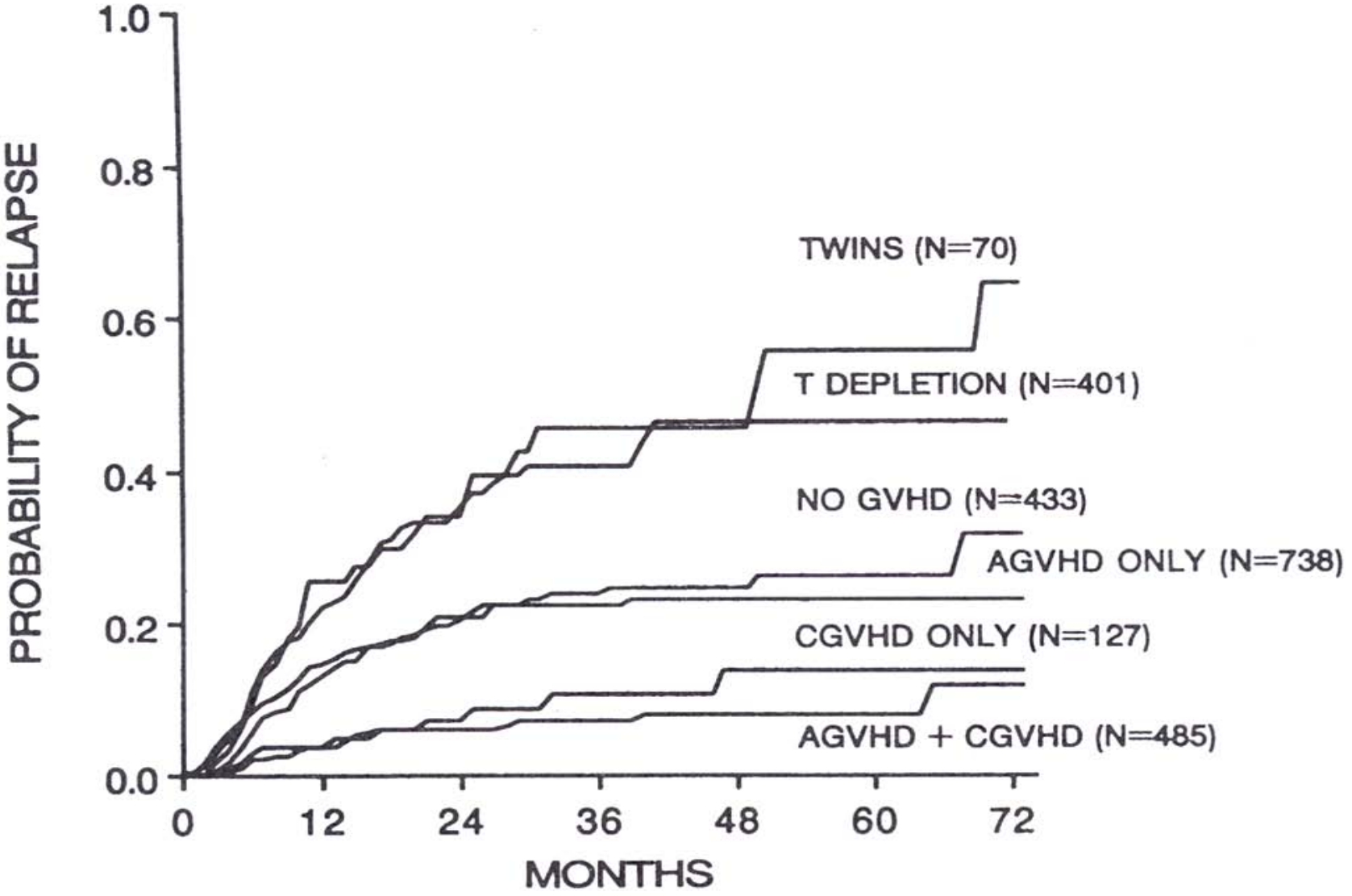
Lorentz Brinch, Department of Blood Diseases, OUS

Diseases treated with allogeneic stem cell transplantation

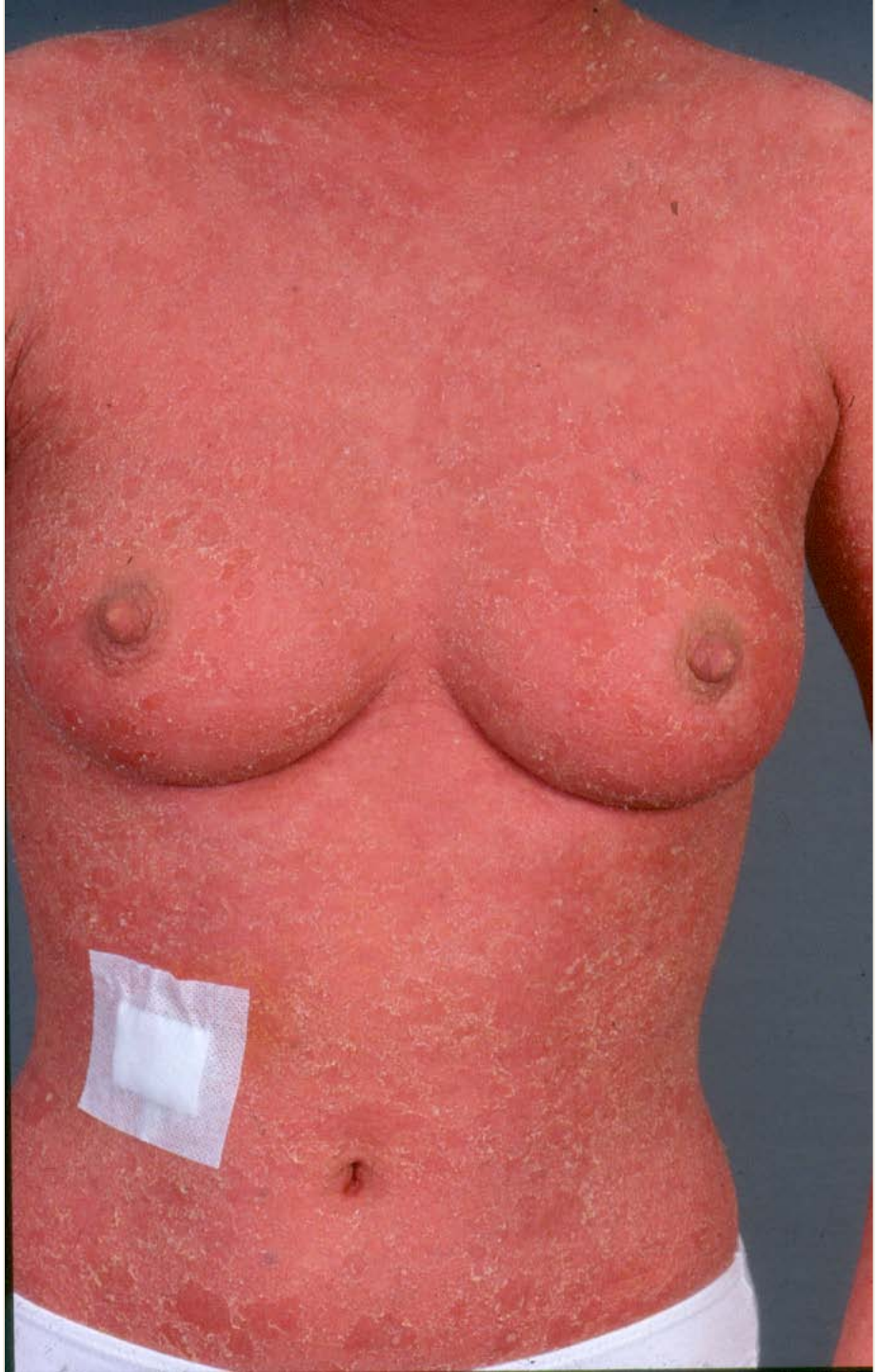


Allogeneic stem cell transplantation in Norway: only performed at Rikshospitalet





Hematopoietic cell transplantation, 2nd edition 1998;319



Tissue engineering

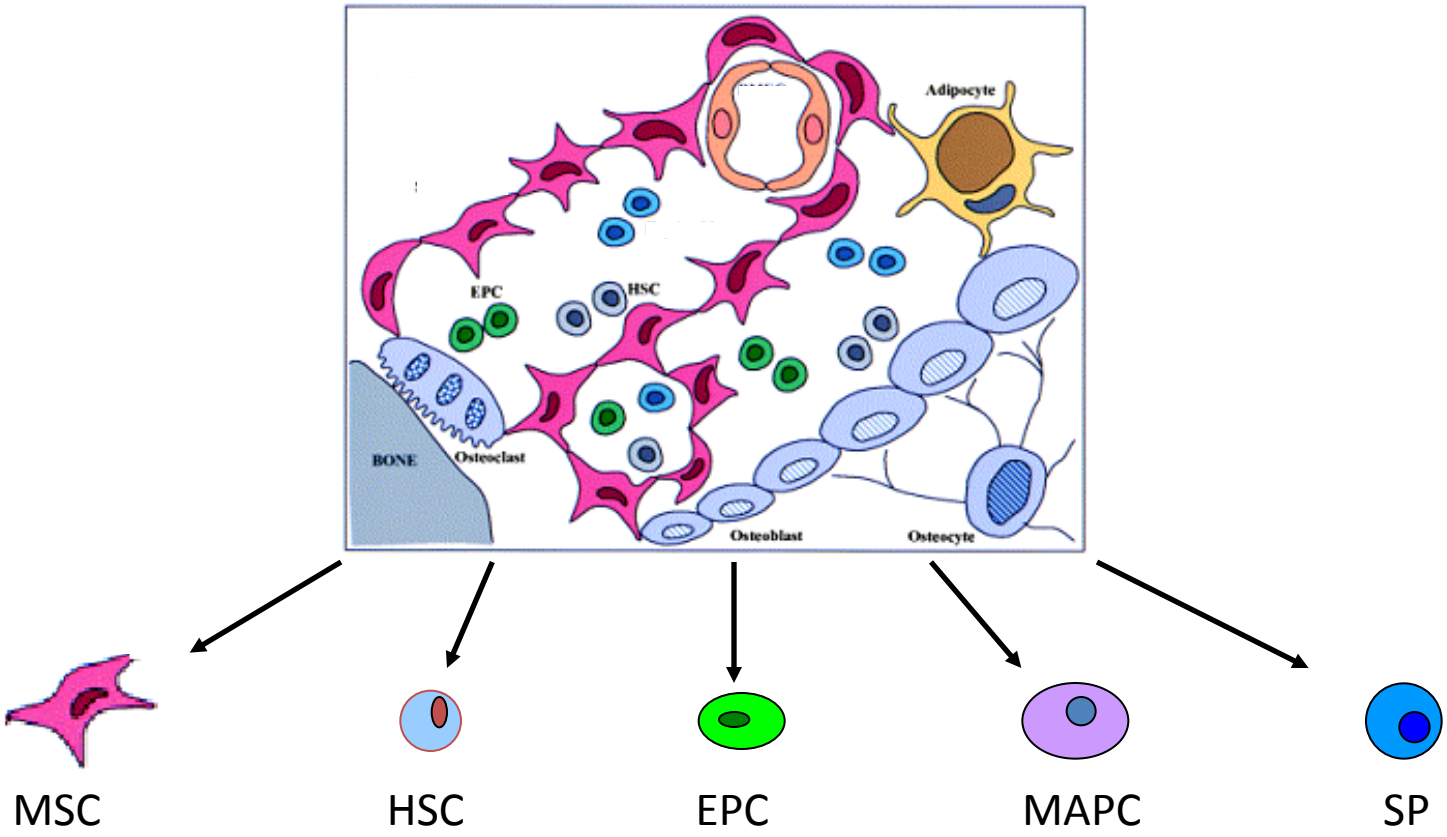
Elements:

- Cells
- Biomaterials
- Imaging
- Advanced surgery

In the clinic:

- Heart
- Cartilage
- Bone
- Eye

Stem/progenitor cells in the bone marrow



Repair of Infarcted Myocardium by Autologous Intracoronary Mononuclear Bone Marrow Cell Transplantation in Humans

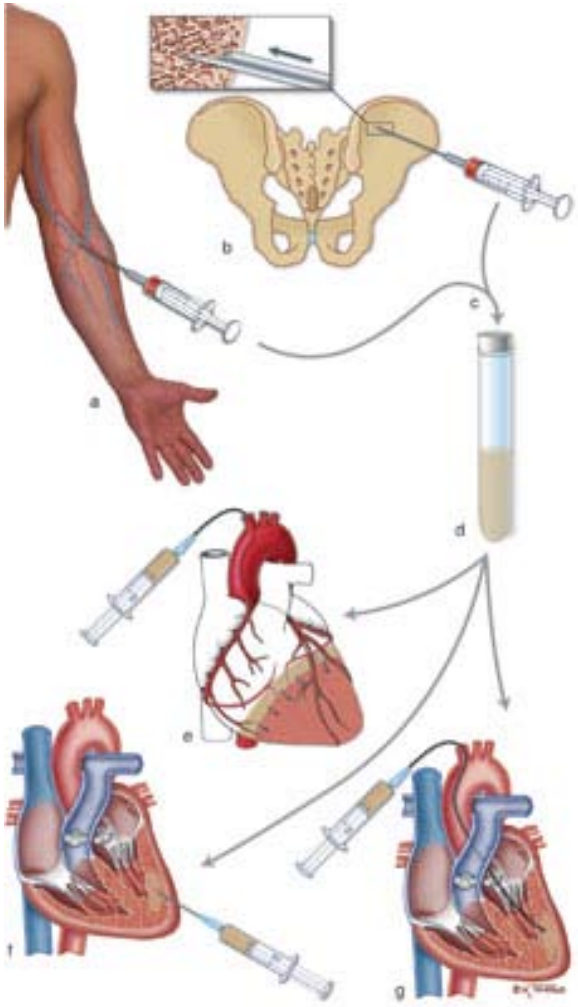
Bodo E. Strauer, MD; Michael Brehm, MD; Tobias Zeus, MD; Matthias Köstering, MD; Anna Hernandez, PhD; Rüdiger V. Sorg, PhD; Gesine Kögler, PhD; Peter Wernet, MD

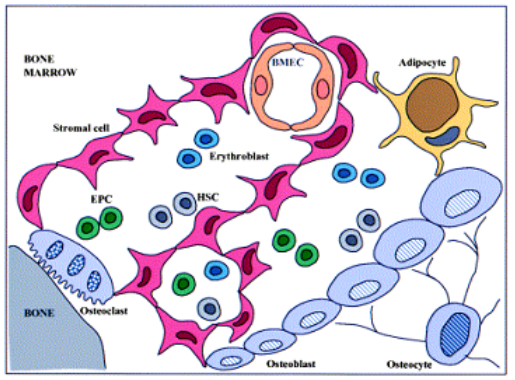
Background—Experimental data suggest that bone marrow–derived cells may contribute to the healing of myocardial infarction (MI). For this reason, we analyzed 10 patients who were treated by intracoronary transplantation of autologous, mononuclear bone marrow cells (BMCs) in addition to standard therapy after MI.

Methods and Results—After standard therapy for acute MI, 10 patients were transplanted with autologous mononuclear BMCs via a balloon catheter placed into the infarct-related artery during balloon dilatation (percutaneous transluminal coronary angioplasty). Another 10 patients with acute MI were treated by standard therapy alone. After 3 months of follow-up, the infarct region (determined by left ventriculography) had decreased significantly within the cell therapy group (from 30±13 to 12±7%, $P=0.005$) and was also significantly smaller compared with the standard therapy group ($P=0.04$). Likewise, infarction wall movement velocity increased significantly only in the cell therapy group (from 2.0±1.1 to 4.0±2.6 cm/s, $P=0.028$). Further cardiac examinations (dobutamine stress echocardiography, radionuclide ventriculography, and catheterization of the right heart) were performed for the cell therapy group and showed significant improvement in stroke volume index, left ventricular end-systolic volume and contractility (ratio of systolic pressure and end-systolic volume), and myocardial perfusion of the infarct region.

Conclusions—These results demonstrate for the first time that selective intracoronary transplantation of autologous, mononuclear BMCs is safe and seems to be effective under clinical conditions. The marked therapeutic effect may be attributed to BMC-associated myocardial regeneration and neovascularization. (***Circulation*. 2002;106:1913-1918.**)

Cardiac repair: can bone marrow cells improve myocardial function in patients with acute myocardial infarction (AMI)?





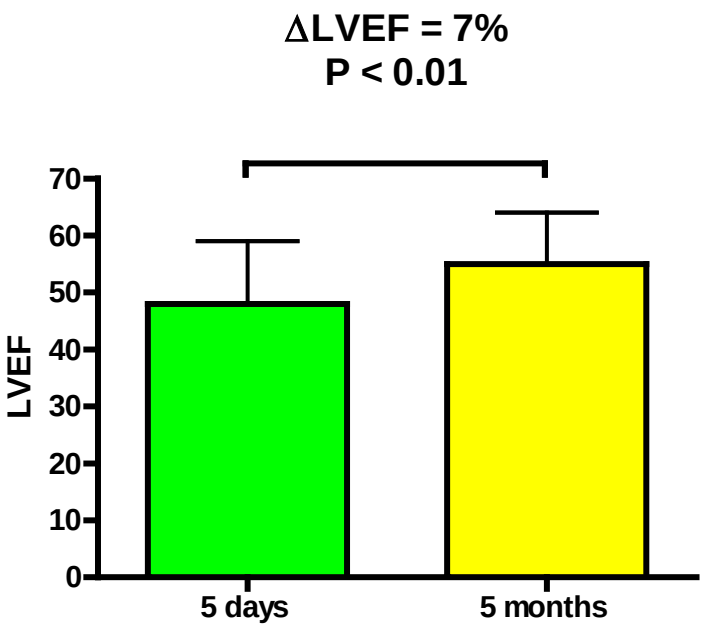
MSC HSC EPC MAPC SP

a) Blood is aspirated to get serum

b) Bone marrow aspiration day 4 - 5

Injection into the affected coronary artery or into the myocardium

Expected improvement in LVEF after AMI by routine treatment

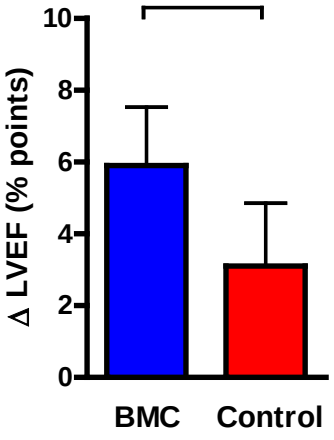


Baks et al, Eur Heart J 2005;26:1070

Results on LVEF in clinical trials with Bone Marrow Cells in AMI

BOOST
n = 60

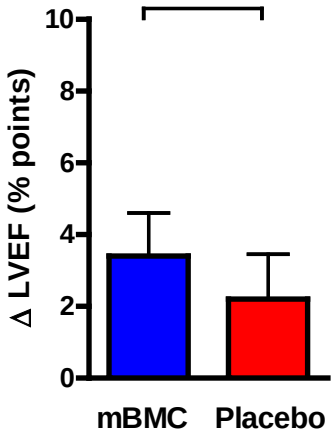
P = 0.27



Meyer et al
Circulation 2006;113:1287-1294

Leuven
n = 67

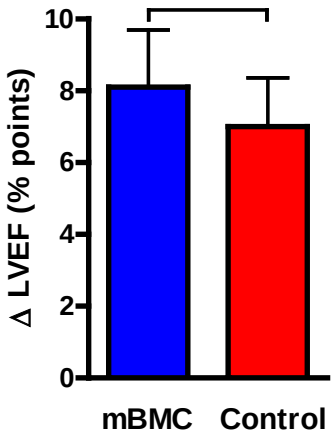
P = 0.36



Janssens et al
Lancet 2006;367:113-21

ASTAMI
n = 100

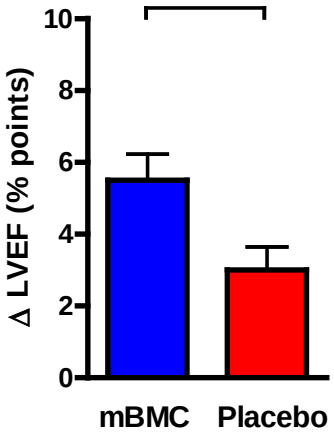
P = 0.77



Lunde et al
NEJM 2006;355:1199-209

REPAIR-AMI
n = 204

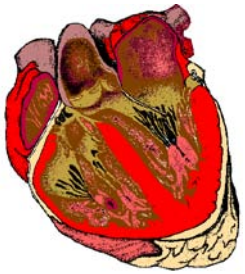
P = 0.01



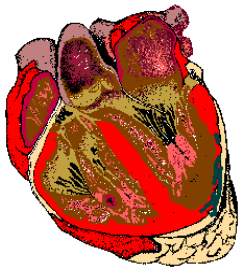
Schächinger et al
NEJM 2006;355:1210-21

What is the reason for the limited success?

The human left ventricle contains
~ 4-5 x10^9 cardiomyocytes



Normal heart

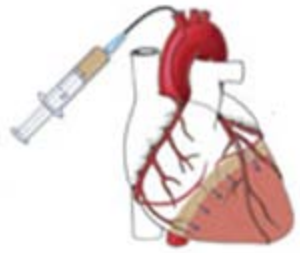


AMI

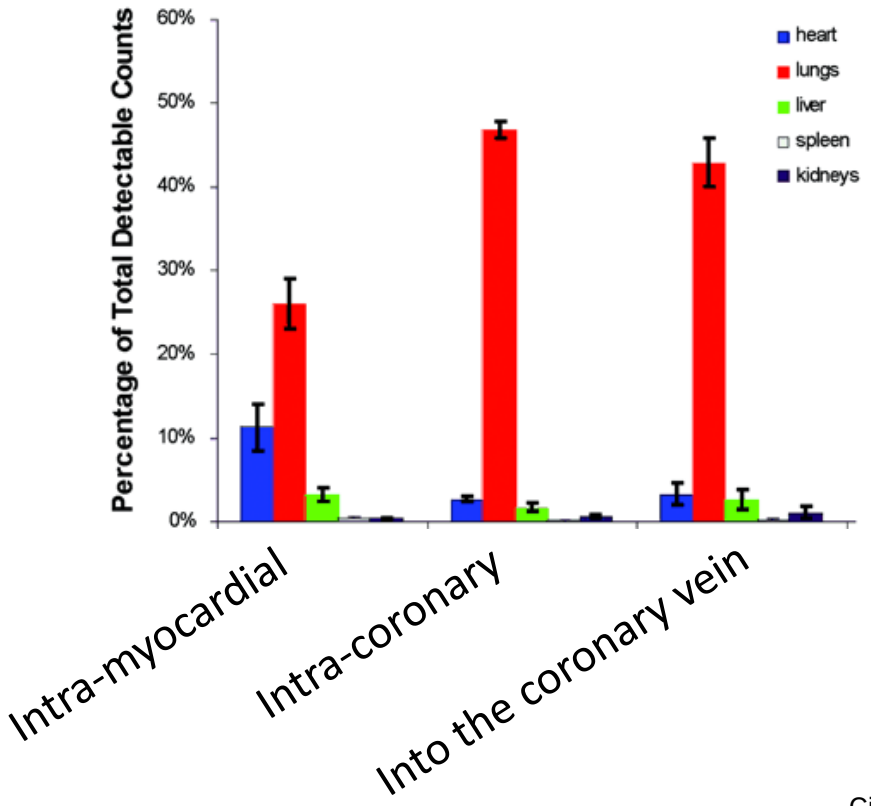
25% MI destroys ~ 1x10^9 cardiomyocytes

Approximately 1% HSC in BM-MNC

Injection of 150x1x10^6 BM-MNC $\rightarrow$ 1.5x10^6 HSC



Very few of the injected cells home to or remain in the myocardium



Analysed 1 hr after injection

Hou et al

Circulation 2005;112[suppl I]:I-150-I-156

ARTICLES

nature medicine

Bone marrow–derived hematopoietic cells generate cardiomyocytes at a low frequency through cell fusion, but not transdifferentiation

Jens M Nygren[1], Stefan Jovinge[1,2], Martin Breitbach[3], Petter Säwén[1], Wilhelm Röll[4], Jürgen Hescheler[5], Jalal Taneera[1], Bernd K Fleischmann[3] & St

Nat Med 2004;10:494-501

Haematopoietic stem cells adopt mature haematopoietic fates in ischaemic myocardium

Leora B. Balsam[1], Amy
Theo Kofidis[1], Irving L

Nature 2004;428:668-73

Haematopoietic stem cells do not transdifferentiate into cardiac myocytes in myocardial infarcts

Charles E. Murry[1], Mark H. Soonpaa[2], Hans Reinecke[1], Hidehiro Nakajima[2], Hisako O. Nakajima[2], Michael Rubart[2], Kishore B. S. Pasumarthi[2]*, Jitka Ismail Virag[1], Stephen H. Bartelmez[3], Veronica Poppa[1], Gillian Bradford[2], Joshua D. Powell[2], David A. Williams[2]* & L

Nature 2004;428:664-8

Bone marrow cells adopt the cardiomyogenic fate *in vivo*

Marcello Rota*, Jan Kajstura*, Toru Hosoda*, Claudia Bearzi*, Serena Vitale*, Grazia Esposito*, Grazia Iaffaldano*, M. Elena Padin-Iruegas*, Arantxa Gonzalez*, Roberto Rizzi*, Narissa Small*, John Muraski†, Roberto Alvarez†, Xiongwen Chen‡, Konrad Urbanek*, Roberto Bolli§, Steven R. Houser‡, Annarosa Leri*, Mark A. Sussman†, and Piero Anversa*¶

*Cardiovascular Research Institute, Department of Medicine, New York Medical College, Valhalla, NY 10595; ‡Cardiovascular Research Center, Temple University, Philadelphia, PA 19140; §Institute of Molecular Cardiology, University of Louisville, Louisville, KY 40292; and †Heart Institute and Department of Biology, San Diego State University, San Diego, CA 92182

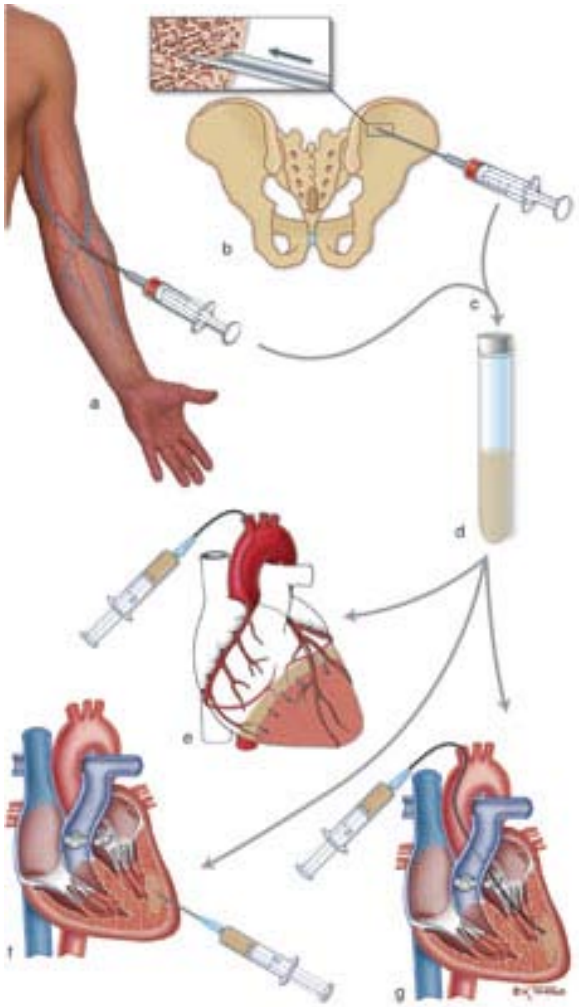
Edited by Andrew R. Marks, Columbia University College of Physicians and Surgeons, New York, NY, and approved September 7, 2007 (received for review July 9, 2007)

PNAS 2007;104:17783-8

Results of Intracoronary Stem Cell Therapy After Acute Myocardial Infarction

Jochen Wöhrle, MD[a,*], Nico Merkle, MD[a], Volker Mailänder, MD[b], Thorsten Nusser, MD[a], Peter Schauwecker, MD[b], Fabian von Scheidt[a], Klaus Schwarz, MD[b], Martin Bommer, MD[c], Markus Wiesneth, MD[b], Hubert Schrezenmeier, MD[b], and Vinzenz Hombach, MD[a]

or LV end-diastolic and end-systolic volume indexes. In conclusion, in this rigorous double-blind, randomized, placebo-controlled trial, we did not observe an evidence for a positive effect for intracoronary BMC versus placebo therapy with respect to LV ejection fraction, LV volume indexes, or infarct size. (Am J Cardiol 2010;105:804–812)



Is it possible to improve myocardial function using cell therapy or tissue engineering following AMI?
Probably

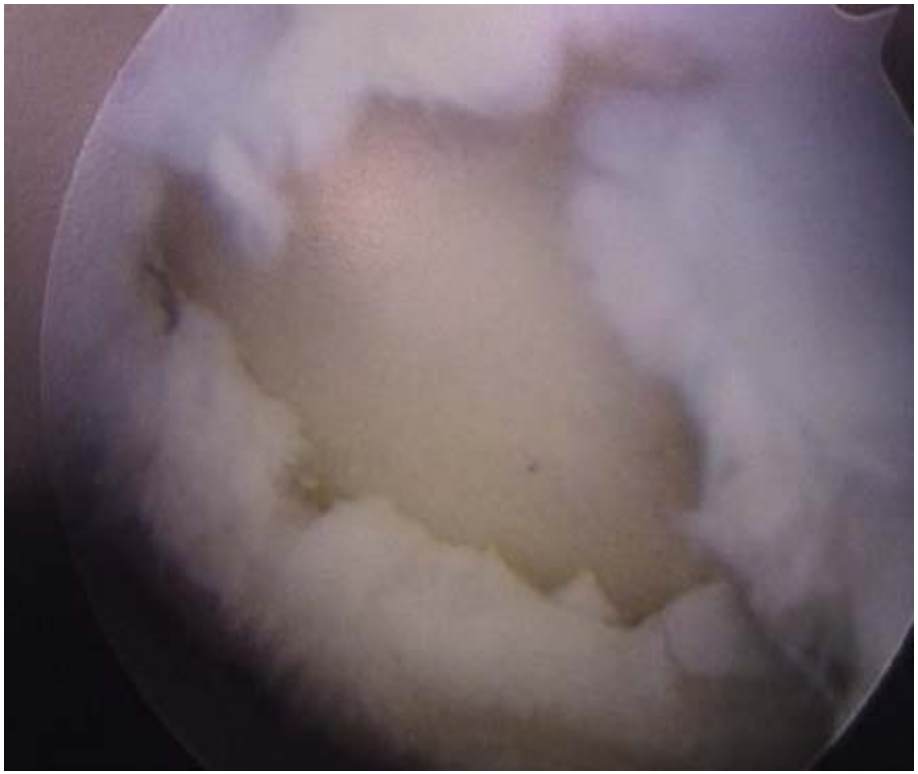
Should this be offered to patients in acute stage MI?
Unlikely, the cells need to be expanded in vitro, and should be autologous

Which are the best cells to use? **Not known, animal studies are ongoing**

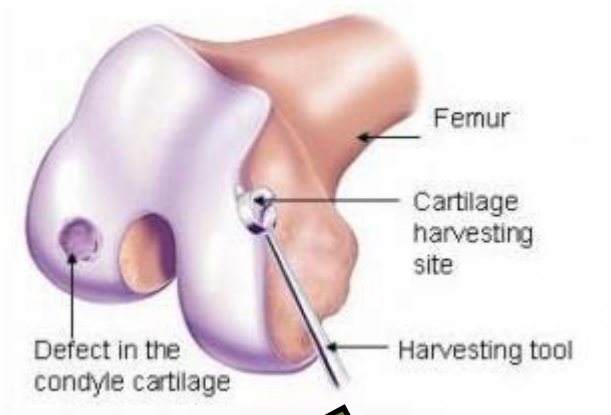
What would be the most likely mechanism for the effect of cell therapy?

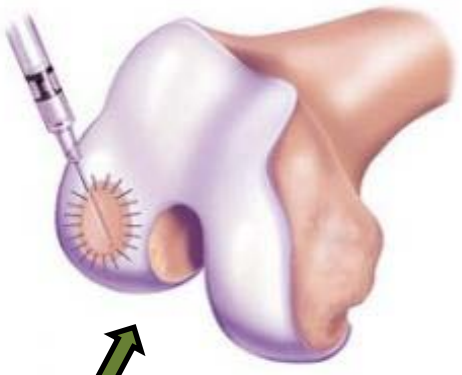
- Transdifferentiation transplanted cells → cardiomyocytes? **Perhaps, but unlikely**
- Stimulation of endogenous repair mechanisms? **More likely**
- Improvement of local blood supply? **Important, may need to include cells specifically for this purpose**

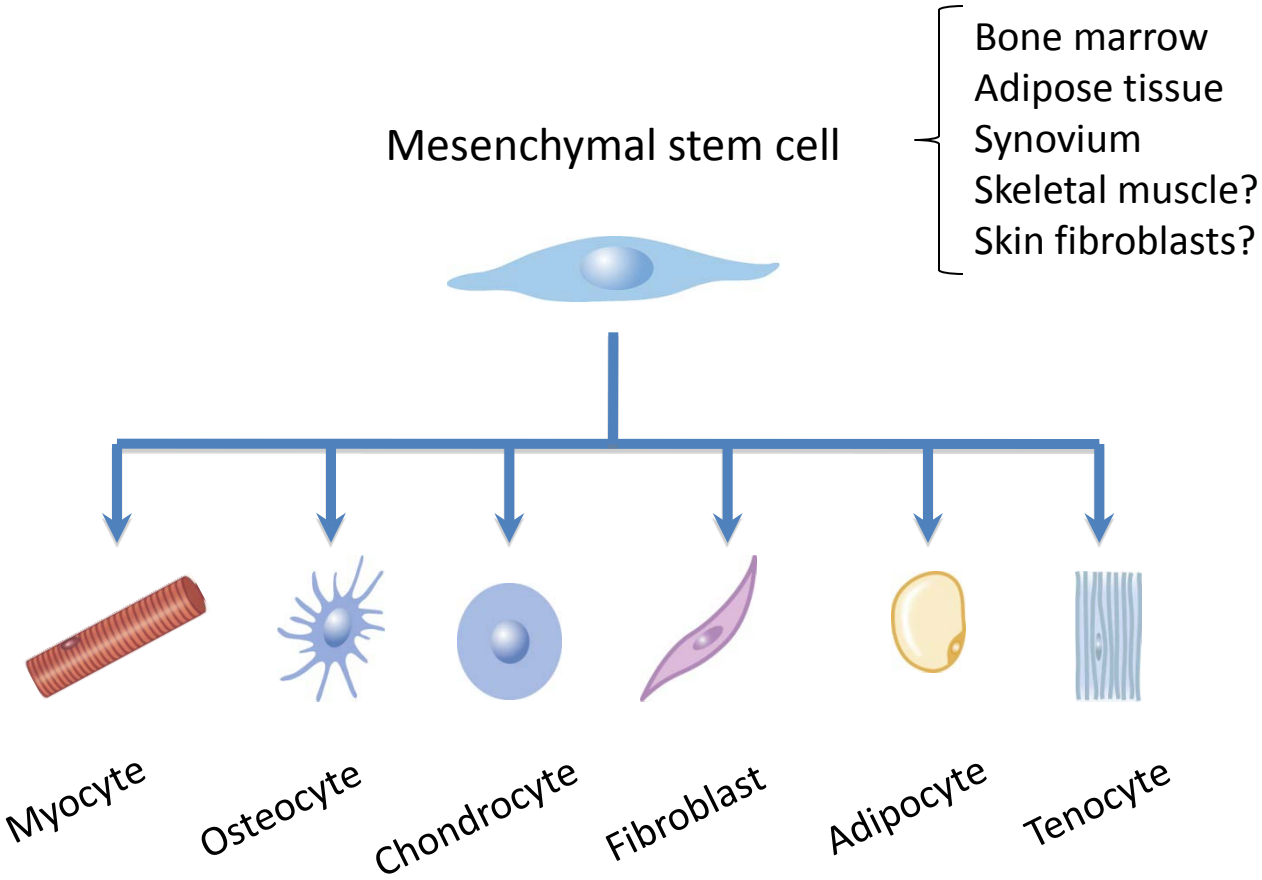
Can adult stem cells be used to treat focal lesions of hyaline cartilage?



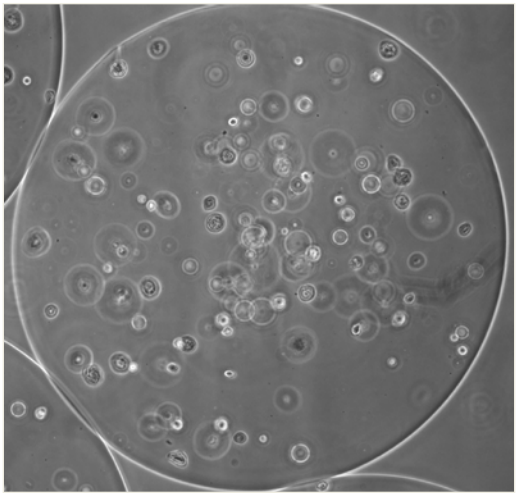
In vitro expanded chondrocytes is used for regeneration of hyaline cartilage, but the result is frequently fibrocartilage





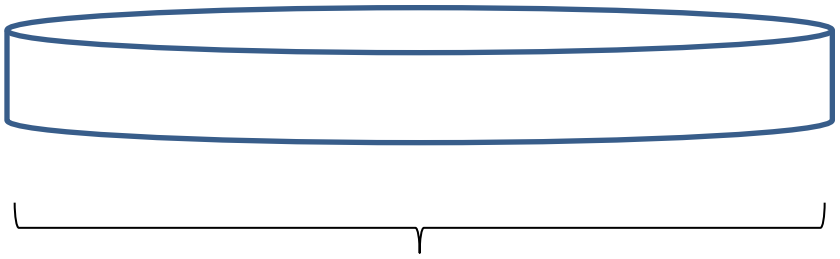

Mesenchymal stem cell
Bone marrow
Adipose tissue
Synovium
Skeletal muscle?
Skin fibroblasts?
Myocyte
Osteocyte
Chondrocyte
Fibroblast
Adipocyte
Tenocyte

Alginate as a scaffold for chondrogenic differentiation of MSC

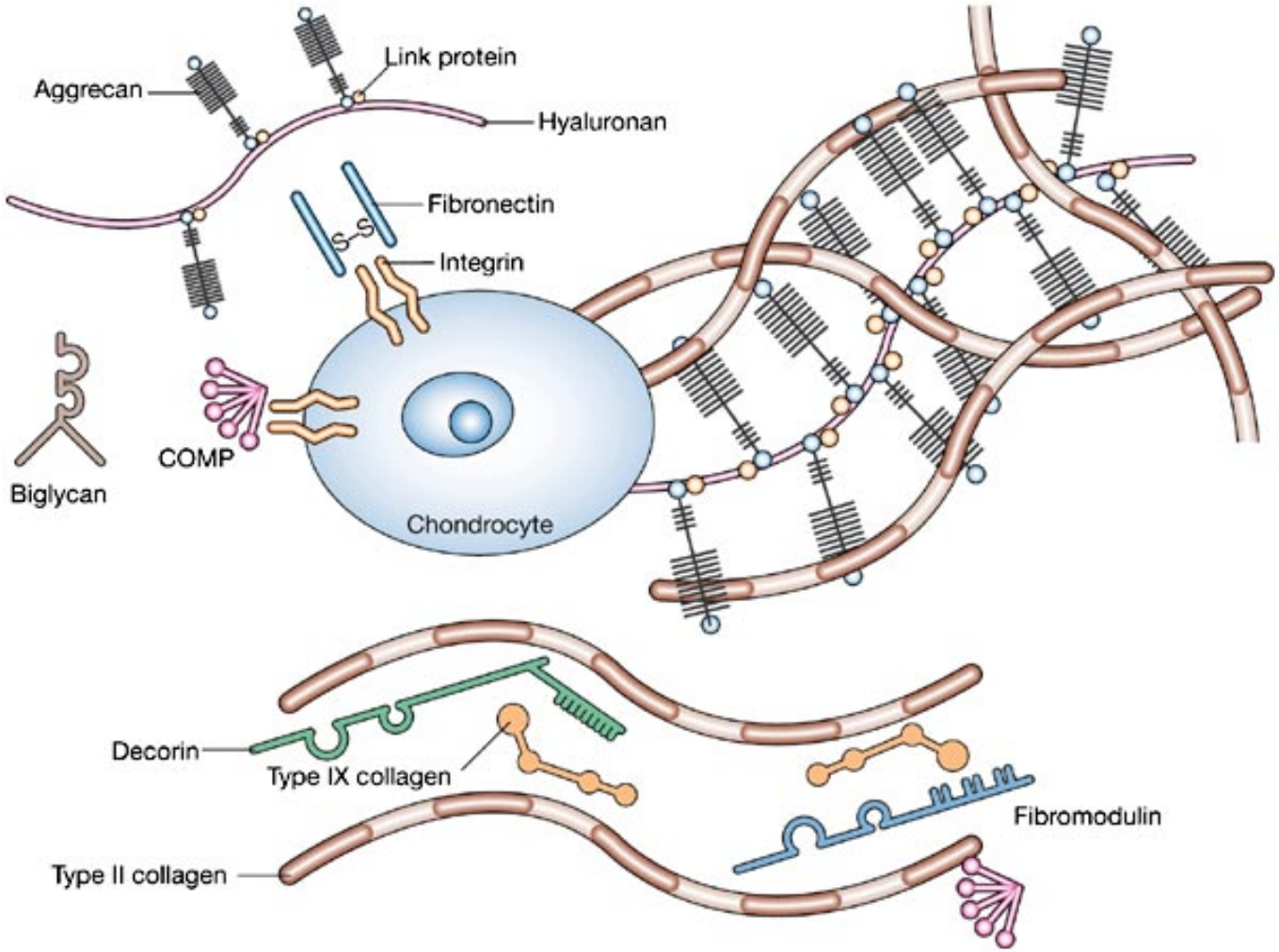


The scaffold can be made to shape of choice

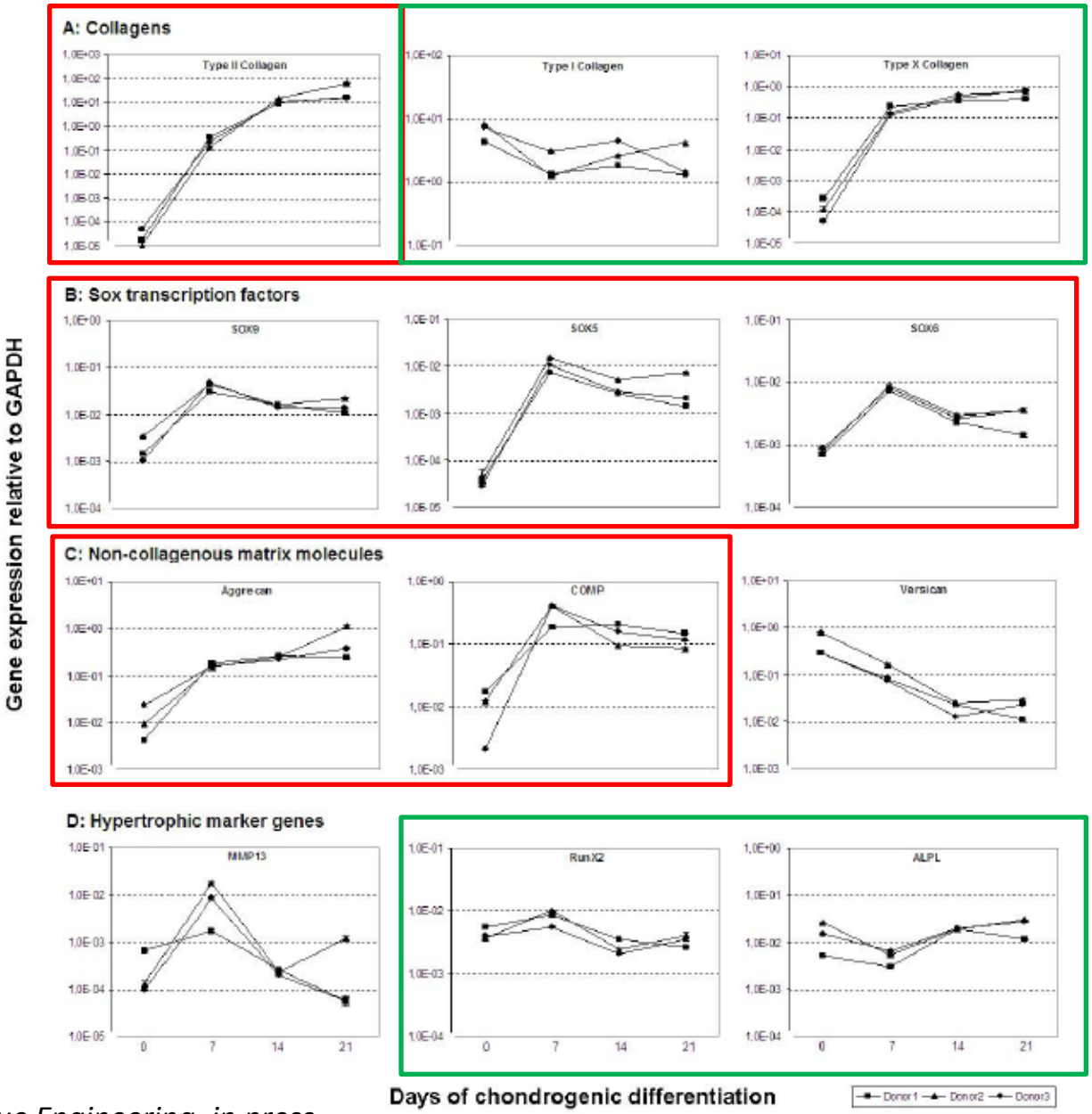
- Cells are quite evenly distributed
- The alginate can be easily removed
- Alginate may be made biodegradable?



Components of normal hyaline matrix

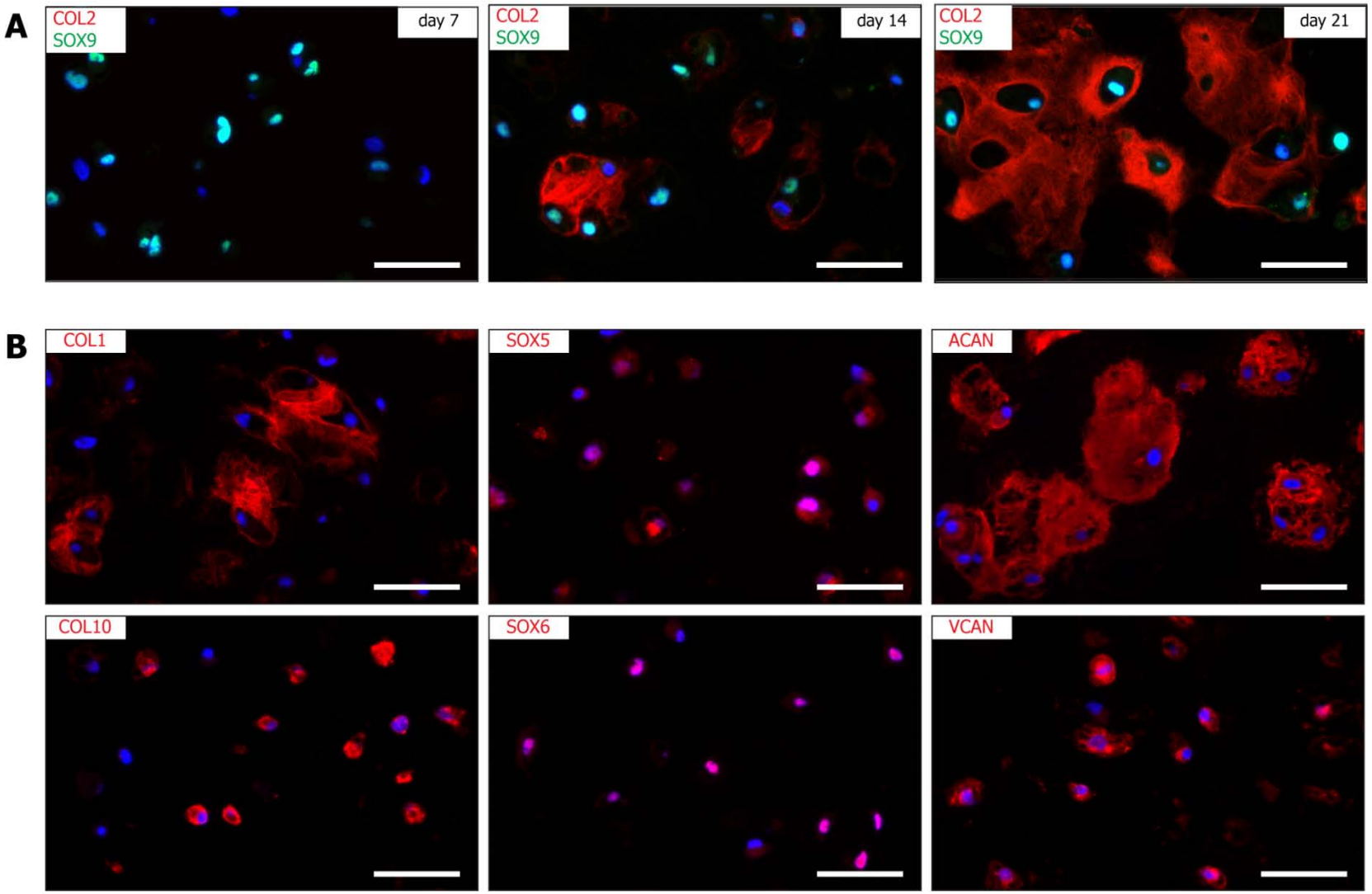


Sarah Herlofsen: Changes in mRNA expression in the course of 3 week differentiation in alginate

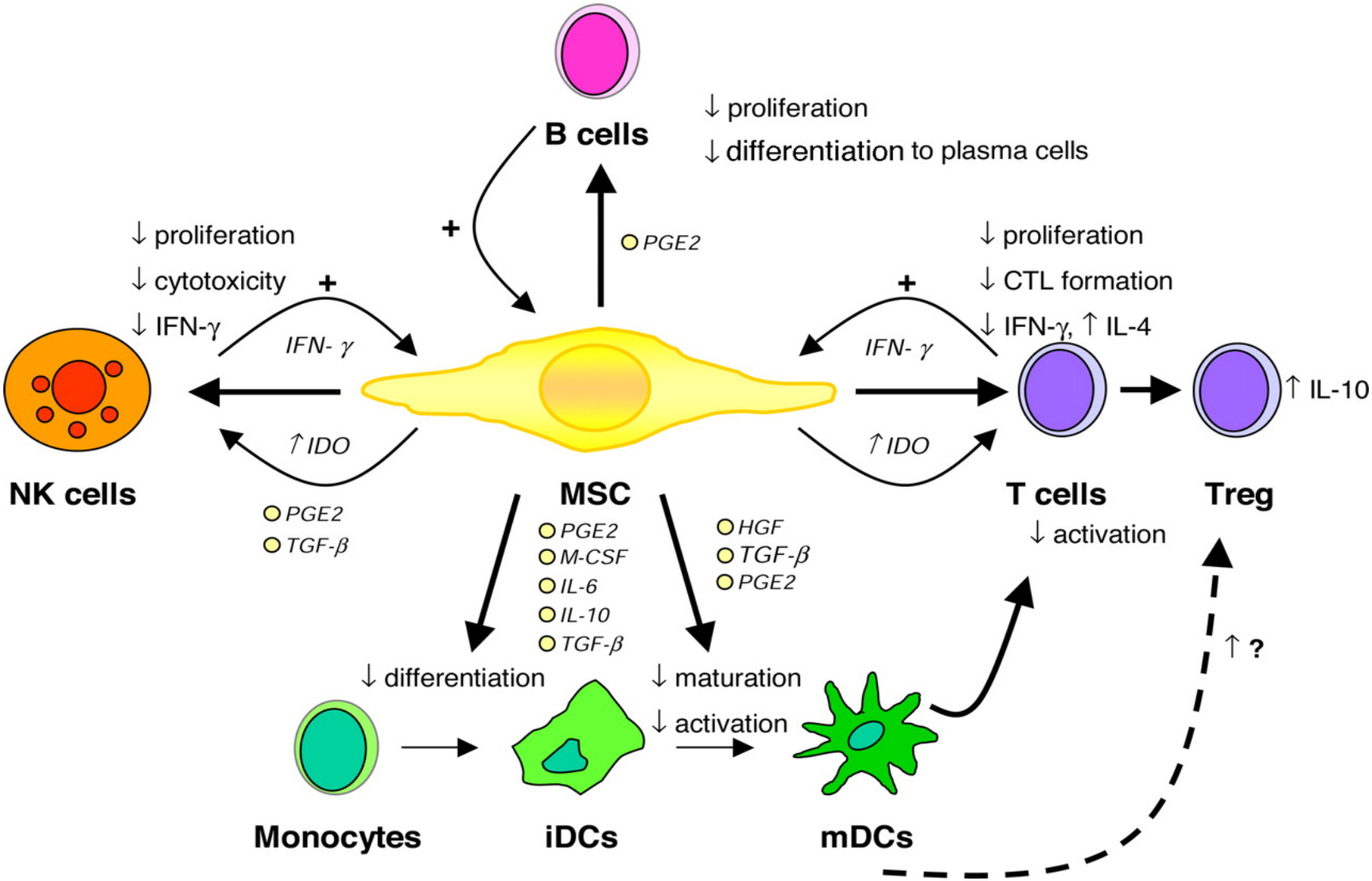


Herlofsen et al, Tissue Engineering, in press

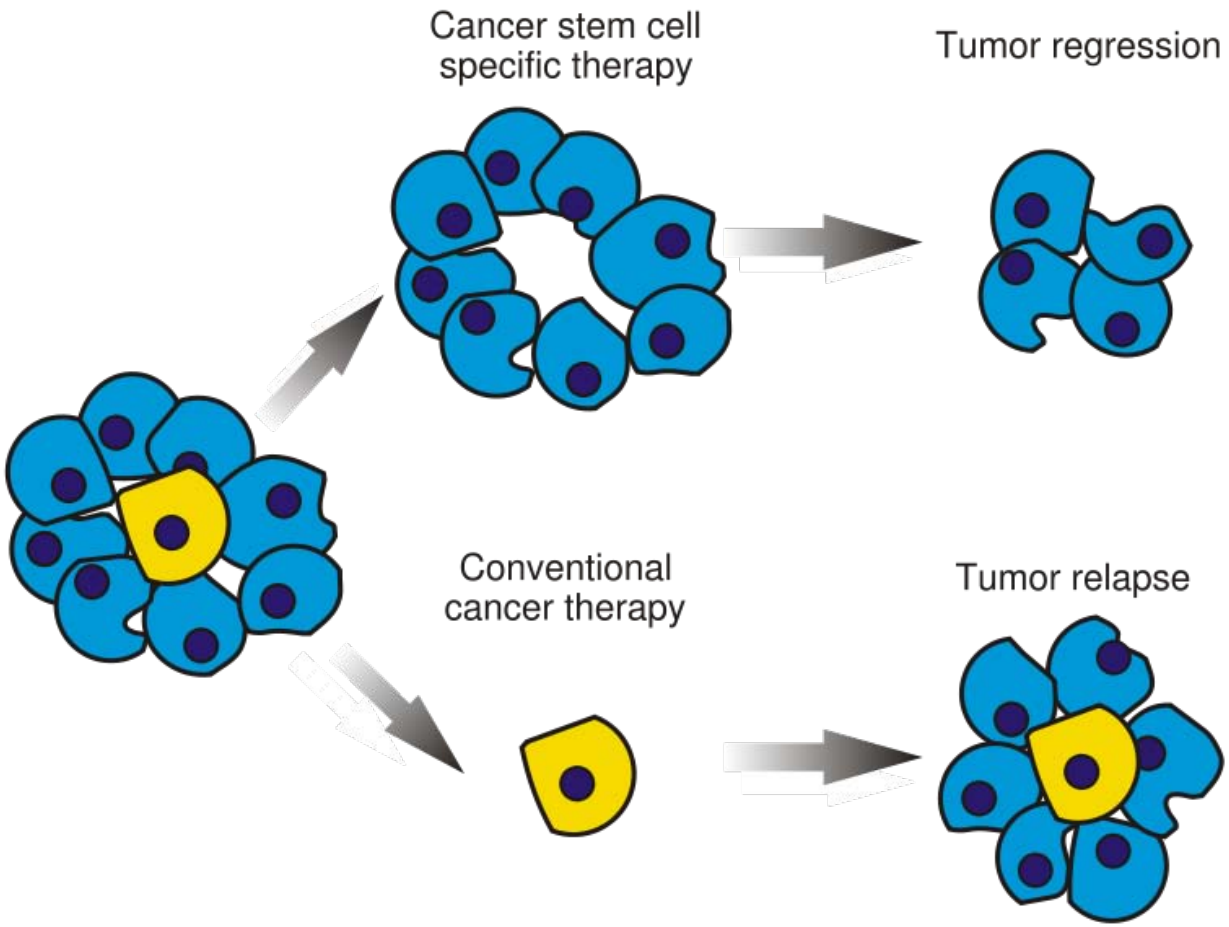
Expression of proteins of importance for chondrogenesis after 21 days of differentiation in alginate discs



MSC may exert immunosuppressive effects

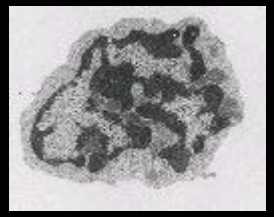


Tumor stem cells



Can expressed genes from glioblastoma stem cells be used in a therapeutic vaccination?

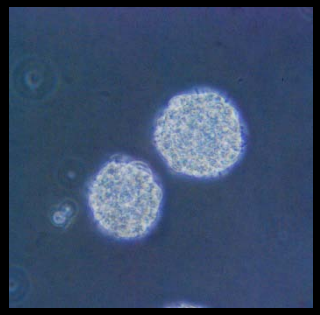
hTERT and survivin mRNA



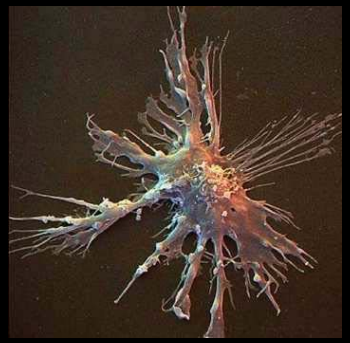
Immature DCs

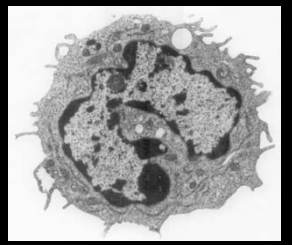
mRNA amplification
and purification

mRNA loading
by electroporation



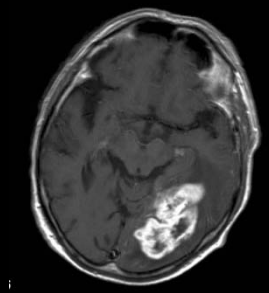
Tumor stem cells



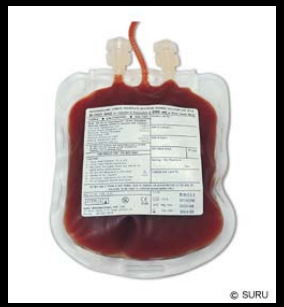


Monocytes

Maturation of DCs



Tumor biopsy



Leukapheresis

The Ex vivo cell laboratory is a GMP regulated production facility for cells for therapeutic trials

Pharmaceutical Net - [Sensor Status]

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27INK-1-B-AL-F	27INK-2-T-RF	28INK-2-B-TS-F	29INK-2-B-O2-F	33-LN2-2-H-F	Lasair II 510
27INK-1-B-CO2 3.3	27INK-2-T-RF-F	28INK-2-T-AL-F	29INK-2-B-RF 0.0	33-LN2-2-HH-F	LII
27INK-1-B-CO2-F	27INK-2-T-TE	28INK-2-T-CO2 0.0	29INK-2-B-RF-F	33-LN2-2-L-F	MINILAZ REMOTE
27INK-1-B-O2 0.0	27INK-2-T-TE-F	28INK-2-T-CO2-F	29INK-2-B-TE 0.0	33-LN2-2-LL 0.0	N2-Sidebytte 0.0
27INK-1-B-O2-F	27INK-2-T-TS	28INK-2-T-O2 0.0	29INK-2-B-TE-F	33-LN2-2-LL-F 0.0	O2-Sidebytte 0.0
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27INK-1-B-TS 37.5	28INK-1-B-O2	28INK-2-T-TE-F	29INK-2-T-CO2-F	365A403 Vent.alarm 0.0	PE27/31 17.1
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27INK-1-T-AL-F 0.0	28INK-1-B-RF 91.9	28INK-2-T-TS-F	29INK-2-T-O2-F	365A403 Vent.svikt 0.0	PE28/31 -16.6
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27INK-2-T-CO2	28INK-2-B-RF-F	29INK-2-B-AL-F	33 LN2-1-TE	FP31L1 16	TE32F2T -22.9
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International Society for Stem Cell Research

Patient Handbook on Stem Cell Therapies

Appendix I of the Guidelines for the Clinical Translation of Stem Cells

December 3, 2008

English

XCell-Center
at the Institute for Regenerative Medicine

Home | About | Treatments | Patient Stories | Request information | Contact

News

Home

The XCell-Center is a private clinic group and institute for regenerative medicine located in Düsseldorf and Cologne, Germany. Bringing together therapeutical use of autologous adult stem cells and medical research, it is our mission to:

- Provide therapeutic application of autologous adult stem cells to patients at the highest medical standard;
- Extend existing knowledge on the effects of autologous adult stem cells by supporting pre-clinical and clinical research.

We offer patients with degenerative diseases the opportunity to undergo an innovative and promising stem cell treatment.

Since the start in January 2007, **more than 2500 patients** have safely undergone our various stem cell treatments.

News

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Therapeutic use

The XCell-Center treats patients with their own autologous adult stem cells. It is the first private clinic worldwide to hold an official license for the extraction and approval of stem cell material for autologous treatment.

Therapy focuses on the treatment of cerebral palsy, spinal cord injuries, diabetes mellitus (types 1 and 2 as well as sequelae) and neurological diseases/disorders such as Parkinson's and stroke. Further indications include multiple sclerosis (MS), amyotrophic lateral sclerosis (ALS), and Alzheimer's as well as arthritis, heart disease, and eye diseases such as macular degeneration.

Advisory board

Learn more about the XCell-Center's Scientific Partners.

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June 16 & 17

Related topics

- Healing potential
- Methods of use
- Physiological mechanism
- Limits of therapy
- No Tumor risk
- Treatment process

Overview of our stem cell treatment

As a patient or the friend or relative of a patient, you have likely consulted this website to learn some basic facts about our stem cell treatment offerings. Therefore, we have carefully compiled relevant information on these pages that we hope will help you.

We would like to point out from the start that there are still some questions concerning the function of stem cells that science has not yet been able to answer, and that despite the advances that have been made recently, there is no guarantee for the success of stem cell therapy. Nevertheless, every week we see this new "medicine" helping a lot of people. Therefore, we offer therapies with adult stem cells *whenever classical treatment does not yield the type of results that are satisfactory for the patient.*

After evaluating important information from each prospective patient's medical history, our medical team decides whether the prospective patient is a suitable

News

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Methods of use - adult stem cells

The use of endogenous adult stem cells is ethical and legally straightforward. Under German law, the extracted stem cells are categorized as drugs. Because they are exclusively for personal use, they are individual drugs, and under German law do not require the same governmental approval as other drugs. Despite this, the clinic still has to obtain a manufacturing license from the surveillance authority. At the XCell-Center, it is guaranteed that the processes of extraction, cleaning and transplantation are all carried out in compliance with Good Manufacturing Practice (GMP) standards, thus guaranteeing maximum quality and safety for the patient.

For the last few years, attempts at therapy with adult stem cells from bone marrow have been carried out at university hospitals. This means that unlike animal testing with embryonic stem cells, adult stem cells are in-part, already being clinically tested. The well-documented success of the cardiologist Prof. Dr. Bodo Strauer from Düsseldorf can be seen as an example. He treated a patient suffering from a series of heart attacks for whom common therapies could not assure any chance of survival with the patient's own bone marrow stem cells. Nine days after the stem cells had been injected into the diseased area, the patient was able to leave the intensive care unit. Up to now, more than 300 patients have been treated in Düsseldorf using this procedure - most of them successfully.

The XCell-Center's treatment is based on the therapy experiences of more

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The world's largest clinical experience in fetal stem cell transplantation
Stem cell treatment for various diseases, conditions and Anti-age treatment

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- Anemi
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- Arteriell hypertension
- Diabetesbehandling
- Muskeldystrofi
- Cancer
- Idiopatisk encefalopati
- Ischemisk hjärtsjukdom
- Leversjukdomar
- Multipel skleros (MS)-behandling
- Parkinson
- SMA - Spinal muskelatrofi
- Ulcerös kolit/Crohns sjukdom

Behandling

Världens största kliniska erfarenheten av foster stamcellstransplantation
Stamceller behandling för olika sjukdomar, villkor och anti-aging

EmCell behandlar olika sjukdomar och rubbningar med en avancerad och patenterad stamcellsbehandling. De fetala stamceller som vi använder i behandlingen är icke-specialiserade celler som kan omvandla sig till alla typer av celler i kroppens olika vävnader och organ. Fetala celler har en enorm potential till differentiering och spridning och de avvisas inte av mottagarens kropp.
mer..

Stamcellsbehandling har visat sig vara effetktivt för "återställning" av organ och vävnad, men också i kampen mot obotliga och kroniska sjukdomar. EmCell behandlar patienter med många olika sjukdomar, till exempel diabetes mellitus, multipel skleros, Parkinsons sjukdom, Duchennes muskeldystrofi, cancer och blodstörningar samt genetiska och ärftliga sjukdomar. En del av våra patienter kommer också till oss för att få anti-agingbehandling. Stamcellsbehandling erbjuder möjligheten att uppnå effekter som är långt utöver vad man kan förvänta sig av någon annan modern behandlingsmetod
mer...

Behandling utomlands

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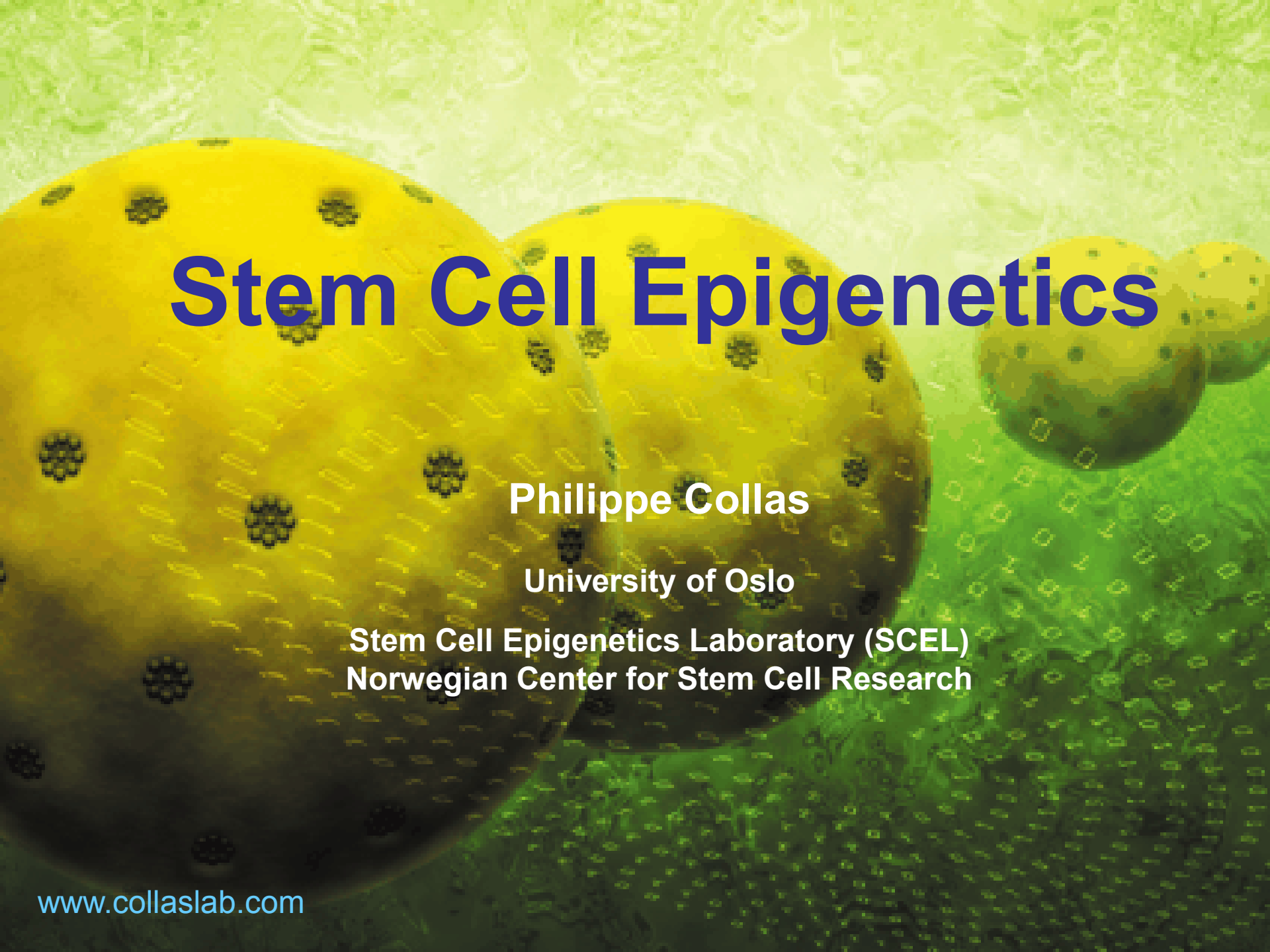
ex: name@gmail.com

Stem cells carry a lot of promise for the development of new therapeutic options, but they should be introduced into the clinic with great caution



Stem Cell Epigenetics

Philippe Collas

University of Oslo

Stem Cell Epigenetics Laboratory (SCEL)
Norwegian Center for Stem Cell Research

www.collaslab.com

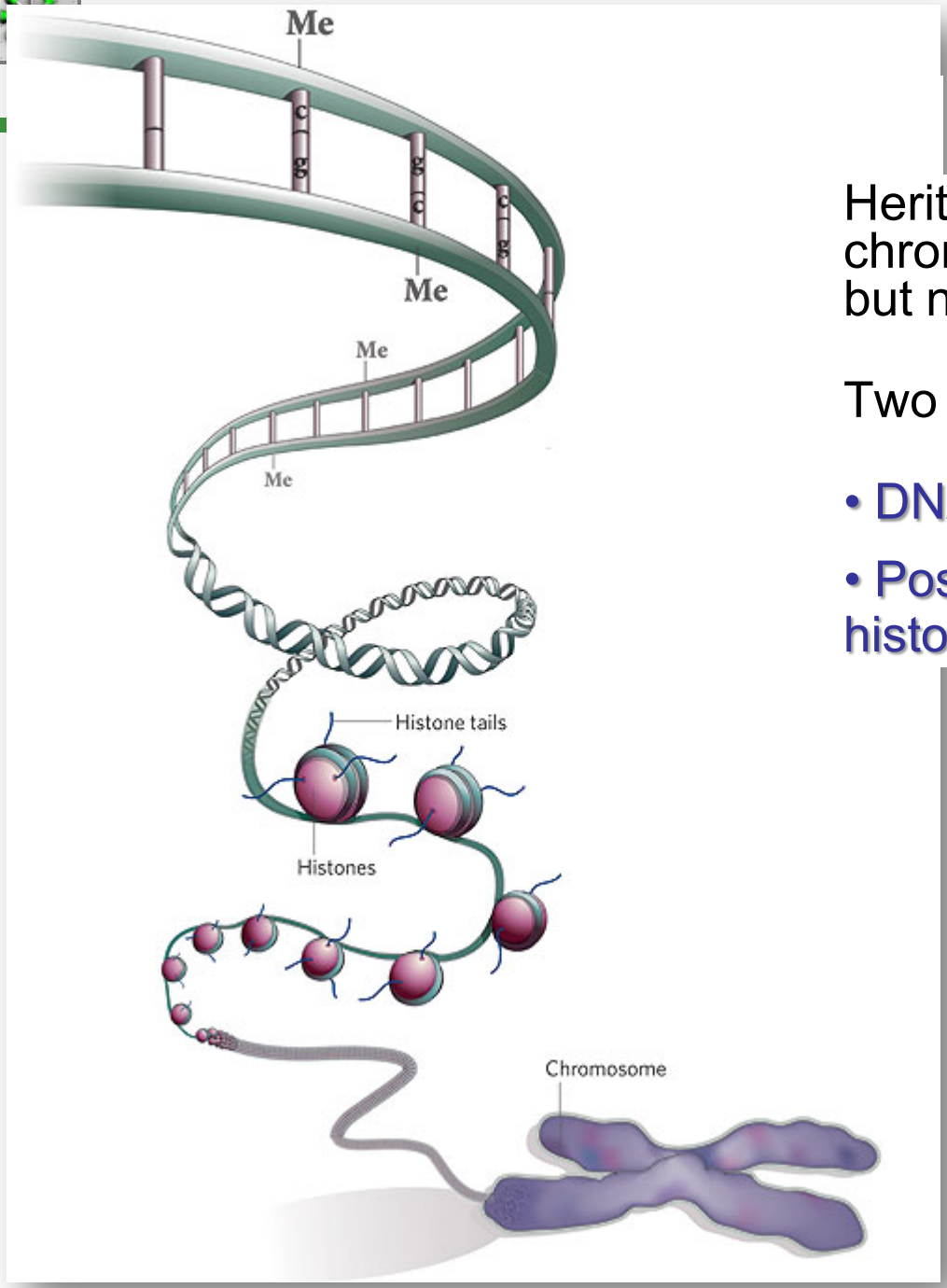
What makes stem cells pluripotent?

- Receptors on their surface, that make stem cells responsive to signals from their environment (the niche)
- Low level expression of genes normally expressed in many different specific cell types (e.g., bone, fat, neurons, muscle, cartilage, etc)
- How genes are packaged in the cell nucleus
  - **active genes**: 'open' configuration (accessible)
  - **inactive genes**: 'closed' configuration (inaccessible)
  - **inactive genes with a potential for activation**:'open' configuration, but with a 'brake on'

Lecture outline

- Introduction to epigenetics
- What provides embryonic stem cells with pluripotent differentiation capacity?
- What about epigenetic states in somatic (adult) stem cells?

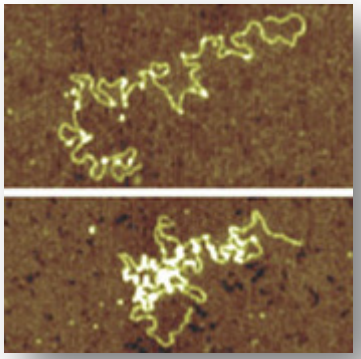
Epigenetics



Heritable modifications of DNA or chromatin that affect gene function, but not DNA sequence.

Two main components:

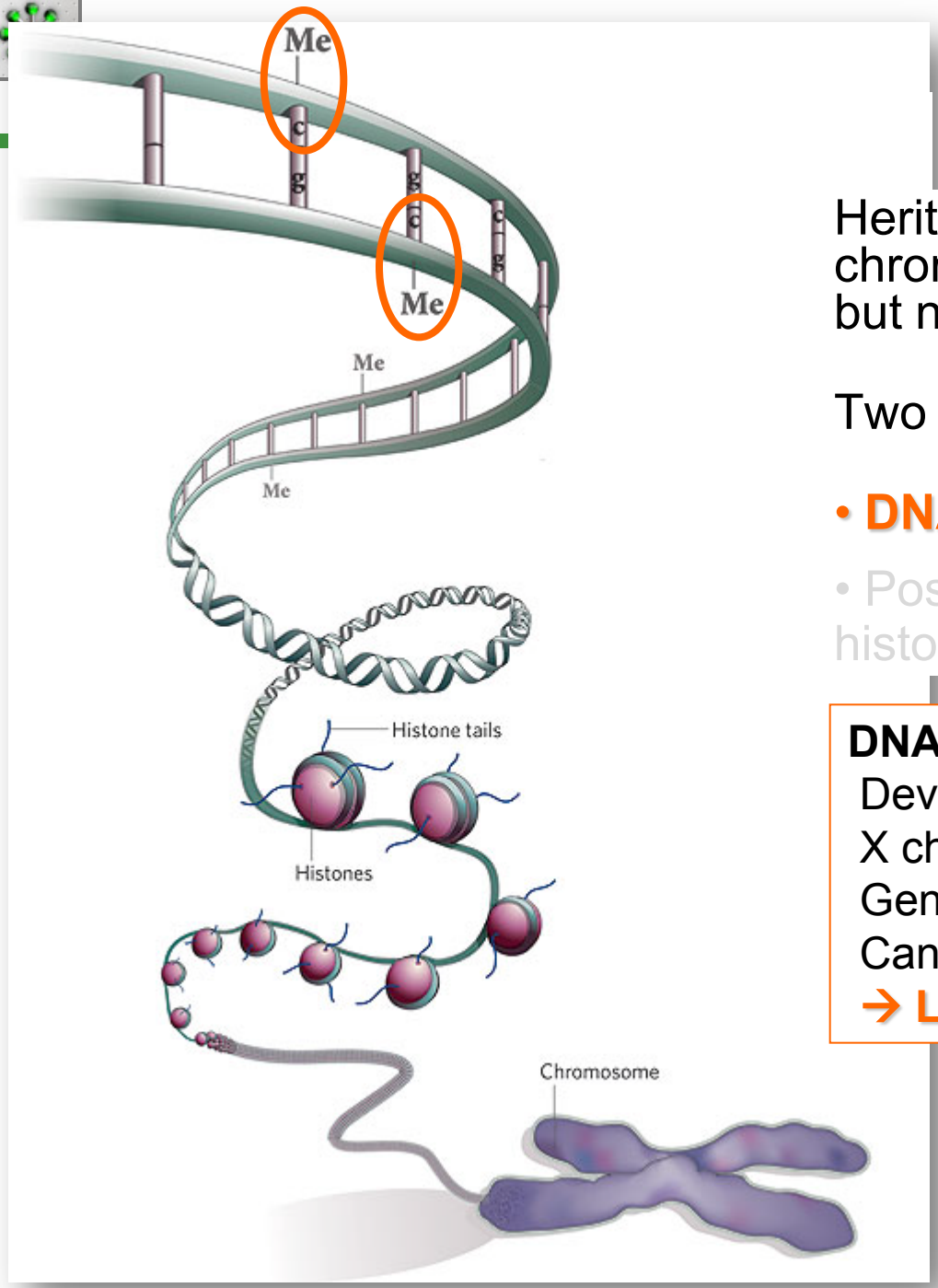
- DNA methylation
- Post-translational modifications of histones



Gene ON

Gene OFF

Epigenetics



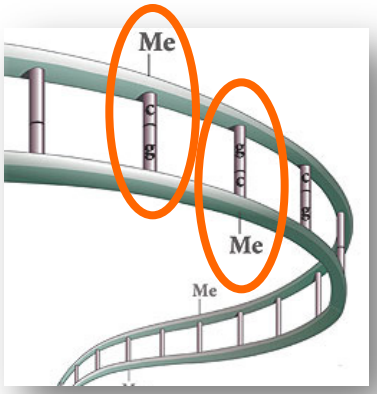
Heritable modifications of DNA or chromatin that affect gene function, but not DNA sequence.

Two main components:

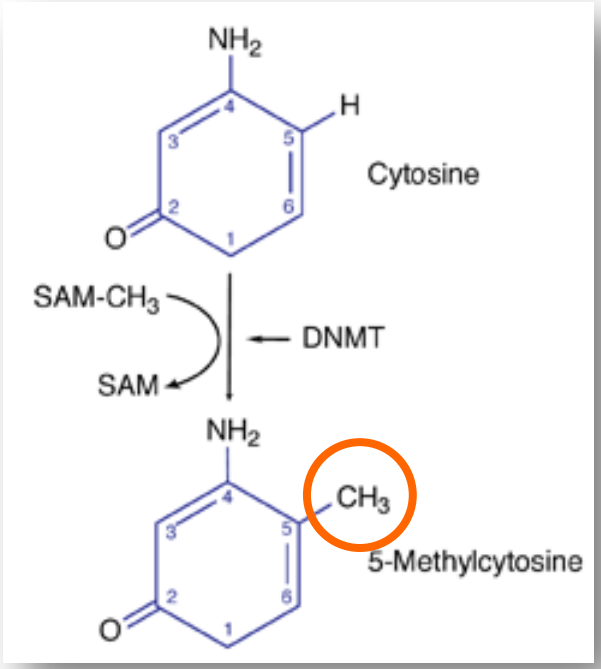
- **DNA methylation**
- Post-translational modifications of histones

DNA methylation is implicated in:
Development
X chromosome inactivation
Genomic imprinting
Cancer: silencing of tumor suppressors
→ **Long-term gene silencing**

A few facts about DNA methylation



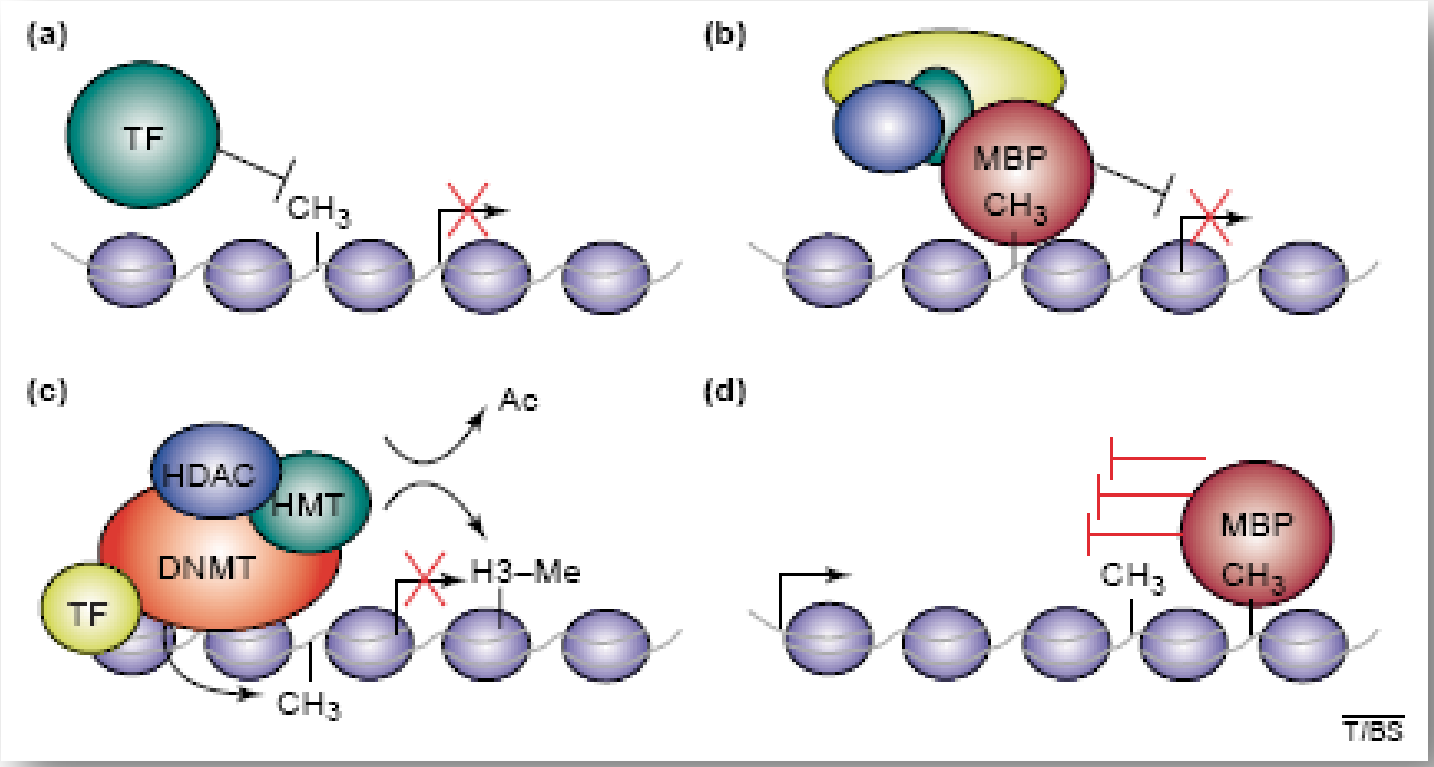
5’ – ^{m}CpG – 3’
3’ – GpC$_{m}$ – 5’



DNA methyl transferases

- **DNMT1**: maintenance methyltransferase; recognizes hemimethylated DNA after replication; ensures fidelity of methylation in daughter cells after cell division
- **DNMT3a/b**: de novo methyltransferase (embryo development, differentiation)
- **DNMT2**: no known DNA methyltransferase activity; methylates RNA

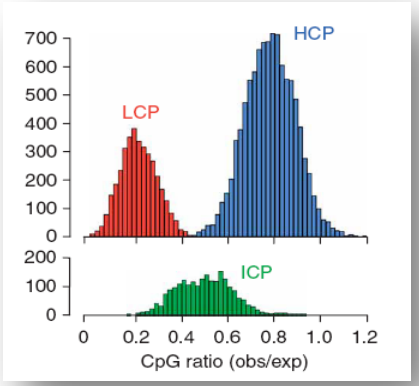
Mechanisms of DNA methylation-mediated gene repression



(a) Inhibition of transcription factor binding to methylated regions
(b) Co-recruitment of a transcriptional co-repressor complex by methyl-binding proteins (MBPs)
(c) Recruitment of histone modifying enzymes (HDACs, HMTs) by DNMTs
(d) MBPs can also bind in the body of genes, inhibiting transcription elongation

Klose and Bird, 2006. Trends Biochem. 31, 89-97

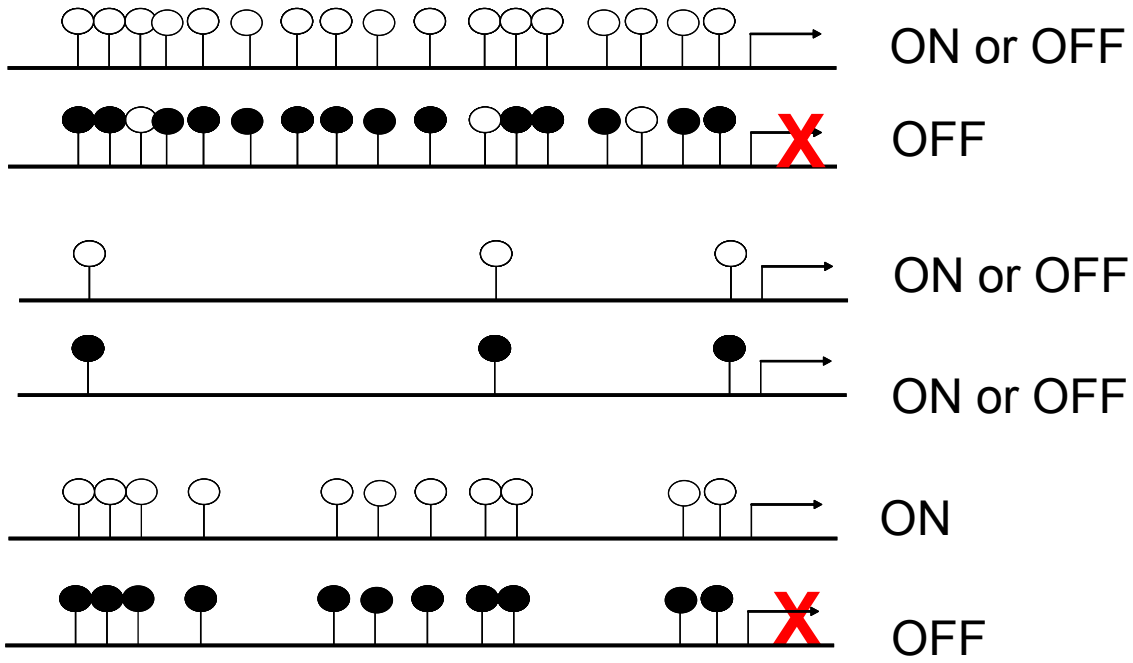
Effect of DNA methylation on promoter activity depends on CpG density in the promoter



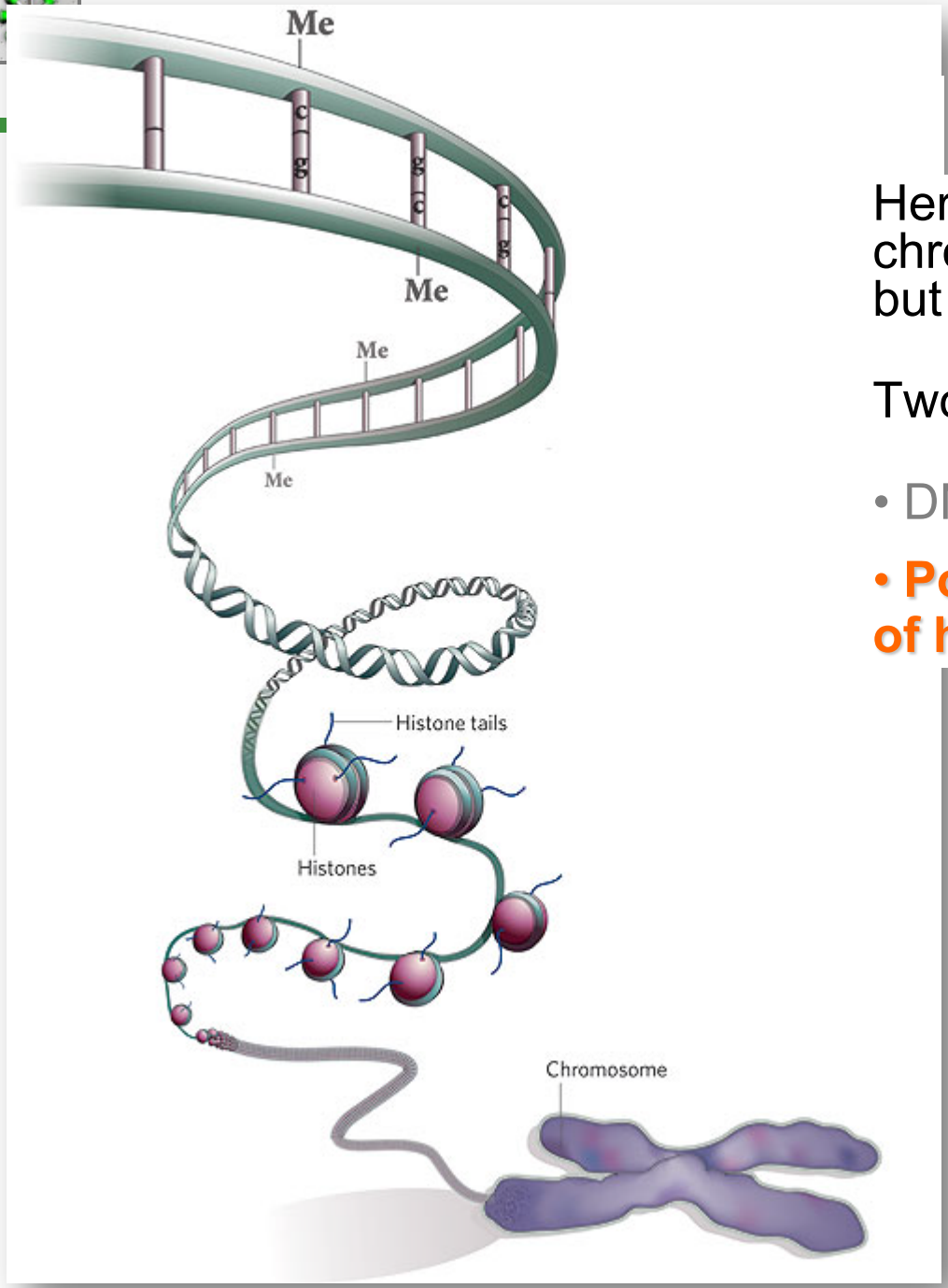
High CpG promoters

Low CpG promoters

Intermediate CpG promoters



Epigenetics

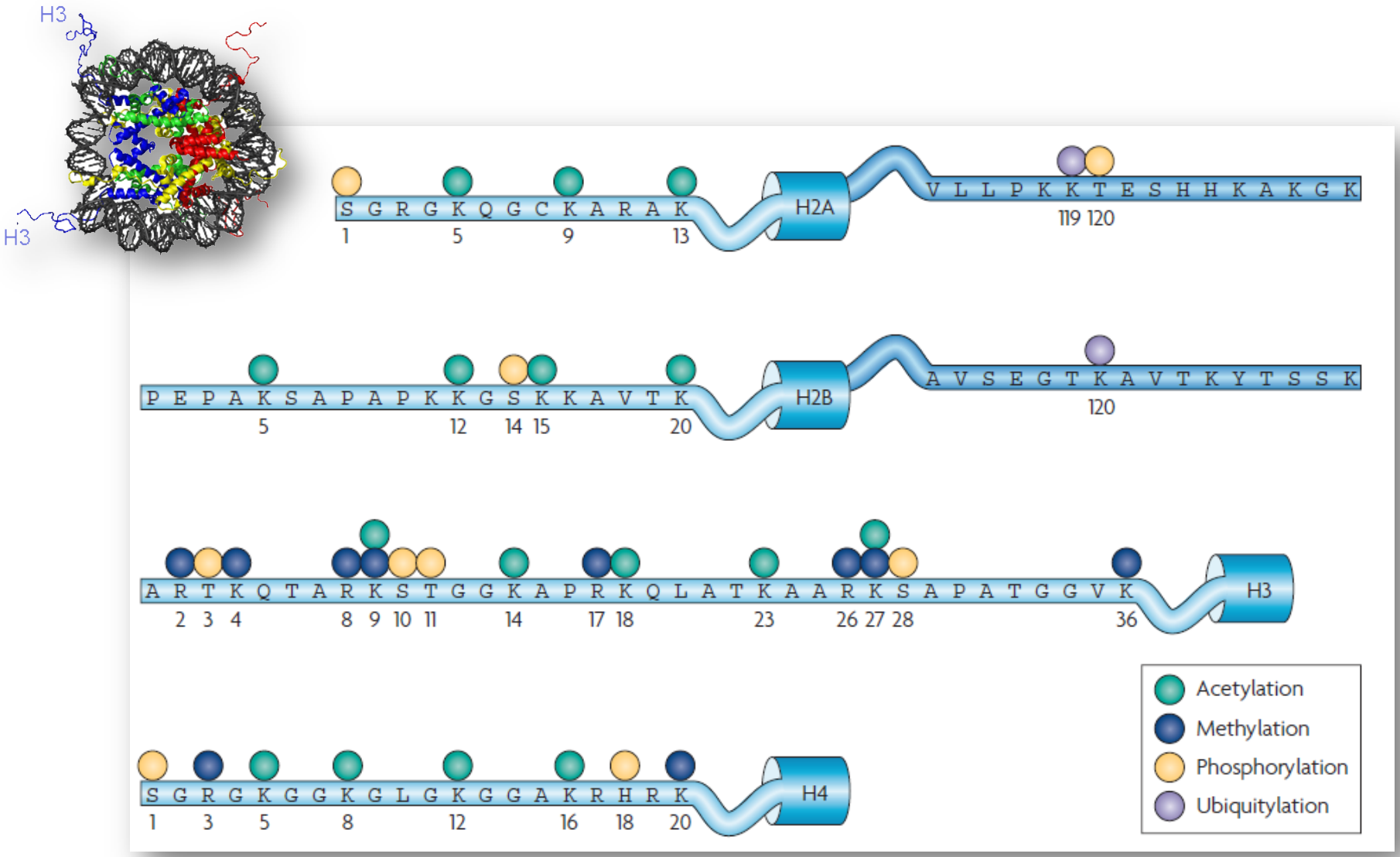


Heritable modifications of DNA or chromatin that affect gene function, but not DNA sequence.

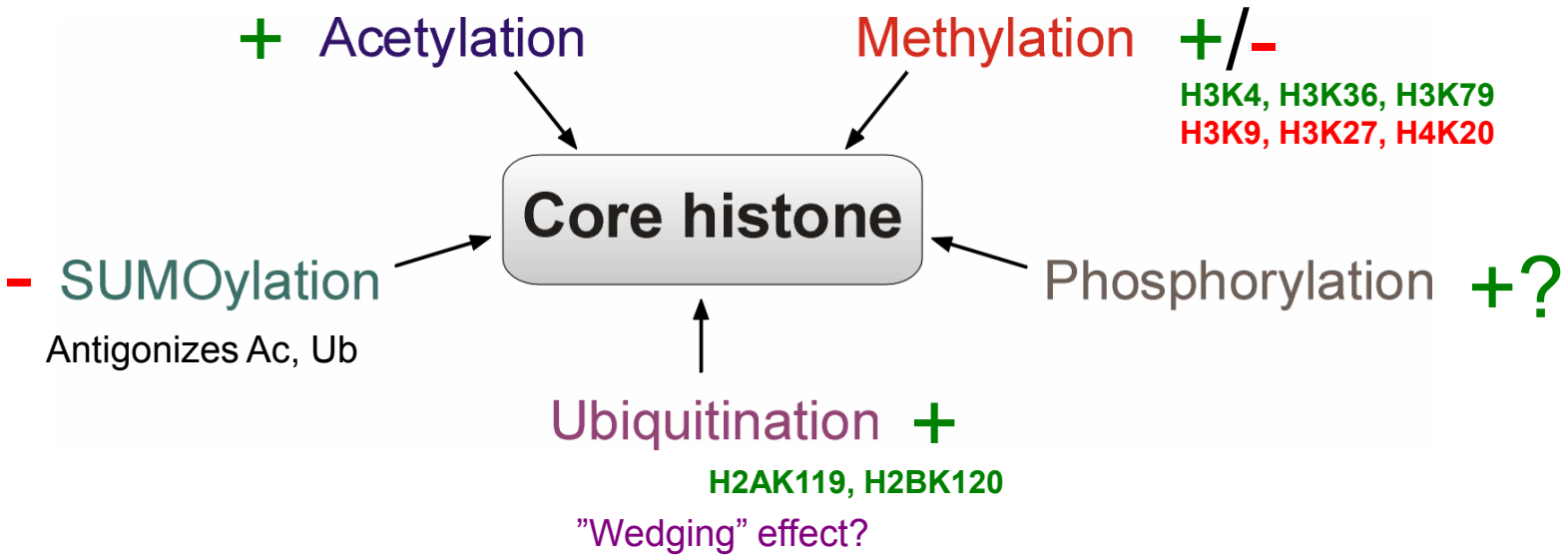
Two main components:

- DNA methylation
- **Post-translational modifications of histones**

Combinations of histone tail modifications make up a 'code'

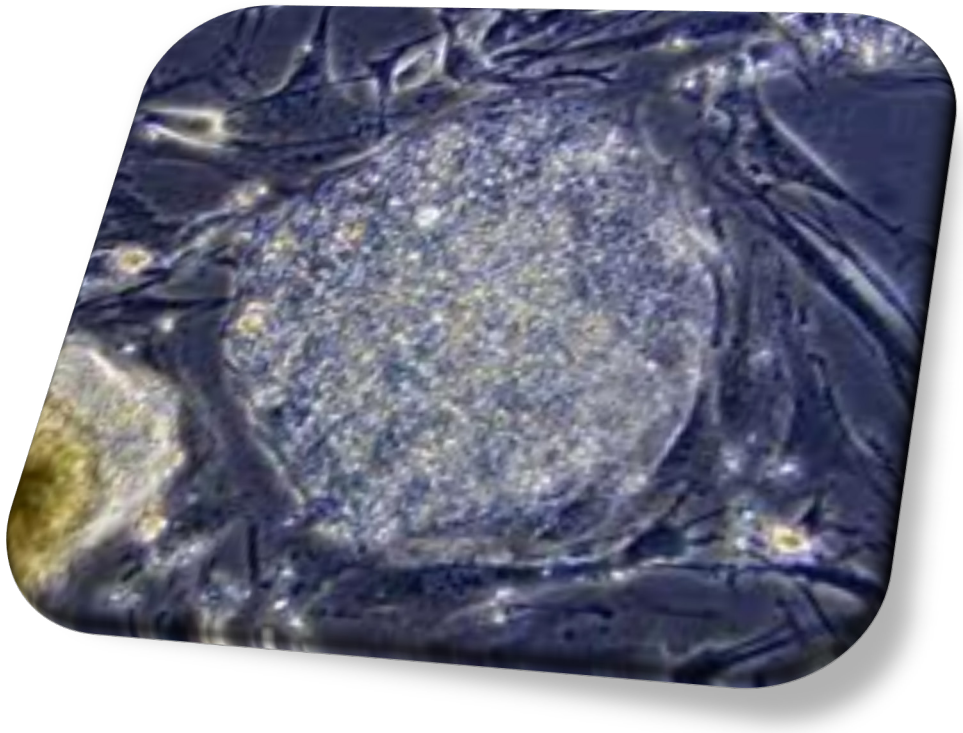


Post-translational modifications of histones



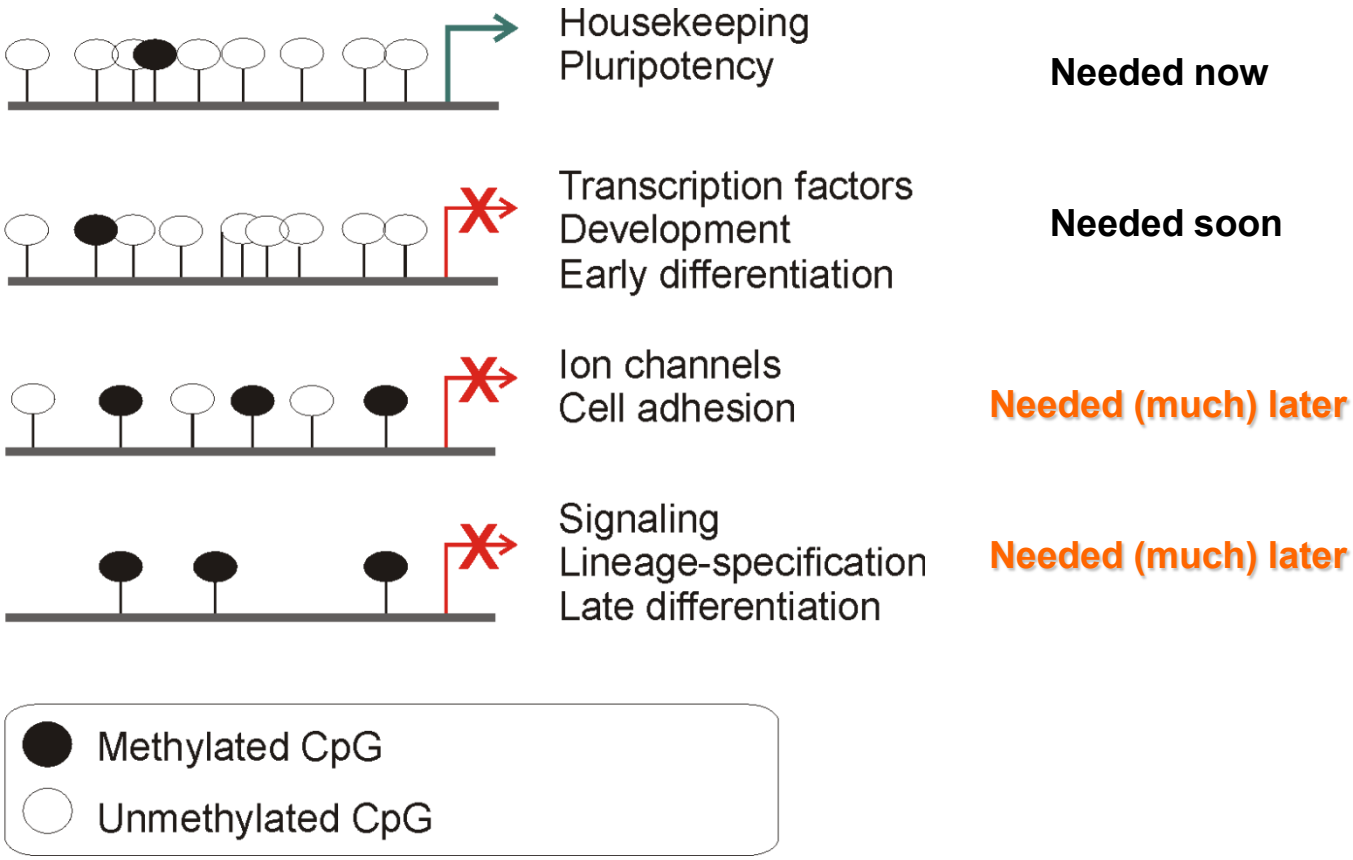
(+/- : effect on gene expression)

Epigenetic states of embryonic stem cells



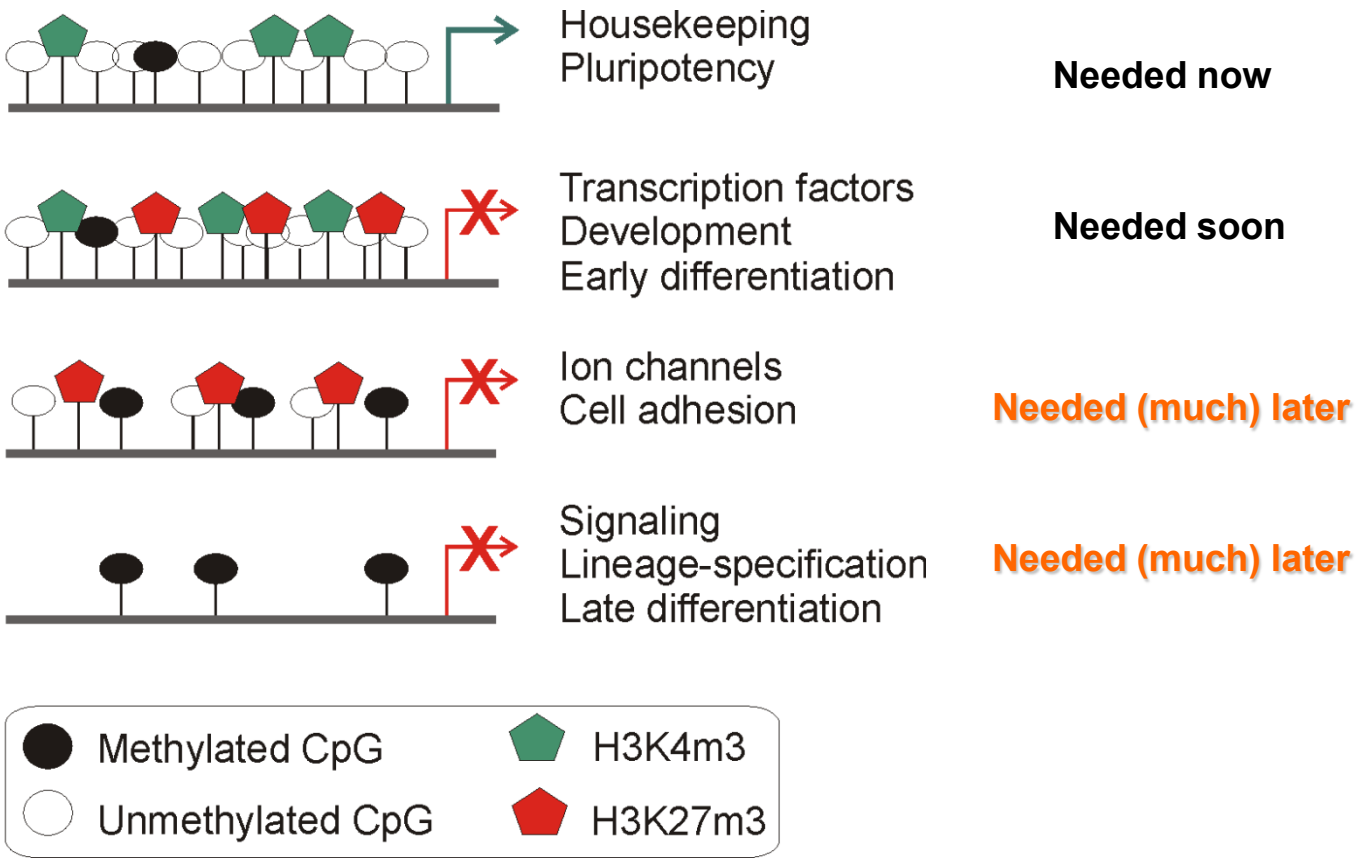
Transcriptional 'posing' of genes important for development and differentiation by co-marking with activating and repressing histone marks

- Overall less DNA methylation than in differentiated cells
- But *not* all genes are unmethylated:



Transcriptional 'posing' of genes important for development and differentiation by co-marking with activating and repressing histone marks

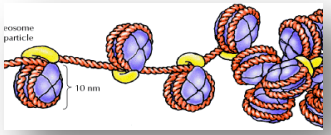
H3K4me3 and H3K27me3 bivalency on unmethylated DNA



Chromatin states in ES cells

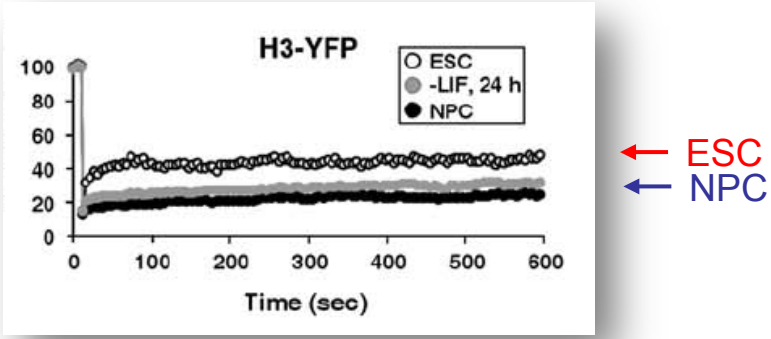
Looser and more dynamic chromatin than in differentiated cells

- Only one histone H1 molecule per 2 nucleosomes



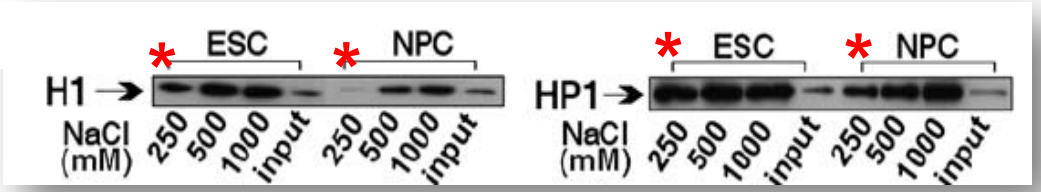
- ES cell chromatin is ”hyperdynamic”: histones are more mobile (not as tightly bound to DNA)

(1) Imaging (FRAP): enhanced histone mobility

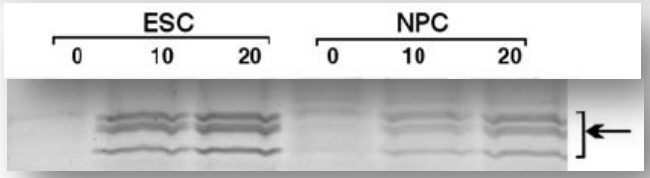


(2) Biochemistry: enhanced histone solublility

Salt extraction



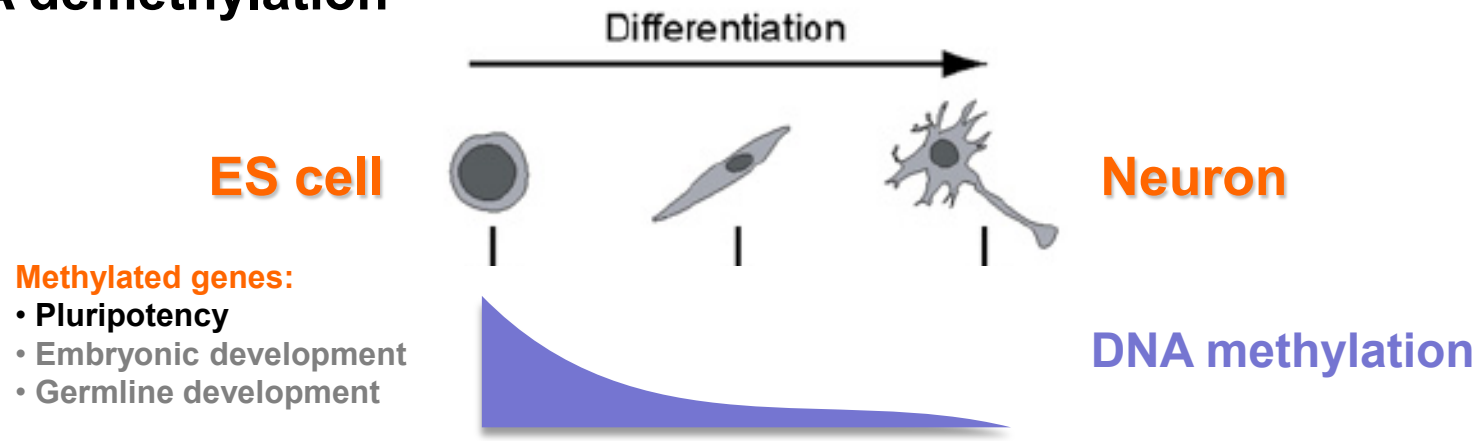
Micrococcal nuclease extraction



Meshorer et al., 2006

DNA methylation changes upon ES cell differentiation

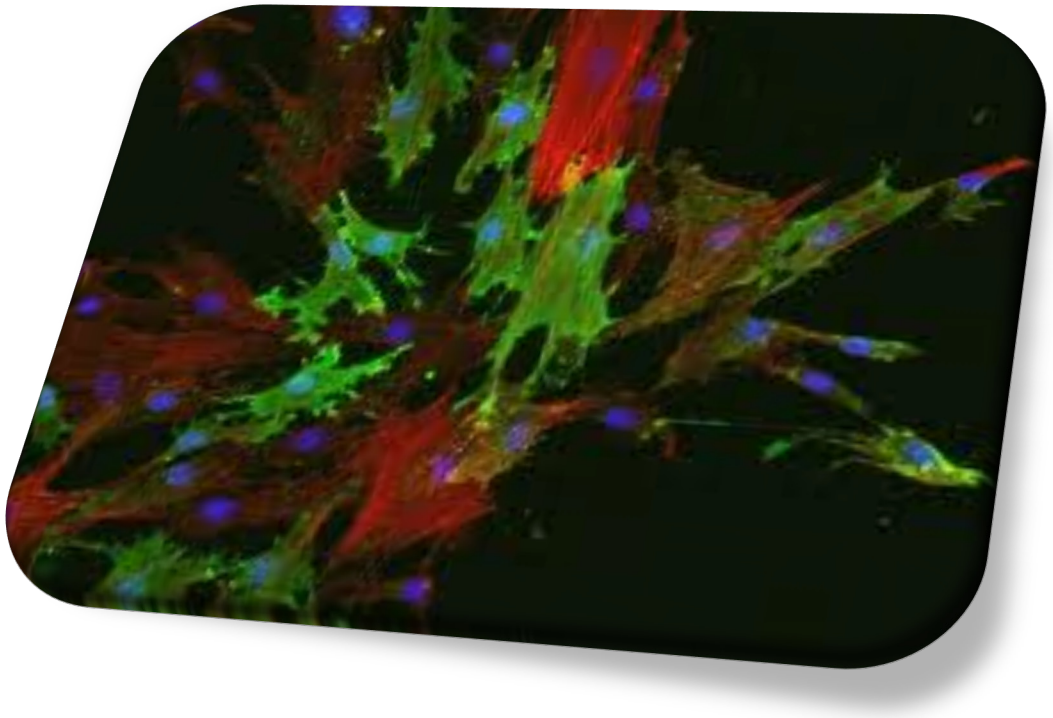
- **DNA demethylation**



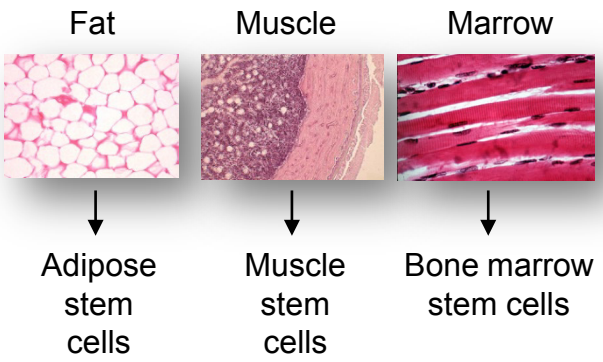
DNA methylation changes correlate with commitment to a progenitor state, when ES cells lose pluripotency

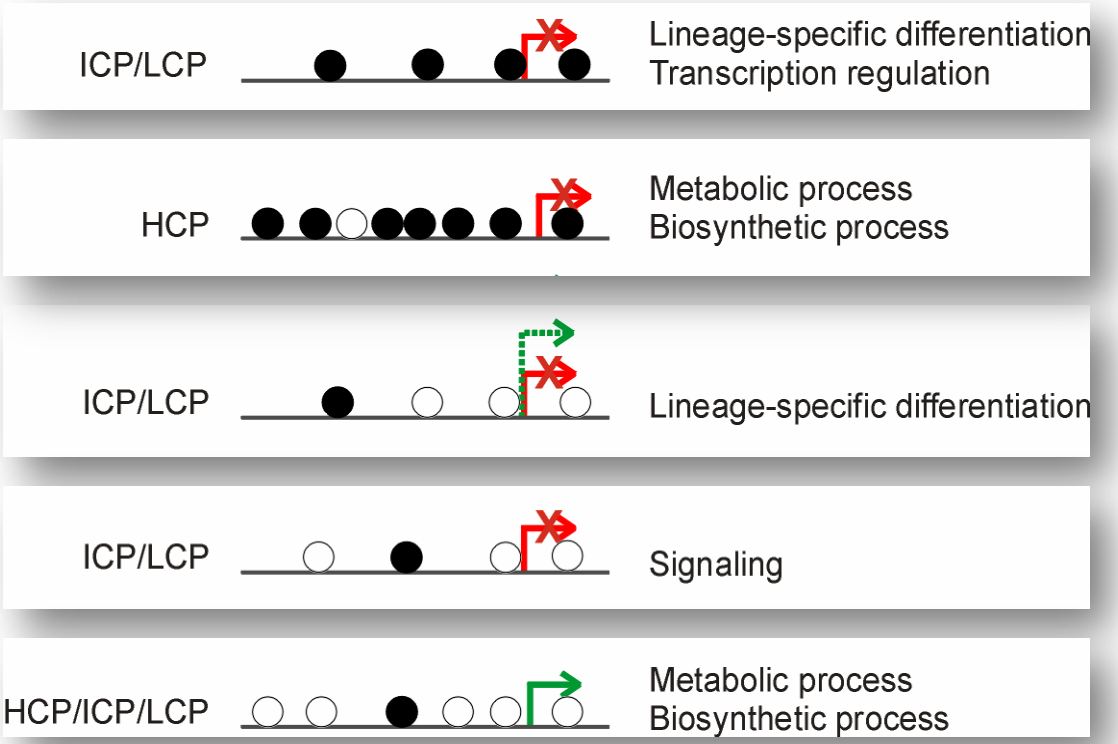
- **H3K27 demethylation (brake release) and H3K9 acetylation (gas on)**

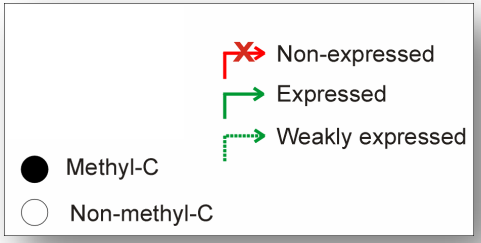
Epigenetic states in somatic stem cells



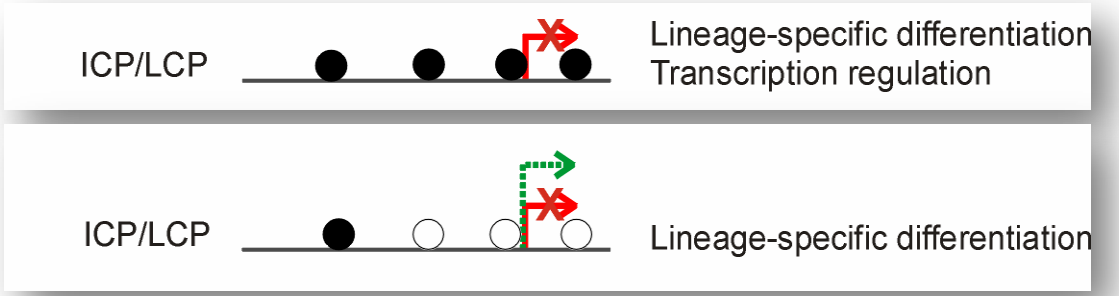
Functional attributions of promoter methylation in mesenchymal stem cells

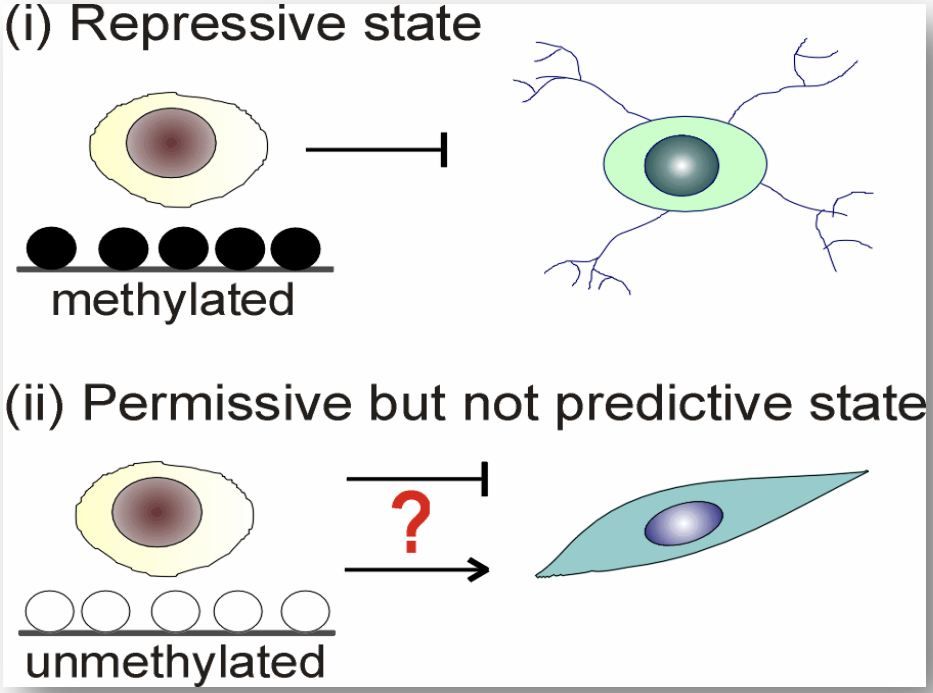




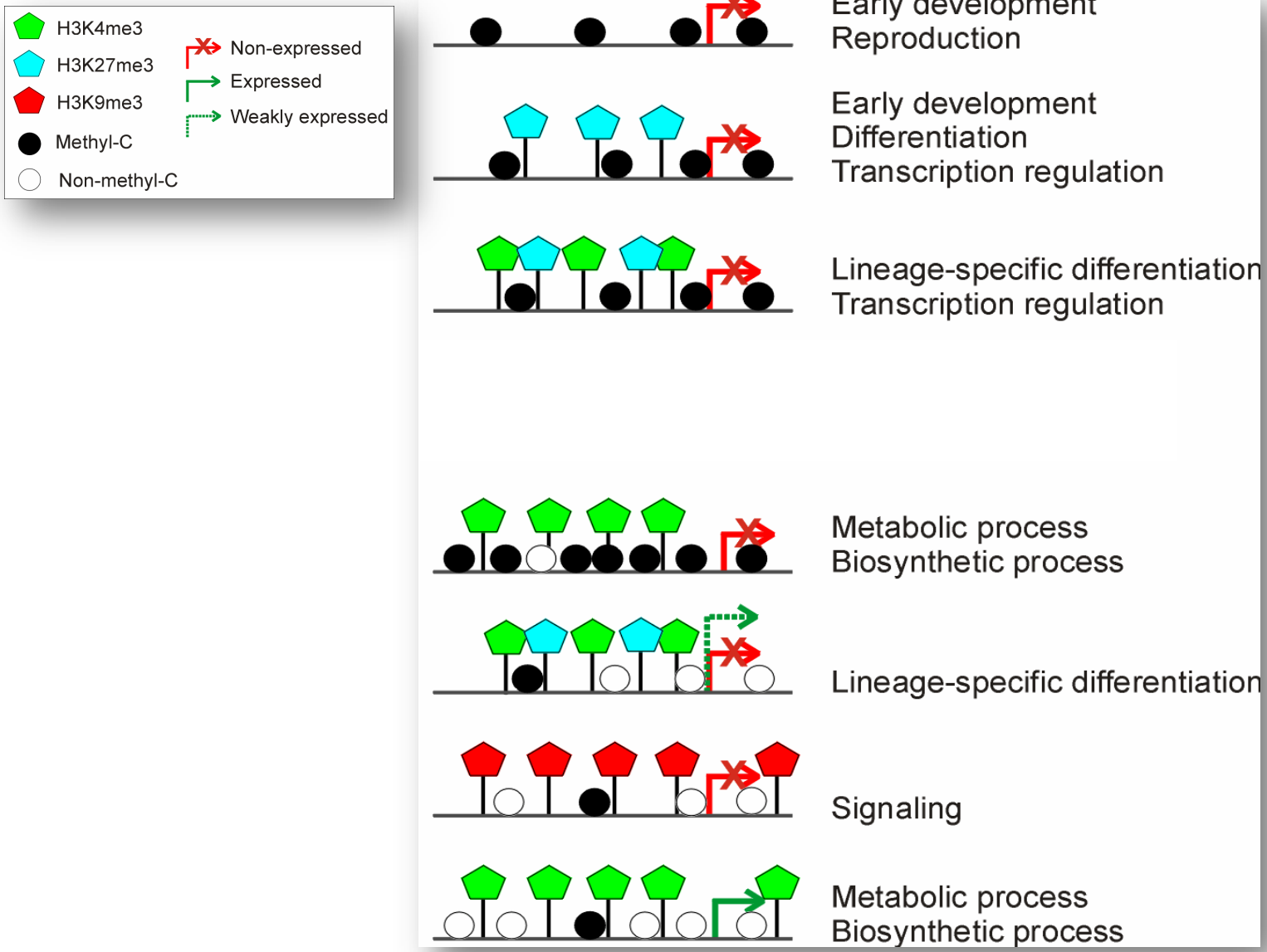


Promoter CpG methylation confers repression, but lack of or weak methylation is not predictive





Combinatorial association of DNA methylation and histone modifications on promoters



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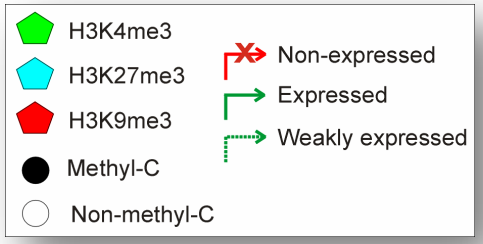
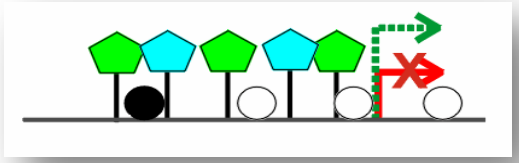
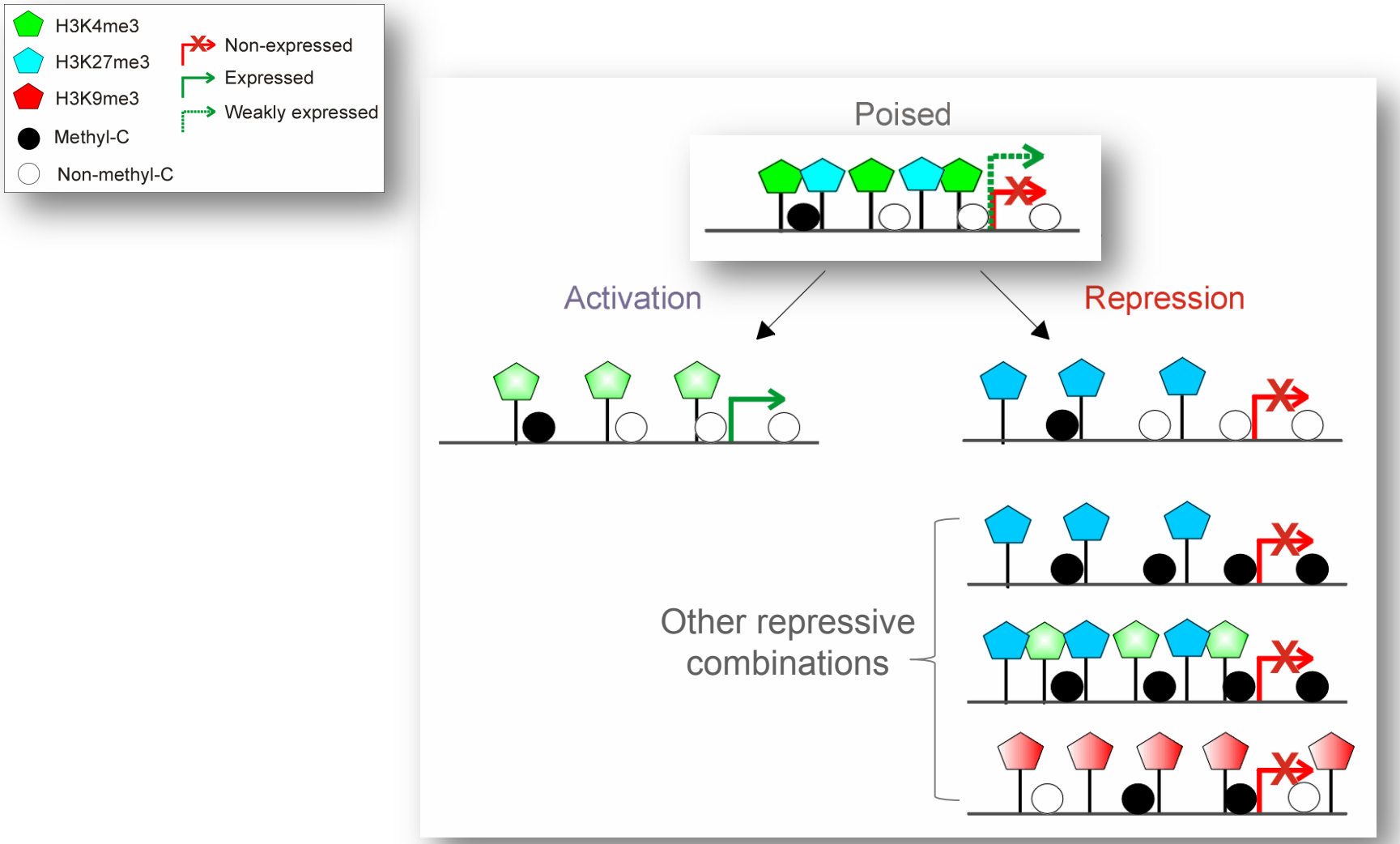
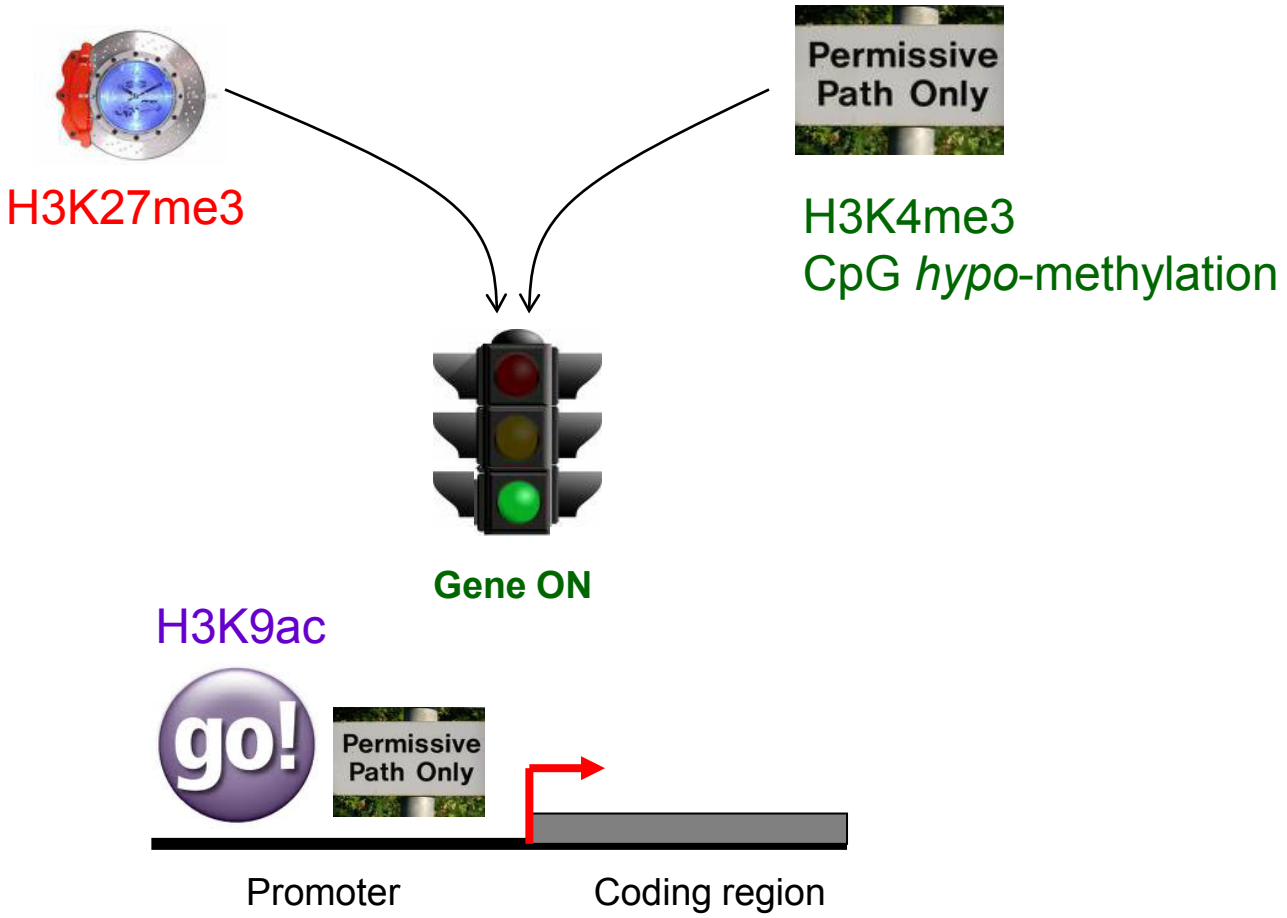




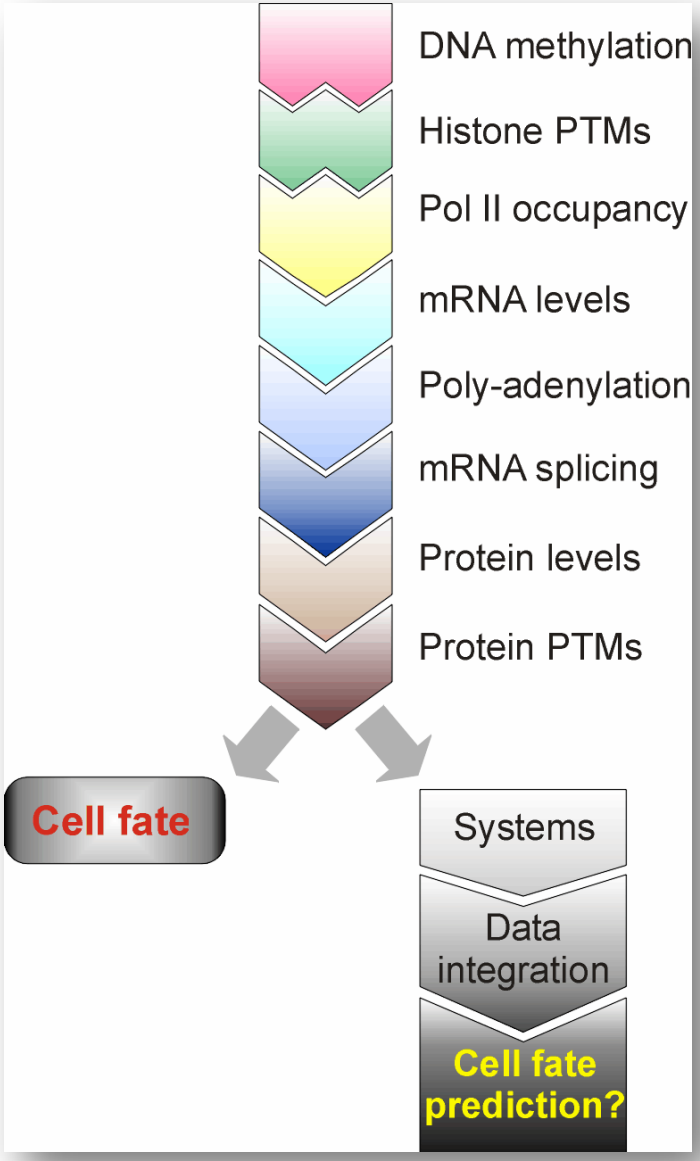
Differentiation segregates the H3K4me3 and H3K27me3 marks



The bottom line (simplified): 'poising' genes for later activation...



Regulatory levels of gene expression and cell fate decisions ('molecular layers')

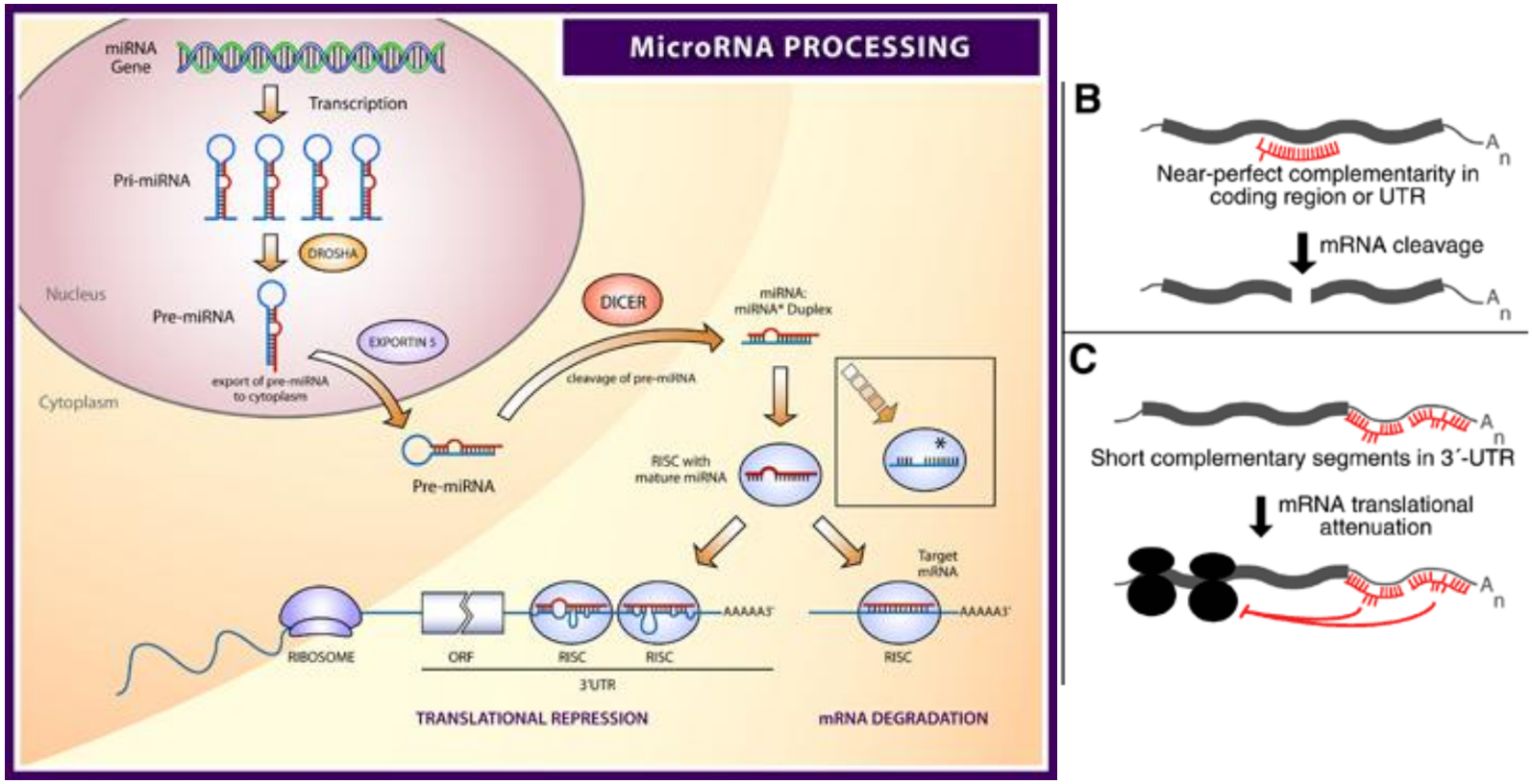


MicroRNA and Stem Cell Differentiation

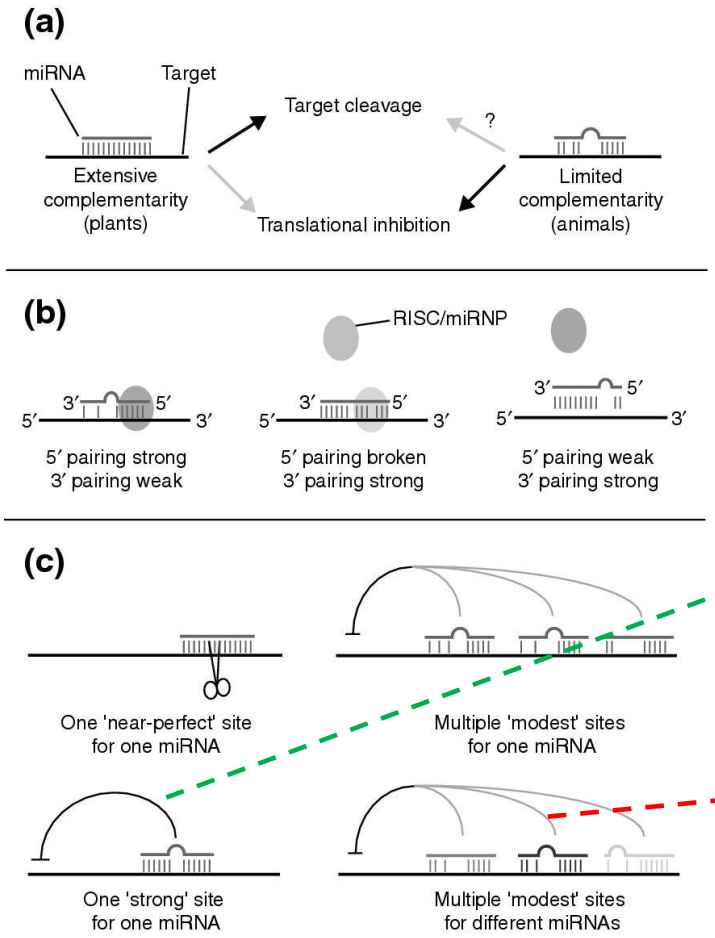
*Jan O. Gordeladze, Hans Yssel, Farida Djouad, Jean-Marc Brondello, Isabelle Duroux-Richard, Daniele Noël, Florence Apparailly, Anthony Lebechec, Charles Lecellier and Christian Jorgensen

IMB, Dept. for Biochemistry, UiO, Norway,
INSERM U844, Montpellier, France
*j.o.gordeladze@medisin.uio.no

The processing of microRNA from gene to RISC complex



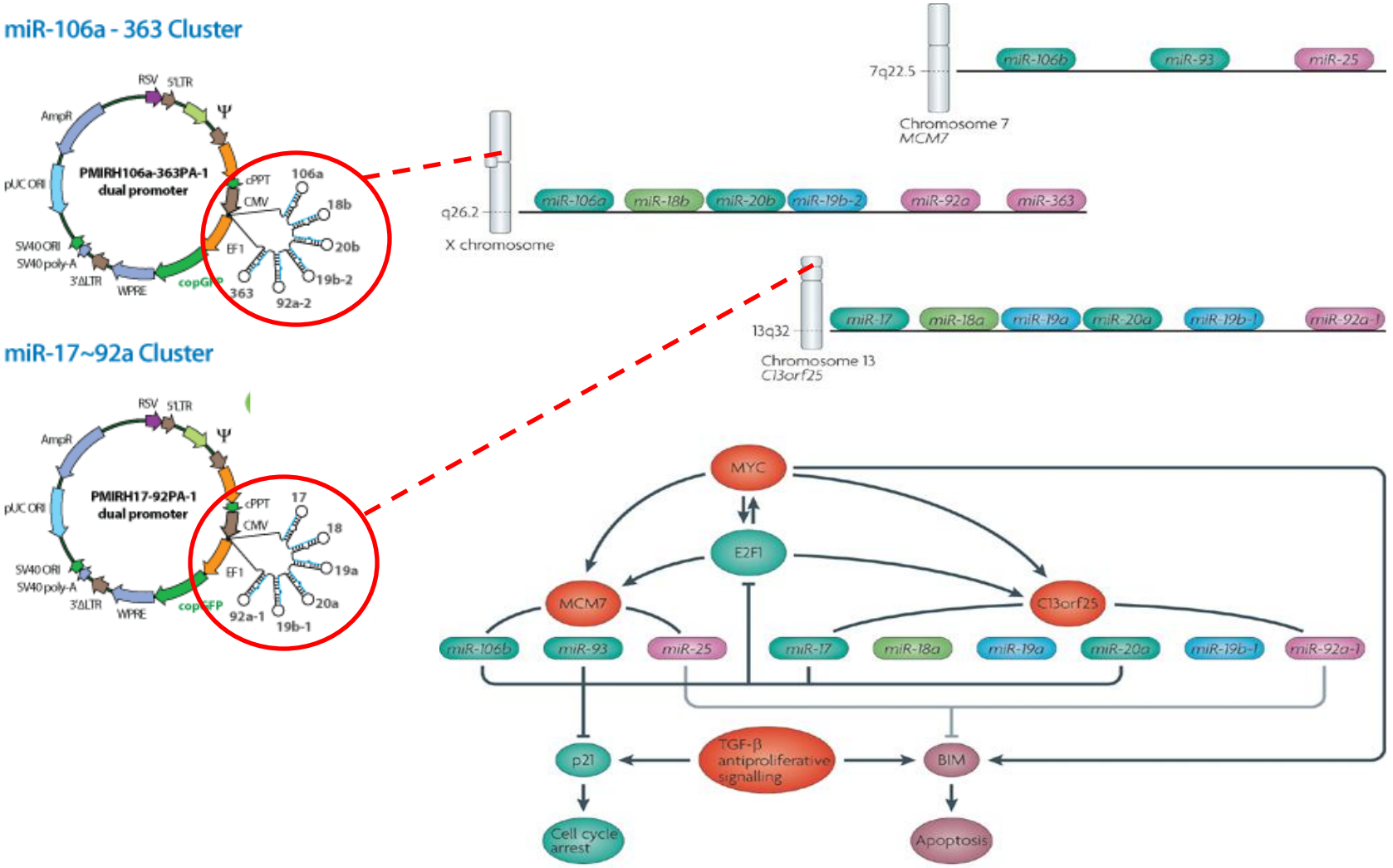
To suppress translation of a transcript; one or more microRNA species?



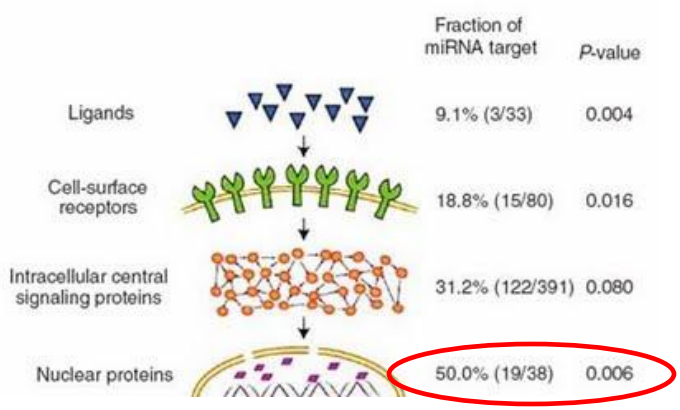
There are two "concepts" advocating the need for microRNAs to control gene expression:

Some people assert that ***only one microRNA*** is necessary and sufficient to alter gene expression/cell phenotype, while others claim that ***5-6 microRNA species*** are necessary to do the same job

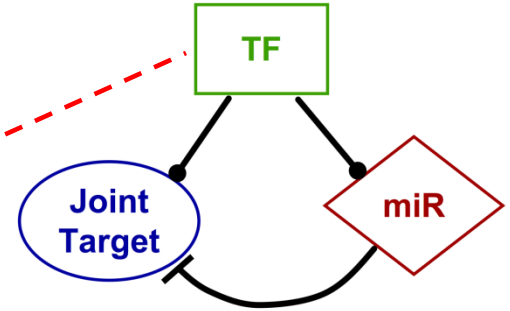
Some microRNAs are located in clusters outside/within genes on given chromosomes and may be organized in hierarchical regulatory sequences or loops encompassing microRNAs, TFs and functional genes

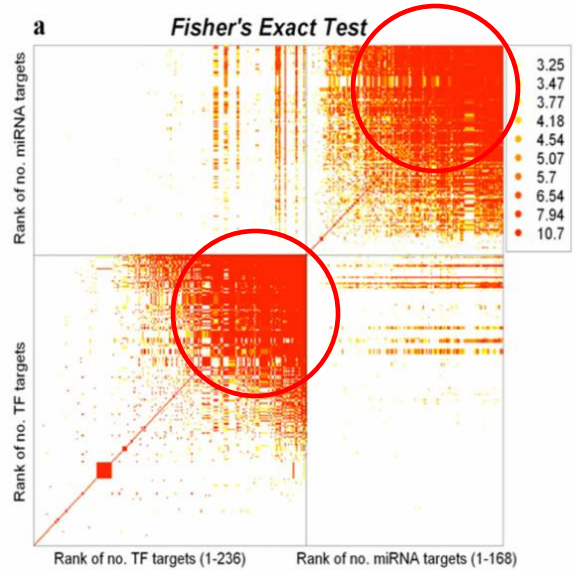


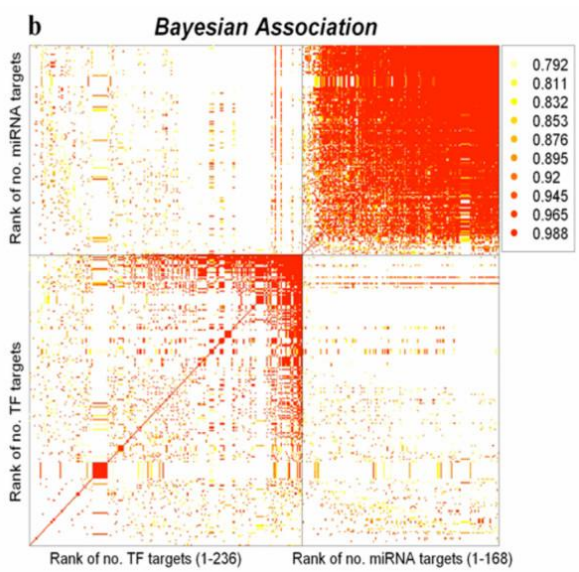
The interrelationship between microRNAs, transcription factors (TFs) and target (functional) genes



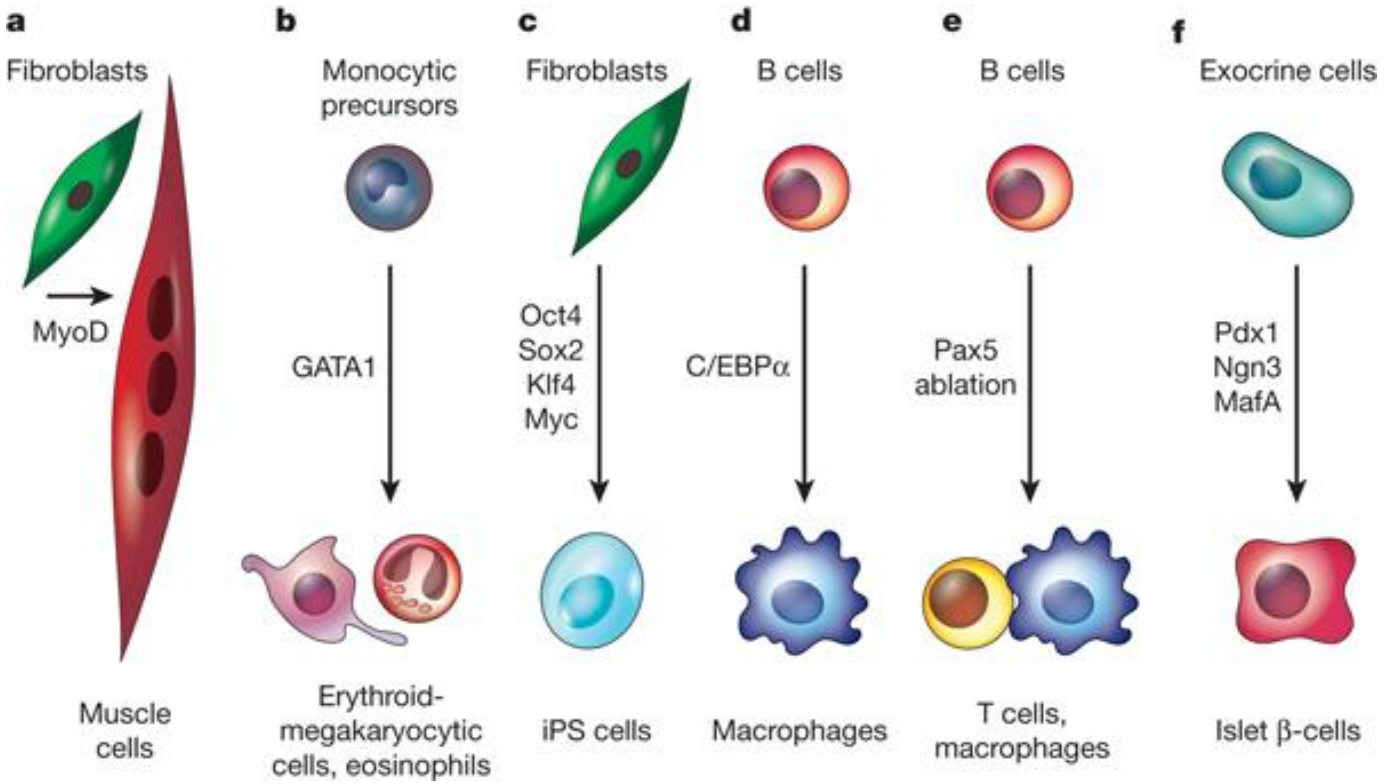
Putatively, TF-TF and microRNA-microRNA interactions are preferred, however, searches for *regulatory loops* may reveal important determinants of cell phenotype







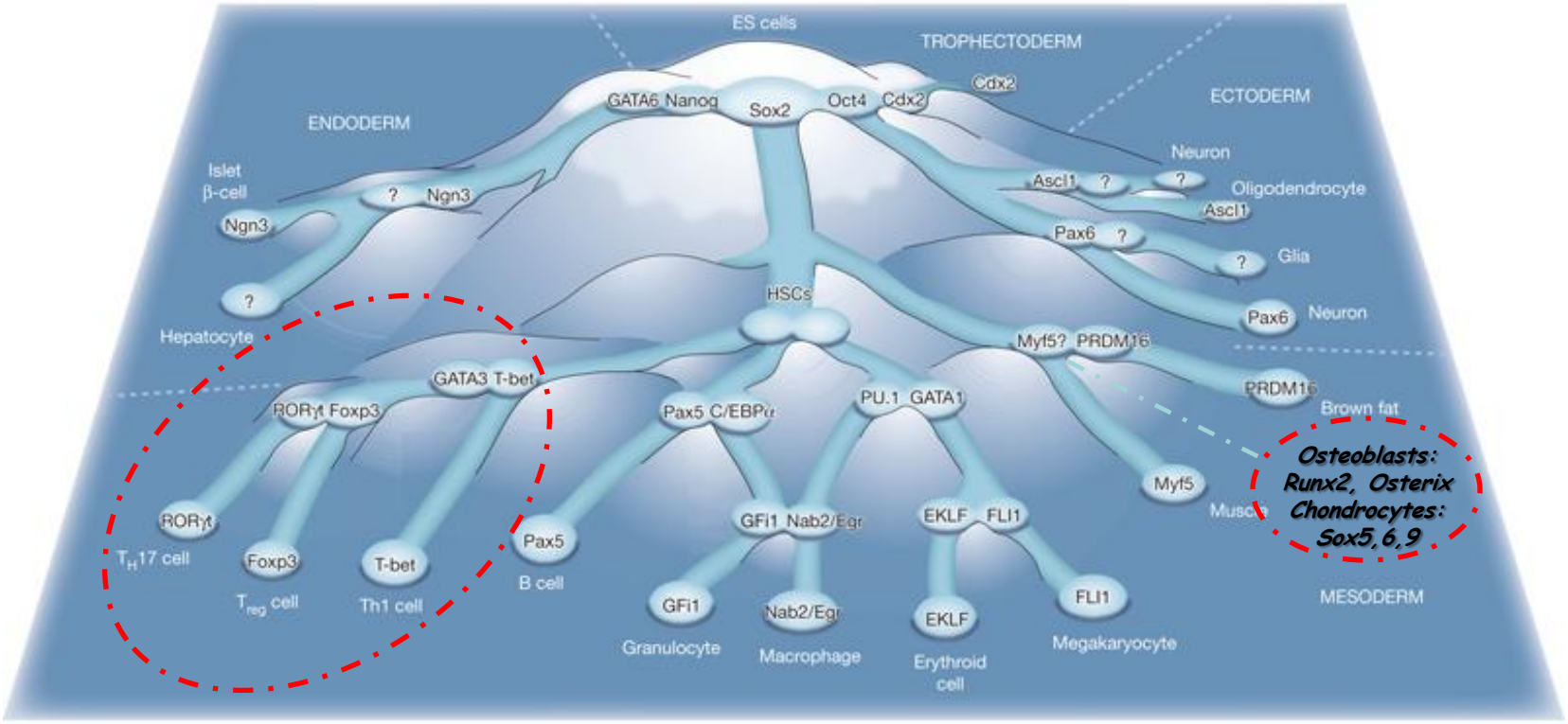
Examples of transcription factor overexpression or ablation experiments that result in cell fate changes



Thomas Graf & Tariq Enver *Nature* **462**, 587-594 (2009)

nature

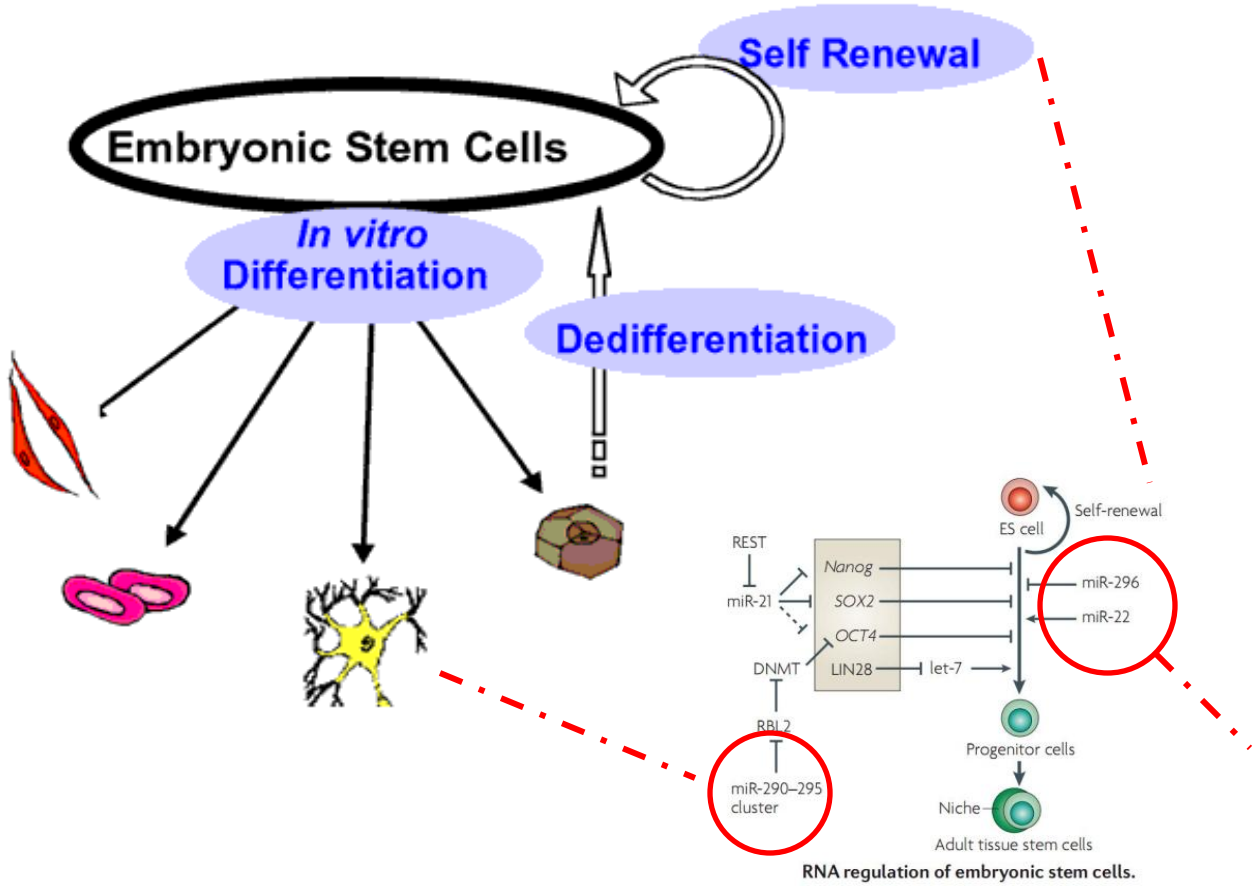
Transcription factor cross-antagonisms in a cascading landscape of unstable and stable cell states



Thomas Graf & Tariq Enver *Nature* **462**, 587-594 (2009)

nature

Manipulering av stamceller med gener (som er viktig for selvfornyelse) og mikroRNA

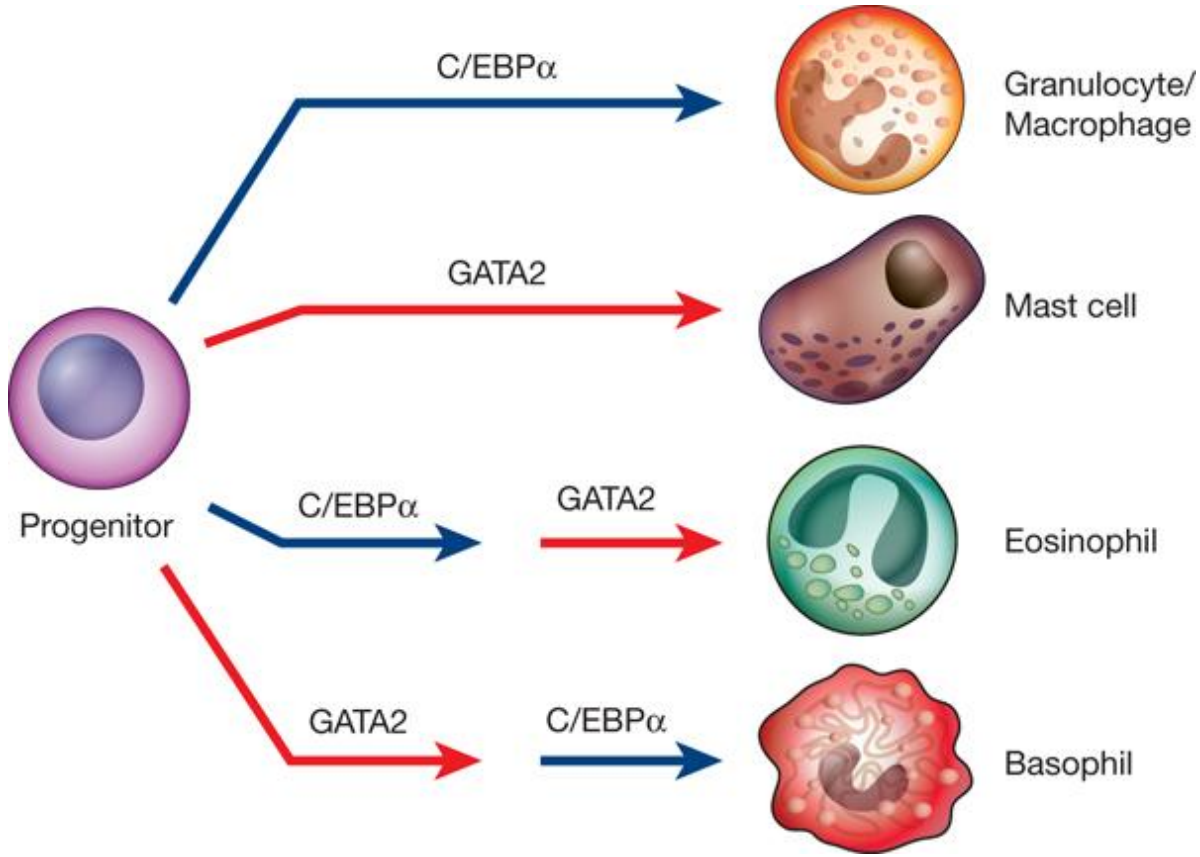


RNA regulation of embryonic stem cells.

- *Man kan dedifferensiere benceller og bruskceller ved å la dem gro i en 2D-struktur i Petri-skåler, eller introdusere (overuttrykke) gener som sørger for selv-fornyelse av stamceller.*
- *Eller man kan også manipulere med cellenes konsentrasjoner av såkalt mikroRNA*

Jan O. Gordeladze, professor, dr. philos, Avd. for Biokjemi, Institutt for Medisinske Basalfag, UiO

Timing of transcription factor expression and lineage outcome

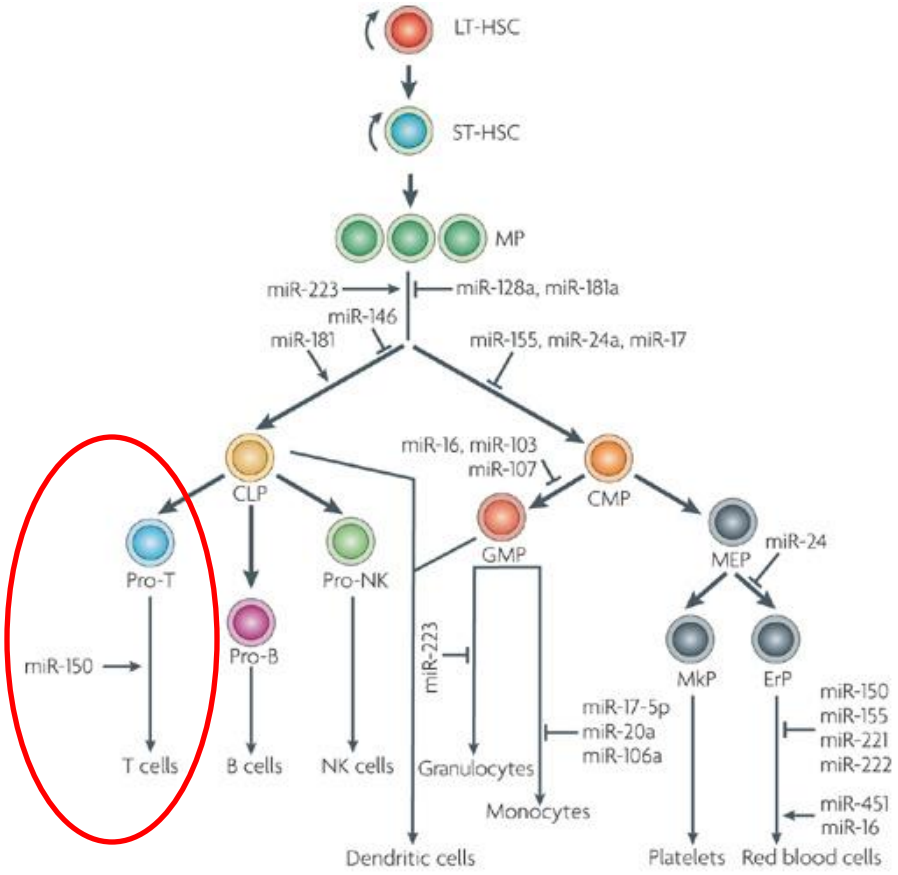


Thomas Graf & Tariq Enver *Nature* **462**, 587-594 (2009)

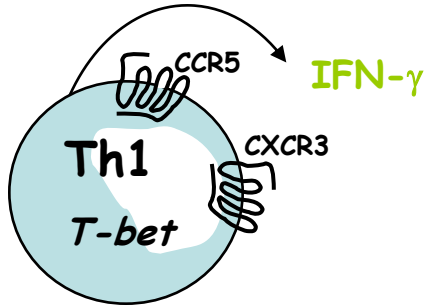
nature

MicroRNA species shown to be involved in hematopoietic stem cell differentiation

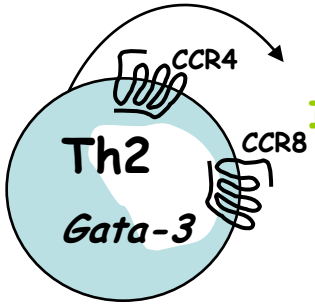
Little is known of the microRNAs responsible for the differentiation and plasticity of naive T cells



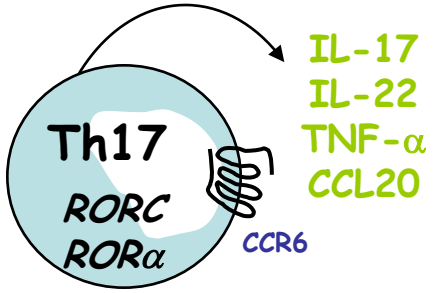
Transcription factors involved in the differentiation of Th-cells from naive T-cells (literature survey, 2009)



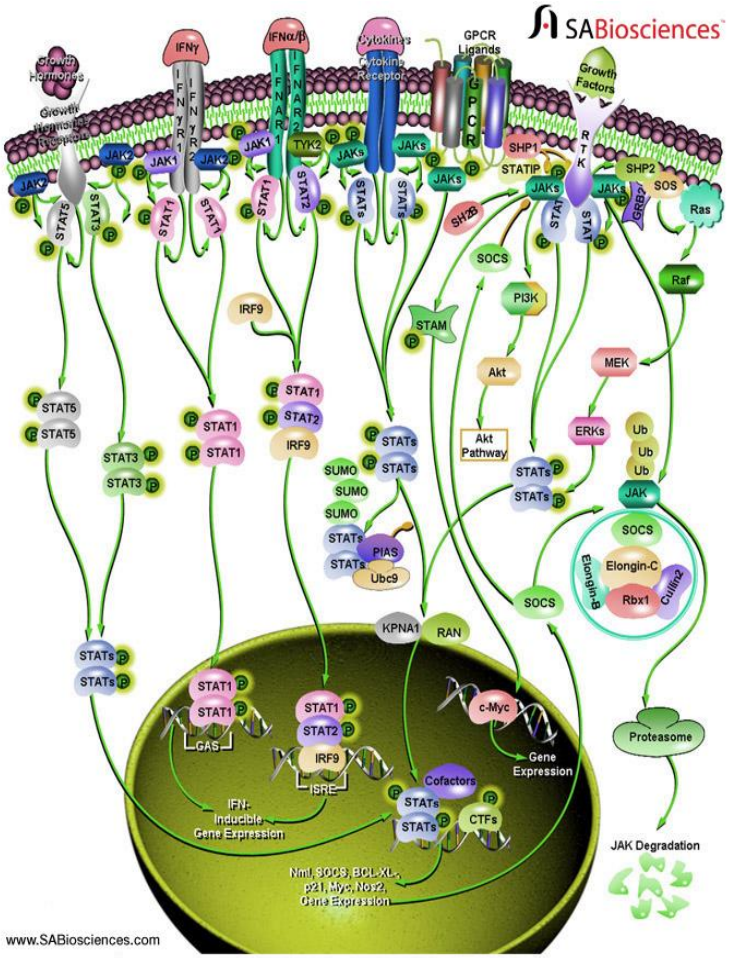
T-bet (TBX21), STAT1, STAT4, IRF1, NFATc1, Runx3



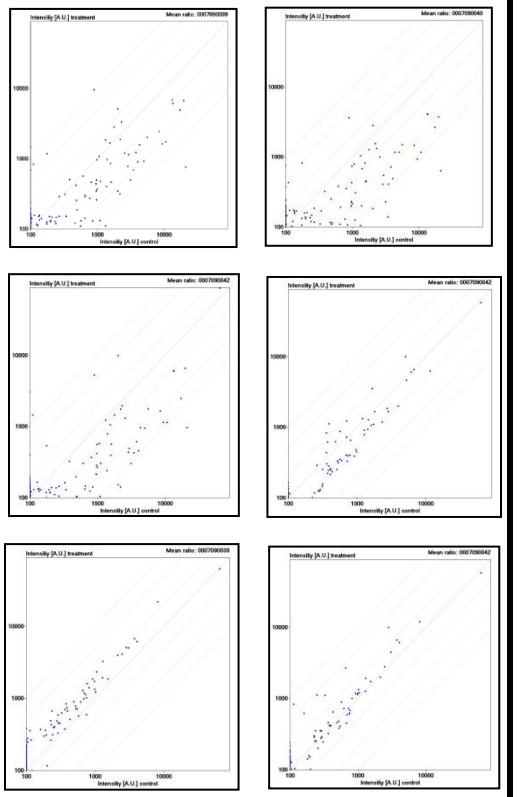
GATA-3, STAT6, c-Maf, c-Jun, NFATc1



RORα (RORA), RORγ (RORC), STAT3, STAT4, IRF4, Act-1, Foxp3, Runx1



Micro-RNA species	*a) Naive T vs Th (1, 2, 17) ratio*	*Micro-RNA species*	*b) Th17 vs Th1 and Th2 ratio*
150	26.8	923	2.78
20a	9.25	638	2.71
30d	9.04	663	3.09
17	8.93		
19b	7.91		
26a	7.64		
106a	6.79		
20b	6.73		
Let-7g	6.52		
Let-7a	5.28		
16	5.13		
19a	4.94		
768-3p	4.93		
142-5p	4.56		
146b-5p	4.43		
155	3.24		
923	0.32		
638	0.12		
663	0.057		



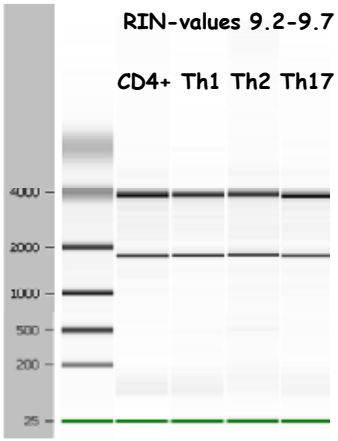
Miltenyi Biotech, France

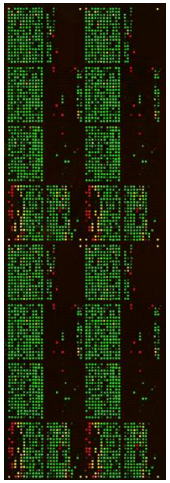
Relative expression of micro-RNA species in:

a) Activated naive T (CD4+) cells vs the average for activated Th1, Th2 and Th17 cells

b) Activated Th17 cells vs the average for activated Th1 and Th2 cells

RNA-solation by *mir*Vana®





Question 3

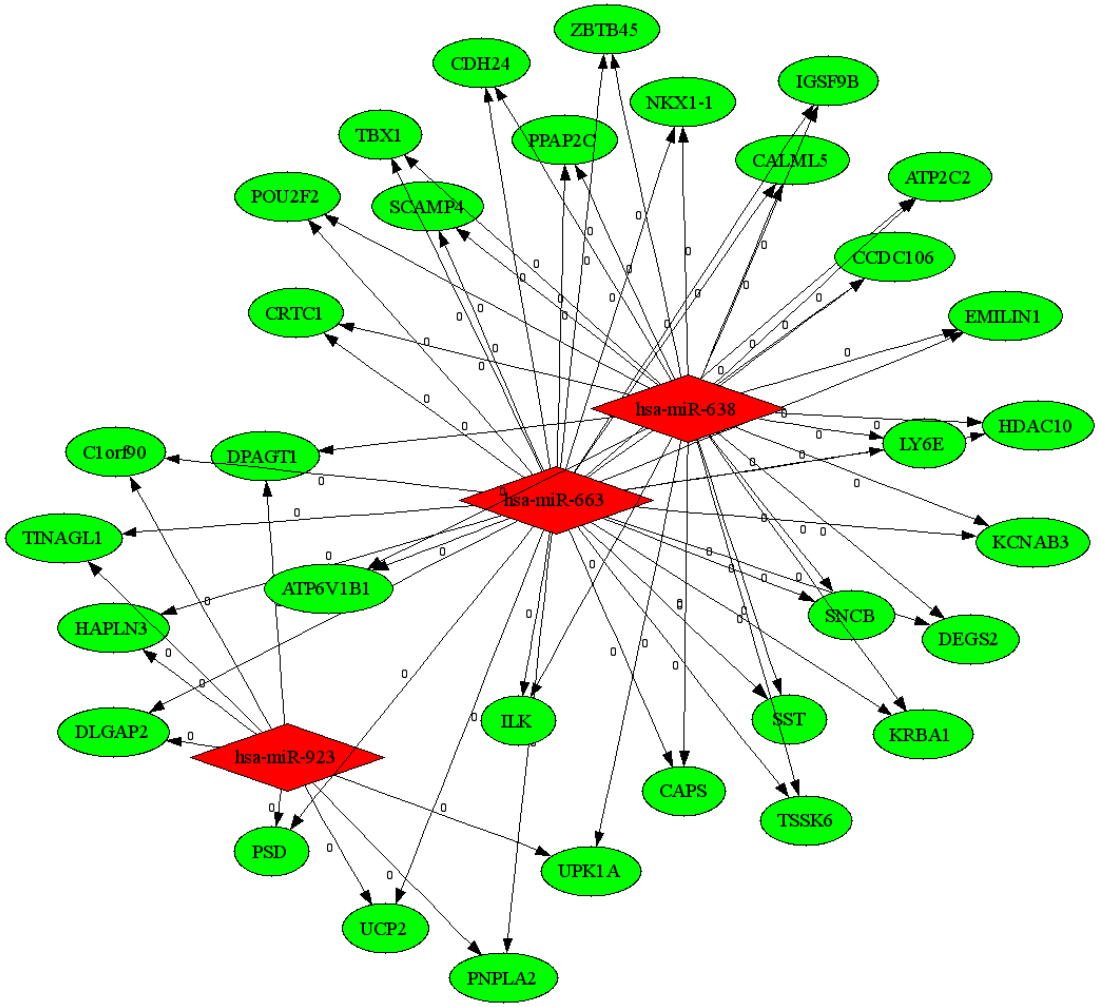
"We would like to know which of the genes, putatively being targeted by the above mentioned microRNAs will have two or more of the subject microRNAs "in common"

Directory: "Common Targets"
I used 3 sets of parameters to find putative target genes: "Stringent", "medium" and "large". Genes are identified by their transcript identifier (from Ensembl). That explains multiple gene occurrences in lists. Lists are ordered by scores, and can be explored using HTML file format.

Stringent list: 57 targeted genes
Score>=18, p-value<=0.001, number of miRNAs on targeted genes >= 2

Medium list: 247 targeted genes
Score>=17, p-value<=0.001, number of miRNAs on targeted genes >= 2

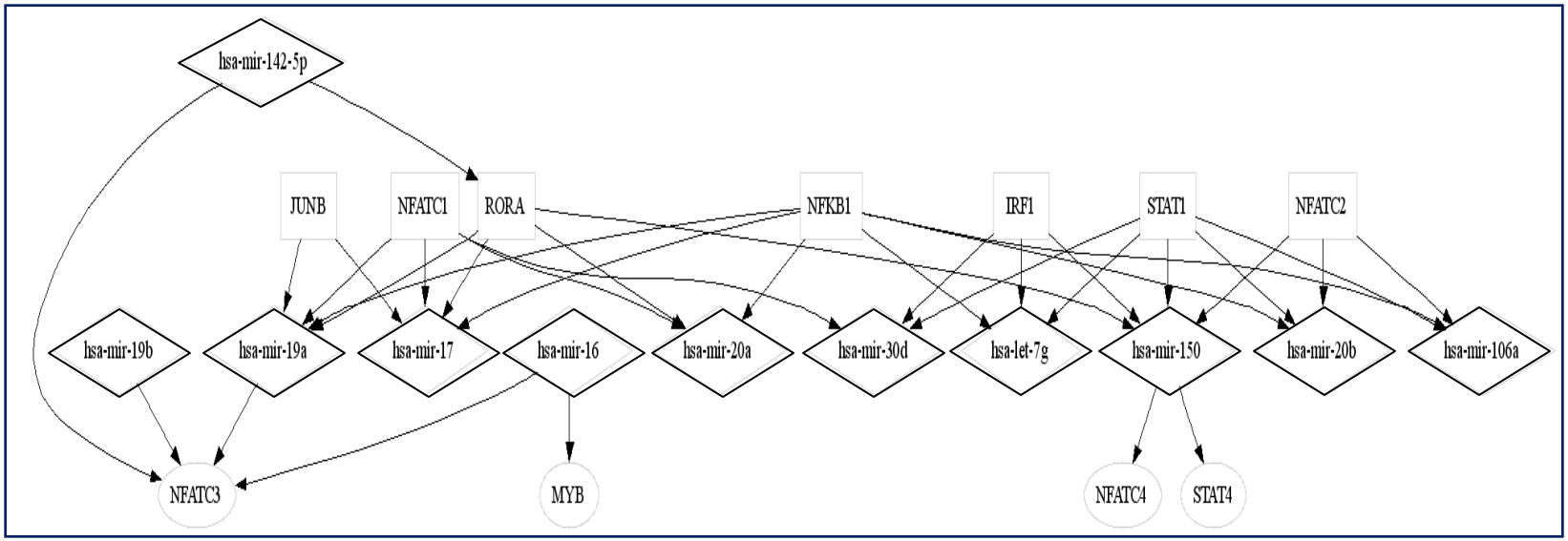
Large list: 620 targeted genes
Score>=17, p-value<=0.01, number of miRNAs on targeted genes >= 2



Question 1, addressed by using the Mir@nt@n database

"We would like to see which microRNAs may target two or more of the transcription factors from the […] complete list"

* Directory: "TF/ListComplete"
2 graphs were generated (Hierarchical and Organic views). TFs found to be targeted by miRNAs: RORA, STAT4, NFATc4, NFATc3 and MYB.



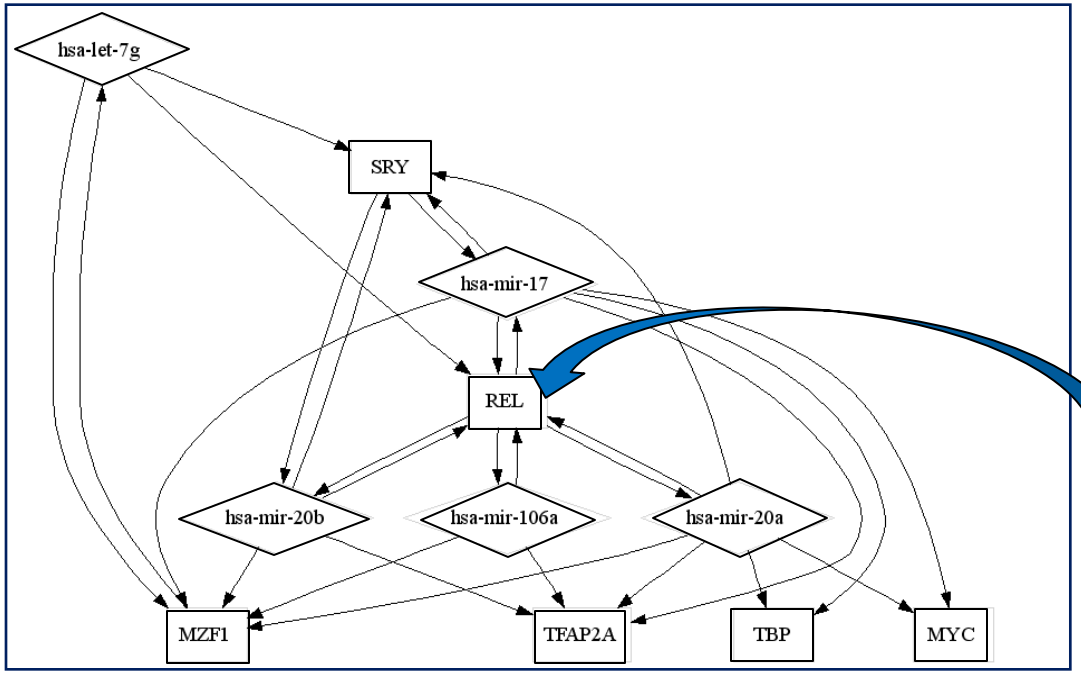
* T-bet (TBX21), STAT1, STAT3, STAT4, STAT6, IRF1, NFATc1, NFATc2, NFATc3, NFATc4, NFATc5, GATA3, c-maf, c-Jun, JunB, RORalpha (RORA), RORgamma (RORC), IRF4, Act-1, Runx1, Runx3, NFkappaB, IkappaB, AP-1, MYB, TOX, Notch, MAML1, p50, p65, Th-POK, Twist

Question 2, addressed by using the Mir@nt@n database

"Can we identify feedback loops using the input microRNA list?"

Directory: "TF/FeedbackLoop"

This question can be answered in one click! Feedback loop is defined as a couple of TF and miRNA that regulate each other. A hierarchical graph was generated and includes 6 TFs and 5 miRNAs.

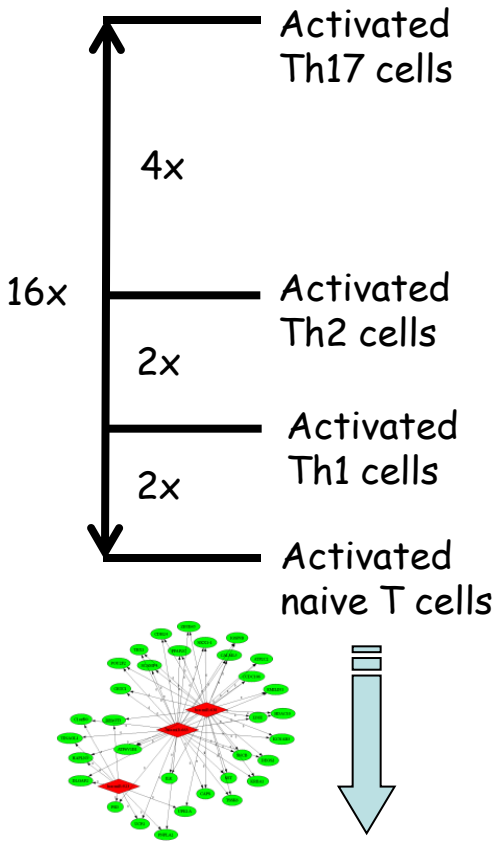


Names of genes involved in feedback loops:

SRY: Testis determining factor
REL: C-rel proto-oncogene protein
MZF1: Myeloid zinc finger 1 (MZF-1)
FAP2A: Transcription factor AP-2
TBP: TATA-box binding protein
MYC: Myc proto-oncogene (transcription factor p64)

REL is heavily involved in lymphocyte proliferation, but also important for T cell function. It interacts with IRF1 and IRF4, as well as the NFκB family of TFs

Relative levels of miRNAs 663, 638 and 923 between T cell species

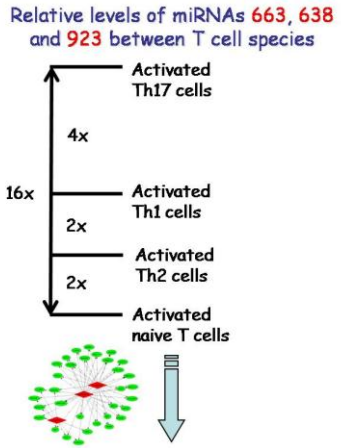


Hypothesis: May these microRNAs determine the polarity/plasticity of activated Th cells solely by endogenous levels?

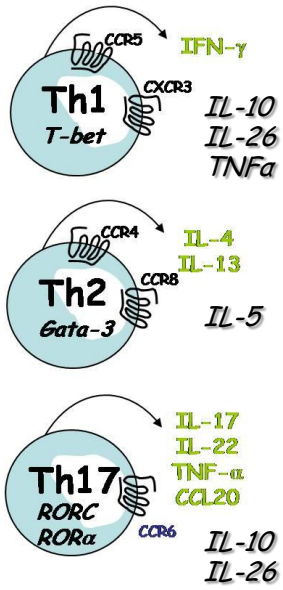
List of genes targeted by microRNAs 663, 638 and 923

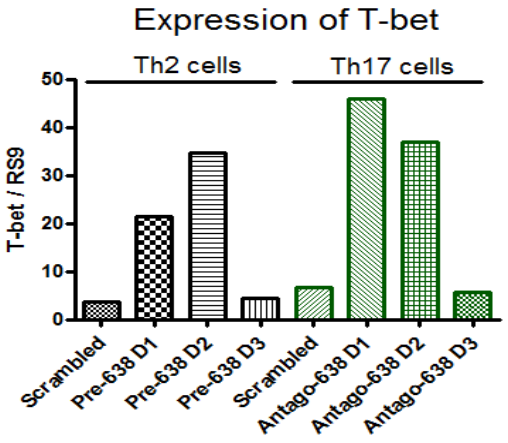
C1orf90: Chromosome 1 open reading frame 90
EMILIN1: Elastin microfibril interfacer 1
DPAGT1: N-acetylglucosaminephosphotransferase 1 (GlcNAc-1-P transf.)
HDAC10: Histone deacetylase 10
IGSF9B: Immunoglobulin superfamily, member 9B
TINAGL1: Tubulointerstitial nephritis antigen-like 1
ATP6V1B1: ATPase, H+ transporting, lysosomal 56/58kDa, V1 subunit B1
CDH24: Cadherin-like 24
CALML5: Calmodulin-like 5
SNCB: Synuclein, beta
PPAP2C: Phosphatidic acid phosphatase type 2C
CAPS: Calcyphosine
PNPLA2: Patatin-like phospholipase domain containing 2
ZBTB455: Zinc finger and BTB domain containing 45
ATP2C2: ATPase, Ca^{2+} transporting, type 2C, member 2
SST: Somatostatin
ILK: Integrin-linked kinase-2
SCAMP4: Secretory carrier membrane protein 4
DLGAP2: Discs, large (Drosophila) homolog-associated protein 2
NKX1-1: NK1 homeobox 1
POU2F2: POU class 2 homeobox 2
CRTC1: CREB regulated transcription coactivator 1
TBX1: T-box 1
UCP2: Uncoupling protein 2 (mitochondrial, proton carrier)
LY6E: Lymphocyte antigen 6 complex, locus E
UPK1A: Uroplakin 1A
KCNAB3: Potassium voltage-gated channel, beta member 3
HAPLN3: Hyaluronan and proteoglycan link protein 3
KRBA1: KRAB-A domain containing 1
TSSK6: Testis-specific serine kinase 6
DEGS2: Degenerative spermatocyte homolog 2, lipid desaturase
PSD: Pleckstrin and Sec7 domain containing
CCDC106: Coiled-coil domain containing 106

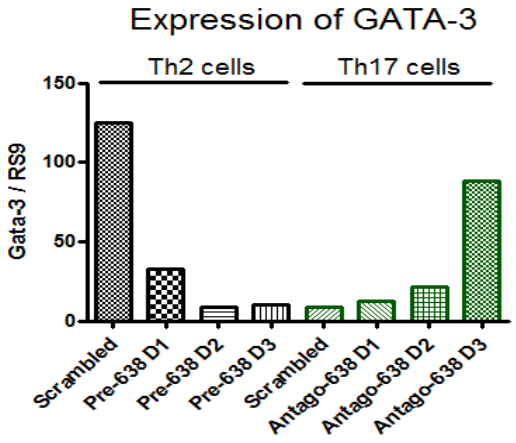
Expression of Th cell "specific" TF s (mRNA) in cells transfected with various amounts of premir-638 or antagomir-638

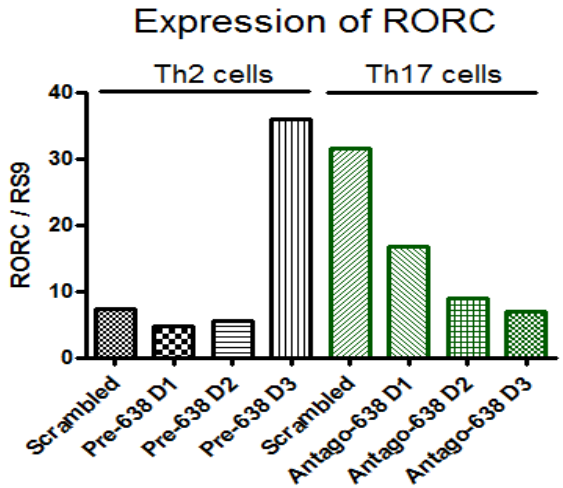


Hypothesis: May these microRNAs determine the polarity/plasticity of activated Th cells solely by endogenous levels?

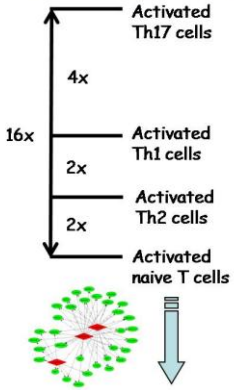




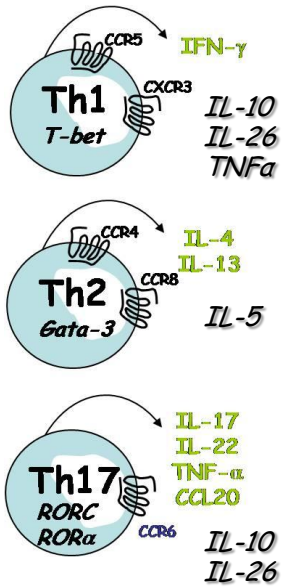




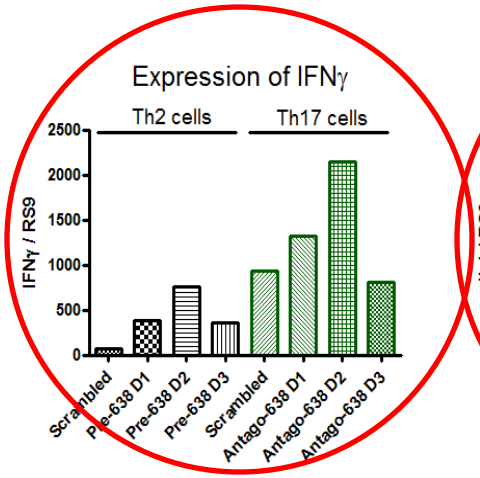
Relative levels of miRNAs 663, 638 and 923 between T cell species

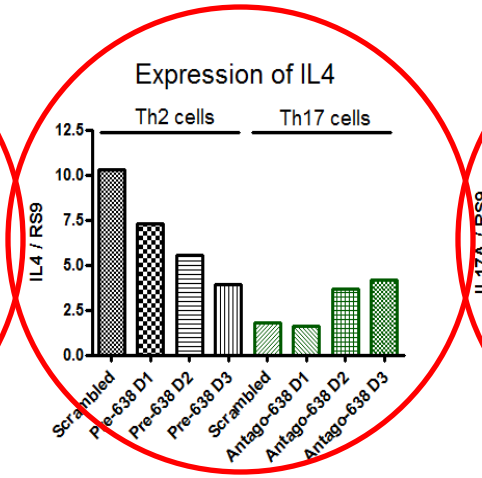


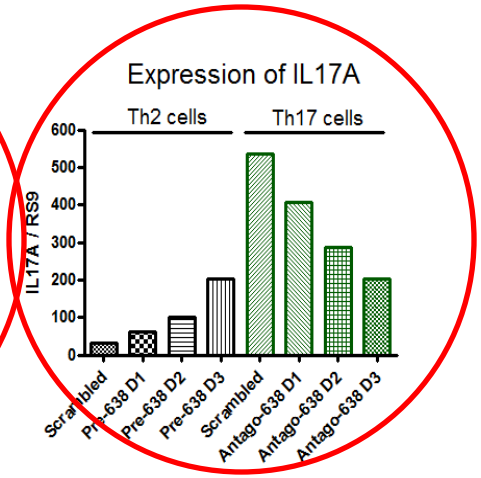
Hypothesis: May these microRNAs determine the polarity/plasticity of activated Th cells solely by endogenous levels?

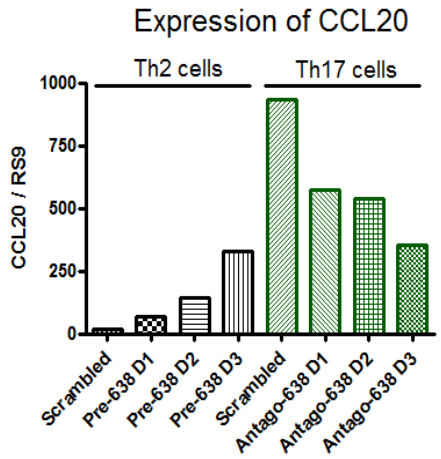


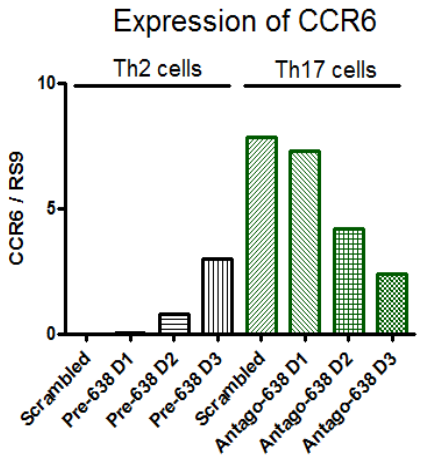
Expression of Th cell "specific" cytokines (mRNA) in cells transfected with various amounts of premir-638 or antagomir-638





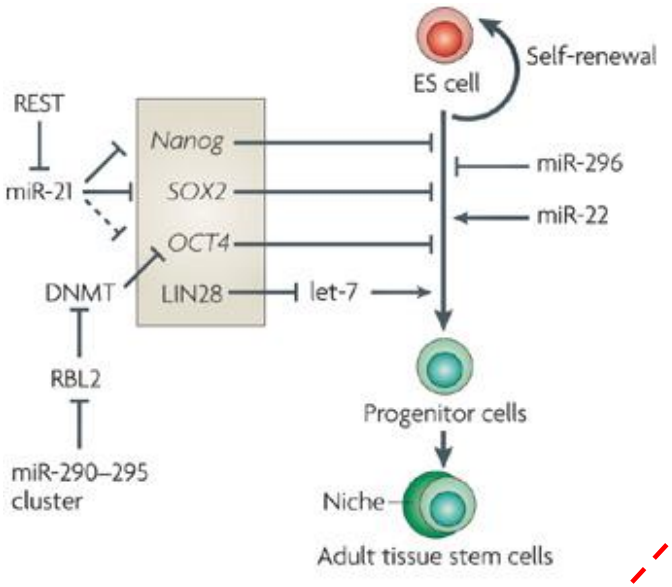






MicroRNAs are heavily involved in self-renewal and differentiation of stem cells

Published microRNAs involved in embryonic stem cell renewal and differentiation



hematopoietic stem cells (HSCs)

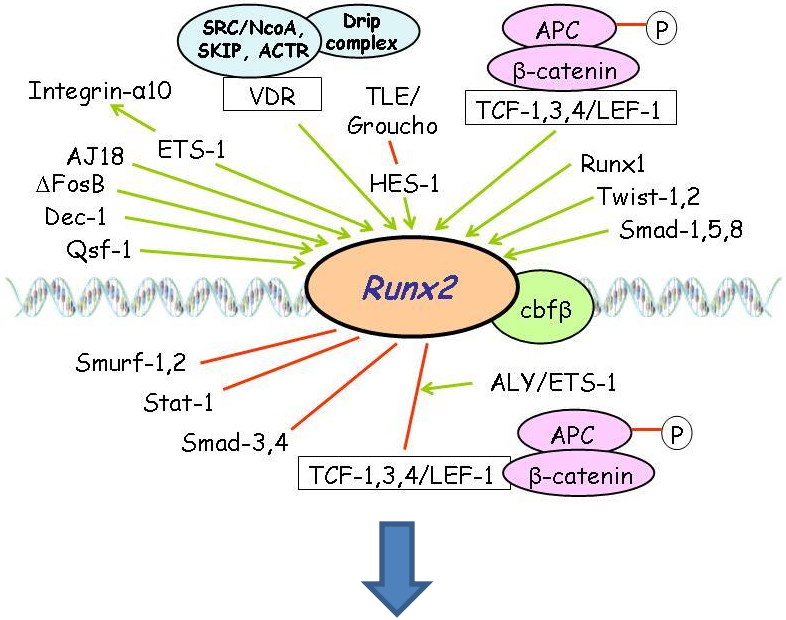
In silico search for microRNA species targeting transcripts of family members of evolutionally conserved and developmental prominent genes (Wnt-, TGFβ-, SHH- Notch- and Homeobox-related) shown to be important for the self-renewal and/or pluripotency of *hematopoietic stem cells* (HSCs)

Gene	***Micro-RNA (according to MiRNA Viewer and PicTar)***
Lef1	22, 24, 26ab, 34abc, 93, 145, 149, 193, 302abcd, 320, 370, 372, 373
BMP4	206, 337
NIK = MAP3K14	17-5p, 19ab, 20, 27ab, 93, 106ab, 130ab, 155, 204, 211, 214, 301, 302abcd, 326, 331, 345, 370, 372, 373
SMO	326, 346, 370
Notch1	15a, 15b, 32, 34abc, 125a, 125b, 139, 195, 223
Hoxa9	Let-7abcefgi, 19b, 26ab, 32, 96, 98, 99, 101, 126, 128ab, 139, 144, 145, 147, 182, 186, 196ab, 199, 205, 301

Many of the microRNAs listed immediately above, like *microRNAs 17-5p, 22, 24, 34ac, 125ab, 128b, 149, 193, 326 and 337* are putatively targeting transcription factors APC, ATF4, Dlx5, ETS-1, HES-1, LEF-1, NFATc1, Sp3, Sp7 (osterix), RNF11, Runx2/cbfa1, Satb2, TAZ, and VDR involved in *osteoblastogenesis*!

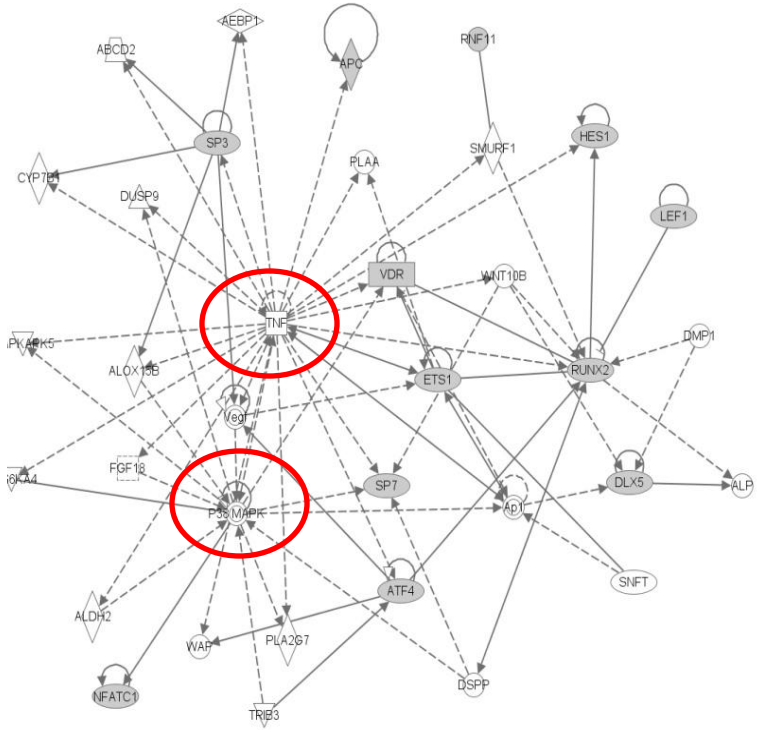
Strategy to ensure blockage of osteogenic differentiation in chondrocytes engineered from hMSCs for cartilage replacement

Focus on *transcription modulators* known to be important for the differentiation of osteoblasts



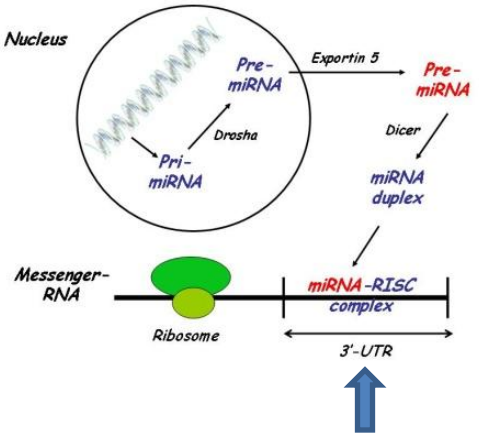
Selected target transcripts:
APC, ATF4, Dlx5, ETS-1, HES-1, LEF-1, NFATc1, Sp3, Sp7 (osterix), RNF11, Runx2/cbfa1, Satb2, TAZ, and VDR

Interrelations between the transcriptional modulators and other genes (the Ingenuity algorithm): *confined to osteoblasts ($p < 5 \cdot 10^{-13}$)*

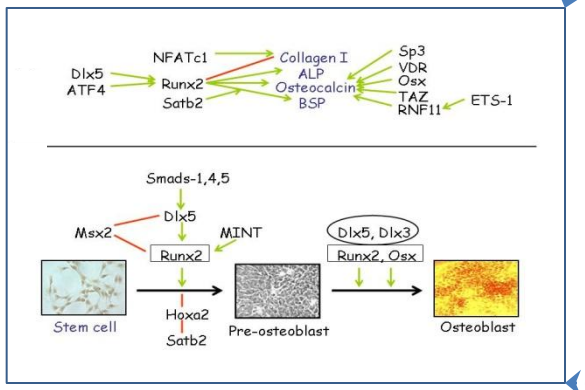


Key junctions: *TNFa* and *p38 MAPK*

Search for putative microRNA species targeting the selected transcriptional modulators



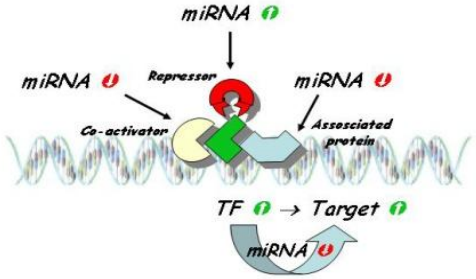
Concept: look for microRNAs *targeting two or more* transcriptional modulators specific for osteoblasts



MiRNA species (ranked by number of hits)	Predicted osteoblast (OB) gene targets	Tentative effect on OB development and function
296	APC, HES-1, NFATc1, Osterix, Runx2, Satb2	Precommitment and differentiation
34c	APC, ETS-1, Sp3, Satb2, Taz, VDR	Precommitment and differentiation
34a	APC, ETS-1, LEF-1, Satb2, VDR	Precommitment and differentiation
124a	Dlx5, ETS-1, RNF11, Sp3, VDR	Precommitment and differentiation
125a	ETS-1, HES-1, Osterix, Satb2, VDR	Precommitment and differentiation
125b	ETS-1, HES-1, Osterix, Satb2, VDR	Precommitment and differentiation
328	APC, ETS-1, Osterix, Runx2, VDR	Differentiation
449	RNF11, Satb2, Sp3, TAZ, VDR	Precommitment and differentiation
128b	APC, LEF-1, NFATc1, Satb2	Precommitment and differentiation
339	ETS-1, Osterix, RNF11, VDR	Differentiation
16	APC, ETS-1, Satb2	Precommitment and differentiation
22	APC, LEF-1, Satb2	Precommitment and differentiation
331	APC, Osterix, RNF11	Differentiation
337	ETS-1, Osterix, VDR	Differentiation
338	APC, ETS-1, Sp3	Differentiation
17-3p	ETS-1, Satb2, VDR	Precommitment and differentiation
24, 149	APC, LEF-1, RNF11	Differentiation
193	APC, ETS-1, LEF-1	Differentiation
328	APC, Runx2, Osterix	Differentiation

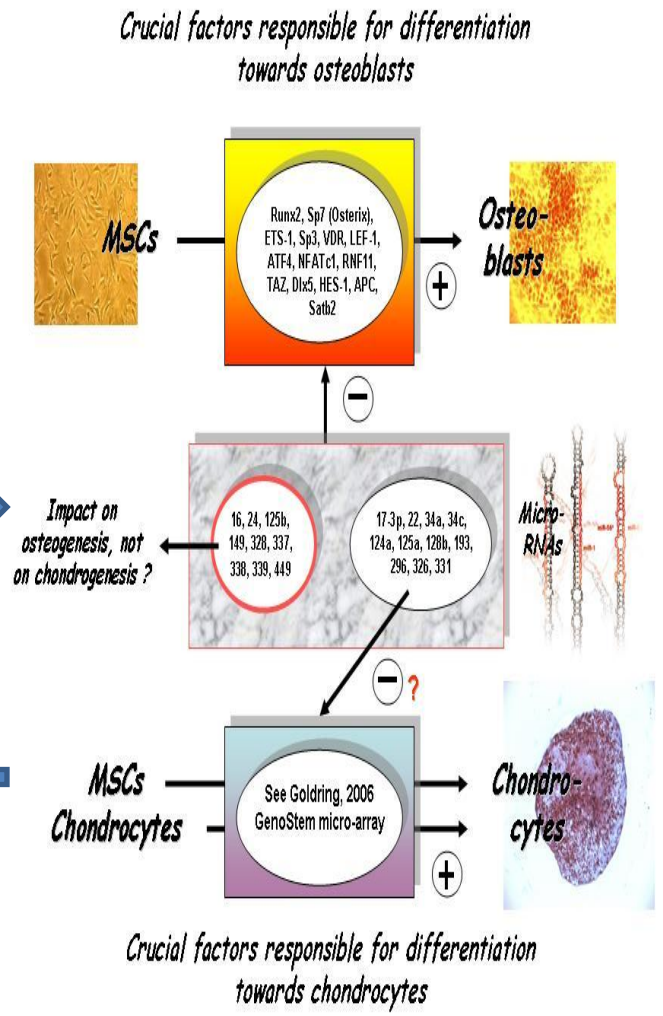
Search for possible detrimental effects of selected microRNA species on chondrogenesis

Watch out for microRNAs putatively affecting repressors of osteoblastic transcriptional modulators and microRNAs negatively affecting chondrogenesis

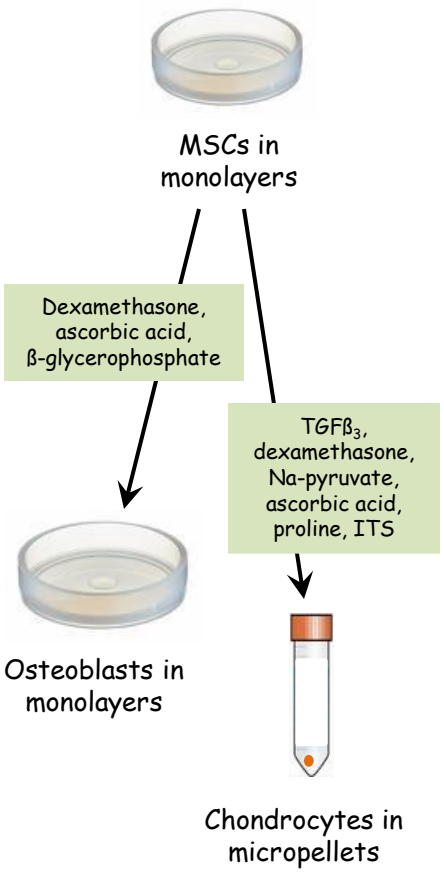


Names of genes (MSC condensation and differentiation; chondrogenesis and terminal differentiation) Goldring et al;, 2006	Aggrecan, Coll10a1, Coll11a1, Coll11a2, Coll2a1, Coll9a1, Fibronectin type 3, Hyaluronan, N-cadherin, Thrombospindin-2, ALK-2, ALK-3, ALK-6, BMPR1A, BMPR1B, CD-RAP, Chordin, FGF-10, FGF-8, FGRF1, FGRF2, FGRF3, Flk-1, GADD45β, Gli1, Gli2, Gli3, Hoxd11, Hoxd13, L-Sox5, Noggin, Npn1, Npn2, Ptc1, Smad1, Smad4, Smad5, Smad8, Smo, Sox6, Sox9, Tak1, Wnt3a, Wnt7a

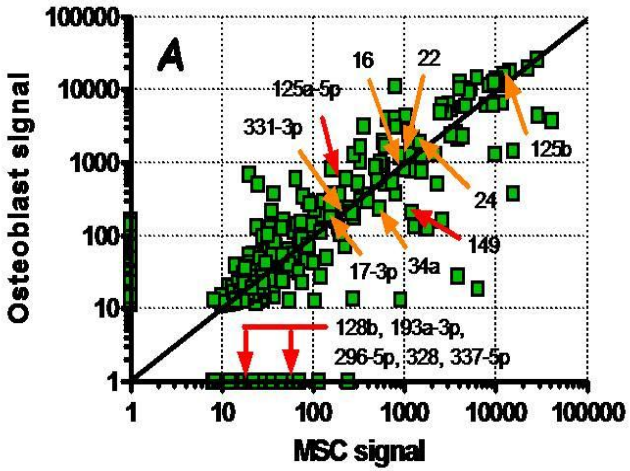
GenoStem transcriptome analysis (chondrocytes from cartilage incubated with TGFβ$_3$ *in vitro*)	**Genes related to transcription**	**Genes related to signalling systems**	**Genes related to matrix/anchoring proteins**
Early up-regulated genes (more than 2-fold on day 1)	Foxo3A, BHLHB2, MXI1, Sox9, Notch3, CEBPD	Wnt5A, STK24, TGFβ1, VEGF, ARL7, THY1, P311, FGF2, IL6, PTP4A1, PARG1, FZD2, NMB, STC1, PENK	CD44, Coll7a1, SPP1, NID, DPT, FN(1)
Early down-regulated genes (more than 2-fold on day 1)	Foxo1A, ID3, SMURF2, RYBP, HMGIY	DKK1, SPRY4, GADD45β, TNFRSF1, YWAH, PDE8A, PTPRC, MKP-L, NDRG1	PRELP, COMP, Coll1a1, ITGA10, MGP

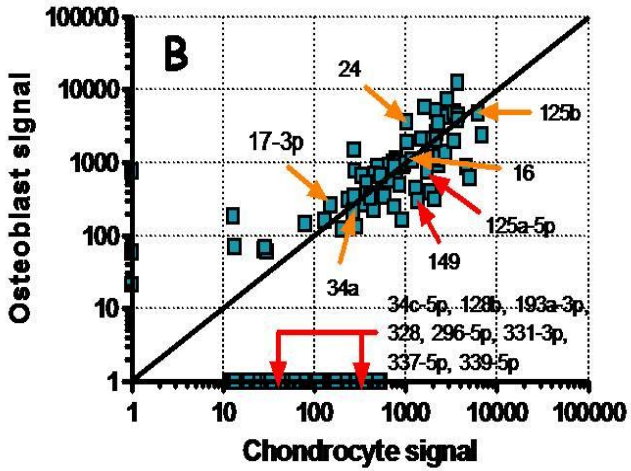


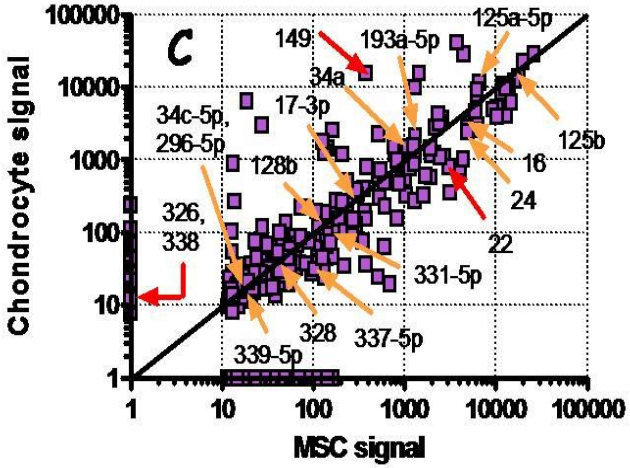
MicroRNA microarray differential display analysis of osteoblasts and chondrocytes differentiated from hMSCs for 3 days

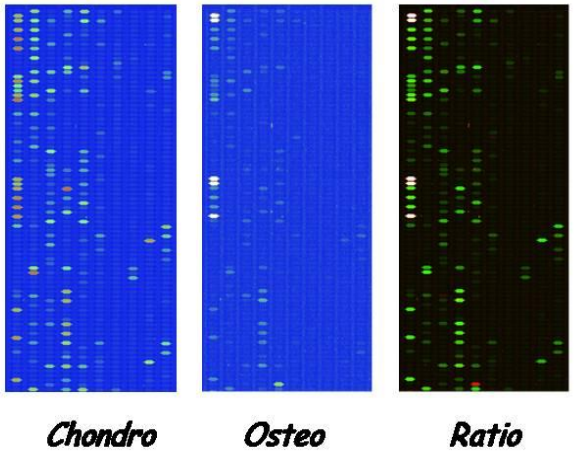


Isolation of total RNA using the *mir*Vana® kit









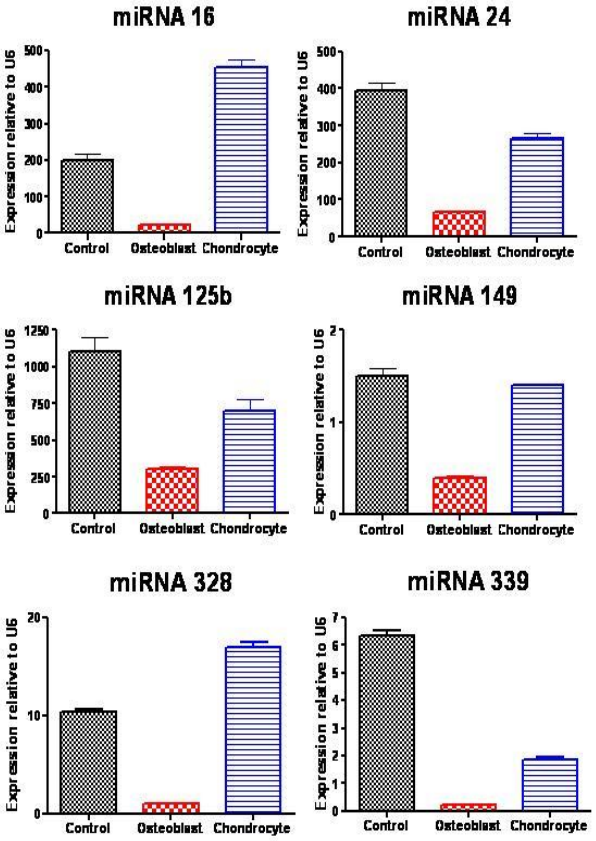
Comparison between the *in silico* search for putative microRNA species and the microRNA microarray analyses

Human miRNAs	Log2 [chondro/osteo] ($p < 0.01$)	Predicted microRNAs	Number of putative targets
34c-5p	Absent in osteo	34c	6
128b	Absent in osteo	128b	4
193a-3p	Absent in osteo	193a	3
328	Absent in osteo	328	3
296-5p	Absent in osteo	296	6
331-3p	Absent in osteo	331	3
337-5p	Absent in osteo	337	3
339-5p	Absent in osteo	339	4
671-5p	5.69		
24-2	4.04	24	3
212	3.68		
26b	3.50		
663	2.98		
29b	2.81		
29c	2.72		
149	2.42	149	3
148a	2.41	148b	1
638	2.38		
15a	2.31	15a	1
923	2.31		
411	2.23		
376c	2.19		
574-3p	2.17		

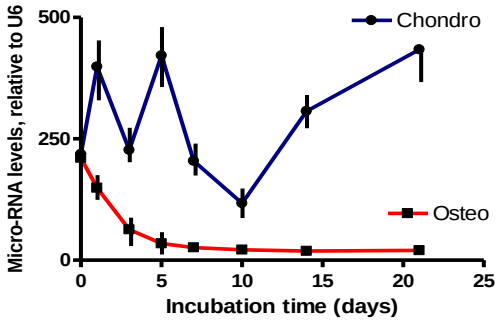
Human miRNAs	Log2 [chondro/osteo] ($p < 0.01$)	Predicted microRNAs	Number of putative targets
99a	2.17		
575	1.62		
1231	1.61		
21	1.60		
Let-7g	1.49	Let-7c	1
494	1.37		
214	1.26	214	1
27b	1.19		
125a-5p	1.10	125a	5
27a	1.03		
199a-3p	0.94	199a	1
100	0.94		
29a	0.91		
		34a	5
		124a	5
		125b	5
		326	5
		449	5
		16	3
		17-3p	3
		22	3
		338	3
		18, 30e-3p, 31, 34b, 103, 107, 128a, 133a, 133b, 205, 330, 365, 368, 370, 422a, 424	1-2

Conclusion: 16 predicted out of 36 analysed microRNA species in common, including miRNAs 149, 328, 337, and 339, putatively not perturbing chondrogenesis

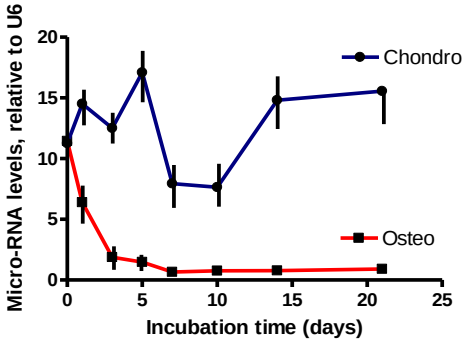
Profile of the microRNA species 16, 24, 125b, 149, 328, and 339 during osteogenic and chondrogenic differentiation from hMSCs for 5 days (left) and up to 21 days (right)



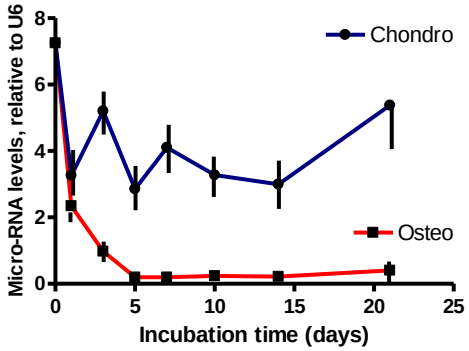
Time-course of *mir-16* expression in hMSCs (P17, PMP7 and P23) differentiated into Chondrocytes or Osteoblasts



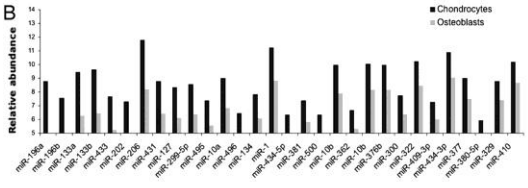
Time-course of *mir-328* expression in hMSCs (P17, MP7 and P23) differentiated into Chondrocytes or Osteoblasts



Time-course of *mir-339* expression in hMSCs (P17, MP7 and P23) differentiated into Chondrocytes or Osteoblasts

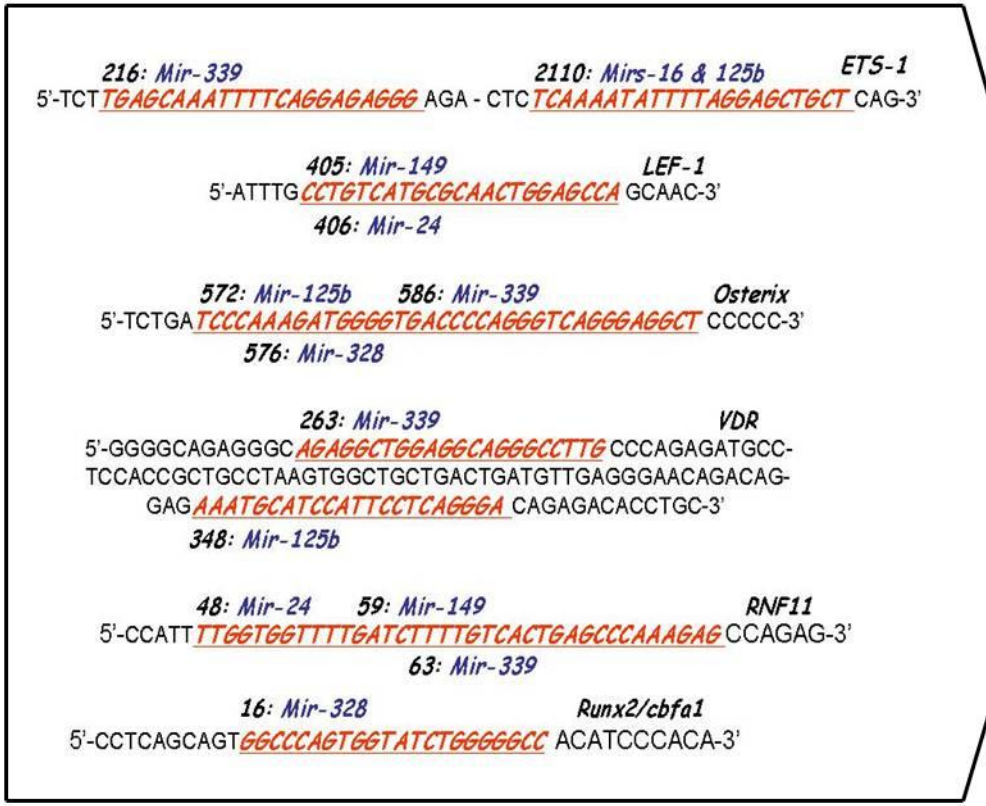


The subject microRNAs are maintained in differentiating chondrocytes, but strongly down-rgulated in differentiating osteoblasts – *"all or none" effect*



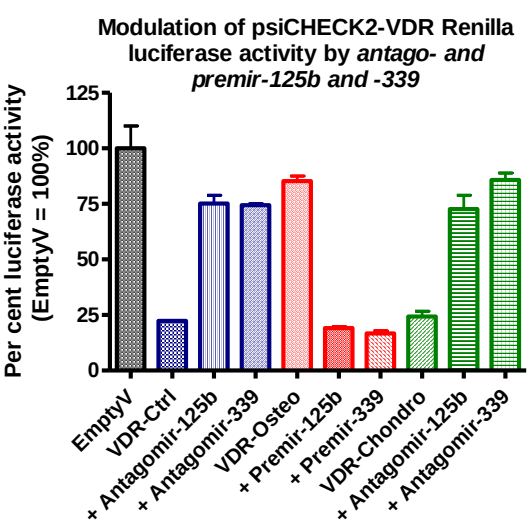
Dicer-dependent pathways regulate chondrocyte proliferation and differentiation
Tatsuya Kobayashi et al., 2009, PNAS

psiCECK2 reporter constructs containing parts (from 473 to 2010 bases) of XhoI/XhoI or XhoI/NotI digests of PCR amplified 3'-UTR sequences

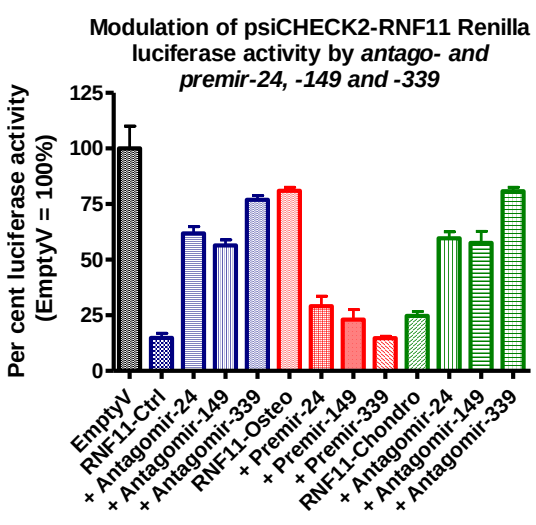


Each construct contains at least one putative target sequence for the osteoblast/chondrocyte signature microRNA species 16, 24, 125b, 149, 328, and 339

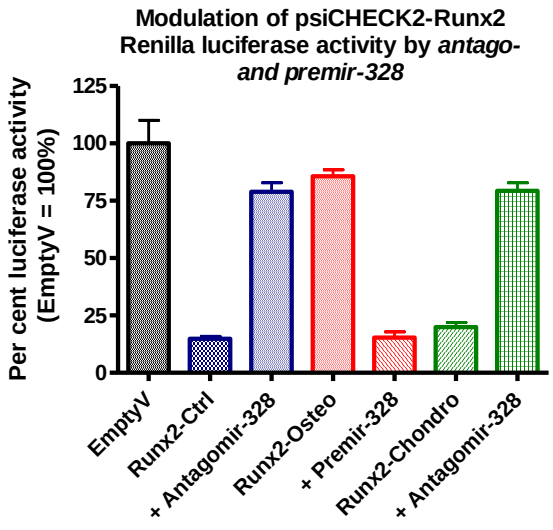
Effect of pre-miRNAs and antago-miRNAs on the luciferase activity of the psiCHECK2 constructs in osteoblasts and chondrocytes differentiated from hMSCs for 3 days (cont.)



MicroRNAs 125b and 339 seem to be equally potent as to their impact on the VDR transcript



MicroRNA 339 seems to be more potent as to its impact on the RNF-11 transcript than miRNAs 24 and 149

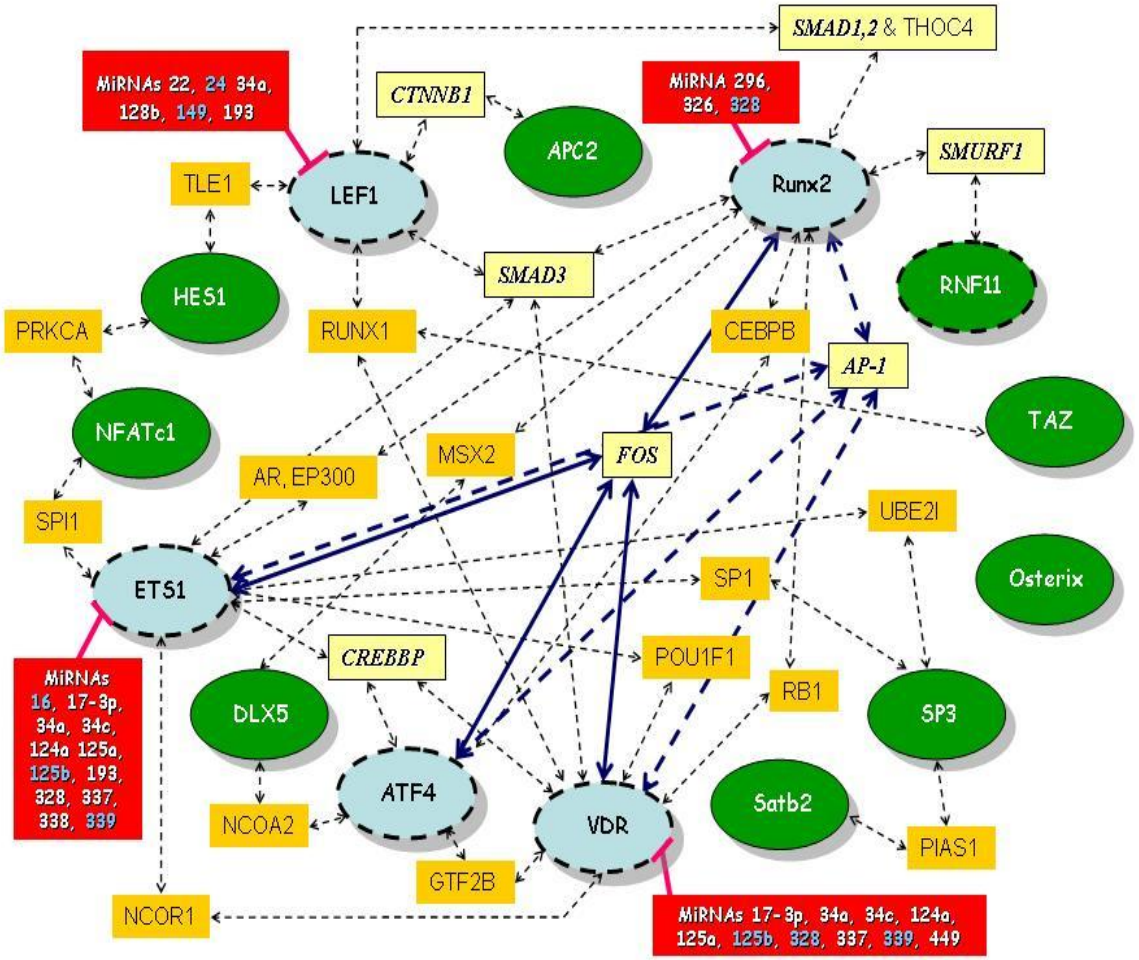


MicroRNA 328 seems to be as potent as its impact on the Runx2 transcript as 339 on the RNF-11 transcript

The transcriptional modulators specific for osteoblasts closely interact with many signalling system molecules

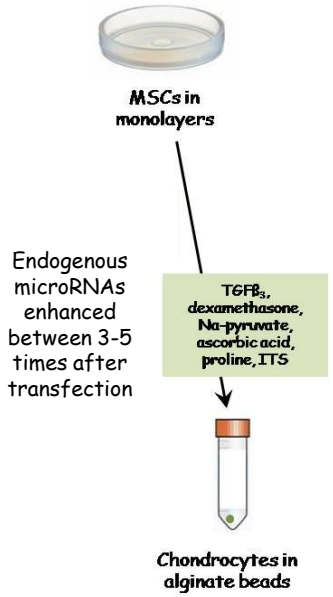
Proteins interacting with at least two of the 14 transcription modulators (according to the«Pina» algorithm) important for osteoblastogenesis:

PRKCA: Protein kinase C alpha type (PKCa)
SPI1: hematopoietic transcription factor PU.1
TLE1: Transducin-like enhancer protein 1 (ESG1)
NCOR1: Nuclear receptor corepressor 1 (N-CoR1)
RUNX1: Runt-related transcription factor 1
AR: Androgen receptor (DHT receptor)
EP300: Histone acetyltransferase p300 (p300 HAT)
NCOA2: Nuclear receptor coactivator 2 (NCoA-2)
CTNNB1: Catenin β1
SMAD3: TGFβ-signaling protein 3
MSX2: Homeobox protein MSX-2 (Hox-8)
CREBBP: CREB-binding protein
GTF2B: Transcription initiation factor IIB
FOS: Proto-oncogene protein c-fos
CEBPB: CCAAT/enhancer-binding protein beta (C/EBPβ)
SP1: Transcription factor Sp1
POU1F1: Pituitary-specific positive TF factor 1 (Pit-1)
SMAD1: TGFβ-signaling protein 1
SMAD2: TGFβ-signaling protein 2
THOC4: THO complex subunit 4 (incl. AML1& LEF1)
SMURF1: SMAD ubiquitination regulatory factor 1
AP-1: Adaptor protein complex AP-1
UBE2I: Ubiquitin-conjugating enzyme E2 I
RB1: Retinoblastoma-associated protein (pRb)
PIAS1: Protein inhibitor of activated STAT protein 1



The chondrocyte differentiating potential of the microRNAs shown to block osteoblastogenesis and facilitate chondrogenesis

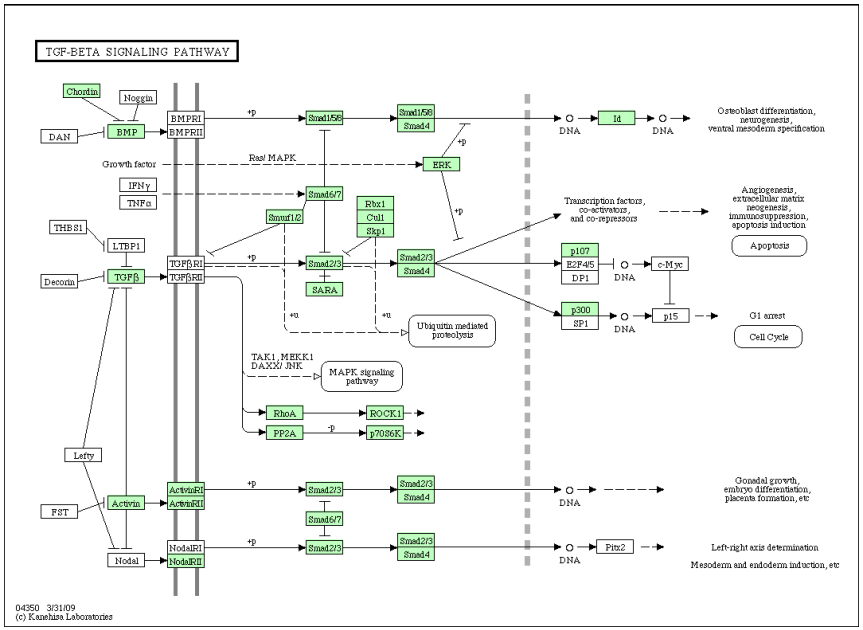
Complete Ambion® transfection kit and protocol. Transfection was performed every 4 days until day 21

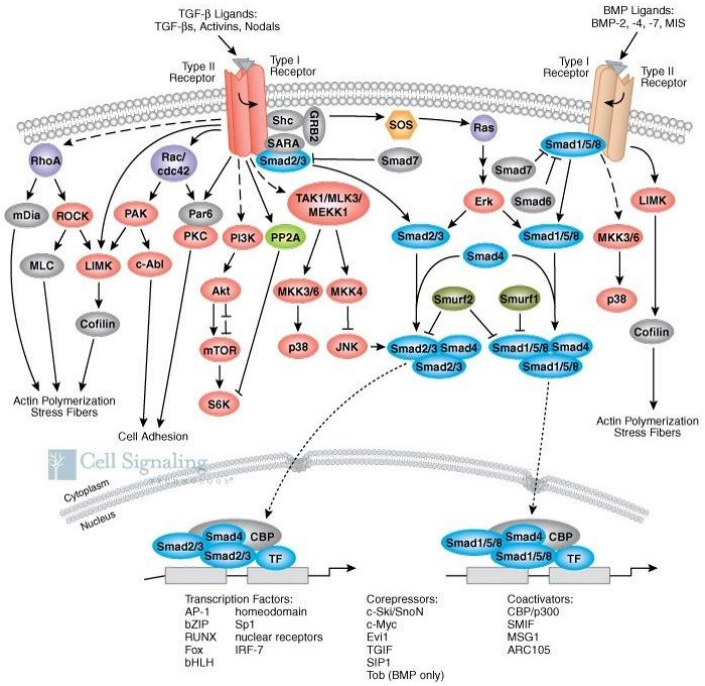


End point measures:
RT-PCR of marker genes (all values expressed relative to controls = $TGFß_3$ = 100%)

Markers	RT-PCR (%) of gene transcripts, GAG/DNA-ratio, and Clinical score (histology, distance between cells, immunihistochemistry)					
Sox9	100	11	23	55	23	63
Wnt5	100	7.6	18	58	16	74
GAG/DNA	100	8.3	21	65	28	62
Clin. Score	100	6.8	26	66	21	66
Aggrecan	100	13	19	55	24	68
Collagen 2a	100	5.1	18	49	16	73
Collagen 10a	100	3.6	24	47	21	61
Cell manipula-tion by						
$TGFß_3$	+					
Premirs 16&125b			+			
Premirs 24&149				+		
Premirs 328&339					+	
All premirs						+

Conclusion: The microRNA species are not able to substitute completely for $TGFß_3$ (with the exception of miRNAs 24&149) in achieving typical chondrocyte differentiation from MSCs

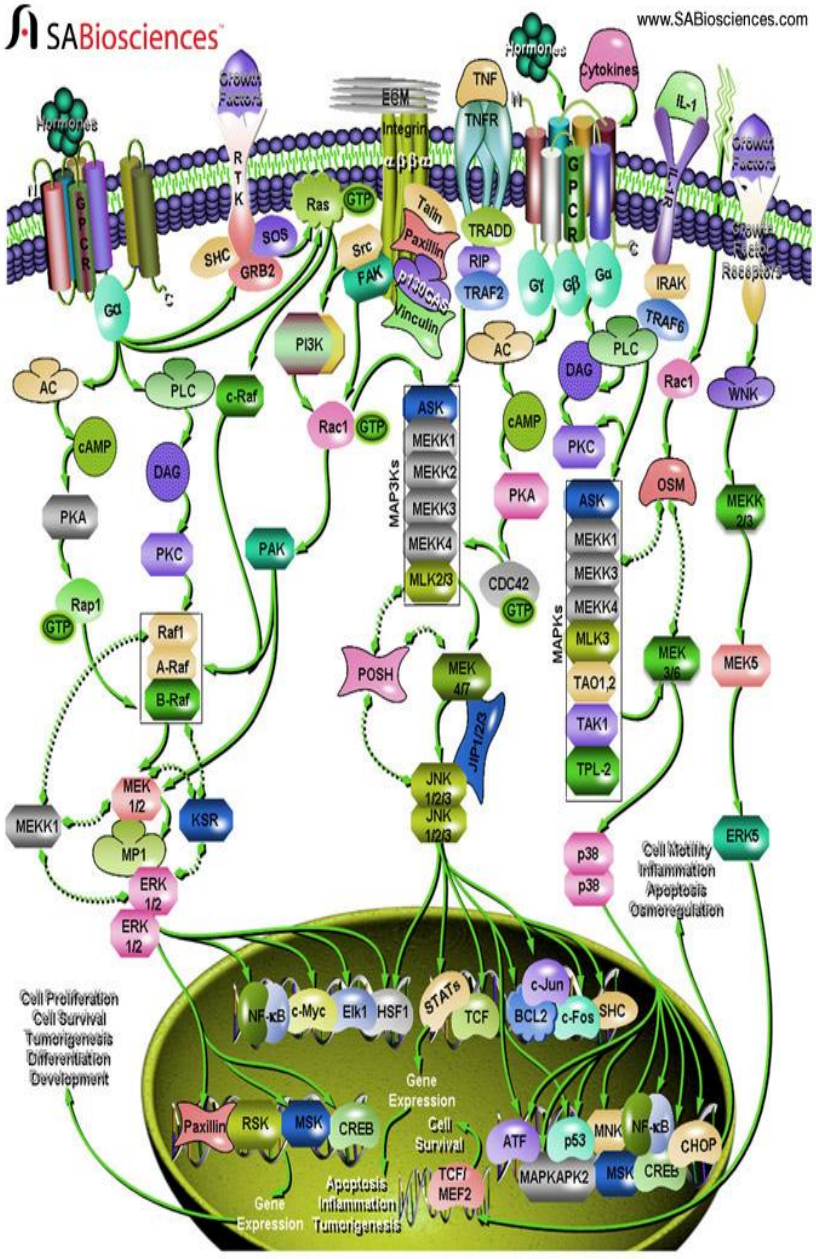




MicroRNAs of the osteo-chondro signature may heavily interfere with antagonists of the chondrocyte differentiation from MSCs

Gene name	Transcript targeted by
Receptor antagonists	
Chordin (CHRD): BMP	24, 125b, 149, 328
Noggin (NOG): BMP	16, 149
THBS1: TGFβ	16, 328
Decorin (DCN): TGFβ	24, 339
TF antagonists	
Smad6: BMP/TGFβ	16, 149
Smad7: BMP/TGFβ	16
Smurf1: TGFβ	16, 125b
Smurf2: TGFβ	16
MAPK14 (p38-MAPK)	24, 125b, 149, 328, 339
Rbx1: vs Smad 2/3 only	16, 149
Cul1: vs Smad 2/3 only	125b
Skp1: vs Smad 2/3 only	125b
Co-repressors of TFBEs	
c-ski/snoN (SKI)	16, 339
c-myc (MYC)	
EvI1	24, 328
TGIF	24, 149
SIP1	16, 125b
Tob: BMP only	16, 149

In silico searches using the Sanger, Viewer, PicTar, Segal and Sloan-Kettering databases



The microRNAs of the osteo-chondro signature are putatively heavily involved in the regulation of the TNFa pathway (i.e. "taking out" its inhibitory impact)

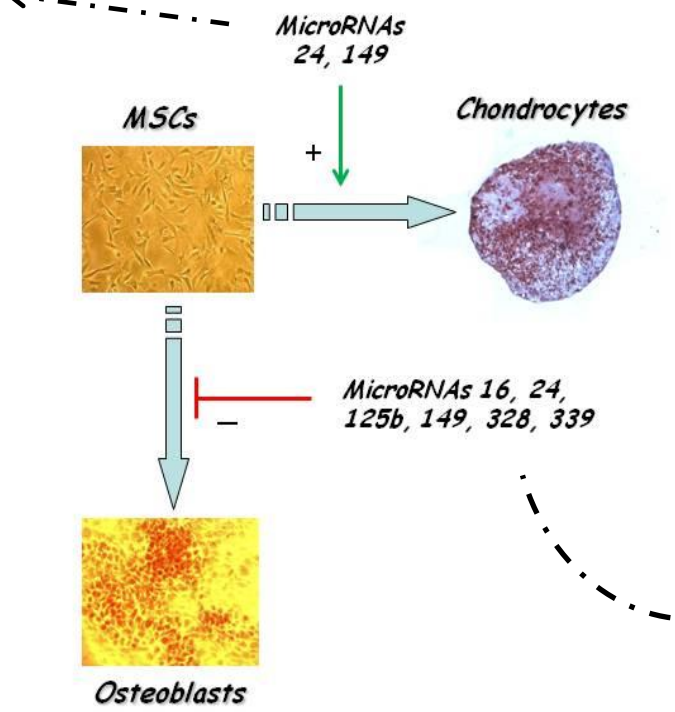
Gene names	*Transcripts targeted by*
Post receptor level	
TRADD	149
RIP = RALBP1	125b
TRAF2	328
ASK = DBF4	
MEKK1 = MAP3K1	16, 24, 125b, 328
MEKK2 = MAP3K2	24
MEKK3 = MAP3K3	16, 24, 125b
MEKK4 = MAP3K4	16, 24
MLK2 = MAP3K10	125b, 328, 339
MLK3 = MAP3K11	125b, 149, 328
MEK4 = MAP2K4	16, 339
MEK7	
JNK1 = MAPK8	24
JNK2 = MAPK9	16, 125b
JNK3 = MAPK10	125b

In silico searches using the Sanger, Viewer, PicTar, Segal and Sloan-Kettering databases

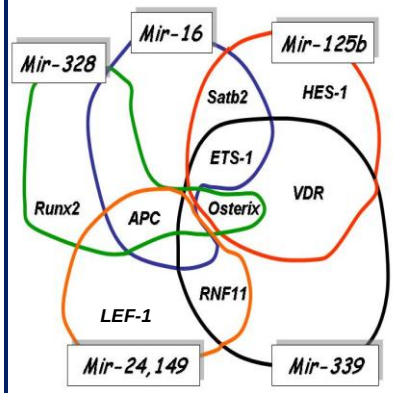
Model for how the microRNA signature affects differentiation of osteoblasts and chondrocytes from hMSCs

MiRNA 149, may serve as switch (since it targets ATF3, which activates Runx2 and inhibits Sox9) between the osteoblast and the chondrocyte phenotypes depending on its endogenous levels and cooperation with other, unidentified, microRNAs

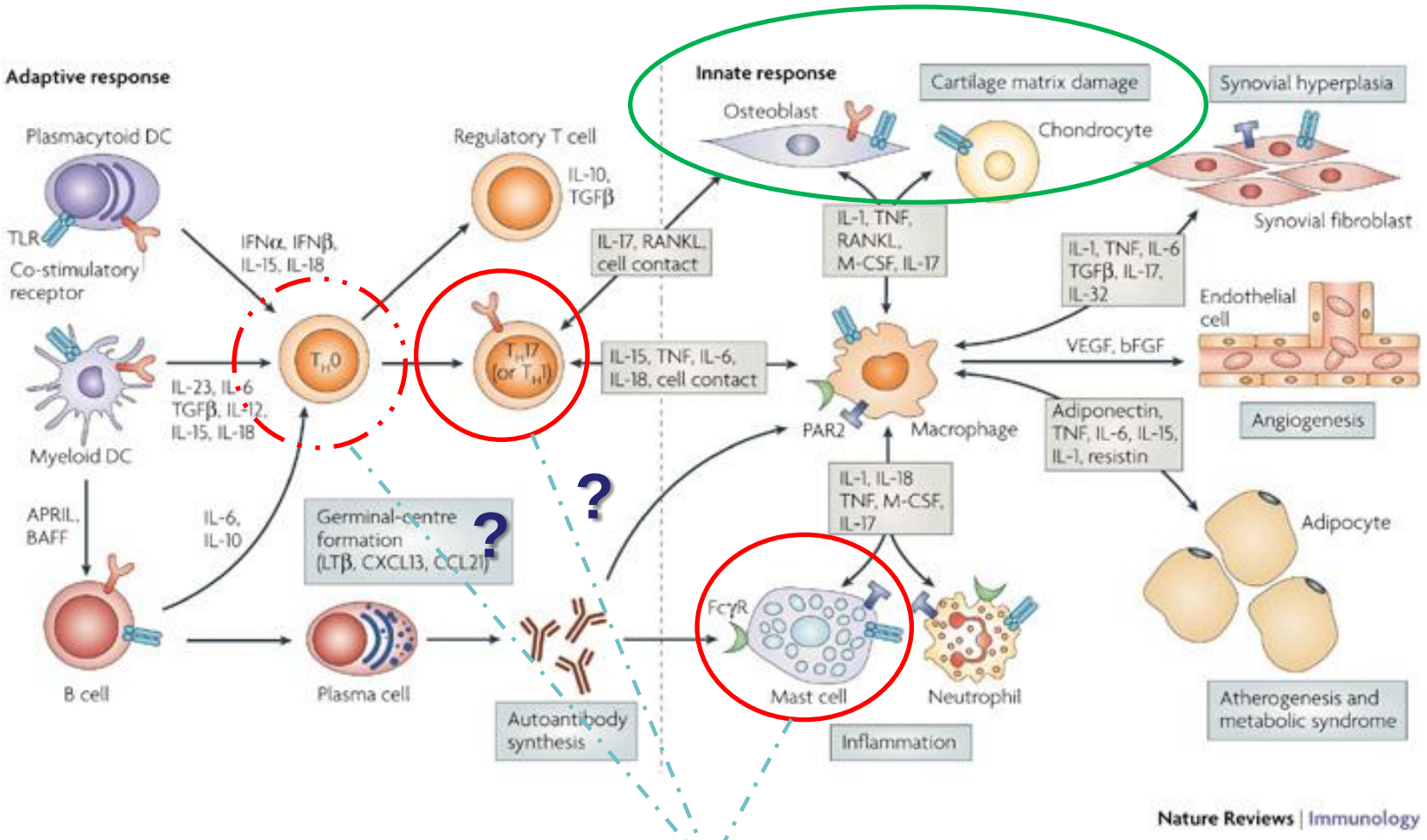
MiRNAs 24 and 149 are putatively interfering with gene transcripts like: PIAS1 (repressing Sox9 through SUMOylation), Stat6 (Sox9 inhibitor), SP1 (inhibitor of CEBPA interacting with Sox9), and PPPIRI6B (TGFß-inhibiting membrane associed protein = protein phosphatase 1 inhibitory subunit 6B) etc.



6 microRNA species specifically block osteoblastogenesis, thereby promoting chondrogenesis, by targeting at least 9 transcriptional modulators:

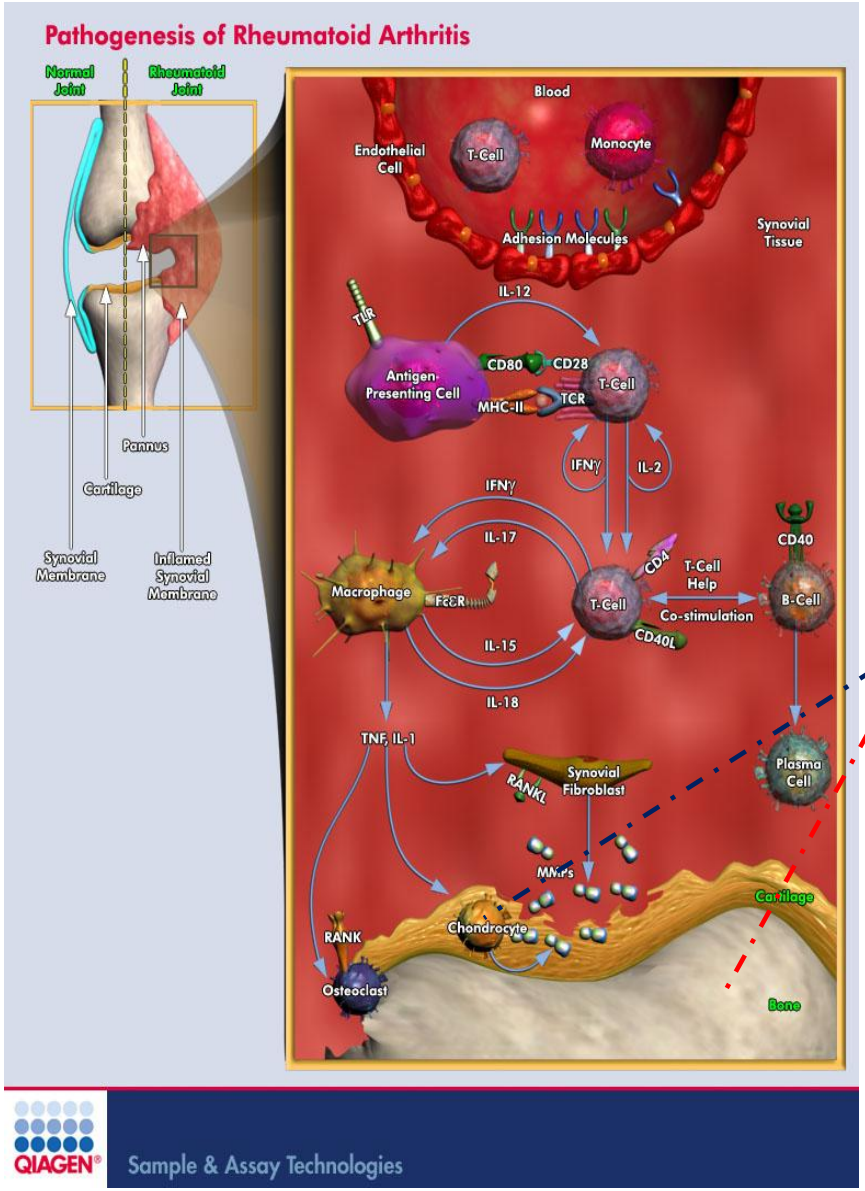


Cells involved in inflammation (e.g. rheumatoid arthritis)



Shedding exosomes containing a plethora of microRNAs

Possible interactions between microRNA-presenting compartments in rheumatic disease



MicroRNAs increased in whole blood from RA-patients:

144, 142-3p, 32, 19a, 340, 7, 101, 142-5p, 19b, 96, 29bc, 424, 125b,

Some microRNAs found in exosomes from mast cells:

451, 10a, 450, 150, 296, 341, 15ab, 24, 20a, ***222***, 324-3p, 23ab, ***21***, ***184***, 500, 29a, 329, ***26a***, 30c, 326, 433,18, 16, 207, 129-5p, 146b, 17-5p, 142-3p, 142-5p, 183, 191, 96, 106b, 291ab, 107, 290, 351, 182, 27b, 468, 300, 470, ***let-7b***, 370, 298, 185, 503

MicroRNAs produced in large amounts in activated Th 17 cells:

21, 22, 638, 663, 34a, 923

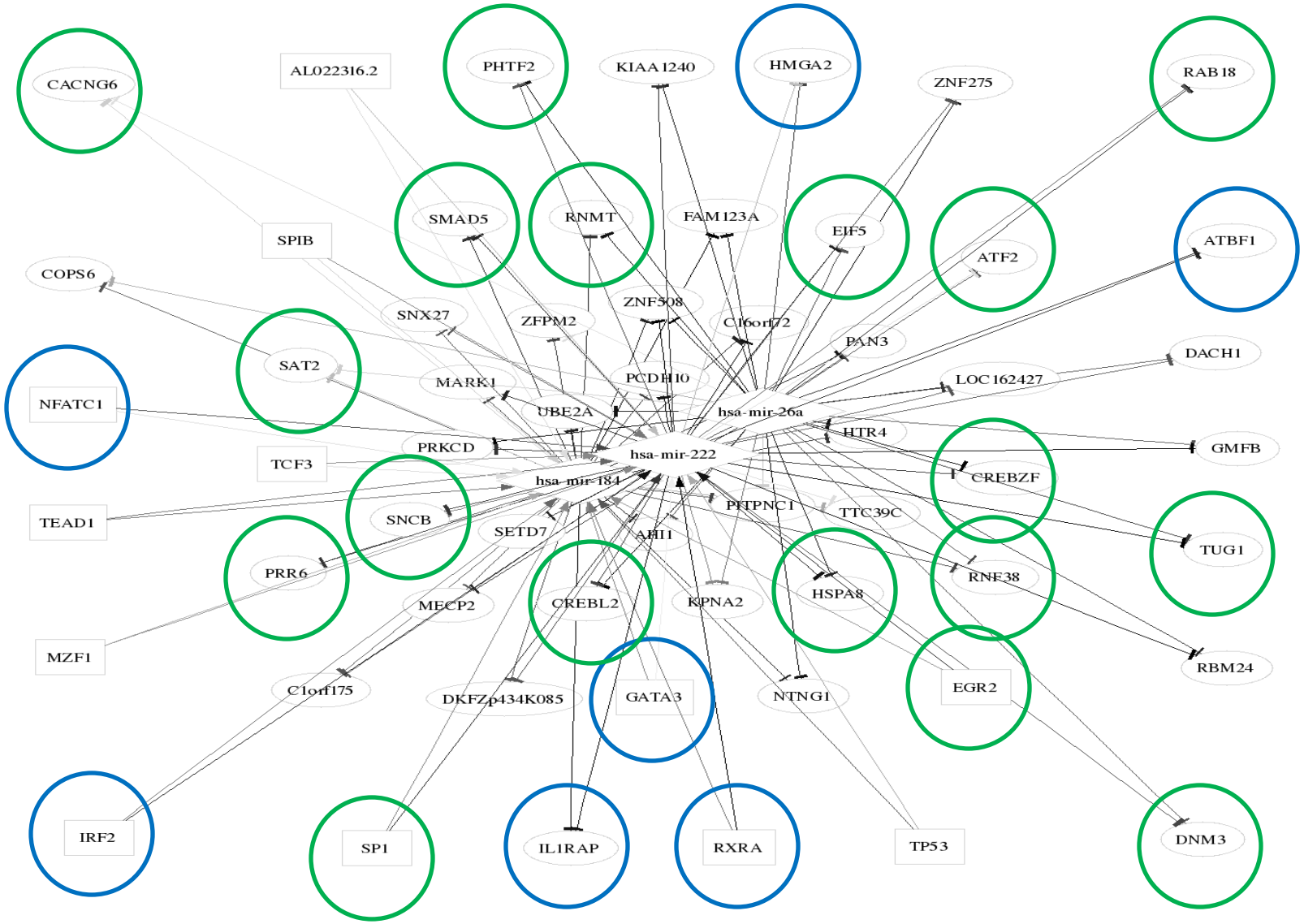
Potential detrimental microRNAs affecting chondrocytes:
26a, 222, 184 and osteoblasts: 21, 22,663, 638, 923, 34a

MicroRNAs produced in small amounts in differentiated chondrocytes: ***26a***, ***222***, 145, 143, ***184***

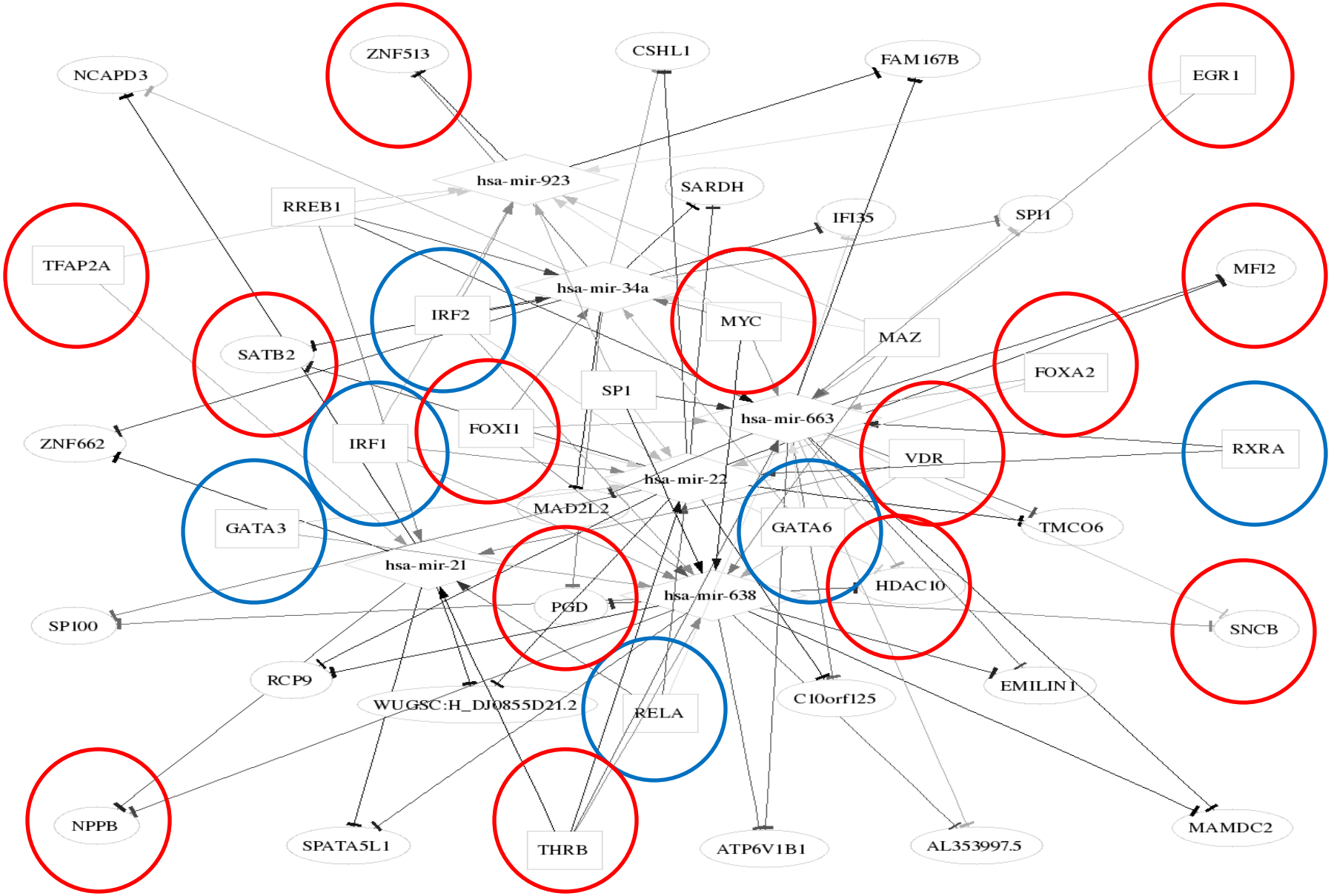
MicroRNAs produced in small amounts in differentiated osteoblasts:

34c-5p, 128b, ***34a***, 193a-3p, 328, 296-5p, 331-3p, 337-5p, 339-59, 671-59, 24, 26b, ***663***, 29bc, 149, 148a, ***638***, 15a, ***923***, 411, 376c, 574-3p, 125ab, 99a, 575, ***21***, 494, 214, 27ab, 199a-3p, ***22***, 100, 29a

Mir@nt@n algorithm: Interaction between microRNAs 26a, 222, and 184, transcription factors and target genes



Putative interrelation between microRNAs 21, 22, 34a, 638, 663, 923 (Mir@nt@n algorithm), transcription factors and target genes



To be commended for their scientific and technical support and never-ceasing enthusiasm:
INSERM U844, Montpellier, France
&
Université Montpellier 1, enabling me to work within the U844 as a guest professor for 3 years

Thank you for your attention!

Human Pluripotent Stem Cells

Susanne Ström, PhD

NORWEGIAN CENTER FOR STEM CELL RESEARCH

NDEVOR

laboratory of Neural Development and Optical Recording

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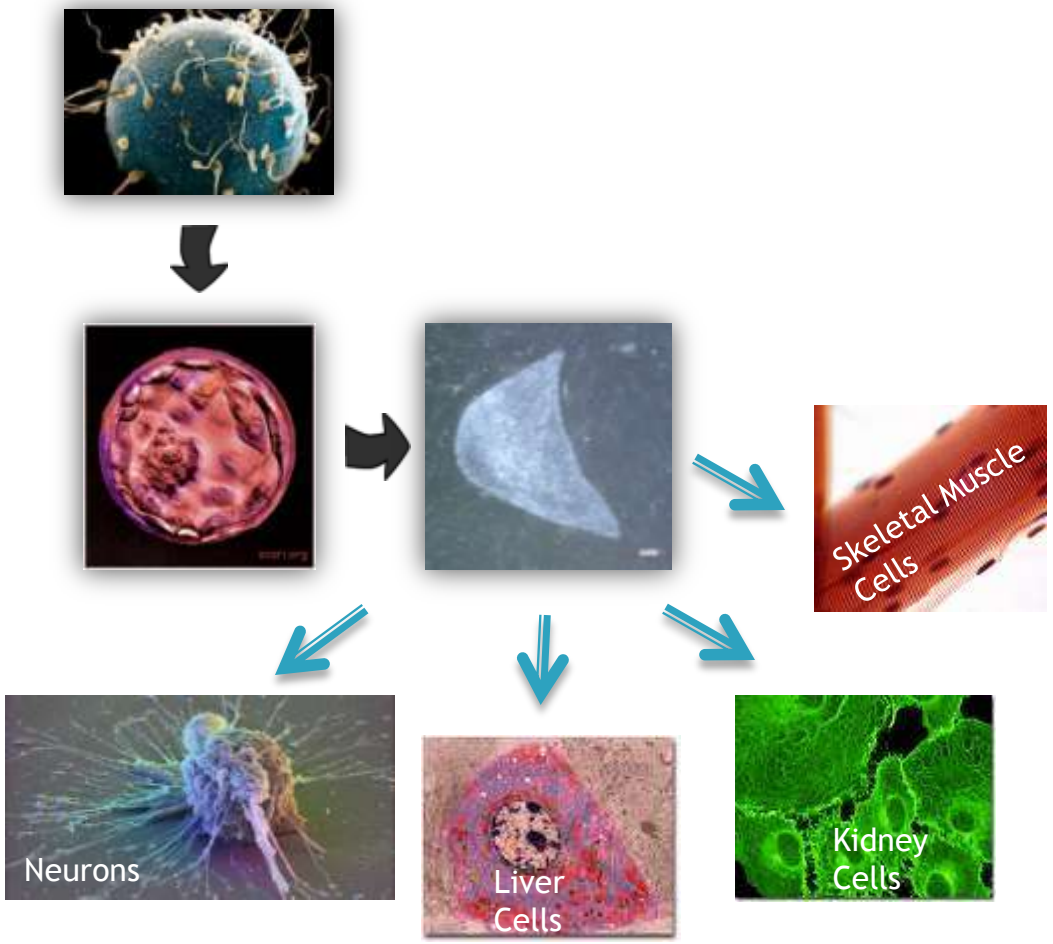
Background

- hESC derived from the ICM of blastocysts
- Have the potential to differentiate to any of the cell types of the body
- The hope is that these cells can be used in cell replacement to cure diseases like; Parkinson's, HD, MS, spinal cord injuries, myocardiac infarctions, diabetes...



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Background

- 1981 First mouse ESC line. **Evans and Kaufman.**
- 1994 First culture of human ICM. **Bongso et al.**
- 1998 First human ESC lines, **Thomson et al.,** 2000 **Reubinoff et al.**
- 2006 First mouse iPS cell line. **Takahashi and Yamanaka.**
- 2007 First human iPS cell lines. **Yamanaka** and **Thomson** groups.

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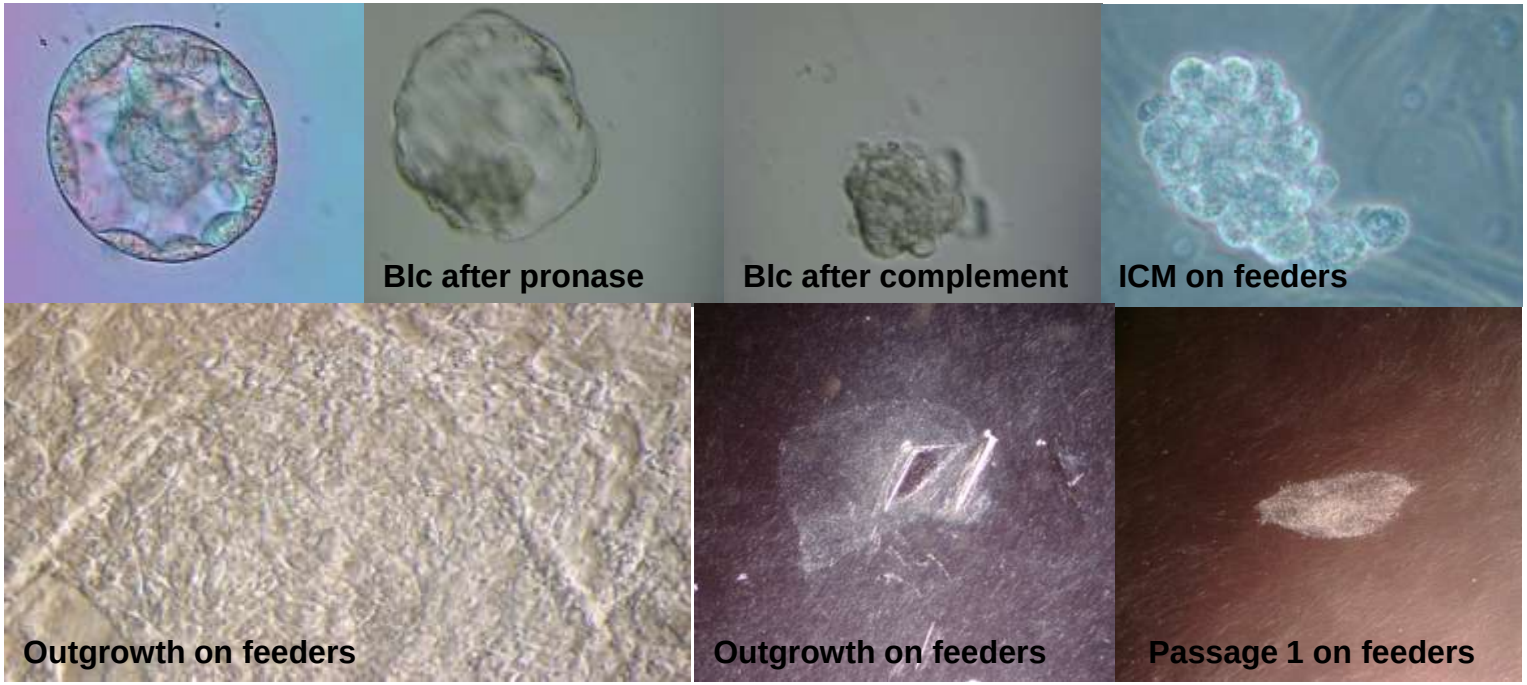
Derivation by Immuno-Surgery



- Pronase to remove the ZP
- Immunosurgery will remove the trophectoderm, rabbit anti-human whole serum and guinea pig complement serum.

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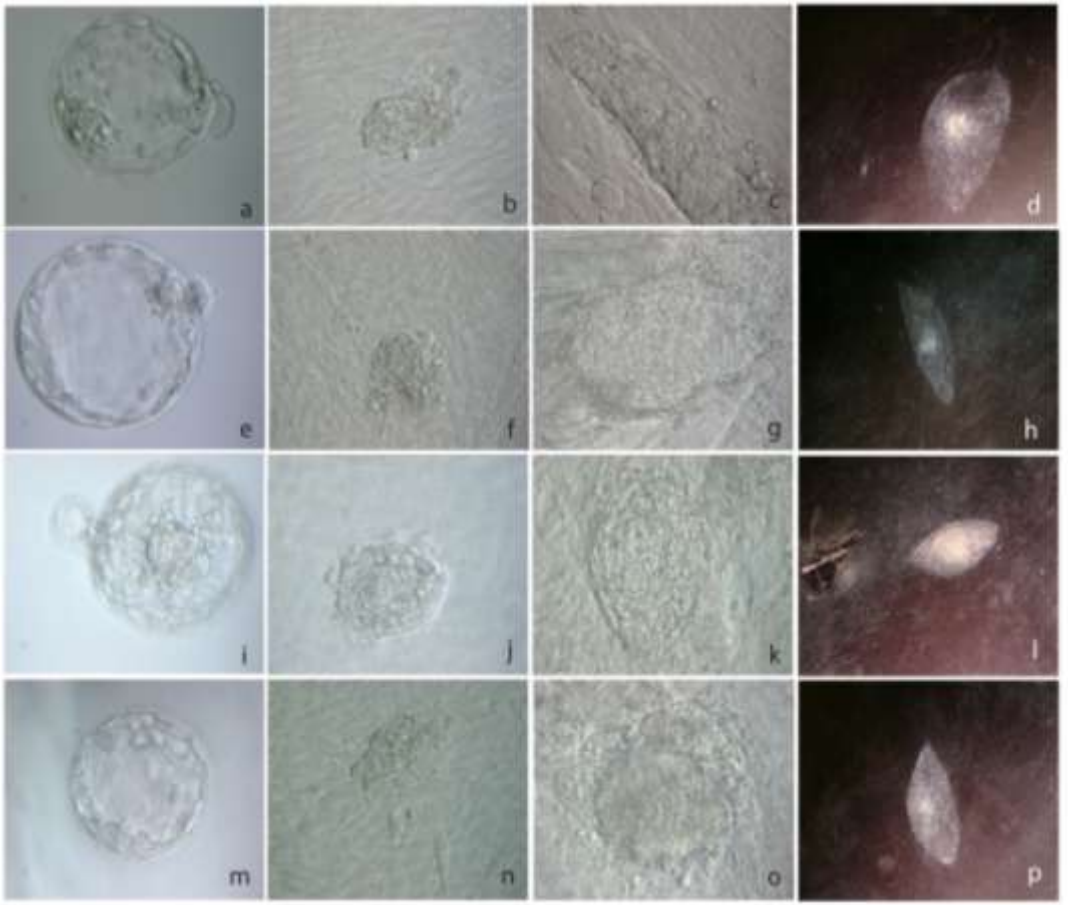
Derivation by Mechanical Isolation of ICM

- ZP is removed with sharp needles and the ICM is cut out and placed on feeder cells or ECM
- Laser dissection has also been published



Ström et al. 2007

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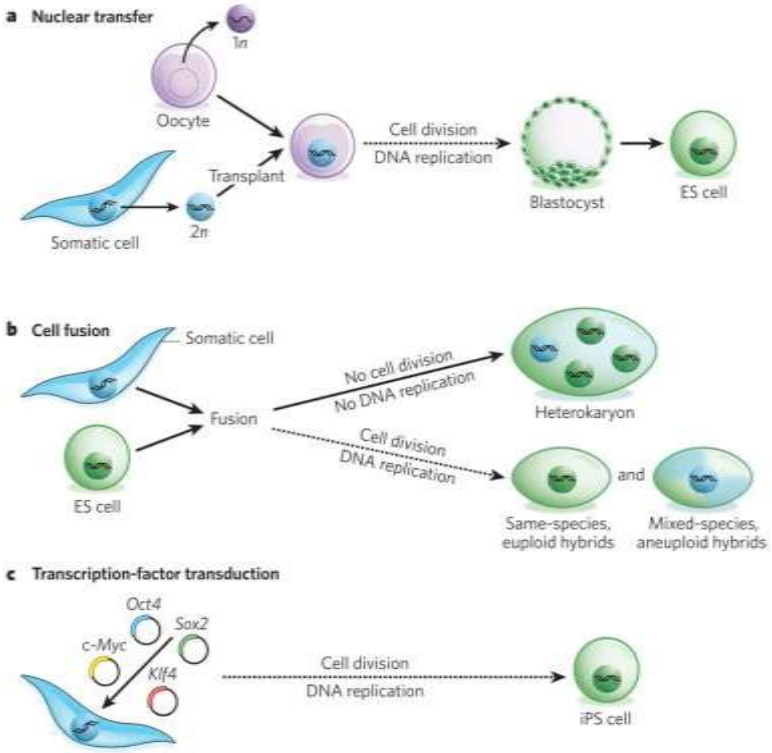
Induction of Pluripotency



- Cloning/SCNT
  - Briggs and King 1952, (tadpoles).
  - Dolly the sheep. Wilmut et al., 1997
  - The first cloned mouse in 1998. Wakayama et al.
- Cell fusion. Ex. Mouse muscle cell fused with human amniotic cell and resulted in heterokaryon expression human muscle proteins (Blau et al., 1983, 1985)
- Transcription factor induction

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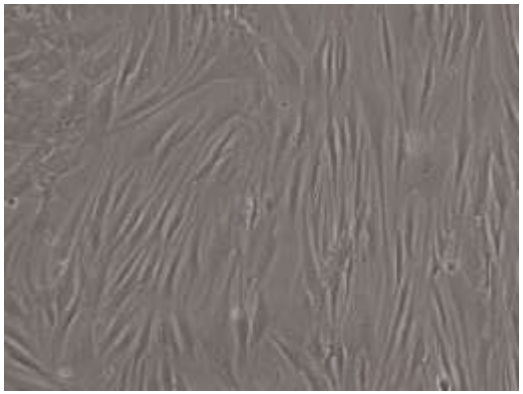
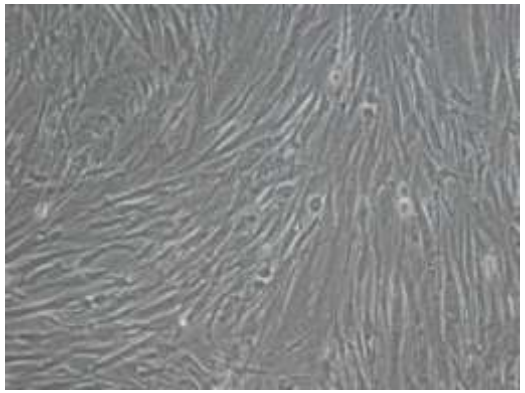
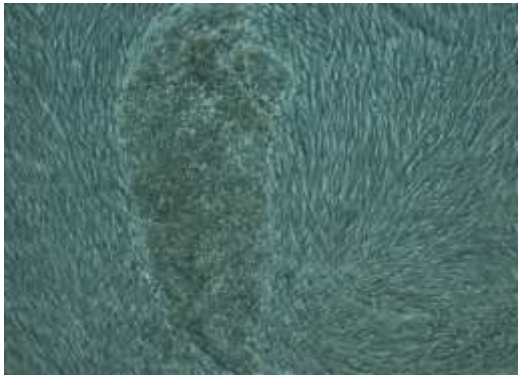
Induction of Pluripotency

Day 0

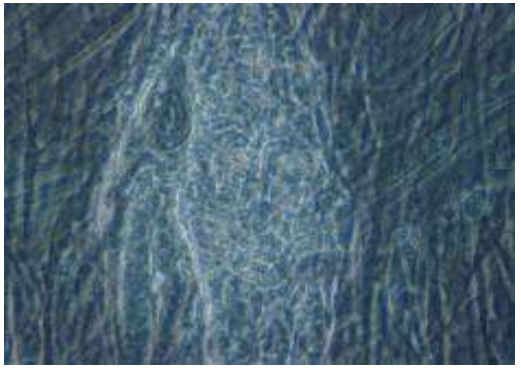


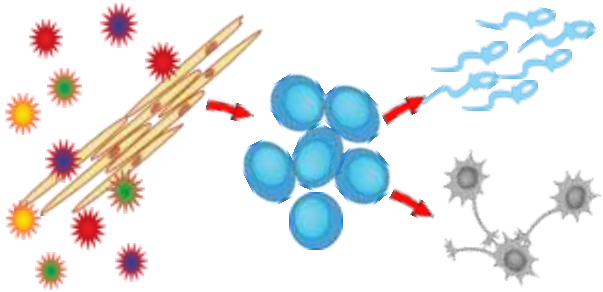
Day 4





Day 10 (p.0 ChiPS 22)





HESC: HS475, 5 days post derivation
Rosita Bergström

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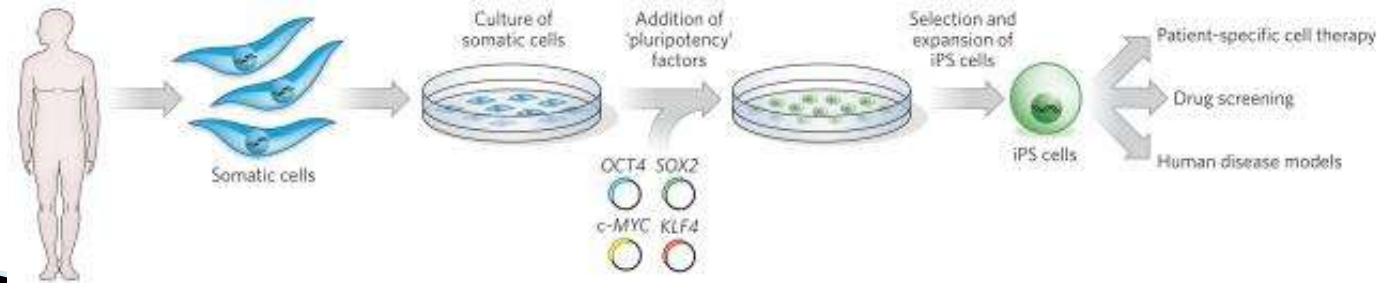
hES cells vs hiPS cells

- hiPS cells: No embryo destruction
- hES cells: Less manipulation
- hiPS cells: Patient specific
- Both can be used for disease modeling, but hiPS cells can be made from any disease. hES cells can be derived from PGD embryos.



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Morphology and Function

- Continuously self-renewing
- High levels of telomerase activity up to 300 passages
- Ability to form any adult cell (higher plasticity than adult SCs)
- Unlimited source of specific cell type
- Provide a tool for studying the molecular mechanisms
  - Early embryonic developmental pathways
  - The pathological basis of genetic disorders
  - Toxisity testing
  - Drug screening

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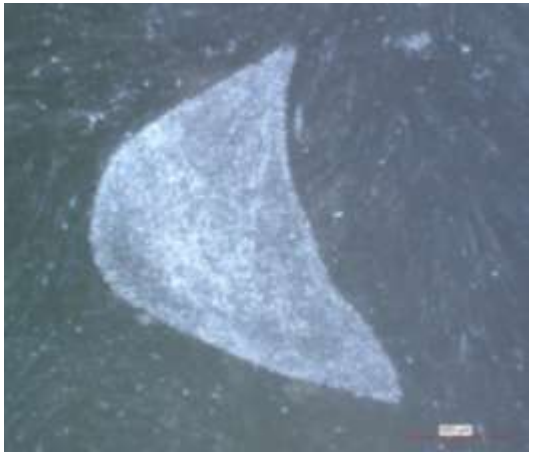
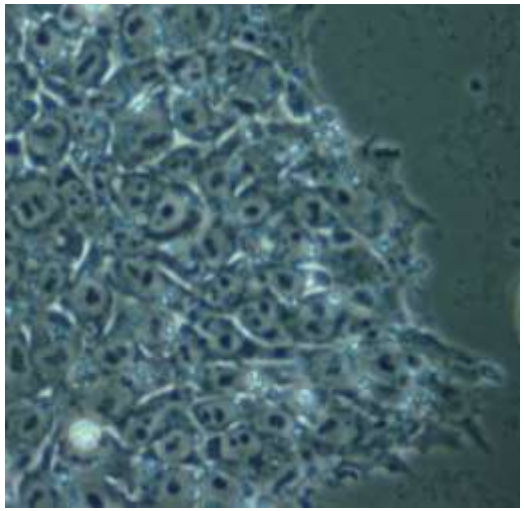
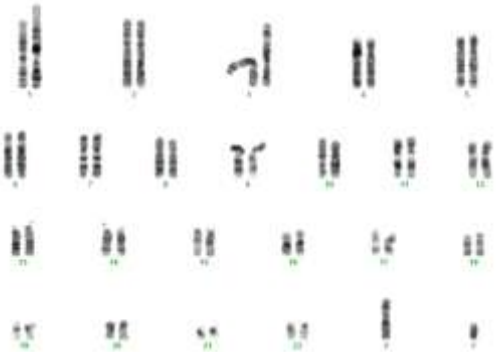
Morphology and Function

- Express high levels of telomerase activity
- Should be able to diffrentiate into all three germ layers (endoderm, ectoderm and mesoderm) *in vivo* and *in vitro.*
- hESC form relatively flat and compact colonies with sharp borders, large nucleus, small cytoplasm and prominent nucleolous
- Should have normal karyotype
- A population doubling period takes 24-36 h







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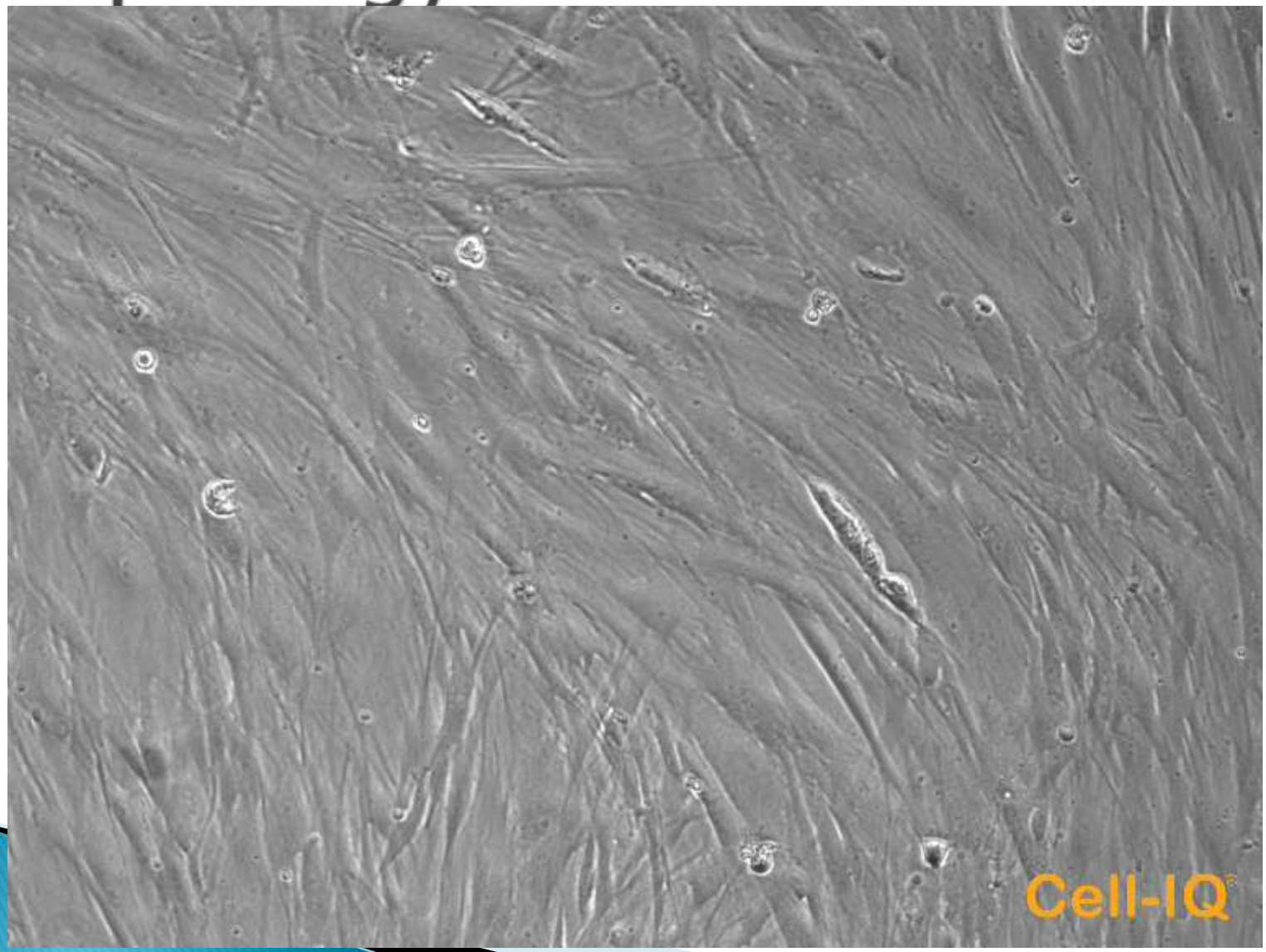
Laboratory of Neural Development and Optical Recording

Morphology and Function



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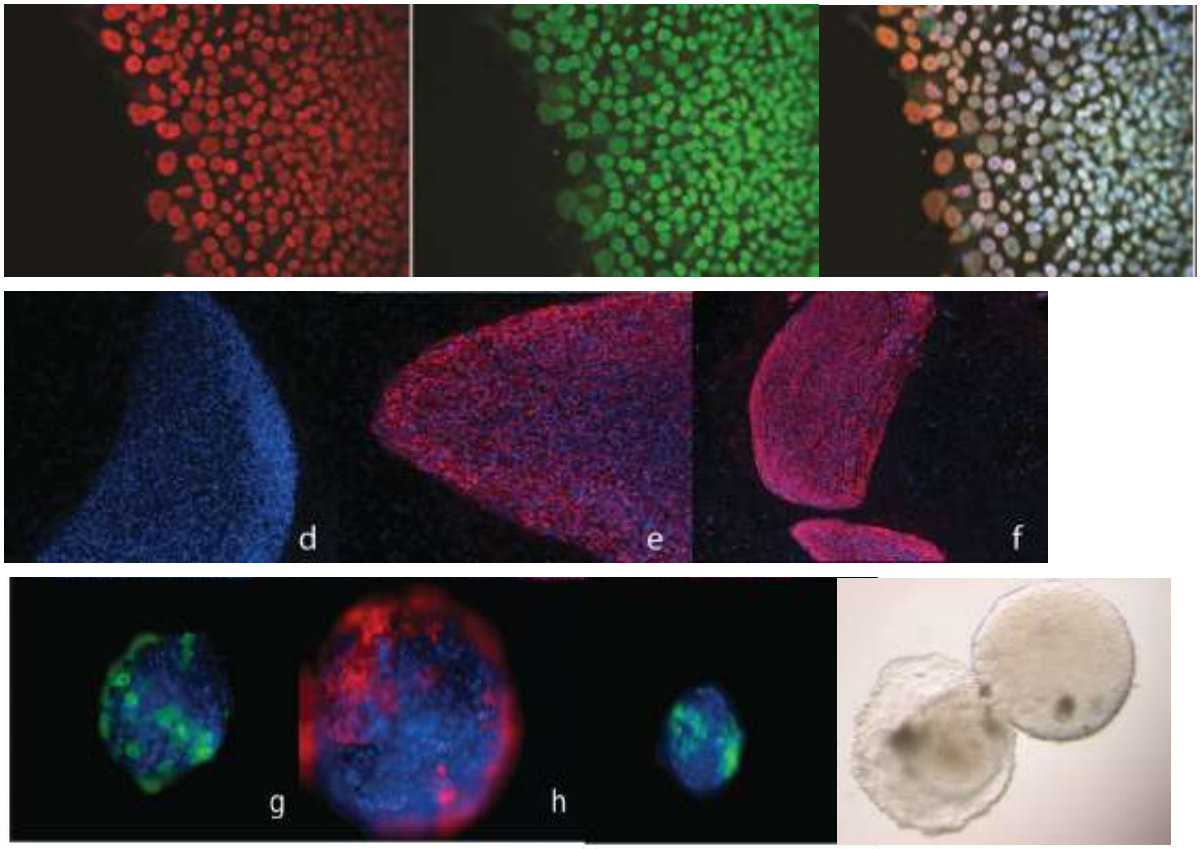
Characterization of hPS cells

- hiPS cells should express transcription factors; Oct-4, Sox-2, Nanog
- Supface antigen; SSES3/4, TRA1-60 and TRA1-81
- Negative for SSEA-1
- Embryoid bodies (EBs)
- Teratoma formation



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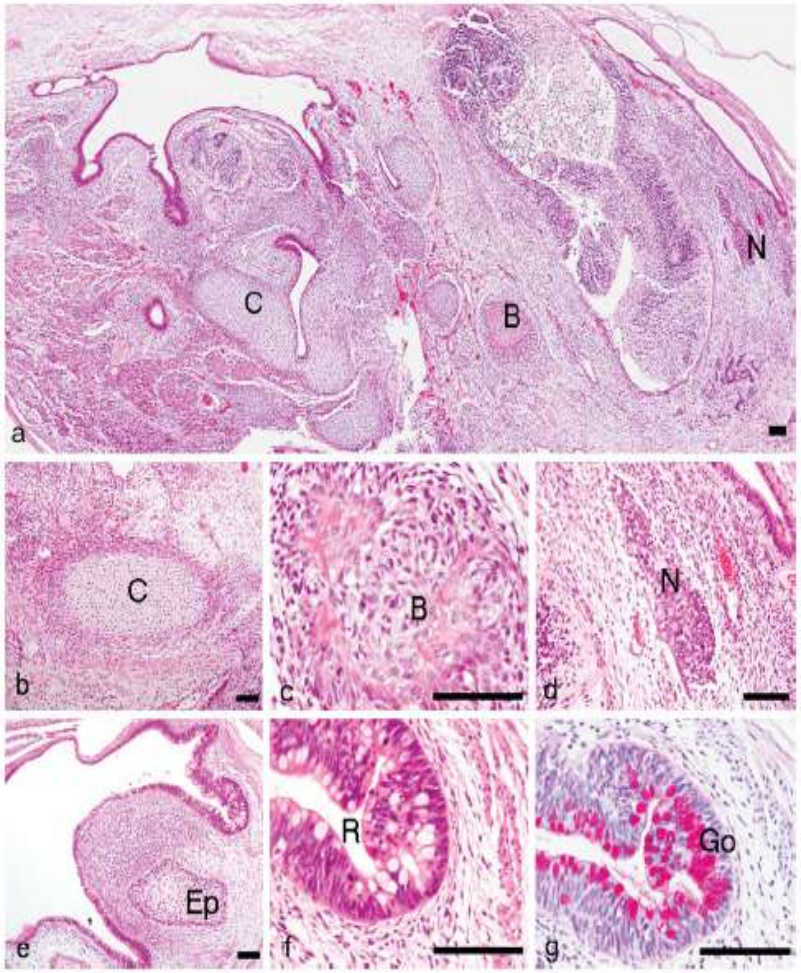
Characterization of hPS cells

***In Vivo* differentiation. hESC are injected subcutanously into SCID (severe combined immunodeficiency) mice and teratomas are formed of all three different tissue types, endo-, meso- and ectoderm.**



(a) mesodermal cartilage (C), bone formation (B) neural tissue (N). (b) Immature cartilage (C)surrounded bya perichondrium. (c)intramembranous type bone (B) and a ganglion. (d) Focal aggregation of cells resembling a ganglion(N). In (e)–(g), a cystic structure is shown lined by cuboidal to columnar epithelium. (e) Note an area of epithelium (Ep) showing squamous differentiation and (f) and (g) is the neighbouring smooth muscle. Respiratory type (R) goblet cells stain positive with Periodic acid Schiff

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Challenges for the use of hPS cells

- Teratoma formation
  - Improved protocols for differentiation
  - Removal of pluripotent cells
- GMP production
- Rejection of implanted cells
  - hES cell for cell therapy will require lifelong immunosuppression
  - Enginering of hES cells for tolerance
- Large scale production

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Regulations in Norway

- Since 2008 it has been legal to do research on surplus embryos fron IVF and on hES cells in Norway. (Bioteknologiloven)
- Surplus embryons can only be used for reseach if;
  - Reseach intended to improve methods and techniques for IVF
  - Reseach intended to improve methods and techniques for PGD
  - Achieve new knowledge regarding serious human diseases
- Not allowed to produce human embryos for reseach in Norway
- Research on embryos must not be made later than 14 days after the egg was fertilized. The time embryos are stored frozen is not included
- Research involving genetic changes that can be inherited in humans, is not allowed

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Acknowledgement

- Joel Glover
- Athina Samara
- Margareth Tamburstuen
- Hege Fjerdingstad
- Amila Topalovic
- Jean-Luc Boulland
- François Lambert
- Mrinal Joel
- Magne Sivertsen
- Nedim Kusamacic
- Orsolya Kreneisz
- Kobra Sultani
- Trang Huynh