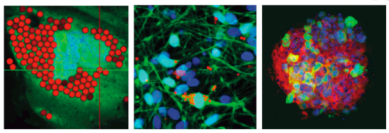
Principles of Stem Cell Biology



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

CAST
CANCER • STEM CELL INNOVATION CENTER

NORWEGIAN CENTER FOR STEM CELL RESEARCH

"Principles of Stem Cell Biology" is offered by the Norwegian Center for Stem Cell Research (www.stemcellnorway.org) and the Cancer Stem Cell Innovation Center (http://csc.rr-research.no/).

Lectures:

0830 – 0930	Basics of stem cell biology (Joel C. Glover)
0930 – 1030	Tumor stem cells (Stefan Krauss)
1030 – 1130	Stem cell epigenetics (Philippe Collas)
1130 – 1200	**Break/Lunch**
1200 – 1300	MicroRNAs and stem cell regulation (Jan Oxholm Gordeladze)
1300 – 1400	Current clinical applications of stem cells in Norway (Jan E. Brinchmann)
1400 – 1415	**Break**

Current stem cell research:

1415 – 1515	Presentations
1515 – 1530	**Break**
1530 – 1630	Presentations
1630	Concluding remarks

STEM CELLS - BASIC CONCEPTS

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http://stemcells.nih.gov/info/basics/
http://www.stemcellresearchfoundation.org
http://www.stemcell.no

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

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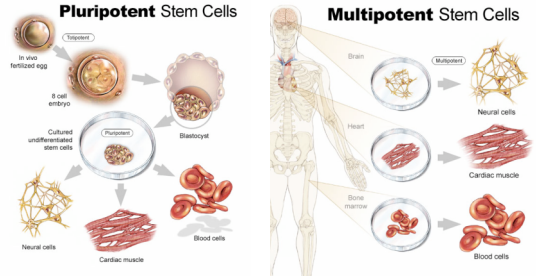
THE CONCEPT OF STEM CELL POTENCY

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



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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

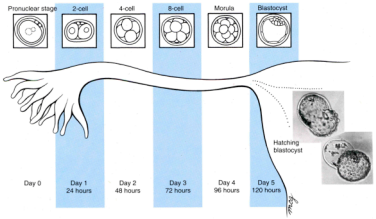

Fig. 1-11. Cleavage and transport down the oviduct. Fertilization occurs in the ampulla of the oviduct. During the first five days, the zygote undergoes cleavage as it travels down the oviduct and enters the uterus. On day 5, the blastocyst hatches from the zona pellucida and is then able to implant 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.

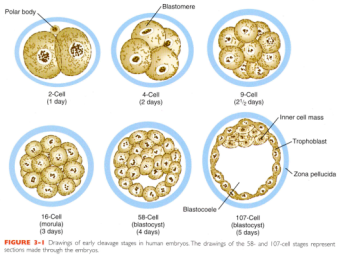

FIGURE 3-1 Drawings of early cleavage stages in human embryos. The drawings of the 58- and 107-cell stages represent sections made through the embryos.

fra Carlson, 3. utg.

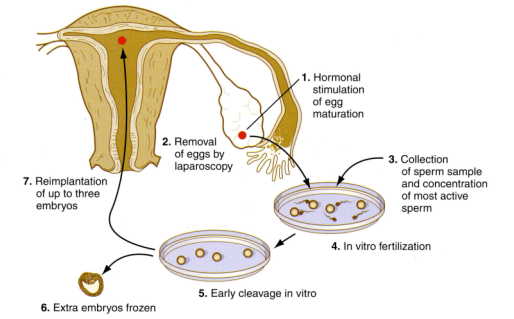

FIGURE 2-7 Schematic representation of a typical in vitro fertilization and embryo transfer procedure in humans.

In vitro fertilization – typical procedure

fra Carlson, 3. utg.

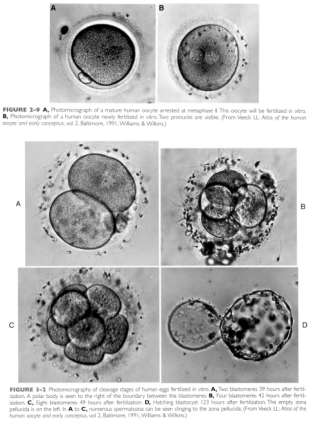

FIGURE 3-2 Photomicrographs of a mature human oocyte arrested at metaphase II. This oocyte will be fertilized in vitro. **B.** Photomicrograph of a human oocyte newly fertilized in vitro. Two pronuclei are visible. (From Veeck LL. Atlas of the human oocyte and early conceptus, vol 2, Baltimore, 1991, Williams & Wilkins.)

FIGURE 3-3 Photomicrographs of cleavage stages of human eggs fertilized in vitro. **A.** Two blastomeres 29 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 48 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.

www.sciencemag.org SCIENCE VOL 282 6 NOVEMBER 1998

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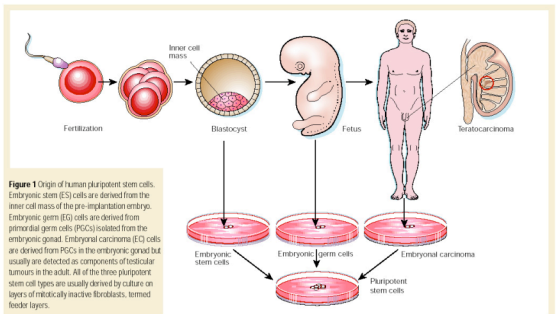


Figure 1 Origin of human pluripotent stem cells. Embryonic stem (ES) cells are derived from the inner cell mass of the pre-implantation embryo. Embryonic germ (EG) cells are derived from primordial germ cells (PGCs) isolated from the embryonic gonad. Embryonal carcinoma (EC) cells are derived from PGCs 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*,†, Elisabet Andersson*,†, Lachlan H. Thompson‡, Marie E. Jönsson‡, Josephine B. Hebsgaard‡, Evanthia Nanou‡, Zhanna Alekseenko§, Ulrika Marklund§, Susanna Kjellander*, Nikolaos Volakakis*, Outi Hovatta*, Abdeljabbar El Manira§, Anders Björklund‡, Thomas Perlmann*,¶,‖, and Johan Ericson*,‖

*The Ludwig Institute for Cancer Research and ‡Departments of Cell and Molecular Biology, §Neuroscience, and ¶Biosciences and Nutrition, Karolinska Institutet, 17177 Stockholm, Sweden; and †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

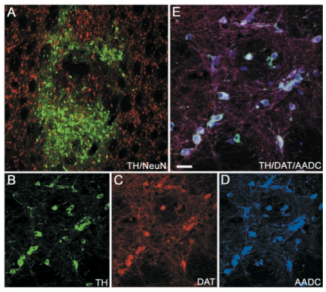


Fig. 1. Immunohistochemical staining of a graft 16 weeks after implantation of a low concentration (1,000–2,000 cells per μl) of D3 ES cells into adult 6-OHDA lesioned striatum. Numerous TH-positive neurons were found within the graft (*A* and *B*, green). All TH-positive profiles coexpressed the neuronal 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

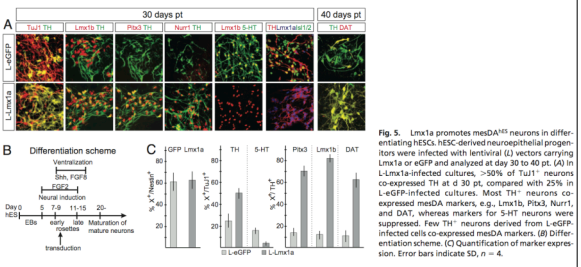


Fig. 5. Lmx1a promotes mesDA^{185} neurons in differentiating hESCs. hESC-derived neuroepithelial progenitors were infected with lentiviral (*L*) vectors carrying Lmx1a or eGFP and analyzed at day 30 to 40 pt. (*A*) In L-Lmx1a-infected cultures, >50% of Tuj1$^+$ neurons co-expressed TH at d 30 pt, compared 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

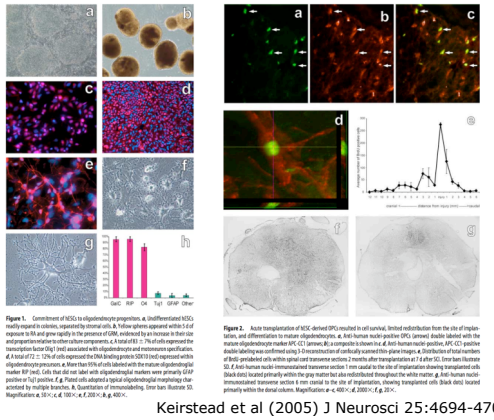
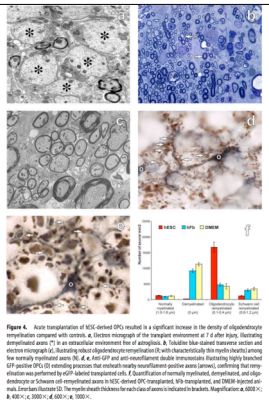


Figure 1. Commitment of hESCs to oligodendrocyte progenitors. **a**, Undifferentiated hESCs readily expand in colonies, separated by stromal cells. **b**, Yellow spheres appeared within 5 d of exposure to RA and grew rapidly in the presence of GDNF, evidenced by an increase in their size and proportion relative to other culture components. **c**, A total of 83 ± 7% of cells expressed the transcription factor Olig2 (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**, 10×; **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



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

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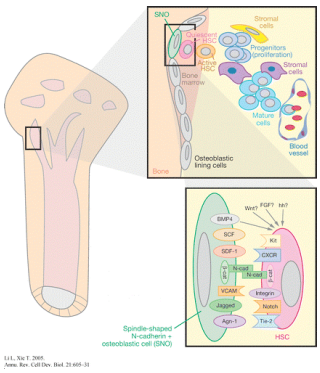
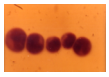
HISTORICAL PERSPECTIVE

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)

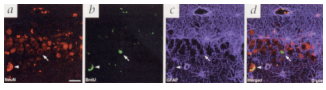




hHSCs in vitro

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.



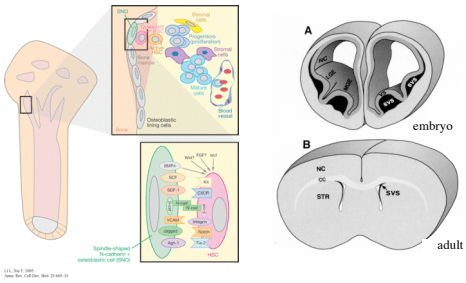
NATURE | VOL 412 | 16 AUGUST 2001 | www.nature.com

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

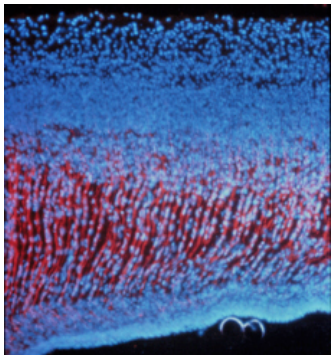
Rodney L. Rietze*, Helen Valcanis†, Gordon F. Brooker*, Tim Thomas*, Anne K. Voss* & Perry F. Bartlett*

** The Walter and Eliza Hall Institute of Medical Research, Royal Parade, Parkville, Victoria 3050, Australia*
† Howard Florey Institute, University of Melbourne, Parkville, Victoria 3010, Australia

CONCEPT OF THE STEM CELL "NICHE"



Somatic stem cells: Remnants of embryogenesis?



"Stages" of development: proliferation versus differentiation

Stem cell → Progenitor cell → Precursor

pluripotent → unipotent (?)

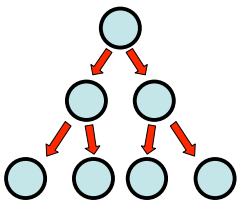
high proliferation → low proliferation

low differentiation → high differentiation

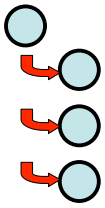
Signals for proliferation ⇄ Signals for differentiation

Role of the microenvironment

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*.

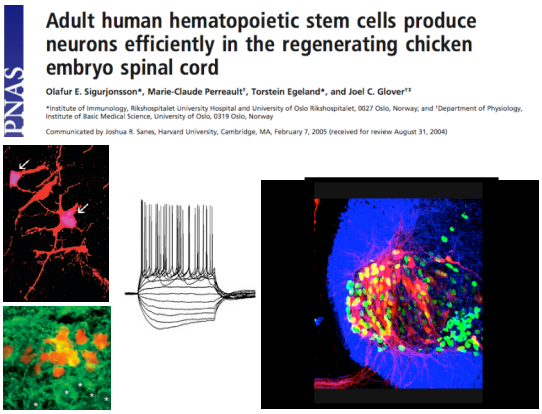
Somatic stem cells: examples of specific uses

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 Cell

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

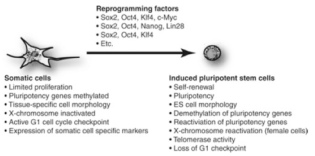


Figure 1 Reprogramming of somatic cells to induced pluripotent stem (iPS) cells. Examples of reprogramming factors are provided along with the characteristics of a typical starting somatic cell and those of an iPS cell

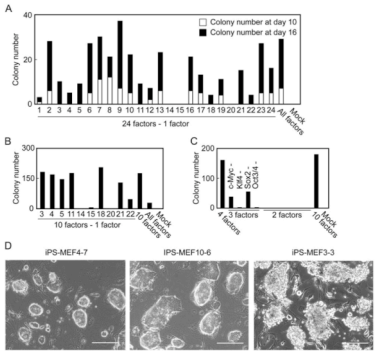


Figure 2. Narrowing down the Candidate Factors

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

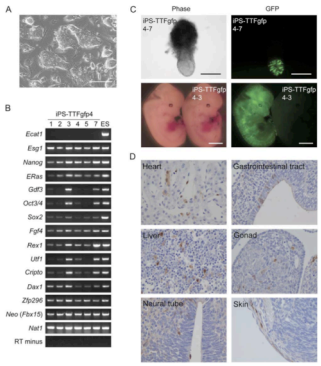


Figure 6. Characterization of iPS Cells Derived from Adult Mouse Tail-Tip Fibroblasts

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

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

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

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

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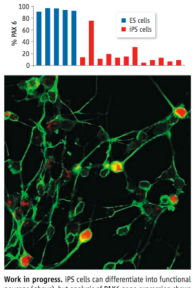
RESPONSE TO DIFFERENTIATION SIGNALS



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