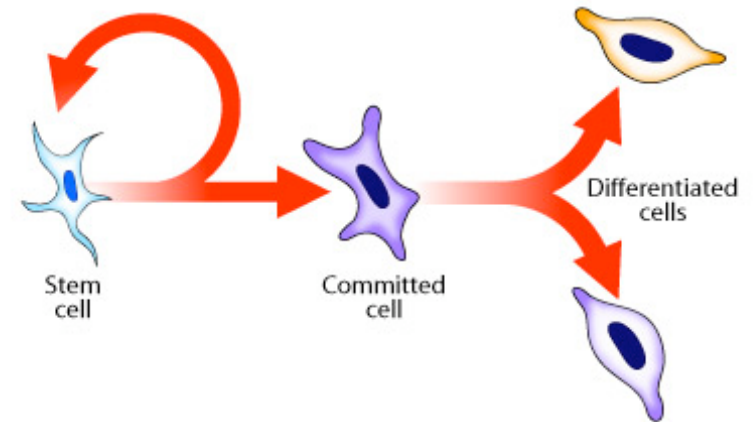
Stem Cell Biology GE4276

- Lecture 1: Introduction to stem cells
- Lecture 2: Embryonic stem cells I
- Lecture 3: Embryonic stem cells II
- Lecture 4: Adult or “tissue specific” stem cells
- Lecture 5: Applications of stem cells in medicine
- Lecture 6: Applications of stem cells in science
- Lecture 7: Induced pluripotent stem cells (iPS)
- Lecture 8: Cancer stem cells



Todays Lecture…..

1. The establishment of mouse (1981) and then human (1998) Embryonic stem cell lines

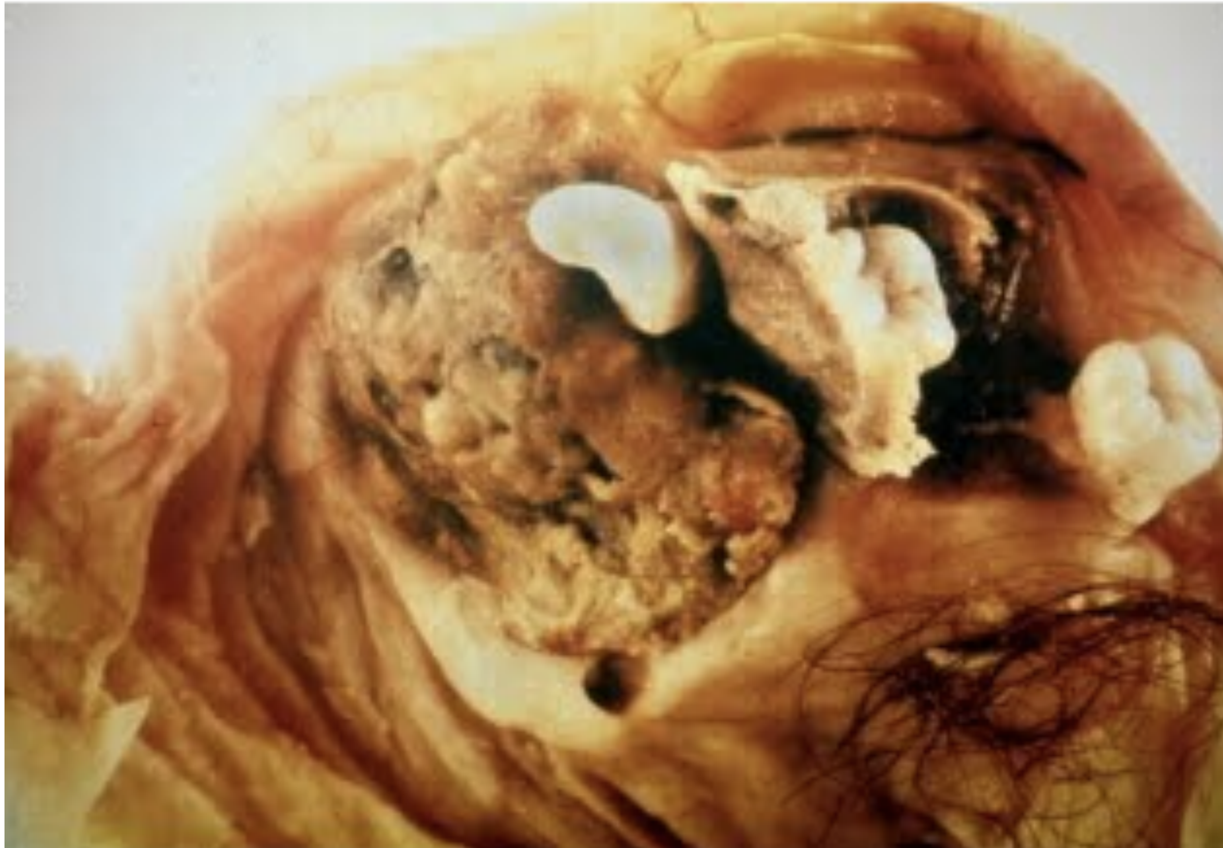
2. The genes required for embryonic stem cell pluripotency

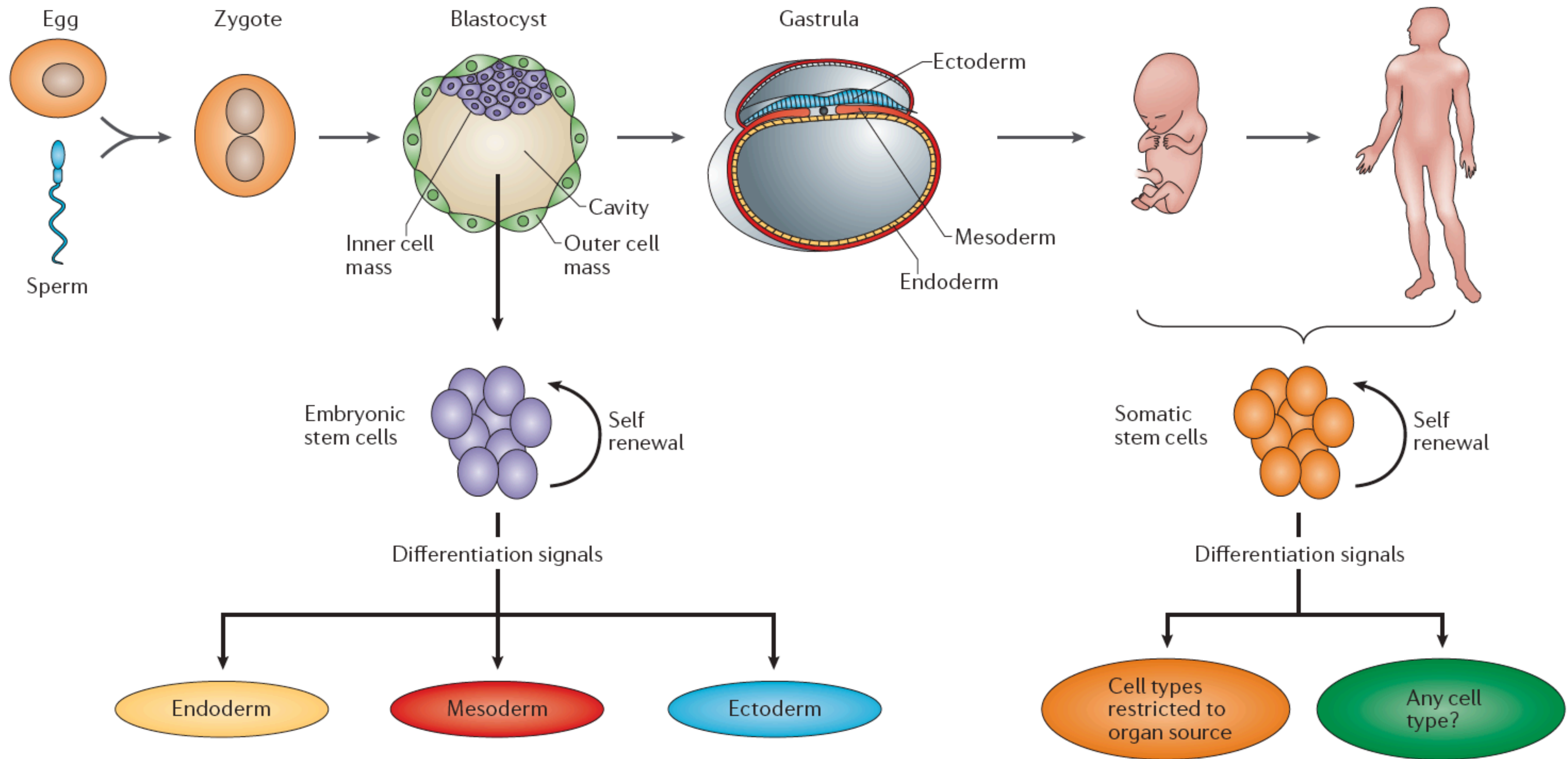
3. The “core transcriptional network of ES cells”

A history of embryonic stem cell research
Prior to the isolation of mouse embryonic stem cells in 1981

1954	Stevens - Description of testicular “teratoma” in 1% of a stain of mice (129).
1961	Propagation and differentiation of Embryonic carcinoma (EC) cells in vitro
1964	Pierce – demonstration that a single cell from a teratoma injected into a donor mouse would develop a new teratoma. This gave rise to the idea that these cells were “pluripotent stem cells” in that they can both self renew and they can give rise to a whole range of different cell types
1980	Solter – Transplantable teratomas containing EC cells can be derived by grafting early post-implantation mouse embryos into host mice.

A Teratoma





Egg
Sperm
Zygote
Blastocyst
Inner cell mass
Cavity
Outer cell mass
Gastrula
Ectoderm
Mesoderm
Endoderm
Embryonic stem cells
Self renewal
Differentiation signals
Endoderm
Mesoderm
Ectoderm
Somatic stem cells
Self renewal
Differentiation signals
Cell types restricted to organ source
Any cell type?

Establishment in culture of pluripotential cells from mouse embryos

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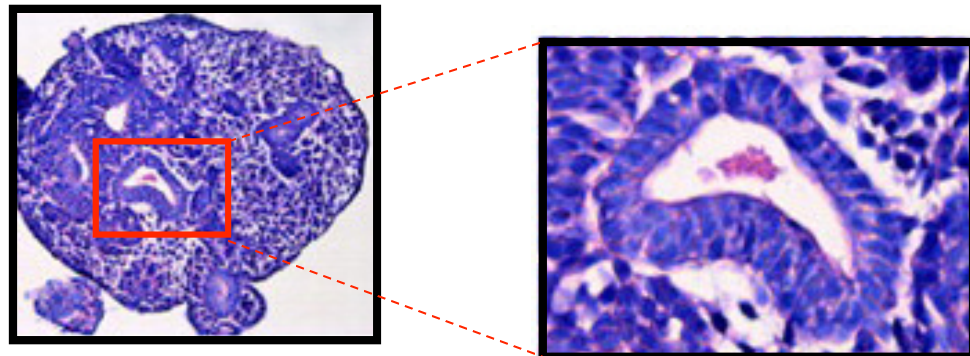
Pluripotential cells are present in a mouse embryo until at least an early post-implantation stage, as shown by their ability to take part in the formation of chimaeric animals[1] and to form teratocarcinomas[2]. Until now it has not been possible to establish progressively growing cultures of these cells *in vitro*, and cell lines have only been obtained after teratocarcinoma formation *in vivo*. We report here the establishment in tissue culture of pluripotent cell lines which have been isolated directly from *in vitro* cultures of mouse blastocysts. These cells are able to differentiate either *in vitro* or after innoculation into a mouse as a tumour *in vivo*. They have a normal karyotype.



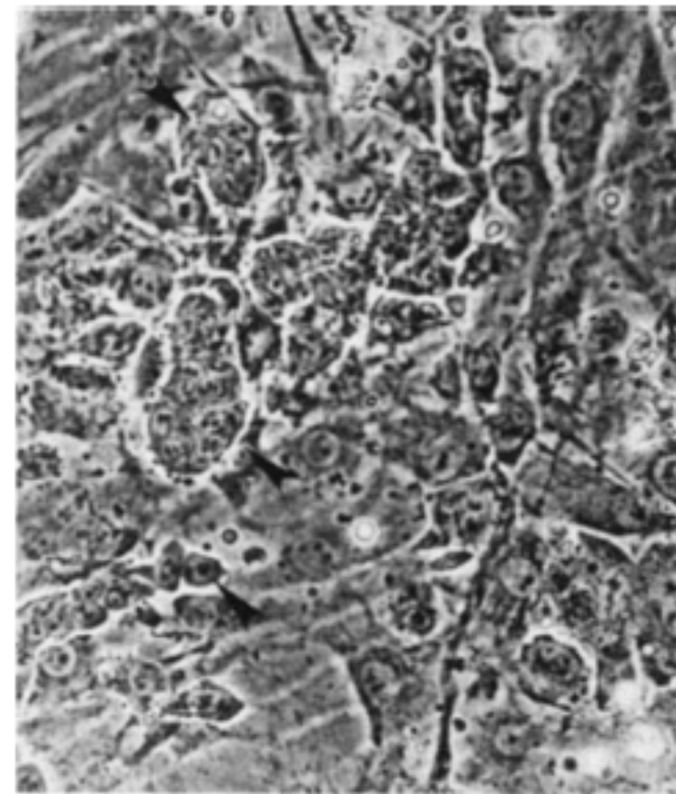
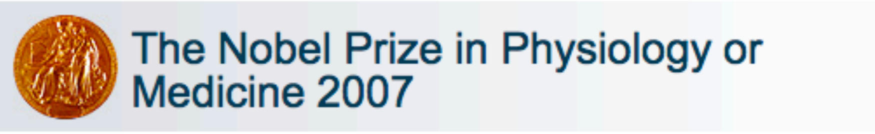


Fig. 1 Groups of pluripotential embryo cells (arrowed) growing in monolayer culture on a background of mitomycin C-inhibited STO cells. The isolation of a definite cell line from a blastocyst takes only ~3 weeks and the pluripotential cell colonies are visible within 5 days of passage. We have had 30% yield of lines from blastocysts in one experiment. Two of the lines have been rigorously cloned by single-cell isolation but most were only colony-picked—this makes no difference.



The Nobel Prize in Physiology or Medicine 2007

"for their discoveries of principles for introducing specific gene modifications in mice by the use of embryonic stem cells"

Photo: U. Montan

Mario R. Capecchi

1/3 of the prize

USA

University of Utah
Salt Lake City, UT, USA;
Howard Hughes Medical Institute

b. 1937
(in Italy)

Photo: U. Montan

Sir Martin J. Evans

1/3 of the prize

United Kingdom

Cardiff University
Cardiff, United Kingdom

b. 1941

Photo: U. Montan

Oliver Smithies

1/3 of the prize

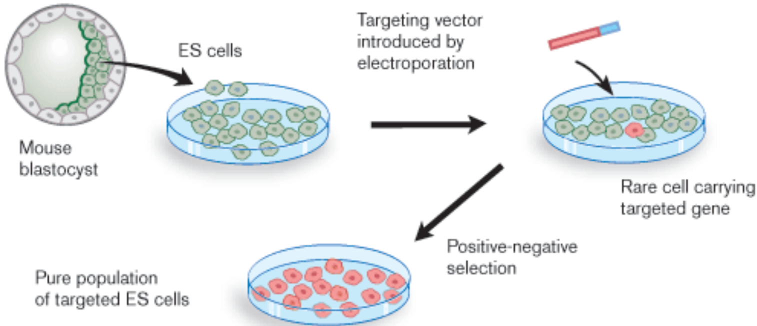
USA

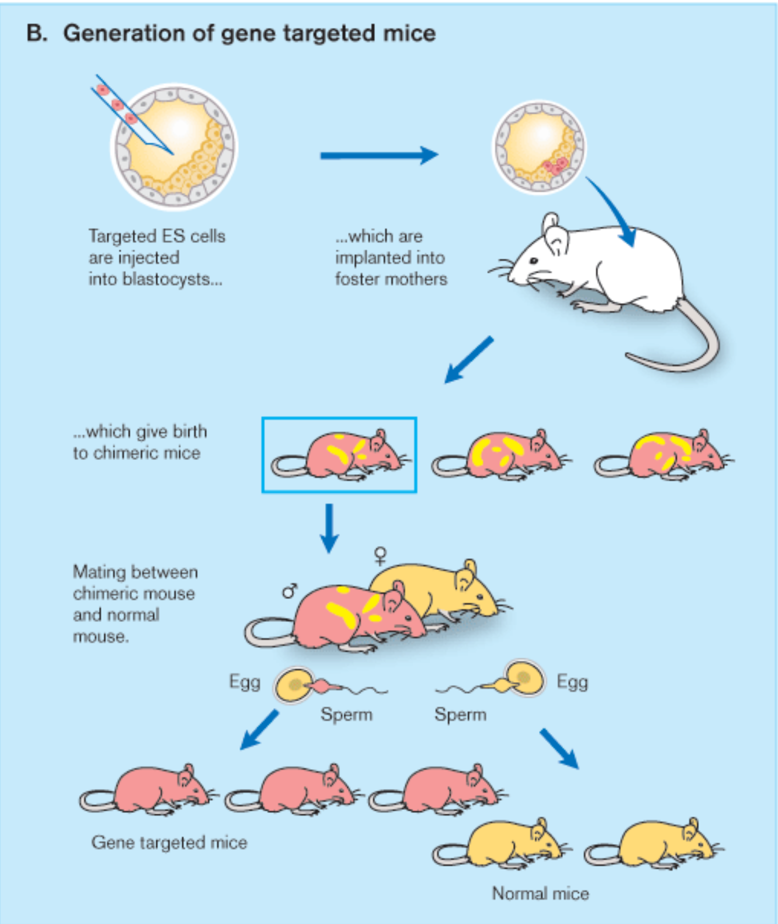
University of North Carolina at Chapel Hill
Chapel Hill, NC, USA

b. 1925
(in United Kingdom)

Titles, data and places given above refer to the time of the award.

Photos: Copyright © The Nobel Foundation





ANNIKA RÖHL

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.

SCIENCE VOL 282 6 NOVEMBER 1998

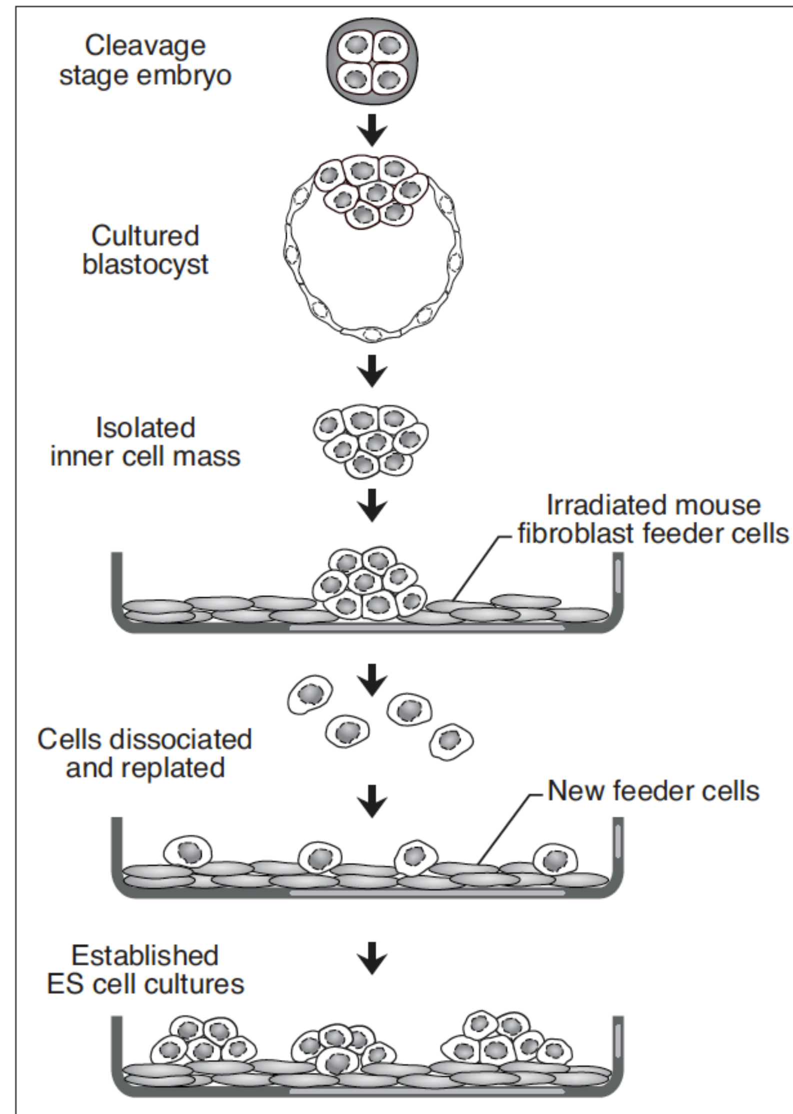


Figure 1. Derivation of human ES cell lines. *Human blastocysts were grown from cleavage-stage embryos produced by in vitro fertilization. ICM cells were separated from trophectoderm by immunosurgery, plated onto a fibroblast feeder substratum in medium containing fetal calf serum. Colonies were sequentially expanded and cloned.*

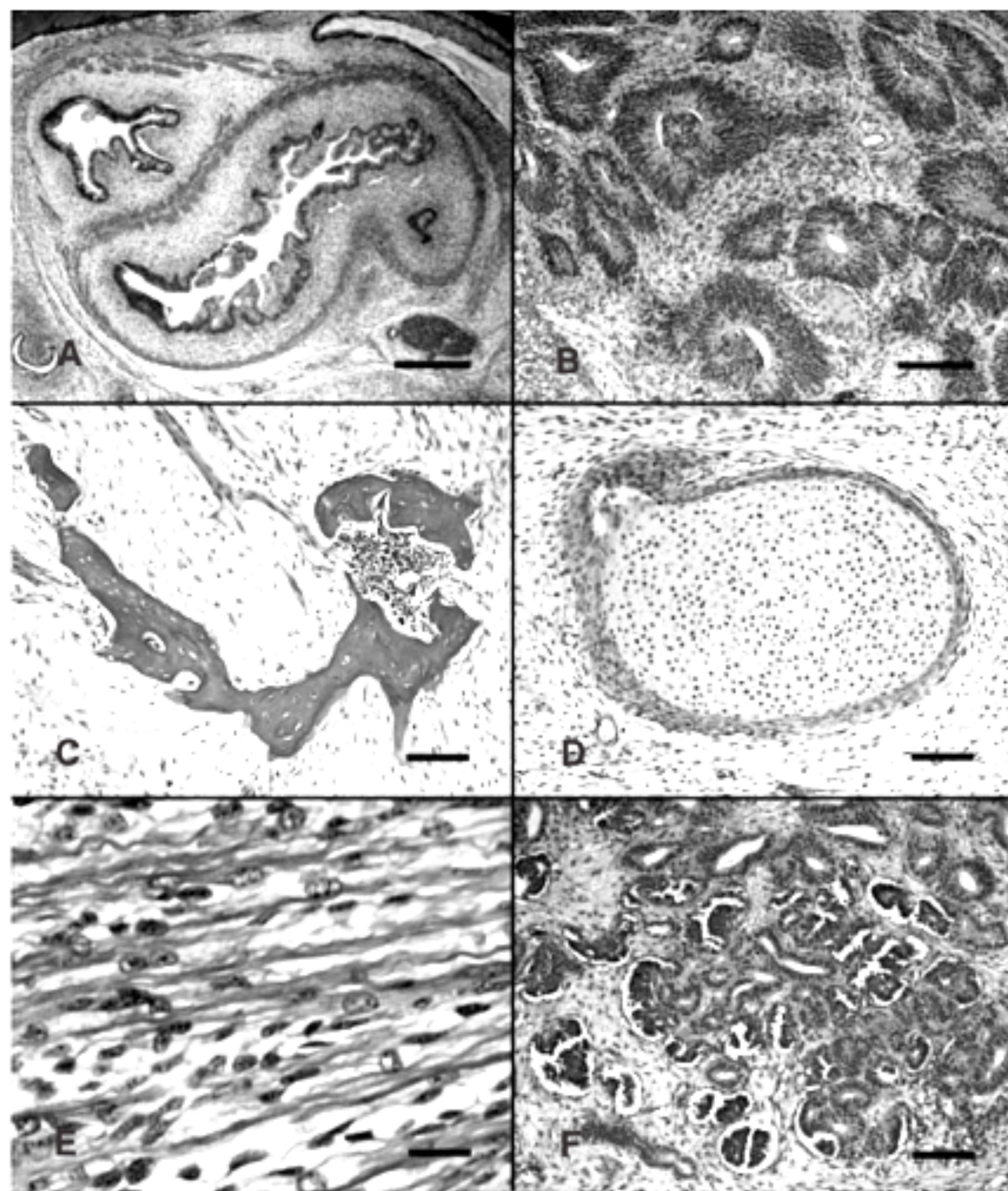
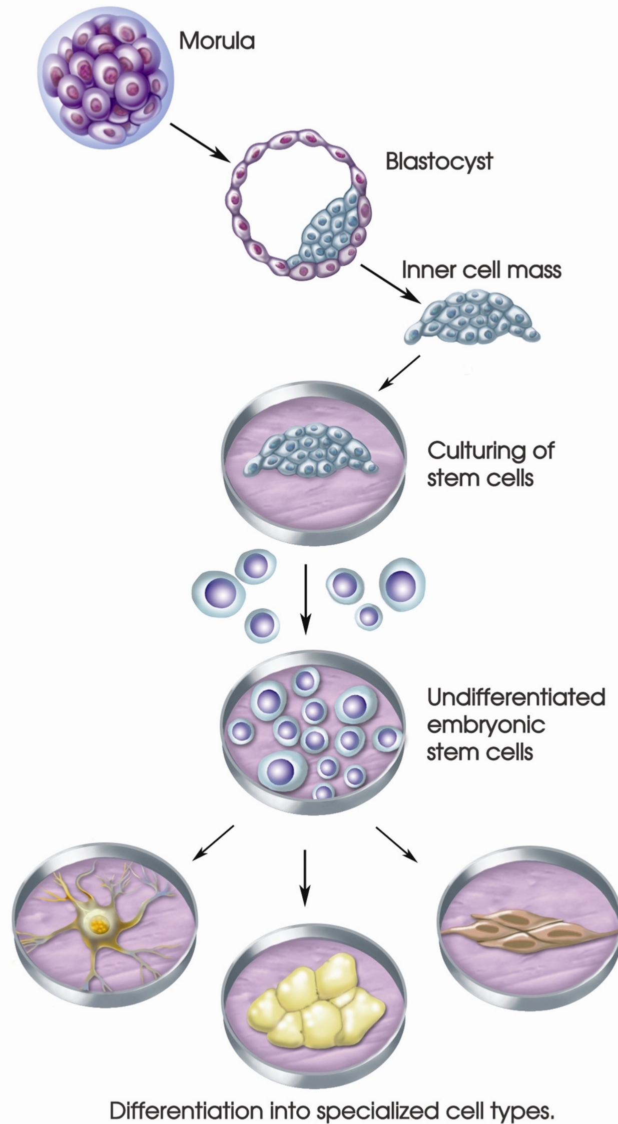


Fig. 4. Teratomas formed by the human ES cell lines in SCID-beige mice. Human ES cells after 4 to 5 months of culture (passages 14 to 16) from about 50% confluent six-well plates were injected into the rear leg muscles of 4-week-old male SCID-beige mice (two or more mice per cell line). Seven to eight weeks after injection, the resulting teratomas were examined histologically. (**A**) Gutlike structures. Cell line H9. Scale bar, 400 μm. (**B**) Rosettes of neural epithelium. Cell line H14. Scale bar, 200 μm. (**C**) Bone. Cell line H14. Scale bar, 100 μm. (**D**) Cartilage. Cell line H9. Scale bar, 100 μm. (**E**) Striated muscle. Cell line H13. Scale bar, 25 μm. (**F**) Tubules interspersed with structures resembling fetal glomeruli. Cell line H9. Scale bar, 100 μm.

Table 1 | **The best-characterized ESC markers***

Undifferentiated state marker	Mouse	Human
Cell-surface and nuclear antigens		
SSEA1[‡]	+	–
SSEA3/4[‡]	–	+
TRA1–60/81[§]	–	+
TRA2-54	–	+
GCTM-2[§]	–	+
TG343[§]	?	+
TG30[‖]	?	+
CD9[‖]	+	+
CD133/prominin	+	+
OCT4	+	+
NANOG	+	+
SOX2	+	+
Enzymatic activities		
AP	+	+
Telomerase	+	+
In vitro *culture requirements*		
Feeder-cell dependent	+	+
LIF dependent	+	–
FGF4	+	–
Growth characteristics		
Ability to form trophoblast	–	+
Teratoma formation *in vivo*	+	+
Growth characteristics *in vitro*	Form tight, rounded, multi-layer clumps; can form EBs	Form flat, loose aggregates; can form EBs
Ability to form germ cells *in vitro*	+	NR

Embryonic Stem Cells (ES or ESC)



- **Can grow indefinitely in the primitive uncommitted state**
- **Can differentiate and give rise to ALL tissues of the human/mouse body**

KEY CHALLENGE:
How do we harness their power for medicine?

Assaying Stem Cell Potential

Developmental potential assays

1) In vitro differentiation
   - Culture dish

2) Teratoma formation
   - Grafted into animal

3) Chimera formation
   - Injected into blastocyst

Smith, Annu Rev Cell Dev Biol 2001.

Cell, Vol. 113, 643–655, May 30, 2003, Copyright ©2003 by Cell Press

Functional Expression Cloning of Nanog, a Pluripotency Sustaining Factor in Embryonic Stem Cells

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Summary

Embryonic stem (ES) cells undergo extended proliferation while remaining poised for multilineage differentiation. A unique network of transcription factors may characterize self-renewal and simultaneously suppress differentiation. We applied expression cloning in mouse ES cells to isolate a self-renewal determinant. Nanog is a divergent homeodomain protein that directs propagation of undifferentiated ES cells. Nanog mRNA is present in pluripotent mouse and human cell lines, and absent from differentiated cells. In preimplantation embryos, Nanog is restricted to founder cells from which ES cells can be derived. Endogenous Nanog acts in parallel with cytokine stimulation of Stat3 to drive ES cell self-renewal. Elevated Nanog expression from transgene constructs is sufficient for clonal expansion of ES cells, bypassing Stat3 and maintaining Oct4 levels. Cytokine dependence, multilineage differentiation, and embryo colonization capacity are fully restored upon transgene excision. These findings establish a central role for Nanog in the transcription factor hierarchy that defines ES cell identity.

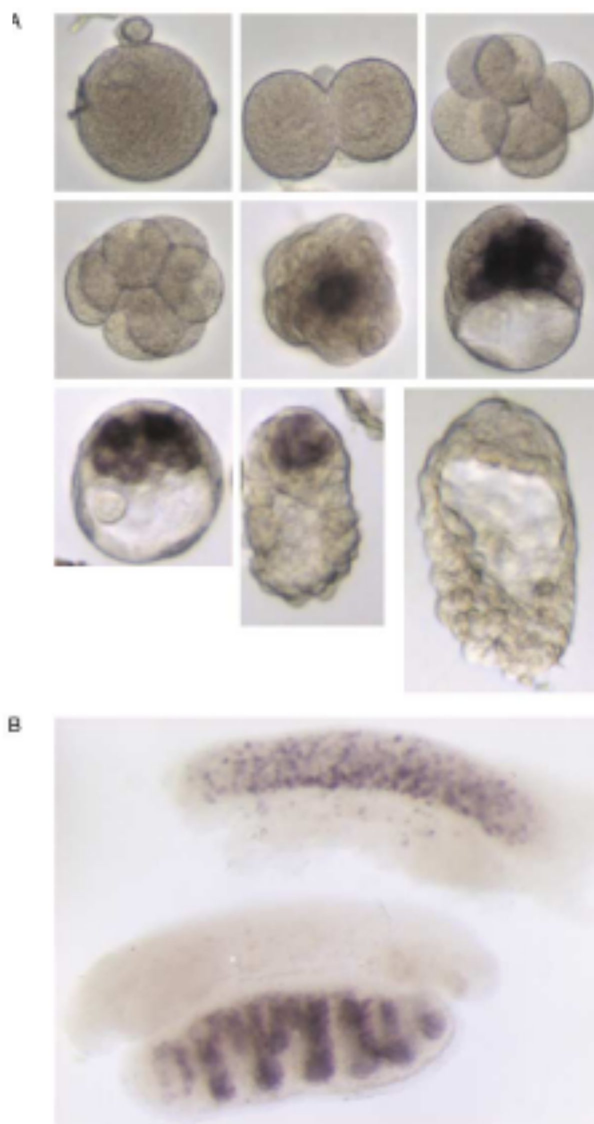
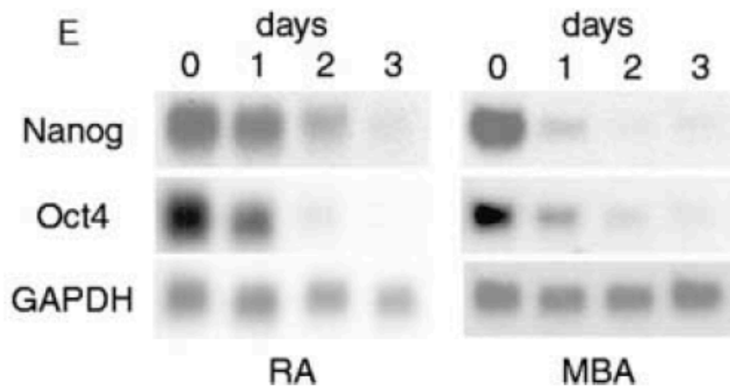


Figure 4. Expression of Nanog In Vivo

(A) Preimplantation embryos. Top: embryos of 1, 2, and 6 cells. Middle: 8-cell embryo, late morula and early blastocyst. Bottom: blastocysts at expanded, hatched, and implanting stages. Embryos were hybridized in the same reaction and stained for the same time. All panels are shown at equal magnification.

(B) E11.5 genital ridges from female (top) and male (bottom) embryos. Hybridization appears localized to the primordial germ cells overlying the somatic tissue.

Nanog is down-regulated during differentiation and is not expressed in differentiated cells



Some of the key molecules required for "Pluripotency"

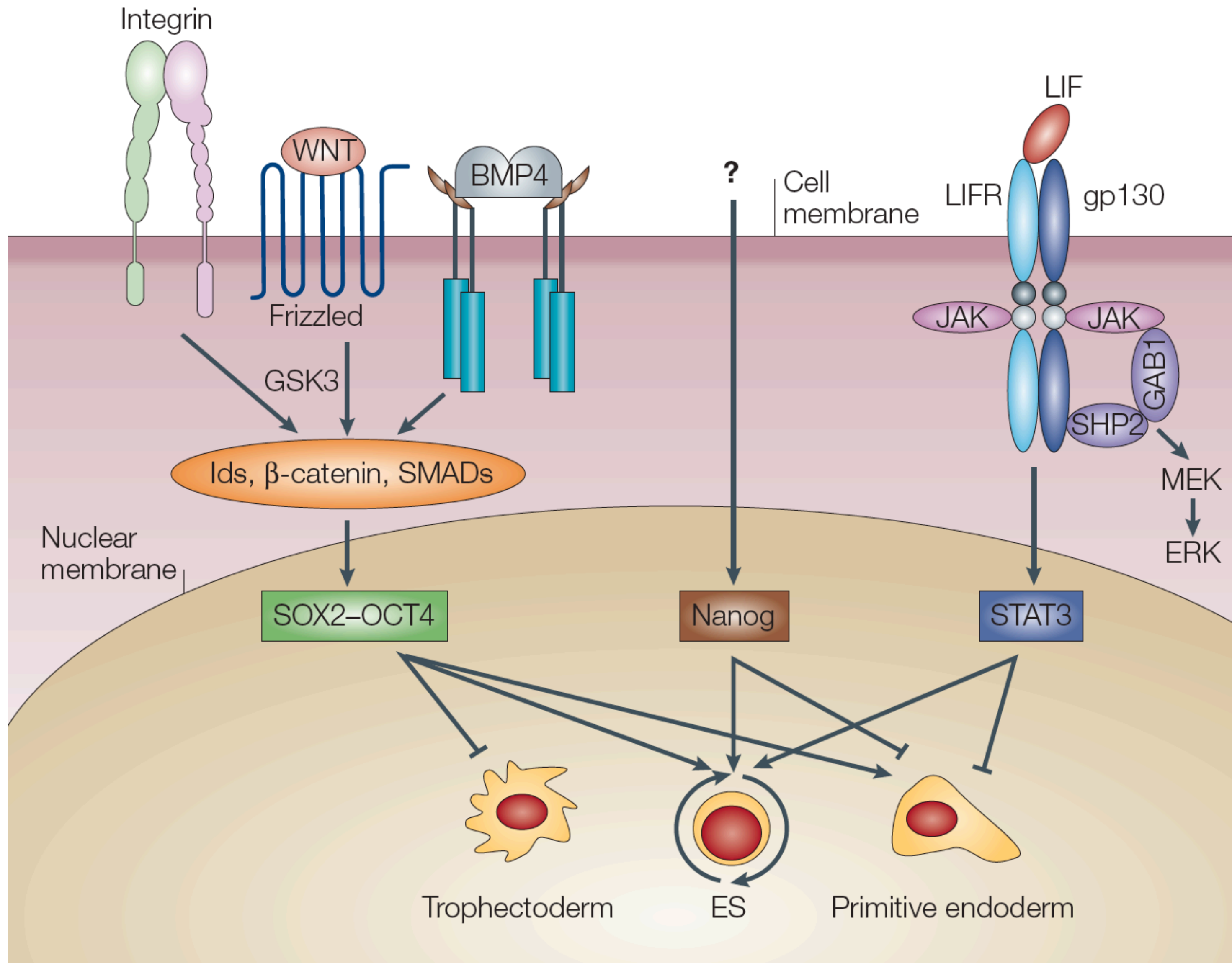
Key Extrinsic Signaling Molecules

- LIF
- FGF signaling
- TGF Beta signaling
- BMP signaling
- WNT signaling

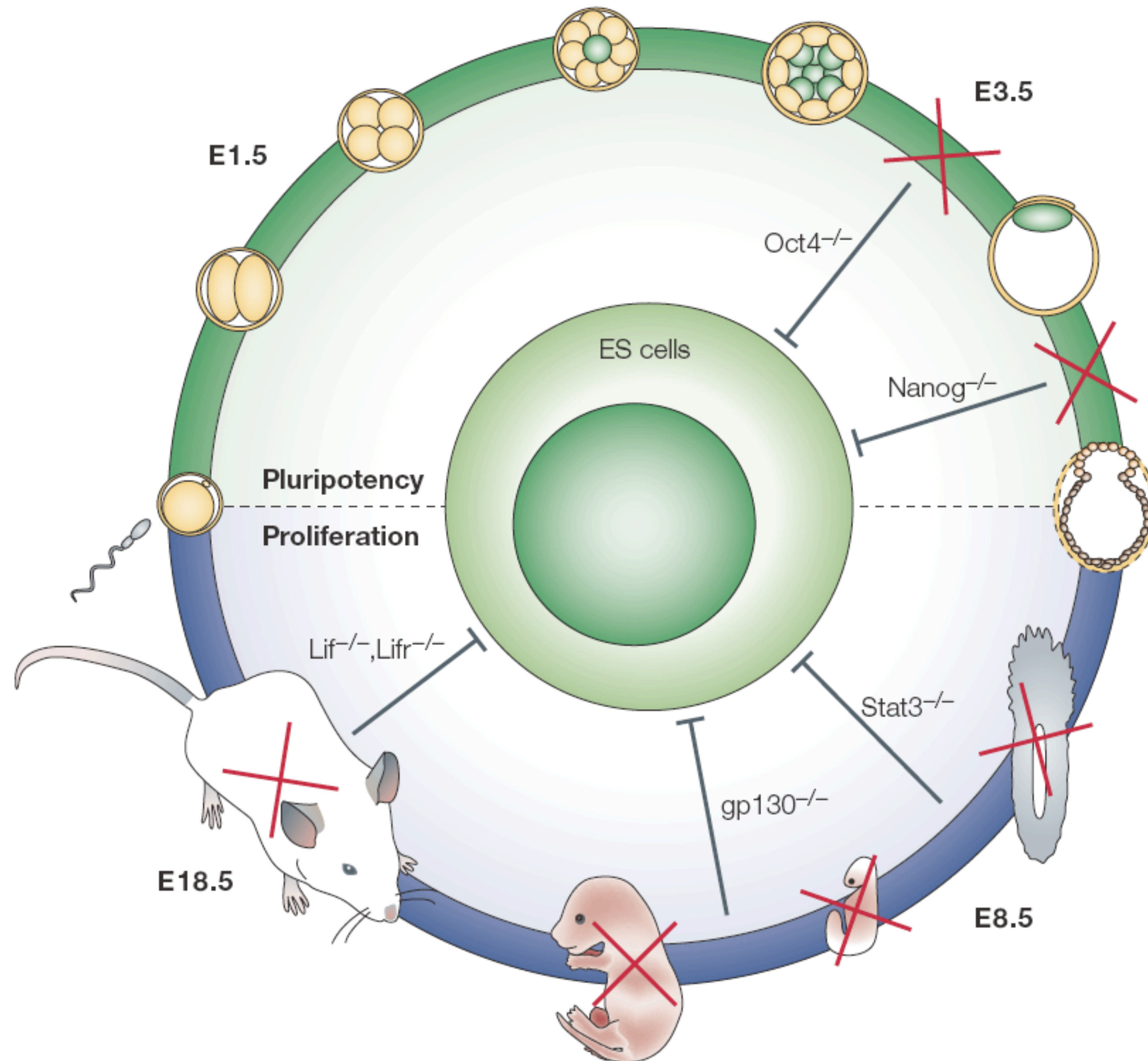
Key Intrinsic Molecules (Transcription factors)

- Oct4
- Nanog
- Sox2
- Stat3

Stem Cell Fate is Regulated by both Extrinsic and Intrinsic Factors



The phenotypic effects of gene-targeting experiments in mice (Mouse Genetics)



Stem Cell Fate is Regulated by both Extrinsic and Intrinsic Factors

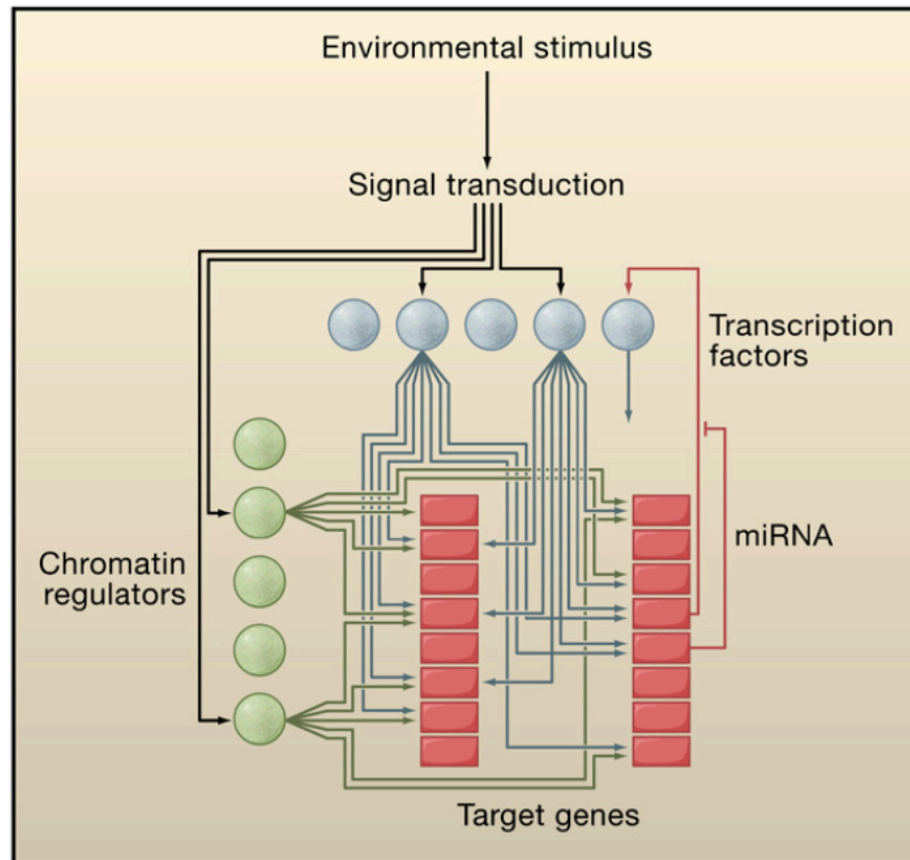


Figure 4. Pluripotency and the Transcriptional Regulatory Circuitry
Cartoon showing hypothetic connections between signal transduction pathways, transcription factors (blue balls), chromatin regulators (green balls), and their target genes (orange squares) to form an image of transcriptional regulatory circuitry. Some target genes produce miRNAs, which function at posttranscriptional levels.

Cell, Vol. 122, 947–956, September 23, 2005, Copyright ©2005 by Elsevier Inc. DOI 10.1016/j.cell.2005.08.020

Core Transcriptional Regulatory Circuitry in Human Embryonic Stem Cells

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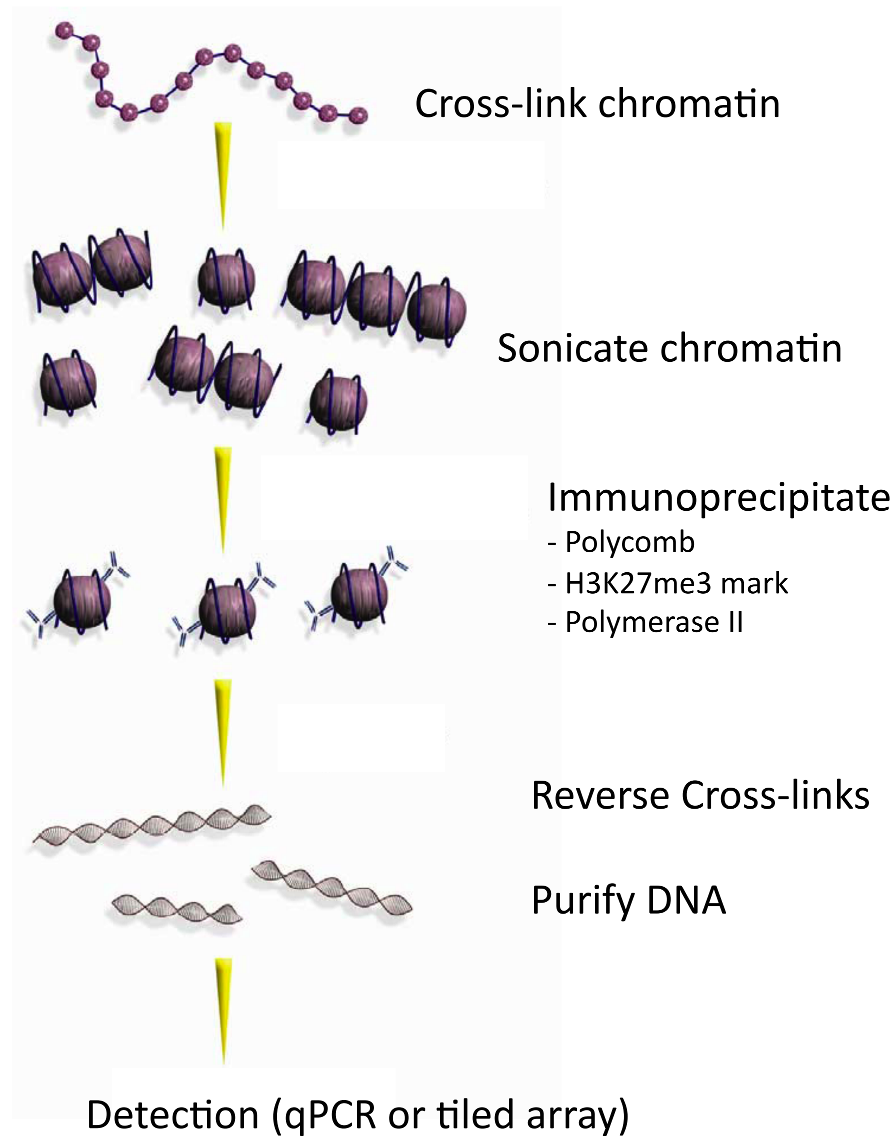
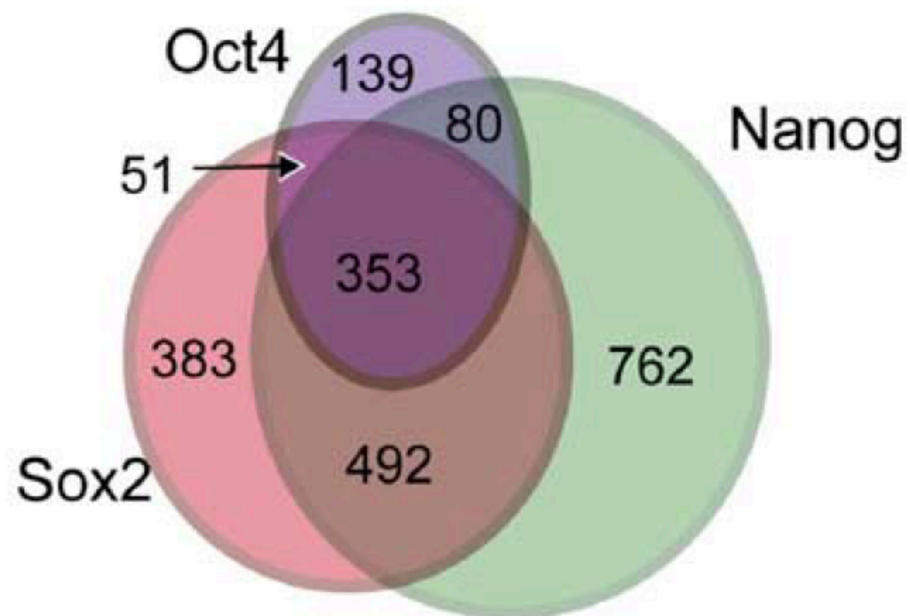
Summary

The transcription factors OCT4, SOX2, and NANOG have essential roles in early development and are required for the propagation of undifferentiated embryonic stem (ES) cells in culture. To gain insights into transcriptional regulation of human ES cells, we have identified OCT4, SOX2, and NANOG target genes using genome-scale location analysis. We found, surprisingly, that OCT4, SOX2, and NANOG co-occupy a substantial portion of their target genes. These target genes frequently encode transcription factors, many of which are developmentally important homeodomain proteins. Our data also indicate that OCT4, SOX2, and NANOG collaborate to form regulatory circuitry consisting of autoregulatory and feedforward loops. These results provide new insights into the transcriptional regulation of stem cells and reveal how OCT4, SOX2, and NANOG contribute to pluripotency and self-renewal.

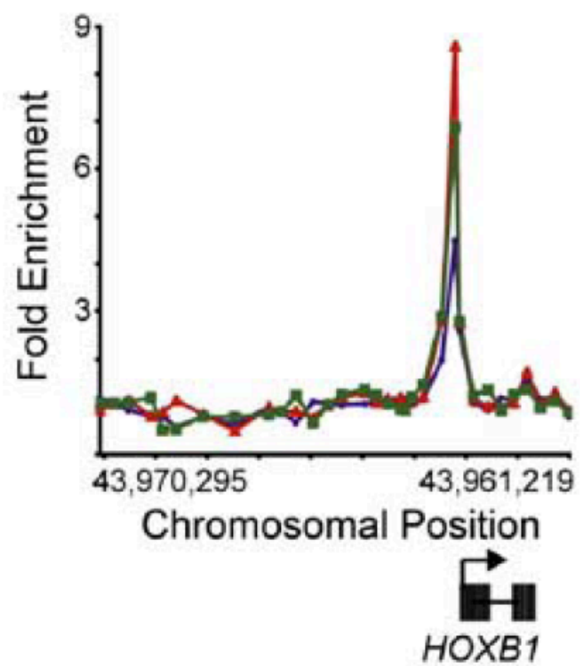
Chromatin Immunoprecipitation or "ChIP"

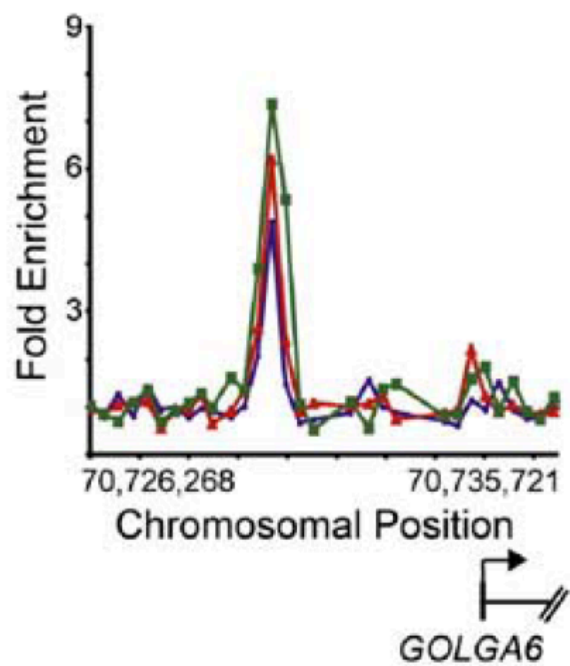


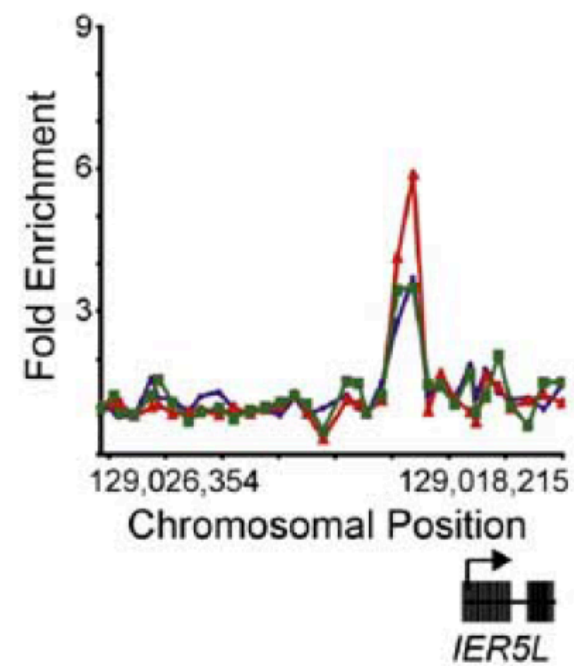
A

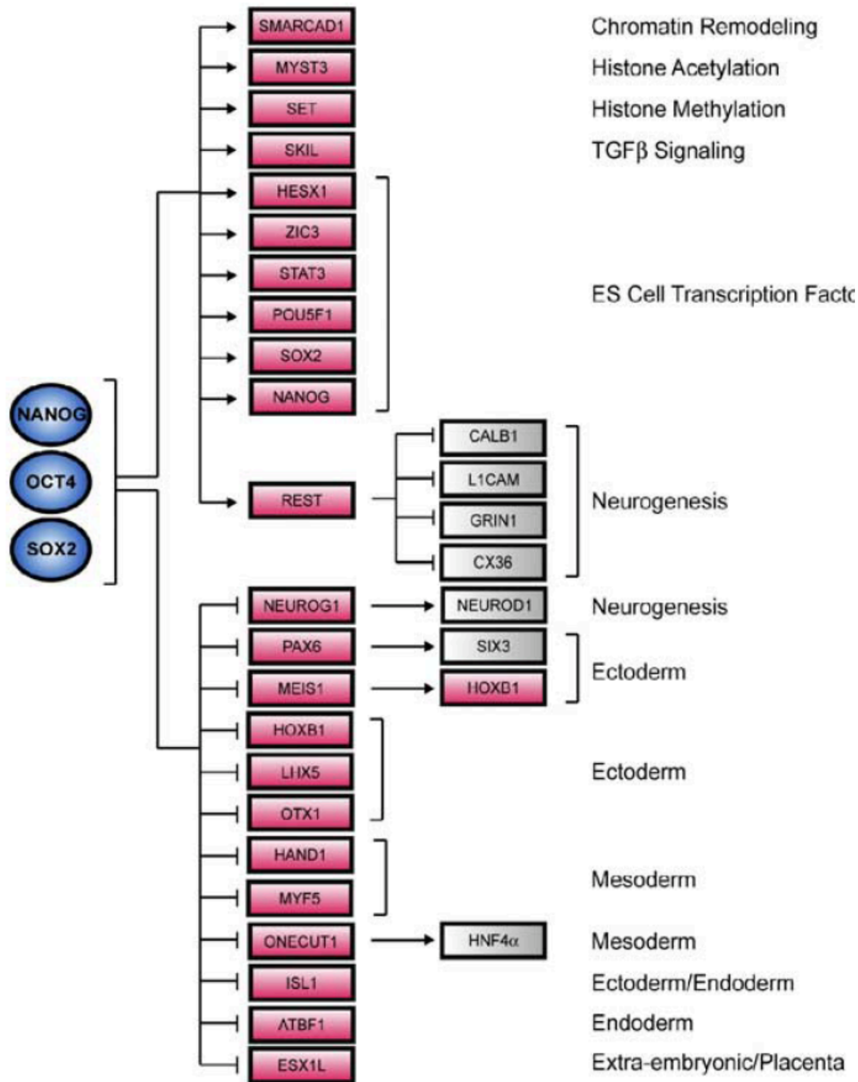


B





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SOX2
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MYST3
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SKIL
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ZIC3
STAT3
POU5F1
SOX2
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REST
CALB1
L1CAM
GRIN1
CX36
NEUROG1
NEUROD1
PAX6
SIX3
MEIS1
HOXB1
HOXB1
LHX5
OTX1
HAND1
MYF5
ONECUT1
HNF4α
ISL1
ATBF1
ESX1L
Chromatin Remodeling
Histone Acetylation
Histone Methylation
TGFβ Signaling
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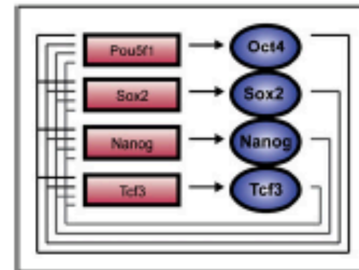
Mesoderm
Ectoderm/Endoderm
Endoderm
Extra-embryonic/Placenta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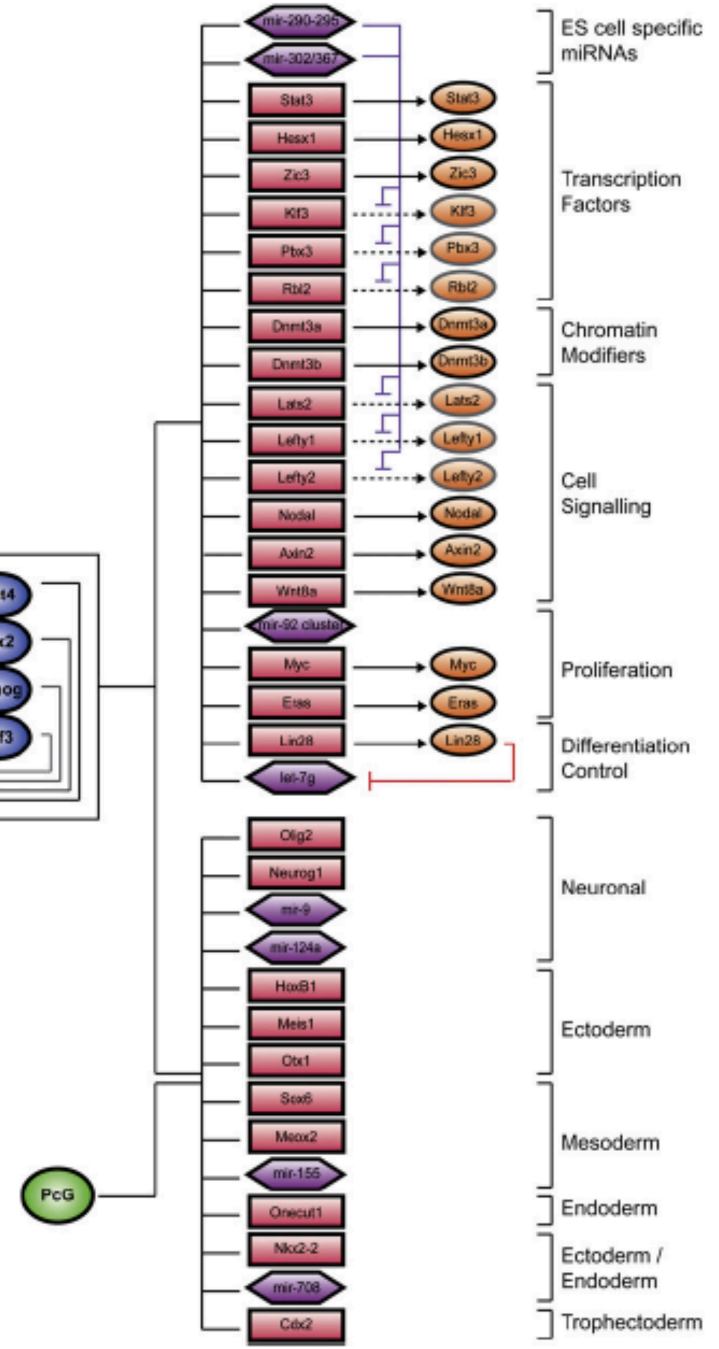
Tcf3
Tcf3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Lefty1
Lefty2
Nodal
Axin2
Wnt8a
mir-92 cluster
Myc
Eras
Lin28
let-7g
Olig2
Neurog1
mir-9
mir-124a
HoxB1
Meis1
Otx1
Sox6
Meox2
mir-155
Onecut1
Nkx2-2
mir-708
Cdx2
PcG
ES cell specific miRNAs
Transcription Factors
Chromatin Modifiers
Cell Signalling
Proliferation
Differentiation Control
Neuronal
Ectoderm
Mesoderm
Endoderm
Ectoderm / Endoderm
Trophectoderm

Recommended reading material

- Essentials of Stem cell biology" Edited by Robert Lanza

- Boiani M, Schöler HR.
  Regulatory networks in embryo-derived pluripotent stem cells.
  Nat Rev Mol Cell Biol. 2005 Nov;6(11):872-84.