iPSCs and cell reprogramming

Julie Martone

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This lesson...

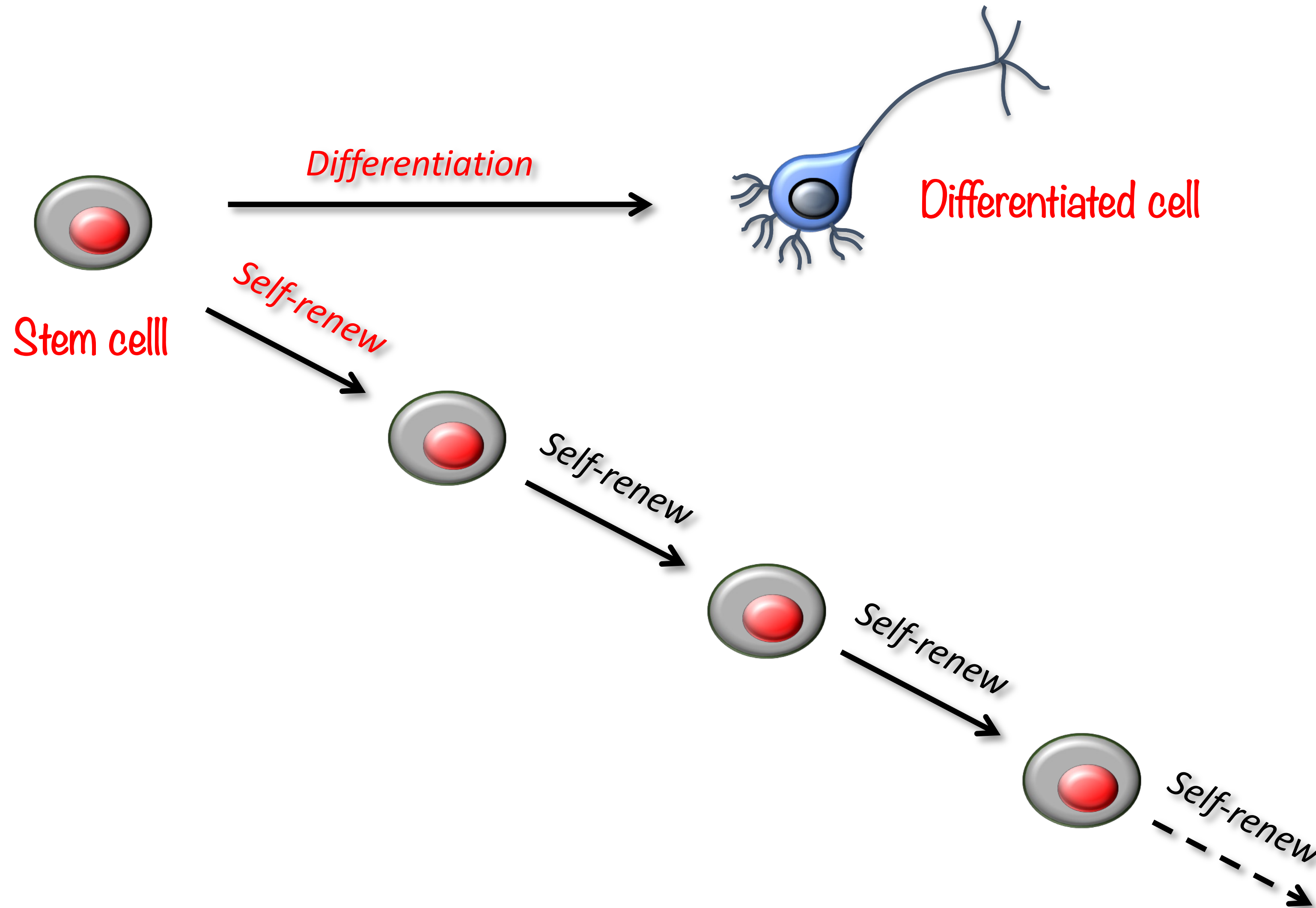
1. What are stem cells

2. A bit of history: which are the key experiments that allowed to arrive at the concept of reporgramming

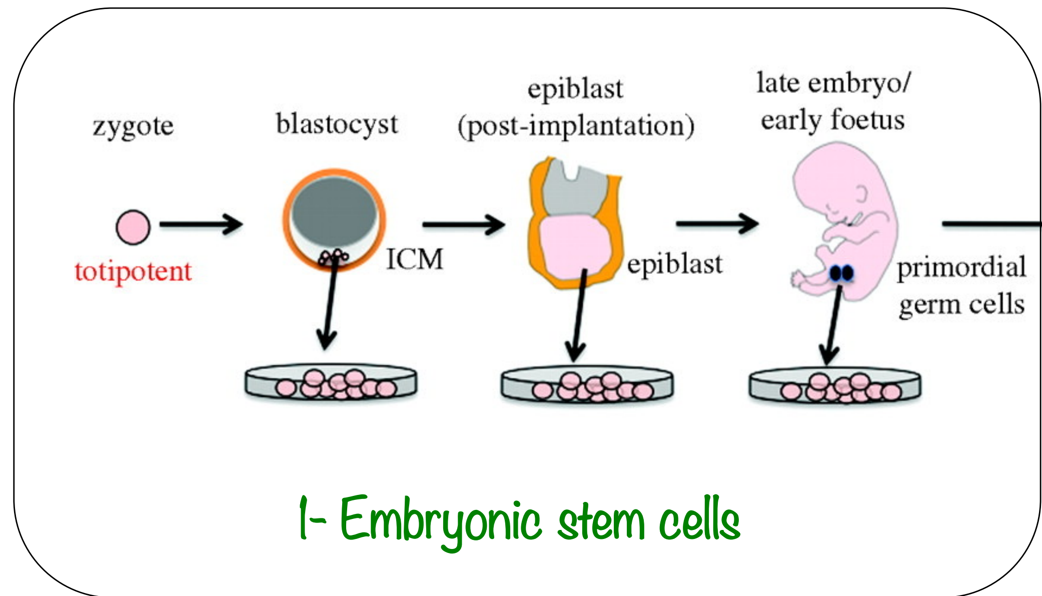
3. Generating pluripotent stem cells from somatic cells through epigenetic reprogramming

WHAT ARE STEM CELLS?

Stem Cells are defined as cells with extensive **self-renewal** capacity and the ability to generate daughter cells that undergo further **differentiation**

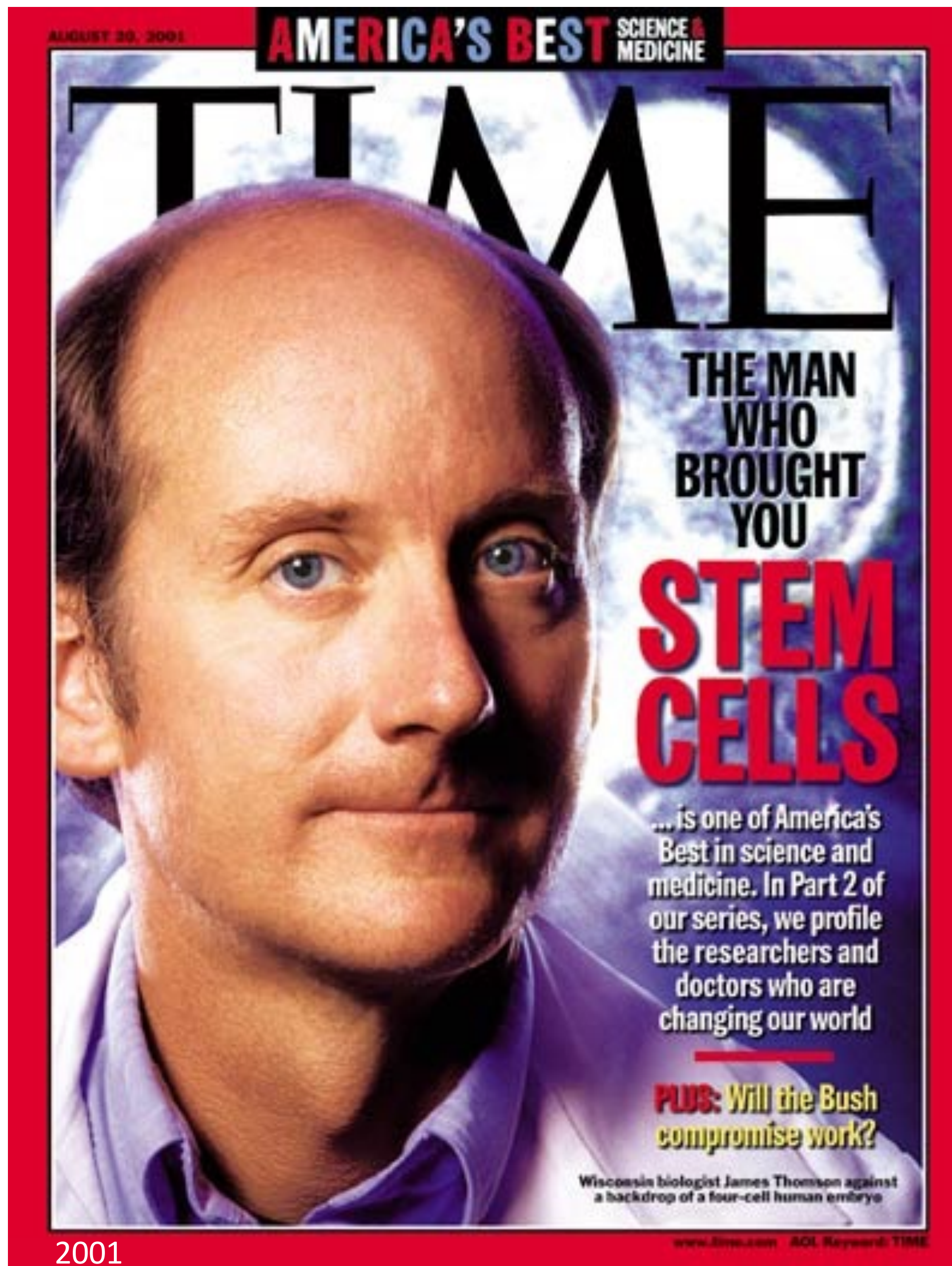


Origin of stem cells



Adapted from Fiona M. Watt, and Ryan R. Driskell Phil. Trans. R. Soc. B 2010;365:155-163

STEM CELLS RESEARCH

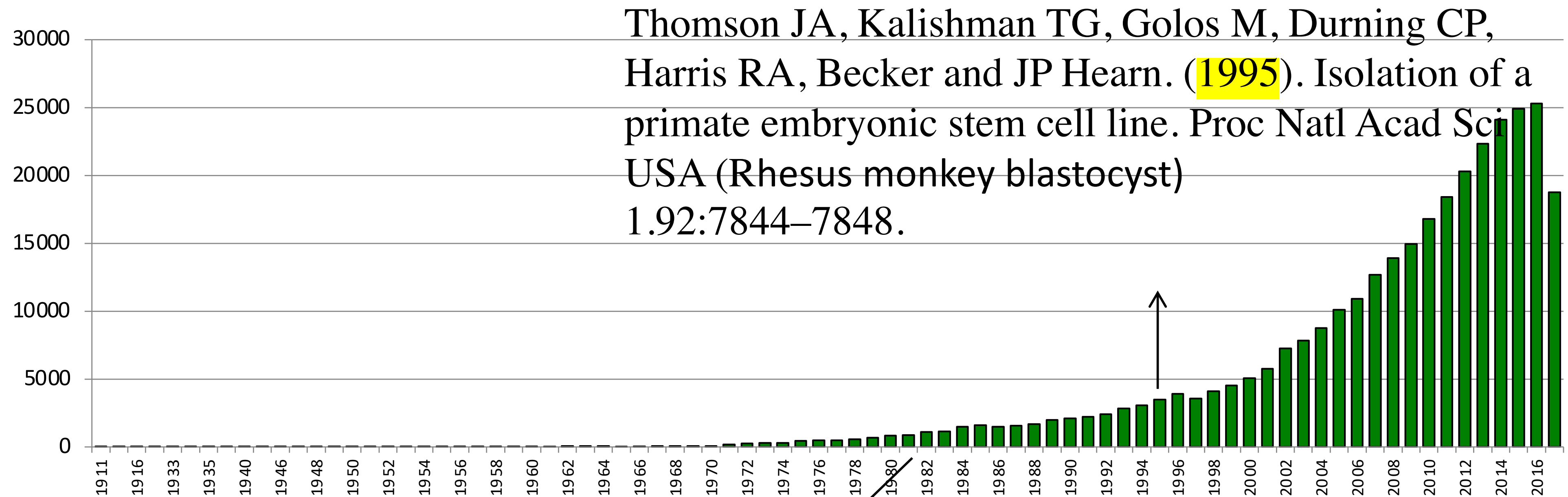


2001

2008

James Thomson

STEM CELLS RESEARCH



Thomson JA, Kalishman TG, Golos M, Durning CP, Harris RA, Becker and JP Hearn. (1995). Isolation of a primate embryonic stem cell line. Proc Natl Acad Sci USA (Rhesus monkey blastocyst) 1.92:7844–7848.

Establishment in culture of pluripotential cells from mouse embryos (1981), *Nature*. Evans & Kaufman

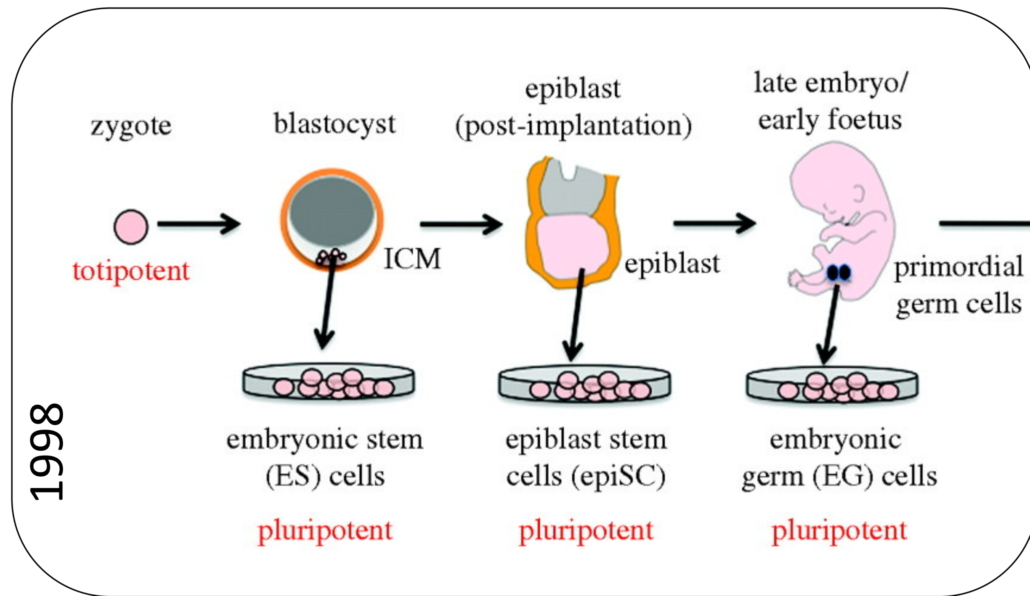
REPORTS

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

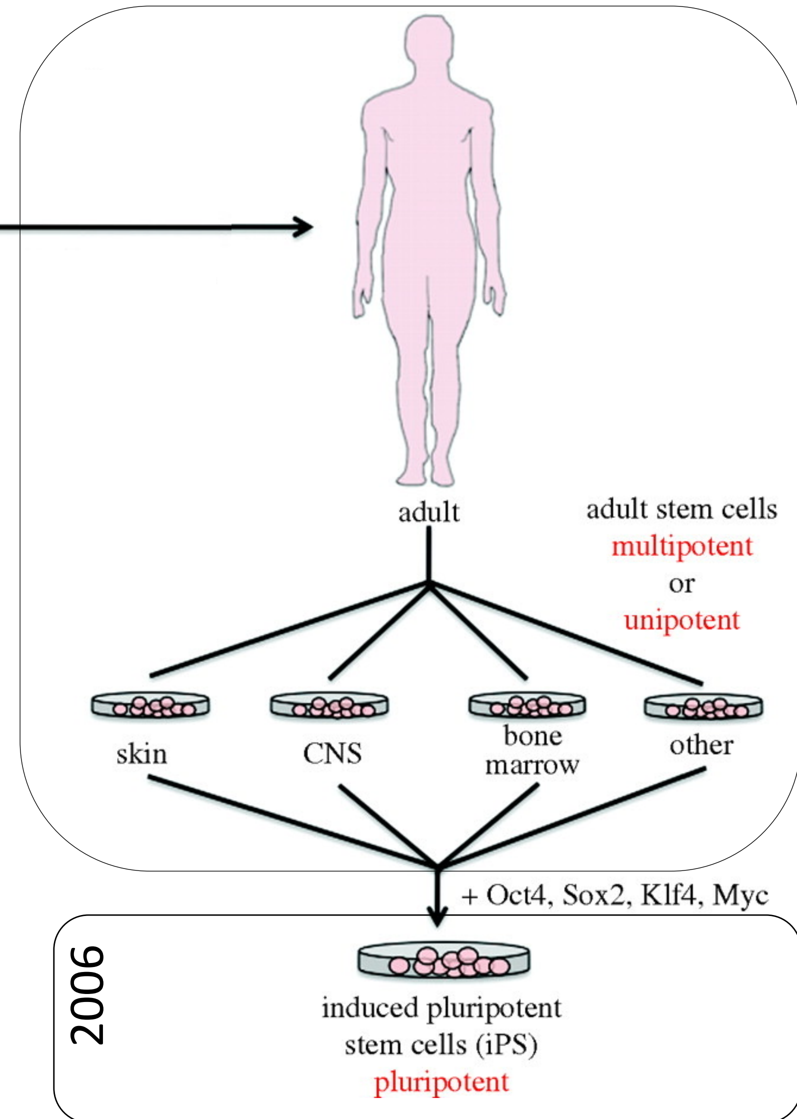
SCIENCE VOL 282 6 NOVEMBER 1998

Origin of stem cells



hEmbryonic stem cells

Adult stem cells



Induced pluripotent stem cell

Cell potency is a cell's ability to differentiate into other cell types. The more cell types a cell can differentiate into, the greater its potency.

Adapted from Fiona M. Watt, and Ryan R. Driskell Phil. Trans. R. Soc. B 2010;365:155-163

DEFINITIONS

Stem cell – Stem cells are distinguished from other cell types by two important characteristics:

1) They are unspecialized cells capable **of continually renewing** themselves through cell division and
2) they have the potential to **develop into many different cell types** of the body.

Given their regenerative potential, stem cells offer new opportunities for treating diseases.

Embryonic stem cell – An unspecialized cell type derived from early-stage embryos. Embryonic stem cells can renew themselves and they are pluripotent, meaning they have the potential to develop into any cell type of the body.

Adult stem cell – An unspecialized cell found among specialized cells in a tissue or organ. Adult stem cells can renew themselves and they are multipotent, meaning they have the potential to develop into a limited number of cells in the body (some or all of the specialized cell types of the tissue or organ from which they were derived).

Induced pluripotent stem cell (iPSC) – An unspecialized, embryonic stem cell-like cell that has been derived from an adult cell through epigenetic reprogramming. (Epigenetics relates to cellular changes caused by external or environmental factors that switch genes on and off and affect how cells read genes instead of being caused by changes in the DNA sequence.) Thus, just like embryonic stem cells, iPSCs can also renew themselves and they are also pluripotent.

NIH National Institutes of Health
Office of Strategic Coordination - The Common Fund

Embryonic and adult stem cells

Embryonic

In vivo, they exist as a very transient population of cells inside the blastocyst. Their self-renew is limited to a short period of time

In vitro, we can keep them undifferentiated indefinitely (self-renew)

Their developmental potential is: **pluripotency**

Adult

In vivo, they reside in niches inside adult organs. Their self-renew capacity is virtually unlimited

Rare

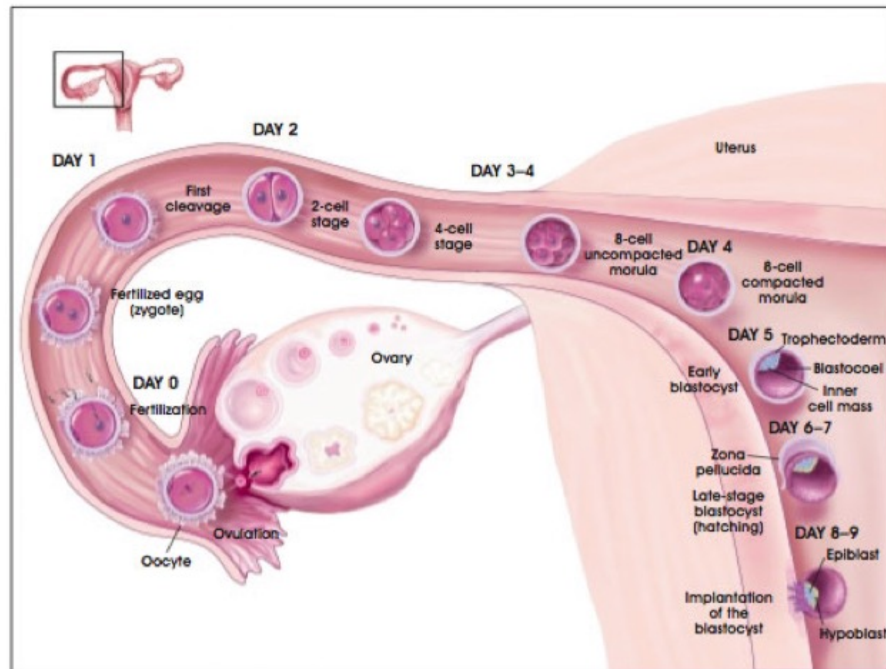
In vitro, not all adult stem cell types can be maintained

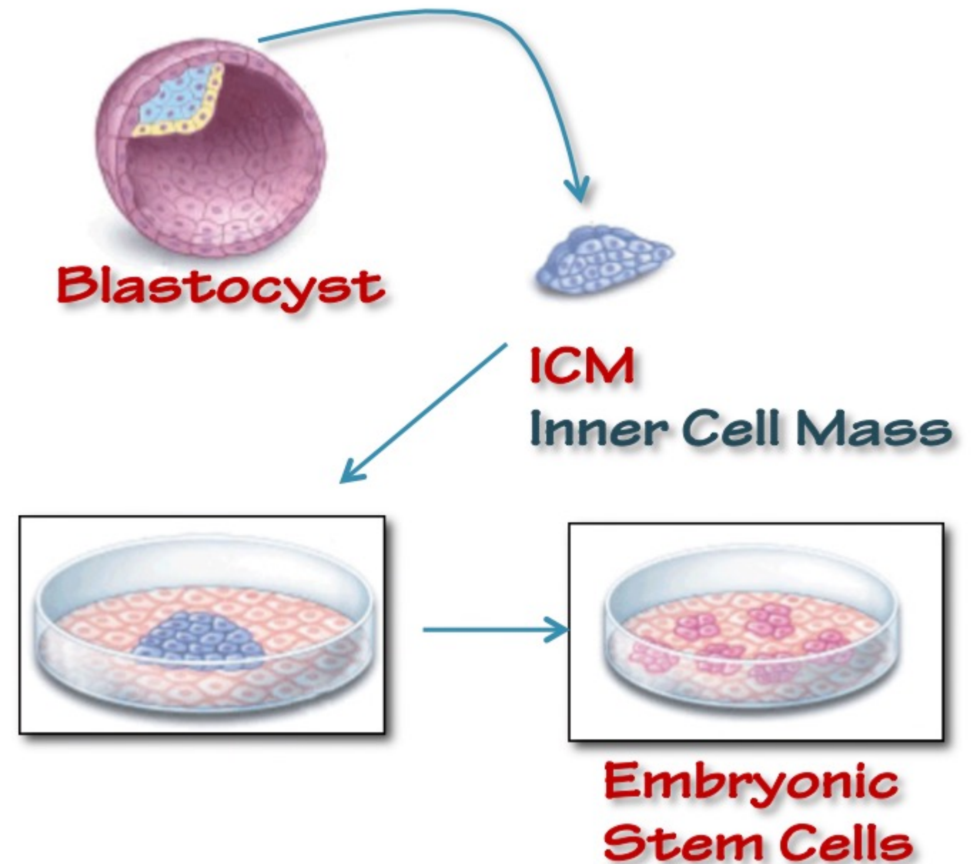
Their developmental potential is: **multipotency** or **unipotency**

EMBRYONIC STEM CELLS

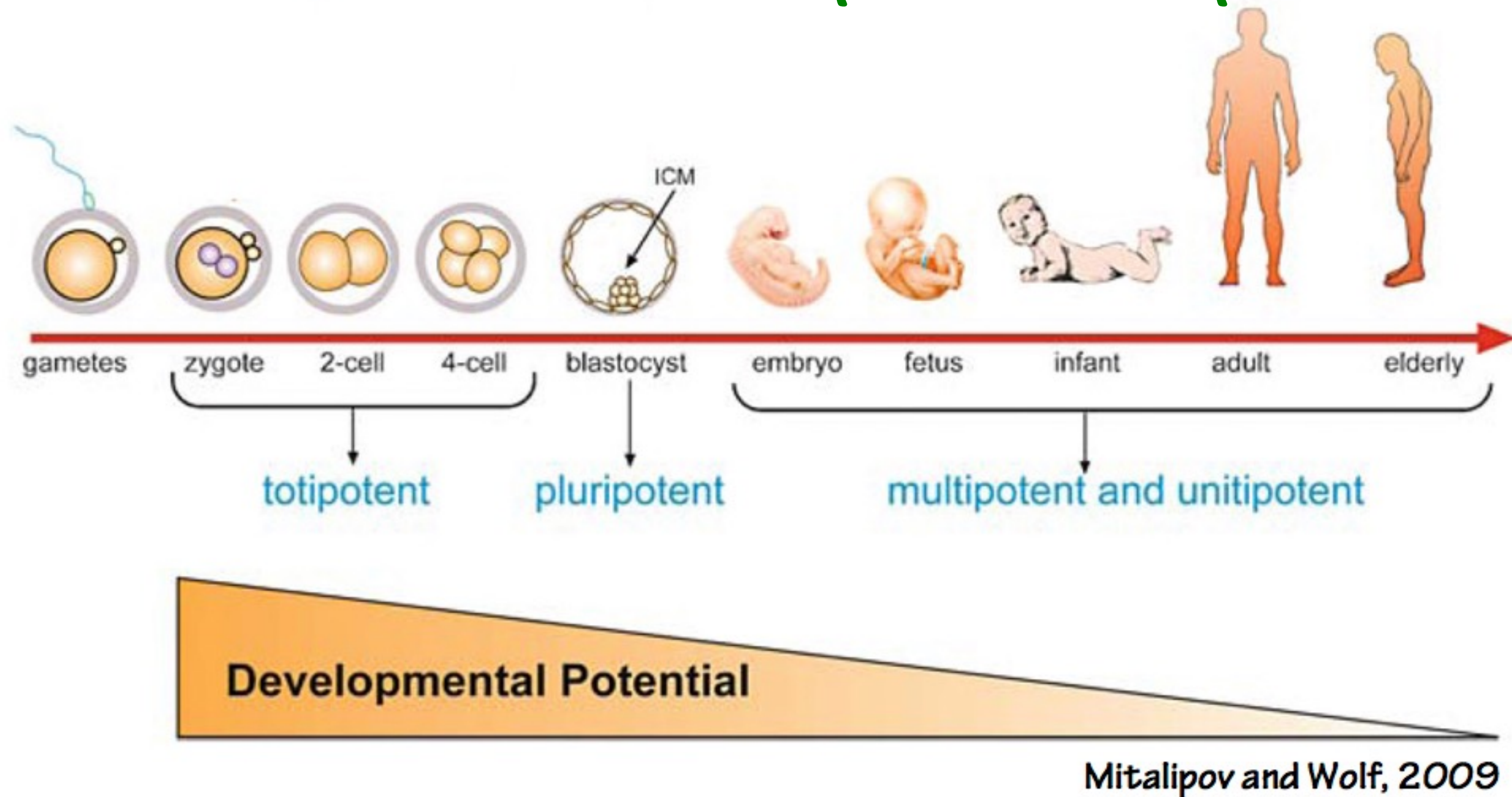
Embryonic Stem Cells derive from the early embryo at the stage of blastocyst.

In vivo, they are a transient population of cells. In vitro, they can self-renew indefinitely.





Decrease of developmental potential

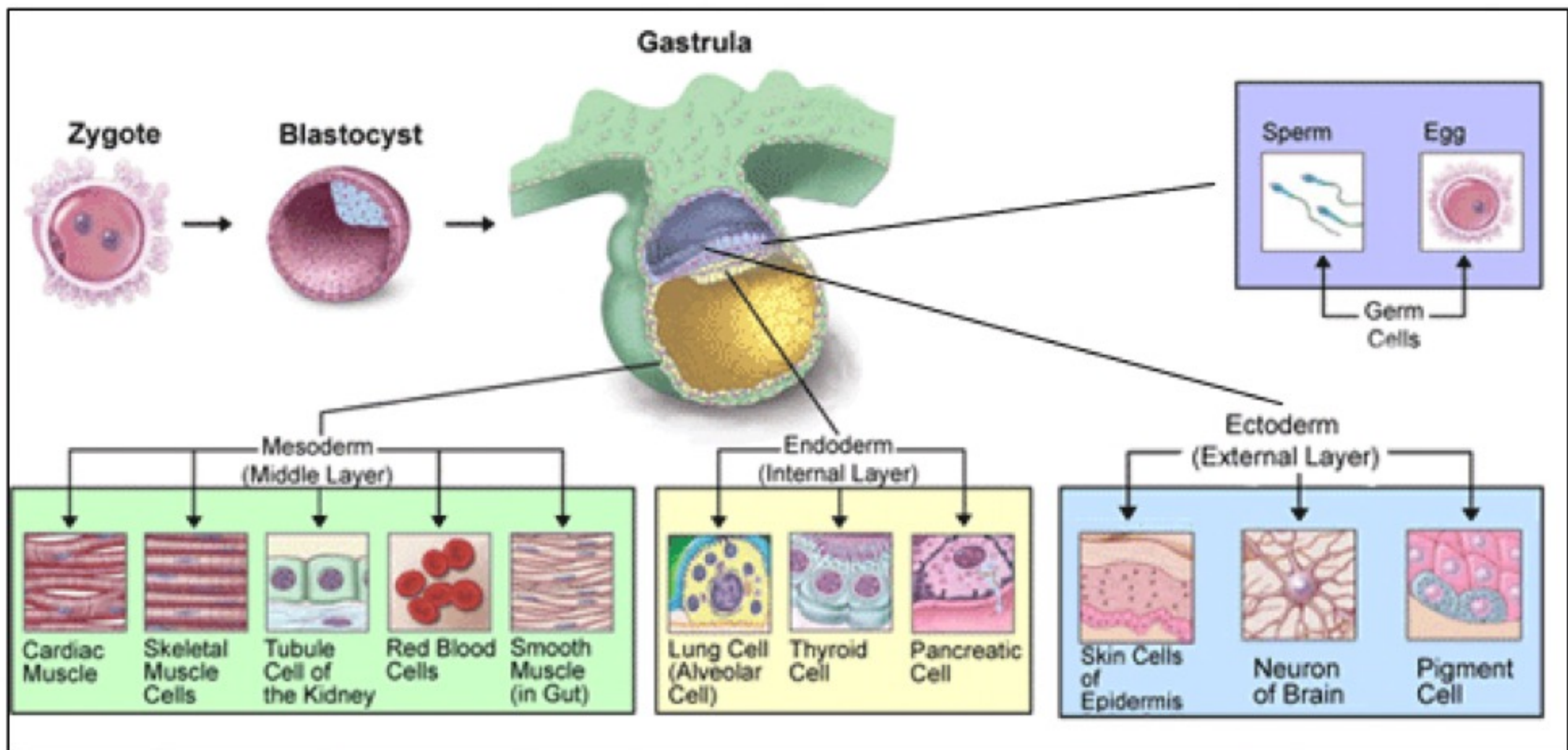


Mitalipov and Wolf, 2009

Potency: number of possible fates open to a cell (decrease with age)

Pluripotency

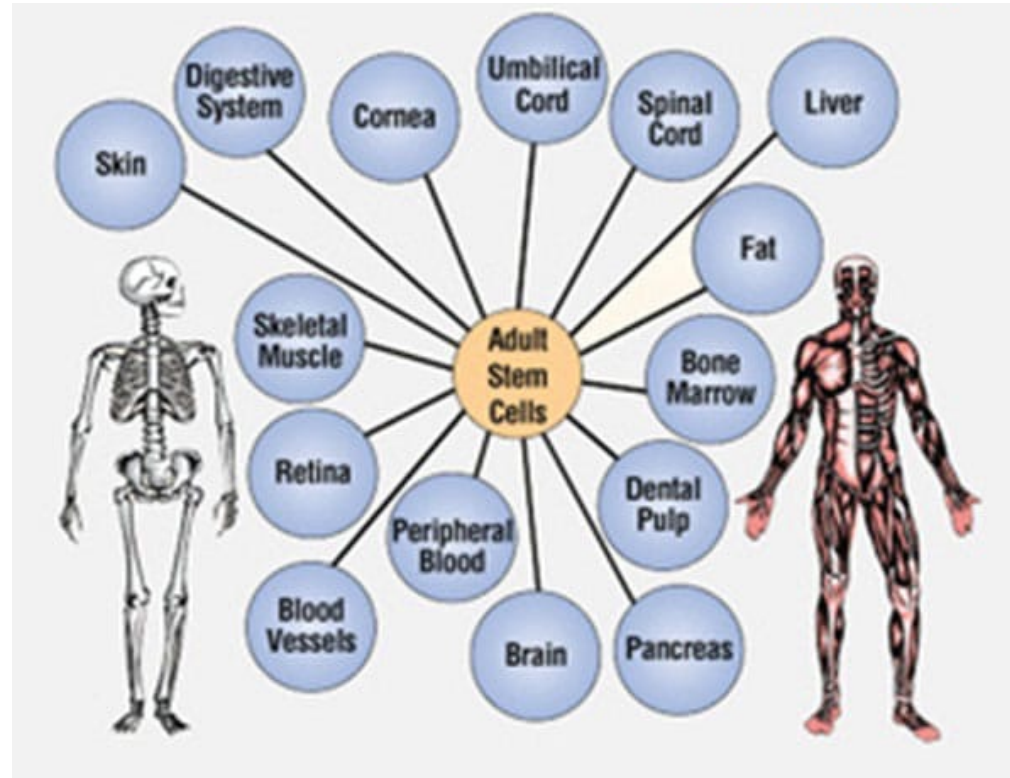
Pluripotency: the ability of a cell to differentiate into any of the three germ layers: endoderm, mesoderm or ectoderm.
Pluripotent stem cells can give rise to any fetal or adult cell type



ADULT STEM CELLS FUNCTIONS

- Rigenerate damaged tissue
- Natural tissue turnover

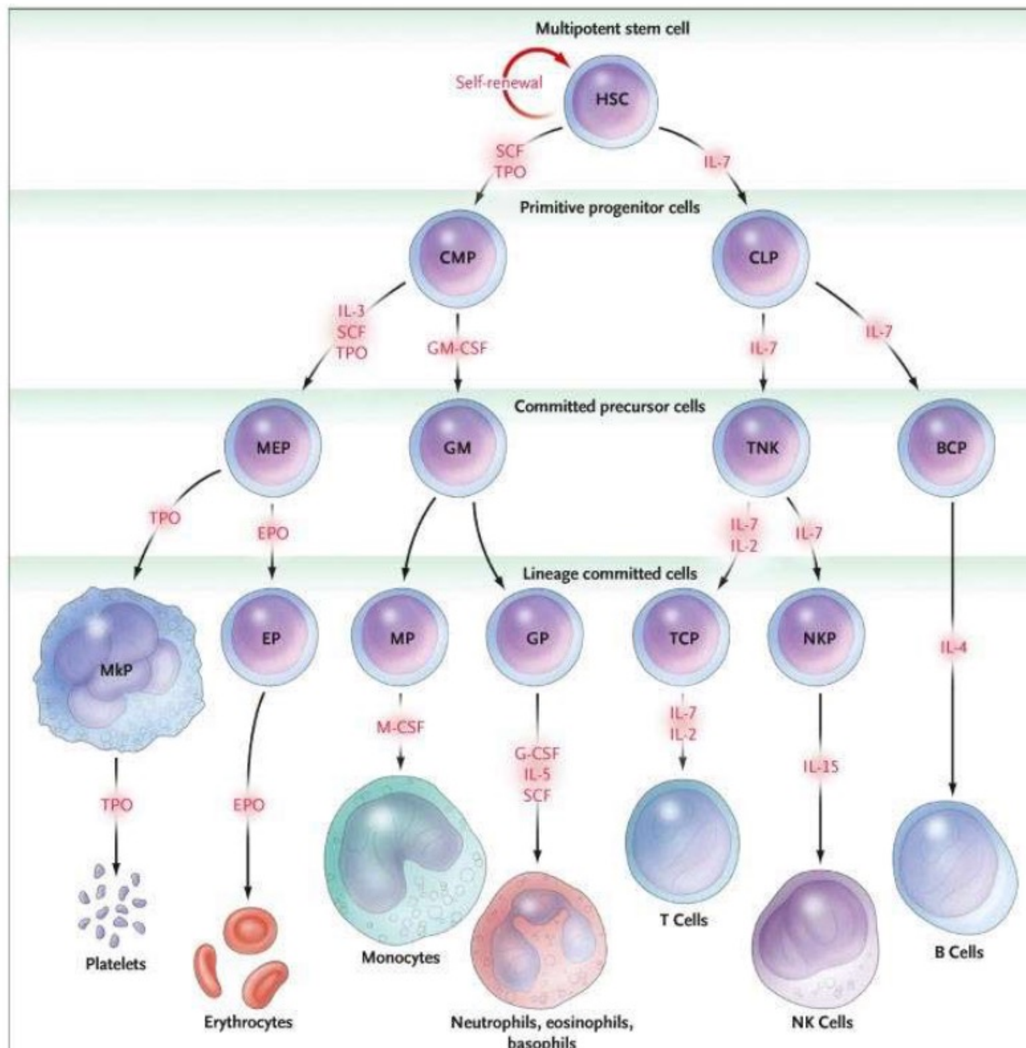
Where are adult stem cells found?



Adult stem cells have been identified in many organs and tissues, including brain, bone marrow, peripheral blood, blood vessels, skeletal muscle, skin, teeth, heart, gut, liver, ovarian epithelium, and testis. They are thought to reside in a specific area of each tissue (called a "stem cell niche").

Multipotent stem cells: All blood cells types come from hematopoietic stem cells

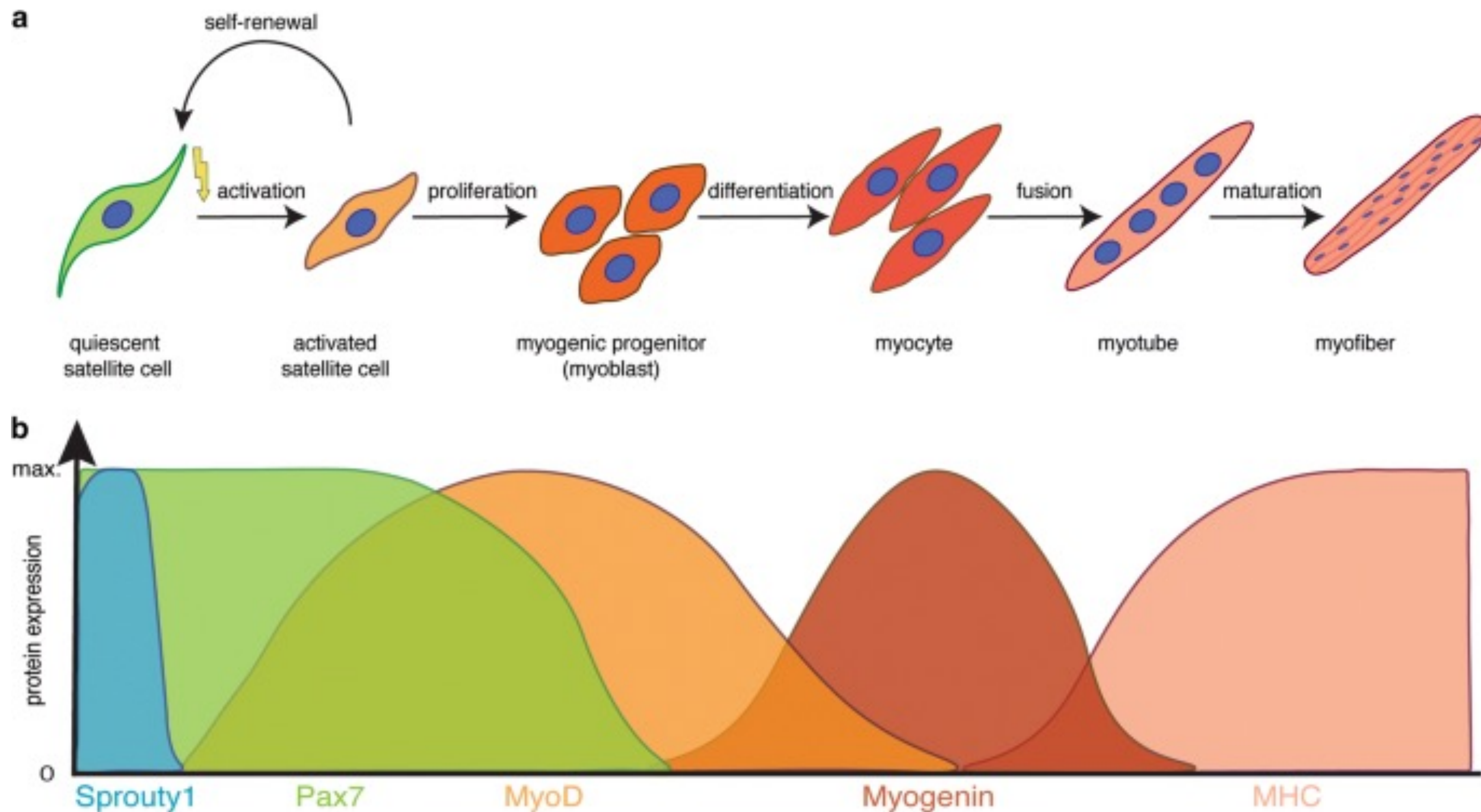
Hematopoiesis is the hierarchical differentiation process that leads to the formation of all blood cells starting from multipotent hematopoietic stem cells (HSCs).



HSCs are the top level of the hierarchy, since they maintain the ability to self-renew and give rise to lineage progenitors all life long.

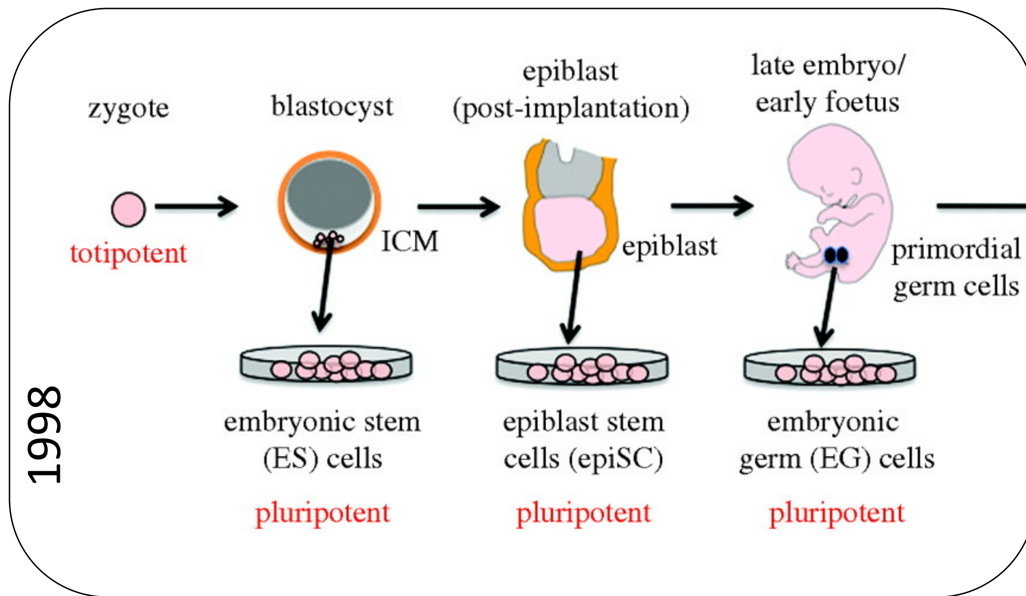
Lineage progenitors are no more able to self-renew, and they pass through increasingly committed intermediates in order to give rise to all the mature blood components.

Unipotent stem cells: satellite cells mediates the long-life maintenance of muscle tissue

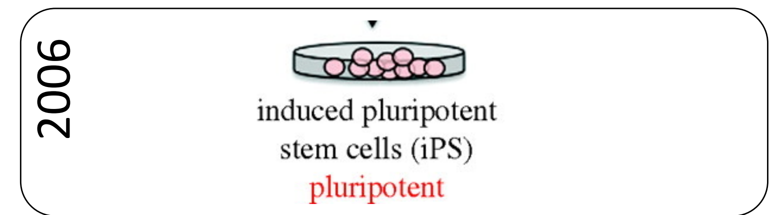


Cellular and Molecular Life Sciences **volume 76**, pages2559–2570(2019)

Pluripotency (the capability to become any somatic cell type) is characteristic of Ebryonic stem cells (ESC) and induced pluripotent stem cells (iPSCs)



Embryonic stem cells



Induced pluripotent stem cell

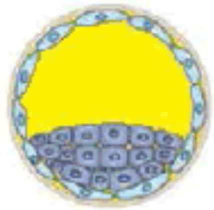
Adapted from Fiona M. Watt, and Ryan R. Driskell Phil. Trans. R. Soc. B 2010;365:155-163

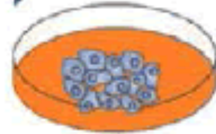
Summary of pluripotency testing methods

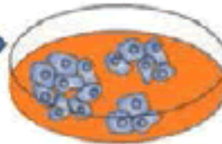
Pluripotency Assay	Purpose	Length of Assay	Nature of Assay	Strength/Definitiveness of Assay
Colony morphology	Verify ESC colony-like morphology of clustered, border-defined colonies.	10 minutes	*in vitro*	Low
Immunohistochemistry	Stain for standard pluripotency markers such as Oct4, Tra-1-60, Sox2, Tra-1-81, Nanog and SSEA.	1–4 days	*in vitro*	Medium
Real-time PCR	Detect and quantify expression levels of selected pluripotency genes; limited by gene number.	4–6 hours (from RNA extraction to RT-PCR	*in vitro*	Medium-High
Embryoid body formation and analysis	Test differentiation capability of PSCs to tissues of all 3 germ layers *in vitro* or *in vivo*; should be coupled with relative quantification expression of gene-expression from the three germ-layers, which can be done by RT-PCR. Genes include: Nanog, Oct4, Sox2, and Klf4 (pluripotency markers); Ncam and NeuroD (ectodermal markers); Runx2, HNF4a, and Nkx2.5 (mesodermal markers); and Sox17, Albumin, Glut2, and Insulin (endodermal markers).	2–3 weeks	*in vitro* or *in vivo*	Medium-High
Microarray	A comprehensive measurement of gene expression levels.	1–2 days	*in vitro*	Medium-High
Teratoma formation	Test differentiation capability into all 3 germ layers *in vivo*.	1–2 months	*in vivo*	High

From: Teratoma formation: A tool for monitoring pluripotency in stem cell research

Teratoma formation







Teratoma assays are considered the gold standard for demonstrating differentiation potential of pluripotent ESC/iPSC

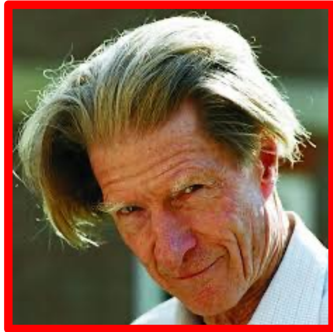
A variety of **animal strains** can be used. Immunodeficient strains (i.e., Nu/Nu nude, BC nude, or SCID for mice; Rowett nude or Athymic for rats), however, form teratomas at a higher incidence. There is a significant chance of cell rejection in non-immunocompromised models.

2012 Nobel Prize

In 2012 Nobel Prize for Medicine was awarded to Sir John Gurdon and Shinya Yamanaka for their groundbreaking contributions to the field of cell reprogramming.

1962

Somatic cell nuclear transfer


Sir John Gurdon

In 1962, in a series of experiments inspired by Briggs and King, Gurdon demonstrated that the nucleus of a frog somatic cell could be reprogrammed to behave like the nucleus of a fertilized frog egg. By inserting the nuclei of intestinal epithelial cells into enucleated eggs, Gurdon was able to create healthy swimming tadpoles. These experiments were the first successful instances of somatic cell nuclear transfer (SCNT) using genetically normal cells.

Success rate=1%

tadpole intestinal cell nucleus

enucleated frog egg

healthy swimming tadpoles

fertile frog adults

Clearly, the frog egg cytoplasm contained factors capable of orchestrating the necessary changes in an incoming nucleus. Interestingly, nuclei from adult frog cells, unlike those taken from tadpoles, were never able to generate viable adult progeny.

1997

DOLLY

Keith Campbell

National Museum of Scotland Edimburg (wikipedia)

NATURE | VOL 385 | 27 FEBRUARY 1997

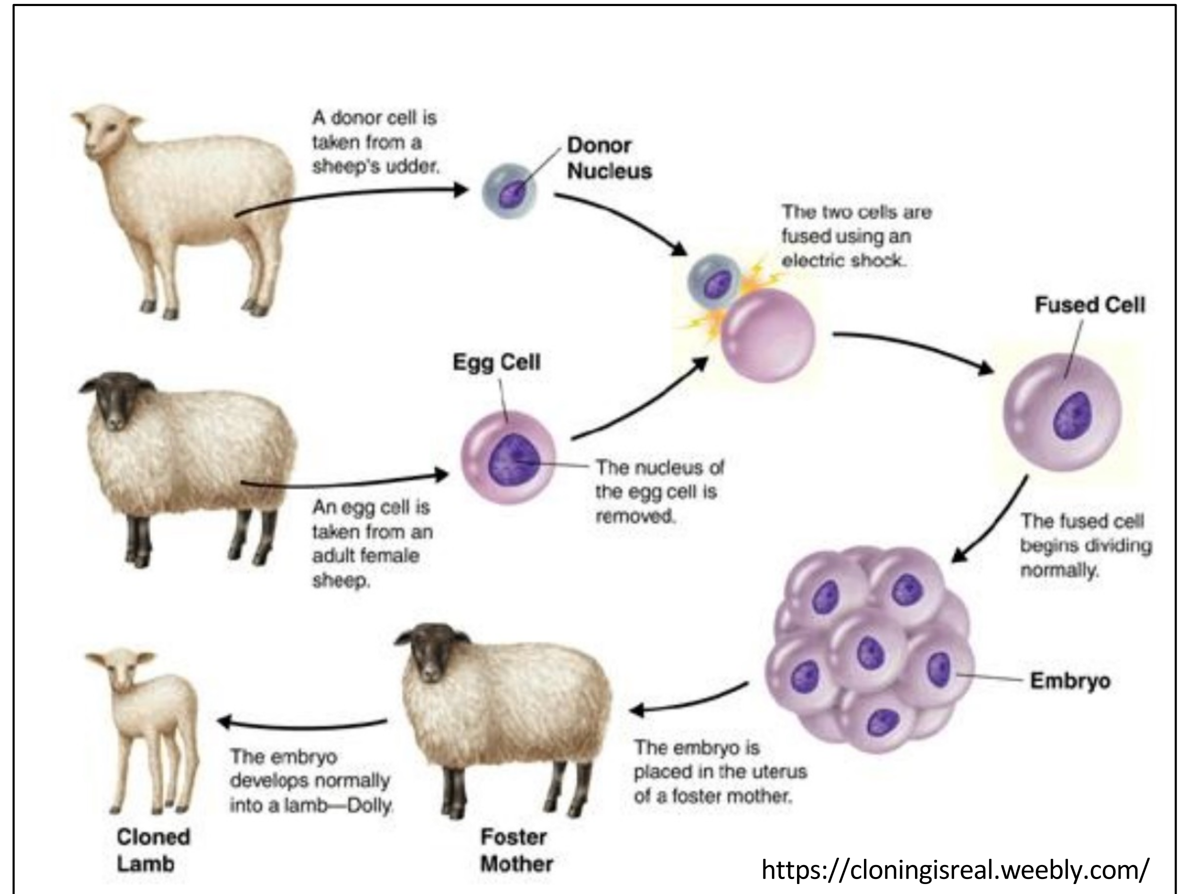
Viable offspring derived from fetal and adult mammalian cells

I. Wilmut, A. E. Schnieke*, J. McWhir, A. J. Kind* & K. H. S. Campbell

Roslin Institute (Edinburgh), Roslin, Midlothian EH25 9PS, UK

letters to nature

Figure 2 Lamb number 6LL3 derived from the mammary gland of a Finn Dorset ewe with the Scottish Blackface ewe which was the recipient.



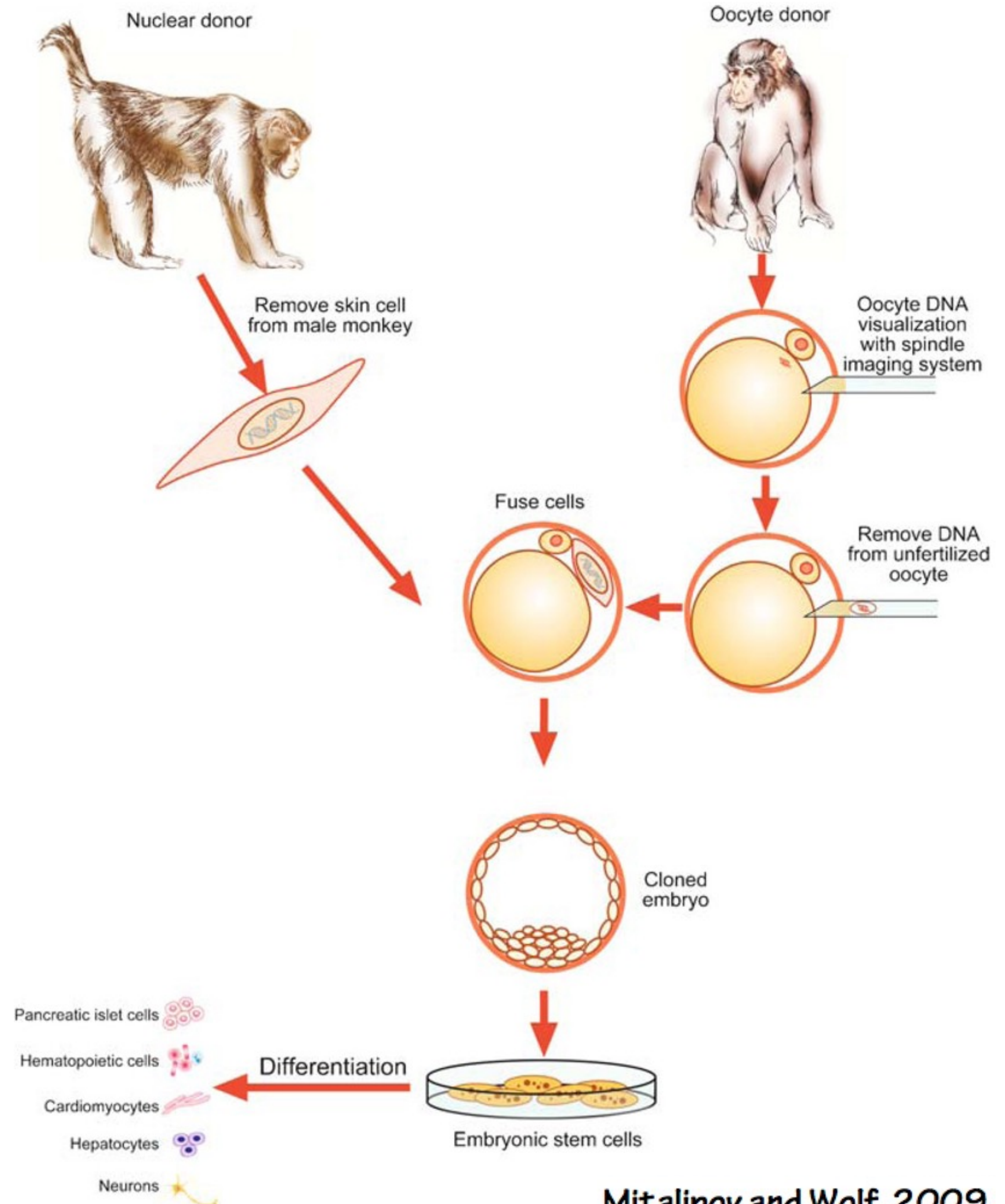
The birth of Dolly the Sheep proved that mammalian clones could be made from adult cell nuclei

2007

Reprogramming of adult primate somatic cells into pluripotent embryonic stem cells via SCNT.

(Byrne et al., Nature 2007)

proof-of-concept for therapeutic cloning in primates



Mitalipov and Wolf, 2009

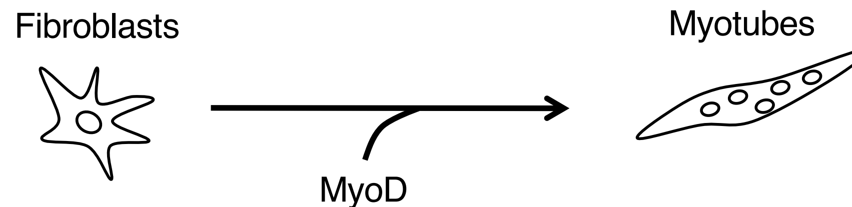
1987

Transcription Factor-Induced Transdifferentiation

Cell, Vol. 51, 987–1000, December 24, 1987, Copyright © 1987 by Cell Press

Expression of a Single Transfected cDNA Converts Fibroblasts to Myoblasts

Robert L. Davis,*† Harold Weintraub,*
and Andrew B. Lassar*



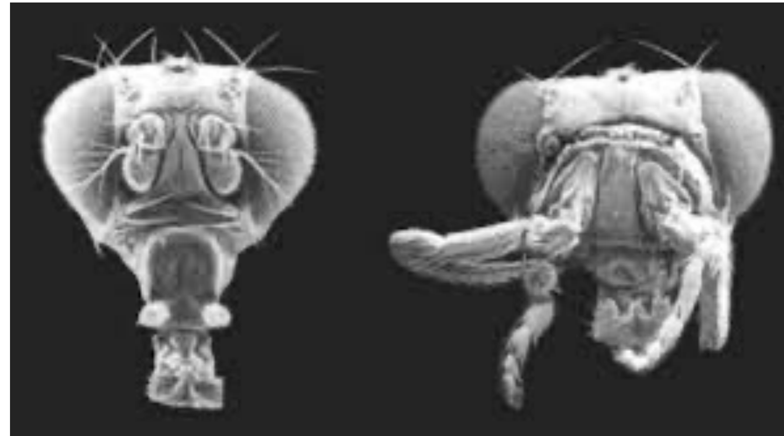
1987

Published: 26 February 1987

Redesigning the body plan of *Drosophila* by ectopic expression of the homoeotic gene *Antennapedia*

Stephan Schneuwly, Roman Klemenz & Walter J. Gehring

Nature **325**, 816–818(1987) | Cite this article



Transcription Factor-Induced Transdifferentiation

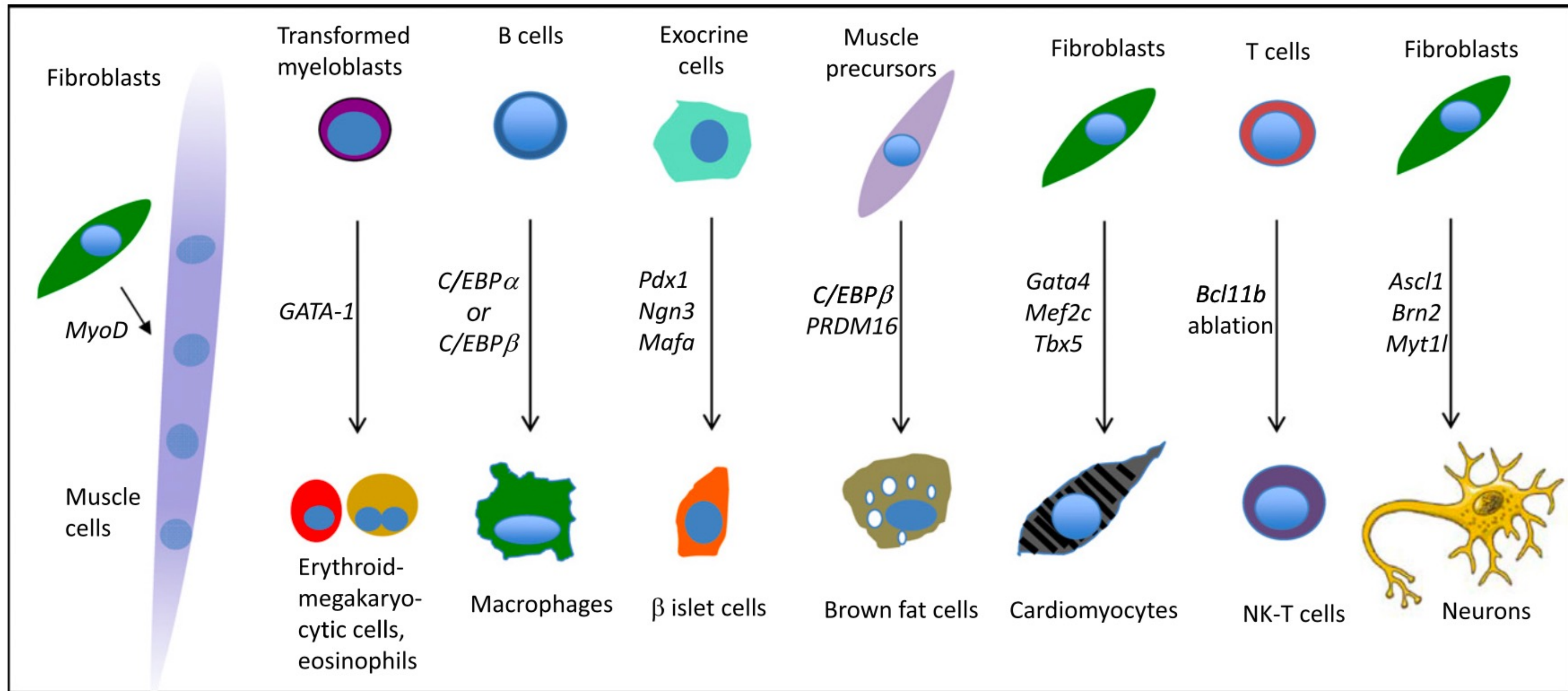


Figure 3. Examples of Transcription Factor-Induced Transdifferentiation
The examples shown are discussed throughout the text. Models (left to right) based on work from Davis et al. (1987), Kulessa et al. (1995), Xie et al. (2004), Zhou et al. (2008), Kajimura et al. (2009), Ieda et al. (2010), Li et al. (2010a) and (2010b), and Vierbuchen et al. (2010).

Thomas Graf, Cell Stem Cell, 2011

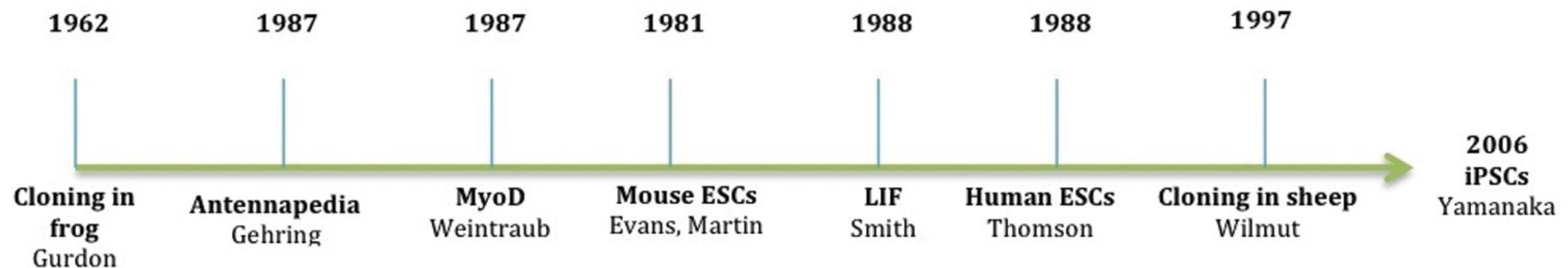
On the way to reprogramming....

Armed with knowledge of:

- ES cell biology (Thomson)

- the history of frog and mammalian SCNT (John Gurdon and Keith Campbell)

- the demonstration in 1987 by Davis et al. that the enforced expression of a single added transcription factor (TF) gene could change fibroblasts into muscle-like cells



Yamanaka set out to reprogram an intact differentiated somatic cell back to the pluripotent state.

Epigenetic landscape

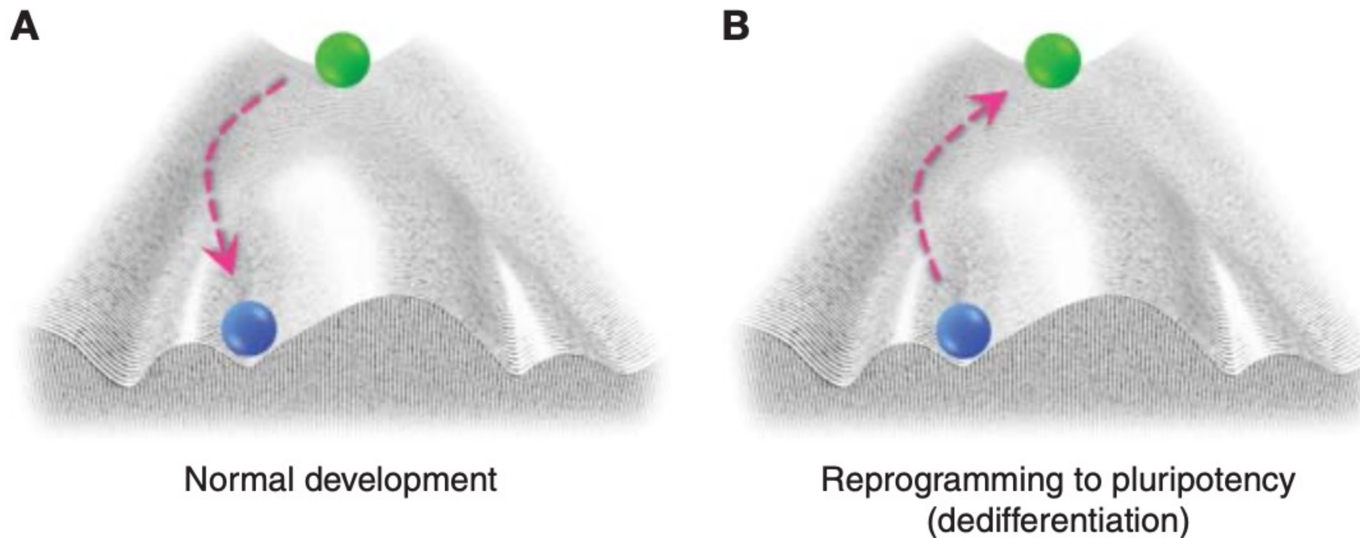
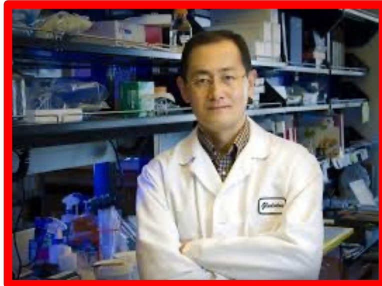


Figure 1. Cellular reprogramming depicted as a trajectory in Waddington's epigenetic landscape. (*A*) A cell's normal developmental trajectory can be traced starting from a pluripotent cell (green ball) at the top of the hill to its final differentiated state (blue ball), illustrating how epigenetics contributes to cell fate determination during development. (*B*) A terminally differentiated cell (blue ball) can be reprogrammed back to pluripotency when exposed to a cocktail of transcription factors.

Takahashi Cold Spring Harb Perspect Biol 2014;6:a018606

Induction of pluripotent stem cells



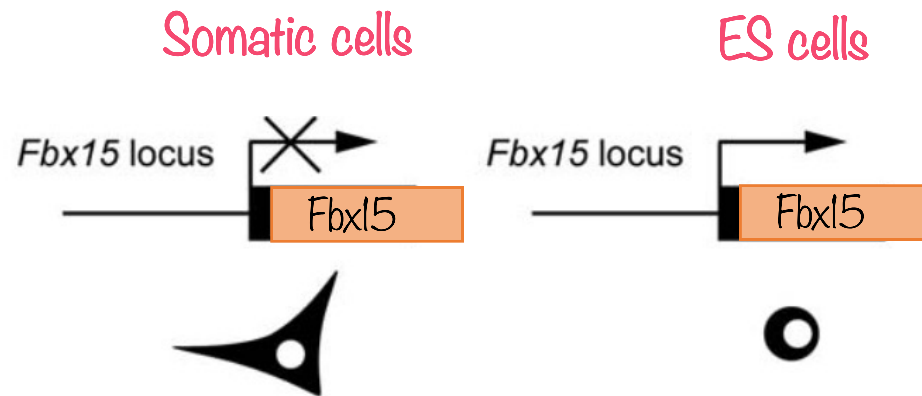
In 2006, Yamanaka made a further conceptual leap. With four defined transcription factors he induced intact mouse somatic cells to revert to a pluripotent state without an egg or embryo as intermediary .

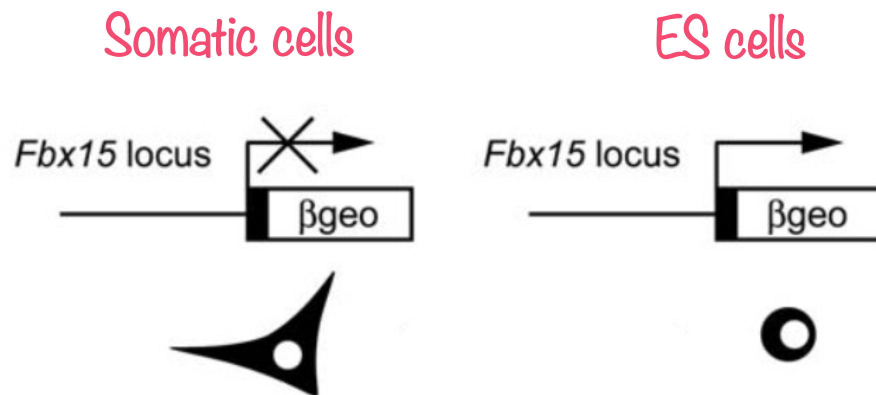
2006

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

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

Strategy to test candidate factors





Fbxl5

- Is specifically expressed in mouse Embryonic Stem Cells (mESCs)
- is dispensable for the maintenance of pluripotency and mouse development

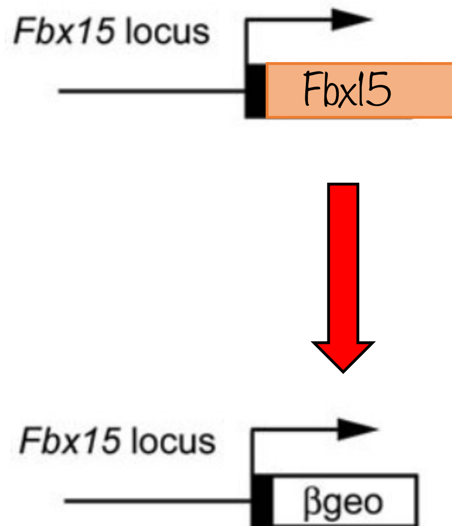
βgeo

- is a fusion of the b-galactosidase and neomycin resistance genes

Takahashi and Yamanaka, 2006, Cell *126*, 663–676

Strategy to test candidate factors

Gene targeting in mESCs



The Nobel Prize in Physiology or Medicine 2007

CRISPR

Charpentier e Doudna.
Nobel Prize in Chemistry 2020

The Nobel Prize in Physiology or Medicine 2007 was awarded jointly to Mario R. Capecchi, Sir Martin J. Evans and Oliver Smithies "for their discoveries of principles for introducing specific gene modifications in mice by the use of embryonic stem cells."

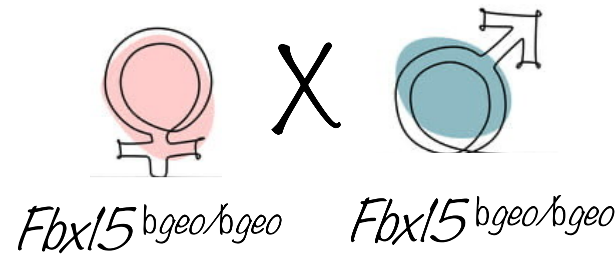
Takahashi and Yamanaka, 2006, Cell *126*, 663–676

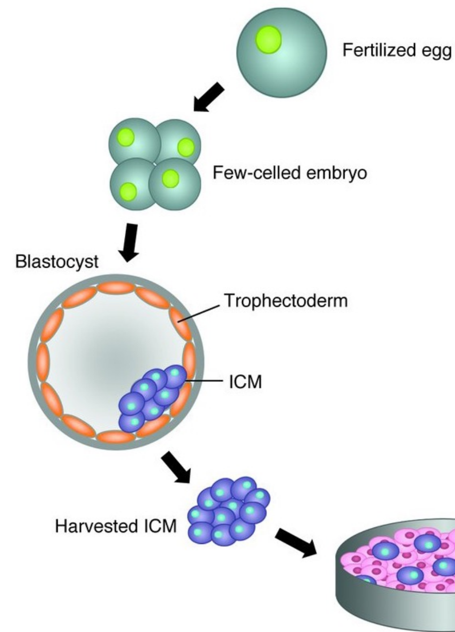
Strategy to test candidate factors

Somatic cells

Somatic cells derived from *Fbxl5* $^{bgeo/bgeo}$ mice were sensitive to neomycin (G418)

ES cells



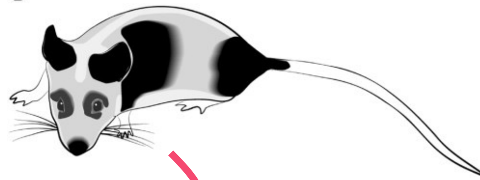


mESCs from *Fbxl5* $^{bgeo/bgeo}$ *mice*

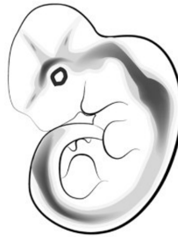
ES cells homozygous for the bgeo knockin construct (*Fbxl5* $_{bgeo/bgeo}$) were resistant to neomycin (G418)

Strategy to test candidate factors

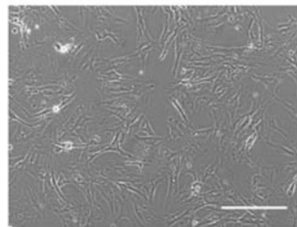
Mouse *Fbxl5*$^{\beta geo/\beta geo}$



Mouse embryo
Fbxl5$^{\beta geo/\beta geo}$

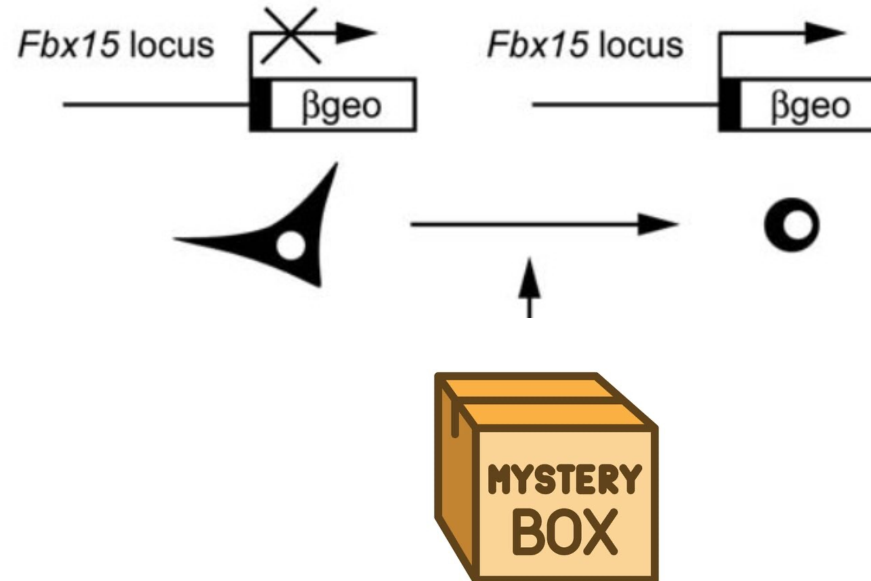


Mouse Embryonic Fibroblasts (MEFs) from *Fbxl5*$^{\beta geo/\beta geo}$ *mice*

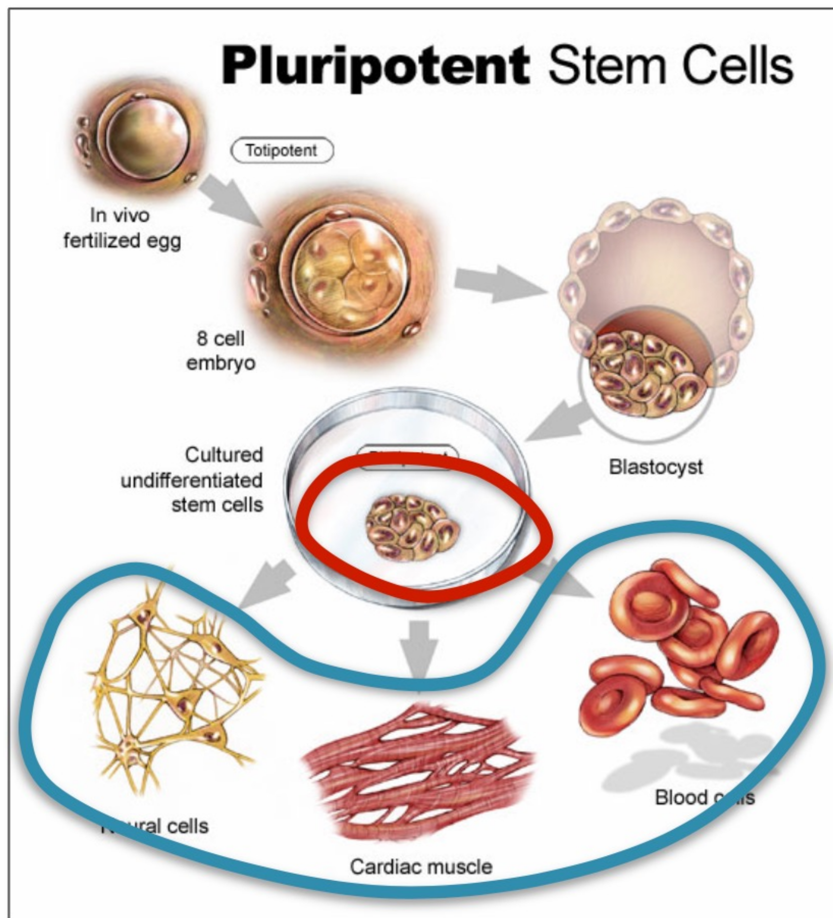


Mouse Embryonic Fibroblasts (MEFs) from *Fbxl5*$^{\beta geo/\beta geo}$ *mice* were sensitive to neomycin (G418)

Strategy to test candidate factors



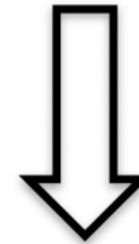
Chasing the reprogramming factors: Yamanaka's strategy



Genes expressed in ESC

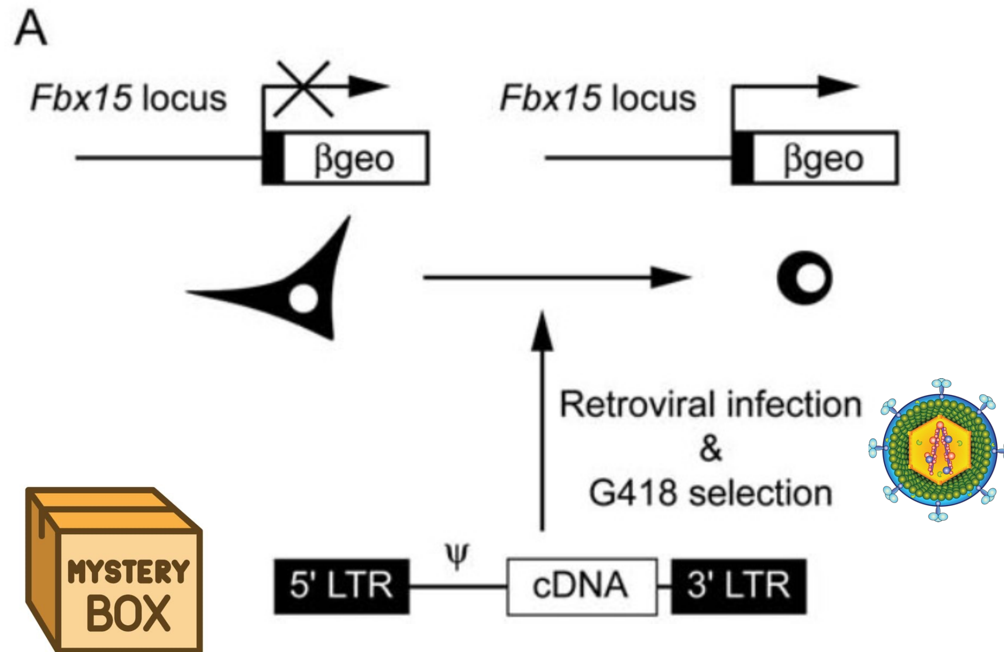
Vs.

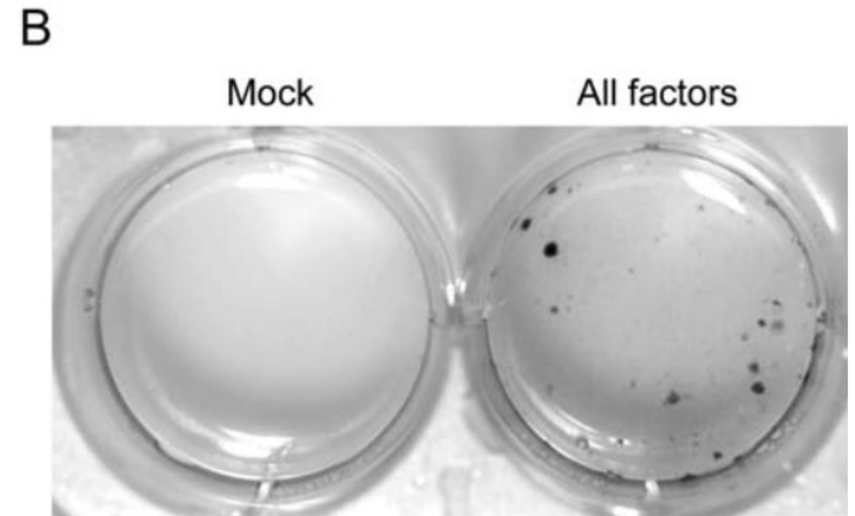
Genes expressed in somatic cells



Candidate Reprogramming Factors

Strategy to test candidate factors



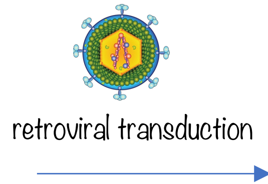


Neomycin -resistant colonies were observed 16 days after transduction with a combination of 24 factors. Cells were stained with crystal violet.

Takahashi and Yamanaka, 2006, Cell *126*, 663–676

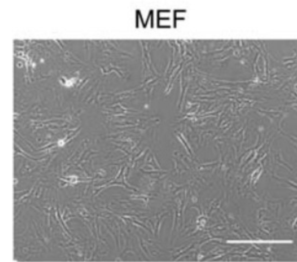
Screening the 24 candidates

Each of the 24 candidate genes into mouse embryonic fibroblasts (MEFs)



Mouse embryonic fibroblasts (MEFs) from *Fbx15bgeo/bgeo*

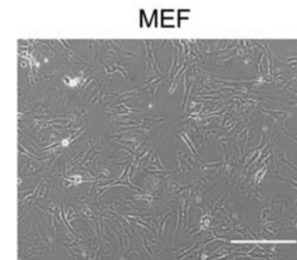
MEF



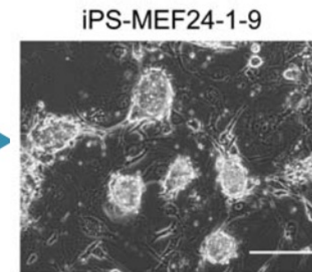
No drug-resistant colonies

All 24 candidate factors at the same time

MEF



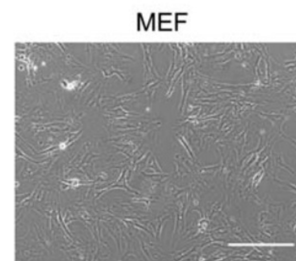
iPS-MEF24-1-9

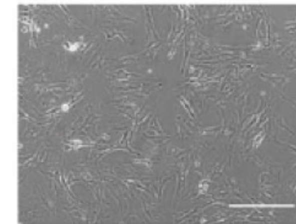


ES-like cells

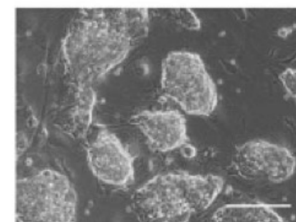
24 candidate factors: withdrawal of individual factors from the pool

MEF





The Factor is essential

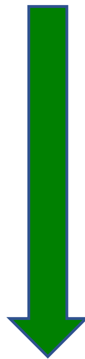


The Factor is <u>not</u> essential

Screening the 24 candidates

10 factors whose individual withdrawal from the bulk transduction pool resulted in no colony formation 10 days after transduction were identified

Combination of these 10 genes alone produced more ES cell-like colonies than transduction of all 24 genes did

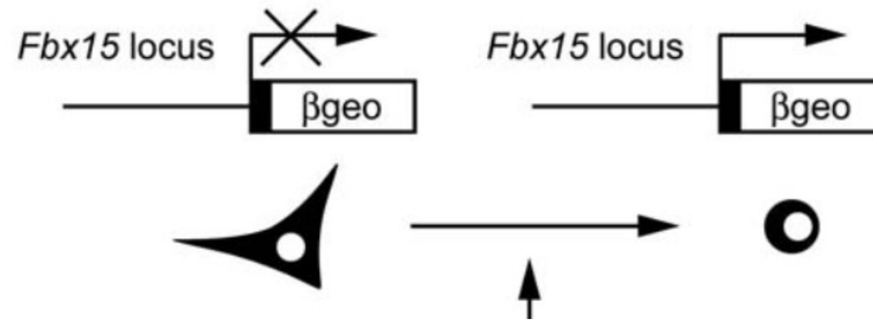


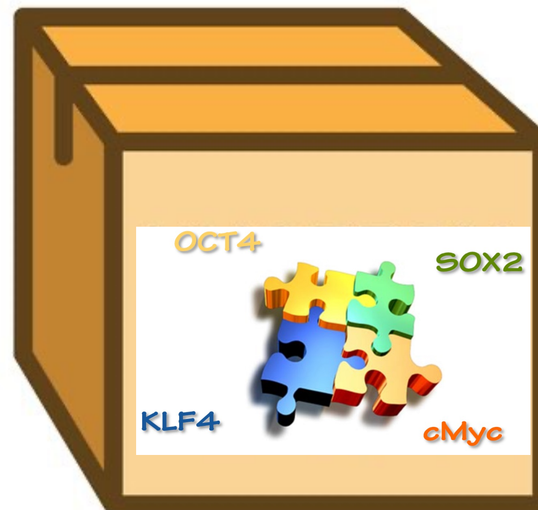
Withdrawal of individual factors from the 10-factor pool transduced into MEFs

The removal of 4 factors Oct3/4, Klf4, Sox2 and c-Myc significantly affected colony numbers. (Removal of the remaining factors did not significantly affect colony numbers).

Oct3/4, Klf4, Sox2, and c-Myc play important roles in the generation of iPS cells from MEFs.

Mouse Embryonic Fibroblasts ES cells





Pluripotency testing methods

Pluripotency Assay	Purpose	Length of Assay	Nature of Assay	Strength/Definitiveness of Assay
Colony morphology	Verify ESC colony-like morphology of clustered, border-definied colonies.	10 minutes	*in vitro*	Low
Immunohistochemistry	Stain for standard pluripotency markers such as Oct4, Tra-1-60, Sox2, Tra-1-81, Nanog and SSEA.	1–4 days	*in vitro*	Medium
Real-time PCR	Detect and quantify expression levels of selected pluripotency genes; limited by gene number.	4–6 hours (from RNA extraction to RT-PCR	*in vitro*	Medium-High
Embryoid body formation and analysis	Test differentiation capability of PSCs to tissues of all 3 germ layers *in vitro* or *in vivo*; should be coupled with relative quantification expression of gene-expression from the three germ-layers, which can be done by RT-PCR. Genes include: Nanog, Oct4, Sox2, and Klf4 (pluripotency markers); Ncam and NeuroD (ectodermal markers); Runx2, HNF4a, and Nkx2.5 (mesodermal markers); and Sox17, Albumin, Glut2, and Insulin (endodermal markers).	2–3 weeks	*in vitro* or *in vivo*	Medium-High
Microarray	A comprehensive measurement of gene expression levels.	1–2 days	*in vitro*	Medium-High
Teratoma formation	Test differentiation capability into all 3 germ layers *in vivo*.	1–2 months	*in vivo*	High

Pluripot

Pluripotency Assay				
Colony morphology	...olo ...ied			
Immunohistochemistry	Stain f... standa... Oct4, Tra-1-60, SSEA.			
Real-time PCR	Detect and quan... pluripotency ge...			
Embryoid body formation and analysis	Test differentiation of all 3 germ layers coupled with relati... of gene-expressio... which can be done Nanog, Oct4, Sox2 ... markers); Ncam a... markers); Runx2, HNF4a, and Nkx2.5 (mesodermal markers); and Sox17, Albumin, Glut2, and Insulin (endodermal markers).	2-		
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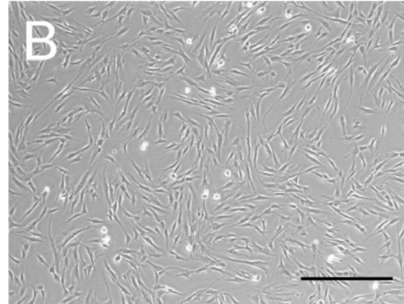
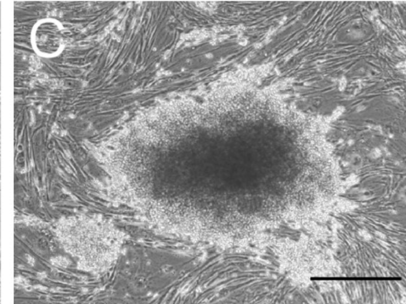
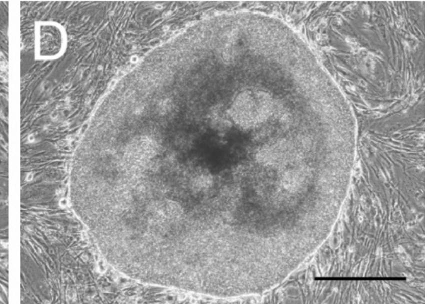
human dermal fibroblasts

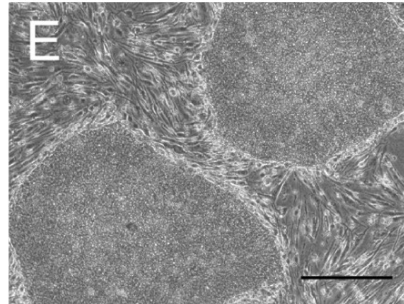


non-ES cell-like colony

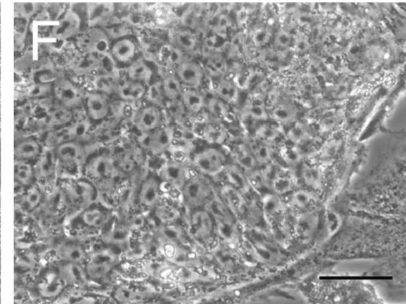


hES cell-like colony

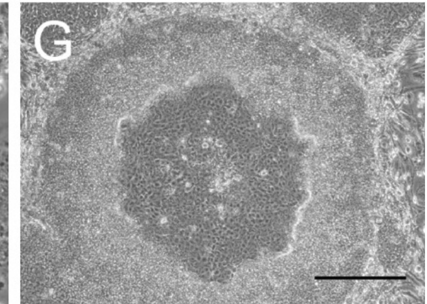




PS cell line at passage number 6



iPS cells with high magnification



Spontaneously differentiated cells in the center part of human iPS cell colonies

Takashi et al., (2007) Cell

Pluripotency testing methods

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Pluripotency Assay				
Colony morphology	Verify ESC borde def			
Immunohistochemistry				
Real-time PCR	Detec and pluripotenc			
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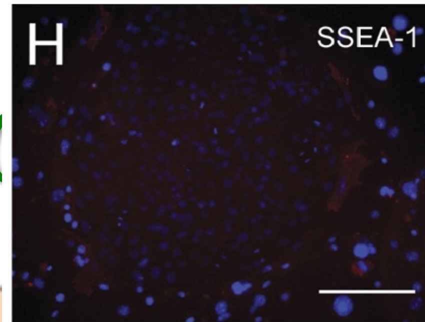

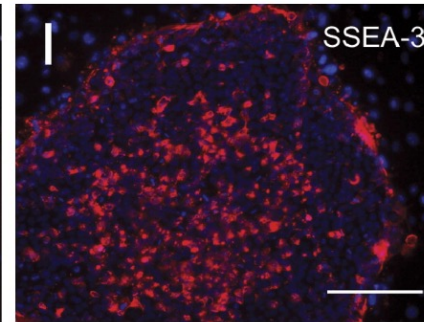

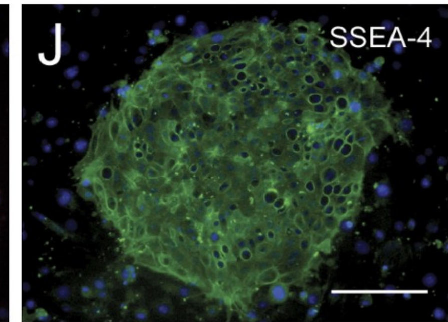

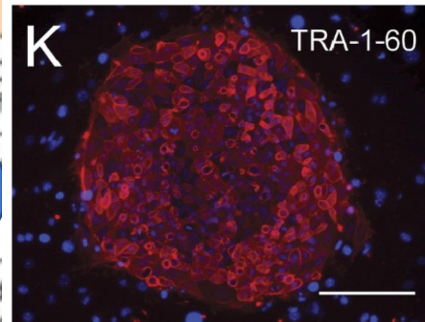

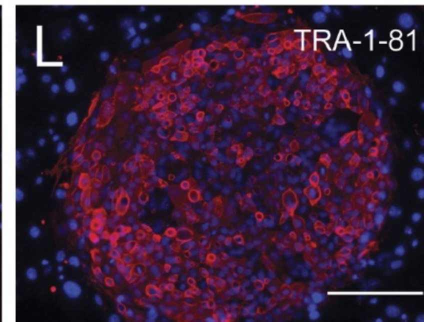

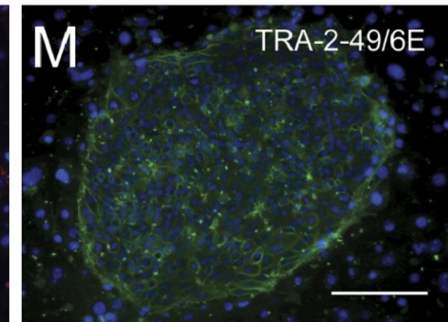

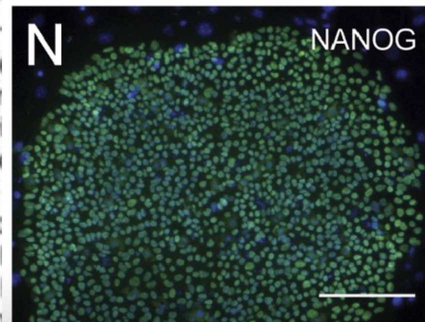

Immunocytochemistry for SSEA-1 (H), SSEA-3 (I), SSEA-4 (J), TRA-1-60 (K), TRA-1-81 (L), TRA-2-49/6E (M), and Nanog (N). Nuclei were stained with Hoechst 33342 (blue).

Takashi et al., (2007) Cell

Pluripotency testing methods

Pluripotency Assay	Purpose	Length of Assay	Nature of Assay	Strength/Definitiveness of Assay
Colony morphology	Verify ESC colony-like morphology of clustered, border-definied colonies.	10 minutes	*in vitro*	Low
Immunohistochemistry	Stain for standard pluripotency markers such as Oct4, Tra-1-60, Sox2, Tra-1-81, Nan[illegible] and SSEA.			
Real-time PCR	[illegible]			
Embryoid body formation and analysis	Test differentiation capability of PSC[illegible]issues of all 3 germ layers *in vitro* or *in vivo*[illegible]hould be coupled with relative quantification expression of gene-expression from the three germ-layers, which can be done by RT-PCR. Genes include: Nanog, Oct4, Sox2, and Klf4 (pluripotency markers); Ncam and NeuroD (ectodermal markers); Runx2, HNF4a, and Nkx2.5 (mesodermal markers); and Sox17, [illegible]umin, Glut2, and Insulin (endodermal mark[illegible]			
Microarray	[illegible]			
Teratoma formation	Test differentiation capability into all [illegible]rm layers *in vivo*.			

Examine whether ES cell marker genes were expressed in iPS cells. Used primers would amplify transcripts of the endogenous gene but not transcripts of the transgene.

Compared the global gene-expression profiles of ES cells and iPS cells using DNA microarrays

Pluripotency testing methods

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Microarray	A comprehensive measurement of gene expression levels.			
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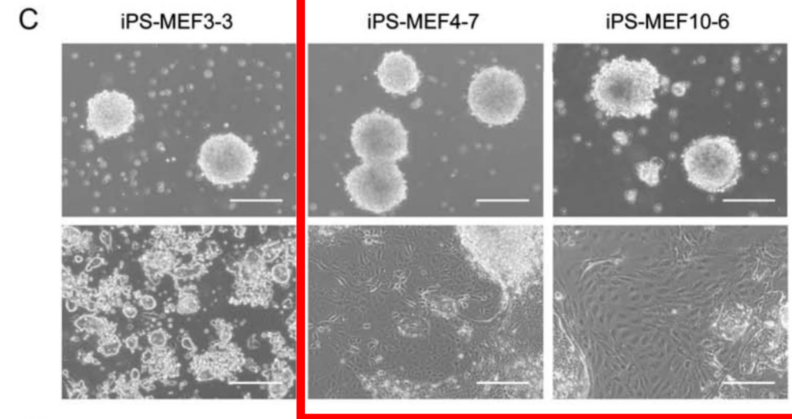
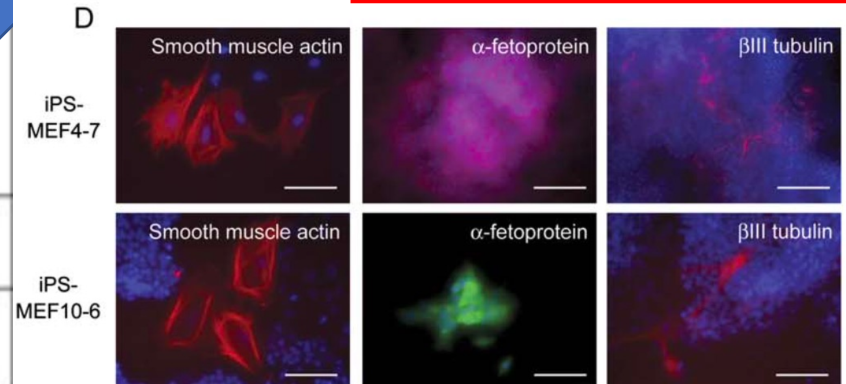
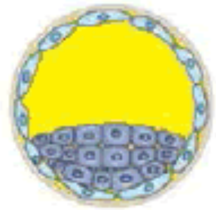
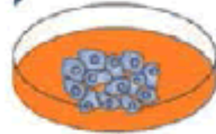


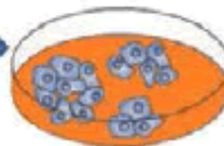


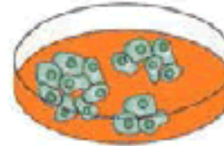
mesoderm marker | endoderm marker | ectoderm marker

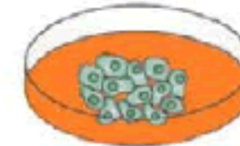
Teratoma formation











Teratoma assays are considered the gold standard for demonstrating differentiation potential of pluripotent ESC/iPSC

A variety of **animal strains** can be used. Immunodeficient strains (i.e., Nu/Nu nude, BC nude, or SCID for mice; Rowett nude or Athymic for rats), however, form teratomas at a higher incidence. There is a significant chance of cell rejection in non-immunocompromised models.

Pluripotency testing methods

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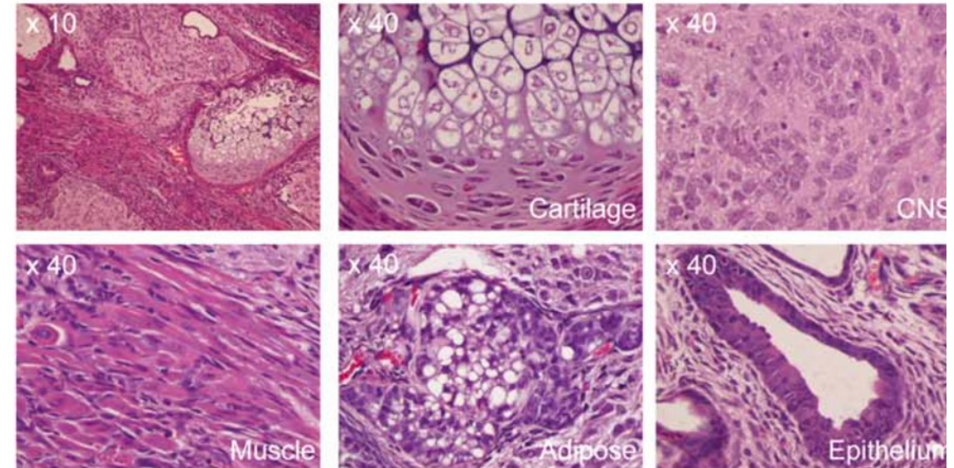
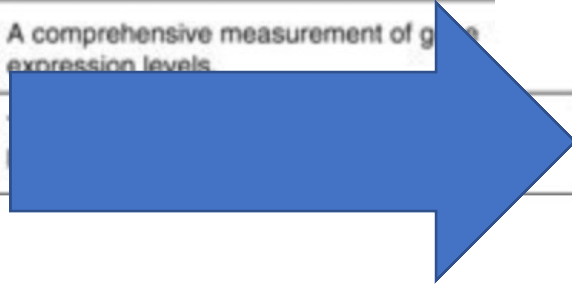
Teratoma formation				



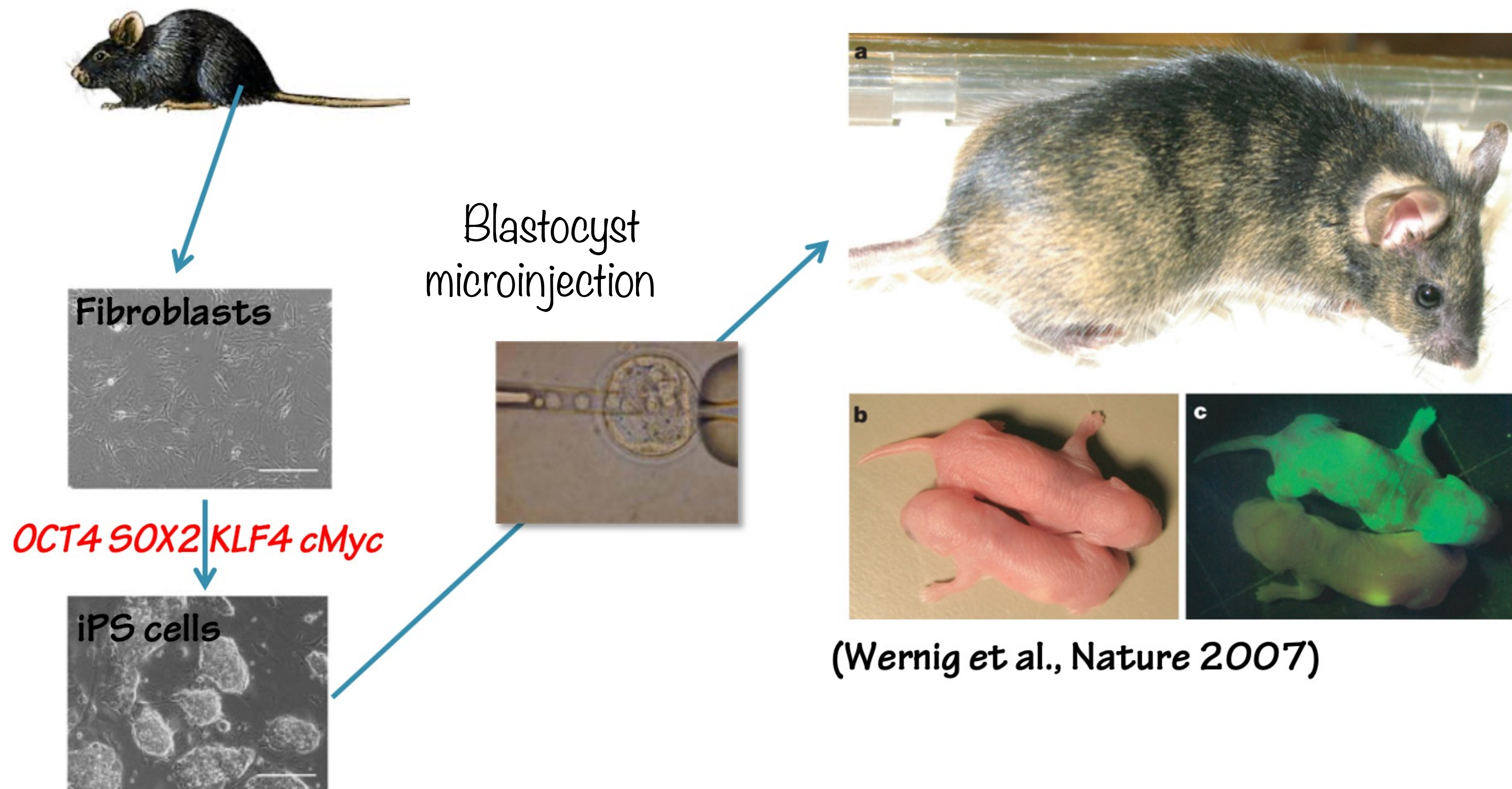


examined the pluripotency of iPS cells by teratoma formation

Pluripotency testing methods: chimeric mouse

2006=Inability to obtain live chimeric mice after blastocyst microinjection of iPS cells

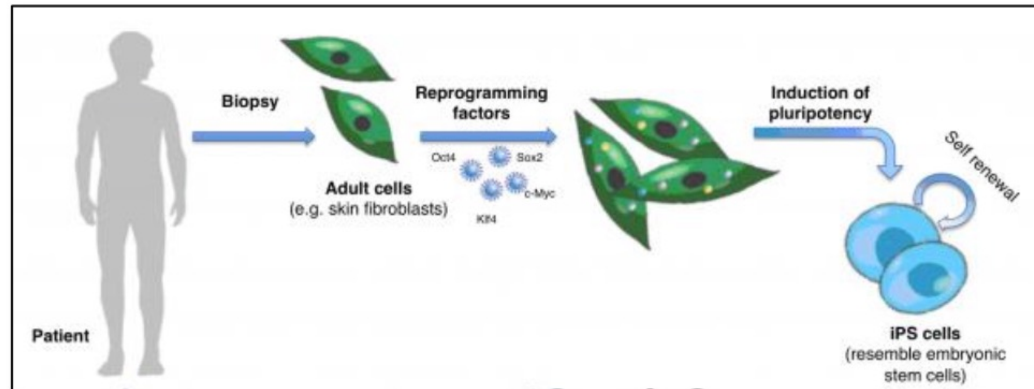
Mouse iPS cells can generate chimeric mice: their developmental potential is equivalent to the potential of Embryonic Stem cells.

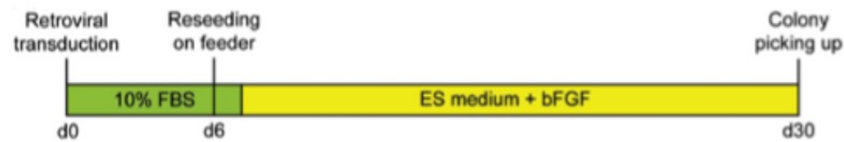


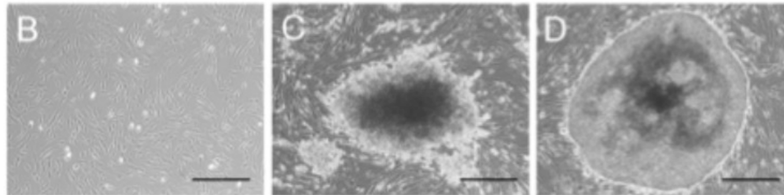
(Wernig et al., Nature 2007)

iPS cells can be derived from human cells

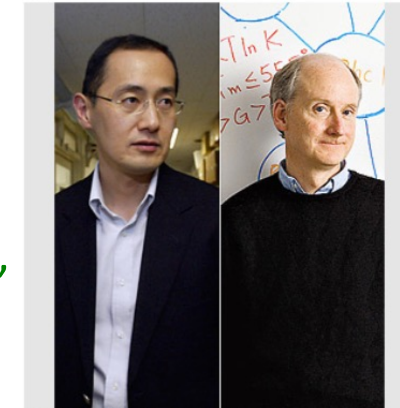
(Takahashi et al., Cell 2007; Yu et al., Science 2007)



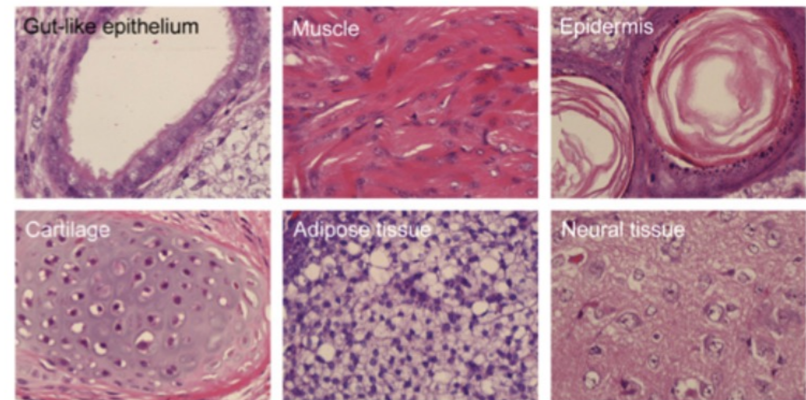




Oct3/4, Sox2, Klf4, and c-Myc

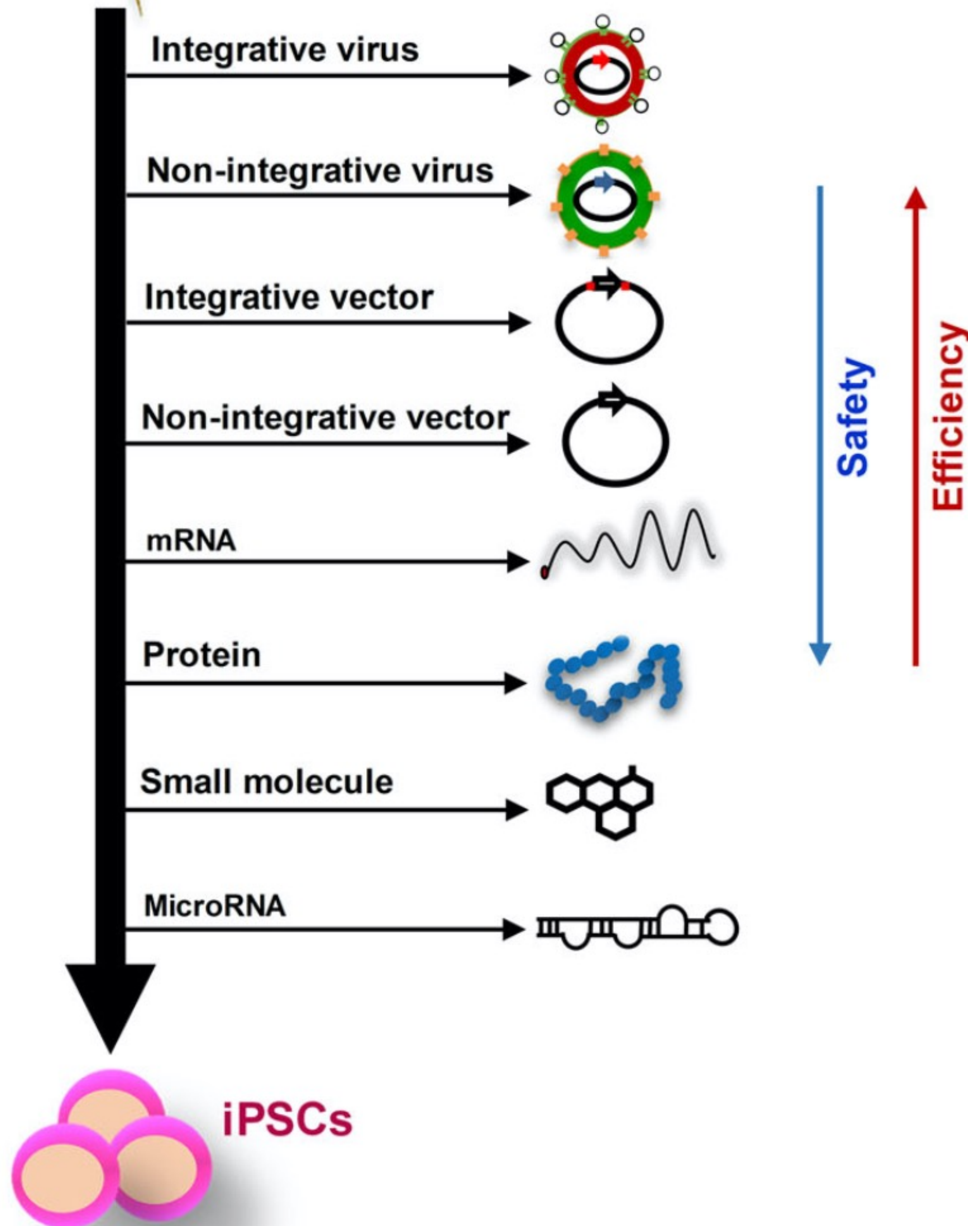


Oct4, Sox2, NANOG, and LIN28



Formation of teratomas (most stringent pluripotency test for human cells)

Generation of iPSCs.



Various methods and approaches are used to convert somatic cells into iPSCs. Integrative methods such as integrative viruses and vectors provide the highest reprogramming efficiency but the lowest safety. In contrast, non- integrative approaches such as the use of small molecules and microRNAs tend to have a less reprogramming efficiency. Notably, episomal vectors, which do not integrate with the host cell's genome, appear to provide both a high efficiency of iPSC generation and sufficient degree of safety. Although all the illustrated approaches could potentially be used to produce iPSCs for applications such as basic research, drug screening, and disease modeling, genomic integration should be avoided for generation of clinical-grade iPSCs.

Moradi et al. Stem Cell Research & Therapy (2019) 10:341

COMMERCIAL KITS…

MERCK

Various methods utilizing viruses, DNA, RNA, miRNA and protein have been developed to generate integration-free induced pluripotent stem cells (iPSCs).
Disadvantages to existing methods include: (1) low reprogramming efficiency (i.e. DNA and protein), (2) a lengthy requirement for negative selection and subcloing steps to remove persistent traces of the virus (i.e. Sendai virus)1 and (3) for daily transfections of four individual in vitro generated mRNAs over a 14 day period (i.e. mRNA based)

Millipore's **Simplicon RNA** Reprogramming Kit is a safe and efficient method to generate integration free, virus-free human iPS cell using a single transfection step. The technology is based upon a positive strand, single-stranded RNA species derived from non-infectious (non-packaging), self-replicating Venezuelian equine encephalitis (VEE) virus3. The Simplicon RNA replicon is a synthetic in vitro transcribed RNA expressing all four reprogramming factors (**OKS-iG; Oct4, Klf4, Sox2 and Glis1**) in a **polycistronic transcript that is able to self-replicate for a limited number of cell divisions.**

Thermo Fisher
SCIENTIFIC

Episomal vectors are a well-described system for producing transgene-free, virus-free iPSCs, providing a source of iPSCs for all stages of your pluripotent stem cell research. Other reprogramming methods, such as lentivirus, contain transgenes that can integrate into the host genome, potentially disrupting the genome or causing unpredictable results.

As oriP/EBNA1 vectors, these **episomal vectors** contain **5 reprogramming factors** (**Oct4, Sox2, Lin28, Klf4, and L-Myc**) and replicate extra-chromosomally only once per cell cycle. At this replication rate, the episomes are lost at a rate of approximately 5% per cell generation. This system shows enhanced iPSC generation through p53 suppression, and the inclusion of L-Myc has been shown to be more potent and specific then c-Myc during human iPSC generation

Retrovirus cocktail: Human induced pluripotent stem cells (iPSCs) can be derived from somatic cells through a reprogramming process driven by ectopic expression of a defined set of reprogramming factors: **Oct4, Sox2, Klf4 and c-Myc.**

A REPROCELL BRAND

Third Generation **RNA** Kit for Cellular Reprogramming of **Fibroblasts, Blood, and Urine (Oct4, Sox2, Klf4, cMyc, Nanog, Lin28** reprogramming factors)

Generation of iPSCs.

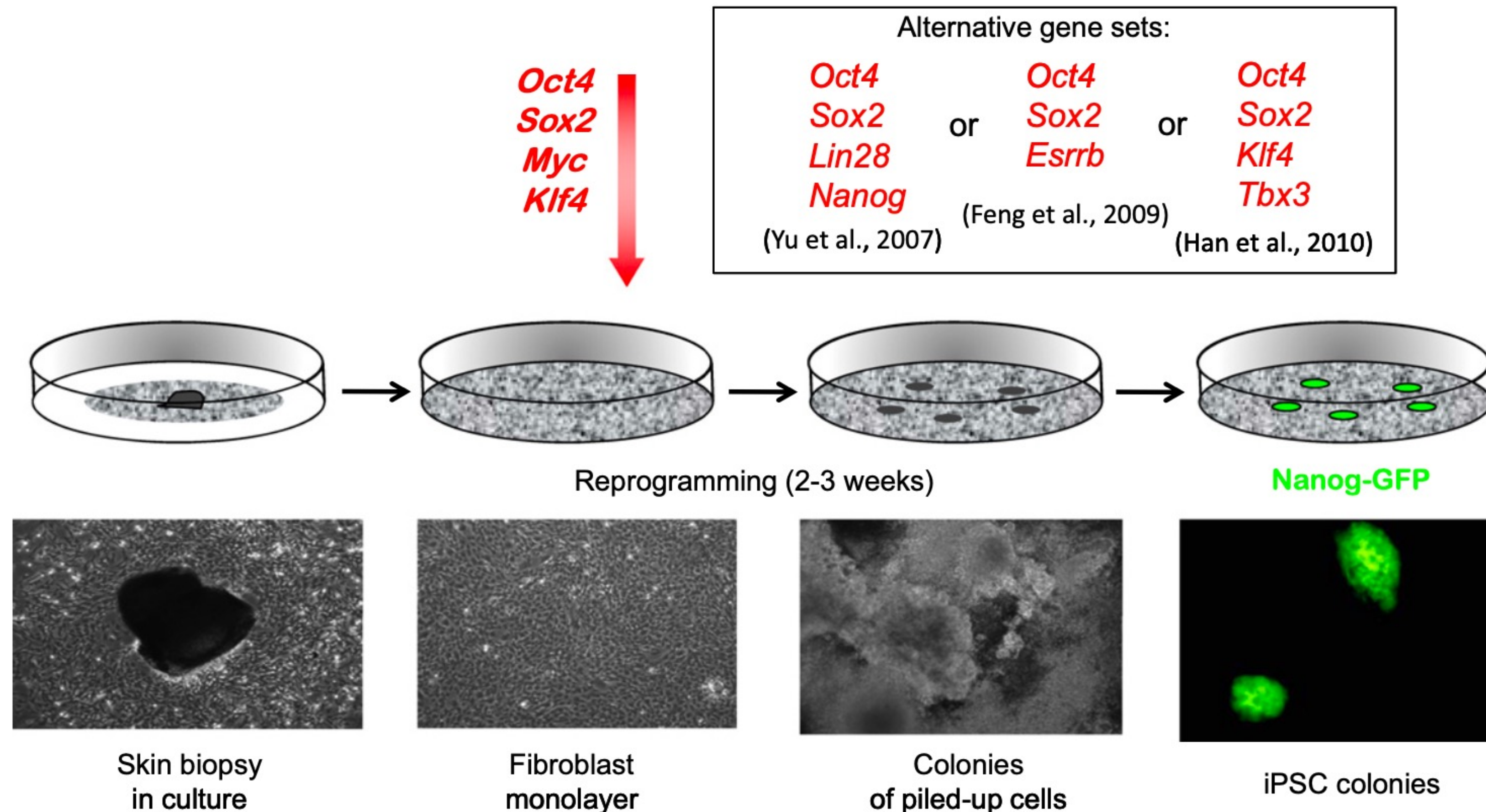
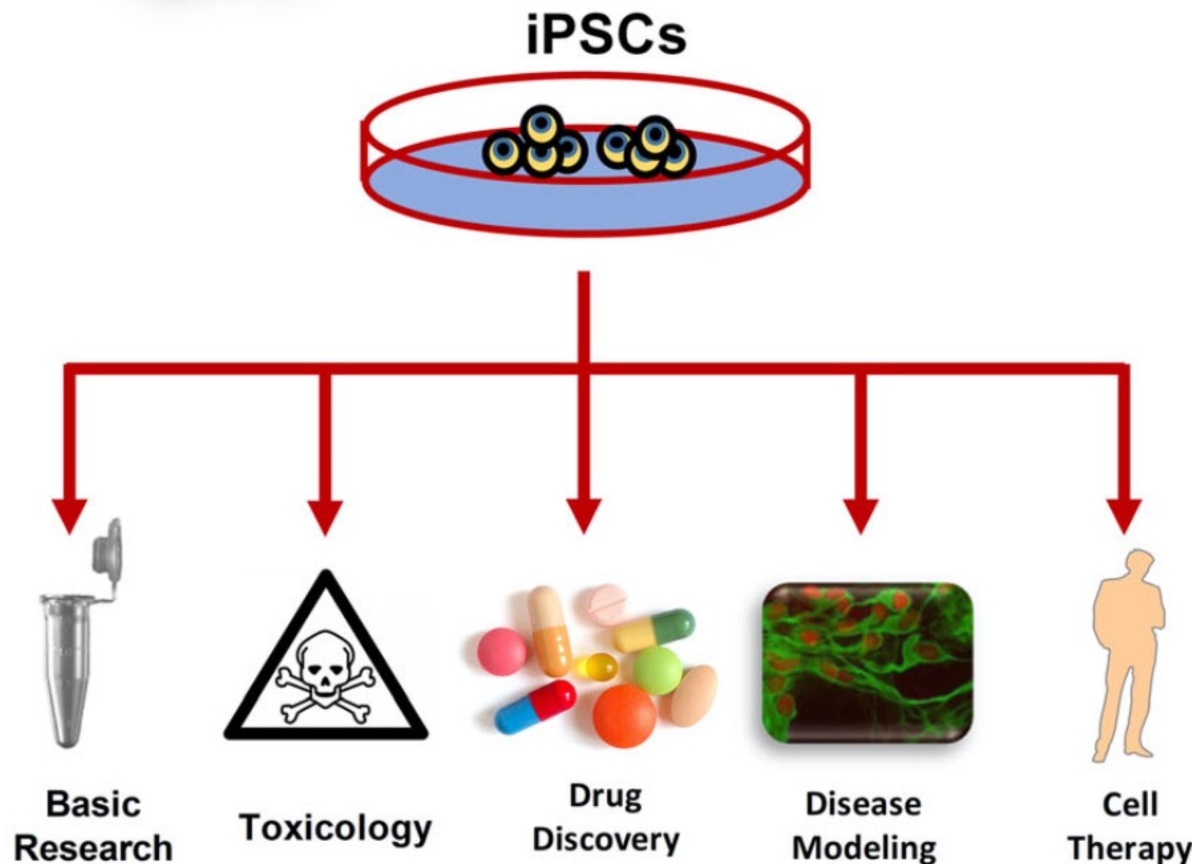


Figure 4. Reprogramming to Pluripotency

The figure outlines the methodology developed by Takahashi and Yamanaka for mice and humans (Takahashi et al., 2007; Takahashi and Yamanaka, 2006) as modified by Maherali et al. (2008). The micrographs (courtesy of Matthias Stadtfeld and Konrad Hochedlinger) illustrate the changes in morphology of skin-derived fibroblasts infected with retroviruses carrying *Oct4*, *Sox2*, *Klf4*, and *Myc*, with a *Nanog*-driven GFP reporter for iPSC formation. The process is very inefficient, typically occurring in less than 1% of the cells. A selection of alternative combinations of transcription factors capable of generating human and mouse iPSCs are indicated in the box (Feng et al., 2009; Han et al., 2010; Yu et al., 2007, 2009).

Thomas Graf, Cell Stem Cell, 2011

Applications of iPSCs.



Because of immortality and immense differentiation potential, iPSCs have all the potential biomedical applications of ESCs. They can be used to model pluripotency and multi-lineage differentiation in vitro, screen and discover new drugs, and establish disease models in a dish. iPSCs also hold a great potential to be used for replacing diseased or lost tissues, which needs specific considerations to provide safe, clinical-grade cells for transplantation into patients

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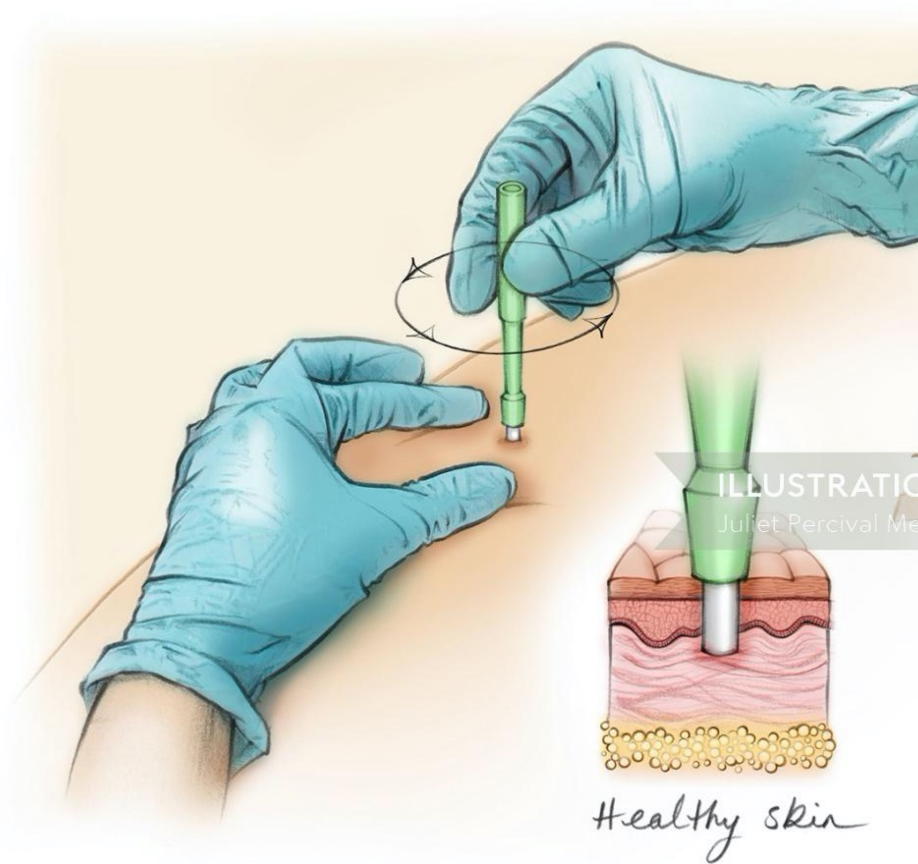
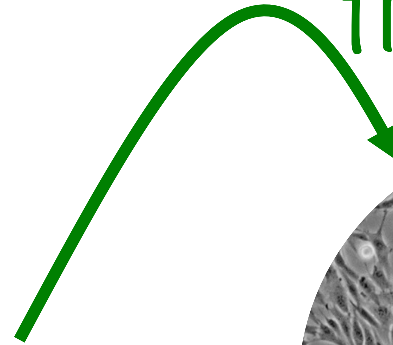
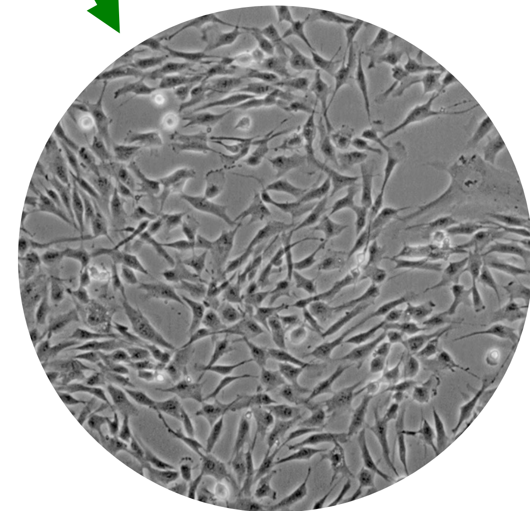
Punch Biopsy





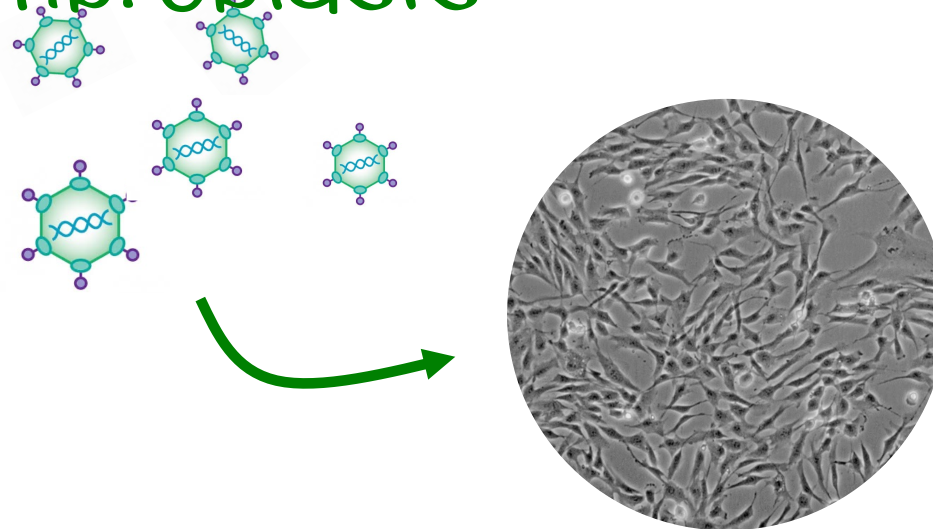
Primary dermal fibroblasts



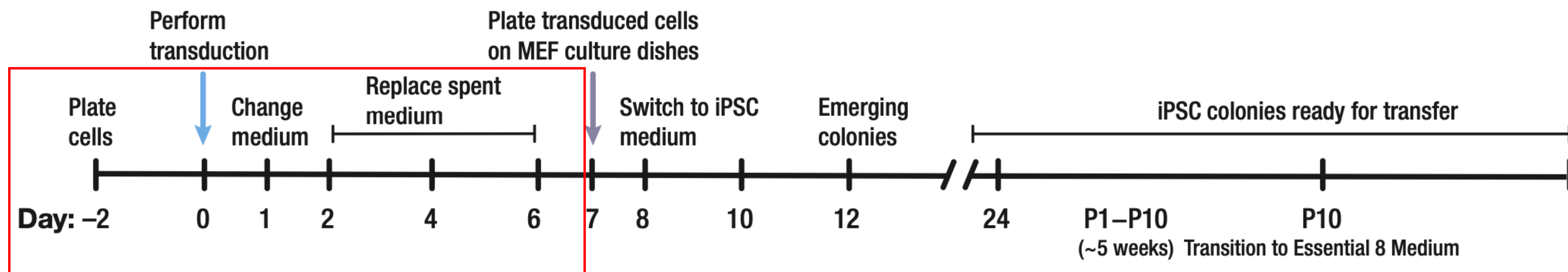
Viral transduction of primary dermal fibroblasts

Day 1-7

Dermal fibroblasts are transduced with single lentiviral vector constitutively expressing the four human reprogramming factors, OCT4, KLF4, SOX2 and cMYC (hSTEMCCA) (Somers et al., 2010).

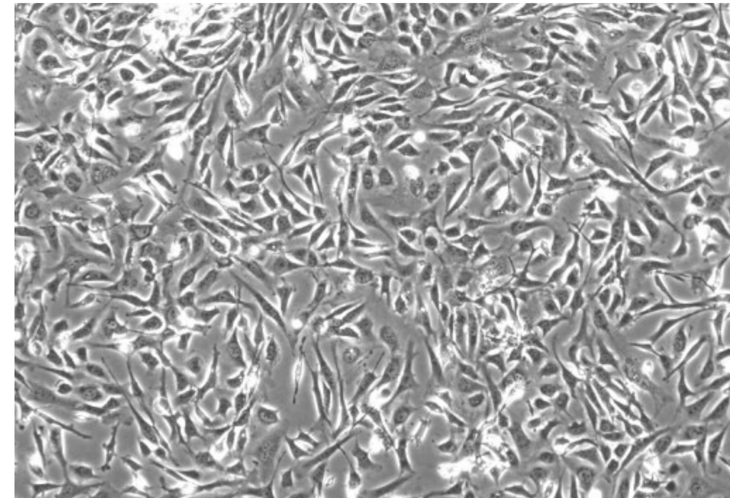


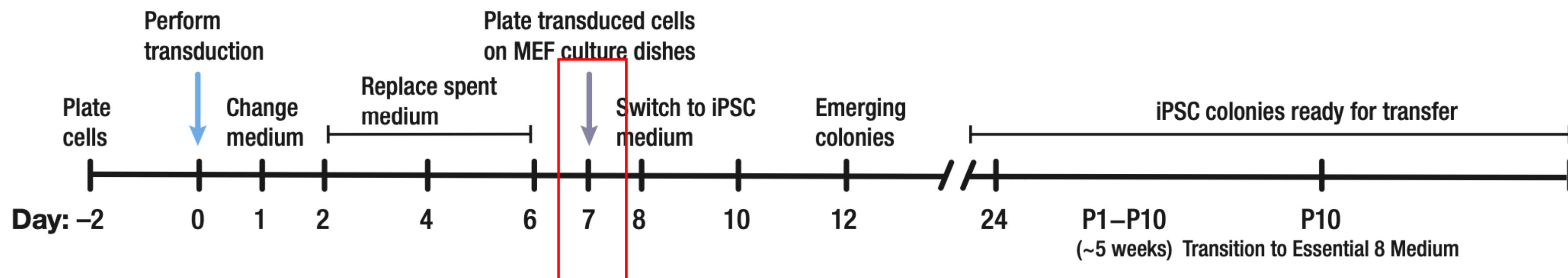
After reprogramming, single iPSC-like colonies with uniform flat morphology and defined borders were selected for expansion as individual clones.



Mouse (ICR) Inactivated Embryonic Fibroblasts

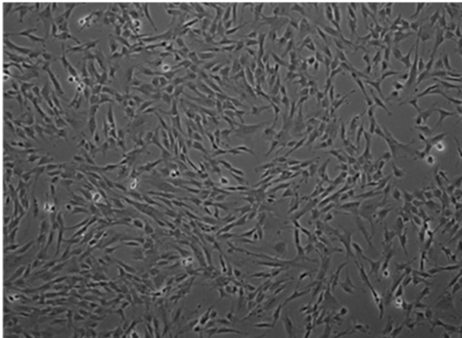
Inactivated Mouse Embryonic Fibroblasts (MEFs) are used as feeder layers for culturing iPSCs in their undifferentiated state. MEFs are mitotically inactivated (by γ-irradiation or mitomycin C treatment). The growth-arrested feeder layer supports the iPSC culture by providing nutrients, growth factors, and matrix components, and it enables PSCs to survive and proliferate more readily in culture.



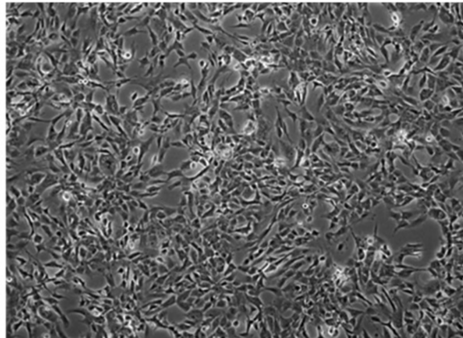


Let's take a close-up look...

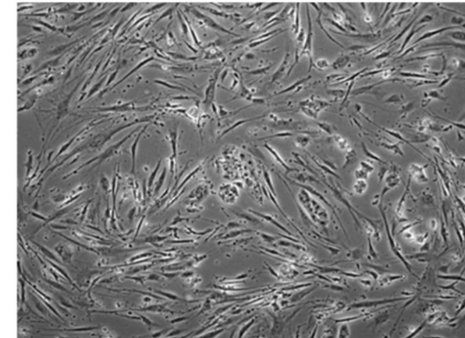
Fibroblasts before transduction (5X)



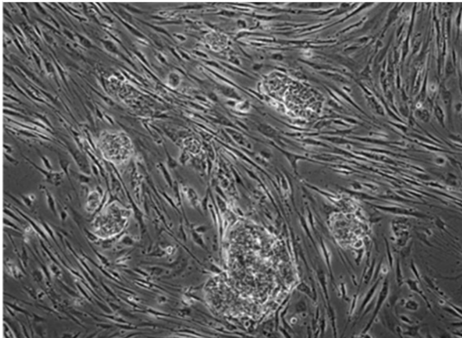
2 days posttransduction



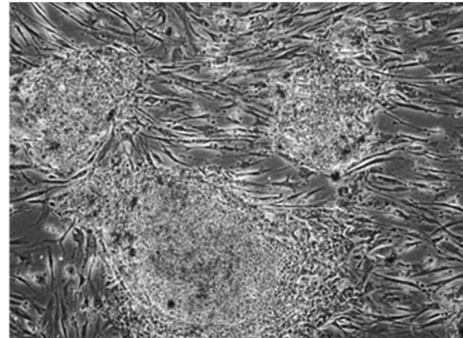
10 days posttransduction



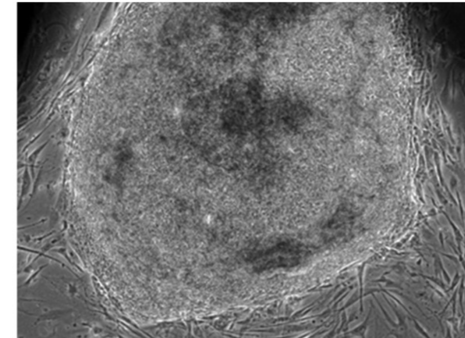
14 days posttransduction (5X)

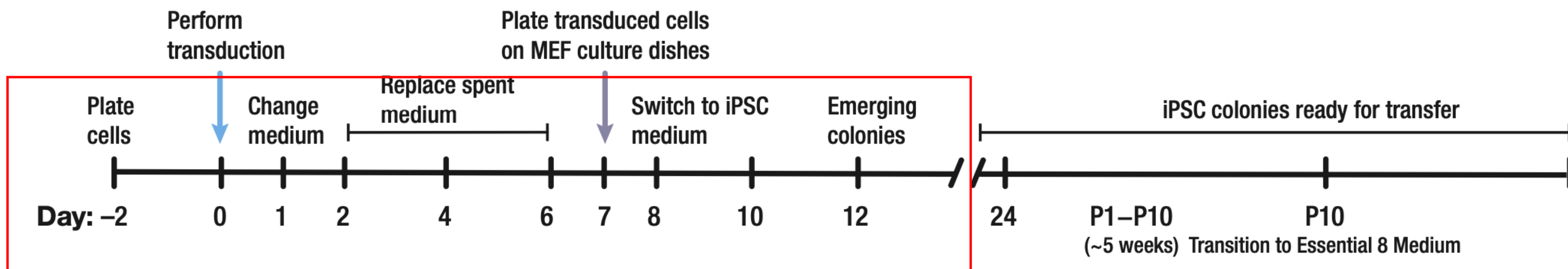


18 days posttransduction

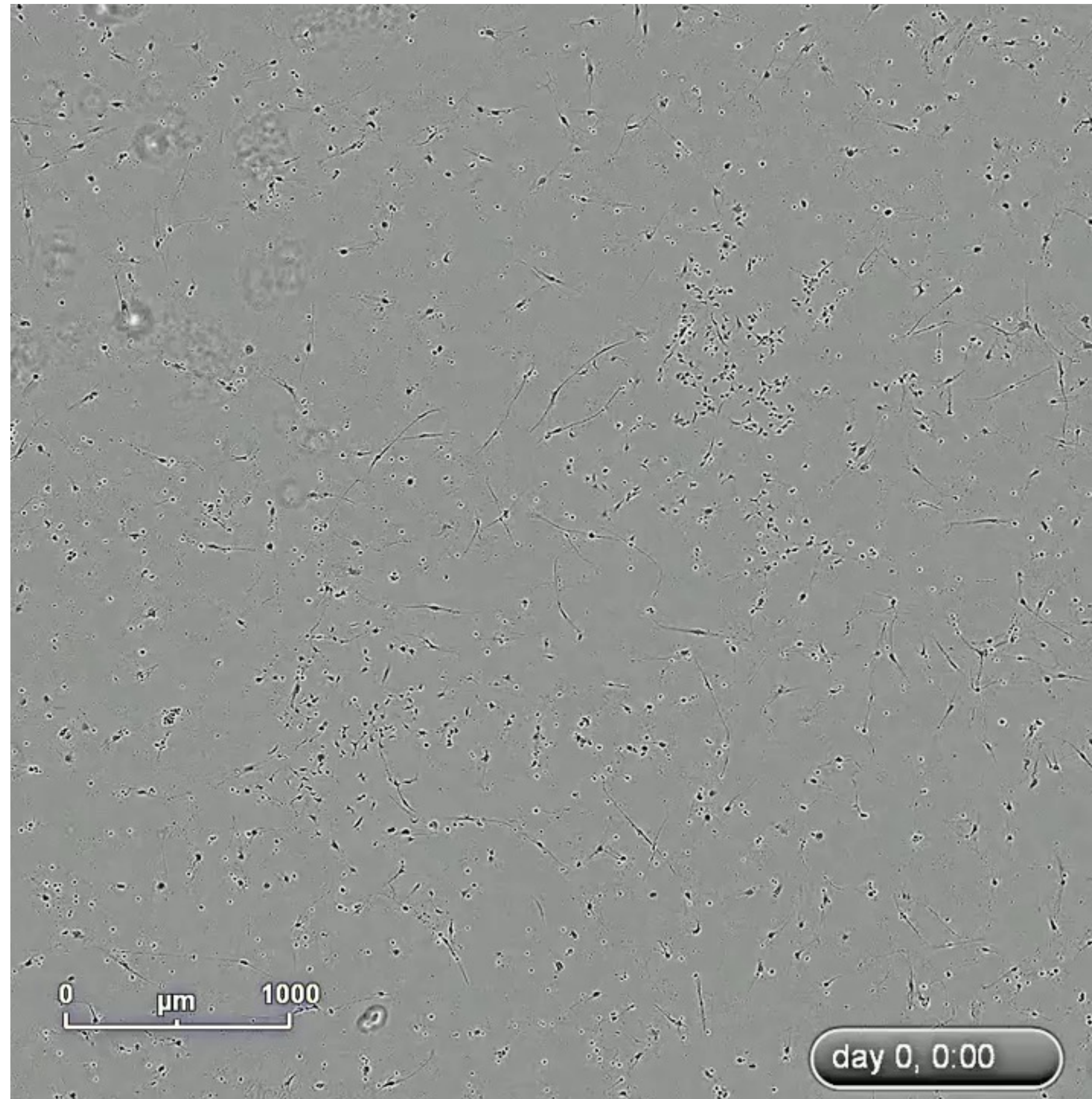


21 days posttransduction

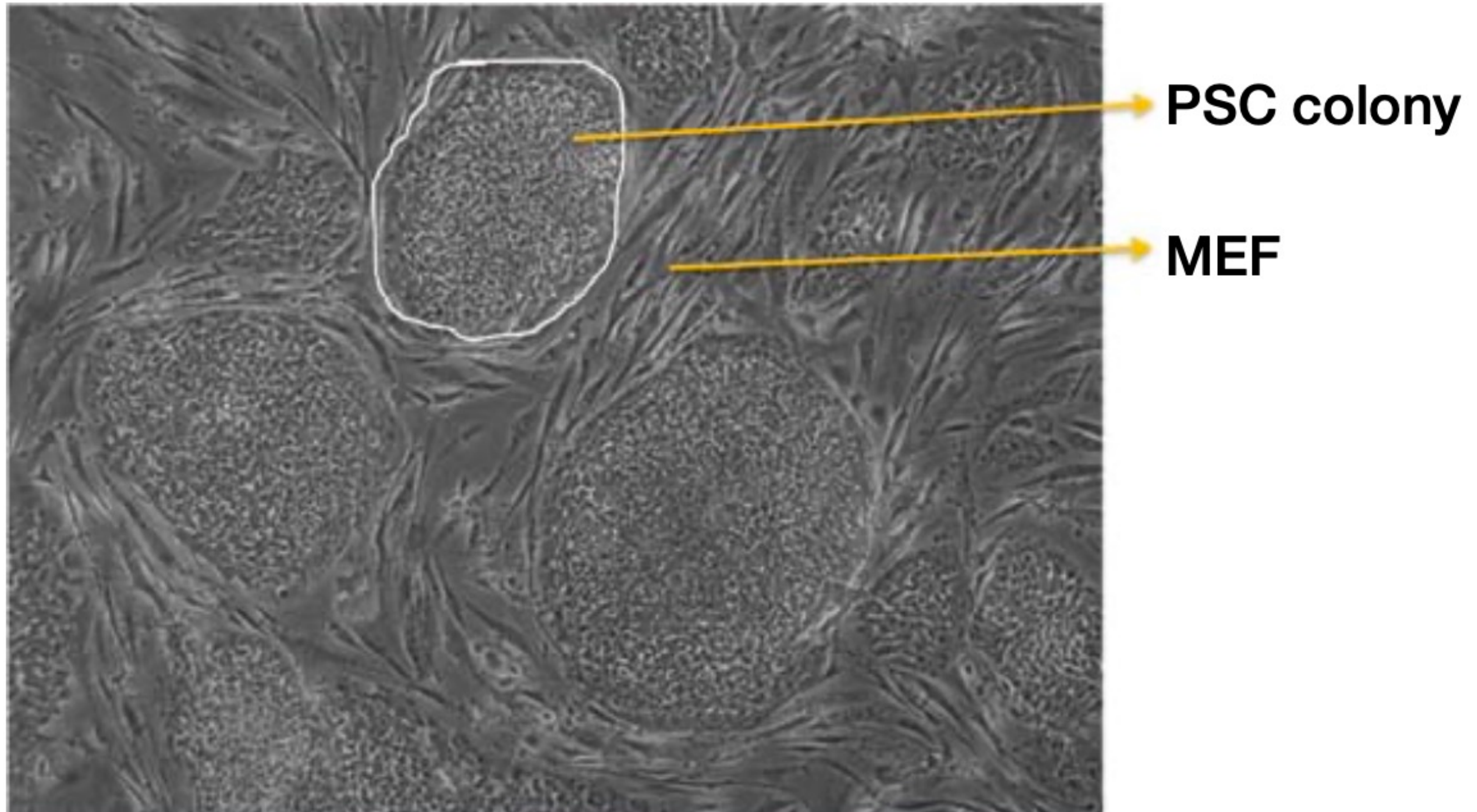




Human fibroblast reprogramming



iPSCs cultured on mitotically inactivated MEF feeder layer

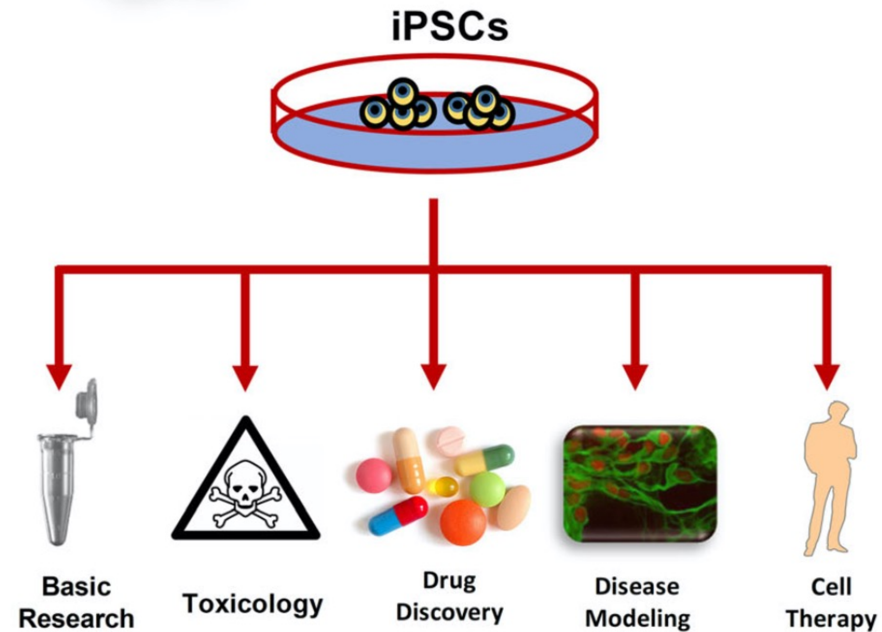


Applications of iPSCs: news from our lab

Basic research

Disease modelling

Drugs discovery



Informative talk given by Yamanaka:

http://www.youtube.com/watch?v=AD1sZU1yk-Y

https://www.youtube.com/watch?v=AD1sZU1yk-Y&t=14s