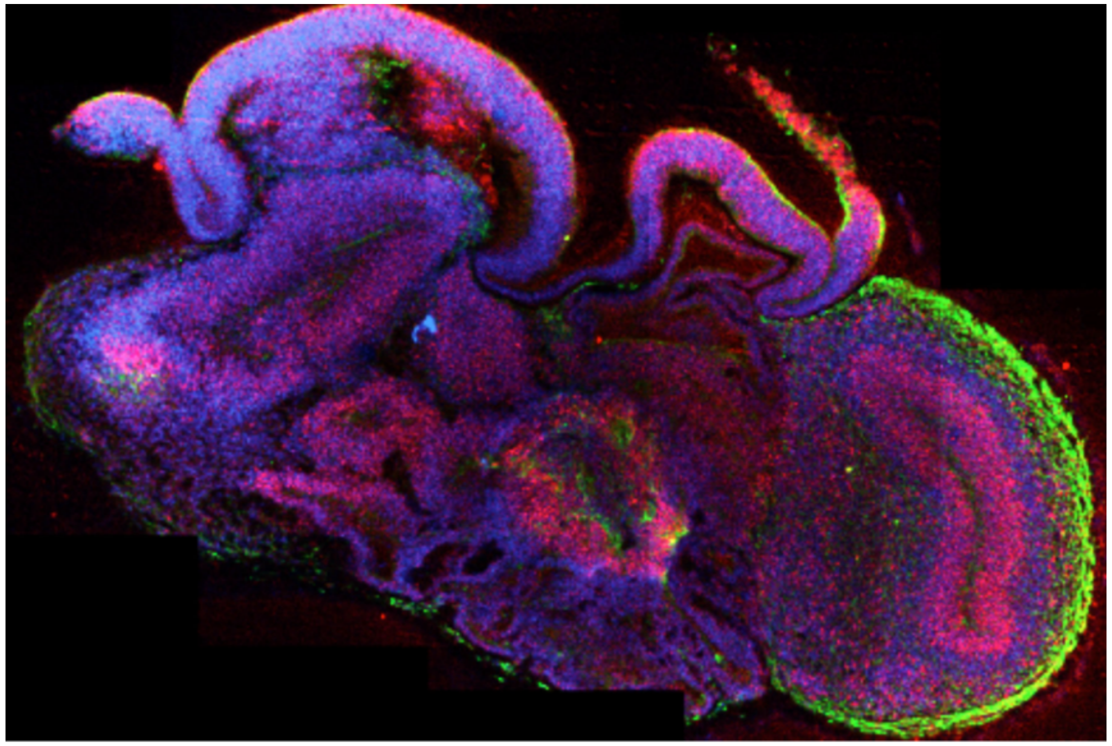
Induced Pluripotent Stem Cells (iPSCs)



"Mini brain"

James Oakley
Literature Talk
05/31/2022

Somatic Cell Nuclear Transfer (SCNT)

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
** PPL Therapeutics, Roslin, Midlothian EH25 9PP, UK*

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

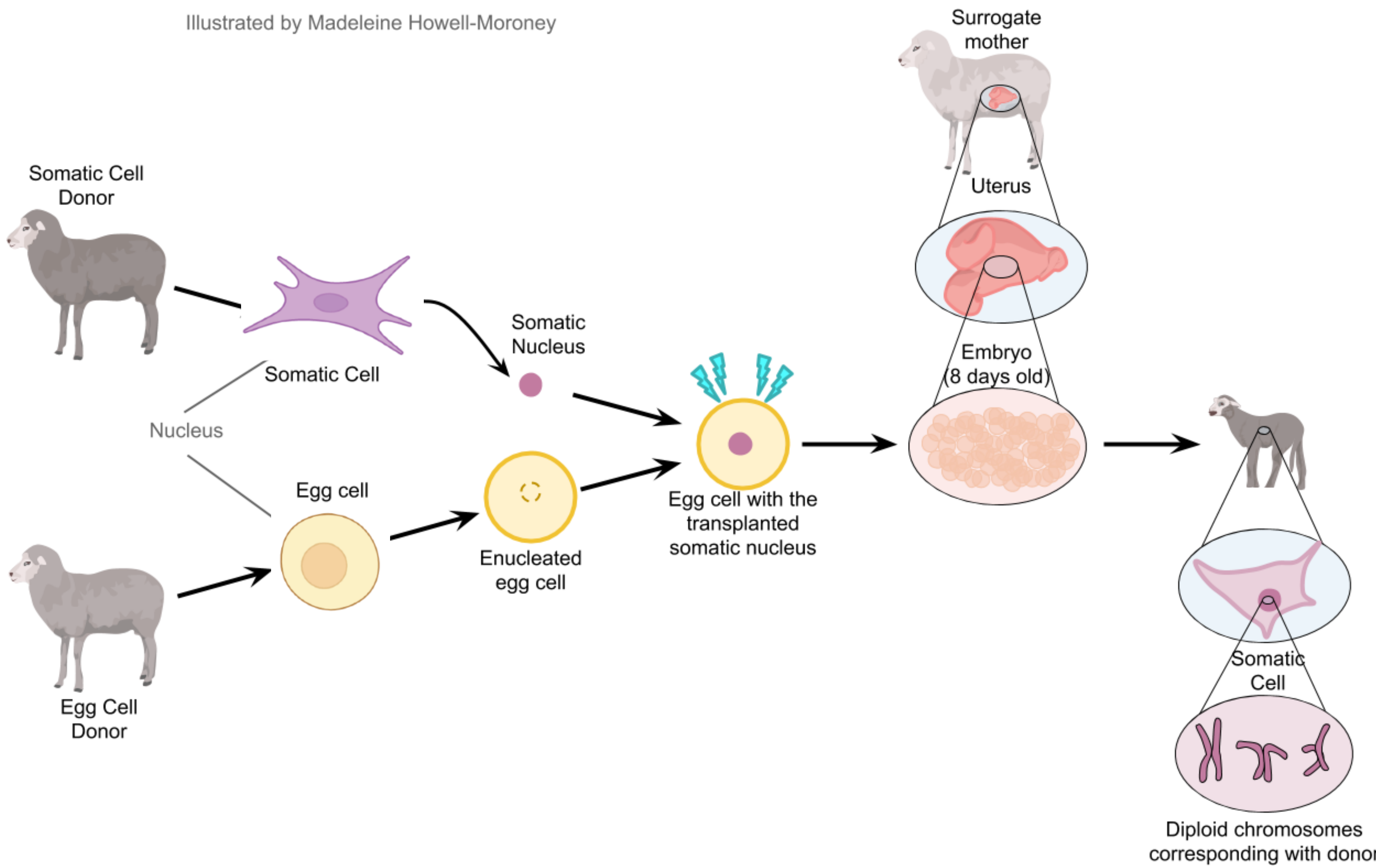
1997: Dolly the Sheep

first mammal cloned from an adult somatic cell

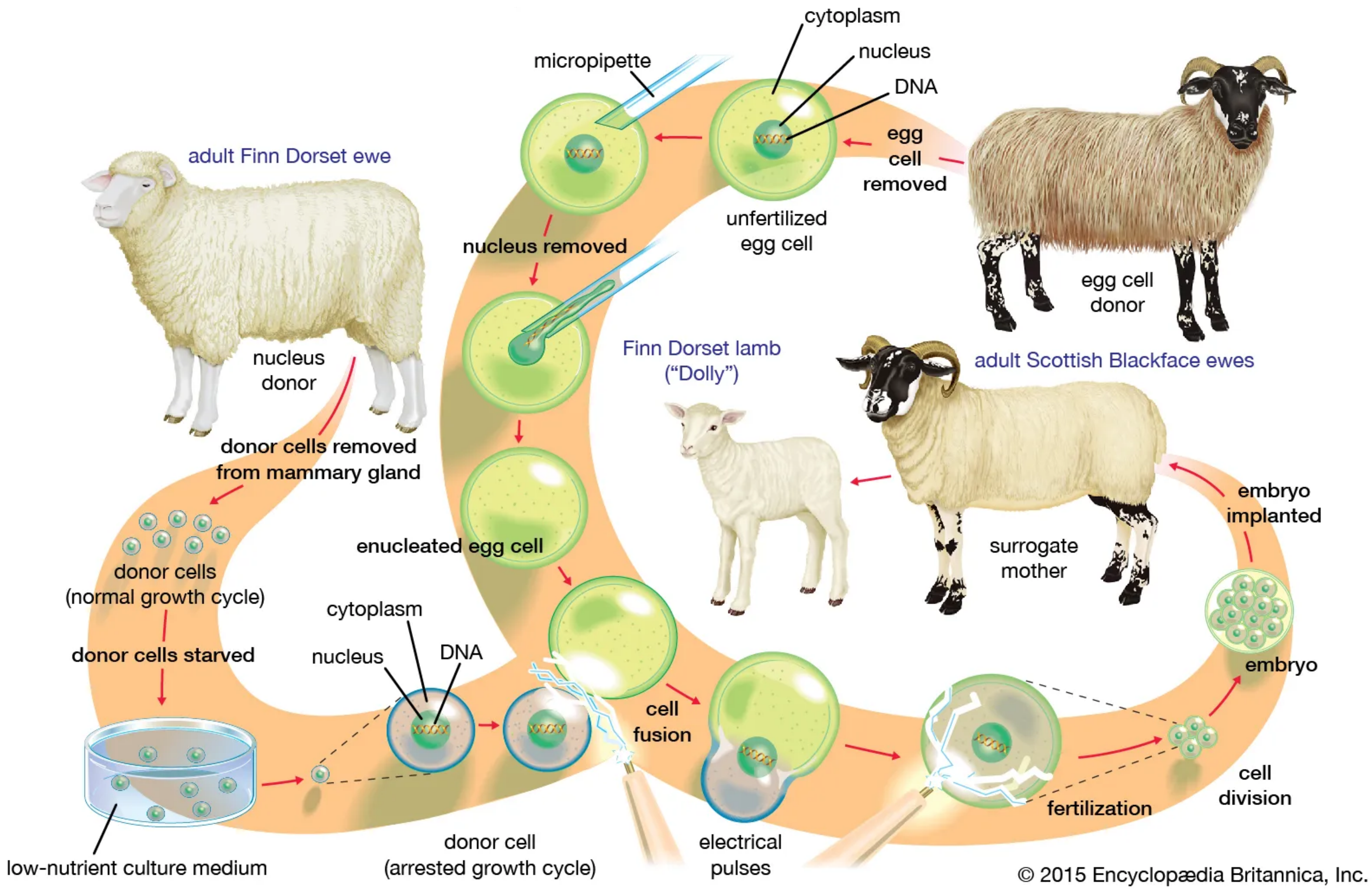
Wilmut, I.; Schnieke, A. E.; McWhir, J.; Kind, A. J.; Campbell, K. H. S. *Nature* **1997***, 385,* 810-813.

Somatic Cell Nuclear Transfer (SCNT)

SCNT: cloning via transfer of nuclei into enucleated oocyte



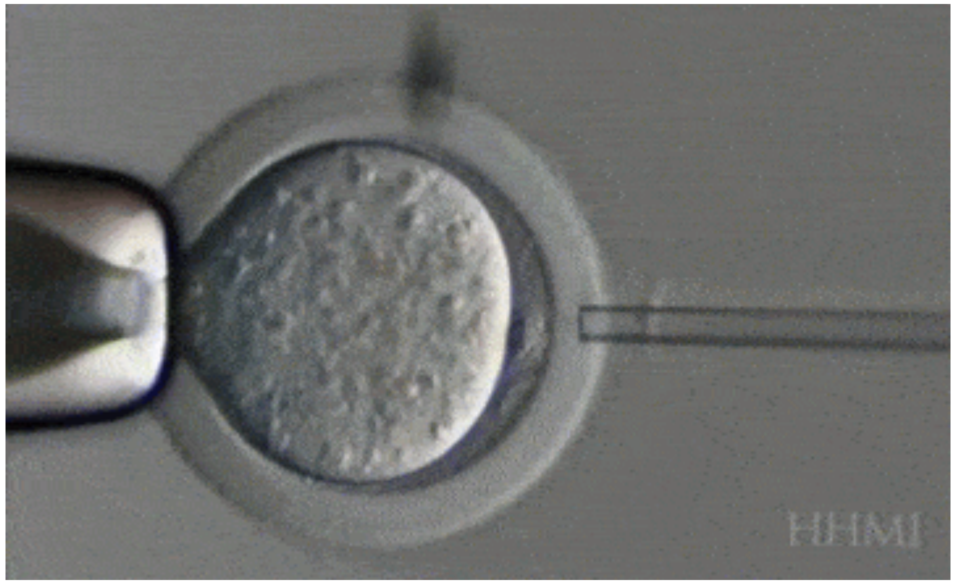
Somatic Cell Nuclear Transfer (SCNT)



Somatic Cell Nuclear Transfer (SCNT)

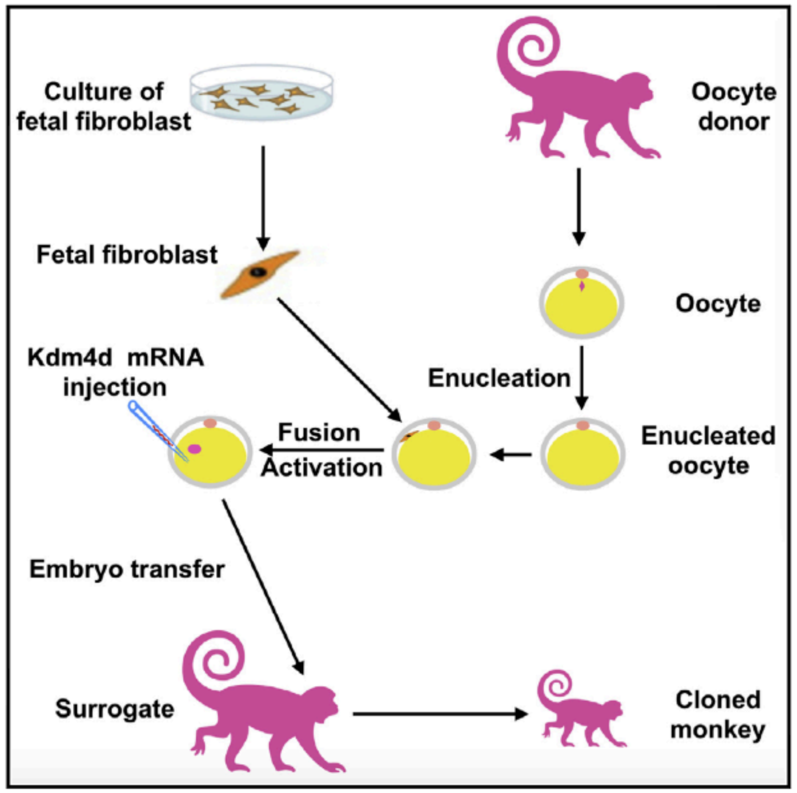
Removal of egg cell nucleus

Injection of somatic cell nucleus

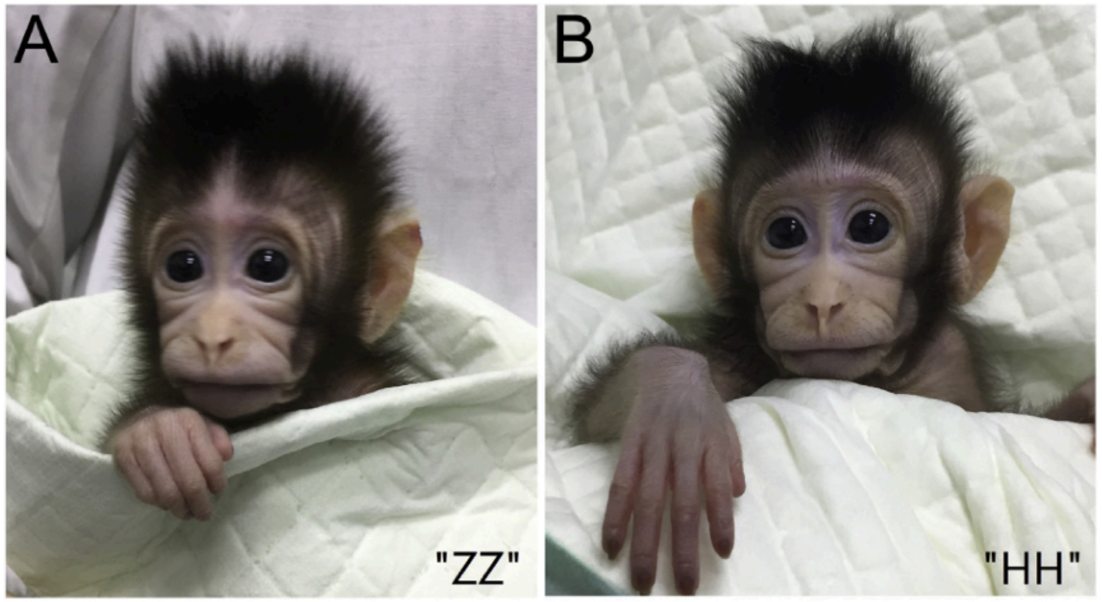


Somatic cell nuclear transfer via glass pipettes

First Cloning of Primates from Somatic Cells



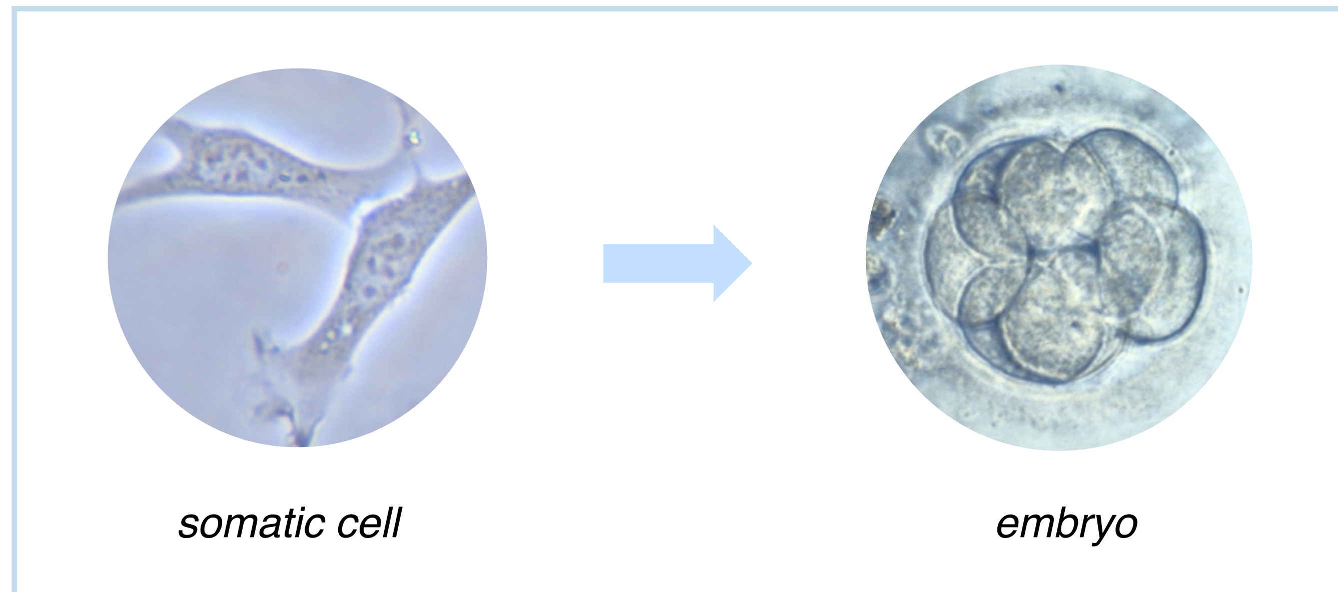
"Zhong Zhong" *"Hua Hua"*



2018: Successful cloning of Macaque monkeys from fetal fibroblasts

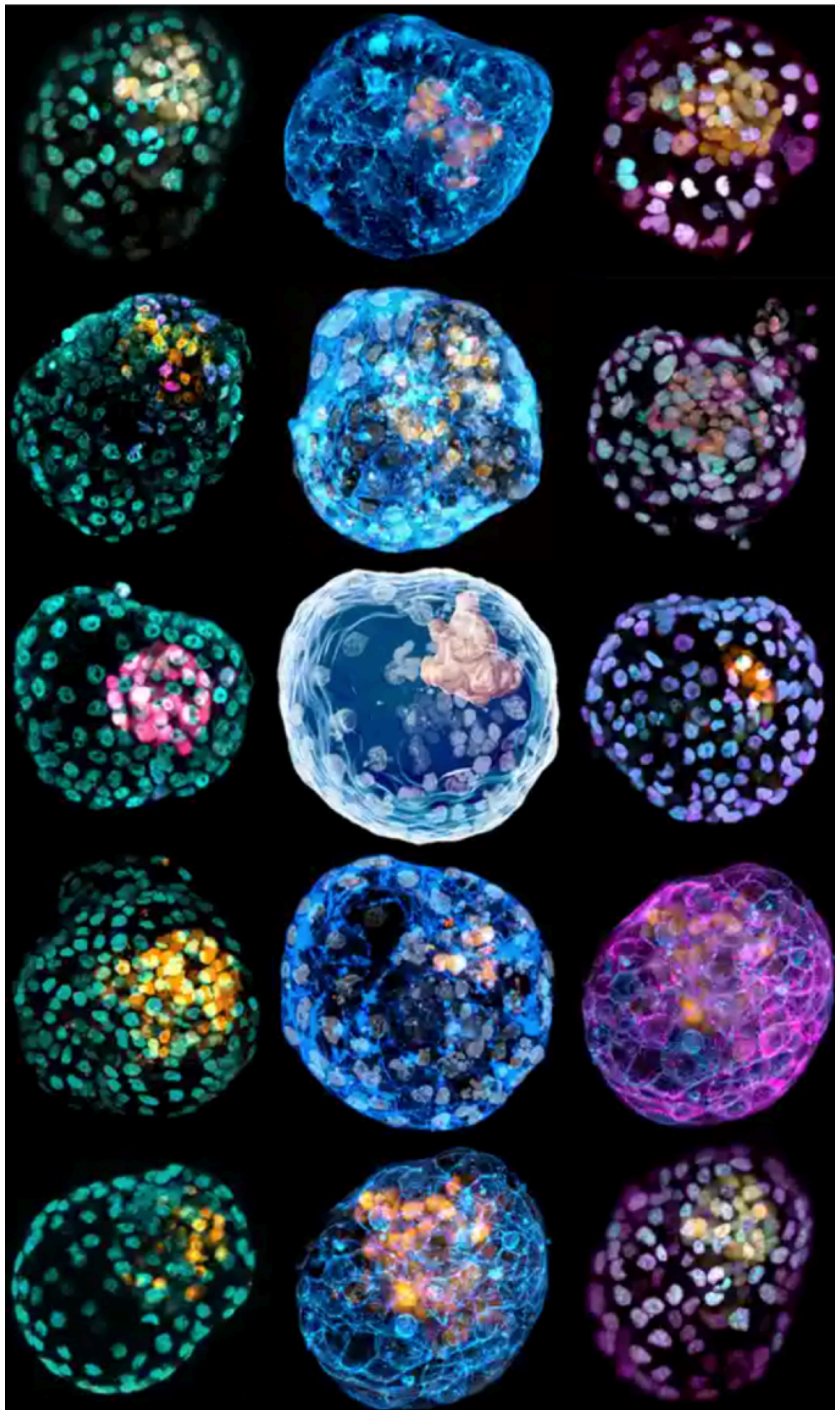
Liu, Z.; Cai, Y.; Wang, Y. *et al. Cell* **2018***, 172,* 881-886.

First Cloning of Primates from Somatic Cells



The genome of adult cells can be reprogrammed to an embryonic state

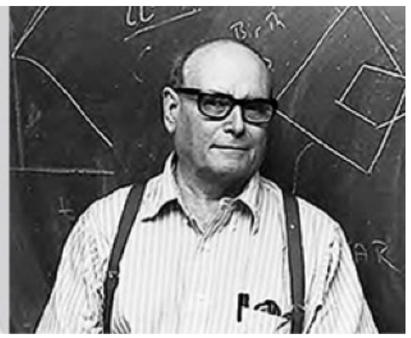
Presentation Overview



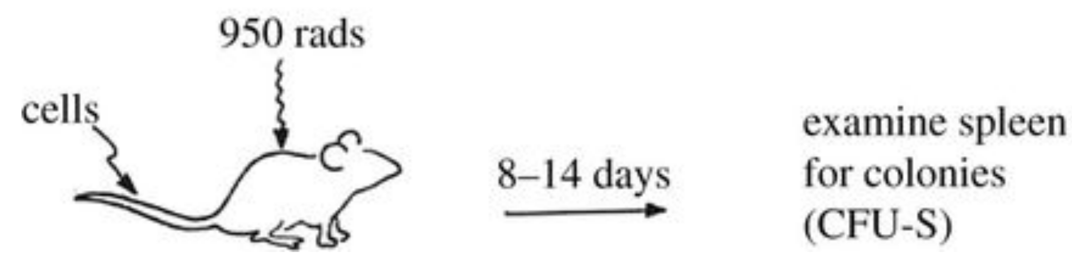
Human blastocysts organoids (iBlastoids) derived from iPSCs

Discovery of Stem Cells

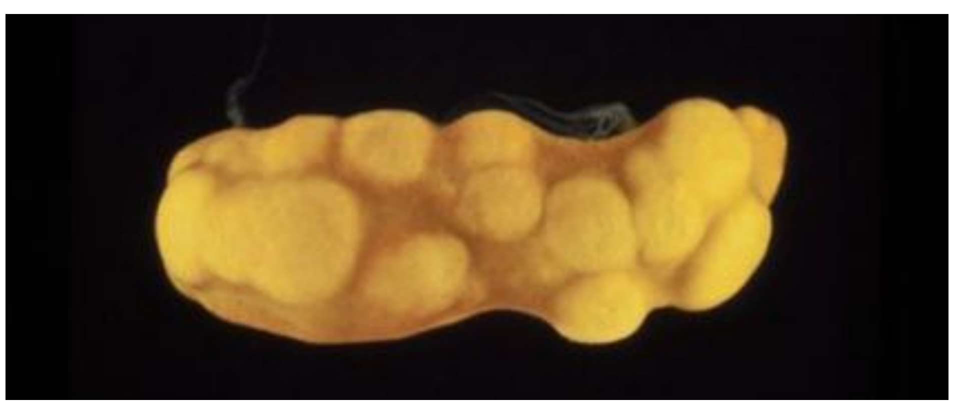
James Till
University of Toronto



Ernest McCulloch
University of Toronto



Early 1960s: What is the effect of radiation on bone marrow?

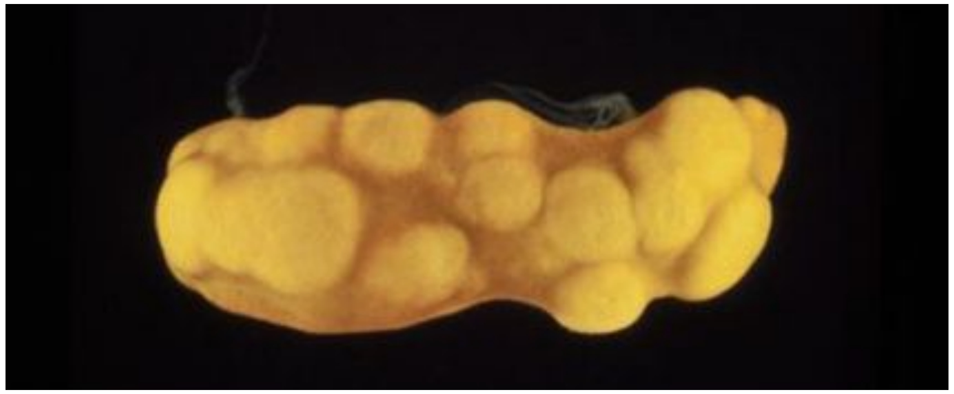


Observation of cell colonies growing on spleen

Colonies were descendants of injected marrow cells

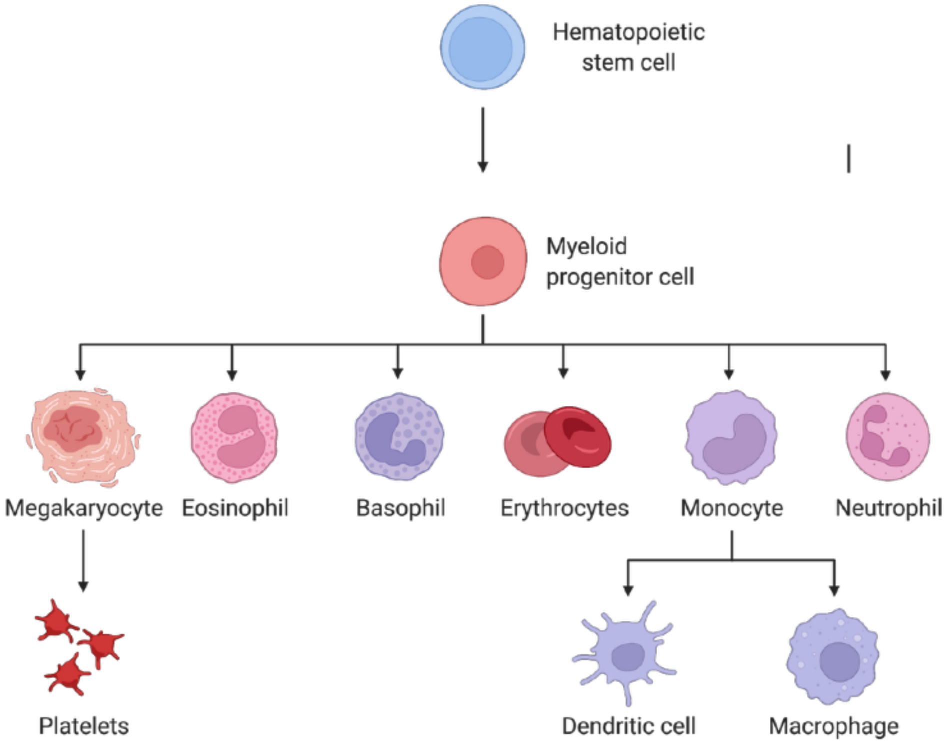
Becker, A.; McCulloch, E. A.L; Till, J. E. *Nature* **1963***, 197,* 452-454.

Discovery of Stem Cells



Observation of cell colonies growing on spleen

Colonies were descendants of injected marrow cells

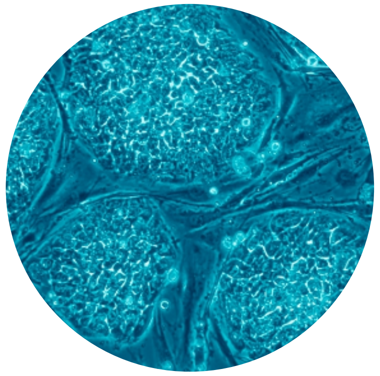


Cells observed to differentiate into blood cell lineages

Some of these cell colonies could self-propagate

Becker, A.; McCulloch, E. A.L; Till, J. E. *Nature* **1963***, 197,* 452-454.

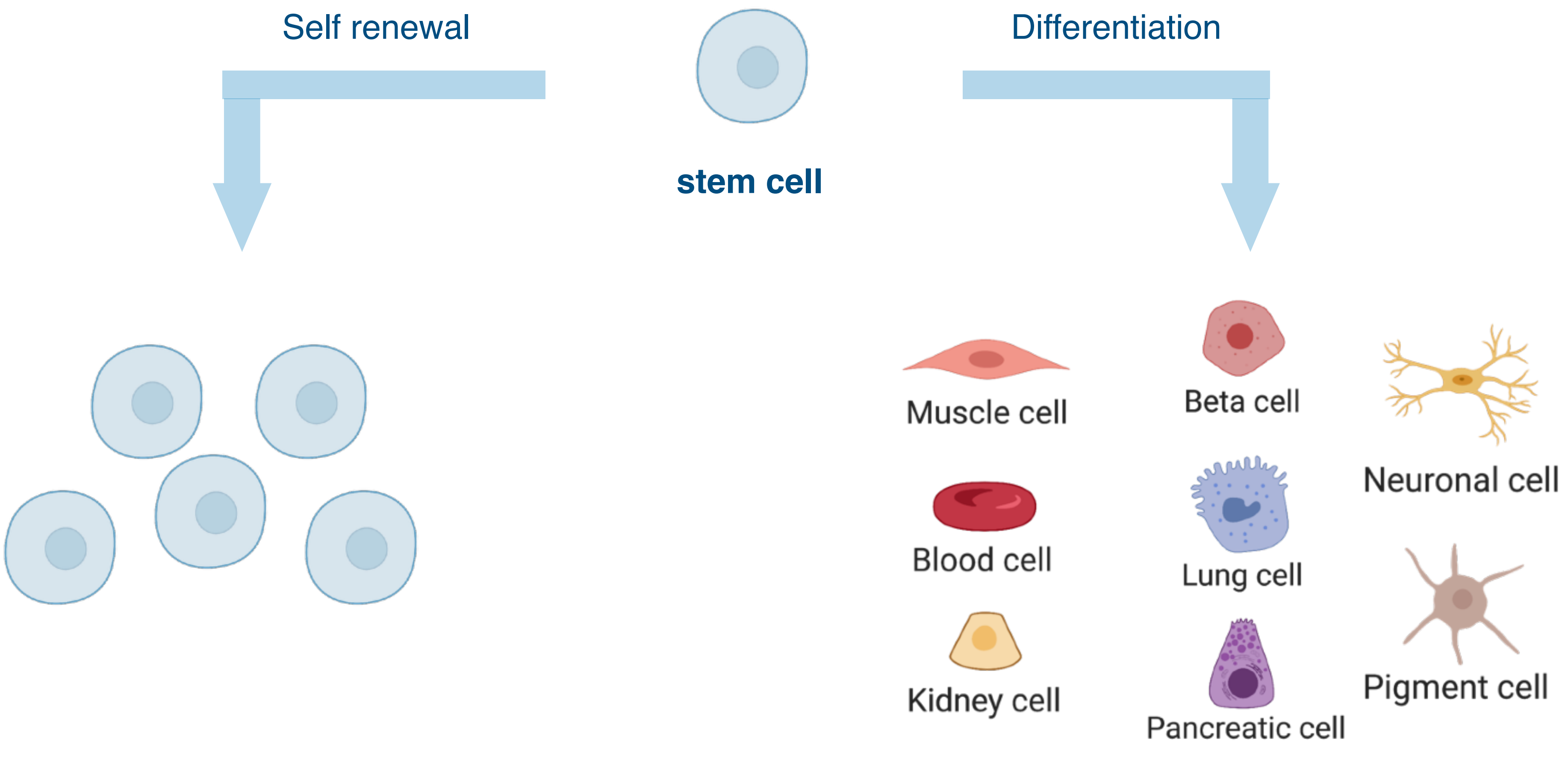
The Promise of Stem Cells



Defining a stem cell

"A stem cell is a cell that can both reproduce itself and generate offspring of different functional cell types"

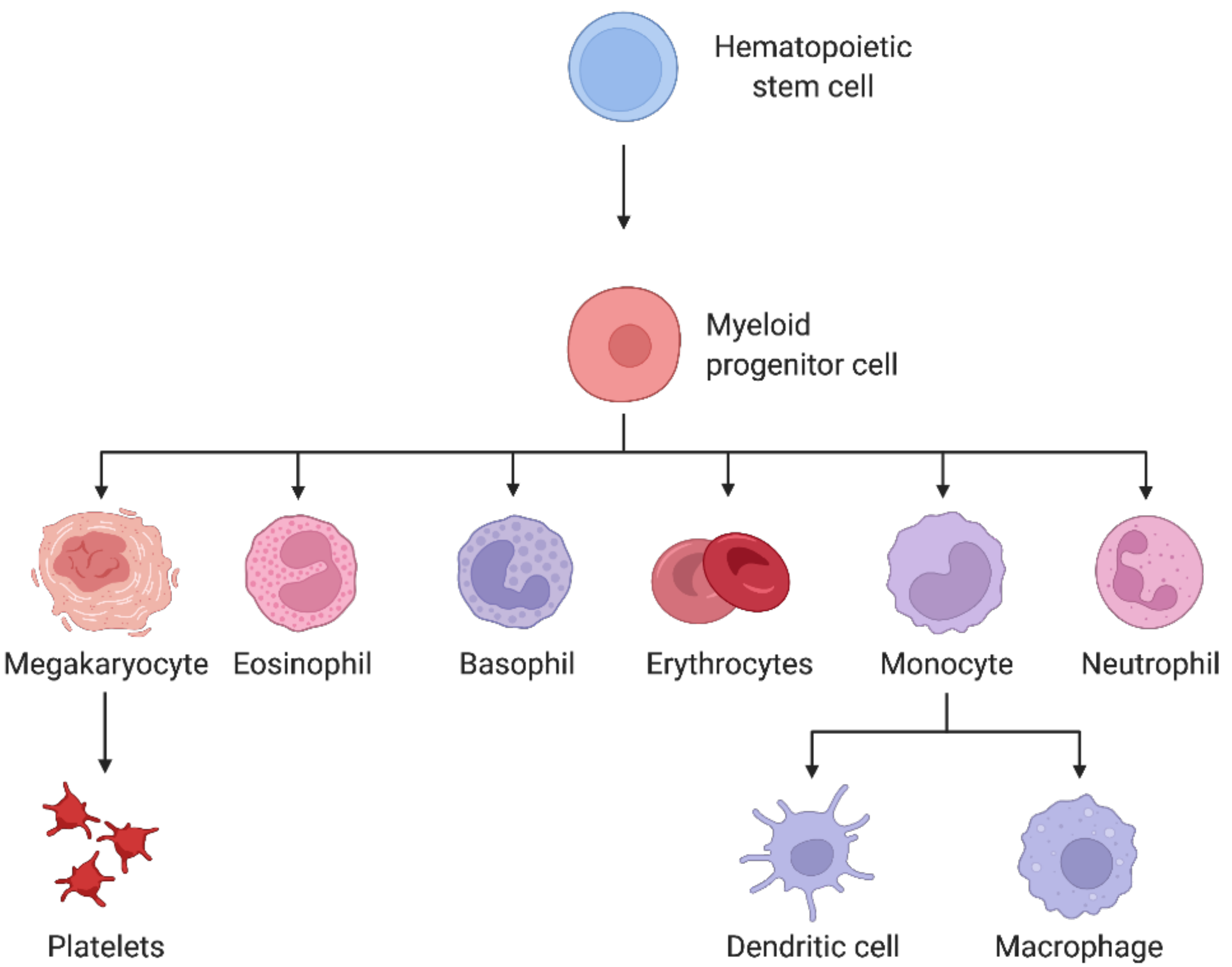
From: Jonathan Slack. "Stem Cells. A Very Short Introduction"



Cell Potency

Cell potency = *a cell's ability to differentiate into other cell types.*

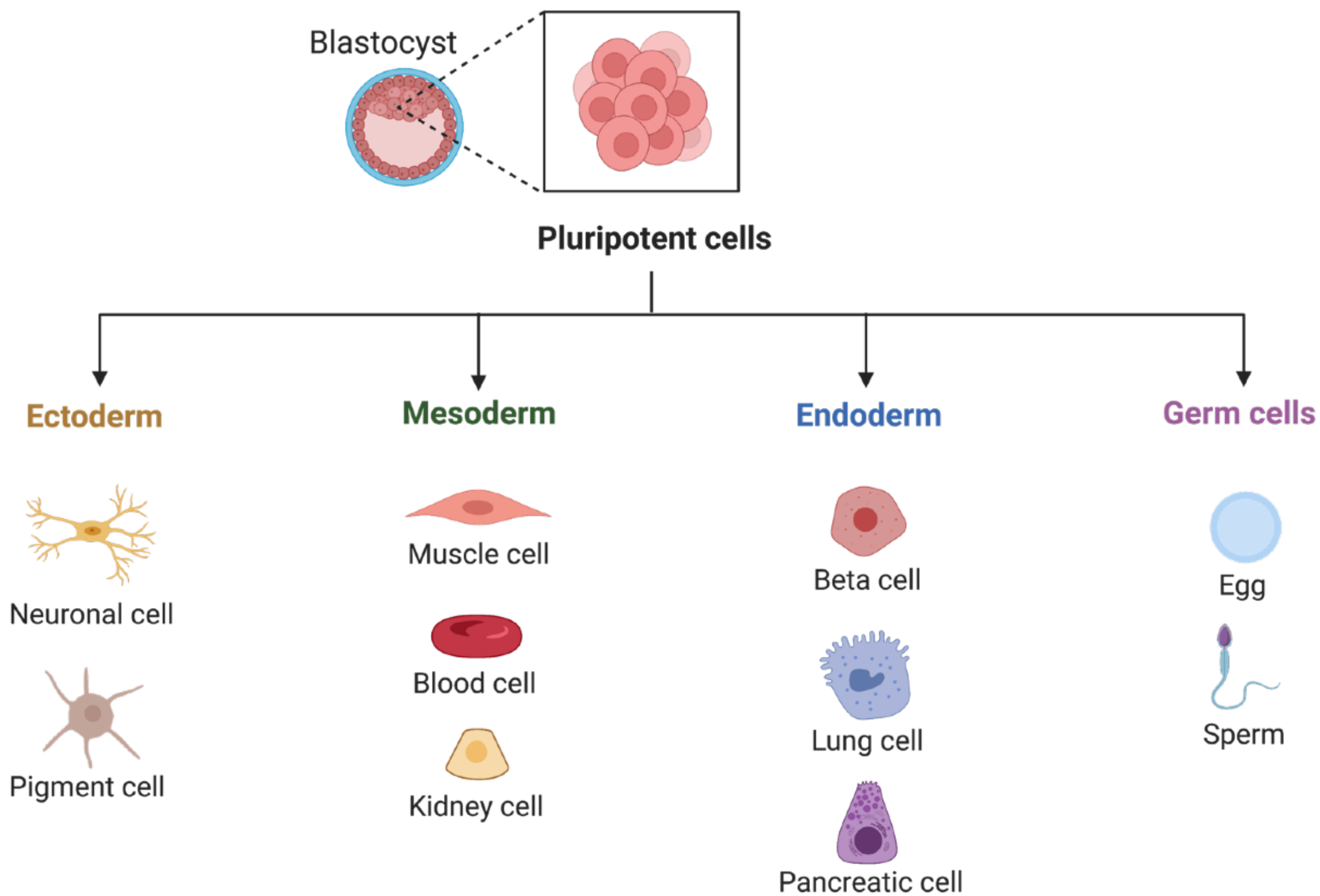
Haematopoietic stem cells are classified as *multipotent*



Cell Potency

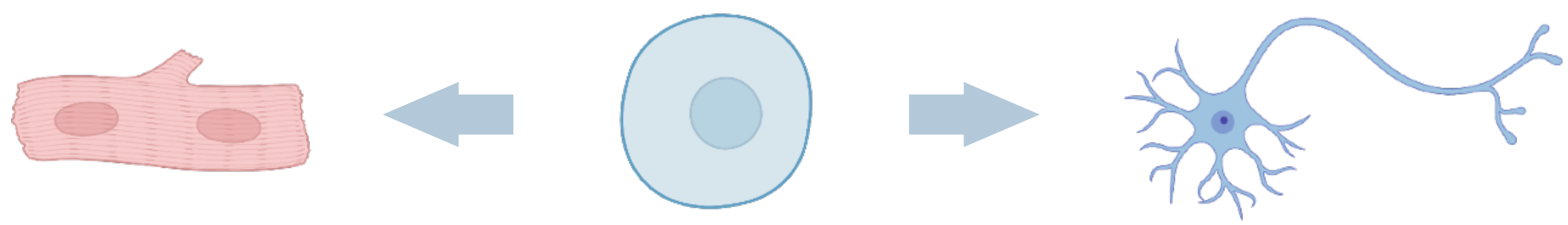
Cell potency = *a cell's ability to differentiate into other cell types.*

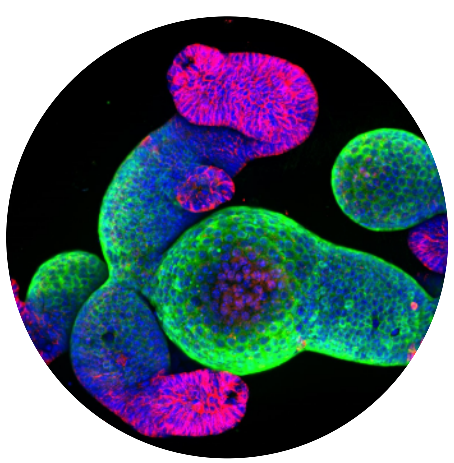
Embryonic stem cells are classified as *pluripotent*



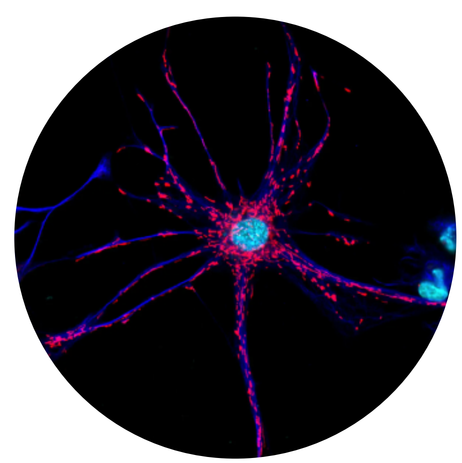
The Promise of Stem Cells

Control over differentiation enables access to cell types and tissues that are difficult or impossible to obtain

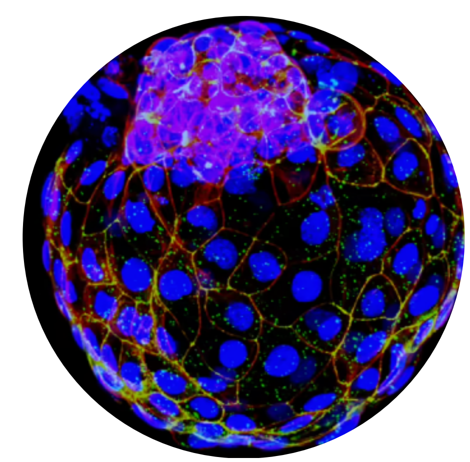




Disease modeling



Regenerative medicine

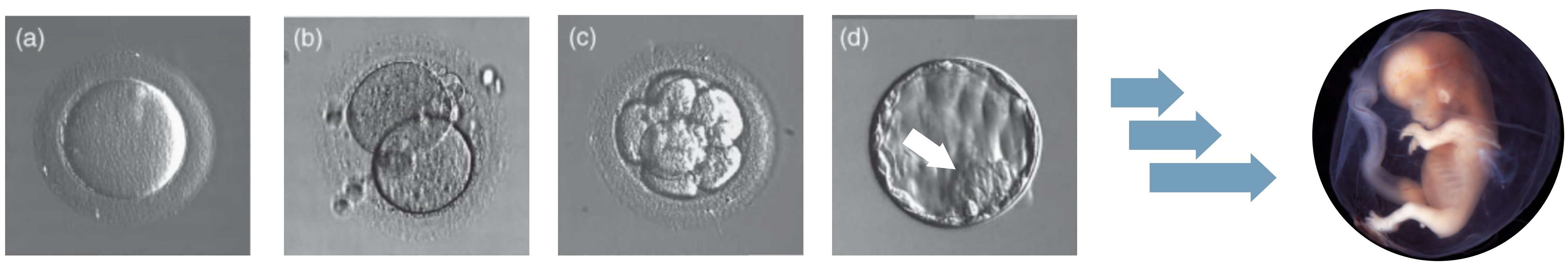


Developmental biology

Embryonic Stem Cells (ES cells)

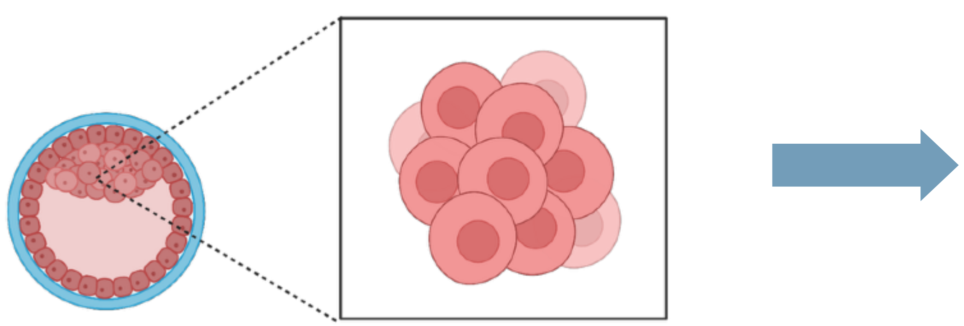
Human embryonic development

day 4-5



Fertilized egg | *Two cell stage* | *Eight cell stage* | ***Blastocyst***

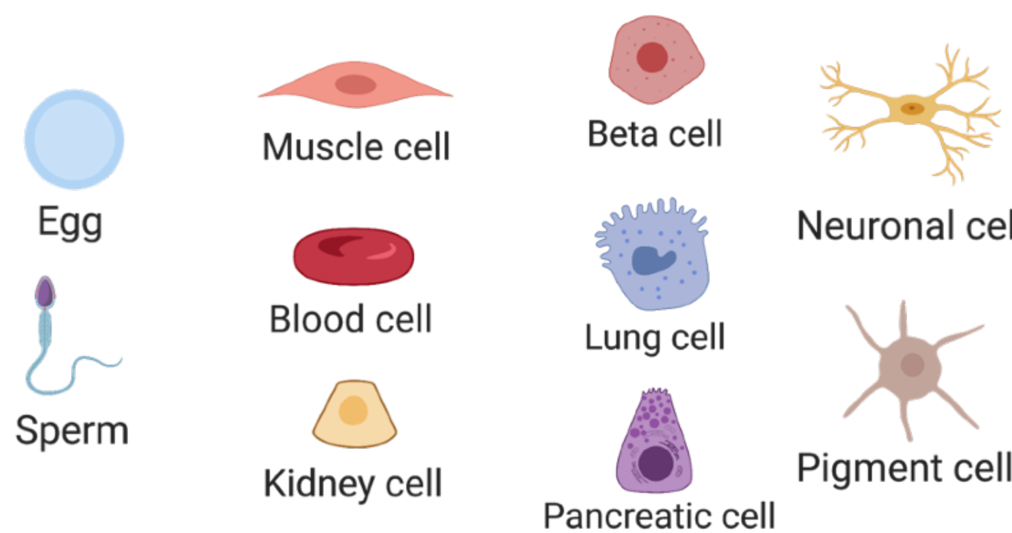
Embryonic stem cells from blastocyst



Blastocyst

Embryonic stem cells (ES cells)

Replications and differentiation to all cell types (pluripiotent)



Embryonic Stem Cells (ES cells)

1981: Mouse ES cells can be isolated from mouse blastocyst and grown without limit *in vitro*

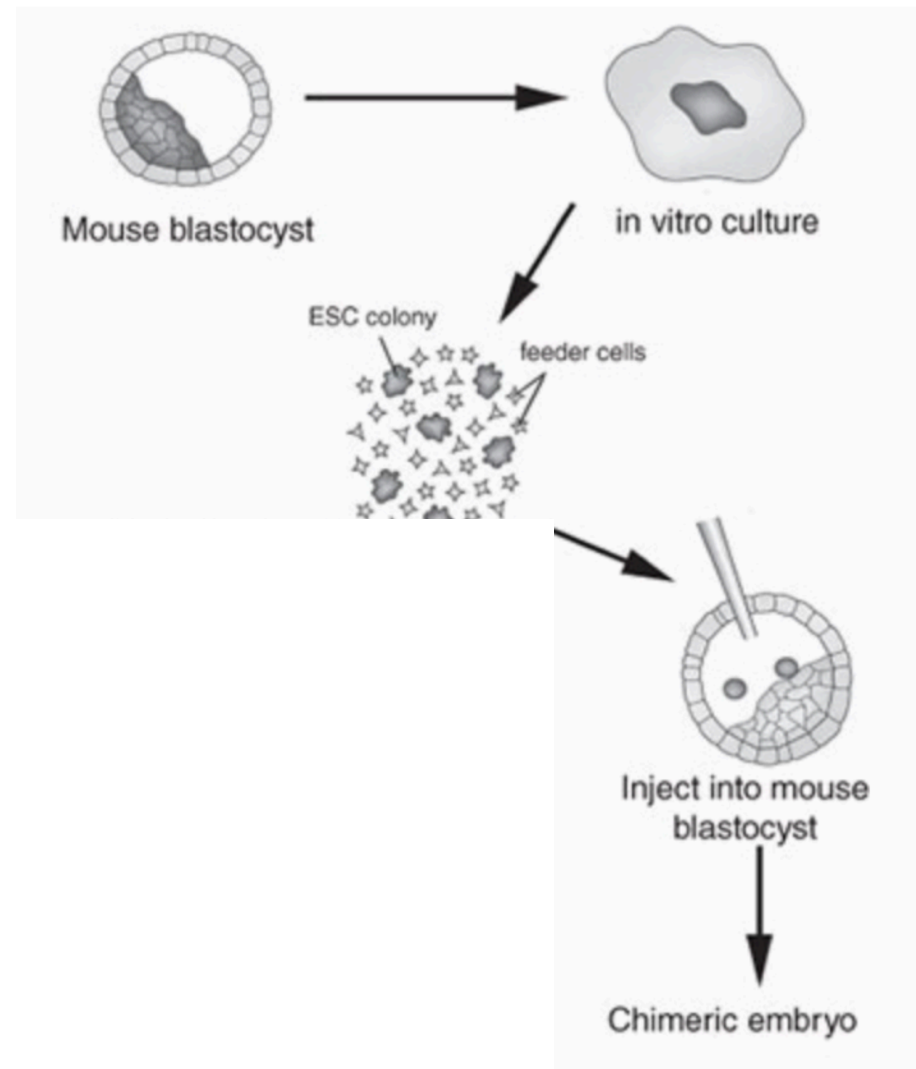
Establishment in culture of pluripotential cells from mouse embryos

M. J. Evans* & M. H. Kaufman†
Departments of Genetics* and Anatomy†, University of Cambridge, Downing Street, Cambridge CB2 3EH, UK

Embryo derived cells display a normal karyotype

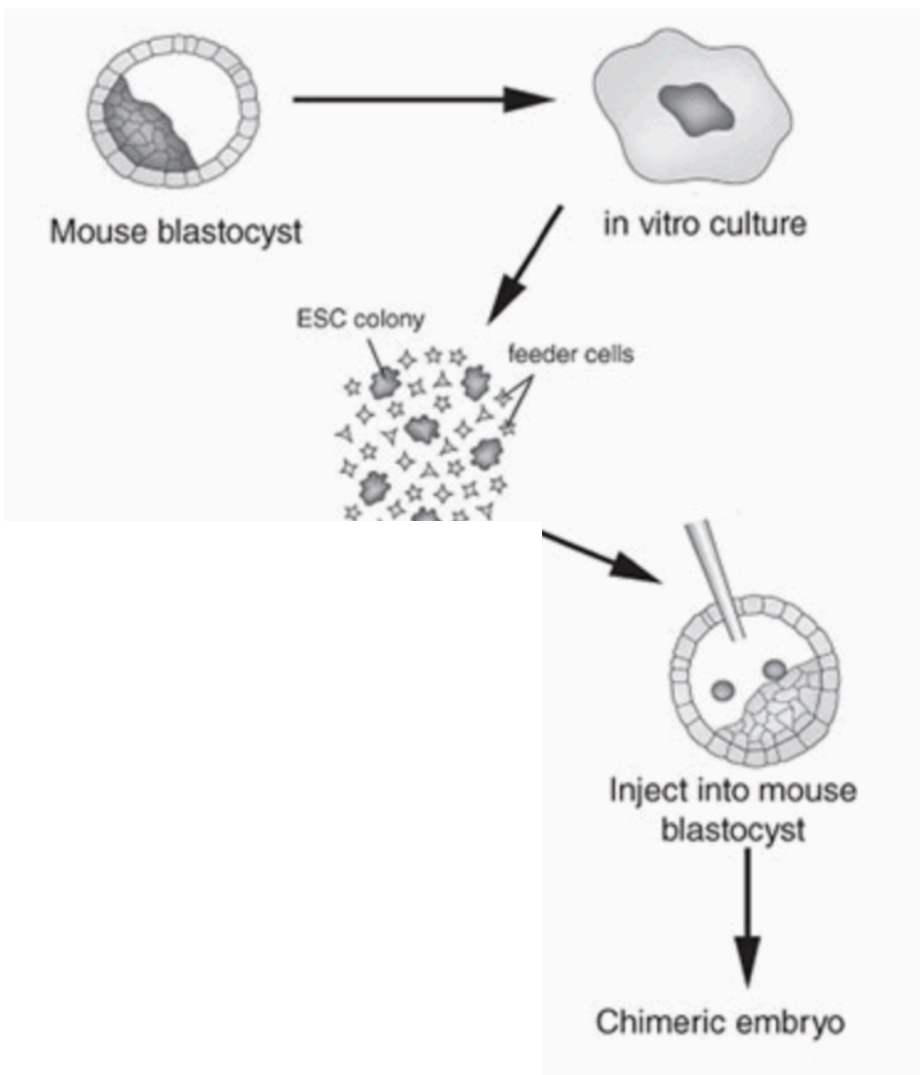
Evans, M.J.; Kaufman, M. H. *Nature* **1981**, *292,* 154-156.

ES cells can be injected into blastocysts



Embryonic Stem Cells (ES cells)

ES cells can be injected into blastocysts

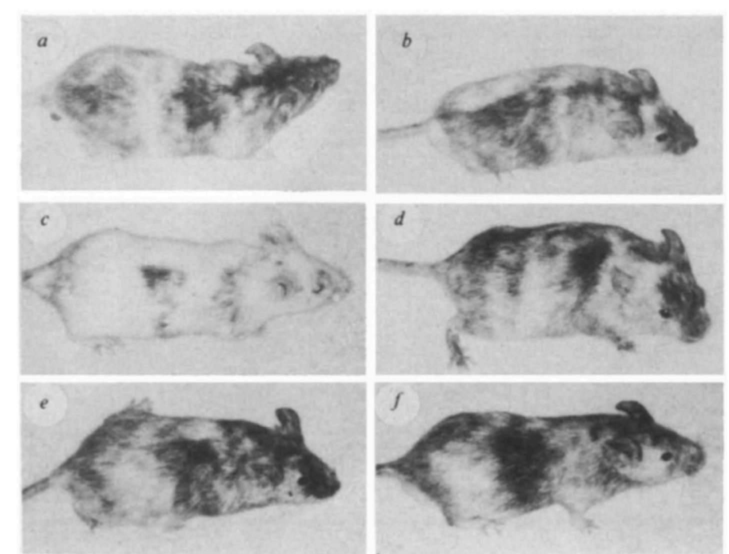


1984: Isolated ES cells can be used to create chimeric mice

Formation of germ-line chimaeras from embryo-derived teratocarcinoma cell lines

Allan Bradley*, Martin Evans*, Matthew H. Kaufman† & Elizabeth Robertson*

* Department of Genetics and † Department of Anatomy, University of Cambridge, Downing Street, Cambridge CB2 3EH, UK



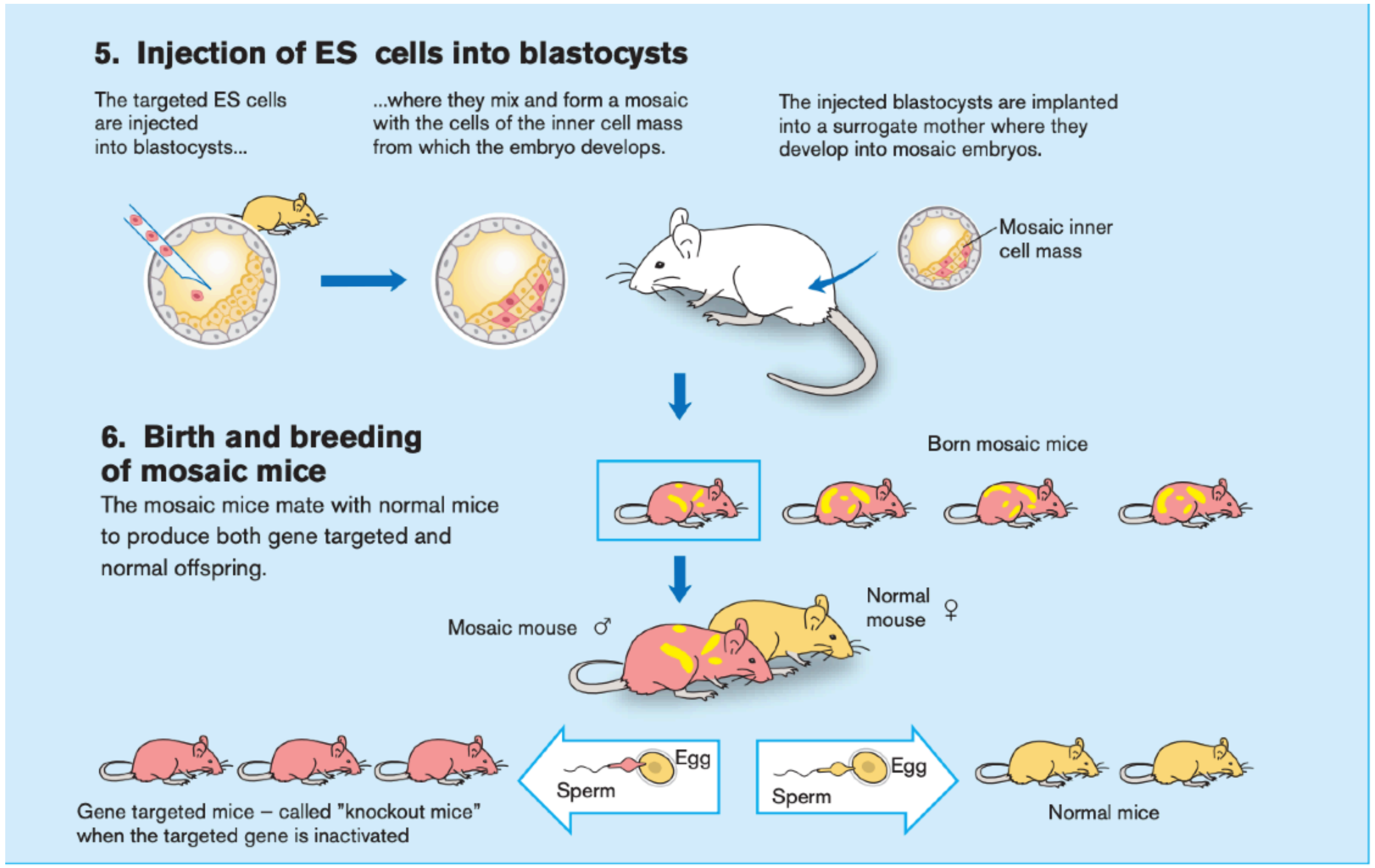
Chimeric mice

Bradley, et al., *Nature* **1984***, 309*, 255-256.

Chimeric: adult organism that is composed of two or more different populations of genetically distinct cells from different zygotes

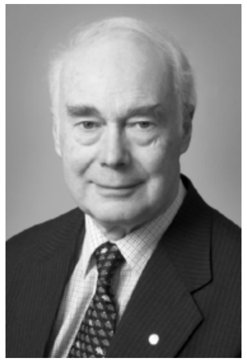
Embryonic Stem Cells (ES cells)

Genes can be added or “knocked out” of mice via injection into blastocysts and subsequent germline transmission



© The Nobel Committee for Physiology or Medicine Illustration: Annika Röhl

Oliver Smithies



Martin Evans

Mario Capecchi

2007 Nobel prize in Physiology or Medicine

“For their discoveries of principles for introducing specific gene modifications in mice by the use of embryonic stem cells.”

>10,000 mouse knockout models have been made using mouse ES cells

Embryonic Stem Cells (ES cells)

1984: Isolated ES cells can be used to create chimeric mice

Formation of germ-line chimaeras from embryo-derived teratocarcinoma cell lines

Allan Bradley*, Martin Evans*, Matthew H. Kaufman† & Elizabeth Robertson*

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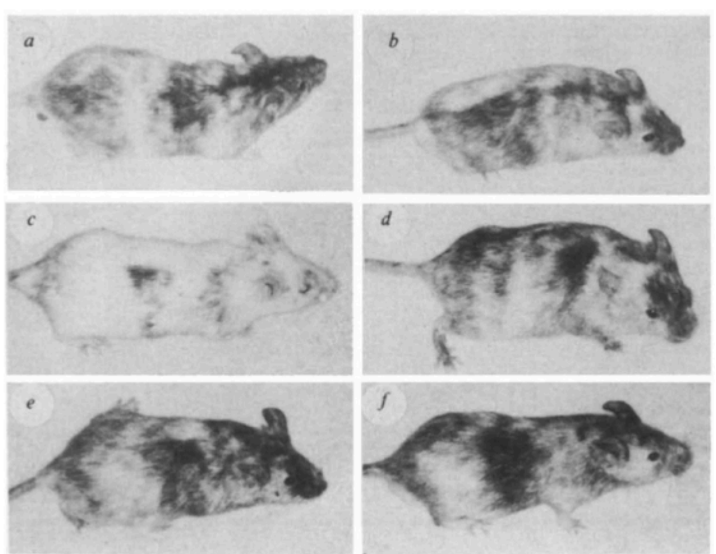


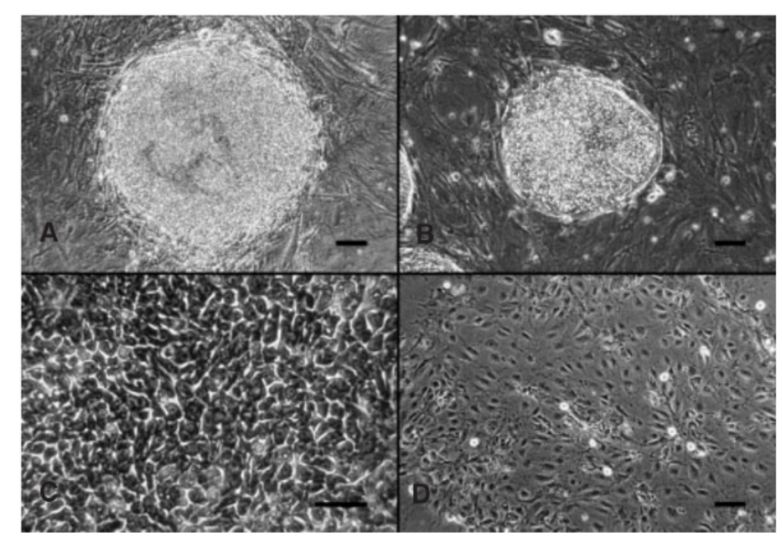
Chimeric mice

Bradley, et al., *Nature* **1984***, 309*, 255-256.

1998: First isolation of human stem cells

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



Maintain undifferentiated proliferation for up to 5 months

Thomson, J. A. *Science* **1998***, 282,* 1145-1147.

Therapeutic Cloning

2013: Generation of human ES cells via SCNT

Shoukhrat Mitalipov

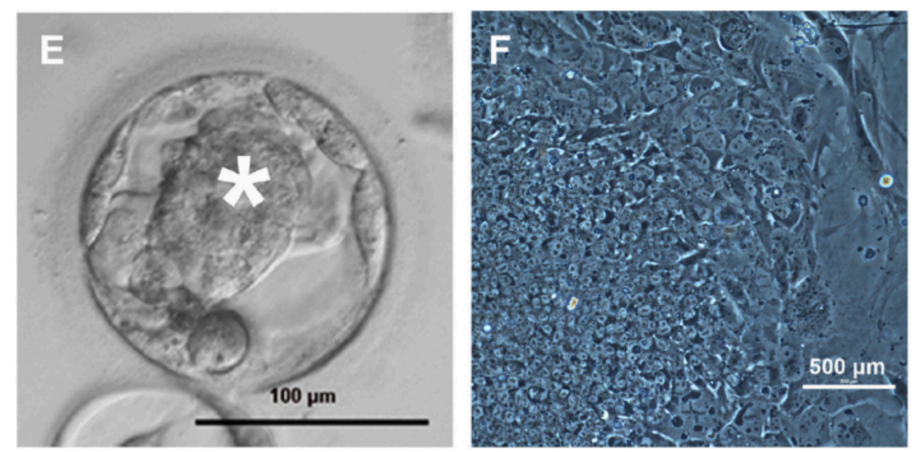
Oregon Health & Science University

The New York Times

Cloning Is Used to Create Embryonic Stem Cells

By Andrew Pollack

May 15, 2013



Implantation of 8-month old baby skin cell nuclei into oocytes led to ES cell generation

Potential for patient matched stem cells for personalized therapies

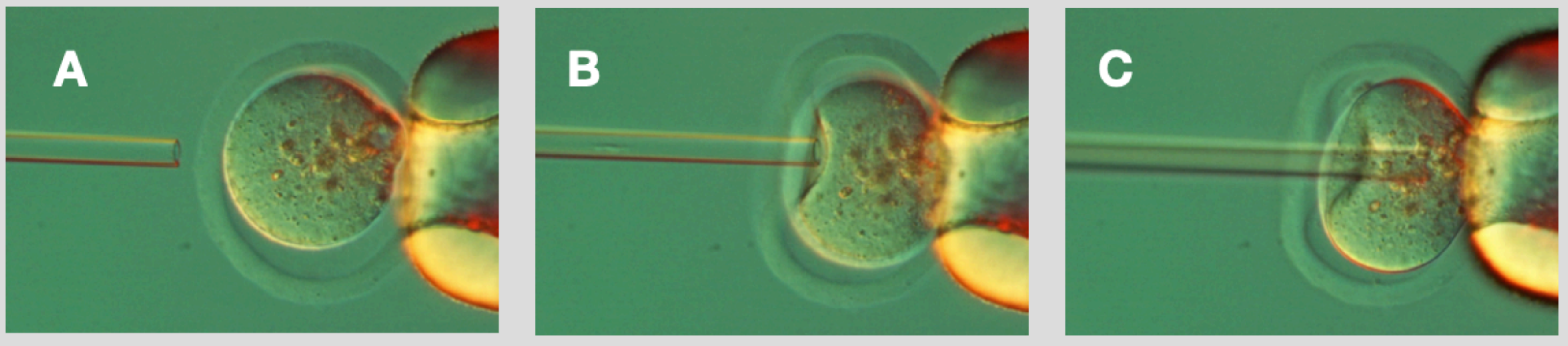
Tachibana, M.; Amato, P.; Sparman, M. *et al*. Cell ***2013**, 153,* 1228-1238.

Discovery of the Yamanaka Factors

Limitations of therapeutic cloning

Low efficiency of reprogramming

Obtaining oocytes is difficult and potentially dangerous



Nuclear transfer from reprograms nucleus of somatic cells to pluripotency

What induces somatic cell reprogramming to pluripotency?

Discovery of the Yamanaka Factors

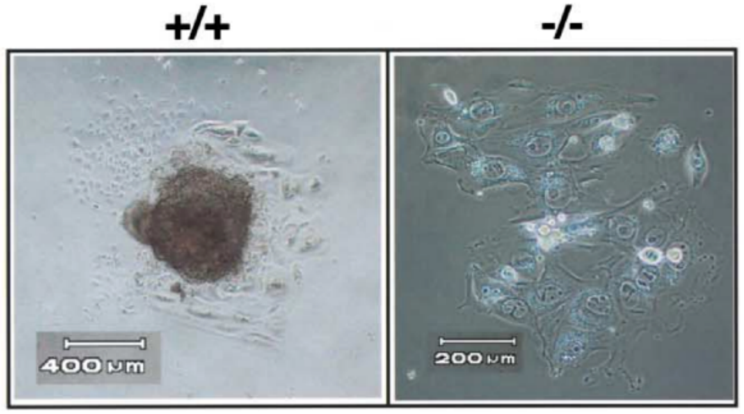
Shinya Yamanaka

Kyoto university

"…it led us to hypothesize that oocytes or ES cells contain intrinsic factors that can reprogram somatic cells into a pluripotent state."

From 2012 Nobel Lecture

What occurs when ES cell-associated transcripts (ECATs) are knocked out of ES cells and mice?



Normal vs. n*anog* deficient mouse blastocysts

n*anog* defficient mouse ES cells lose pluripotency

Takahashi, K.; Mitsui, K.; Yamanaka, S. *Nature* **2003**, *423*, 541-545.

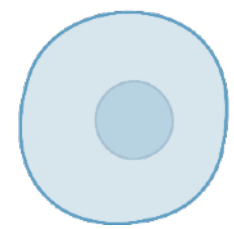
Mitsui, K. *et al. Cell* **2003**, *113*, *631-642*.

By 2004, Yamanaka had identified 24 candidate reprogramming factors

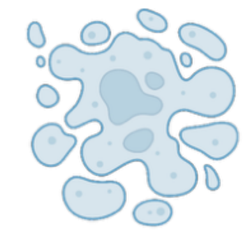
Discovery of the Yamanaka Factors

How can we identify which of these factors induce pluripotency?

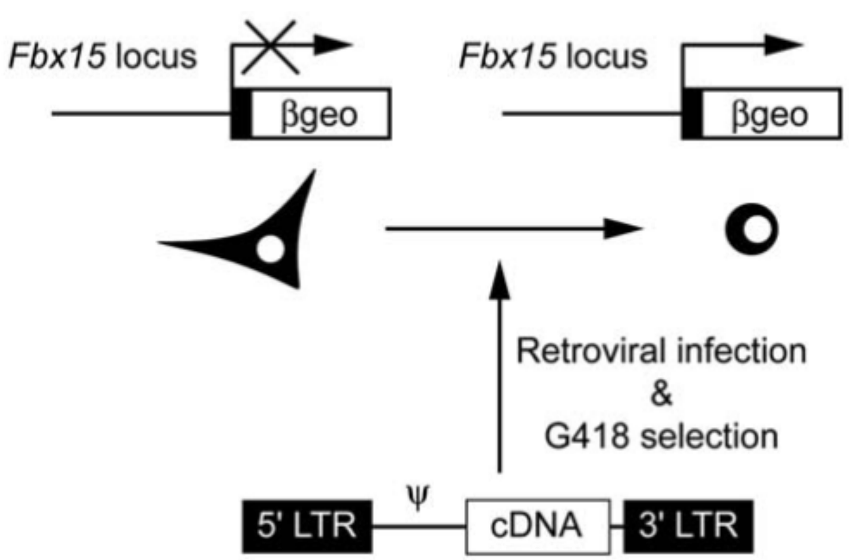
Experimental design: induction of pluripotentcy is linked to cell survival



Pluripotent (cell lives)



Non-pluripotent (cell dies)



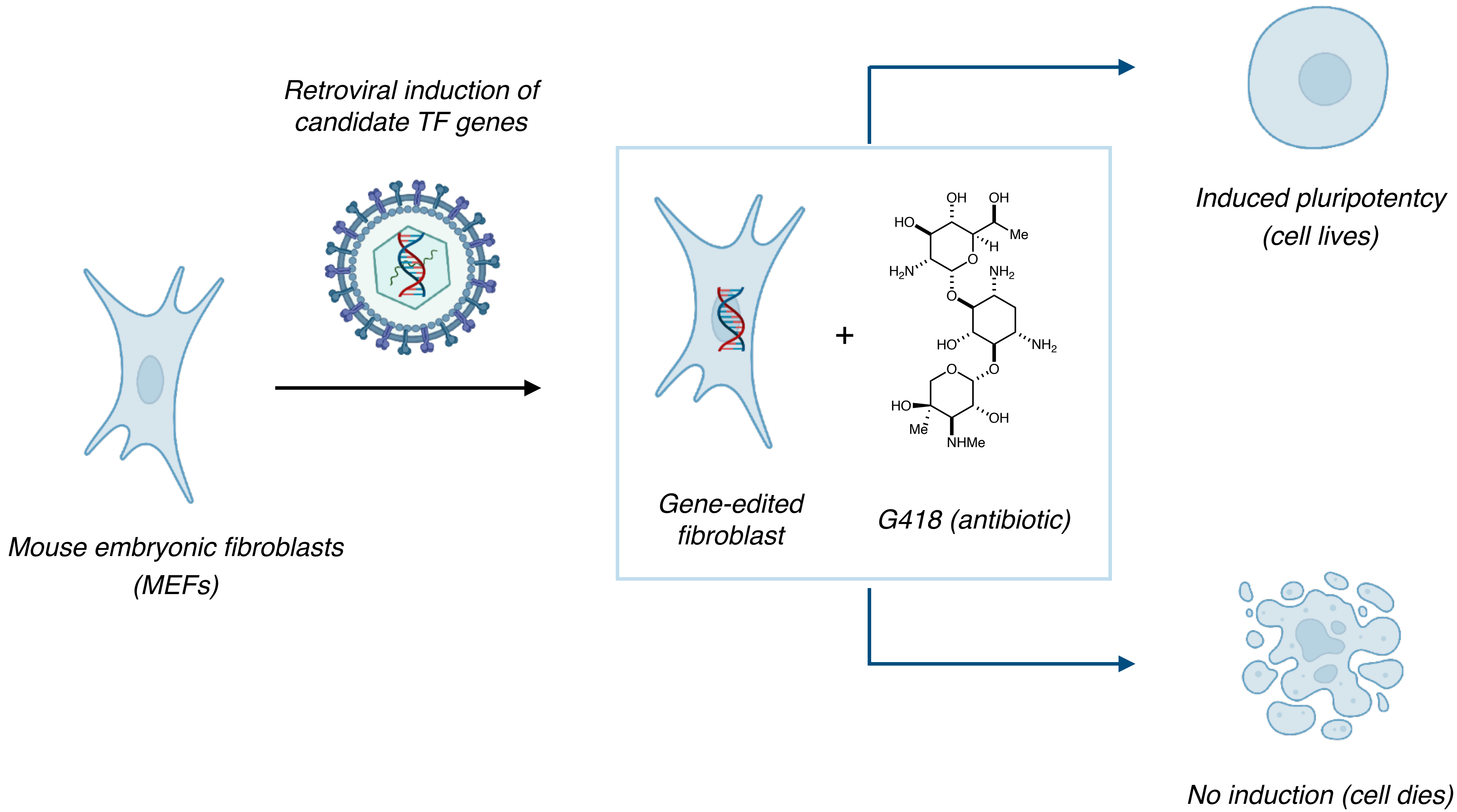
Expression of Fbx15 correlates with pluripotentcy

Candidate TF genes and antibiotic resistance fused to Fbx15 locus

Pluripotent cells will express Fbx15 and have antibiotic resistance

Takahashi, K.; Yamanaka, S. *Cell* **2006***,* 126, *663-676*.

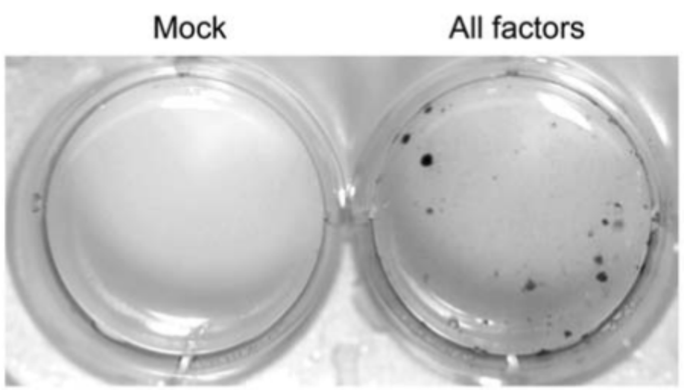
Discovery of the Yamanaka Factors



Induction of pluripotentcy is linked to cell survival

Takahashi, K.; Yamanaka, S. *Cell* **2006***,* 126, *663-676*.

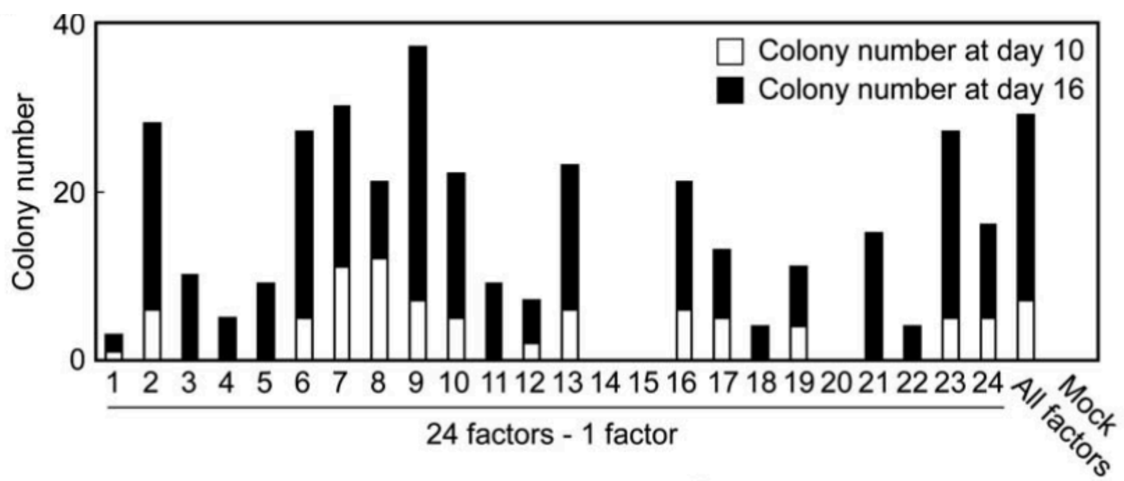
Discovery of the Yamanaka Factors



Addition of all 24 candidate factors induces pluripotency

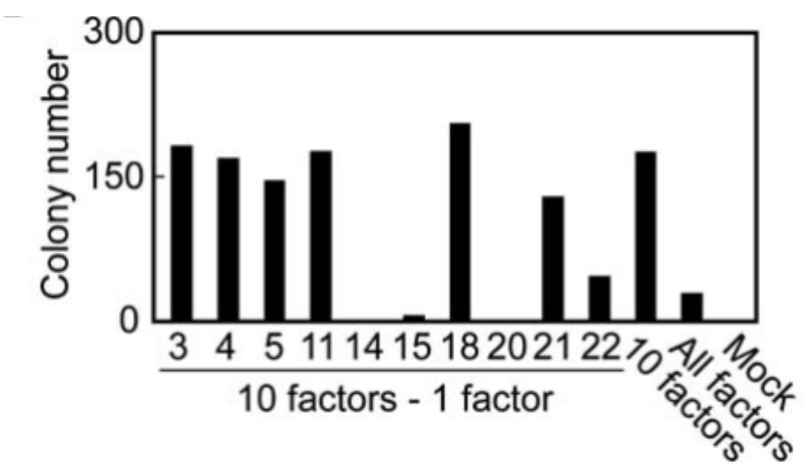
Induced pluripotent cells have similar morphology and replication properties of ES cells

Which factors are critical for pluripotency?



24 factors minus 1 factor screened

10 important factors identified

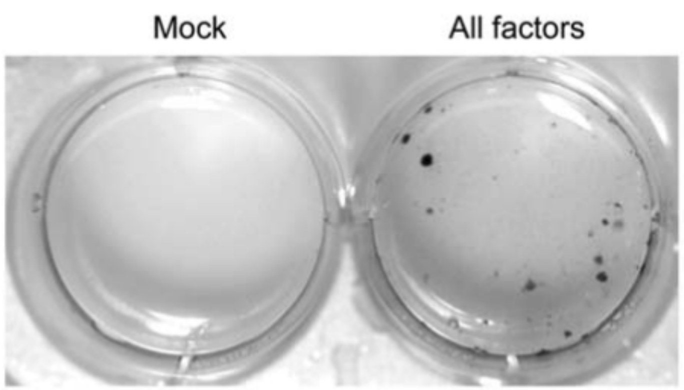


10 factors minus 1 factor screened

4 factors identified

Takahashi, K.; Yamanaka, S. *Cell* **2006**, 126, *663-676*.

Discovery of the Yamanaka Factors

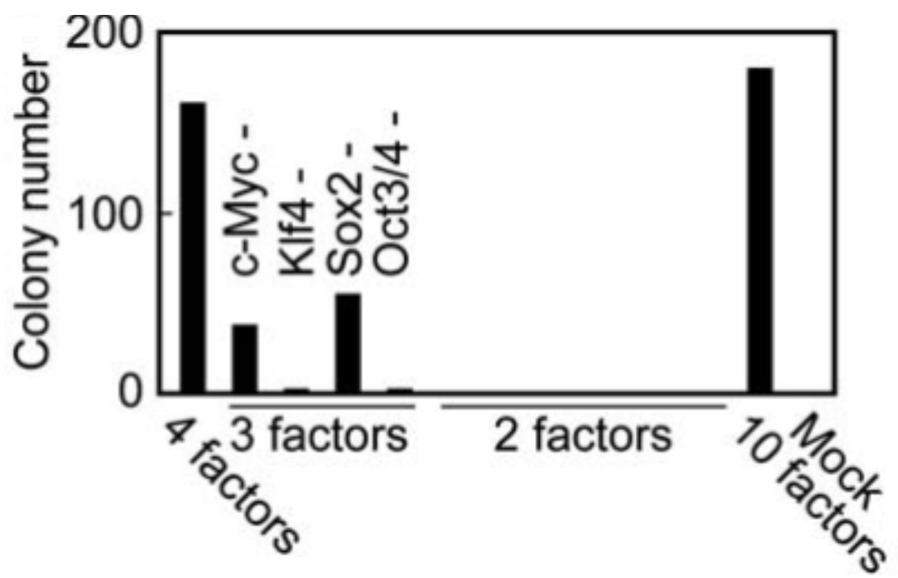


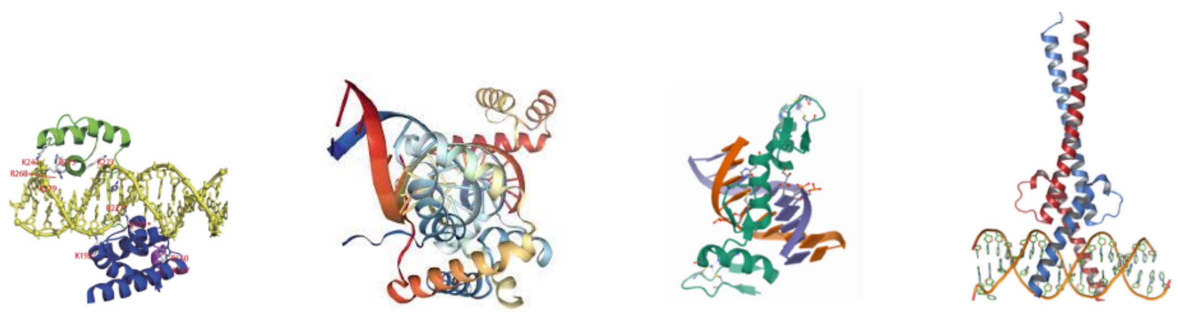
Addition of all 24 candidate factors induces pluripotency

Induced pluripotent cells have similar morphology and replication properties of ES cells

Which factors are critical for pluripotency?

4 transcription factors are required for optimal iPS generation (Yamanaka factors)



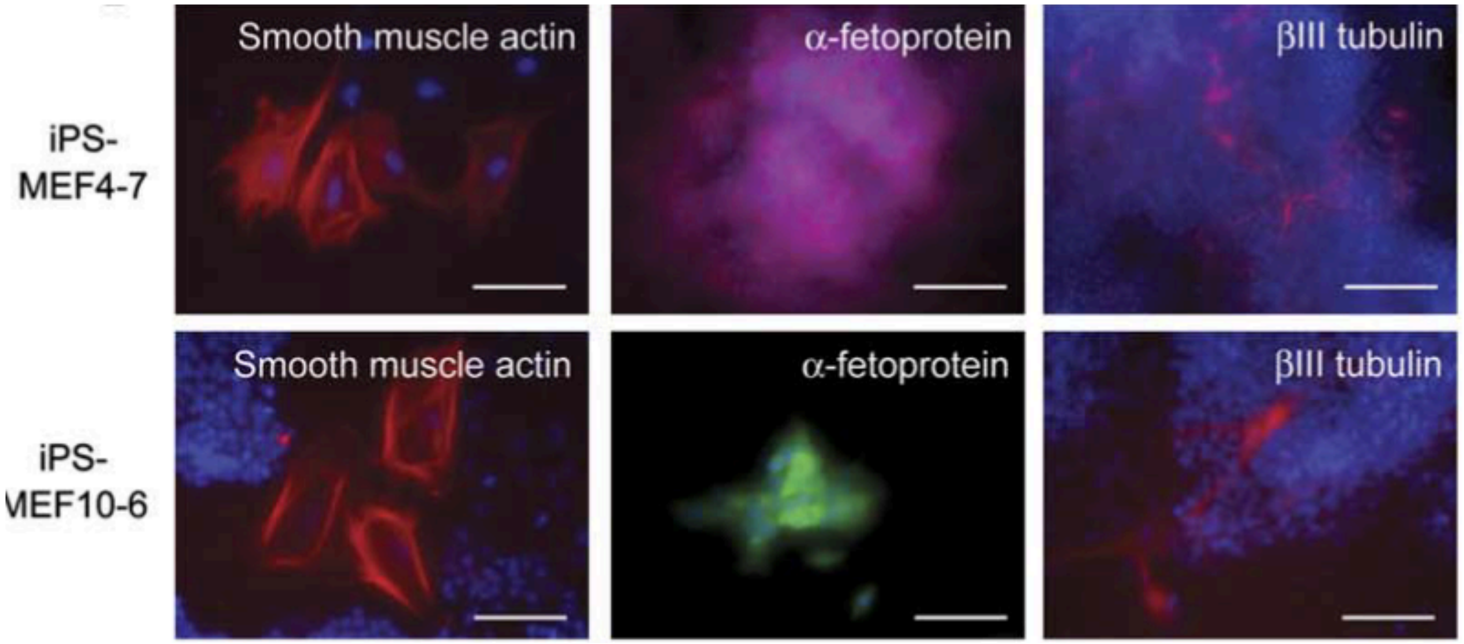


Oct4 **Klf4** **Sox2** **c-Myc**

Oct3/4, Klf4, Sox2, and C-Myc
(OKSM, Yamanaka factors)

Takahashi, K.; Yamanaka, S. *Cell* **2006**, 126, *663-676*.

Discovery of the Yamanaka Factors



Differentiated cells observed from iPS colonies in vitro

iPS can be grown and differentiated into all three germ layers

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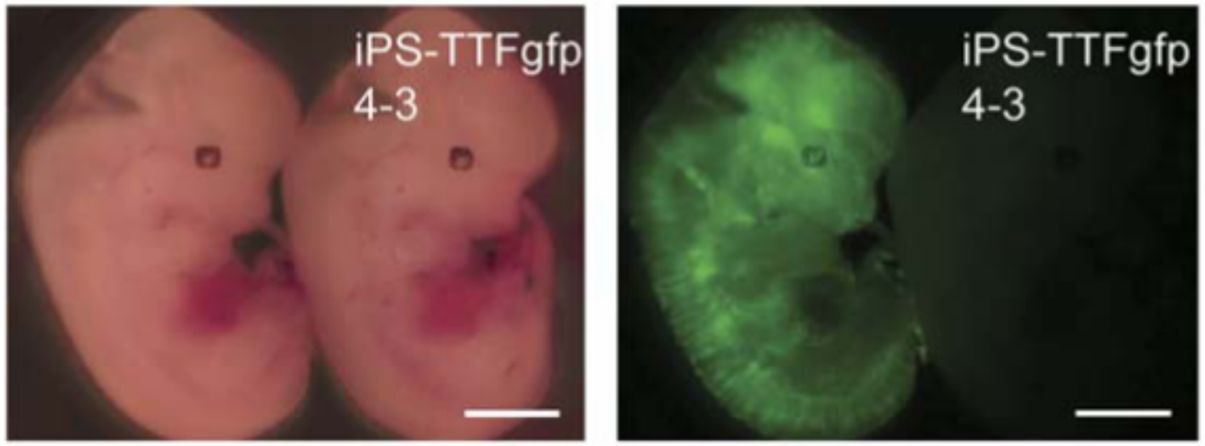
Pluripotent can be induced from adult mouse cells

iPS from adult mice can be implanted into mouse embryos

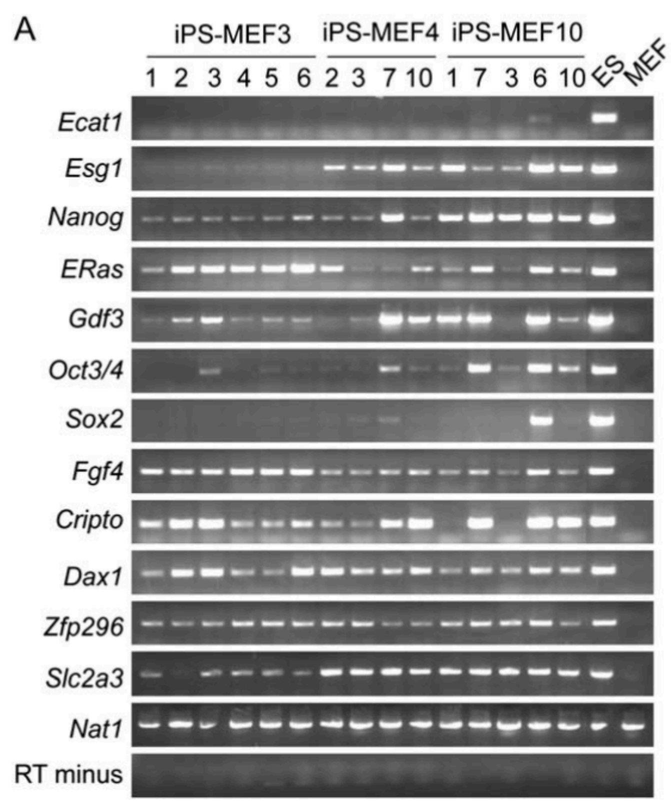


Mouse embryos derived from adult tail tip fibroblast (TTF) GFP-positive iPS cells injected into C57/BL6-129 mouse blastocysts

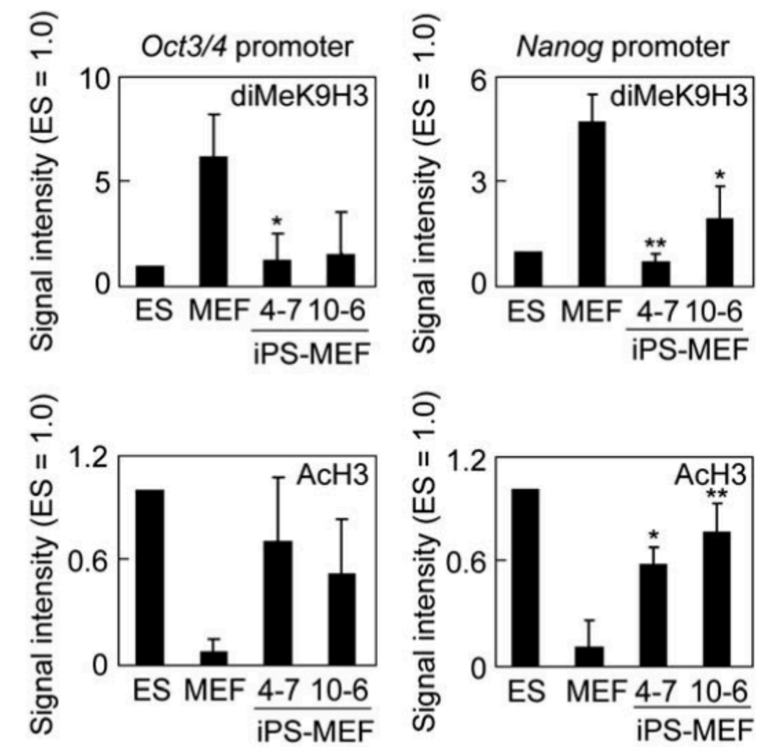
Takahashi, K.; Yamanaka, S. *Cell* **2006**, 126, *663-676*.

Discovery of the Yamanaka Factors

iPS cells display differences in gene expression and DNA methylation compared to ES cells



RT-PCR analysis of endogenous ES marker genes in iPS cells, ES cells, and MEFs.



Dimethylation status of lysine 9 on histone H3 (top) and acetylation of histone 3

iPS cells are similar, but not identical, to ES cells

iPS cells cannot produce adult chimeras

Takahashi, K.; Yamanaka, S. *Cell* **2006**, 126, *663-676*.

Discovery of the Yamanaka Factors

(2007) Selection for Nanog expression allows generation of germline-competent iPS cells, more comparable to ES cells

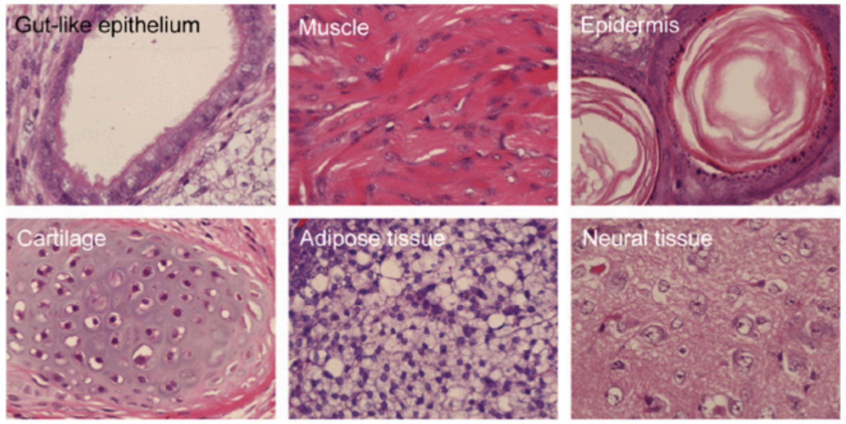
Offspring of mice from iPS-derived chimeras

Chimeric mice from iPS cells are capable of producing offspring, germline transmission

20% of offspring develop tumors due to c-myc retrovirus reactivation

Okita, K.; Ichisaka, T.; Yamanaka, S. *nature* **2007***, 448*, *313-318*.

(2007) Generation of human iPS cells from adult human fibroblasts



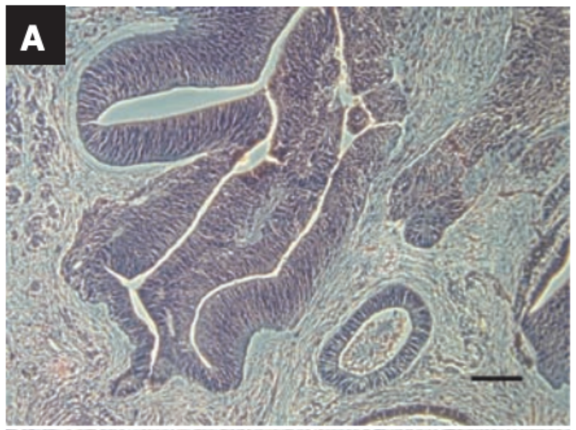
Differentiated human cells observed in tetratoma

Injection of human iPS cells into immunodeficient (SCID) mice resulted in teratomas comprised of various tissues

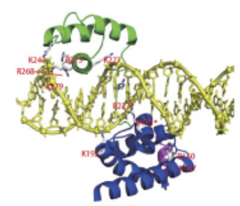
Takahashi, K.; Tanabe, K.; Ohnuki, M.; Narita, M.; Ichisaka, T.; Tomoda, K.; Yamanaka, S. *Cell* **2007***,* 131, *861-872*.

Beyond the Yamanaka Factors

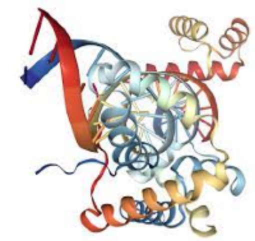
(2007) Generation of human iPS cells from human newborn foreskin fibroblasts with Lin28 in lieu of c-myc



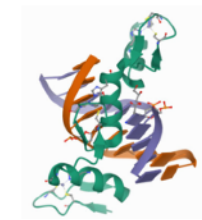
Ectoderm cells from human iPS cells



Oct4



Klf4



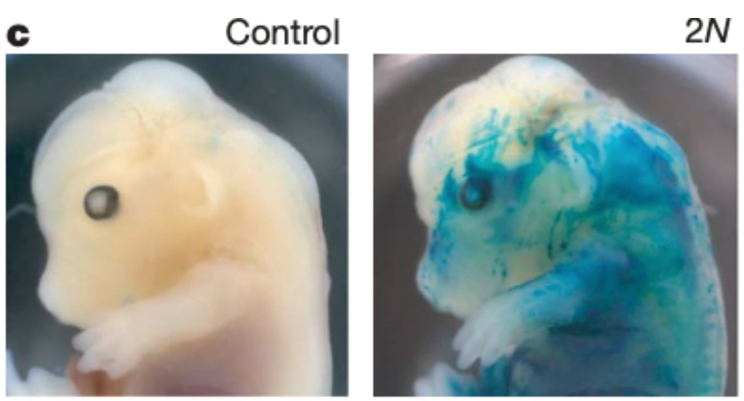
Sox2

Lin28

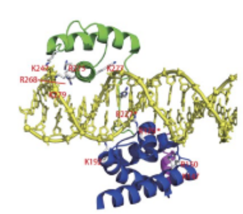
Factors exhibited similar repgroamming efficiency to Yamanaka factors (Thompson factors)

You, J. *et al*., *Science* **2007***, 3*18, 1917-1920.

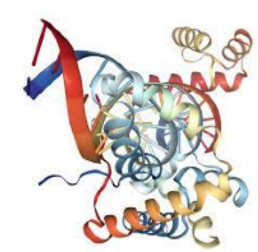
(2008) neural stem cells reprogrammed to iPS via two factors



Chimeric mouse embryos derived from OK iPS cells



Oct4



Klf4

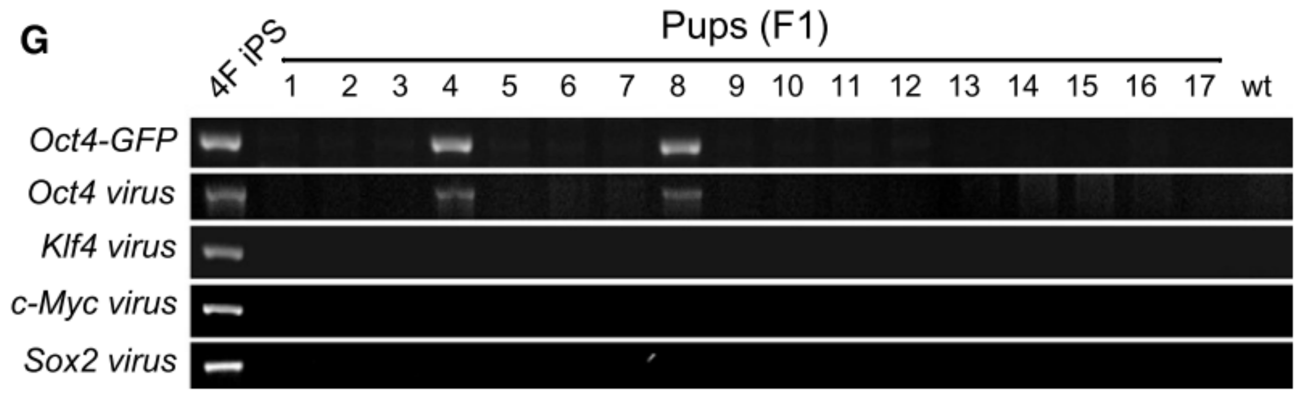
Oct4 and Klf4 sufficient to induce pluripotentcy in adult mouse neuronal cells

No tumors observed in offspring from breeding of chimeric mice

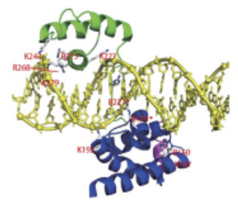
Kim, J. B. *et al*. *Nature* **2008***,* 454, 646-651.

Beyond the Yamanaka Factors

(2009) Generation of iPS cells from neural stem cells with Oct4 alone



PCR confirming germline transmission from 1-factor iPS mice



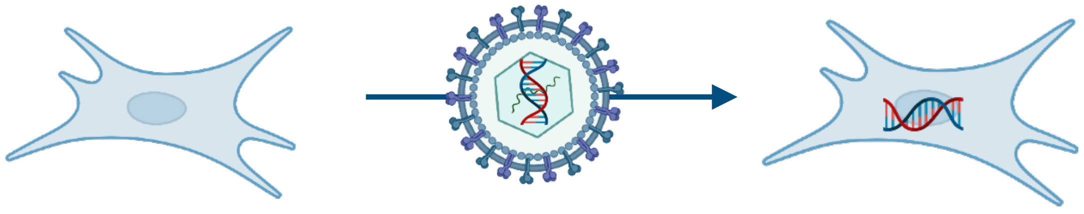
Oct4

Exogenous Oct4 alone is sufficient to induce germline competent iPS cells

Mouse neural cells: Kim, J. B. *et al.*,*Cell* **2009***,* 136, 411-419.

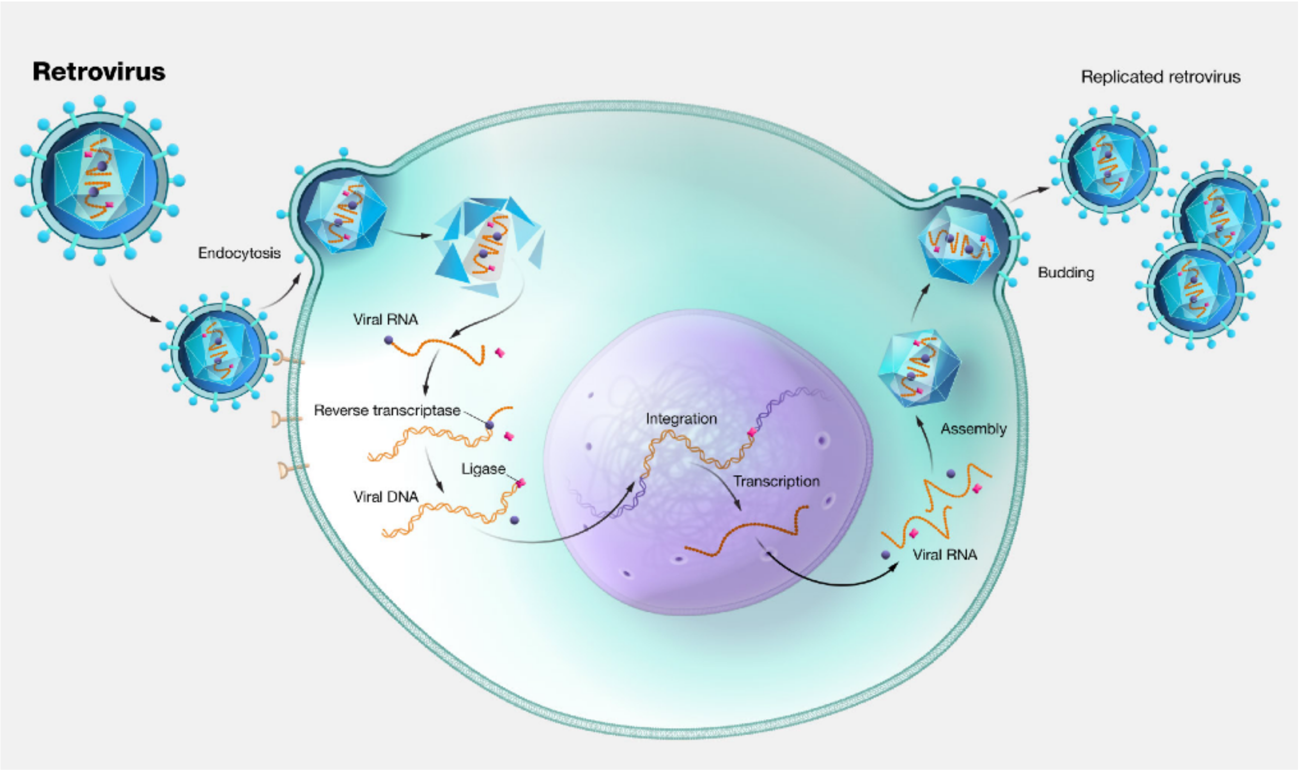
Human neural cells: Kim, J. B. *et al.*, *Nature* **2009***,* 461, 649-653.

Retroviral insertion of reprogramming factors can be problematic…



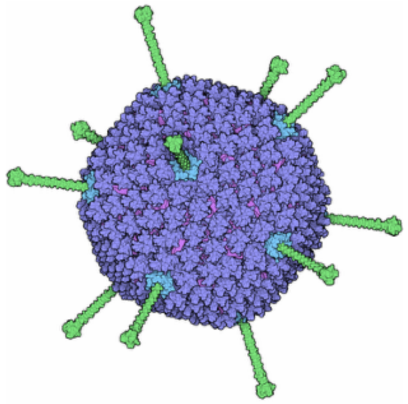
Retroviral vectors can cause cancer by disrupting endogenous gene expression

The Problem with Retroviruses



Posses serious safety issues for basic research and clinical applications

Is there a way to induce pluripotency without non-integrating vectors?

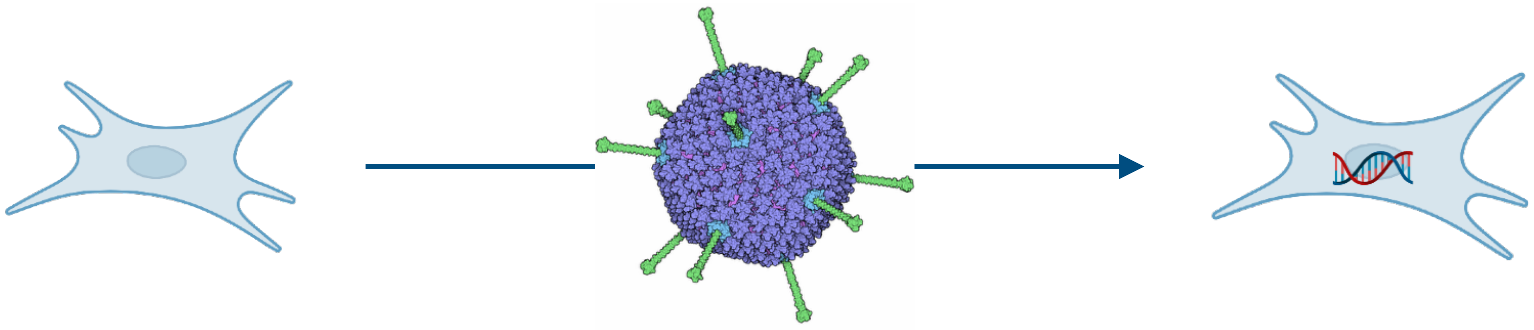


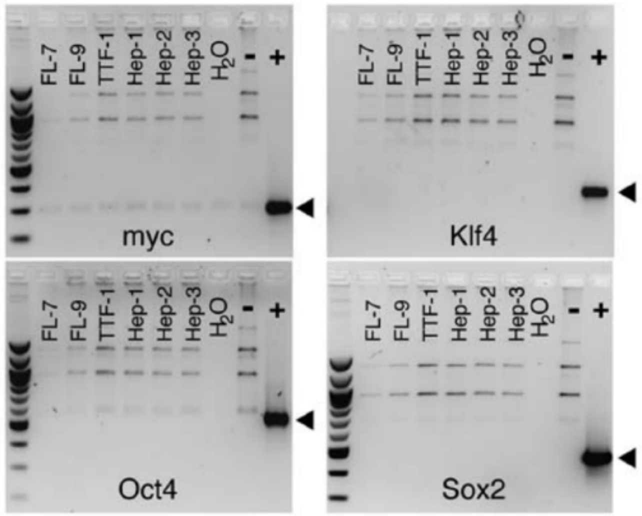
Adenovirus

Introduction of exogenous genes into host cell without genomic integration

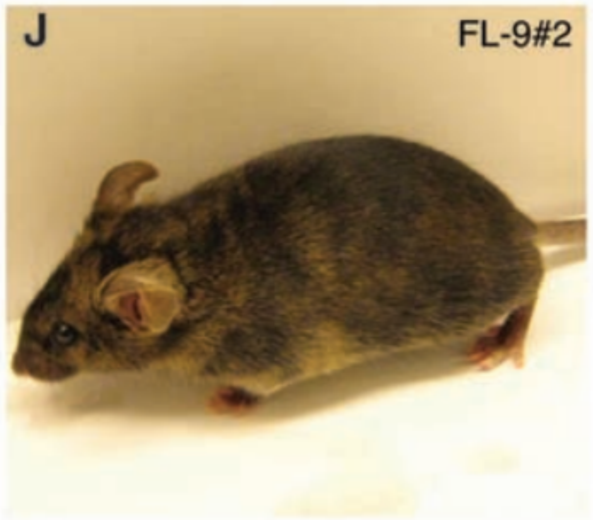
The Problem with Retroviruses

(2008) Adenovirus induced iPS from mouse fibroblasts and liver cells (Adeno-iPS)





Viral DNA not detected in host genome



Adeno-iPS are germline competent

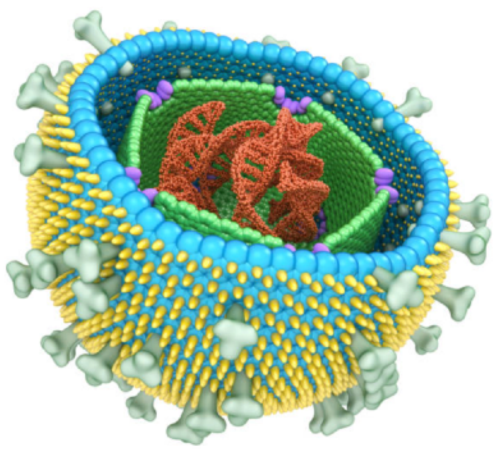
Insertional mutagenesis is not required for reprogramming

Stadtfeld, M.; Nagaya, M.; Utikal, J.; Weir, G.; Hochedlinger, K. *Science* **2008**, *322*, 945-949.

Okita, K.; Nakagawa, M.; Hyenjong, H.; Ichisaka, T.; Yamanaka, S. *Science* **2008**, *322*, 949-952.

Other Transduction Methods

Epstein-Barr virus

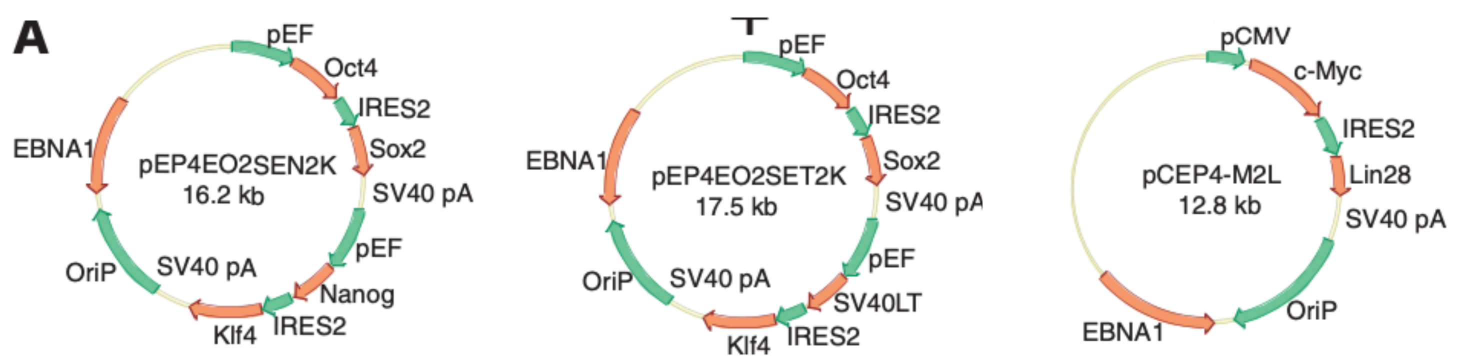


Infected B lymphocytes that can proliferate indefinitely

Episomal viral cDNA that replicates autonomously in host cells and be used to express desired genes

Yates, J. L.; Warren, N.; Sugden, B. *Nature* **1985**, *313*, 812-815.

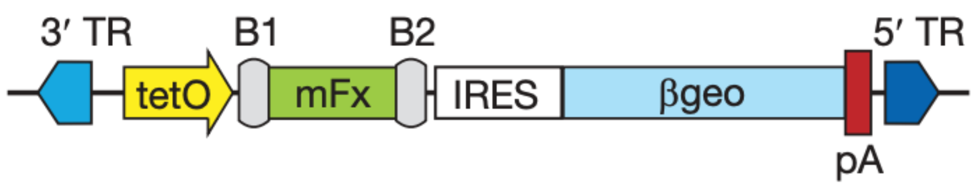
(2009) Generation of iPS from human foreskin fibroblasts using Epstein-Barr derived sequences



Yu, J.; Hu, K.; Smuga-Otto, K.; Tian, S.; Stewart, R.; Slukvin, I. I.; Thomson, J. A. *Science* **2009**, *324*, 797-800.

Other Transduction Methods

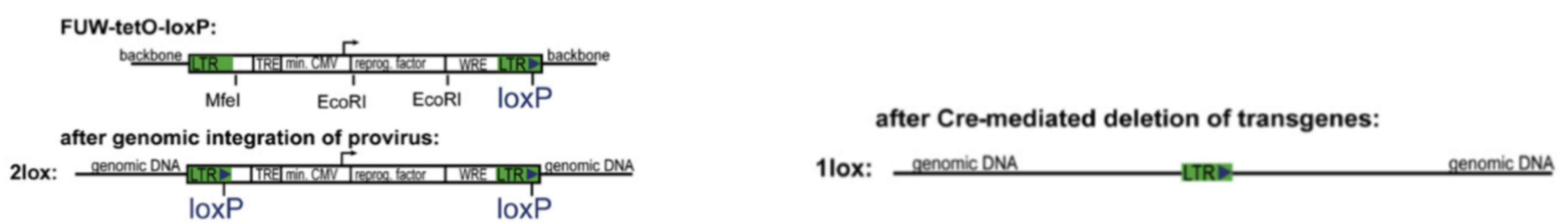
(2009) Generation of iPS using piggyBac transposon/transposase system to deliver RFs



PiggyBac transposon allows traceless removal of transgenes

Woltjen, K. *et al*., *Nature* **2009**, *458*, 766-770.
Kaji, K. *et al*., *Nature* **2009**, *458*, 771-775.

(2009) Generation of dopaminergic neuroms from Parkinson's patient cells using floxed RFs



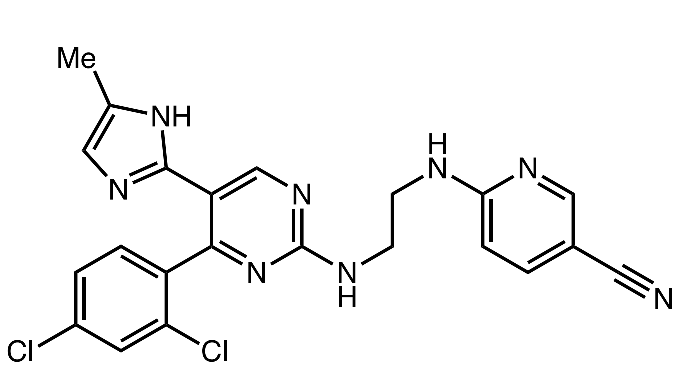
Expression of Cre recombinase results in excision of transgenes

Soldner, F. *et al*., *Cell* **2009**, *136*, 964-977.

Beyond the Yamanaka Factors

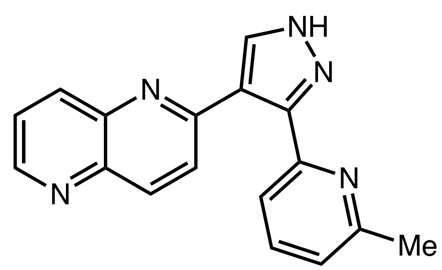
(2013) Reprogramming of mouse adult fibroblasts using only small molecules (CiSPCs)

Addition of small molecules stimulate the downstream expression of endogenous Oct4



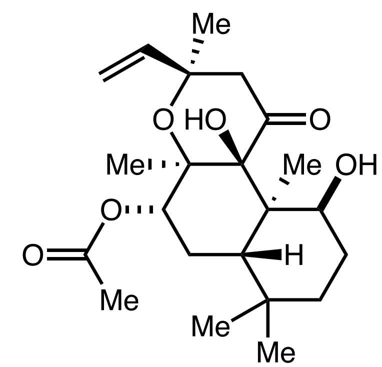
CHIR

Glycogen synthase kinase 3 inhibitor



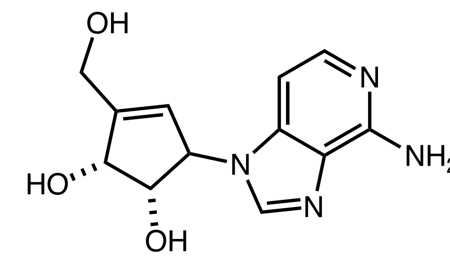
616452

Transforming growth factor-beta inhibitor



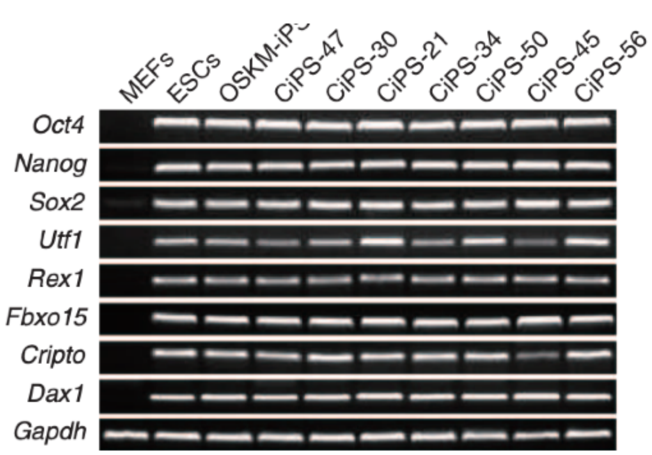
FSK

cAMP agonist

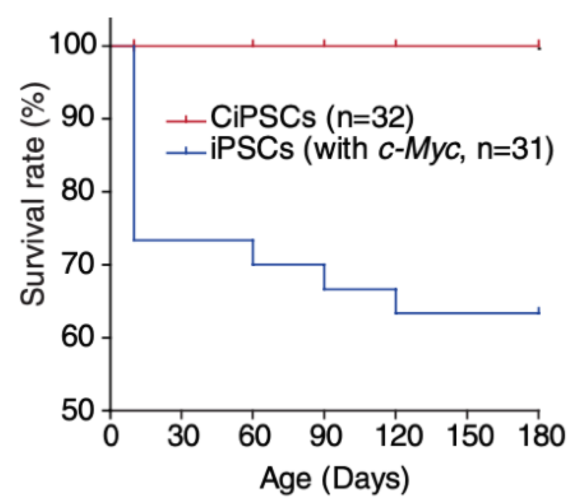


DZNep

S-adenosylhomocysteine inhibitor



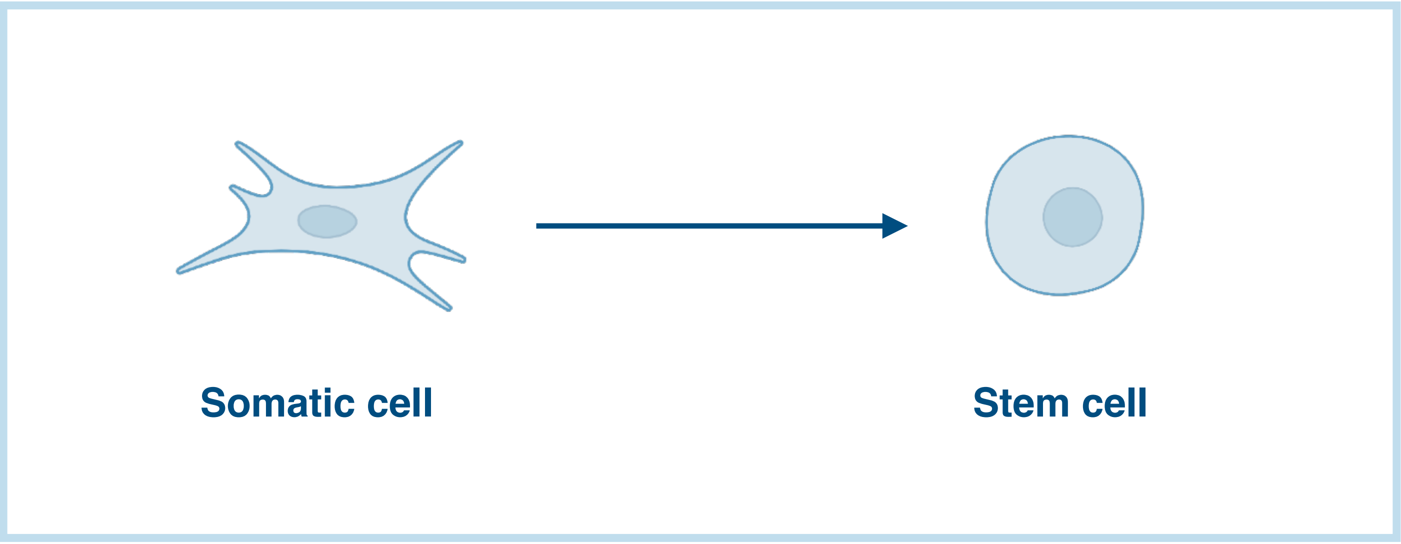
Gene expression profiles of CiSPCs are similar to ESCs



CiPSC mice live longer than virally transduced mice

Hou, P. *et al.*, *Science* **2013**, *341*, 651-654.

Beyond the Yamanaka Factors



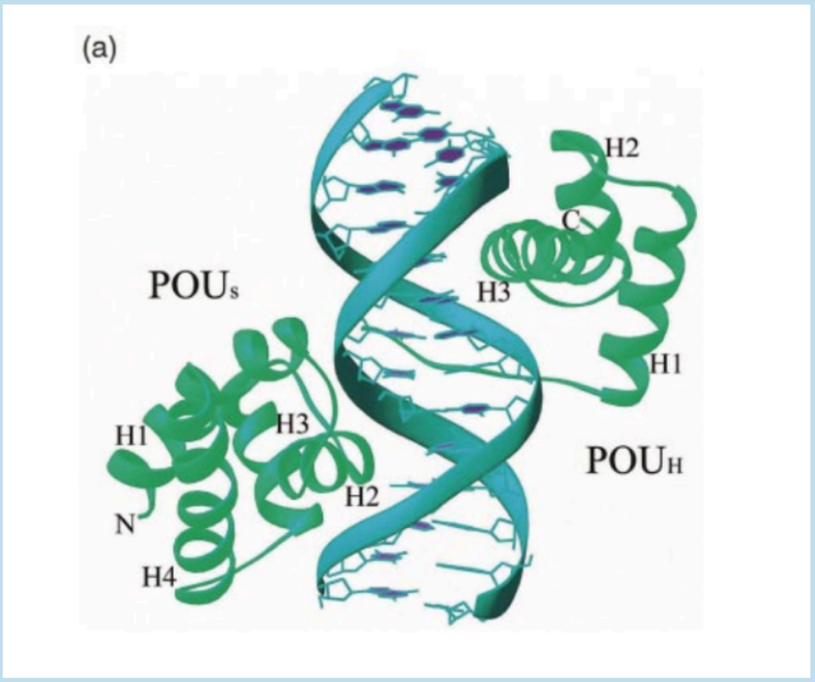
What is the molecular basis for somatic cell reprogramming?

Octamer-binding (Oct) Proteins

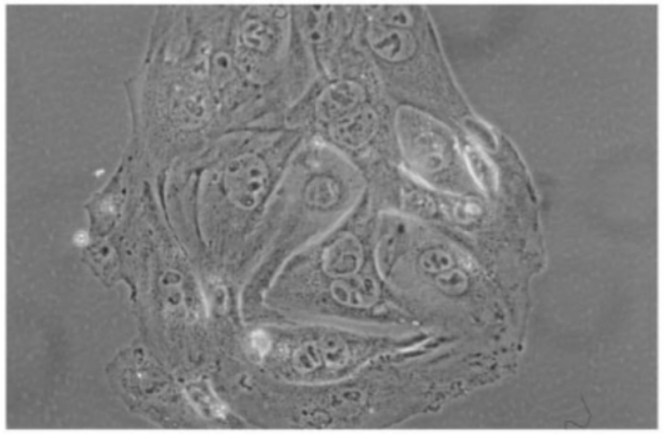
Oct transcription factor family is comprised of 8 members

(Oct1, Oct2, Oct4, Oct6, Oct7, Oct8, Oct9, and Oct11)

Comprised of two linked binding domains (POU_S and POU_H), which each bind 4 DNA base pairs in the major groove



Oct1 binding to its cognate DNA sequence



Oct4-deficient embryos yield only trophoblast cells

Oct4 is only Oct TF family member critical in ES cell self-renewal and pluripotent

(1990) Oct4 was discovered to be expressed in early embryos but not in adult tissues

Okamoto, K. *et al.*,*Cell* **1990***, 60*, 461-472.

(1998) Oct4-deficient embryos do not develop pluripotent inner mast cells

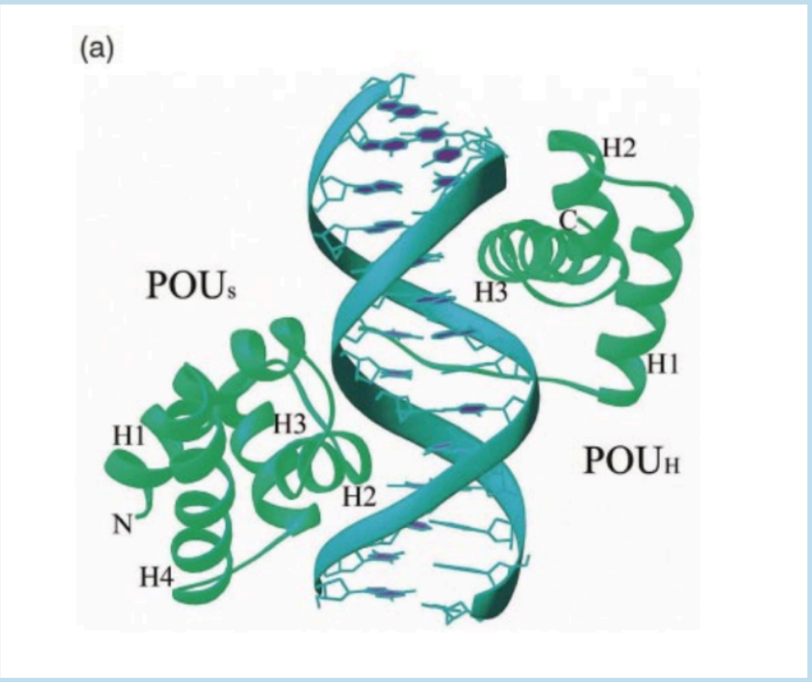
Nichols, J. *et al.*,*Cell* **1998**, 95, 379-391.

Octamer-binding (Oct) Proteins

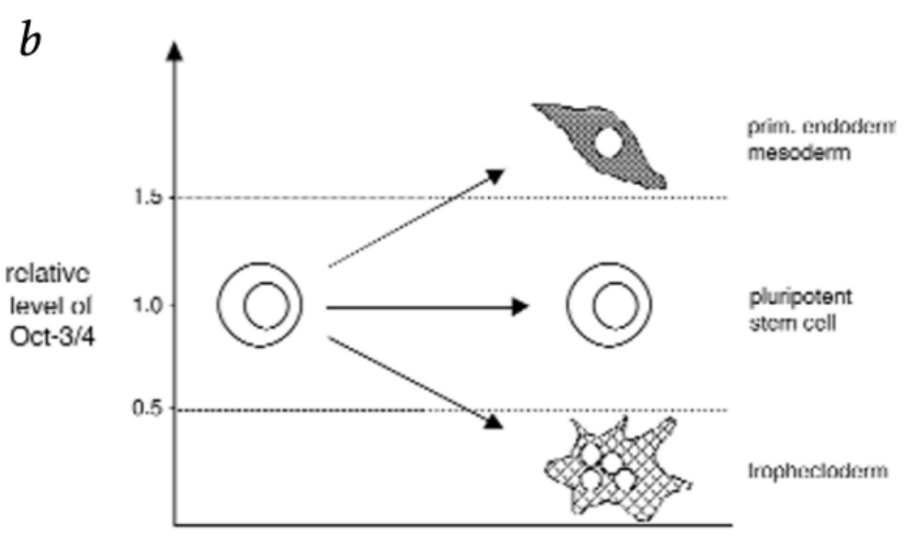
Oct transcription factor family is comprised of 8 members

(Oct1, Oct2, Oct4, Oct6, Oct7, Oct8, Oct9, and Oct11)

Comprised of two linked binding domains (POU_S and POU_H), which each bind 4 DNA base pairs in the major groove



Oct1 binding to its cognate DNA sequence

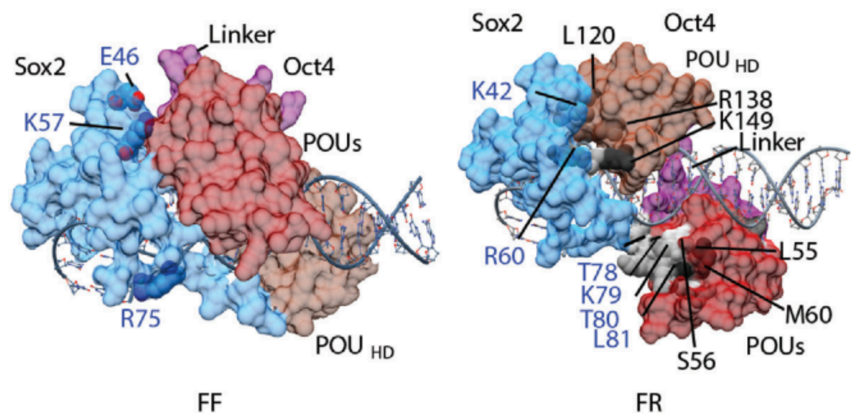


ES cell fate is sensitive to the cellular level of Oct4

(2000) up-or-dowm regulation by 50% leads to ESC differentiation

Niwa, H.; Miyazaki, J.; Smith A. G. *Nat. Genet.* **2000***, 24*, 372.

Octamer-binding (Oct) Proteins



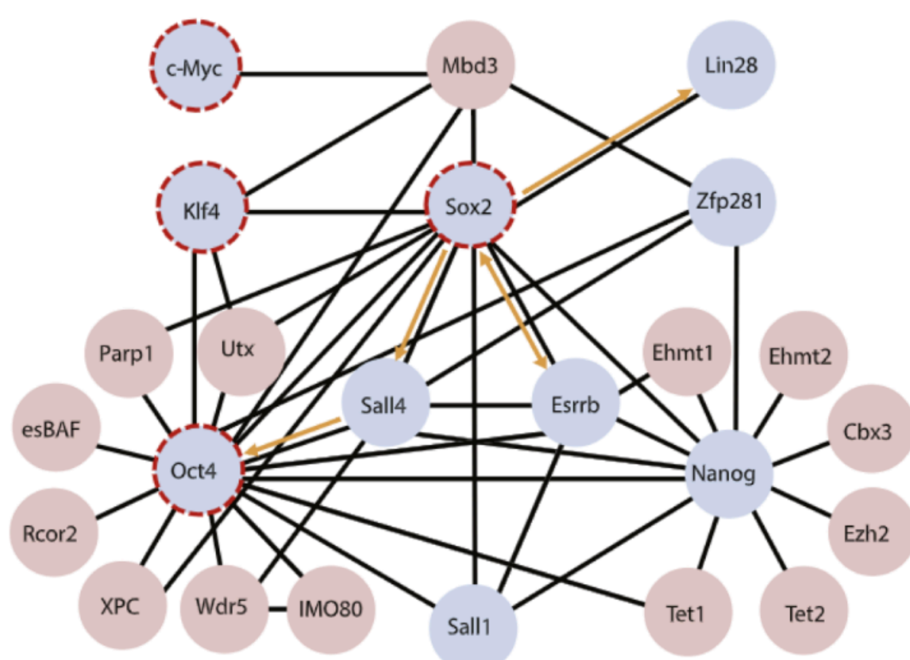
Oct4-Sox2 heterodimer binding to DNA

Oct4,Sox2, and Nanog heterodimer serves as a master regulators of pluripotentcy and self renewal (co-occupy over 300 target genes)

Boyer, L. A. *et al. Cell* **2005**, *122*, 947-956.

stoichiometry of Sox2 and Oxt4 influence efficiency and quality of iPS cells

Papaetrou, E. P. *et al.*,*Proc. Natl. Acad. Sci.* **2009**, *106*, 12759-12764.



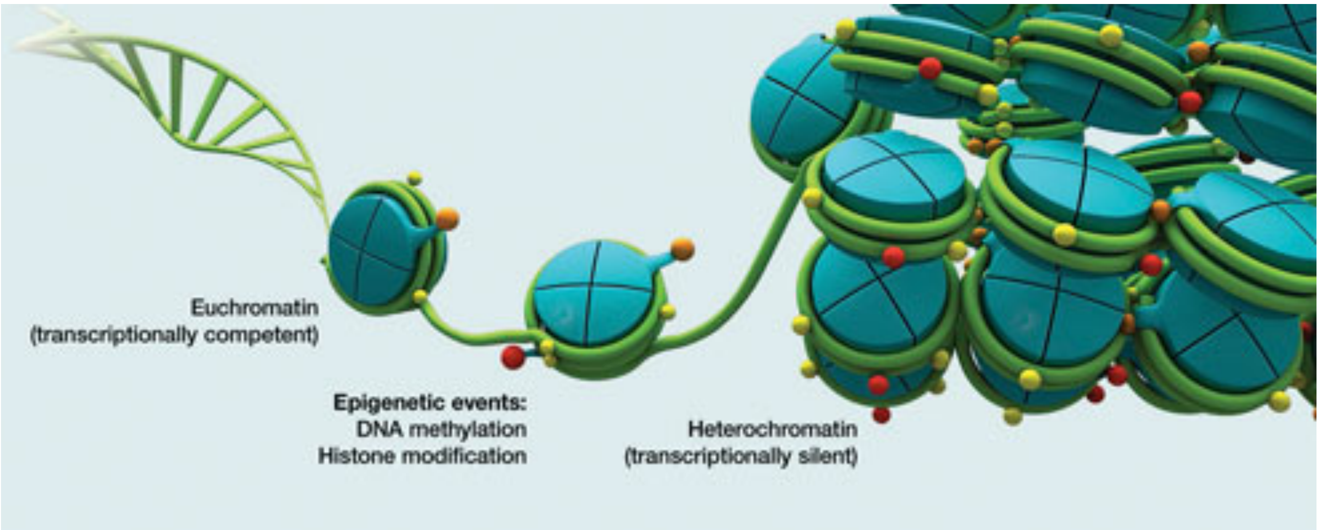
Three core factors engage in an extended interactome to regulate pluripotency

Co-occupy and promote expression of pluripotency genes while repressing differentiation genes

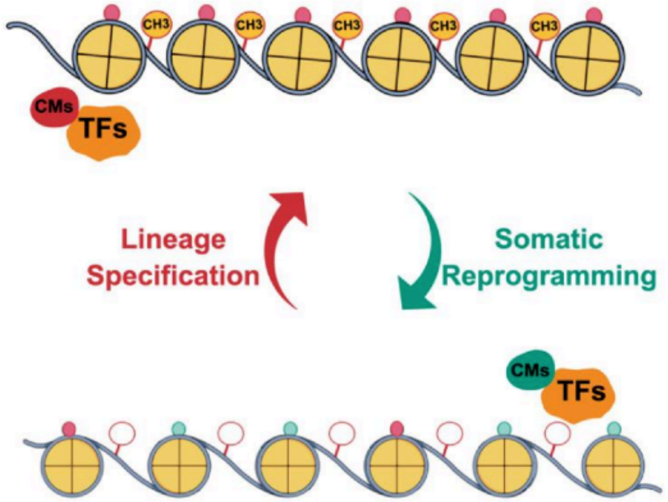
Theunissen, T. W.; Jaenisch, R. *Cell Stem Cell* **2014**, *14*, 720.

Epigenetic Re-writing in Cellular Reprogramming

Chromatin structure and methylation patterns influence gene expression



Erasing of repressive histone marks is critical for reprogramming



Meir, Y. J.; Li, G. *Cells* **2021**, *10*, 2888.

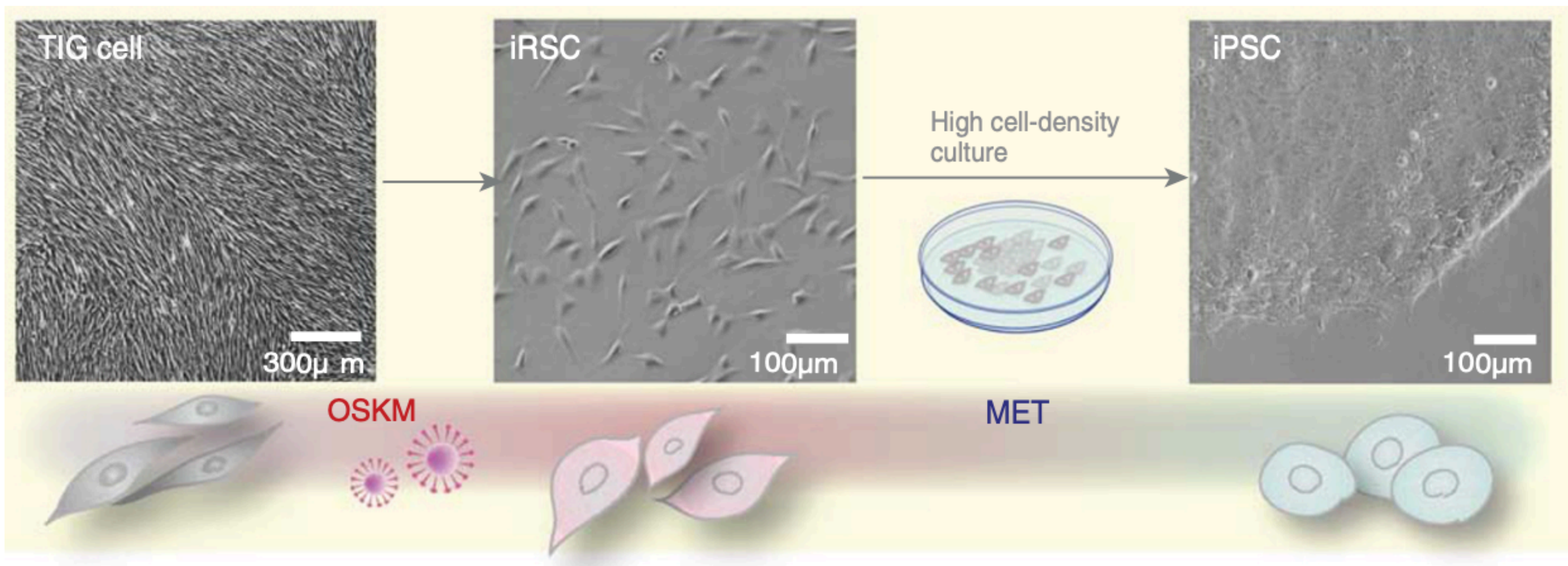
Histone chaperone CAF-1 represents a safeguard of somatic identity

CAF-1 suppression accelerates iPSC reprogramming

Cheloufi, S. *Nature* **2015**, *528*, 218.

Epigenetic Re-writing in Cellular Reprogramming

Reprogramming causes a gradual change in cell morphology



iPSC reprogramming takes 3-4 weeks in human cells, with efficiencies around 0.01-0.1%

Teshigawara, R.; Cho, J.; Kameda, M.; Tada, T. *Lab. Invest.* **2017***, 97*, 1152-1157

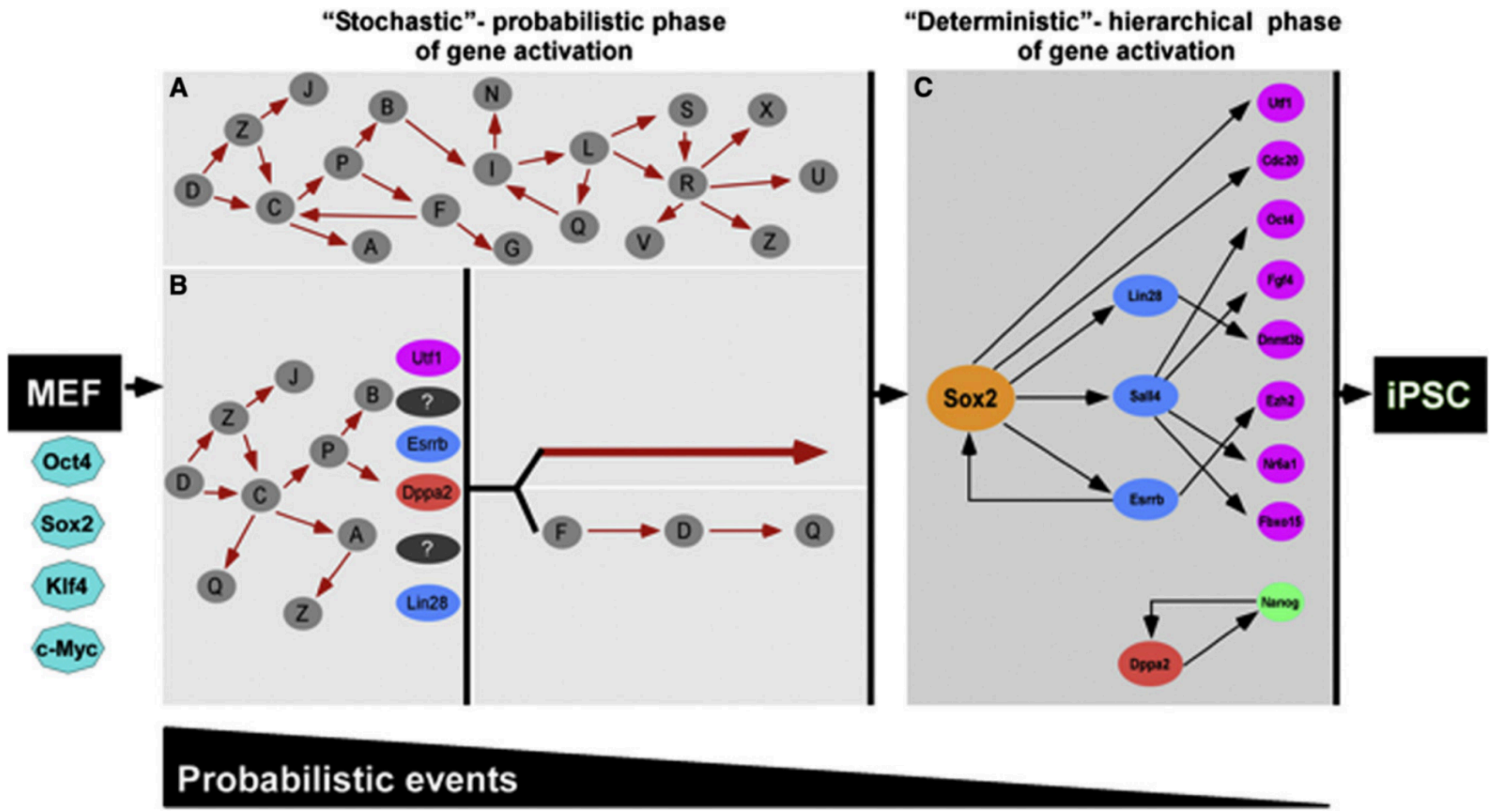
+ OKMS *Transgene Dependent* — - OKMS *Transgene Independent*

	MEF	Initiation	Maturation	Stabilization (iPSC)
Hallmark		• Loss of somatic cell program • Metabolism changes • Increased proliferation rate • Inhibition of apoptosis and senescence • Morphologic changes (MET)	• Gain of a subset of pluripotency associated genes • Preparing for transgenes independency	• Transgene-independant self renewal • Pluripotency • Loss of epigenetic memory • X-reactivation • Telomeres elongation
Markers	Thy1, Zeb1/2, Snai1/2, CD44	Alpl, E-Cadh, EpCam, SSEA1	Nanog, Oct4, Esrrb, ICAM1	Sox2, Dppa4, Pecam

Distinct genetic markers can be found over the course of reprogramming

David, L.; Polo, J. M. *Stem Cell Res*. **2014***, 12*, 754-761

Reprogramming is Initially Stochastic

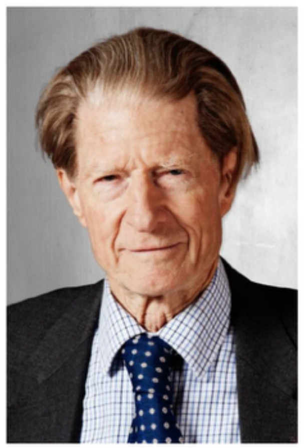


Heterogeneity in gene expression observed between cells after introduction of OKSM

Random initial gene expression may or may not lead to a productive hierarchal mechanism

Buganim, Y., *et al*. *Cell* **2012**, *150*, 1209-1222.

2012 Nobel Prize in Physiology and Medicine



Sir John B. Gurdon

Gurdon institute

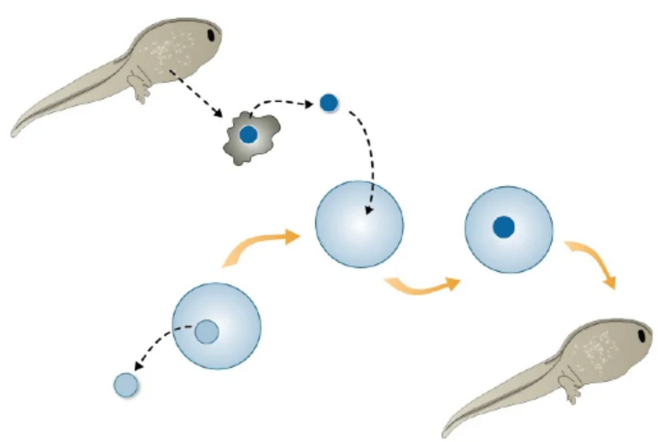
Shinya Yamanaka

Kyoto university

2012 Nobel prize in Physiology or Medicine

"For the discovery that mature cells can be reprogrammed to become pluripotent"

John B. Gurdon: First animal cloning via somatic cell transfer

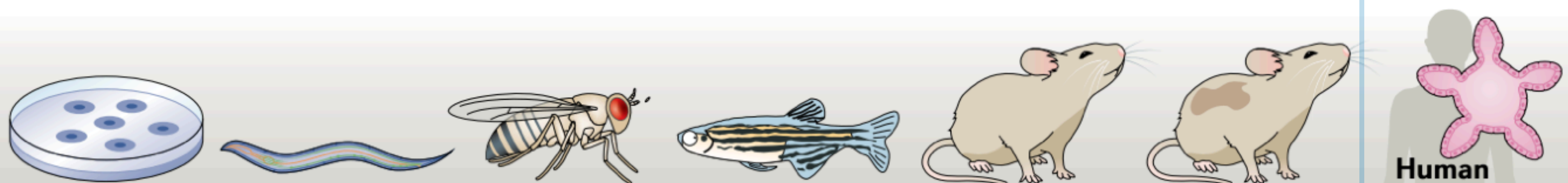


Frog clones can accept skin grafts from one another

Gurdon, J. B. *J. Embryol. Exp. Morphol.* **1962***, 10*, 622-640.

Gurdon, J. B.; Uehlinger, V. *Nature* **1966***, 210*, 1240-1241.

The Need for Human-cell-based Disease Models



	2D cell culture	*C.elegans*	*D. melanogaster*	*D. rerio*	*M. musculus*	PDX	Human organoids
Ease of establishing system	✓ (Partly suitable)/✗ (Not suitable)	✓ (Best)	✓ (Best)	✓ (Partly suitable)	✓ (Partly suitable)	✓ (Partly suitable)	✓ (Good)
Ease of maintenance	✓ (Best)	✓ (Good)	✓ (Good)	✓ (Partly suitable)	✓ (Partly suitable)	✓ (Partly suitable)	✓ (Good)
Recapitulation of developmental biology	✗ (Not suitable)	✓ (Best)	✓ (Good)	✓ (Good)	✓ (Good)	✗ (Not suitable)	✓ (Good)
Duration of experiments	✓ (Best)	✓ (Best)	✓ (Best)	✓ (Best)	✓ (Good)	✓ (Best)	✓ (Best)
Genetic manipulation	✓ (Best)	✓ (Good)	✓ (Good)	✓ (Good)	✓ (Partly suitable)	✗ (Not suitable)	✓ (Best)
Genome-wide screening	✓ (Best)	✓ (Good)	✓ (Good)	✓ (Good)	✗ (Not suitable)	✗ (Not suitable)	✓ (Good)
Physiological complexity	✗ (Not suitable)	✓ (Good)	✓ (Good)	✓ (Best)	✓ (Best)	✓ (Best)	✓ (Partly suitable)
Relative cost	✓ (Best)	✓ (Best)	✓ (Best)	✓ (Partly suitable)	✓ (Partly suitable)	✓ (Partly suitable)	✓ (Good)
Recapitulation of human physiology	✓ (Partly suitable)	✓ (Partly suitable)	✓ (Partly suitable)	✓ (Good)	✓ (Good)	✓ (Best)	✓ (Best)

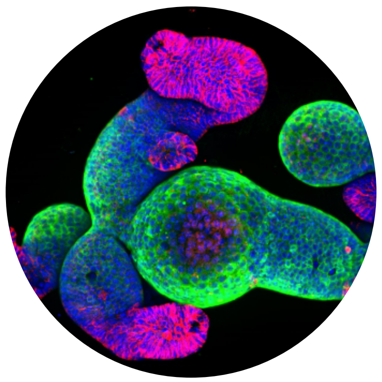
✓ Best ✓ Good ✓ Partly suitable ✗ Not suitable

Certain biological phenomena cannot be reproduced in animal models

Organoids can offer a representative, patient specific model of in vivo biology

Kim, J.; Koo, B.; Knoblich, J. A. *Nat. Rev. Mol. Cell Biol.* ***2020****, 21*, 571-584.

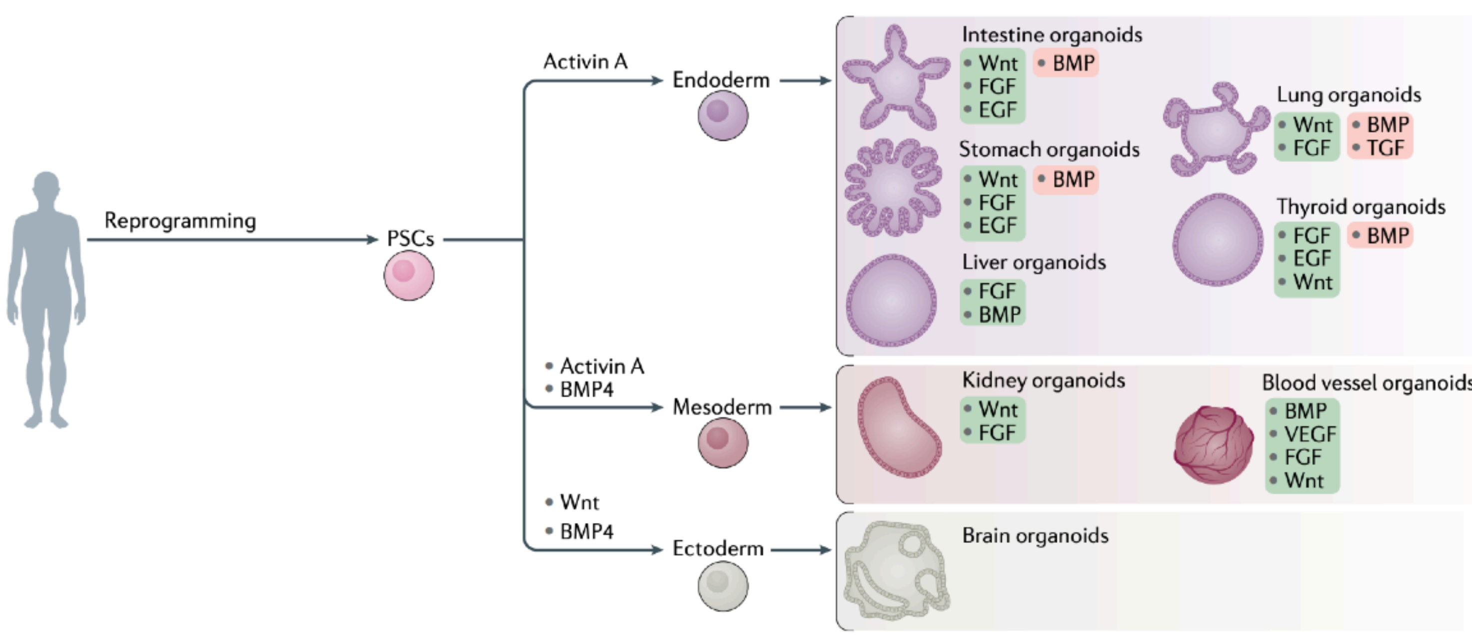
Organoids



Organoids: self-organized 3D assembly of cells generated in vitro

Highly similar, in both histology and function, to organs

Synthesis of organoids from iPSCs

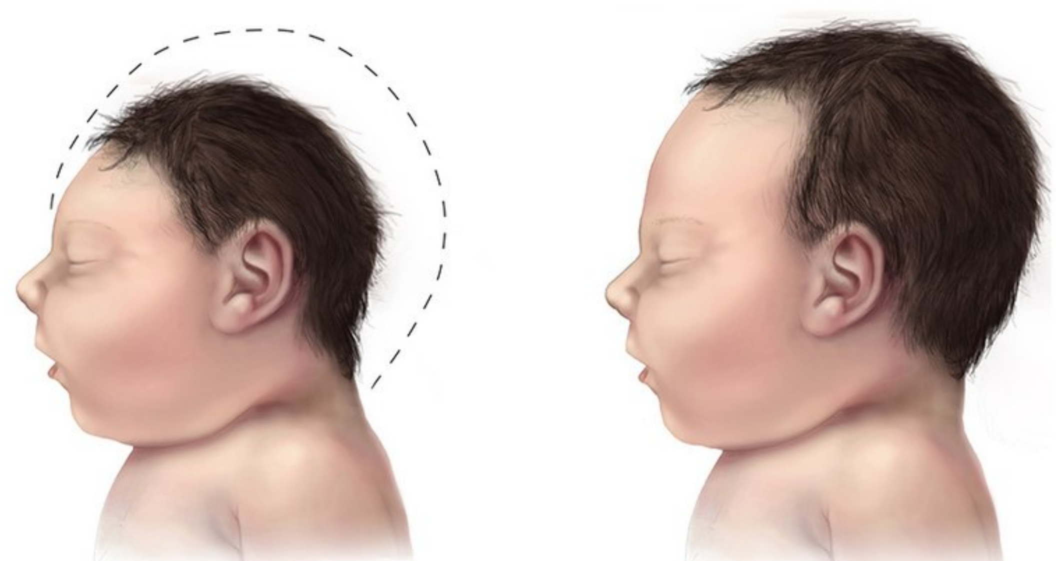


Subjecting iPSCs to a series of specific growth and signaling factors allows for specified differentiation

Kim, J.; Koo, B.; Knoblich, J. A. *Nat. Rev. Mol. Cell Biol.* ***2020****, 21*, 571-584.

iPSC-derived Organoids to Model Developmental Brain Disorders

Microcephaly: small skull size from impeded brain development during pregnancy



Disorders affecting human brain development have often proved difficult to recapitulate in animal models

Can organoids be used to model microcephaly?

Lancaster, M. A.; Renner, M.; Martin, C. *et al.*, *Nature* ***2013****, 501*, 373-379.

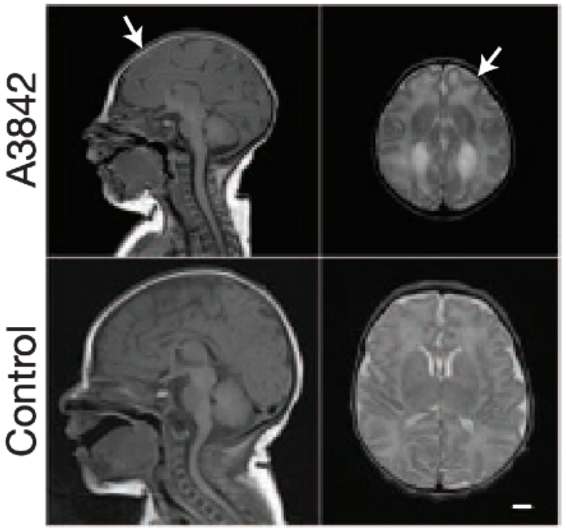
iPSC-derived Organoids to Model Developmental Brain Disorders

2013: First human clinical trial for iPS-cell-based therapy

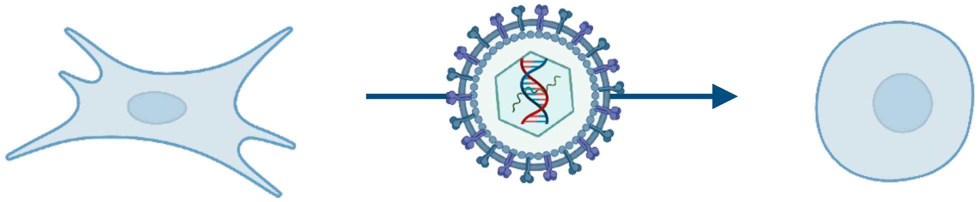
Institute of Molecular Biology (IMBA), Vienna

Jürgen Knoblich

Patient with severe microcephaly



Patient-specific iPSCs derived from skin fibroblasts

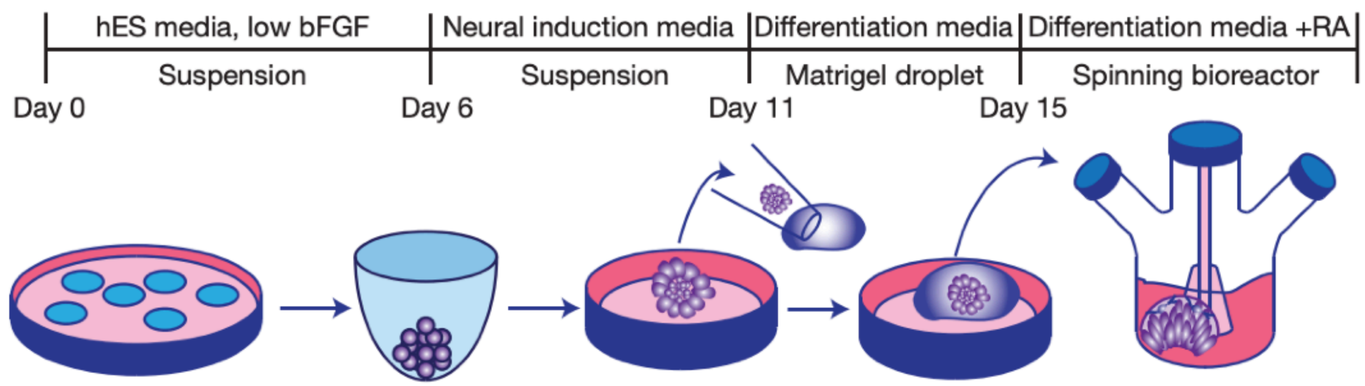


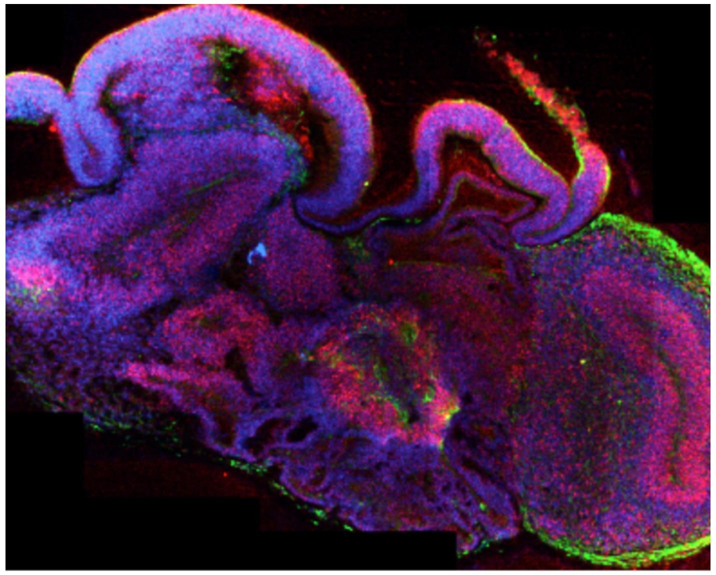
Lentiviral transfection of Yamanaka factors

Lancaster, M. A.; Renner, M.; Martin, C. *et al.*, *Nature* ***2013**, 501*, 373-379.

iPSC-derived Organoids to Model Developmental Brain Disorders

Patient-specific iPSCs grown into cerebral organoid via specialized culture protocol



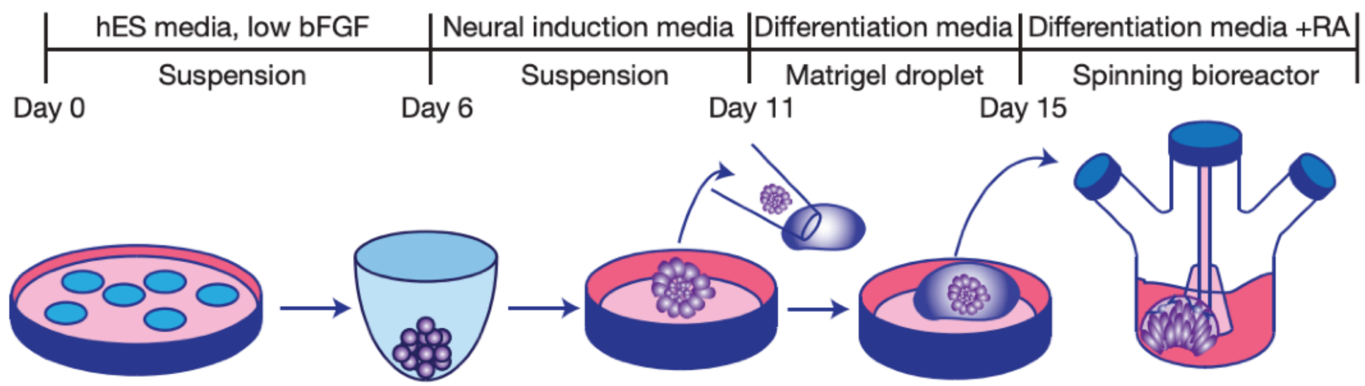


organoids display regions similar to natural brain regions (cerebral Cortex, choroid plexus, retina, and meninges)

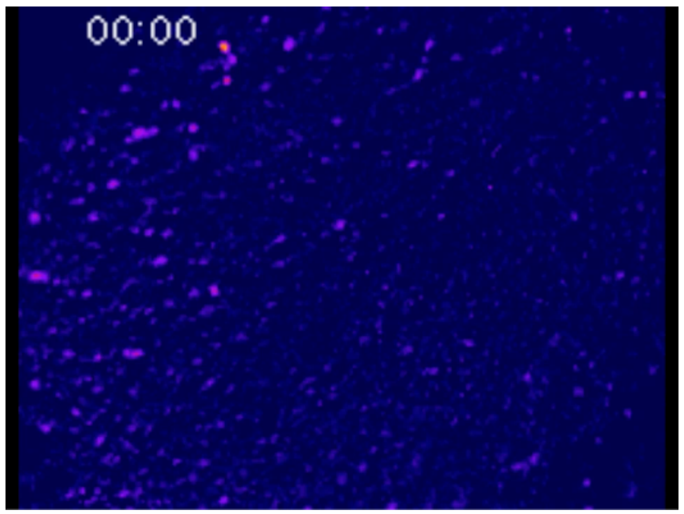
Lancaster, M. A.; Renner, M.; Martin, C. *et al.*, *Nature* ***2013****, 501*, 373-379.

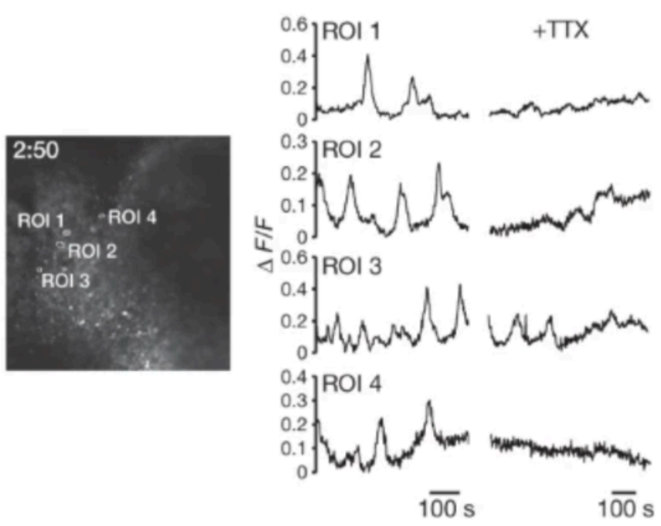
iPSC-derived Organoids to Model Developmental Brain Disorders

Patient-specific iPSCs grown into cerebral organoid via specialized culture protocol



Ca^{2+} imaging reveals that neurons in organoids are electrically active





Lancaster, M. A.; Renner, M.; Martin, C. *et al.*, *Nature* ***2013**, 501*, 373-379.

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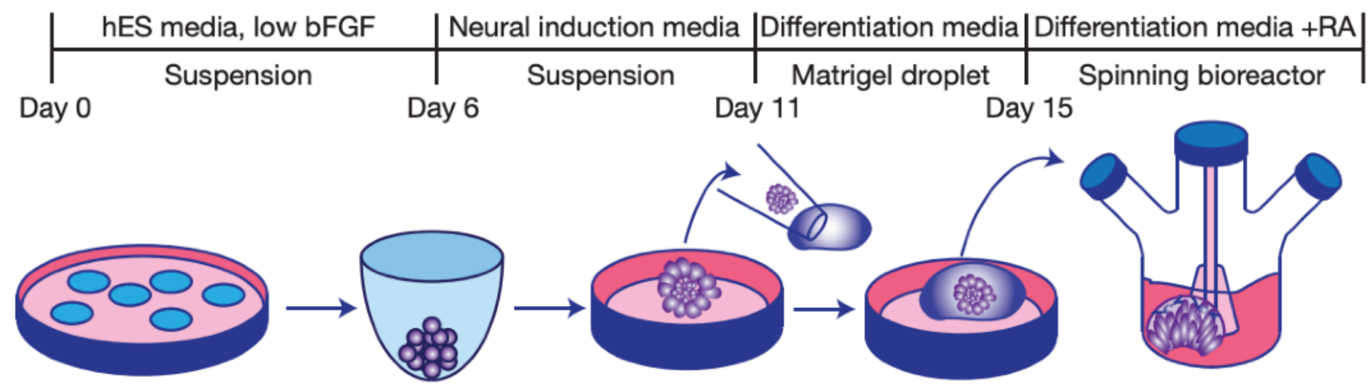
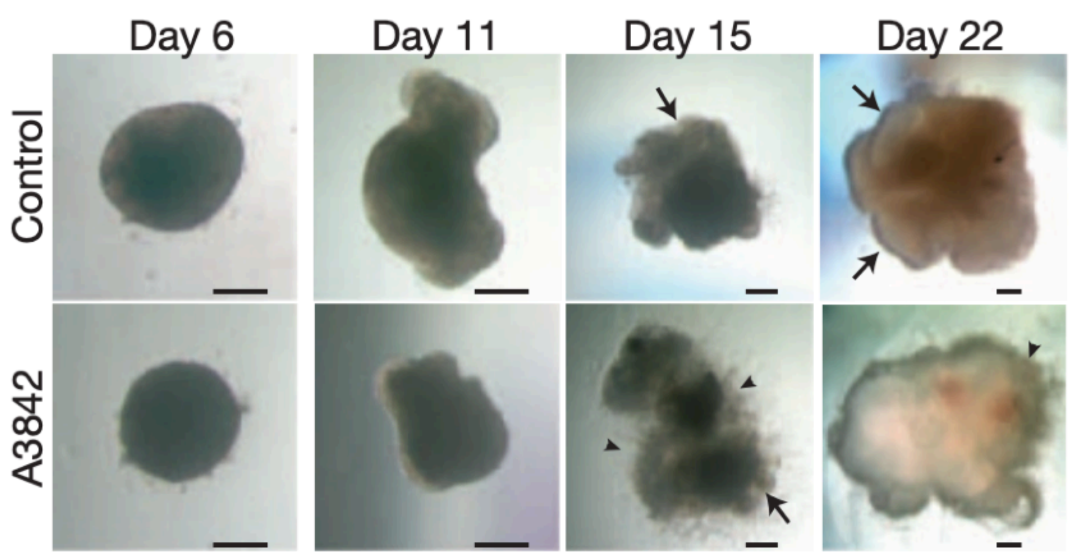
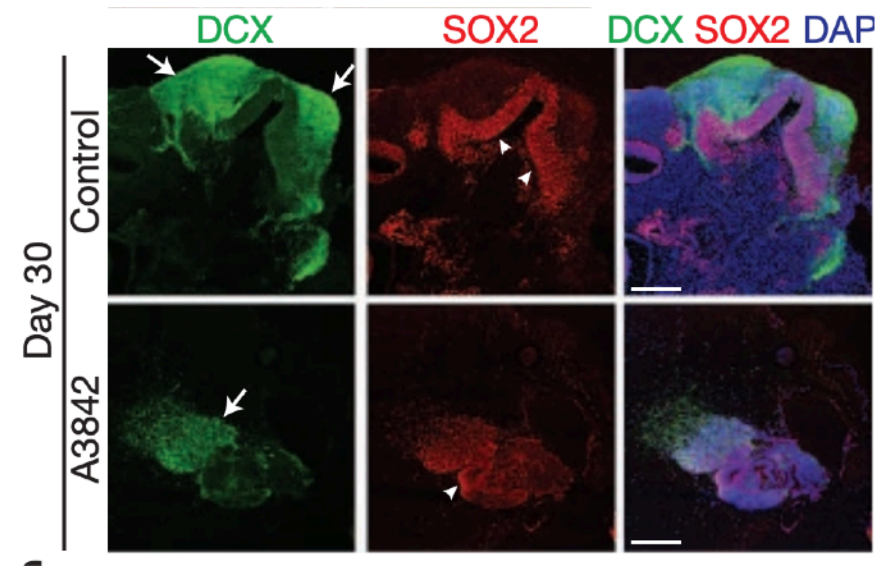


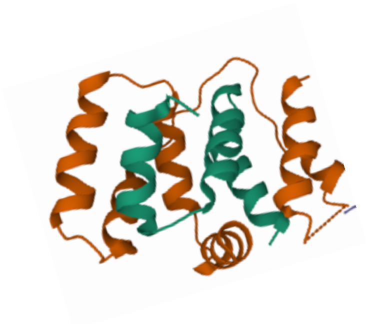




Patient organoid displayed altered morphology and stunted neural growth relative to control

Lancaster, M. A.; Renner, M.; Martin, C. *et al.*, *Nature* ***2013***, *501*, 373-379.

iPSC-derived Organoids to Model Developmental Brain Disorders

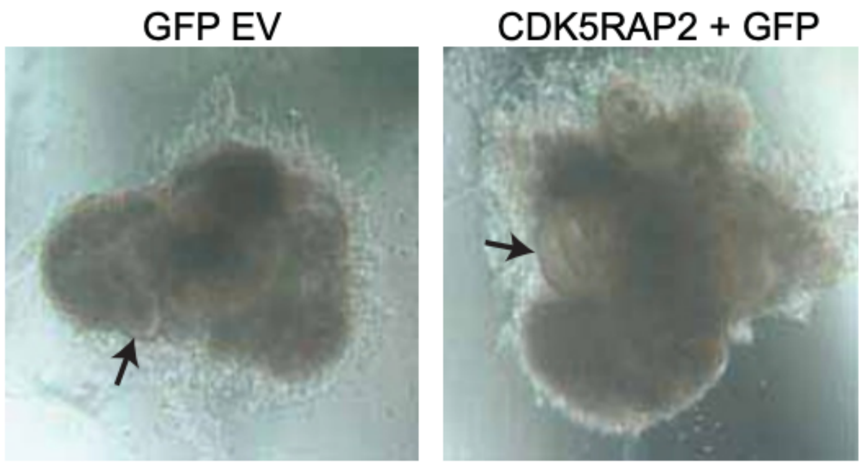


CDK5RAP2

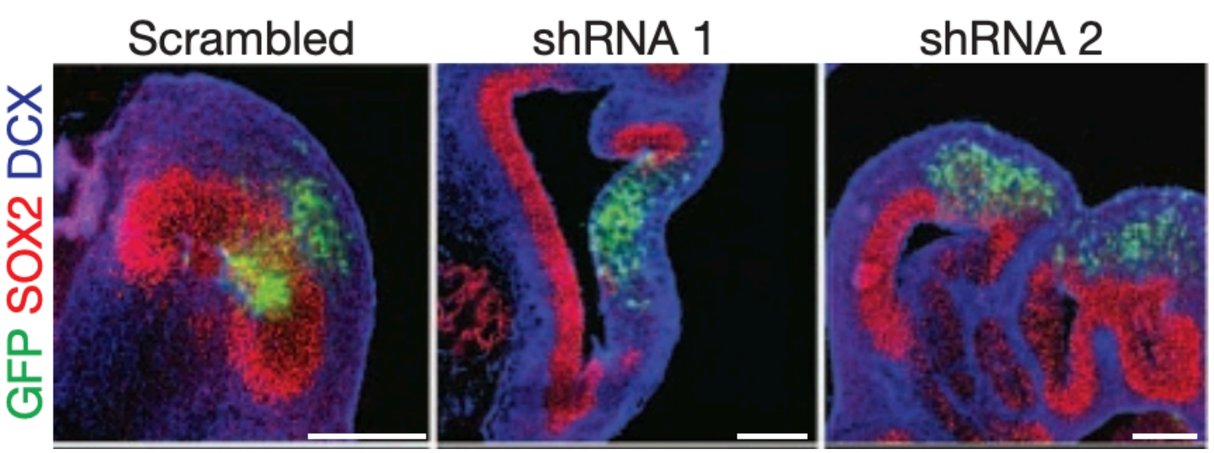
(CDK5 regulatory subunit-associated protein 2)

Loss of function mutations of CDK5RAP2 are associated with microcephaly

Transfection of CDK5RAP-2 into patient-derived organoid



Knock-down of CDK5RAP-2



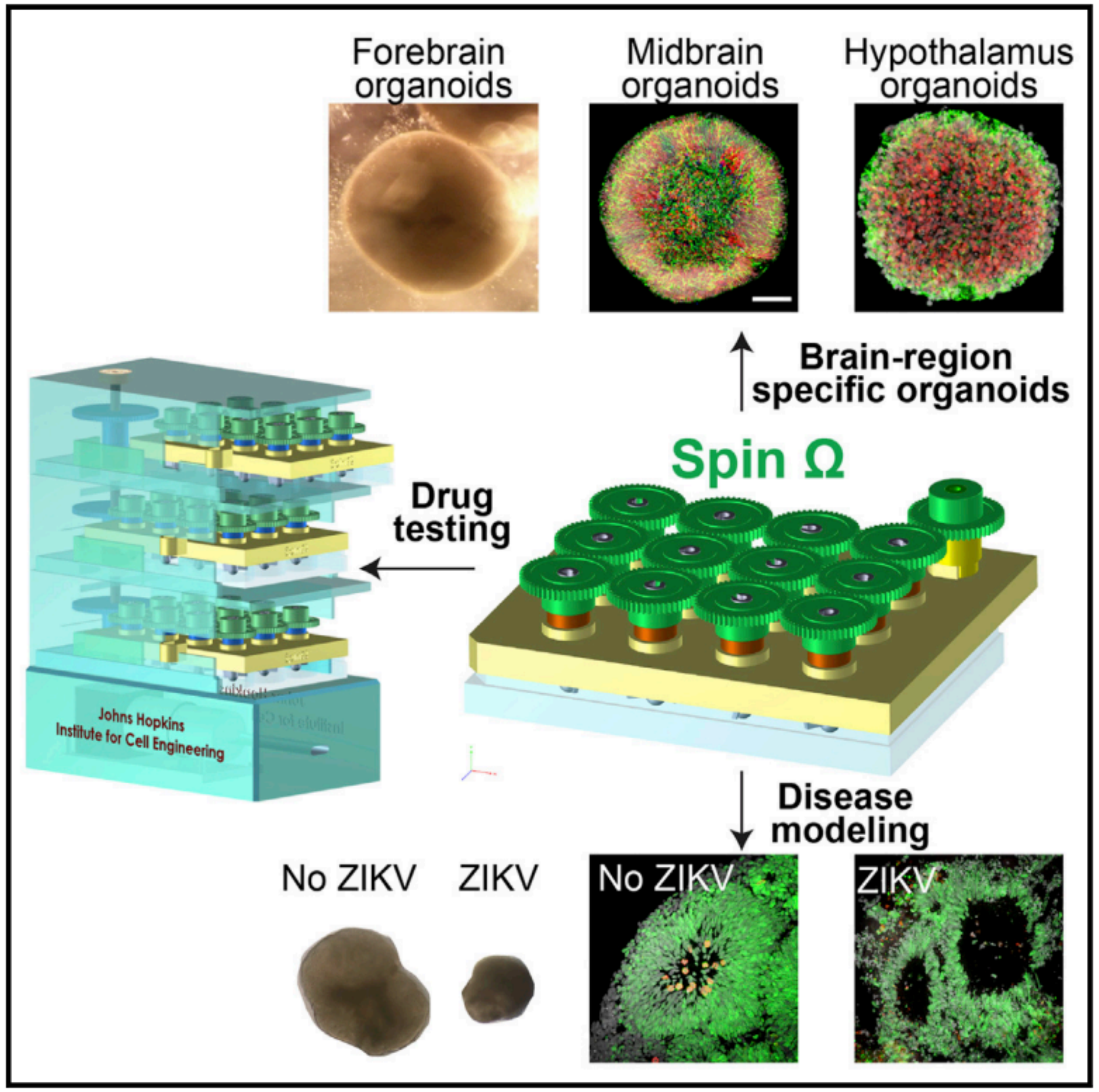
Microcephaly phenotype is specific to loss of CDK5RAP2

Loss of CDK5RAP2 causes premature cellular differentiation

Lancaster, M. A.; Renner, M.; Martin, C. *et al.*, *Nature* ***2013****, 501*, 373-379.

iPSC-derived Organoids to Model Developmental Brain Disorders

Use of brain-region-specific organoids to model Zika virus exposure



ZIKV infection leads to a decrease in brain organoid volume

Qian, X.; Nguyen, H. N. *et al.*, *Cell* ***2016****, 165,* 1238-1254.

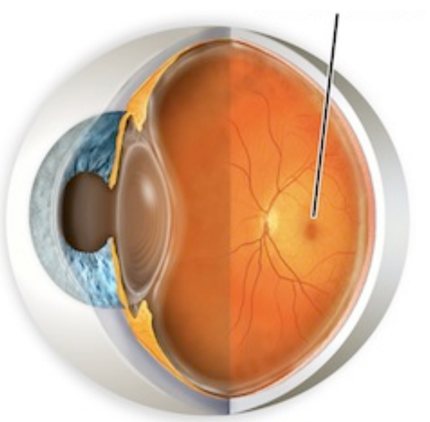
iPSC-derived Retinal Cells for the Treatment of ARMD

ARMD: degeneration of macula over time, leading to loss of vision


Normal Vision

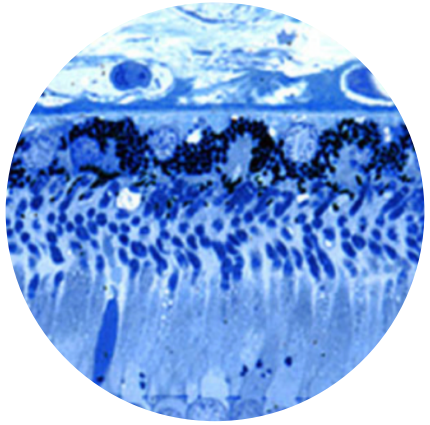

The same scene affected by age-related macular degeneration

Leading cause of blindness in the elderly - 288 million cases worldwide by 2040



***Macula**: 5 mm pigmented area responsible for visual resolution*

*Proper function and health maintained by **retinal pigment epithelium (RPE)***



Can RPE cells from iPSCs be used to treat ARMD?

iPSC-derived Retinal Cells for the Treatment of ARMD

2013: First human clinical trial for iPS-cell-based therapy

RIKEN

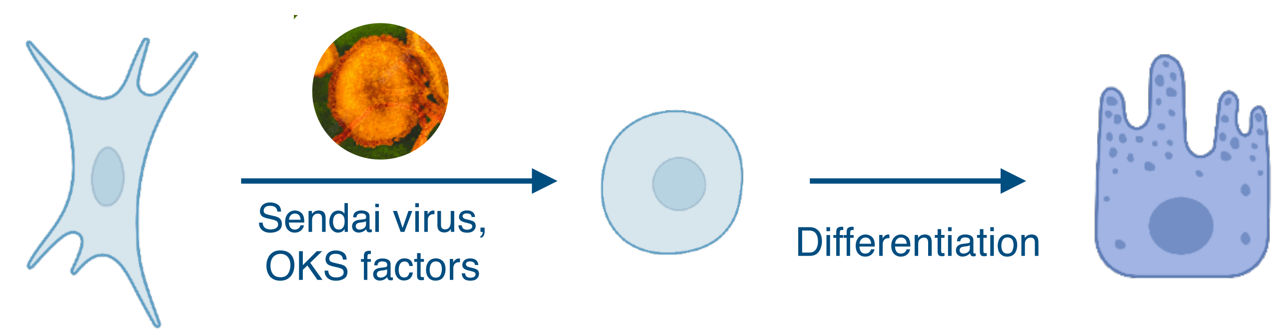
RIKEN Center for Developmental Biology (CDB)
Kobe, Japan

Masayo Takahashi, MD, PhD

Patient 1 | **Patient 2**

77 year old woman | 68 year old man

Patient-specific RPE cells via iPSC technology



Skin fibroblasts | **RPE sheet**

Liu, X.; Tan, J. P. *et al.*, *Nature* ***2021***, *591*, 627-632.

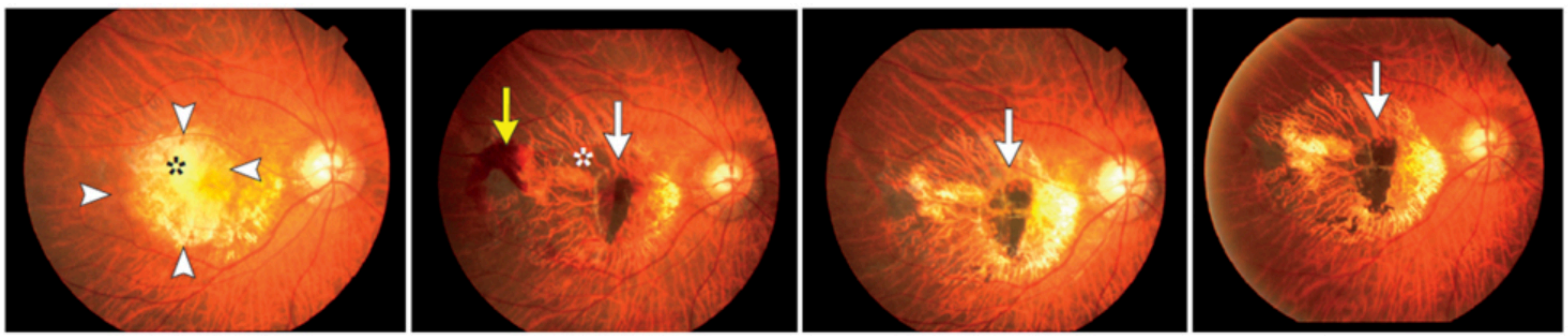
iPSC-derived Retinal Cells for the Treatment of ARMD

Patient 1 underwent surgery on September 12th, 2014

ectopically formed blood vessels removed from damaged RPE area

iPSC-derived RPE cell sheet (1.3 X 3.0 mm) transplanted under retina

Photographs of macular region



Before surgery | 3 days after surgery | 8 weeks after surgery | 1 year after surgery

Transplanted RPE sheet visible after 1 year (arrow)

Mandai, M.; Watanabe, A. *et al.*, *N. Engl. J. Med.* ***2017****, 376*, 1038-1046.

iPSC-derived Retinal Cells for the Treatment of ARMD

Patient 1 underwent surgery on September 12th, 2014

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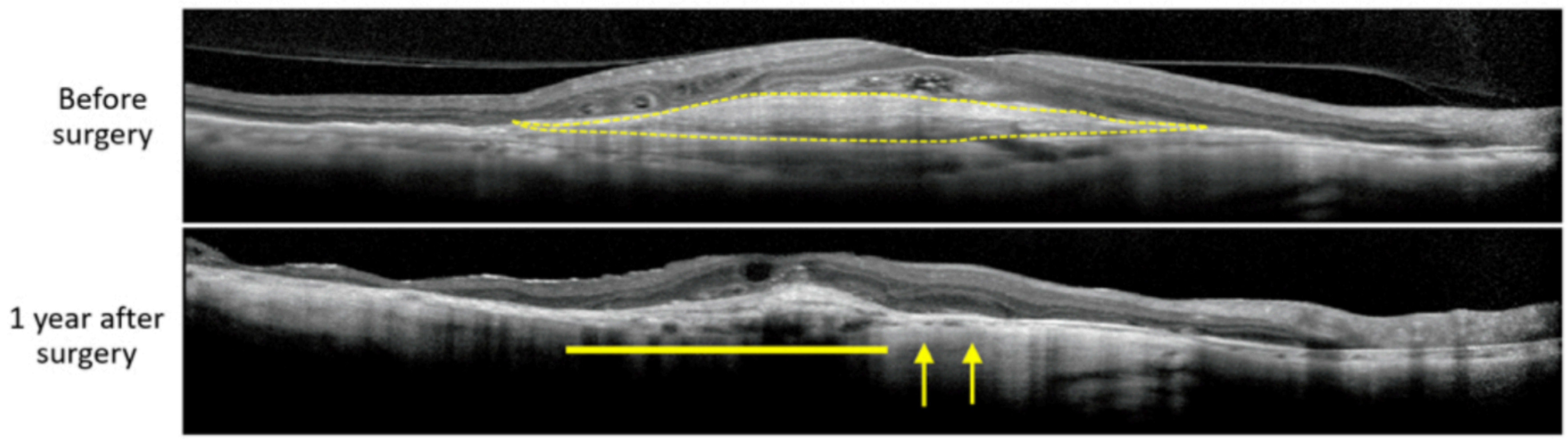
iPSC-derived RPE cell sheet (1.3 X 3.0 mm) transplanted under retina

Vertical sectional view of macular region by optical coherence tomography (OCT)



Transplanted RPE (solid line) Retinal tissue (arrows)

Present day: no adverse effects detected. Vision has not improved or worsened

Mandai, M.; Watanabe, A. *et al.*, *N. Engl. J. Med.* ***2017****, 376*, 1038-1046.

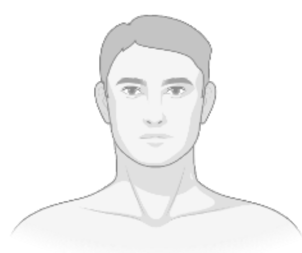
iPSC-derived Retinal Cells for the Treatment of ARMD

Patient 1 underwent surgery on September 12th, 2014

ectopically formed blood vessels removed from damaged RPE area

iPSC-derived RPE cell sheet (1.3 X 3.0 mm) transplanted under retina

Patient 2 did not undergo surgery in lieu of safety concerns



Whole genome analysis revealed genetic changes in iPSC-derived RPE cell lines
(3 SNV and 3 CNV not present in original fibroblasts)

Unknown affect of changes raised safety concerns

NEWS

RIKEN suspends first clinical trial involving induced pluripotent stem cells

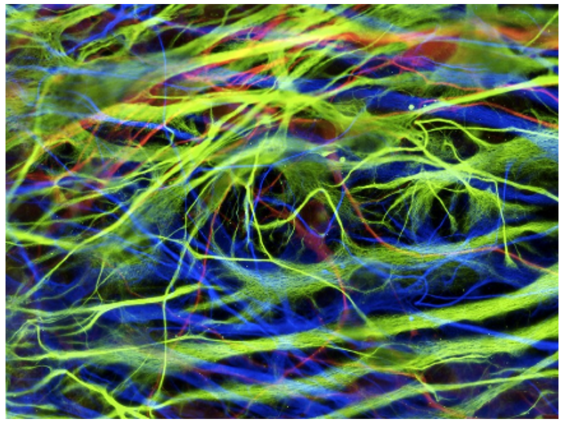
Nat. Biotech. ***2015***, *33*, 890.

Mandai, M.; Watanabe, A. *et al.*, *N. Engl. J. Med.* ***2017***, *376*, 1038-1046.

Ongoing Clinical Trials Using iPSCs

Ongoing clinical trials for iPSCs as of September 2020

Parkinson's Disease[1)]	(1) https://upload.umin.ac.jp/cgi-open-bin/ctr/ctr_view.cgi?recptno=R000038278
Macular degeneration[2)]	(2) https://upload.umin.ac.jp/cgi-open-bin/ctr/ctr_view.cgi?recptno=R000013279, https://upload.umin.ac.jp/cgi-open-bin/ctr/ctr_view.cgi?recptno=R000029894, https://ClinicalTrials.gov/show/NCT04339764, https://ClinicalTrials.gov/show/NCT02464956
Retinitis pigmentosa[3)]	(3) https://jrct.niph.go.jp/en-latest-detail/jRCTa050200027
Corneal disorder[4)]	(4) https://jrct.niph.go.jp/en-latest-detail/jRCTa050190084
Heart failure[5)]	(5) https://jrct.niph.go.jp/en-latest-detail/jRCT2053190081, https://ClinicalTrials.gov/show/NCT04396899, https://ClinicalTrials.gov/show/NCT03763136
Spinal cord injury[6)]	(6) https://jrct.niph.go.jp/en-latest-detail/jRCTa031190228
Platelet transfusion[7)]	(7) https://jrct.niph.go.jp/en-latest-detail/jRCTa050190117
Graft versus host disease[8)]	(8) https://ClinicalTrials.gov/show/NCT02923375
Cartilage defect[9)]	(9) https://jrct.niph.go.jp/en-latest-detail/jRCTa050190104
Cancer immunotherapy[10)]	(10) https://ClinicalTrials.gov/show/NCT03407040, https://ClinicalTrials.gov/show/NCT04106167, https://ClinicalTrials.gov/show/NCT03841110, https://jrct.niph.go.jp/en-latest-detail/jRCT2033200116



Jan 2021, iPSC-derived dopaminergic neurons for the treatment of advanced Parkinson's begins Phase I in USA

Yamanaka, S. *Cell Stem Cell.* ***2020**, 27*, 523-531.

Questions?