CHAIRE ÉPIGÉNÉTIQUE ET MÉMOIRE CELLULAIRE

Année 2013-2014 :

“Reprogrammations développementales, induites et pathologiques ”

Cours III

Reprogrammation experimentale – les cellules pluripotentes induites

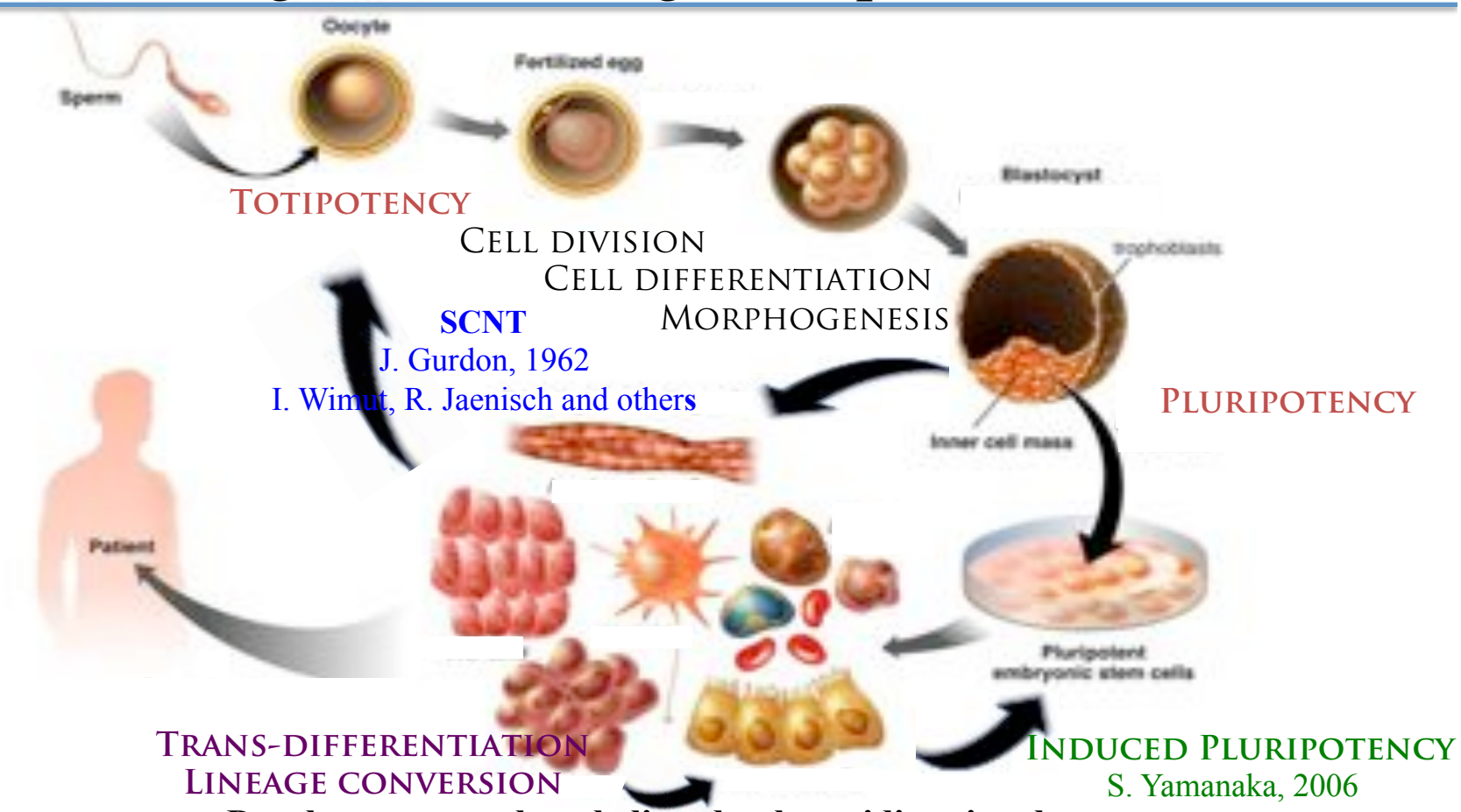
24 mars 2014

Seminaire:

Dr Claire Rougeulle à 17h30

“Inactivation du chromosome X, pluripotence et reprogrammation, de la souris à l’homme”.

All cells have the capacity to form a whole organism – through differential gene expression



Development was long believed to be <u>unidirectional:</u>

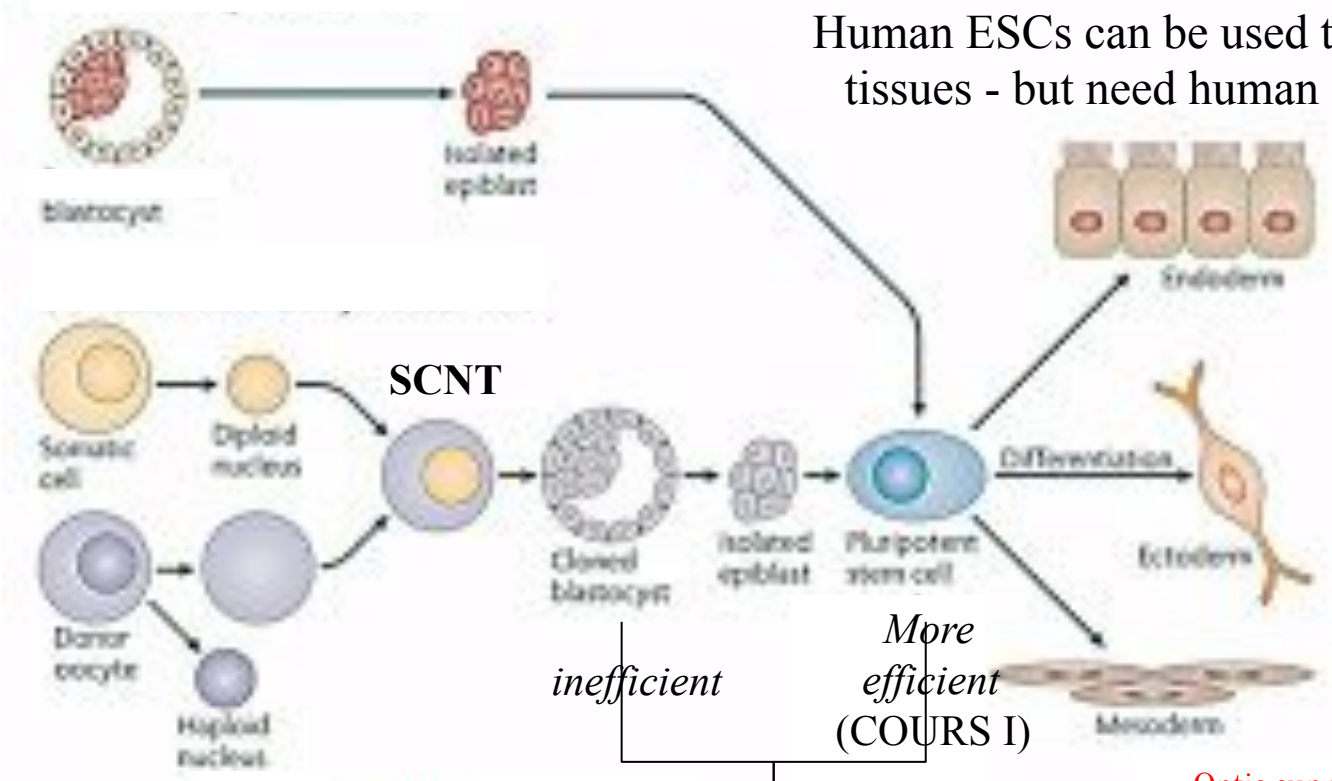
Transcription factors dictate gene expression states, epigenetic marks perpetuate them stably

We now know that there is remarkable plasticity during development and even in somatic cells (following SCNT, ES cell fusion, *trans*-differentiation): epigenetic barriers can be overcome!

⇒ Differentiated cells are NOT irreversibly committed to their fate
but can be REPROGRAMMED and/or REPURPOSED

Perspectives brought by ES cells and SCNT

Human ESCs can be used to generate all tissues - but need human embryos…!

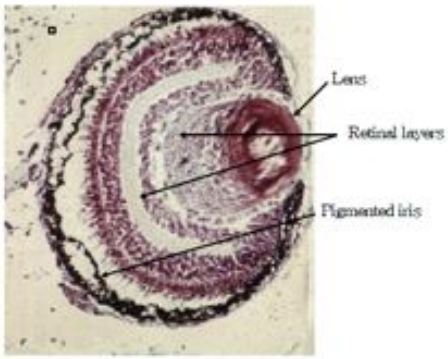


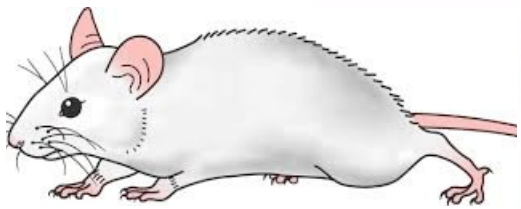
Therapeutic Cloning

inefficient

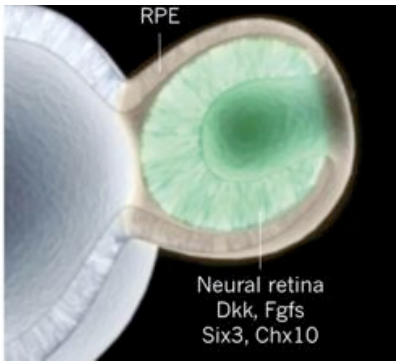
More efficient (COURS I)

Normal eye
by cloning from a muscle cell





Optic cup formation by *in vitro* differentiation of ES cells & 3D culture (*Eiraku et al, 2011*)



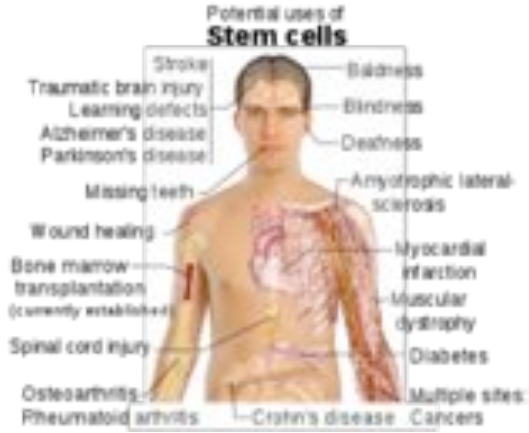
Therapeutic Cloning?

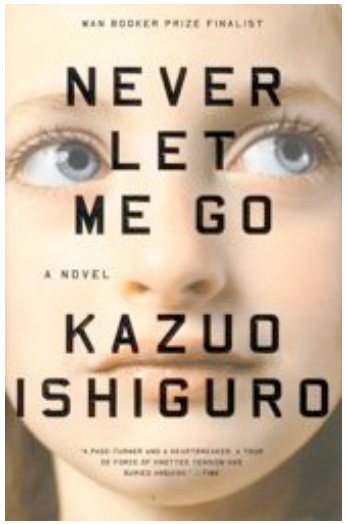
Derivation of **human ES cells** by Jamie Thomson in 1998 and more recent work by Noggle et al 2011 showing that it is, in principle, possible to reprogram human somatic cells up to the blastocyst stage at least.

=> "**Therapeutic Cloning**" became a real possibility: Patient-specific pluripotent ESCs by somatic cell nuclear transfer into human eggs, which could then be differentiated to the cell type that was defective in the patient *For organ transplant replacement, skin grafts, degenerative diseases (eg Parkinson's), diabetes, spinal cord repair, leukemia…)*

Serious ethical issues in humans!!!

Ideally, one would like to derive human ESCs from patient's own somatic cells?
Or to change one cell type into another cell type from the same patient?





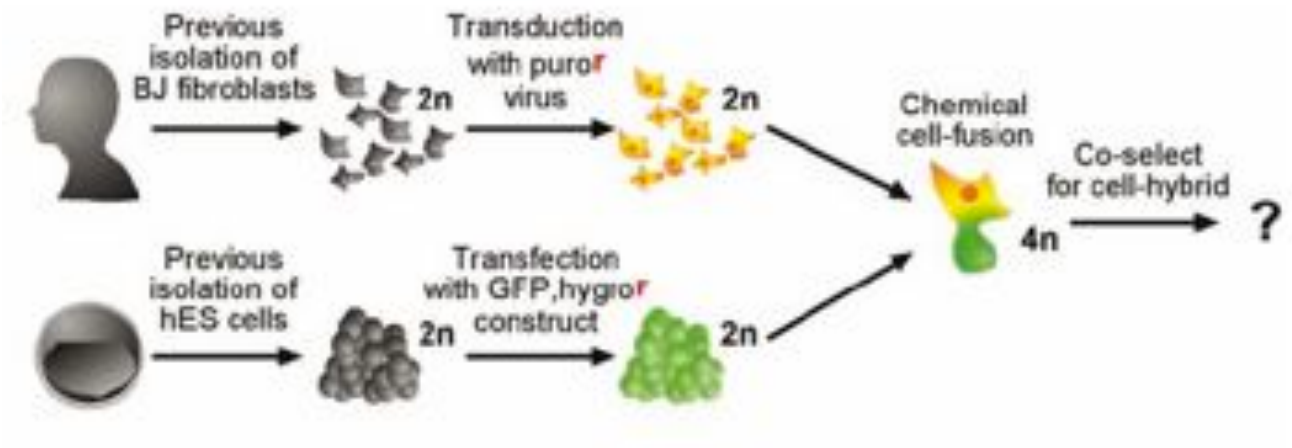
In this novel, the Hailsham school children are clones, created to be "donors" that provide vital organs for "normals" through a series of "donations" that eventually lead to the donor's death

ES cells have the capacity to reprogram somatic cells: "ES Cell Dominance"

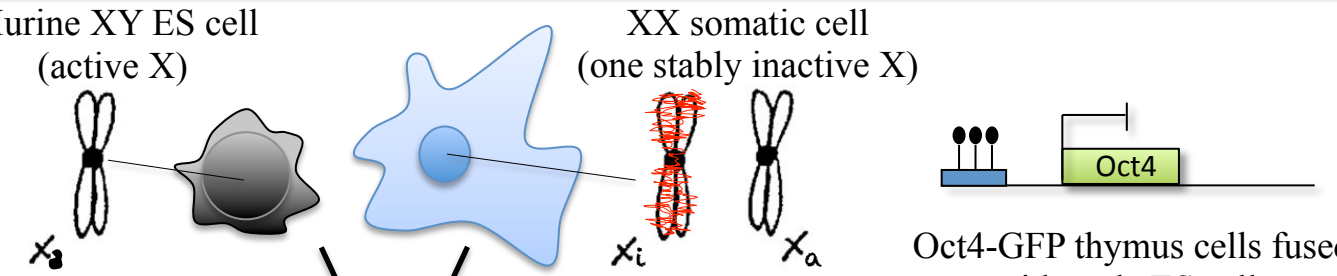
Experiments in the 1970s and 80s demonstrated the reprogramming capacity of one cell over another by cell fusion:

• EC cells were fused with thymus cells and then injected into mice – forming teratocarcinomas => **pluripotency must be dominant** (Miller and Ruddle 1976)

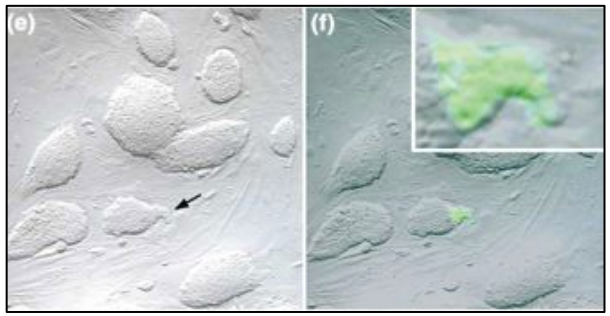
• Subsequently mouse ESCs shown to impose pluripotency onto hybrids generated using various somatic cell fusion partners eg T cells (Tada et al., 2001), splenocytes (Matveeva et al., 1998), bone marrow (Terada et al., 2002), & neural progenitors (Ying et al., 2002).

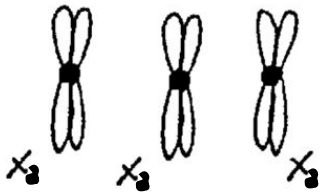


The inactive X chromosome in a somatic cell can be reactivated after cloning and after fusion with ES cells



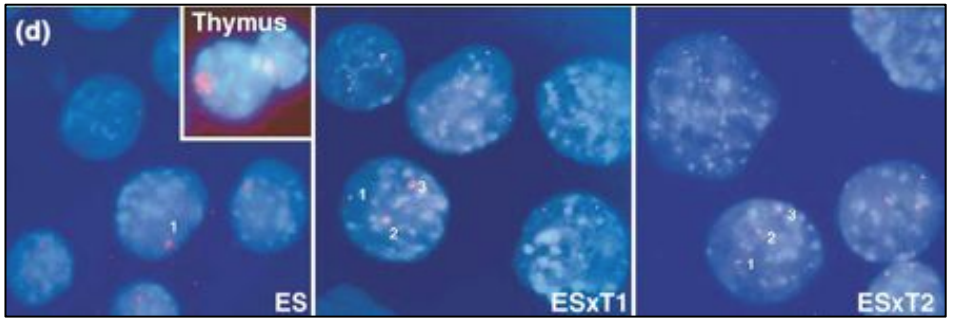
Oct4-GFP thymus cells fused with male ES cells





Oct4-GFP activation
48h post-fusion

Reversal of:
Xist RNA coating,
Gene silencing,
Chromatin marks
DNA methylation
Late replication



Also *Nanog*, *Cripto*, *Esrrb*, *Tle1* and *Rex1* as early as 24-30h after fusion
(Pereira et al, 2008; Bhutani et al, 2010)

Reactivation of the Xi following fusion of Female thymus cells with male ES cells
(*Xist* down regulation; shift to synchronous replication timing)

Tada et al, 2002 "Nuclear reprogramming of somatic cells by in vitro hybridization with ES cells", Curr.Biol.

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ES cells:
⇒ can rewire the gene regulatory network of any cell type?
⇒ can reprogram the inactive X chromosome, as well as pluripotency factors (*Oct4*)
⇒ must contain trans-acting factors capable of reprogramming somatic cell nuclei?

What could these be?

• Overexpression of *Nanog*, a pluripotency transcription factor, substantially enhanced fusion-based nuclear reprogramming (Silva et al, 2006)

• Heterokaryon-based reprogramming of human B lymphocytes for pluripotency requires Oct4 (Pereira et al, 2008).

⇒ Key transcription factors required for ES cell pluripotency are *also* required for reprogramming to pluripotency?

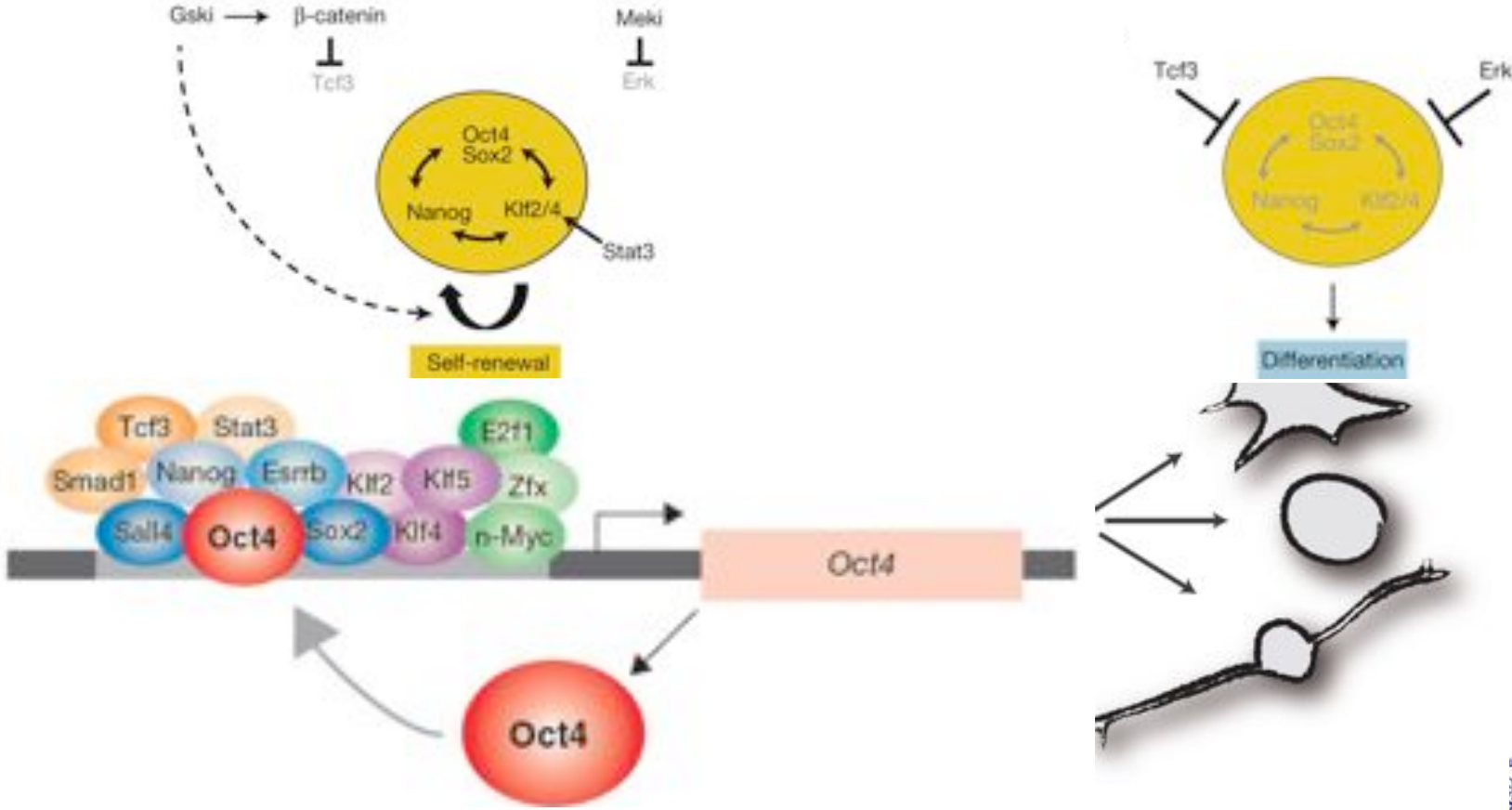
!

Pluripotency TF network and Embryonic Stem (ES) cells

Pluripotency transcriptional network: driven by core transcription factors (TFs) Oct4, Nanog, Sox2, Klf4 – maintain pluripotency and self-renewal.

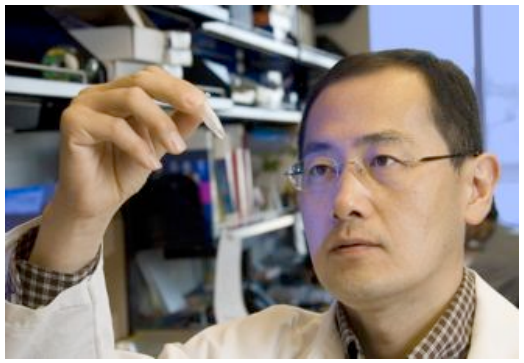
This network activates genes required for ES cell survival and proliferation & represses target genes that are only activate during differentiation.

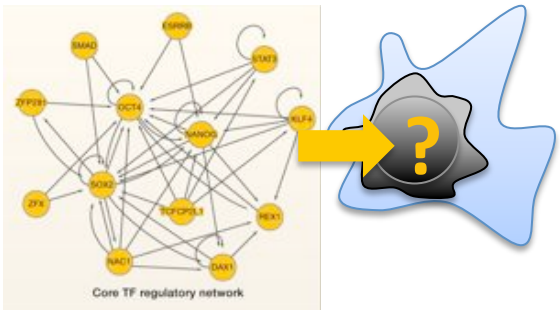
In vivo, these TFs play roles in early development and lineage specification



Transcription factor-mediated repurposing of somatic cells into pluripotent stem cells?

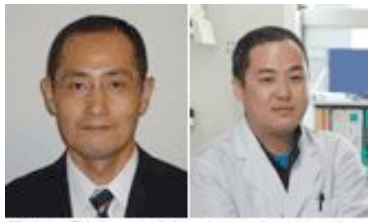
"When I saw the embryo, I suddenly realized there was such a small difference between it and my daughters. I thought, we can't keep destroying embryos for our research. There must be another way." s. Yamanaka - while looking through a microscope at a friend's fertility clinic.





Based on what was known about ES cells, Shinya Yamanaka reasoned that <u>forcing</u> the expression of ES cell-specific genes, particularly transcription factors, in somatic cells might induce them to take on a more embryonic character…

The Yamanaka Strategy



Drs. Shinya Yamanaka and Kazutoshi Takahashi

• Select 24 "ES-cell" gene candidates

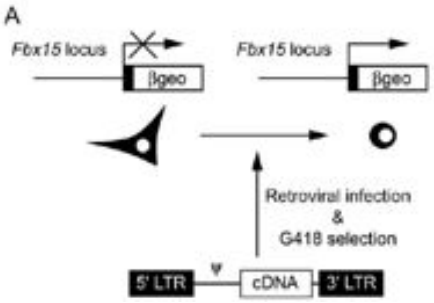
Transcription factors involved in self-renewal (*Oct3/4*, *Sox2*, and *Nanog*), or known to be upregulated specifically in ES cells (Mitsui et al., 2003), and some that are associated with transformation but that have also been implicated in the maintenance of ES cell pluripotency (*c-Myc*, *Eras*, and *Klf4*).

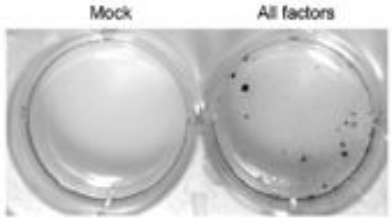
• Retroviral infection

(Morita et al, 2000) of 24 cDNAs, together, alone, or in combinations

• Mouse embryonic ("young") or adult tail tip fibroblasts ("old")

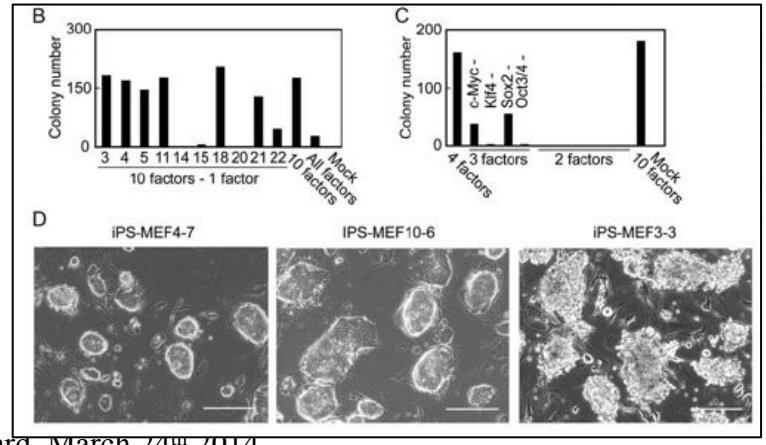
Harboring selectable marker (b-geo) under control of a promoter active only in ES cells (*Fbx15*). *Fbx15* activation results in Neomycin (G418) resistance (cell survival – colony) and β-galactosidase activity





- Transduction of 24 genes ->NeoR and b-*gal*+ colonies
- Individual genes alone -> no colonies
- Narrowed down to ***Oct4, Sox2, Klf4 and c-myc*** the "magic" cocktail" **(OSKM)** (NB not *Nanog*…

Narrowed down factors from 24 to 10 to 4…



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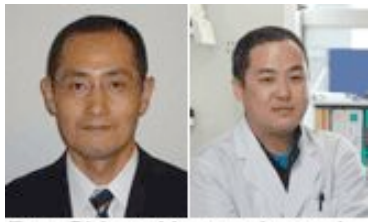
The Yamanaka Strategy



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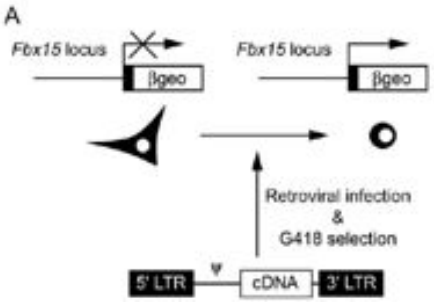

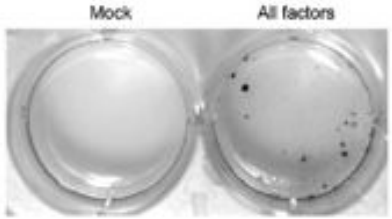

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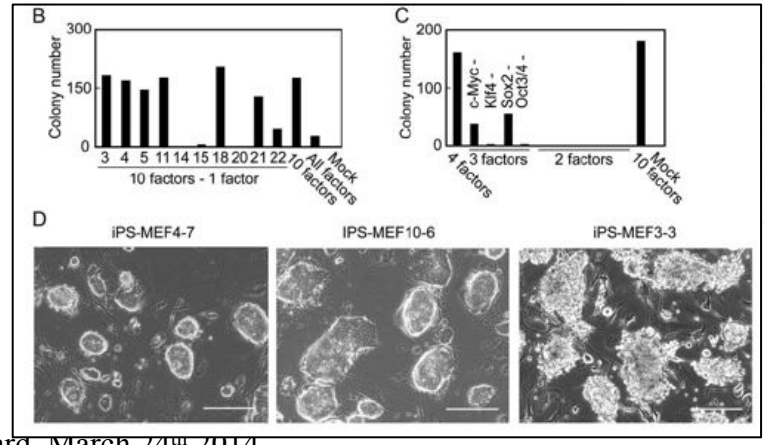
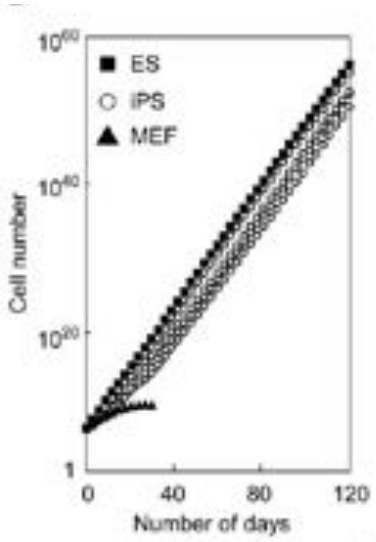


Criteria for "stemness"?

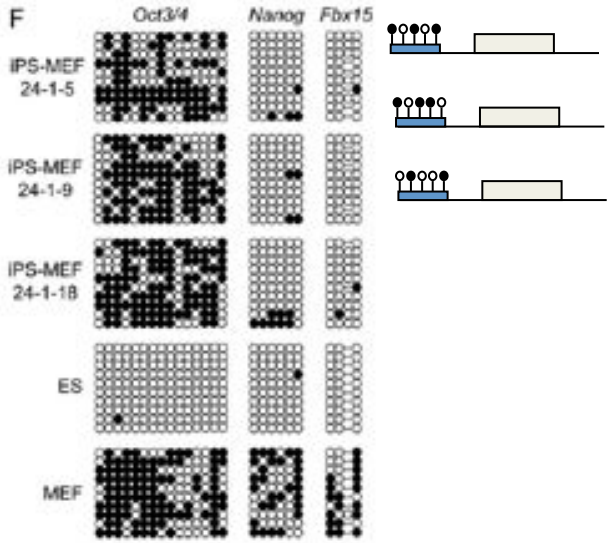
- ES cell morphology
- Self renewal (continuous cell division)
- Expression of endogenous pluripotency factors
- Transcriptomes (general gene expression pattern)
- Pluripotency (differentiation into all three germ layers)
- Teratomas
- Germ line transmission

These first iPS cells did NOT pass all the tests in fact

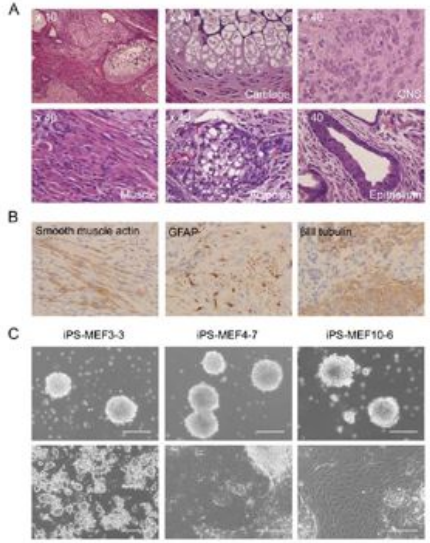
Do these "induced" pluripotent stem cells (iPS) fit the criteria for "stemness"?

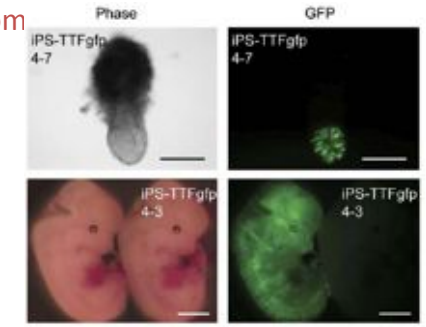


Immortal growth



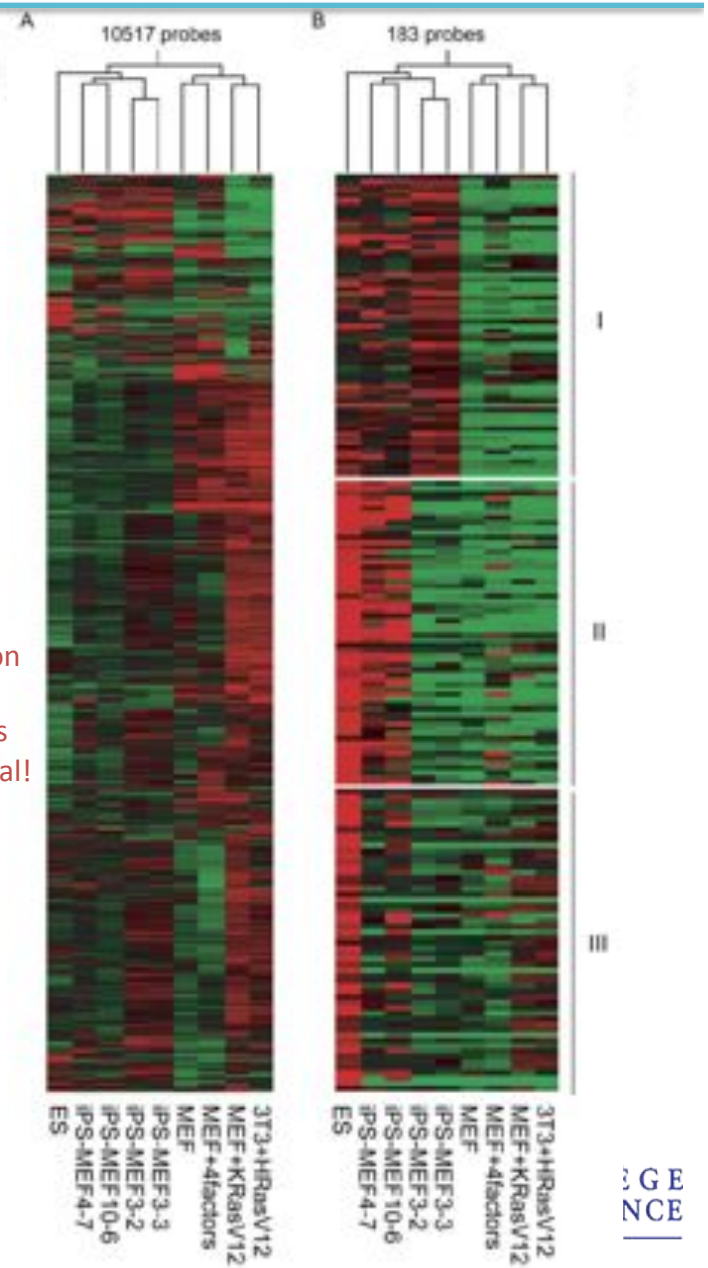
Lose DNA Methylation of





Differentiation *in vitro* into 3 germ layers and contribution to diverse tissues in chimeric embryos up to embryonic day 13.5.

Gene expression patterns?
Similar to ESCs
But NOT identical!



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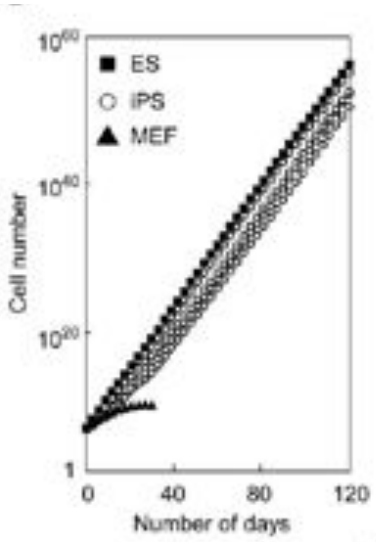
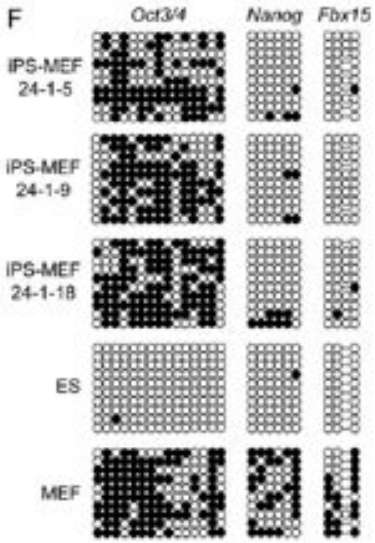
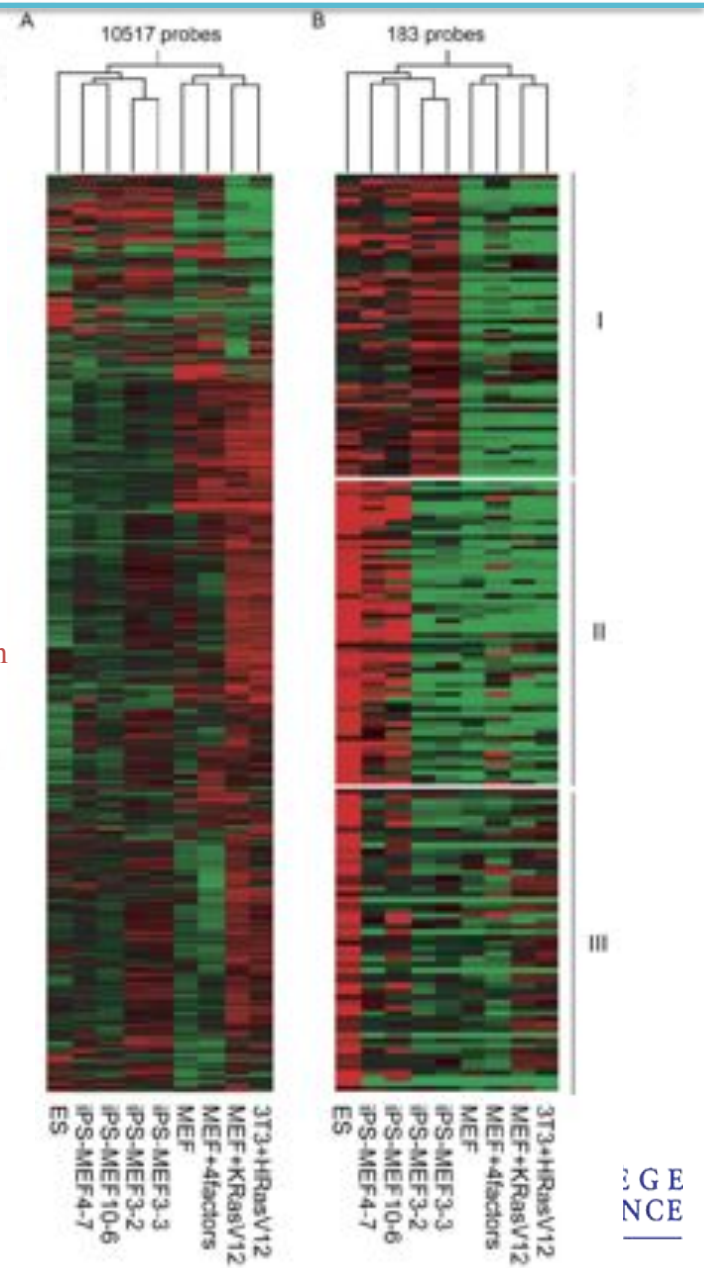


Immortal growth characteristics of genuine, self-renewing ES cells.



Lose DNA Methylation of promoters of Nanog and Fbx15 (but not Oct4!)

Gene expression patterns?



iPS cells fit many of the criteria of ES cells – BUT:

- Incomplete demethylation of the *Oct4* promoter.
- Low level of endogenous *Oct4* and *Sox2* expression.
- Expression profiles – similar but NOT identical to ES cells
- No chimeras, no germ line transmission

No contribution from iPS-derived cells to postnatal animals

⇒ **Incomplete Reprogramming?**

Germ-line competent murine iPS cells

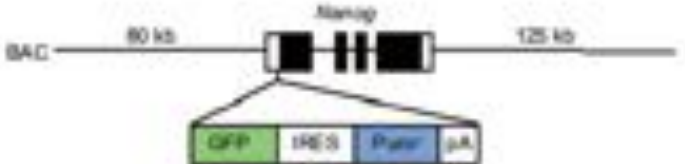
Yamanaka group (Okita et al, 2007) produced **germ-line competent murine iPS cells**

Vol 448 | 19 July 2007 | doi:10.1038/nature05934 nature

ARTICLES

Generation of germline-competent induced pluripotent stem cells

Keisuke Okita[1], Tomoko Ichisaka[1,2] & Shinya Yamanaka[1,2]



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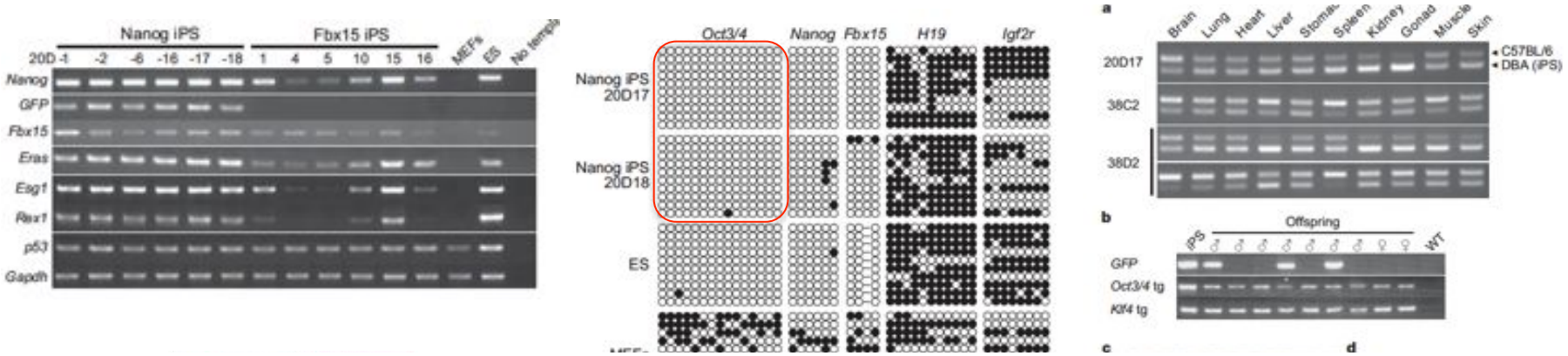
Germ-line competent murine iPS cells

Yamanaka group (Okita et al, 2007) produced **germ-line competent murine iPS cells**

Selection for *Nanog* expression (rather than *Fbx15*) resulted in germ line-competent iPS cells, with increased ES-cell-like gene expression and DNA methylation patterns

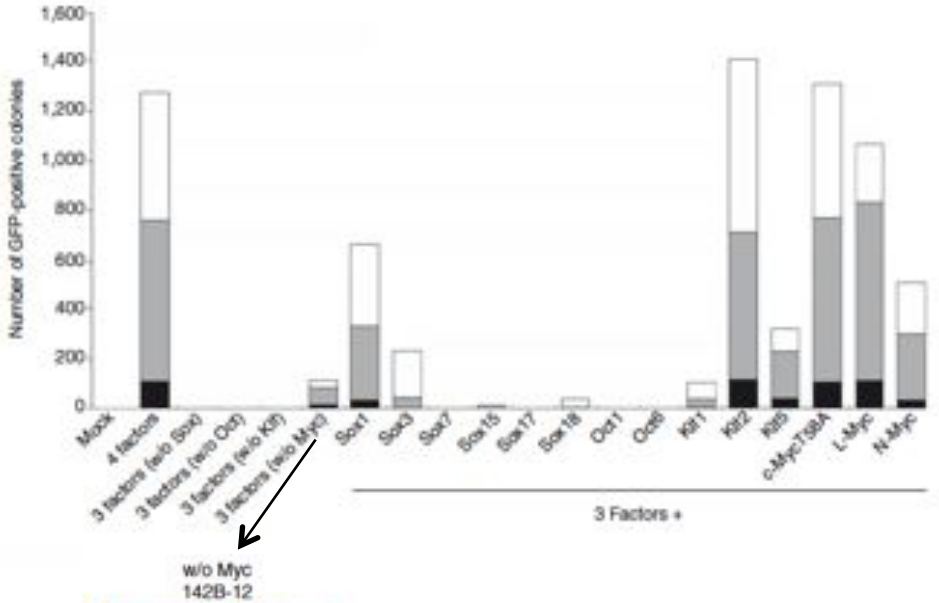
- Longer time allowed for reprogramming before selection
- Selection for *Nanog* expression = better readout than *Fbx15* for pluripotent state
- Efficiency of iPS clones still low: 0.1%, but clone quality less variable than *Fbx15* iPS

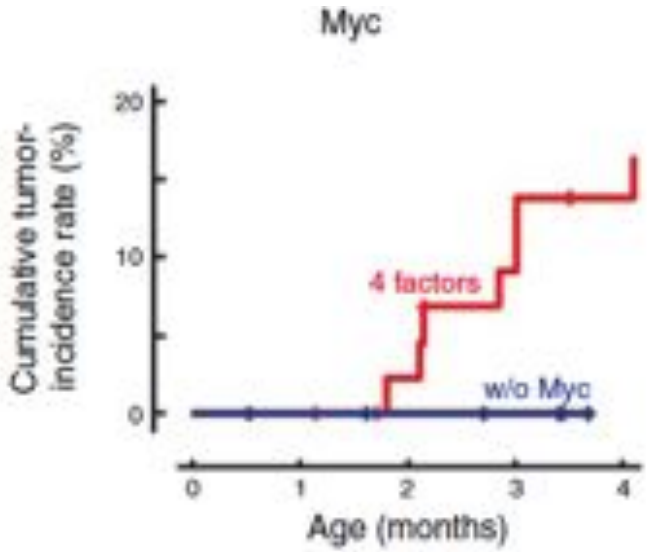


"Out of 121 F1 mice (aged 8–41 weeks) derived from the Nanog-iPS-20D17 cell line, 24 died or were killed because of weakness, wheezing or paralysis. Necropsy of 17 mice identified neck tumours in 13 mice and other tumours in five mice, including two mice with neck tumours….In these tumours, retroviral expression of c-myc, but not Oct3/4, Sox2, or Klf4, is reactivated"

Generation of iPS cells without the Myc oncogene

Yamanaka group (Nakagawa et al, 2008) produced **iPS cells without exogenous *Myc*** to overcome the tumorigenicity problems – less efficient, but clearly safer.





"Our study does not argue that Myc is dispensable for iPS cell generation. We found that MEFs expressed c-Myc from the endogenous gene at ~20% of the levels observed in mouse ES cells. This expression continues in iPS cells. Thus, Oct4, Sox2

Yamanaka, Hochedlinger and others also produced **iPS cells without retroviruses** to limit the mutagenic effects of retrovrial integration (eg Okita et al "Generation of mouse induced pluripotent stem cells without viral vectors". Science 322, 949–953 (2008) and Nature Protocols (2009).

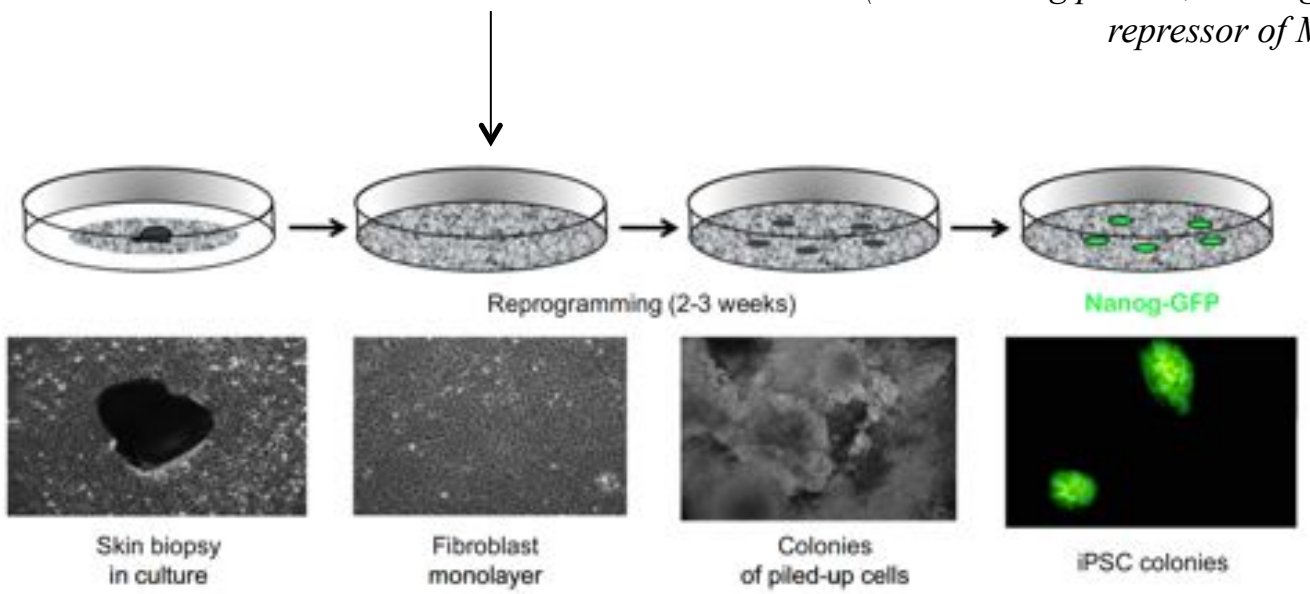
E. I

How general is the Yamanaka strategy?
Can other mammalian cells be reprogrammed?

Yamanaka group (Takahashi et al, 2007) and Thomson group (Yu et al, 2007) produced **iPS cells** from **Adult Human Fibroblasts**

Yamanaka : OKSM (OCT4, SOX2, KLF4, c-MYC)
Thomson: OSLN (OCT4, SOX2, LIN28, NANOG)

NB LIN28 probably functionally replaces c-MYC (RNA binding protein, that regulates Let7 miRNA - a repressor of MYC))



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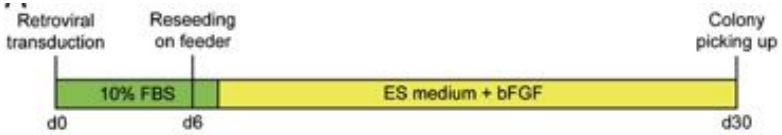
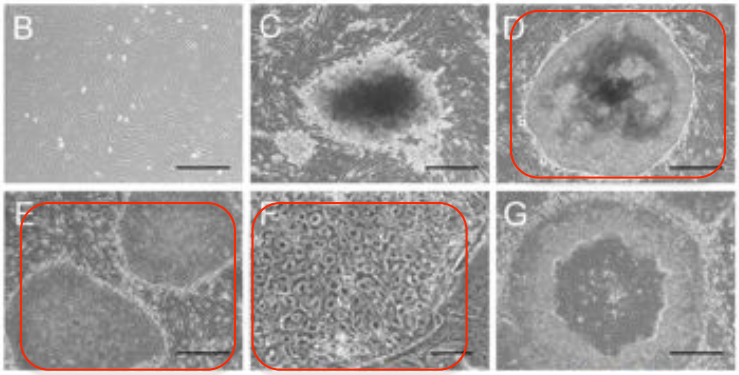
Can other mammalian cells be reprogrammed?

Yamanaka group (Takahashi et al, 2007) and Thomson group (Yu et al, 2007) produced **iPS cells** from **Adult Human Fibroblasts**

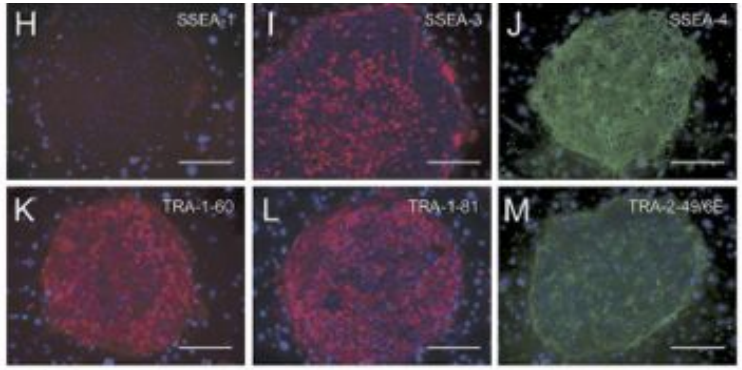
- Human iPS morphology similar to hESC
- Self renewal
- Similar Transcriptomes in iPS cells and hESCs
- ES cell marker expression, OCT4 hypomethylation
- Teratoma induction in SCID mice

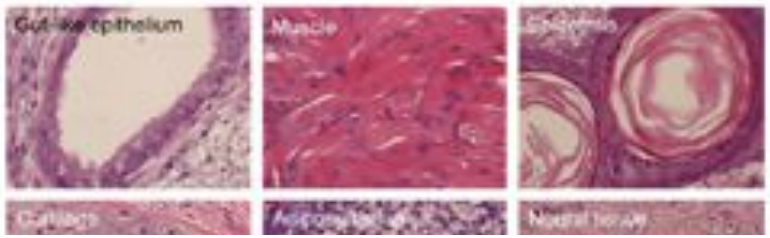
Yamanaka : OKSM (OCT4, SOX2, KLF4, c-MYC)
Thomson: OSLN (OCT4, SOX2, LIN28, NANOG)





(B) Morphology of human dermal fibroblasts
(C) Typical image of non-ES cell-like colony.
(D) Typical image of hES cell-like colony.
(E) Morphology of established iPS cell line
(F) Image of iPS cells with high magnification.
(G) Spontaneously differentiated cells in the





Human iPS cells resemble human ES cells – however they are rather different to mouse ES and iPS cells **-- SEMINAR, CLAIRE ROUGEULLE**

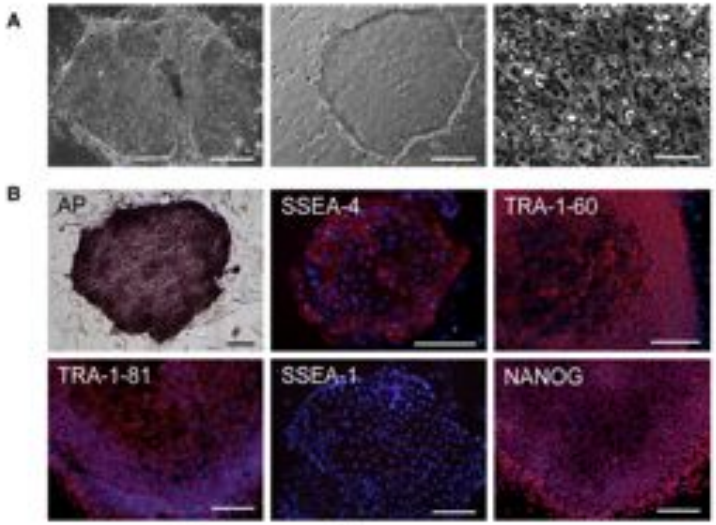
How general is the Yamanaka strategy?
Can other mammalian cells be reprogrammed to pluripotency?

Rats, Cats, and Elephants, but Still No Unicorn: Induced Pluripotent Stem Cells from New Species

Li et al, 2009 and Liao et al 2009: Rat primary cells reprogrammed using lentiviral vectors that expressed OSKM.

Generation of Induced Pluripotent Stem Cells from Adult Rhesus Monkey Fibroblasts

Haisong Liu,[1,2,6] Fangfang Zhu,[1,2,6] Jun Yong,[1,2,6] Pengbo Zhang,[1] Pingping Hou,[1] Honggang Li,[1] Wei Jiang,[1] Jun Cai,[1] Meng Liu,[1,2] Kai Cui,[1] Xiuxia Qu,[1] Tingting Xiang,[1] Danyu Lu,[3] Xiaochun Chi,[3] Ge Gao,[4] Weizhi Ji,[5] Mingxiao Ding,[1] and Hongkui Deng[1,2,*]



Retrovirus-mediated transduction of OSKM into monkey fibroblasts:

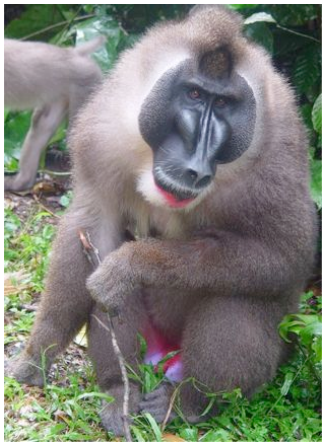
- Monkey iPS morphology similar to hESC
- Self renewal
- Similar Transcriptomes in iPS cells and hESCs
- ES cell marker expression, OCT4 hypomethylation
- Differentiation into 3 germ layers and teratoma induction in SCID mice.

How general is the Yamanaka strategy?
Can other mammalian cells be reprogrammed to pluripotency?

Rats, Cats, and Elephants, but Still No Unicorn: Induced Pluripotent Stem Cells from New Species

Self-renewing and pluripotent iPSCs
- Powerful alternatives for animal research
- Hope for endangered species ?

- Therapeutic applications for captive animals.
- For nearly extinct species, iPSCs may be a means to rescue species from extinction?
- Preserving the genomes of individual animals as pluripotent stem cells opens the possibility of producing iPSC-derived germ cells (COURS V), which could be used in conjunction with advanced assisted reproduction efforts to increase the size and diversity of the population.



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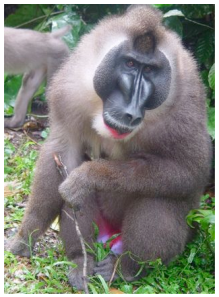
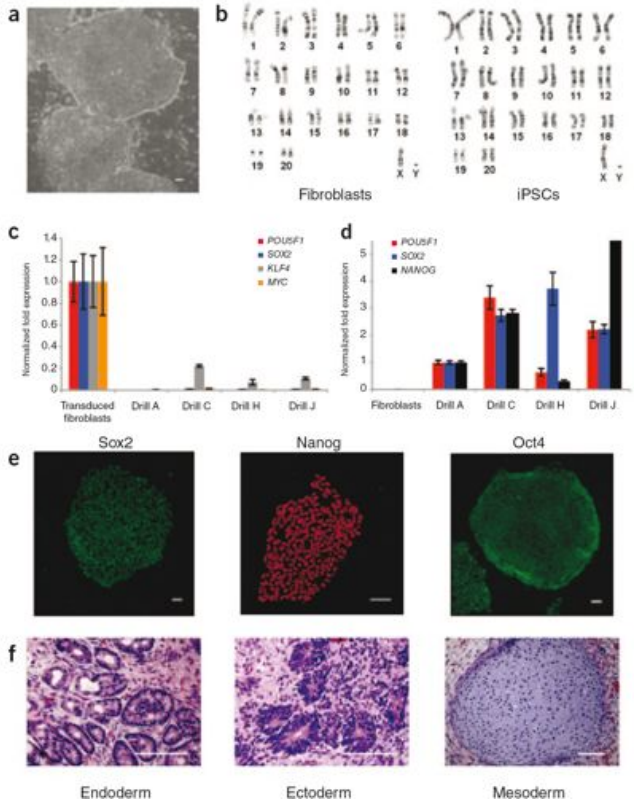
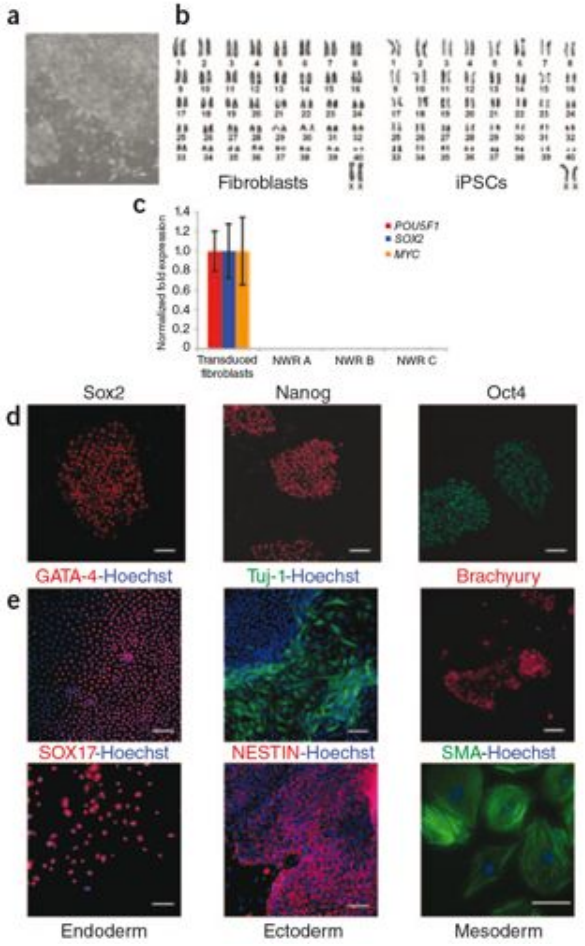




Ben-Nun et al, Nature Methods, 2011



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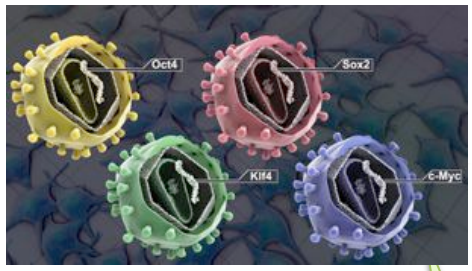
Summary and open questions from the first iPS papers

• **Four transcription factors** (Oct4, Sox2, Klf4 and Myc) are sufficient for nuclear reprogramming of somatic cells into "induced pluripotent stem" iPS cells.

• **Inefficient** (0.1-2%) **but highly reproducible** : three subsequent studies from different labs obtained reprogramming to pluripotency within one year!

• **iPS resemble ESCs** (self renewing, pluripotent -> give rise to all three germ layers *in vitro*) but are they truly equivalent? ? Do they have any "memory" of their somatic history?



OSKM
Or other TF combinations

Differentiated cell
(embryonic or adult)

Self-renewing,
"iPS" pluripotent cell

Embryoid bodies,
Teratomas

Chimeras

Gene expression patterns?
Epigenetic marks?

Summary and open questions from the first iPS papers

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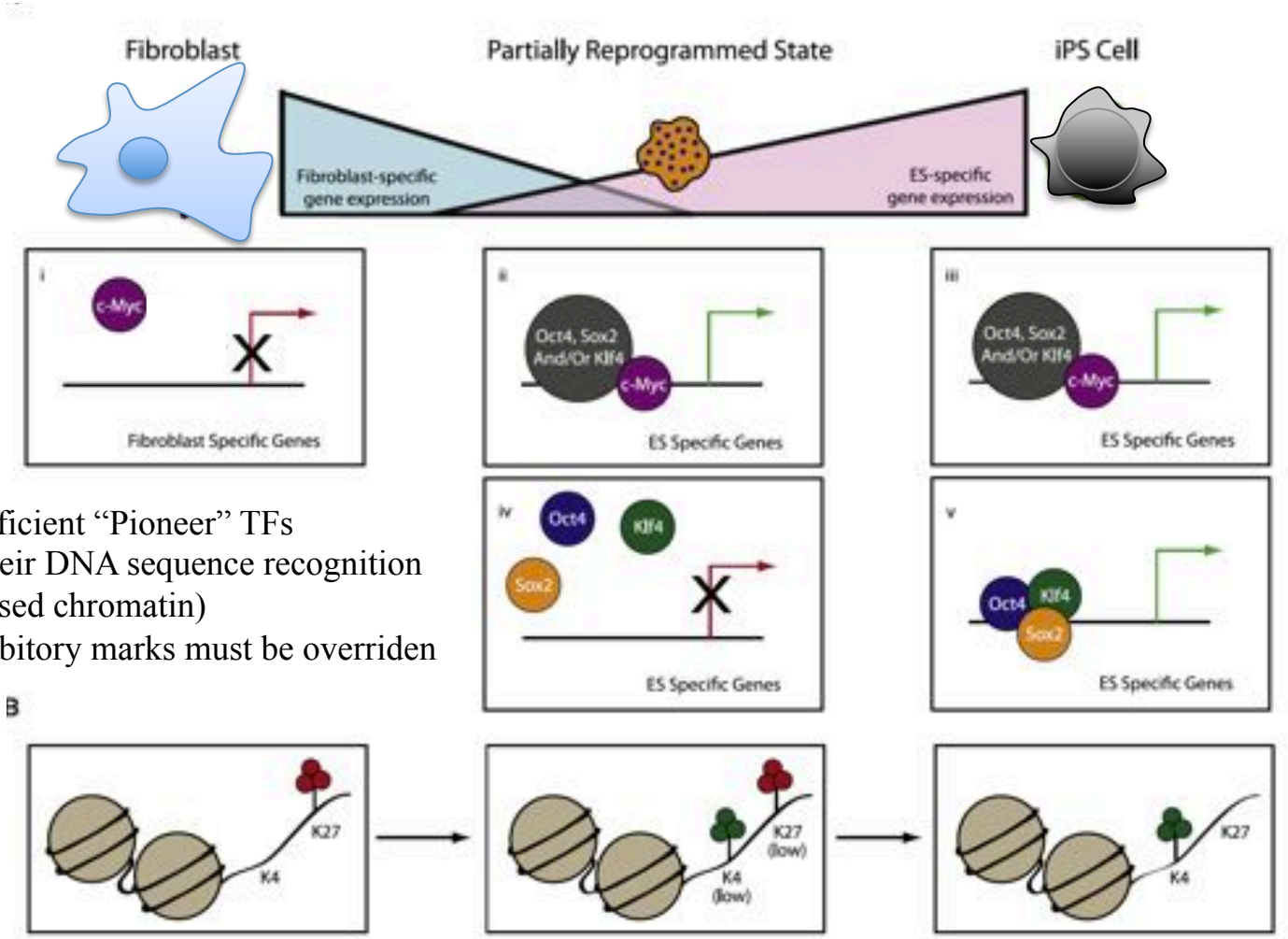
• **iPS resemble ESCs** (self renewing, pluripotent -> give rise to all three germ layers *in vitro*) but are they truly equivalent? ? Do they have any "memory" of their somatic history?

• **Why do these <u>particular</u> factors achieve reprogramming**?

- Oct4, Sox2, Klf4 TFs cooperatively suppress lineage specific genes and activated ES-related genes – leading to self-sustaining pluripotency network essential TFs for pluripotency (Boye et al, 2005; Loh et al, 2006; Wang et al 2006; Orkin and Hochedlinger, 2011 for review)
- c-*Myc* = Facilitator of reprogramming? It increases expression of many genes important for proliferation and self-renewal (eg Telomerase - Cartwright et al., 2005).

• **Why is induced pluripotency so slow and inefficient?**

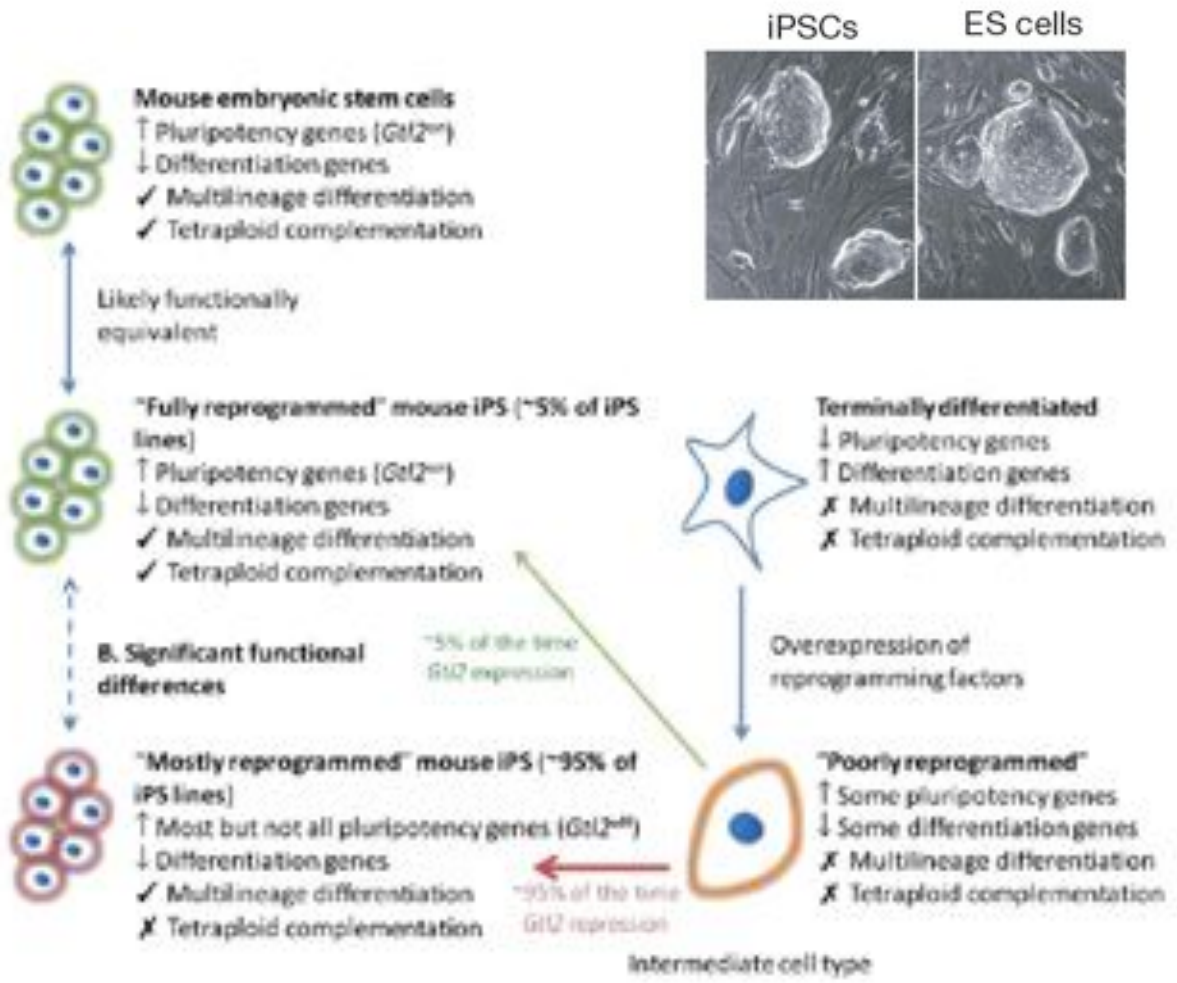
Role(s) of the Murine Reprogramming Factors in the Induction of Pluripotency?



- OKS are not efficient "Pioneer" TFs
ie cannot bind their DNA sequence recognition sites even in closed chromatin)
- Epigenetic inhibitory marks must be overriden first?

➢ Each (and all) of the OKSM factors can be functionally replaced by <u>other TFs, by miRNAs, small compounds…</u>**(=> COURS IV)**

Are iPS cells truly equivalent to ES cells?



Why is induced pluripotency so inefficient?

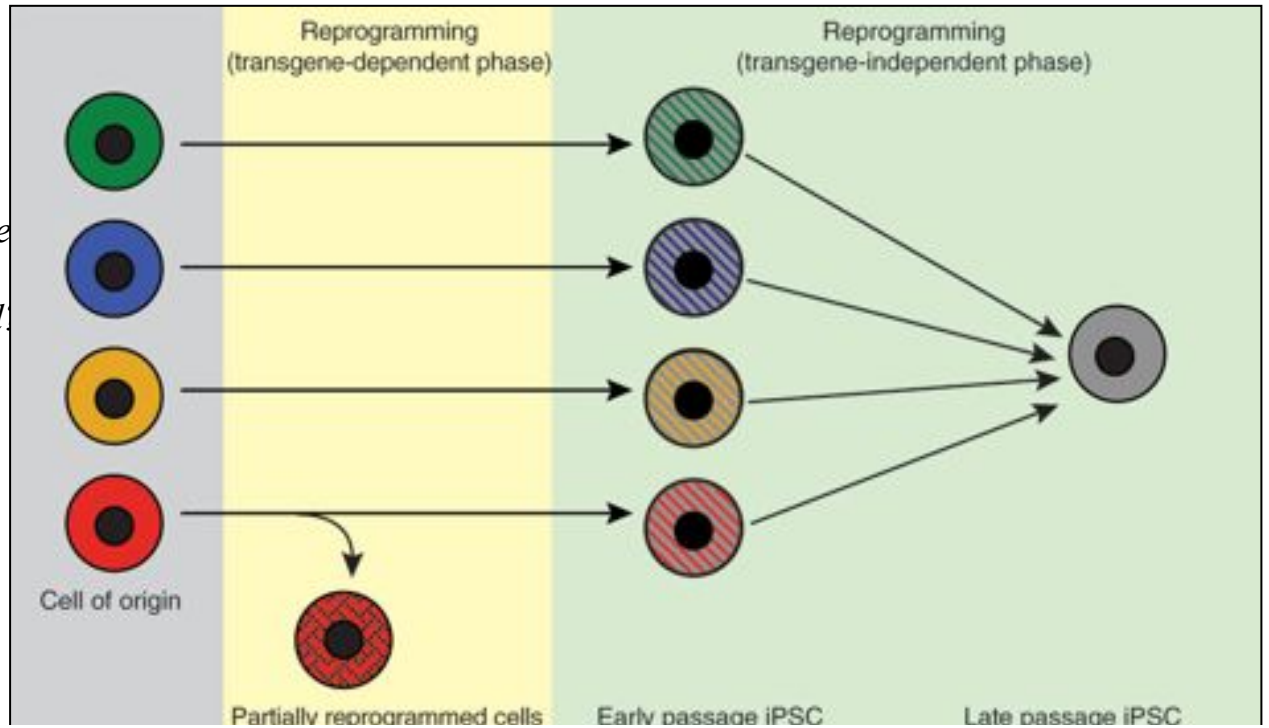
Adapted from Loh and Lim, 2010

Induced Pluripotency is slow and inefficient

WHY?

Passaging improves iPS cell pluripotency
Dividing and amplifying the cells allows epigenetic memory to be erased

Only rare « elite » cells can be reprogrammed! ***Unlikely….***



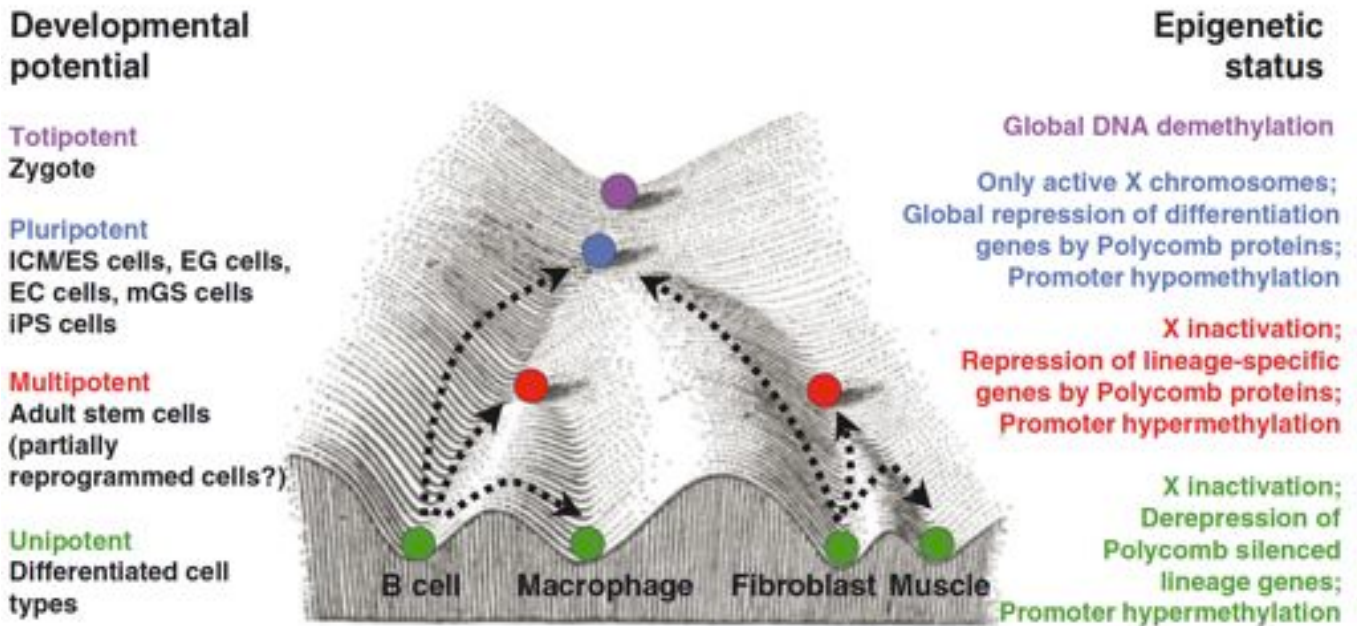
Must have similar steps to normal reprogramming (eg in the germ line):

- Silencing of somatic cell program and activation of self-renewing/pluripotency program
- Need to override epigenetic barriers:

Interfering with epigenetic processes increases frequencies up to 10% or more! **(COURS IV)**

Induced Pluripotency is slow and inefficient

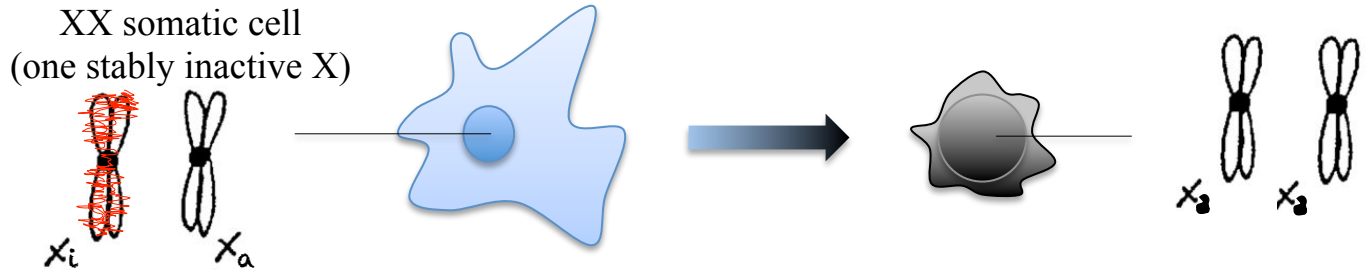
Epigenetic barriers, that were imposed on the genome during differentiation, to stabilize cell identity and prevent aberrant cell fate changes, must be overcome during reprogramming



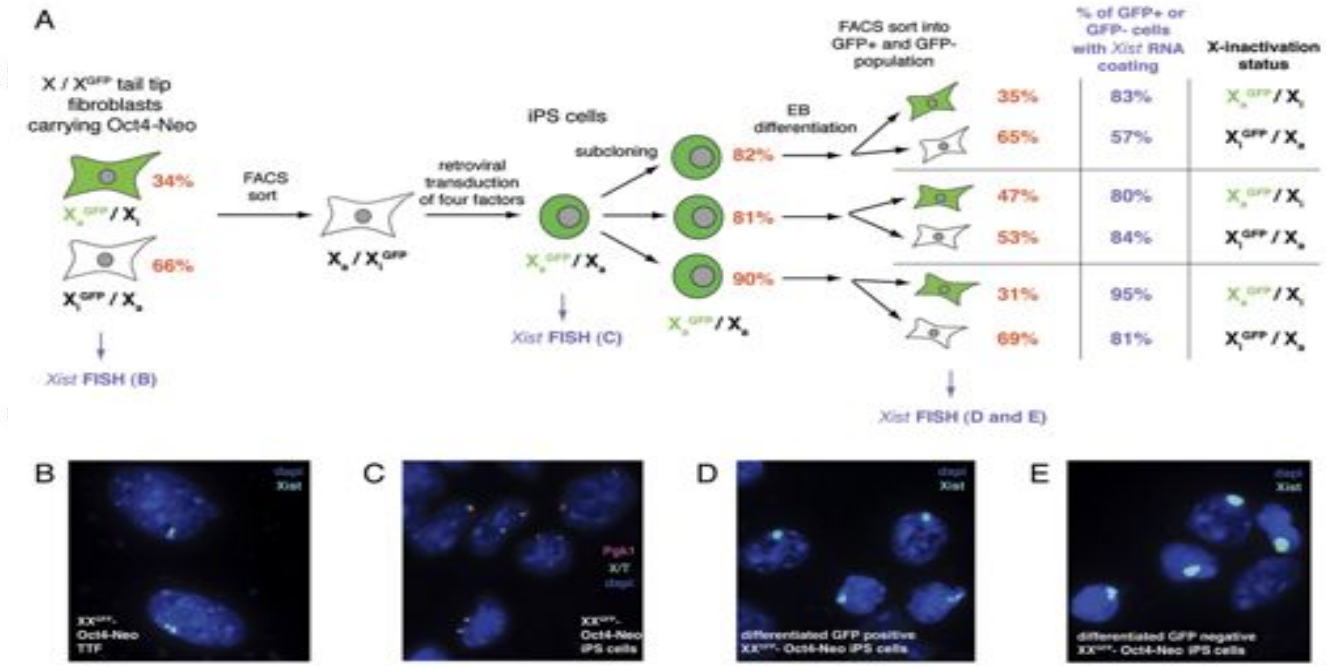
Nature of this Epigenetic Memory? Ways to overcome it? (COURS IV)

X-chromosome reactivation as a “gold standard” for efficient reprogramming?

iPS and epigenetic reprogramming of mouse cells
Xi Reactivation?

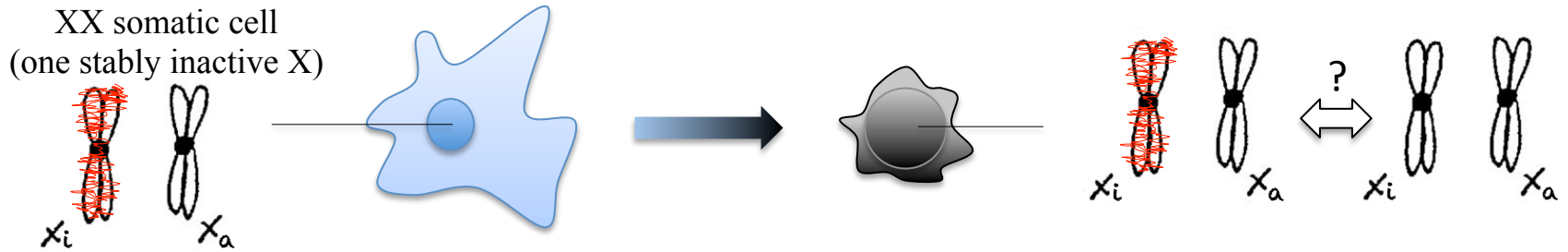


Mouse female iPS cells showed reactivation of a somatically silenced X chromosome and undergo random X inactivation upon differentiation.



Maherali et al, 2008

iPS and epigenetic reprogramming of human cells
Xi Reactivation?

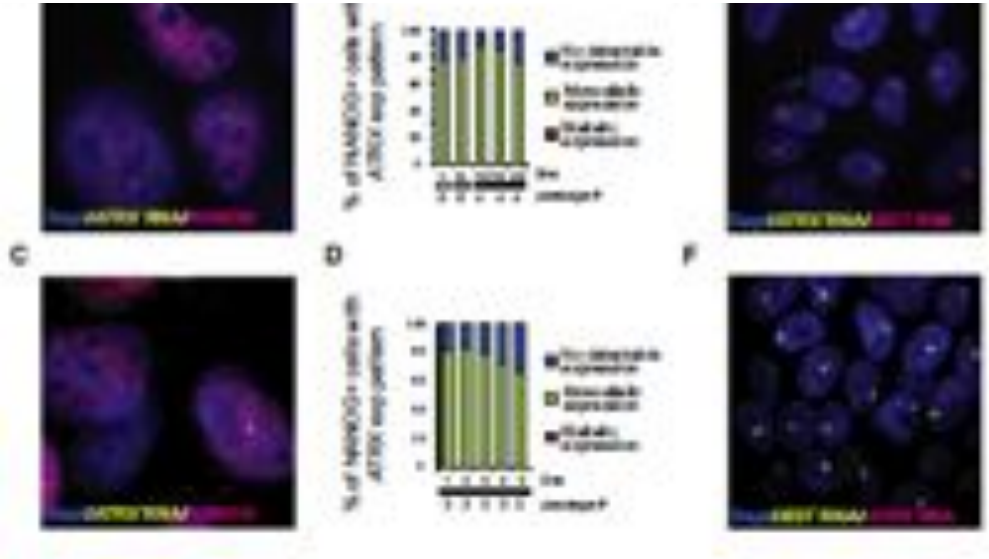


Female Human iPSCs Retain an Inactive X Chromosome

The inactive X is not reactivated during human iPS cell induction

Culture conditions, Xi status in hESCs, Xi status in human embryos (ICM)

SEMINAR, CLAIRE ROUGEULLE



Tchieu et al, 2010

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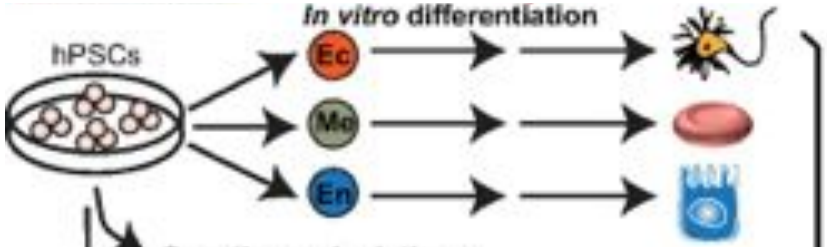
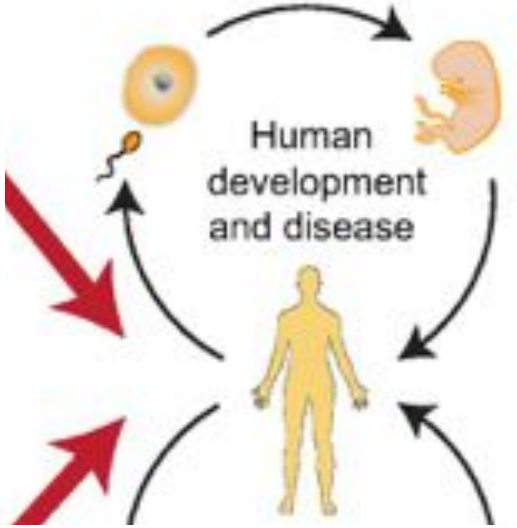
Perspectives brought by iPS cells

- Cell and Tissue therapy - *without use of human embryos* and reduces problems associated with compatibility (COURS V)
- Enables study of differentiation and development *in vitro*
- Powerful tool for drug screening
- Enables genetic engineering for functional investigation of development and disease *in vitro* and *in vivo* (production of chimeric animals)





Issues/problems:

- Obtaining iPS cell lines swifly and then sufficient cell numbers upon *in vitro* differentiation can be a problem (in a therapeutic context)
- iPS still brings a risk of cancer (even avoiding *myc* and integrative vectors…) **(***Why does the iPS cell production process create oncogenically transformed cells)*

Can safety and efficiency be improved?

Can iPS efficiency and safety be improved?

IMPROVED DELIVERY OF TFs

OVERCOMING EPIGENETIC BARRIERS

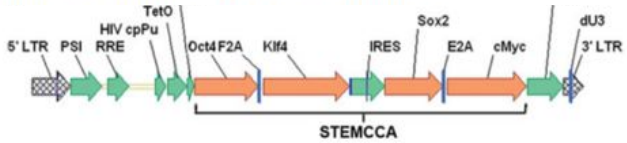
IMPROVED CULTURE CONDITIONS (COURS V)

Can iPS efficiency and safety be improved?

IMPROVED DELIVERY OF TFs

Induced Pluripotent Stem Cell Generation Using a Single Lentiviral Stem Cell Cassette

CESAR A. SOMMER,[a] MATTHIAS STADTFELD,[b] GEORGE J. MURPHY,[c] KONRAD HOCHEDLINGER,[b] DARRELL N. KOTTON,[d,e] GUSTAVO MOSTOSLAVSKY[a]



Stem Cells 2009;27:543–549

Human Induced Pluripotent Stem Cells Free of Vector and Transgene Sequences

Junying Yu,[1,2,3*] Kejin Hu,[3] Kim Smuga-Otto,[1,2,3] Shulan Tian,[1,2] Ron Stewart,[1,2] Igor I. Slukvin,[3,4] James A. Thomson[1,2,3,5*]

Stem Cell Reports
Article
ISSCR
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Reprogramming to Pluripotency Using Designer TALE Transcription Factors Targeting Enhancers

Xuefei Gao,[1] Jian Yang,[1] Jason C.H. Tsang,[1] Jolene Ooi,[1] Donghai Wu,[2] and Pentao Liu[1,*]

A more efficient method to generate integration-free human iPS cells

Keisuke Okita[1], Yasuko Matsumura[1], Yoshiko Sato[1], Aki Okada[1], Asuka Morizane[1,2], Satoshi Okamoto[3], Hyenjong Hong[1], Masato Nakagawa[1], Koji Tanabe[1], Ken-ichi Tezuka[4], Toshiyuki Shibata[5], Takahiro Kunisada[4], Masayo Takahashi[1,3], Jun Takahashi[1,2], Hiroh Saji[6] & Shinya Yamanaka[1,7–9]

We report a simple method, using p53 suppression and nontransforming L-Myc, to generate human induced pluripotent stem cells (iPSCs) with episomal plasmid vectors. We generated human iPSCs from multiple donors, including two putative human leukocyte antigen (HLA)-homozygous donors who match ~20% of the Japanese population at major HLA loci; most iPSCs are integrated transgene-free. This method may provide iPSCs suitable for autologous and allologous stem-cell therapy in the future.

OVERCOMING EPIGENETIC BARRIERS

Can iPS efficiency and safety be improved?

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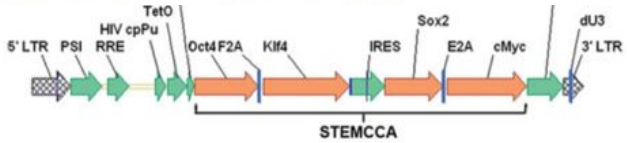

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OVERCOMING EPIGENETIC BARRIERS

Vitamin C modulates TET1 function during somatic cell reprogramming

Jiekai Chen[1,2,6], Lin Guo[1,2,6], Lei Zhang[3,6], Haoyu Wu[1,4], Jiaqi Yang[1,2], He Liu[1,2], Xiaoshan Wang[1,2], Xiao Hu[3], Tianpeng Gu[3], Zhiwei Zhou[1,2], Jing Liu[1,2], Jiadong Liu[1,5], Hongling Wu[1,2], Shi-Qing Mao[3], Kunlun Mo[1,2], Yingying Li[1,2], Keyu Lai[1,2], Jing Qi[1,2], Hongjie Yao[1,2], Guangjin Pan[1,2], Guo-Liang Xu[3] & Duanqing Pei[1,2]

How microRNAs facilitate reprogramming to pluripotency

Frederick Anokye-Danso[1,*], Melinda Snitow[2,*] and Edward E. Morrisey[1,2,3,4,‡]

The use of small molecules in somatic-cell reprogramming

Alexander J. Federation[1,2,3], James E. Bradner[1,2,4], and Alexander Meissner[2,5,6]

Derivation of novel human ground state naive pluripotent stem cells

Ohad Gafni[1*], Leehee Weinberger[1*], Abed AlFatah Mansour[1*], Yair S. Manor[1*], Elad Chomsky[1,2,3*], Dalit Ben-Yosef[4,5], Yael Kalma[4], Sergey Viukov[1], Itay Maza[1], Asaf Zviran[1], Yoach Rais[1], Zohar Shipony[2,3], Zohar Mukamel[2,3], Vladislav Krupalnik[1], Mirie Zerbib[1], Shay Geula[1], Inbal Caspi[1], Dan Schneir[1], Tamar Shwartz[4], Shlomit Gilad[6], Daniela Amann-Zalcenstein[6], Sima Benjamin[6], Ido Amit[7], Amos Tanay[2,3], Rada Massarwa[1], Noa Novershtern[1] & Jacob H. Hanna[1]

Removing Reprogramming Roadblocks: Mbd3 Depletion Allows Deterministic iPSC Generation

Justin Brumbaugh[1,2] and Konrad Hochedlinger[1,2,3,*]

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Can iPS efficiency and safety be improved?

IMPROVED DELIVERY OF TFs

OVERCOMING EPIGENETIC BARRIERS

NO EXOGENOUS FACTORS

Pluripotent Stem Cells Induced from Mouse Somatic Cells by Small-Molecule Compounds

Pingping Hou,[1*] Yanqin Li,[1*] Xu Zhang,[1,2*] Chun Liu,[1,2*] Jingyang Guan,[1*] Honggang Li,[1*] Ting Zhao,[1†] Junqing Ye,[1,2†] Weifeng Yang,[3†] Kang Liu,[1†] Jian Ge,[1,2†] Jun Xu,[1†] Qiang Zhang,[1,2†] Yang Zhao,[1‡] Hongkui Deng[1,2‡]

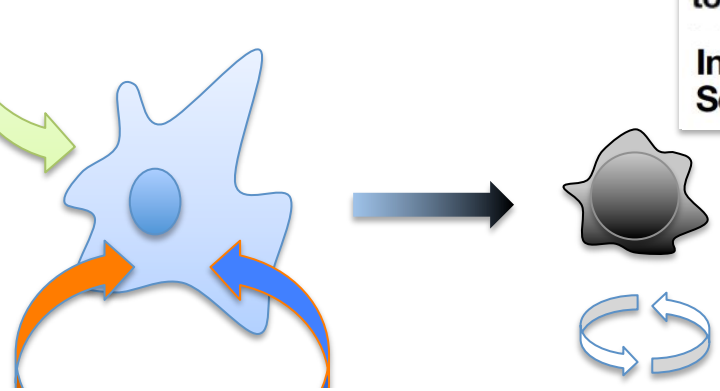
Stimulus-triggered fate conversion of somatic cells into pluripotency

Haruko Obokata[1,2,3], Teruhiko Wakayama[3†], Yoshiki Sasai[4], Koji Kojima[1], Martin P. Vacanti[1,5], Hitoshi Niwa[6], Masayuki Yamato[7] & Charles A. Vacanti[1]

Beyond the Yamanaka concept: Induced Pluripotency by Lineage Specifiers

OSKM

OCT4 and SOX2, can be replaced by lineage specifiers involved in mesendodermal (ME) specification and in ectodermal (ECT) specification, respectively (Shu et al, 2013)



Mesodermal TFs Ectodermal TFs

Reprogramming of Human Fibroblasts to Pluripotency with Lineage Specifiers

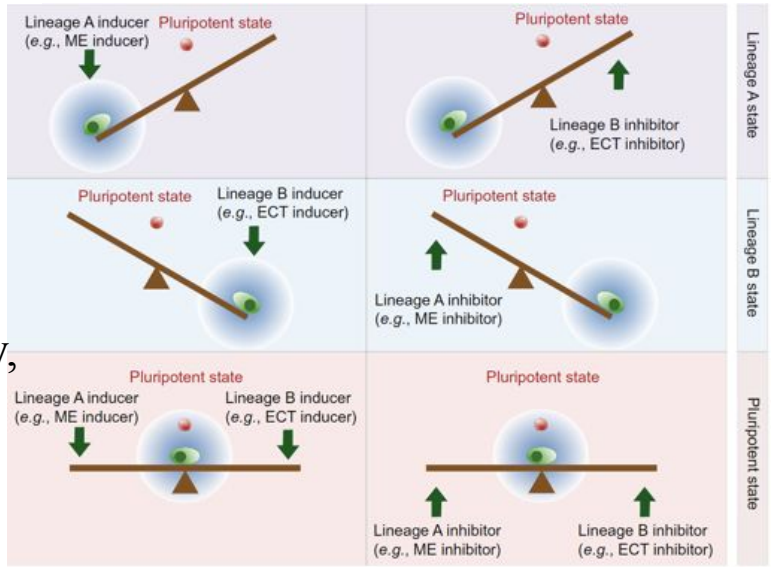
Induction of Pluripotency in Mouse Somatic Cells with Lineage Specifiers

Montserrat et al, 2013
Shu et al, 2013

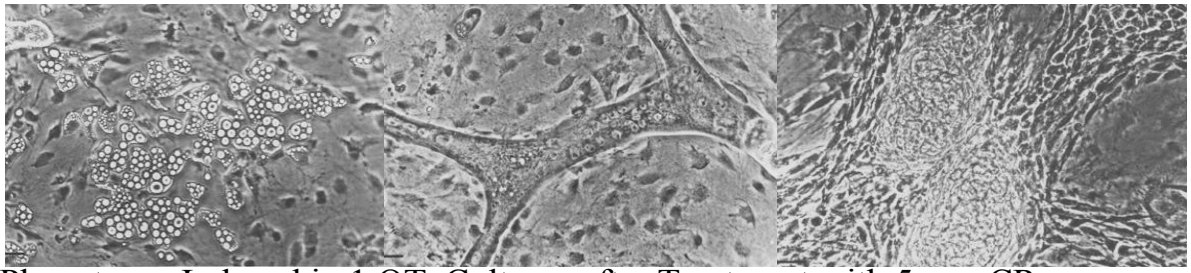
OCT4 and SOX2 counteract the expression of lineage specification genes
(Loh and Lim, 2011; Thomson et al., 2011; Wang et al., 2012).

"Alternative-lineage" specifiers can reprogram mouse and human cells to plutipotency!

Pluripotency may not represent a discrete cellular entity, but rather a functional state established by a balance between opposite differentiation forces
(Loh and Lim, 2011; Zipori, 2004).



Using TFs to induce *direct* lineage conversion (Trans-differentiation)

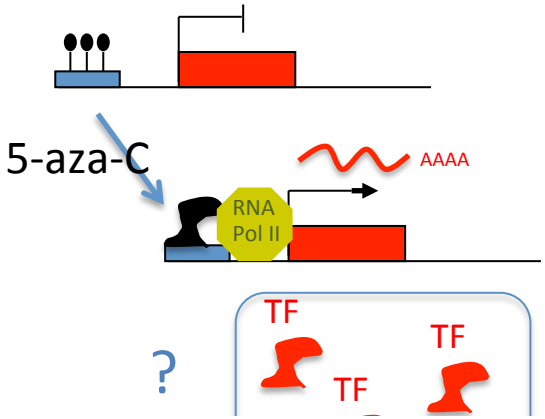


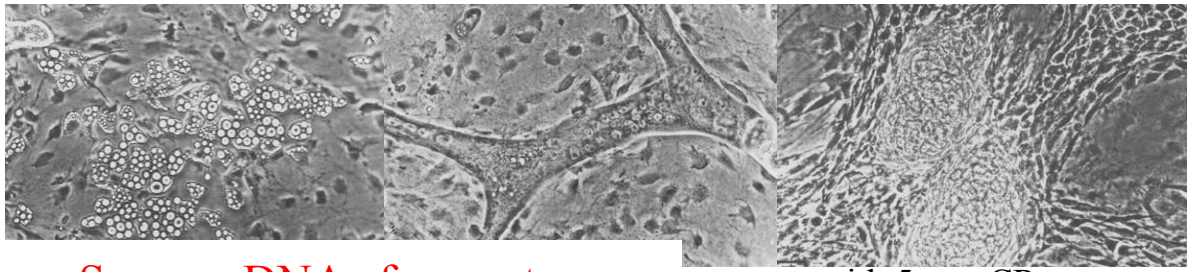
Phenotypes Induced in 1 OT: Cultures after Treatment with 5-aza-CR
(a) Adipocytes (4 weeks after treatment); (b) myotubes (2 weeks after treatment); (c) chondrocytes (5 weeks after treatment). *Taylor and Jones, 1979.*

Observations by Peter Jones and colleagues in the 1970's
5-aza-C treated fibroblasts sometimes gave rise to cells with "new phenotypes"(adipocytes, myotubes, chondrocytes…)

"possibly by inducing a reversion
to a more pluripotential state from which the new phenotypes subsequently differentiate..?"

Using TFs to induce *direct* lineage conversion (Trans-differentiation)

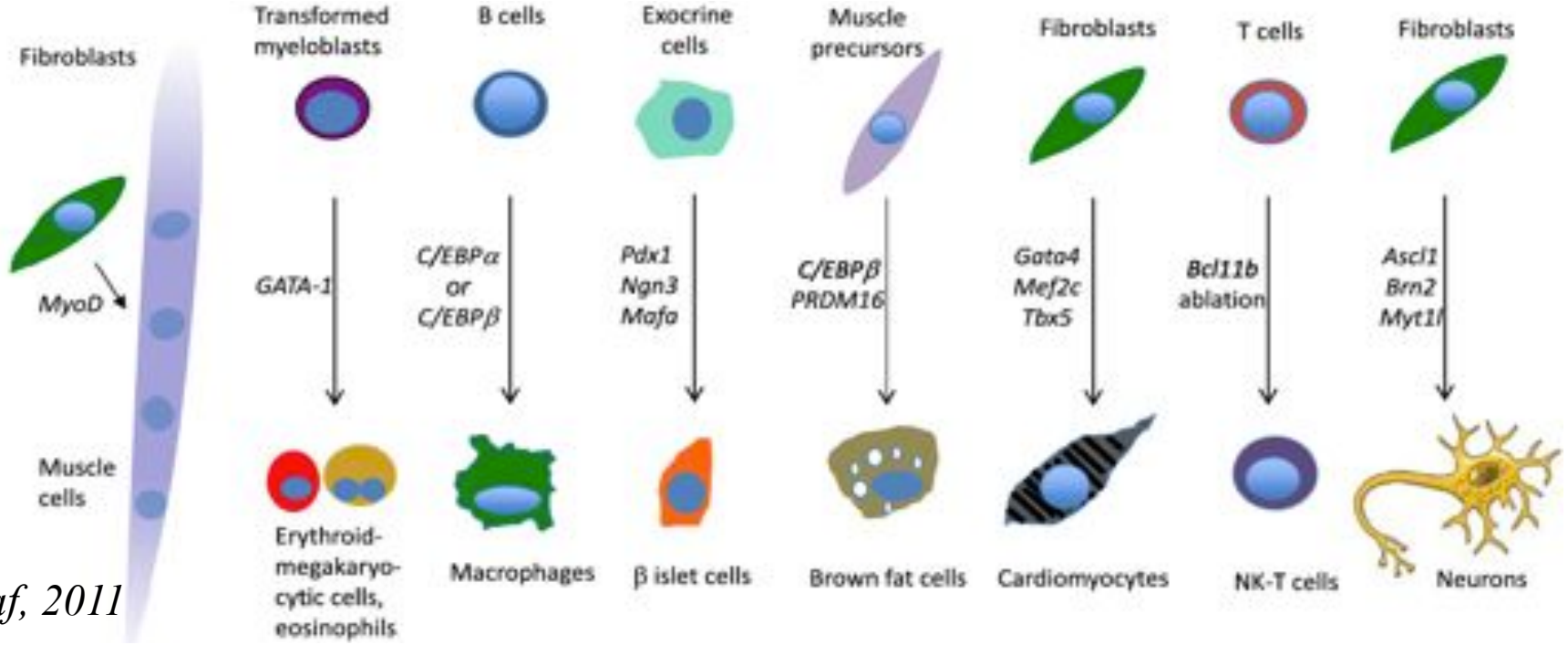




Screen cDNAs for master regulator(s) that could convert fibroblast to muscle cells?

reatment with 5-aza-CR
nyotubes (2 weeks after
eatment). *Taylor and Jones, 1979.*

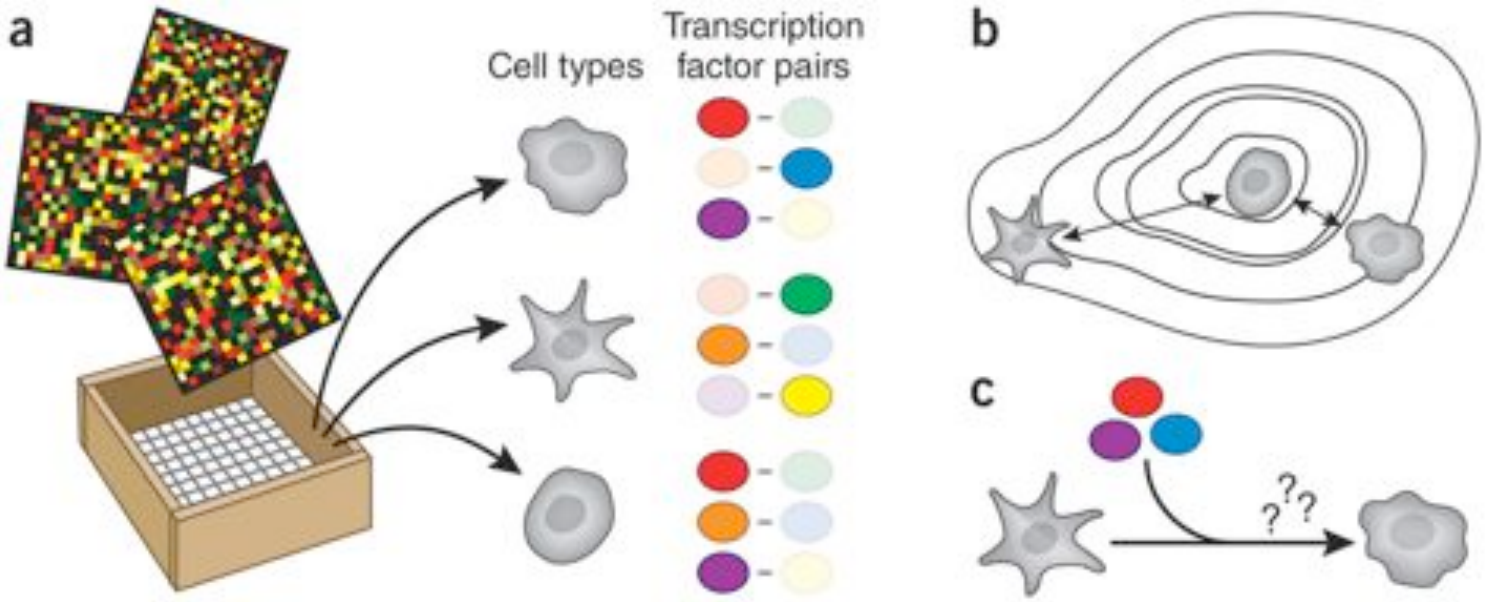
TFs can direct changes in cell fate without a need to transit through pluripotency



Graf, 2011

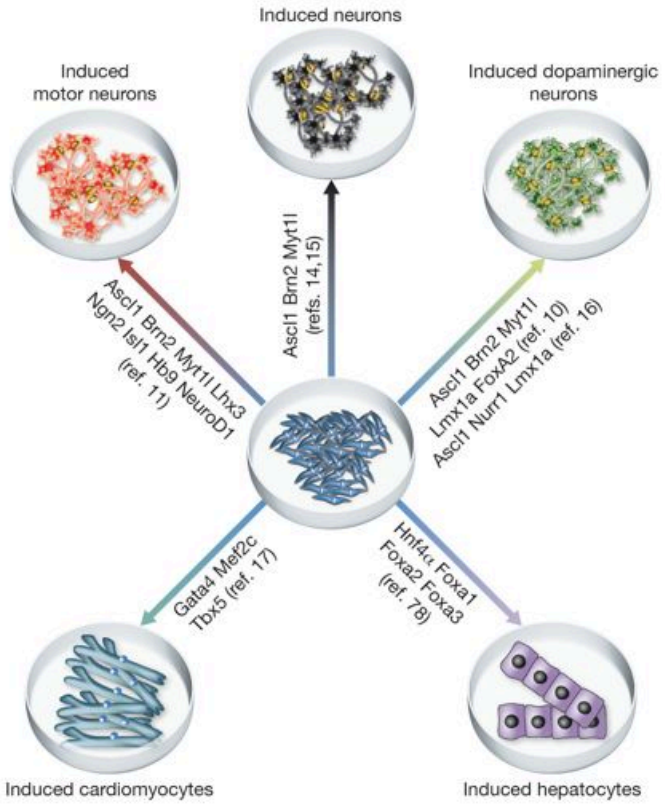
Identify key TFs that induce lineage conversion

A computational biology approach to find regulators of cell fate (Heinäniemi et al, 2013): Bioinformatics approach that searches public gene-expression data sets for candidate transcriptional regulators for many human cell types.
⇒ a new resource for experiments aimed at direct lineage conversion



Pairwise comparison of TF expression levels extracted from many gene array data sets yields candidate cell type–specific master regulators. Solid-colored ovals represent the dominant TF in a given pair for a given cell type. (b) Quantification of pairwise relationships between TFs can be used to visualize lineage relationships in a topographical manner. (c) Identified TFs may be candidate factors to reprogram one cell type into another.

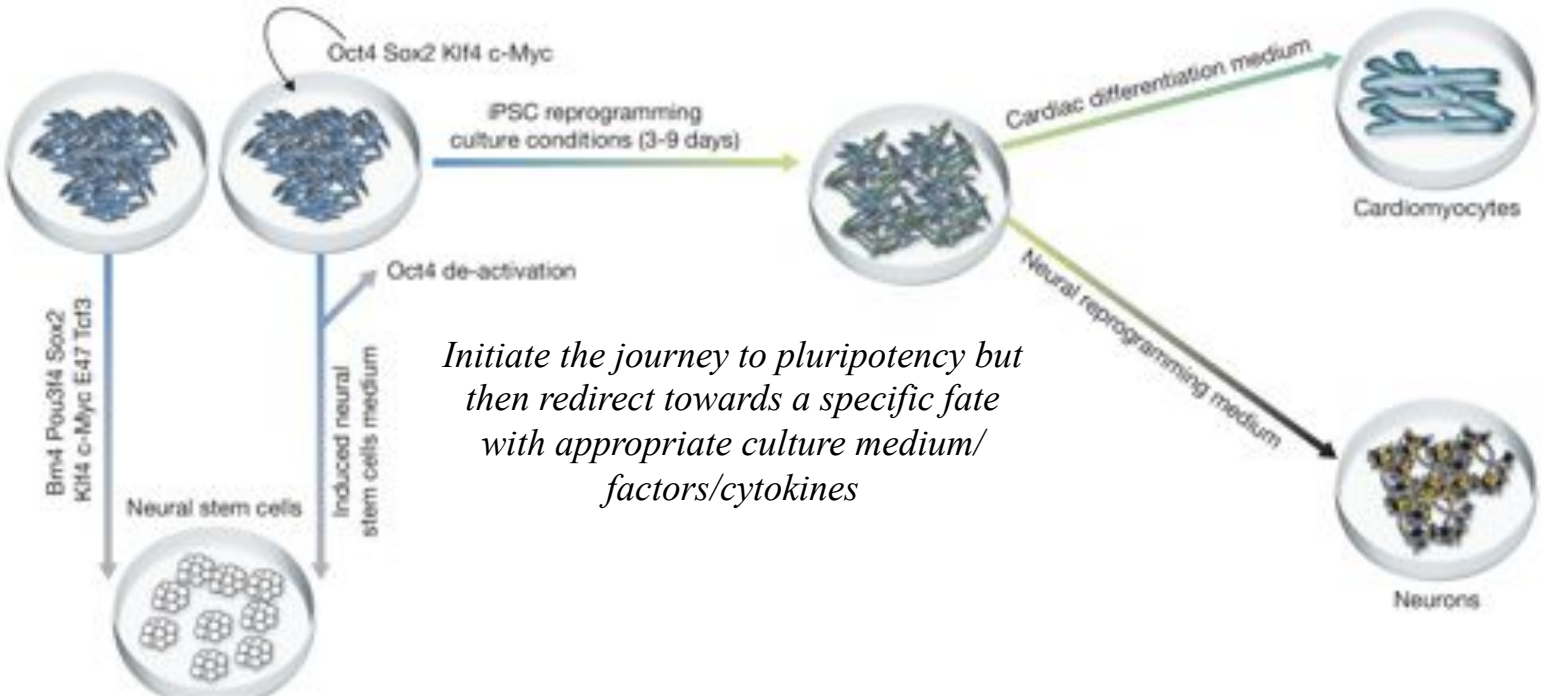
Direct lineage conversion for generation of specific cell types



- Identification of specific transcription factors controlling differentiated cell identity allows for the **forced conversion of one lineage into another in the absence of cell proliferation.**
- Direct conversion of numerous cell types now shown - including neurons, cardiomyoctes and hepatocytes. Direct lineage conversion is sufficient for the generation of distantly related cell types crossing germ layer boundaries.

From Sancho-Martinez et al, Nat. Cell Biol. 2012

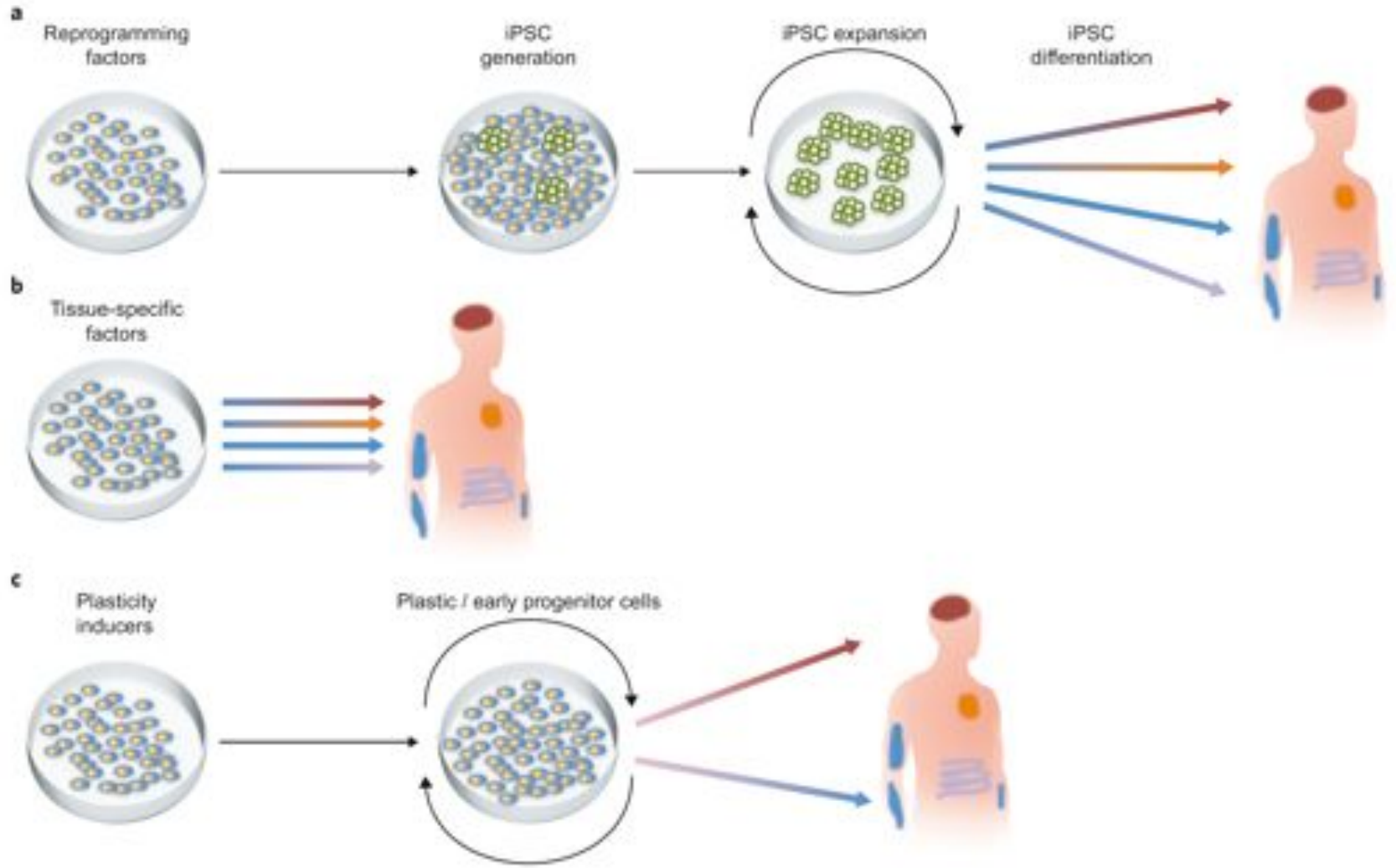
Indirect lineage conversion: for generation of specific cell types in absence of specific TFS



Initiate the journey to pluripotency but then redirect towards a specific fate with appropriate culture medium/ factors/cytokines

- Indirect lineage conversion: a more general approach in the absence of specific transcription factors.
- Relies on the use of pluripotency TFs (OSKM) initially.
- On forced OSKM expression, this first leads to removal of differentiated marks, creating an unstable state suitable for further differentiation on exposure to appropriate signals.

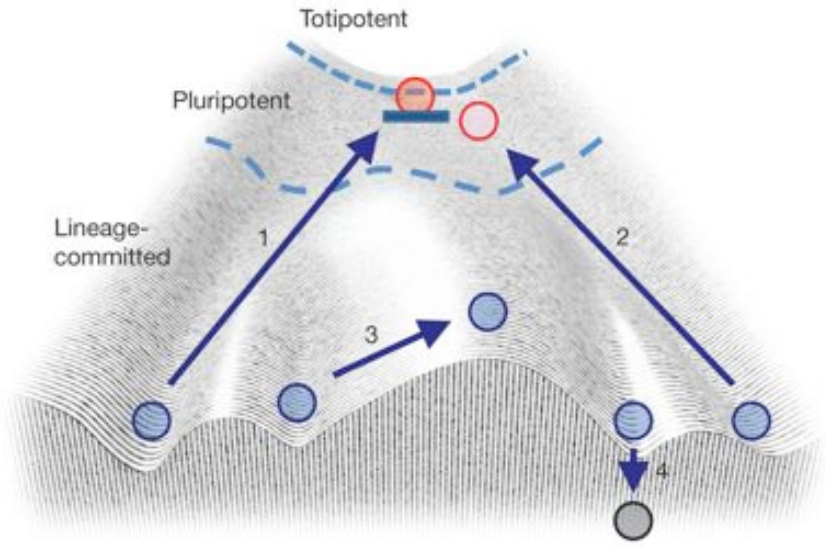
Different conversion methodologies to generate cells of a given fate



From Sancho-Martinez et al, Nat. Cell Biol. 2012

Multiple Reprogramming Perspectives

- Cell and tissue therapy perspectives using iPS, direct or indirect cell conversions
- Study of development and disease *in vitro*
- Powerful tools for drug screening
- Genetic engineering for functional investigations



Seminaire

Dr Claire Rougeulle

Inactivation du chromosome X, pluripotence et reprogrammation, de la souris à l'homme