Reprogramming of Somatic Cells

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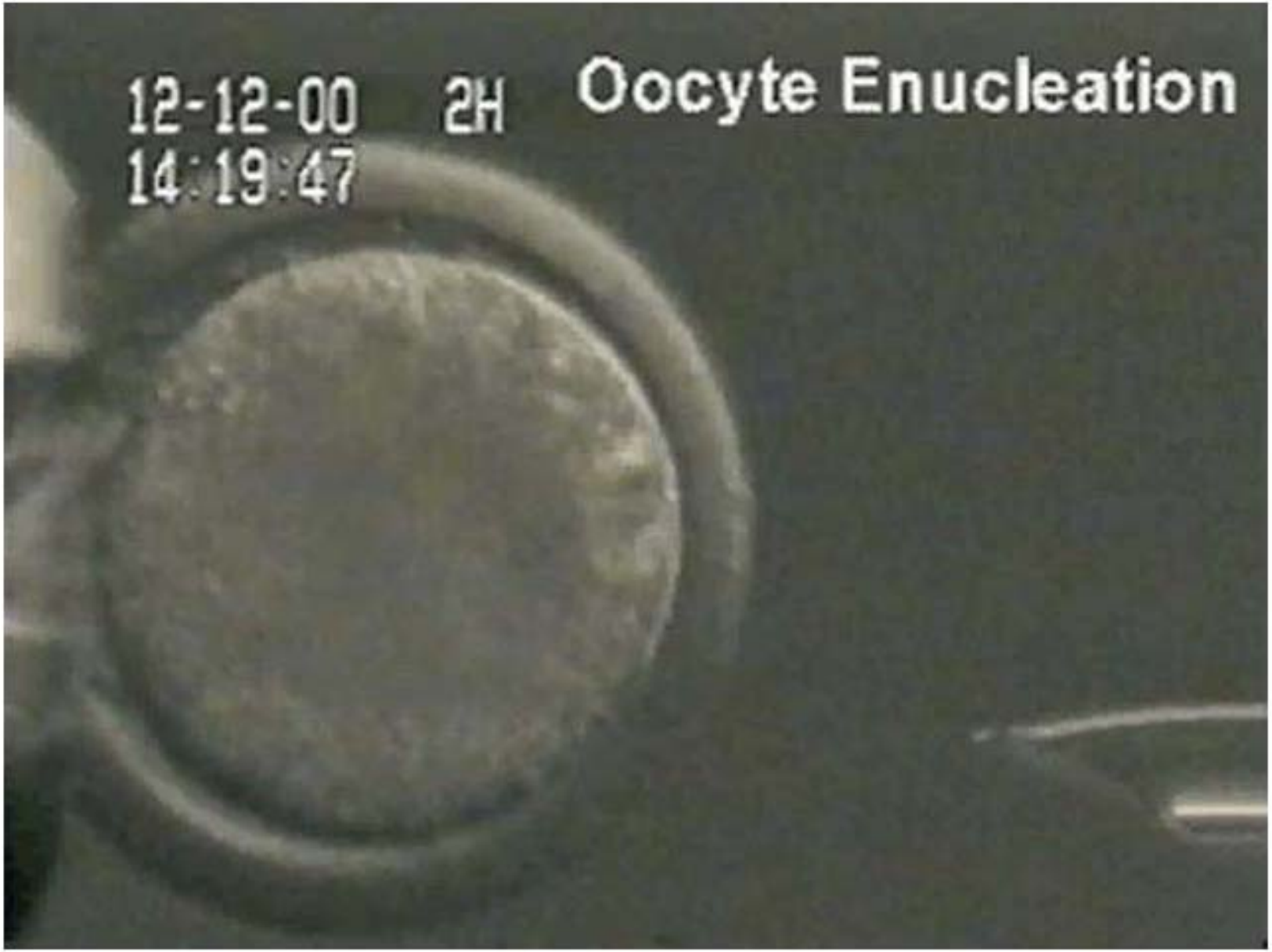
Stem Cells Class-3

Reprogramming

- remodeling of epigenetic marks, such as DNA methylation.

- Various methods have been developed to reprogram cells to a pluripotent state:

1. somatic cell nuclear transfer (SCNT). uses material from oocytes,
2. Fusion of somatic cells with pluripotent cells. resultant cells are tetraploid.
3. induced pluripotent stem cells (iPSCs)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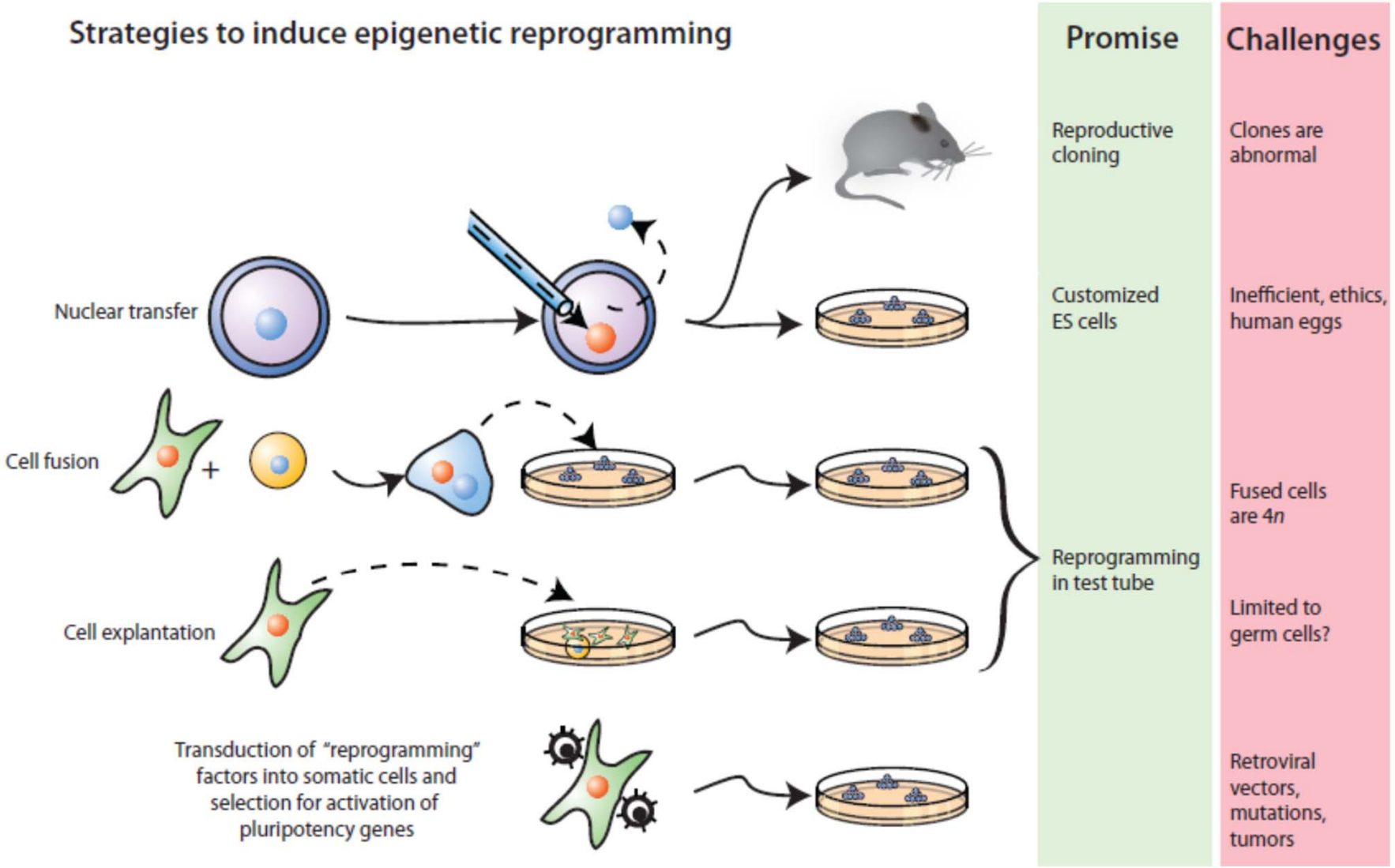
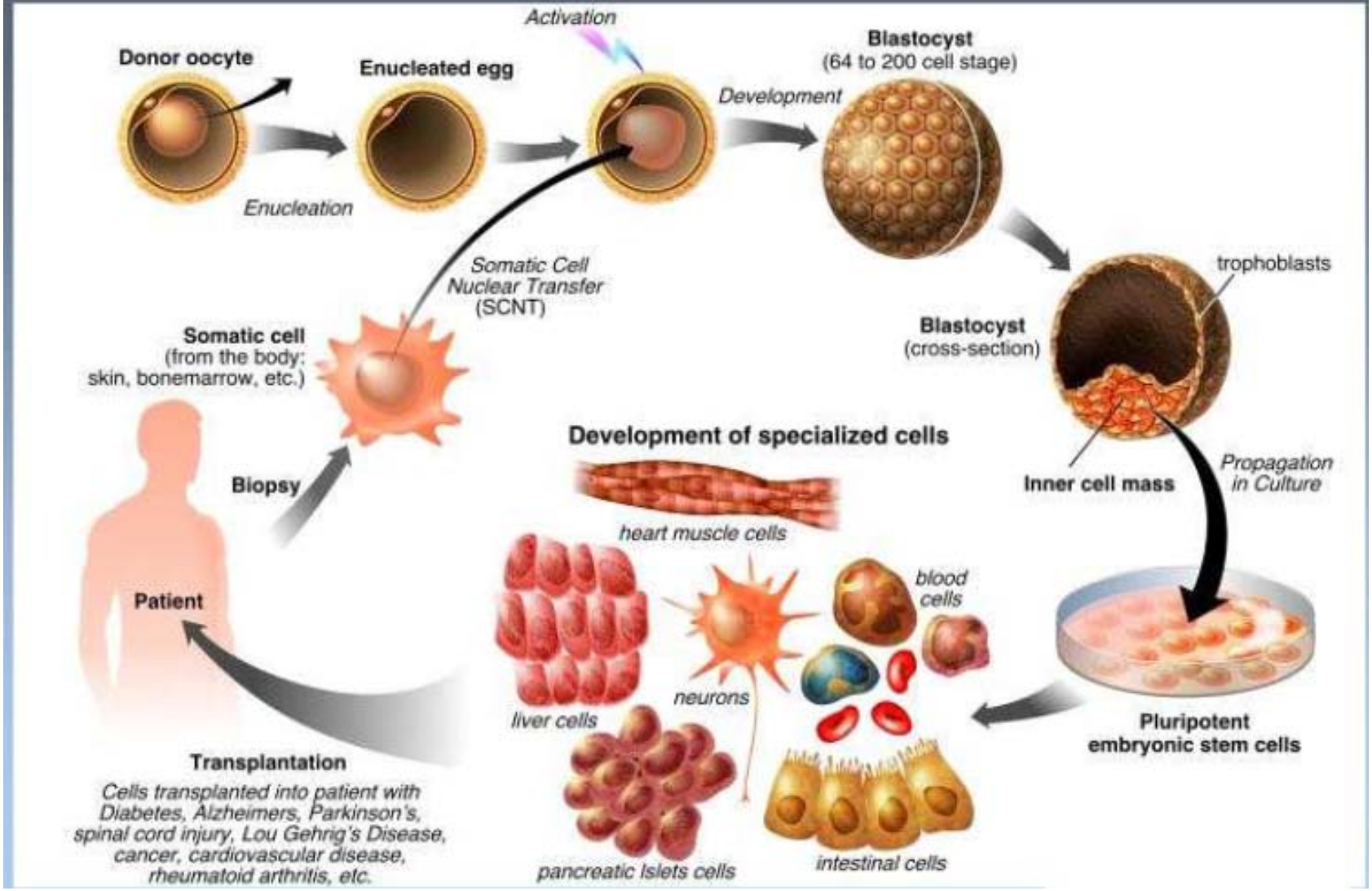
Oocyte Enucleation

Methods for Reprograming the Somatic Cells

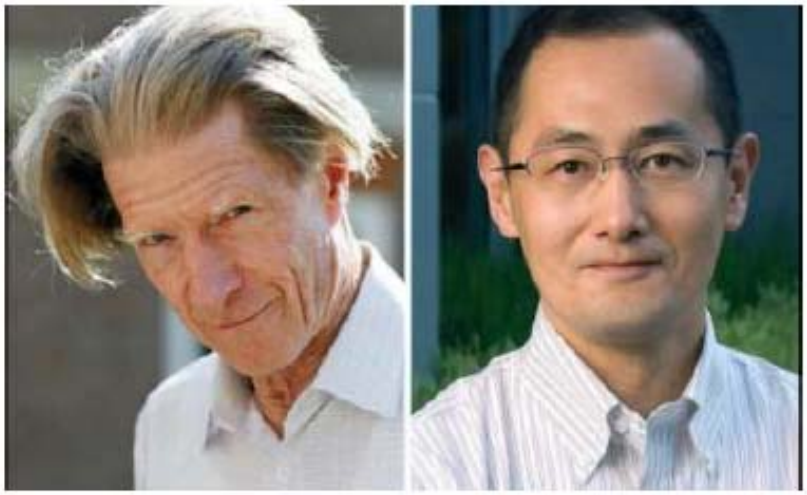


Nuclear transplantation



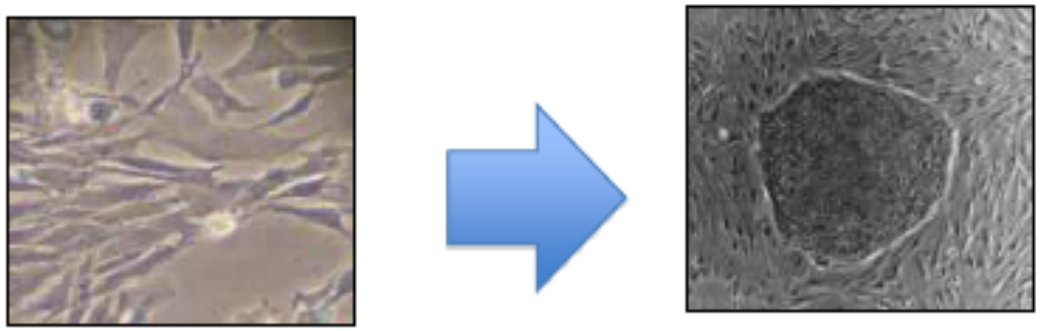
Somatic cells are reprogrammed to induced pluripotent stem cells (iPSCs) by ectopic expression of the pluripotent transcription factors (TFs) Oct4, Sox2, Klf4, and c-Myc

The Nobel Prize in Physiology or Medicine 2012 was awarded jointly to Sir John B. Gurdon and Shinya Yamanaka *"for the discovery that mature cells can be reprogrammed to become pluripotent"*



SCNT iPS

Induced pluripotent stem cells (iPS) generation



- The first iPS cell line generated with 24 factors. (Takahashi K & Yamanaka S. Cell 2006)

- The Classical 4 factors cocktail: **Oct4/3, Sox2, c- myc & KlF4 or Oct4/3, Sox2, Lin28 & Nanog** (Takahashi K & Yamanaka S. Cell 2006, Takahashi K et al, Cell 2007, Yu et al, Science NY, 2007Park et al, Nature 2008)

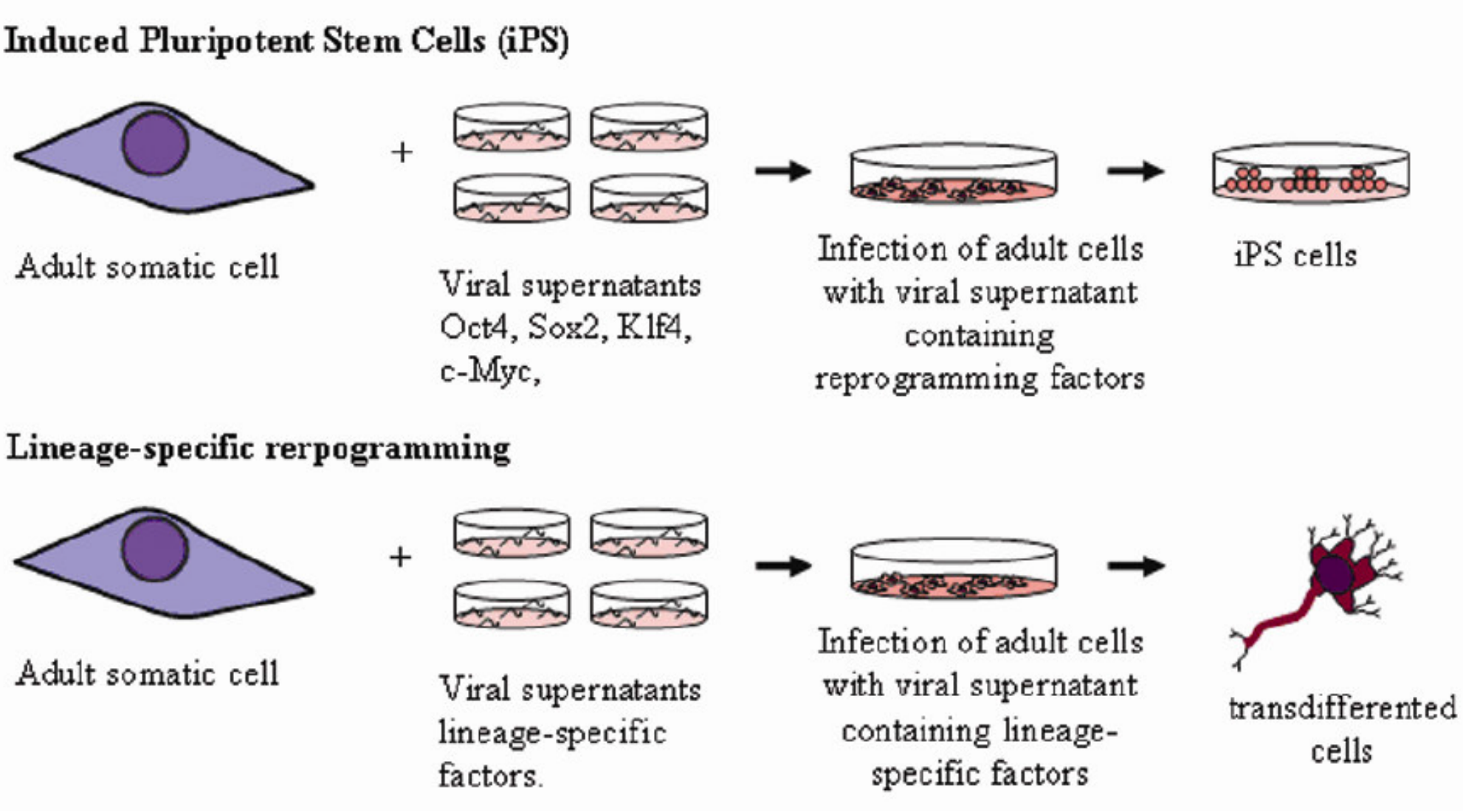
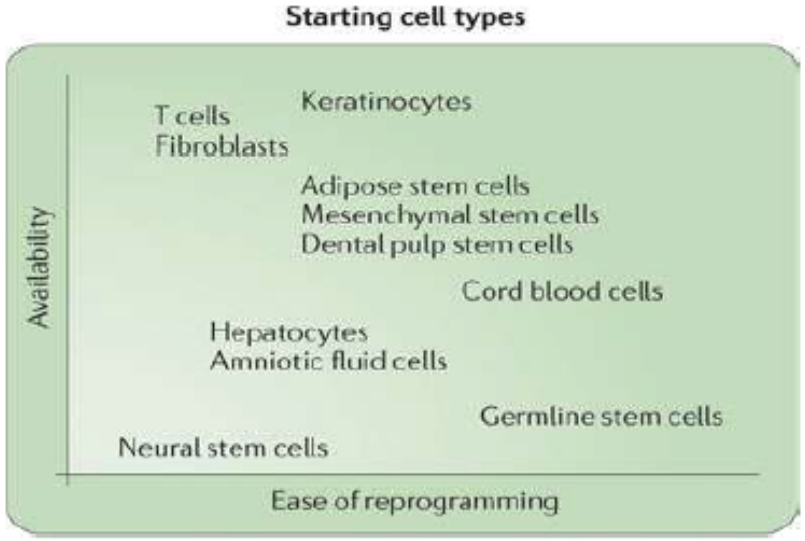
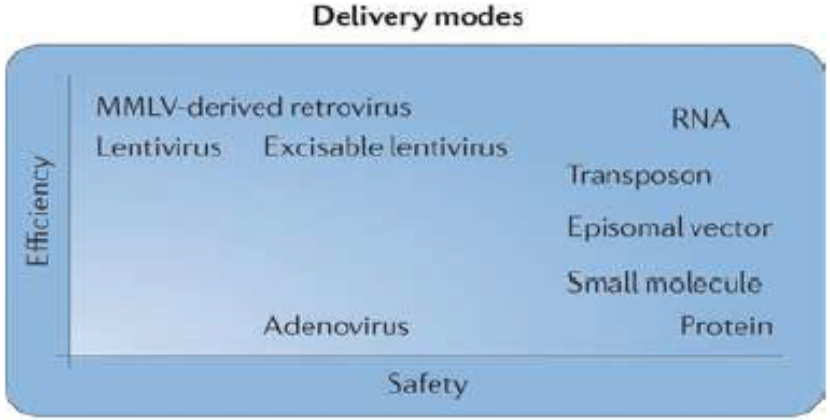


Fig. 1—Different methods of reprogramming somatic cells.





F. González et al *2011*

Nature Reviews | Genetics

iPS GENERATION

- Choice of starting cell types
- Choice of methods of factor delivery
- Choice of factors

Factors

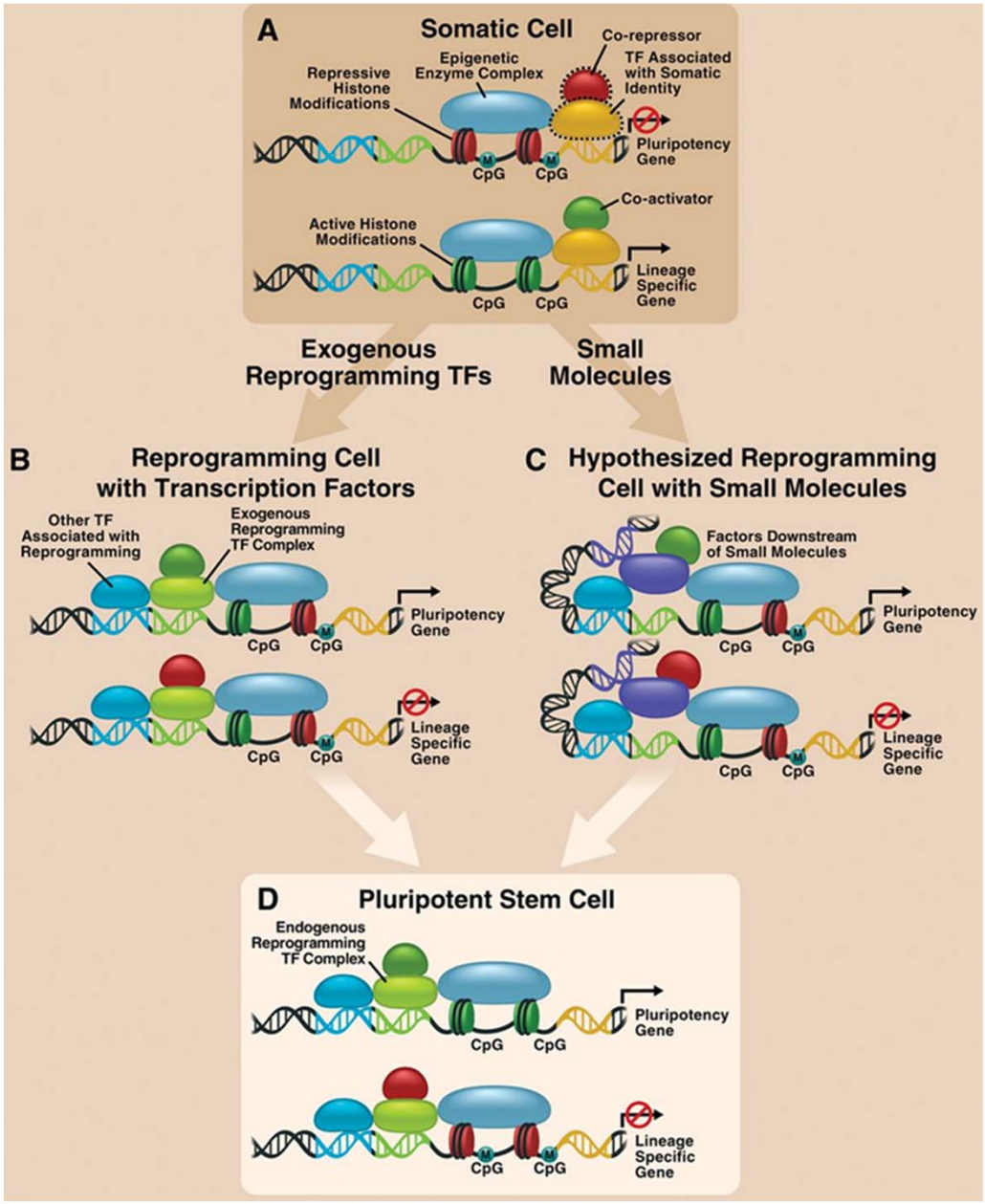
To express/overexpress	To repress
Important for embryonic development: OCT4, SOX2, NANOG, UTF1, LIN28, SALL4, NR5A2, TBX3, ESSRB, DPPA4	Apoptosis, cell cycle and senescence: $p16^{INK4A‡}$, p53‡, microRNA, p21
Proliferation and cell cycle: MYC*, KLF4*, SV40LT*, REM2, MDM2*, cyclin D1*	Epigenetic regulators: histone deacetylase, histone demethylase, G9a, DNMT1*
Epigenetic regulators: CHD1, PRC2	Signalling pathways: TGFβ, WNT, ERK–MAPK
Others: vitamin C, hypoxia, E-cadherin, miR-294, TERT*	*Potential oncogene ‡Potential tumour suppressor gene

- Lentivirus/retrovirus mediated reprogramming methods are still major approaches for generation of iPS

iPS

- In practice, combined with efficient differentiation strategies, iPSCs would be valuable not only to derive functional cells for transplantation but also to establish patient-specific disease models for drug discovery and development.
- Similar to the capability of cell- type-specific TFs to maintain cell identity by binding to specific DNA sequences across the genome and achieving additional sequence-binding specificity and transcriptional/epigenetic regulation by forming complexes with coregulatory factors, exogenous iPSC TFs (TFs overexpressed in generation of iPSCs) cooperatively remodel chromatin to activate expression of genes in the pluripotency network and to suppress expression of genes that promote differentiation.

- In addition, through both co-occupancy with downstream effectors of various signaling pathways and recruitment of diverse epigenetic enzymes over the whole genome, specific chromosomal binding patterns of exogenous iPSC TFs during the reprogramming process contribute to the establishment of iPSC-specific signal transduction, transcriptional circuitry, and epigenetic pattern.
- Although iPSC reprogramming is technically simpler than SCNT and cell fusion, it only induces a stochastic and non- specific reprogramming process and is therefore less efficient and slower than SCNT and cell fusion.


A
Somatic Cell
Co-repressor
Epigenetic Enzyme Complex
TF Associated with Somatic Identity
Repressive Histone Modifications
Pluripotency Gene
CpG
CpG
Co-activator
Active Histone Modifications
Lineage Specific Gene
CpG
CpG
Exogenous Reprogramming TFs
Small Molecules
B
Reprogramming Cell with Transcription Factors
Other TF Associated with Reprogramming
Exogenous Reprogramming TF Complex
Pluripotency Gene
CpG
CpG
Lineage Specific Gene
CpG
CpG
C
Hypothesized Reprogramming Cell with Small Molecules
Factors Downstream of Small Molecules
Pluripotency Gene
CpG
CpG
Lineage Specific Gene
CpG
CpG
D
Pluripotent Stem Cell
Endogenous Reprogramming TF Complex
Pluripotency Gene
CpG
CpG
Lineage Specific Gene
CpG
CpG

Bad Face

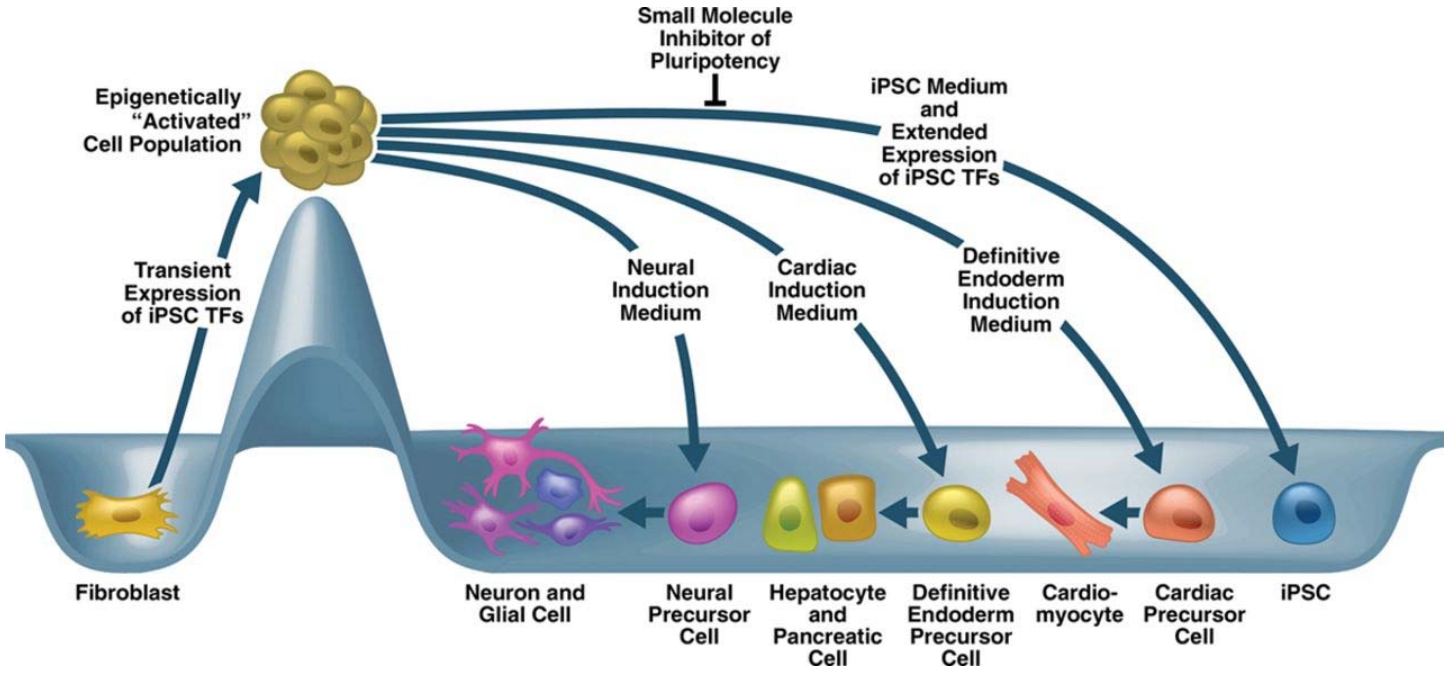
- iPSCs generated by conventional methods raise concerns about their safety (eg, immunogenicity7 and the risk of tumorigenesis) for clinical applications, as they use virus-mediated gene delivery that results in genomic integration of exogenous sequence and enforced expression of oncogenes.

Improvments

- Several specific cell types were shown to enable reprogramming with higher efficiency and less number of exogenous iPSC TFs. However, nearly all of them rely on overexpression of exogenous iPSC TFs and extra manipulations (eg, administration of cytokines or small molecules) to get reprogrammed efficiently and rapidly.
- In addition to reprogramming using different starting cell types, methods using virus-free, removable PiggyBac transposons or episomal systems have been developed. Despite their success in generating iPSCs, often without a genetic footprint, the use of
- DNA constructs leaves the possibility of genomic integration of exogenous sequence. Other attempts to generate iPSCs by nonintegrating virus-mediated gene delivery cannot preclude the safety concerns raised by using viruses.

- In addition, generation of iPSC with small molecules alone is being attempted. This promising strategy might eliminate many of the drawbacks (eg, the risk of tumorigenesis from genomic integration of exogenous sequence or overexpression of oncogenes) of conventional and other improved iPSC reprogramming methods.
- Although the final outcome of iPSC reprogramming induced by any method is the establishment of pluripotency-associated gene expression profile and epigenetic pattern, the small molecule approach would use a different process/mechanism from other methods to launch reprogramming process.
- In details, in other methods, exogenously introduced and pluripotency-associated elements (eg, TFs) trigger iPSC reprogramming by directly participating in and directing pluripotency-specific chromatin remodeling in somatic cells, whereas the small molecule approach indirectly initiates iPSC reprogramming by mediating endogenous, nonpluripotency-specific components in somatic cells (Figure slide 12).

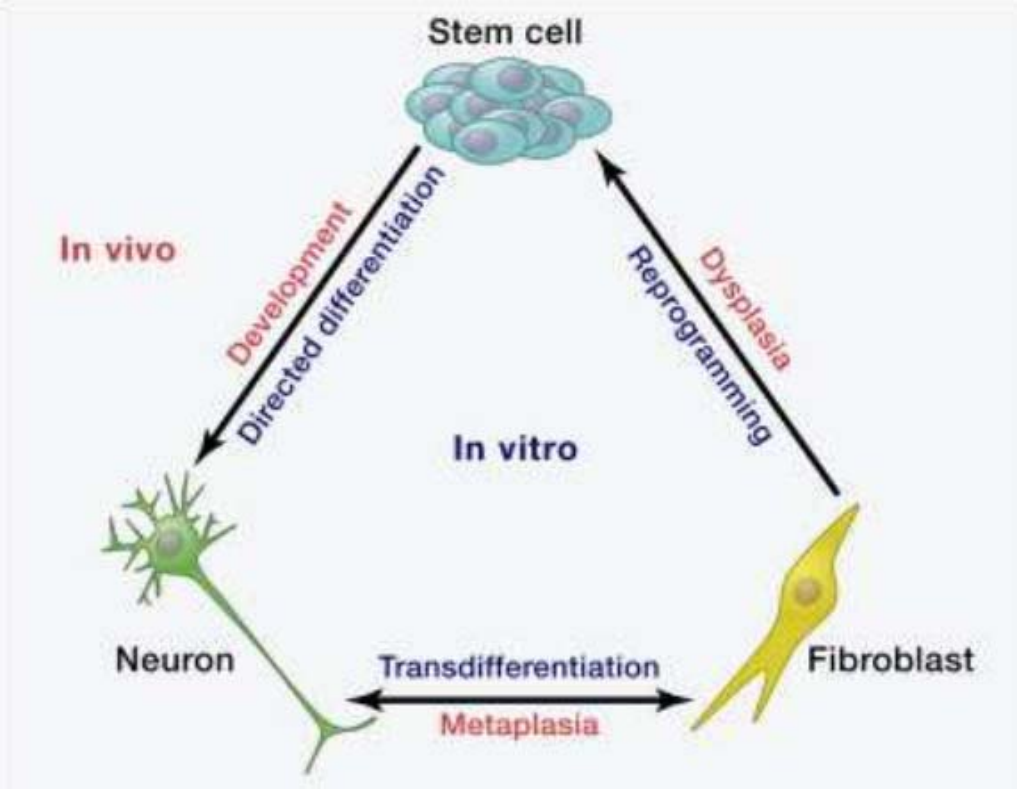
A simplified and conceptual paradigm of induced pluripotent stem cell (iPSC) transcription factor (TF)–based transdifferentiation.



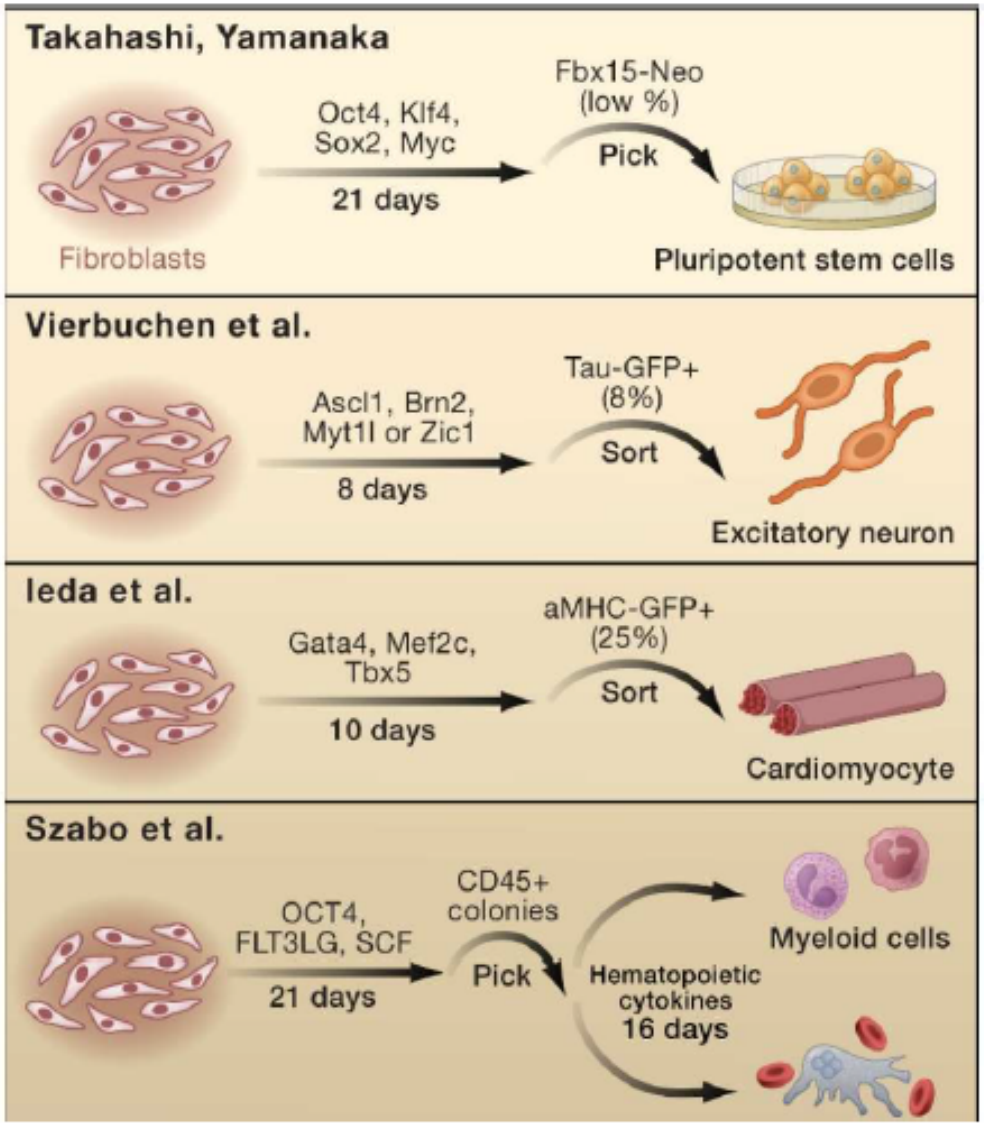
Tianhua Ma et al. Circulation Research. 2013;112:562-574

Transdifferentiation

It is possible to convert one differentiated cell type into another without having to reverse differentiation all the way back to a pluripotent state by using methods broadly similar to that used to induce complete reprogramming .



TRANSDIFERENTIATION



References

1- Ma T, Xie M, Laurent T, Ding S. Progress in the reprogramming of somatic cells. Circulation research. 2013 Feb 1;112(3):562-74.

2- Cieślar-Pobuda A, Knoflach V, Ringh MV, Stark J, Likus W, Siemianowicz K, Ghavami S, Hudecki A, Green JL, Łos MJ. Transdifferentiation and reprogramming: Overview of the processes, their similarities and differences. Biochimica et Biophysica Acta (BBA)-Molecular Cell Research. 2017 Jul 1;1864(7):1359-69.

3- NIH Stem Cell Information Home Page. In Stem Cell Information [World Wide Web site]. Bethesda, MD: National Institutes of Health, U.S. Department of Health and Human Services, 2016 [cited February 1, 2019] Available at < //stemcells.nih.gov/info.htm>