“Techniques in Cellular and Molecular Neurobiology”

International Master’s Degree in Neuroscience

Lesson 4

DIPARTIMENTO DI SCIENZE DELLA VITA

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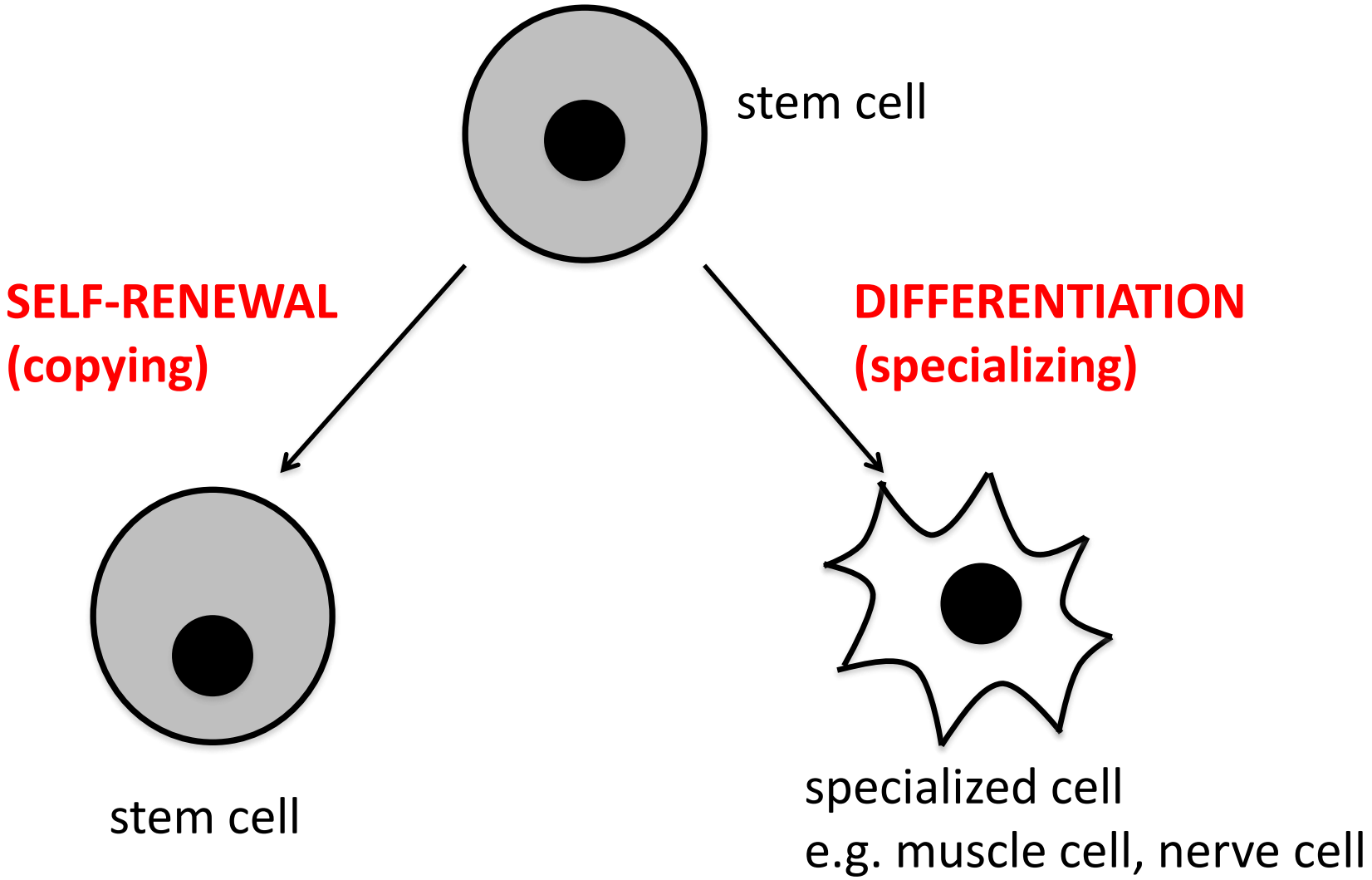
What is a stem cell?



What is a stem cell?

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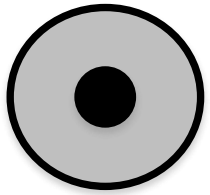
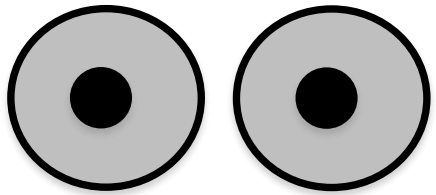
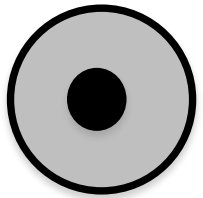
Stem cell



SELF-RENEWAL
(copying)

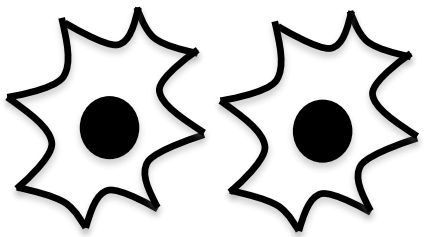


Identical stem cells



Stem cell

DIFFERENTIATION
(specializing)



Specialized cells

Why self-renew AND differentiate?

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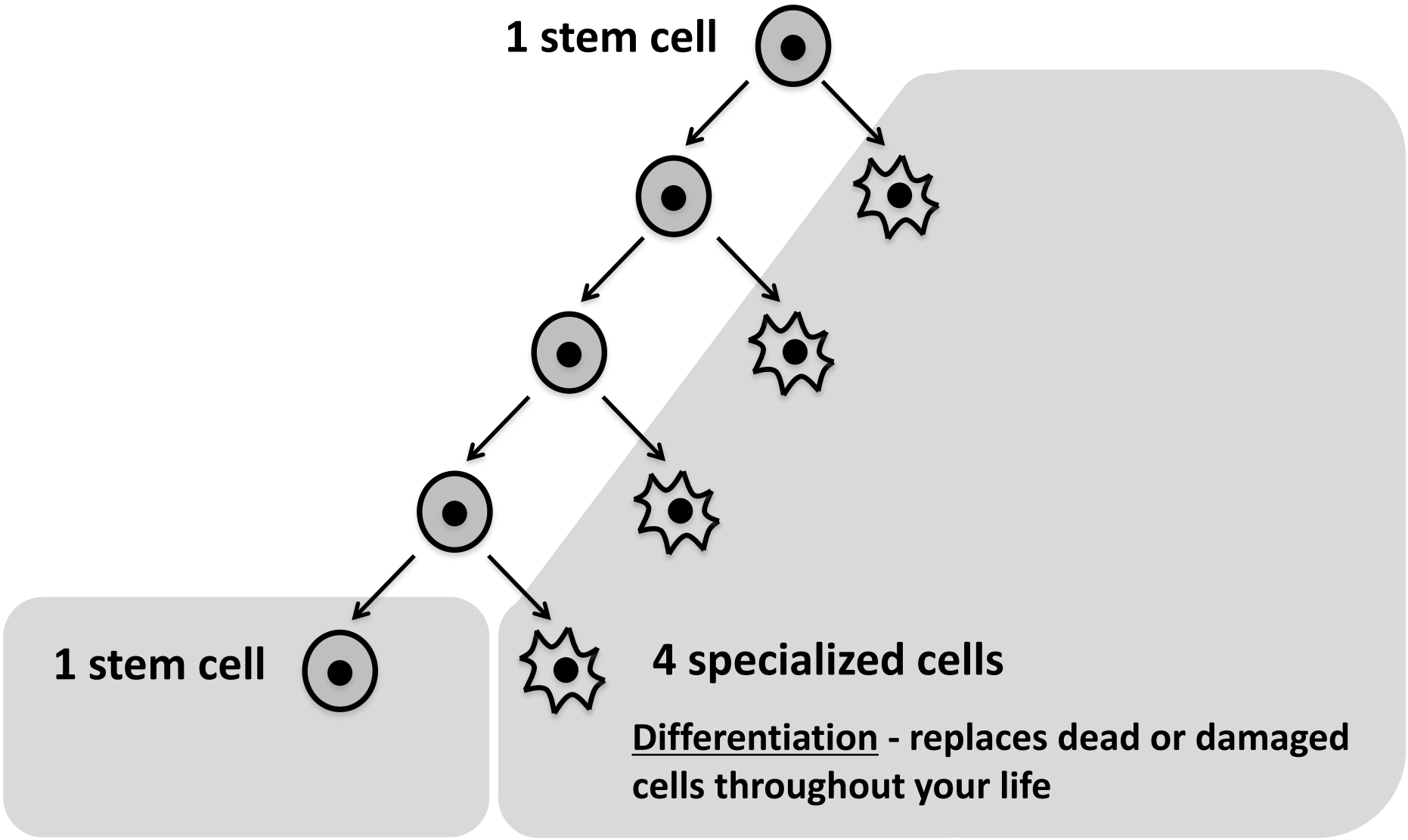

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Stem Cell Reviews and Reports (2020) 16:3–32
https://doi.org/10.1007/s12015-019-09935-x

Advances in Pluripotent Stem Cells: History, Mechanisms, Technologies, and Applications

Gele Liu[1] · Brian T. David[1] · Matthew Trawczynski[1] · Richard G. Fessler[1]

Published online: 23 November 2019

1

Embryonic stem cells (ESCs)

Pluripotent stem cells derived from the inner cell mass of a blastocyst (embryo)

Very Small Embryonic-Like Stem Cells (VSELs)

Pluripotent stem cells derived from adult tissues

Nuclear transfer stem cells (NTSCs)

One new single cell is produced by the transplantation of the donor nucleus into an enucleated oocyte of a donor egg. Reprogramming occurs to form blastocyst.

3

Reprogramming Stem Cells (RSCs)

Pluripotent stem cells generated by reprogramming adult cells. Derived by applying manual laboratory methods to reprogram adult cells (except SCNT). RSCs include iPSCs and direct reprogramming stem cells.

2

Adult stem cells (ASCs)

A type of cell in close proximity to rich, nutrient-full microenvironment such as vessels, bone marrow, or organs (heart and brain, etc) in the mature or adult organism; they are able to respond to tissue-specific stimulation to produce stem cells.

Types of stem cell:
1) Embryonic stem cells

Embryonic stem (ES) cells: Where we find them

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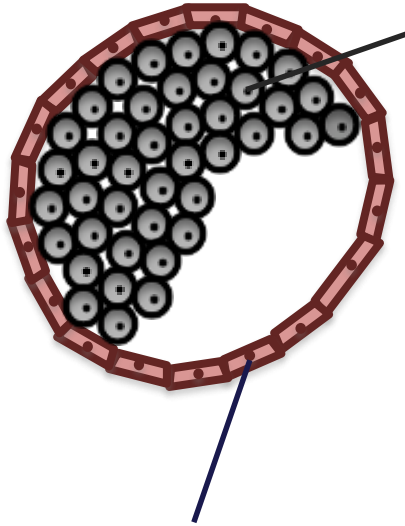
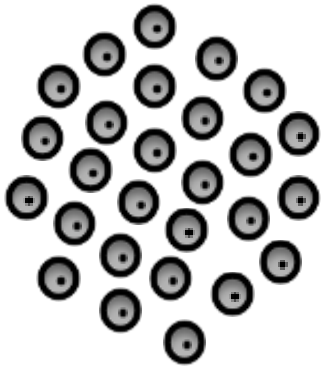
blastocyst

cells inside
= 'inner cell mass'

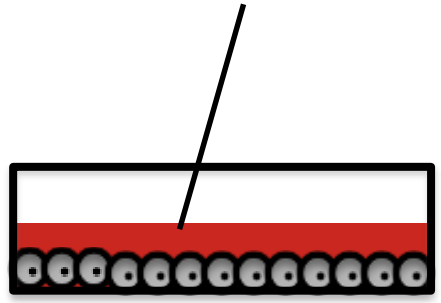


outer layer of cells
= 'trophectoderm'



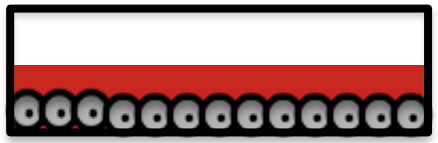
embryonic stem cells taken from the inner cell mass

fluid with nutrients



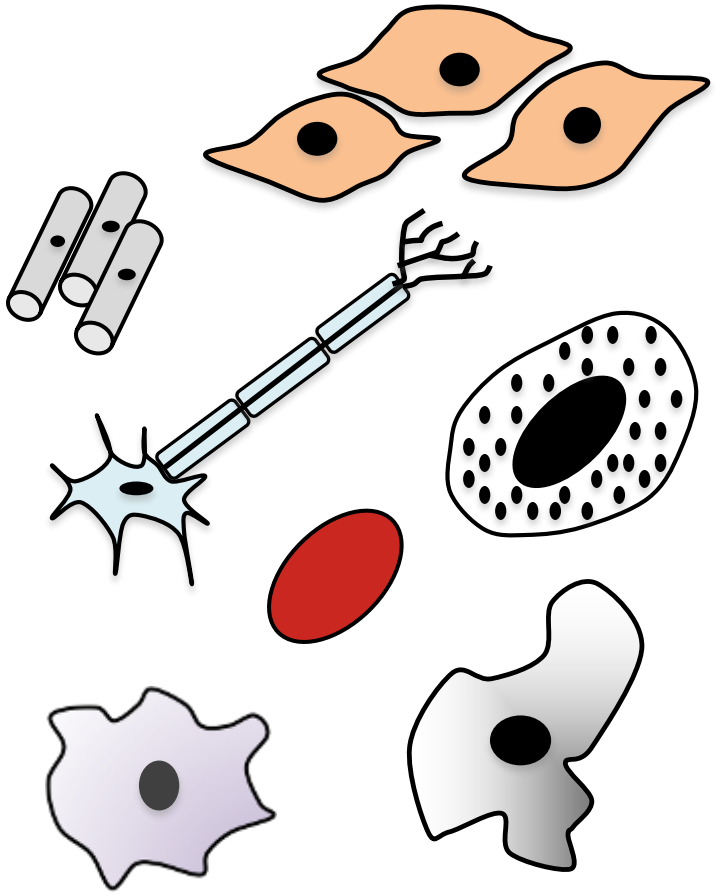
culture in the lab
to grow more cells

Embryonic stem (ES) cells: What they can do



embryonic stem cells

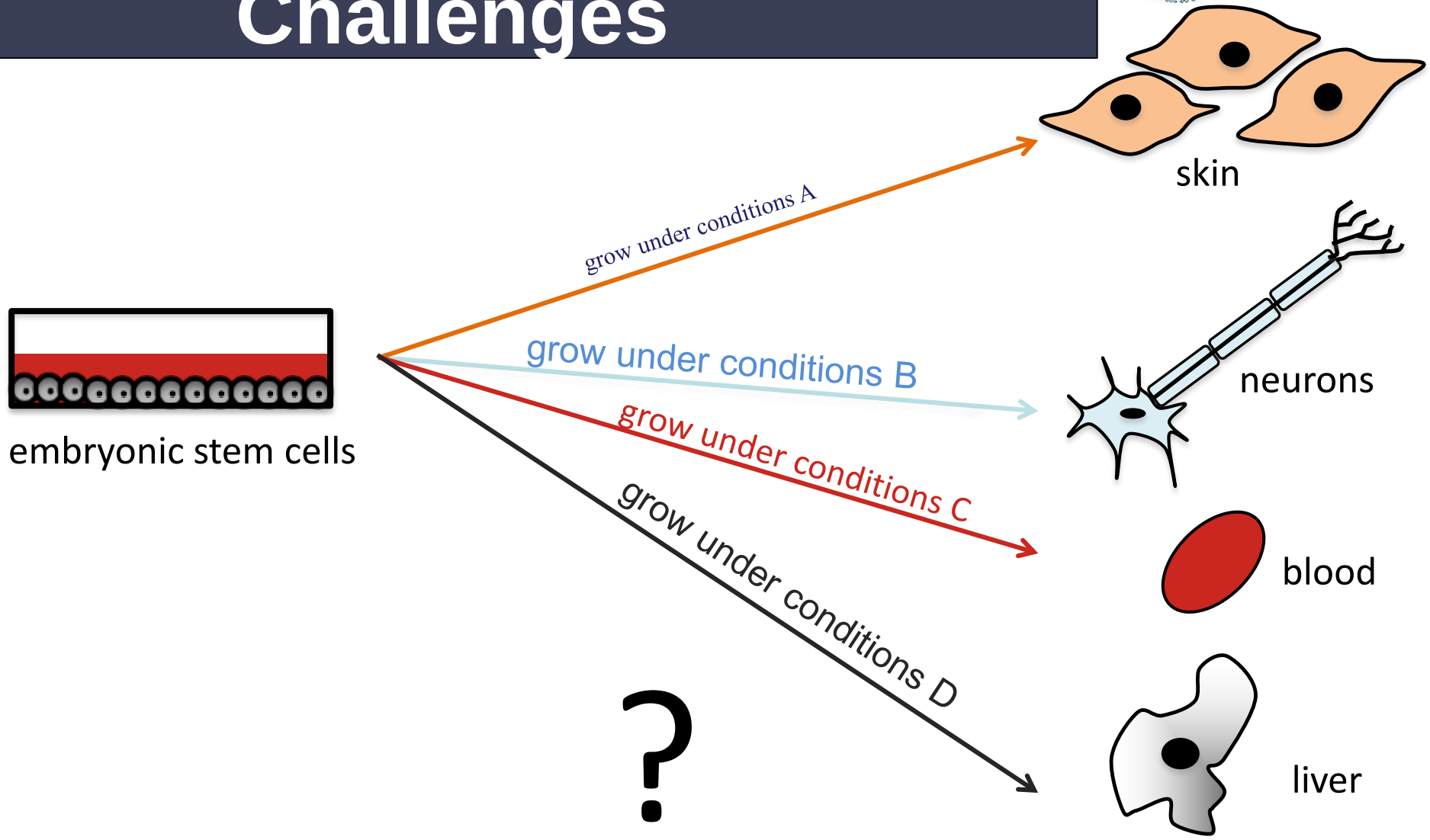
differentiation



all possible types of specialized cells

PLURIPOTENT

Embryonic stem (ES) cells: Challenges



Human Stem Cell Research and Regenerative Medicine

Focus on European policy and scientific contributions

EUROPEAN SCIENCE FOUNDATION

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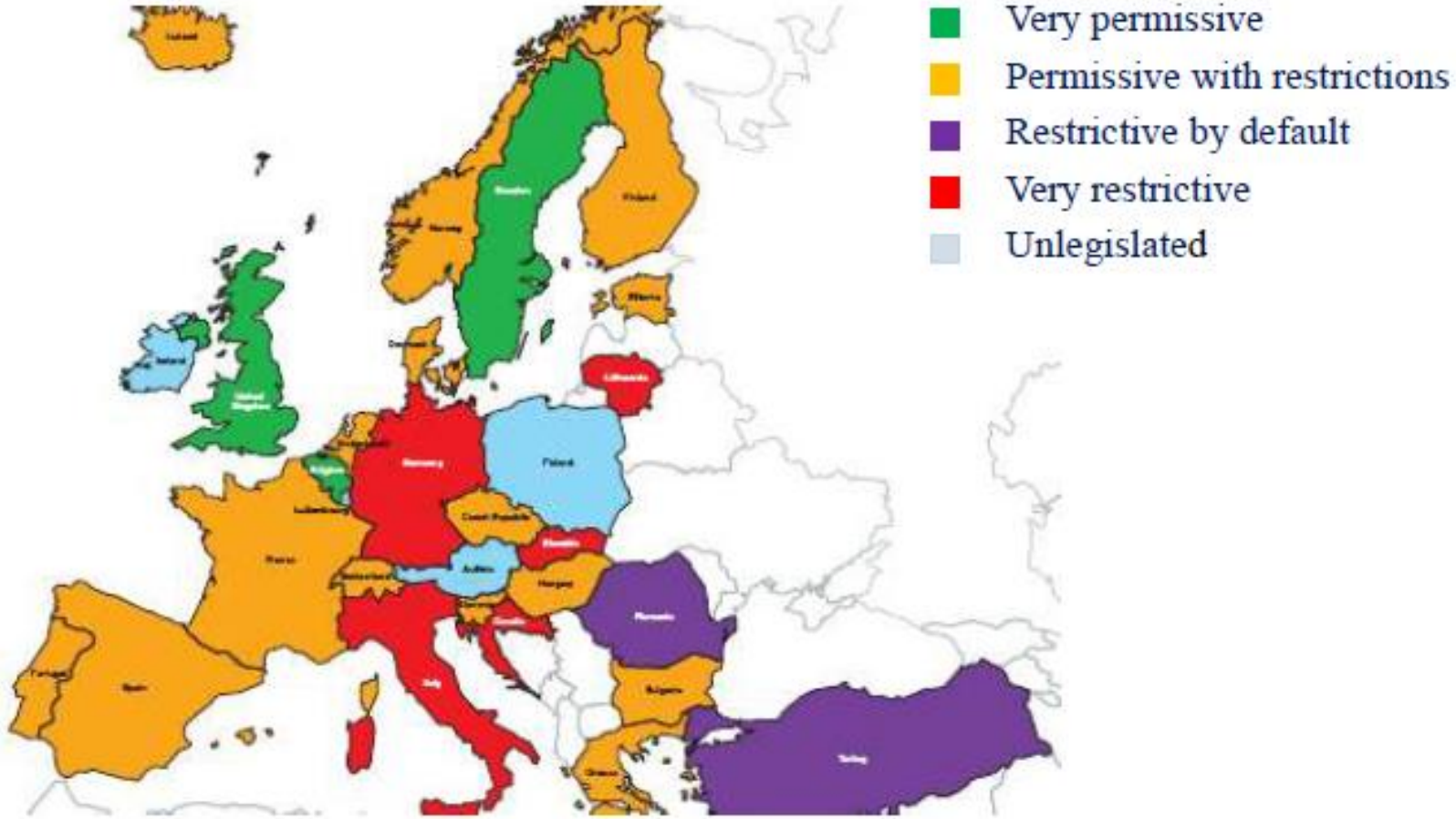
DI DI TRIESTE



National positions on human embryonic stem cell research policy and regulatory framework in Europe

Types of stem cell:
2) Tissue stem cells

Tissue stem cells: Where we find them

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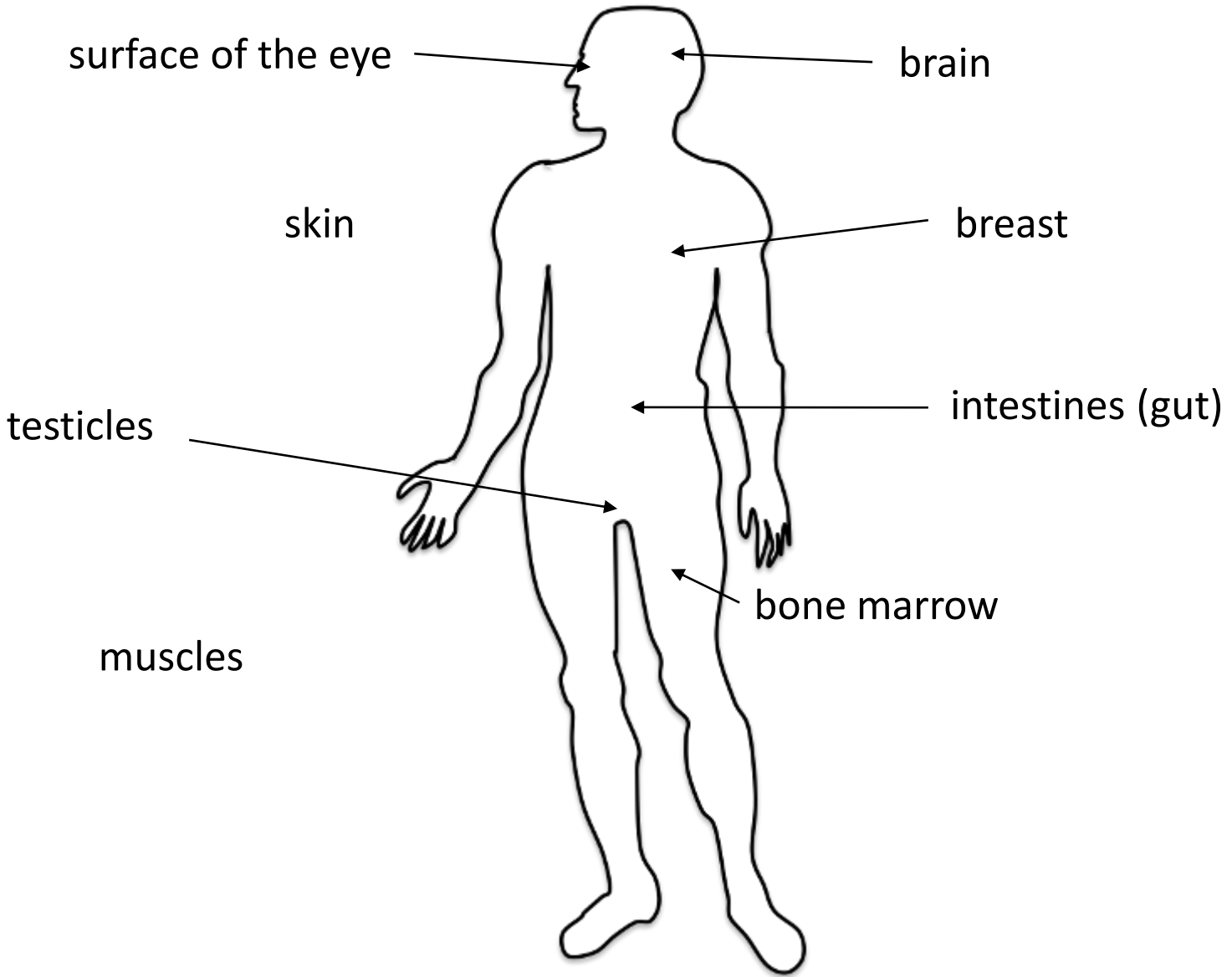
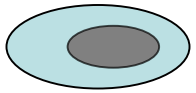
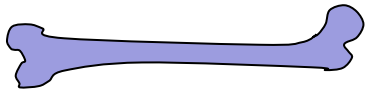


Tissue stem cells: What they can do



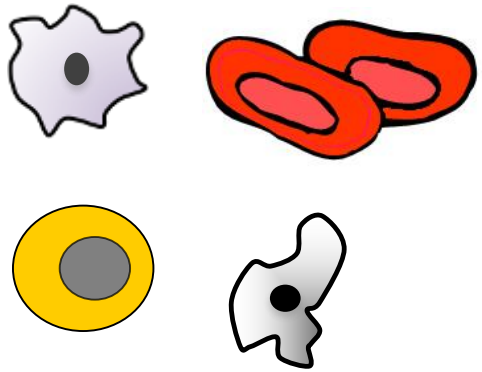
blood stem cell



found in
bone marrow

MULTIPOTENT

differentiation



only specialized types of blood cell:
red blood cells, white blood cells,
platelets

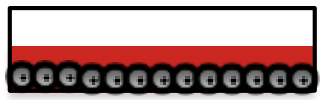
Types of stem cell:
3)Induced pluripotent (iPS) stem cells

Induced pluripotent stem cells (iPS cells

adult cell

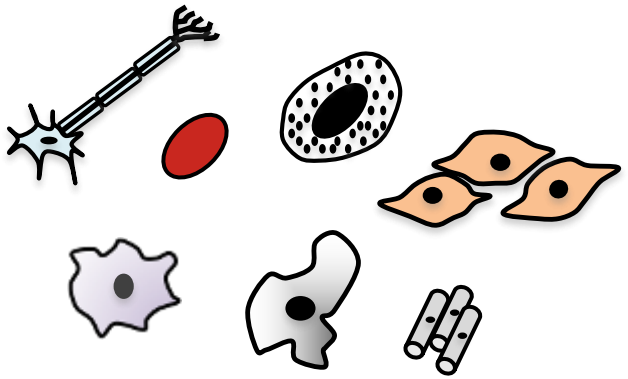
'genetic reprogramming'
= add certain genes to the cell

induced pluripotent stem (iPS) cell
behaves like an embryonic stem cell



culture iPS cells in the lab

differentiation



all possible types of
specialized cells

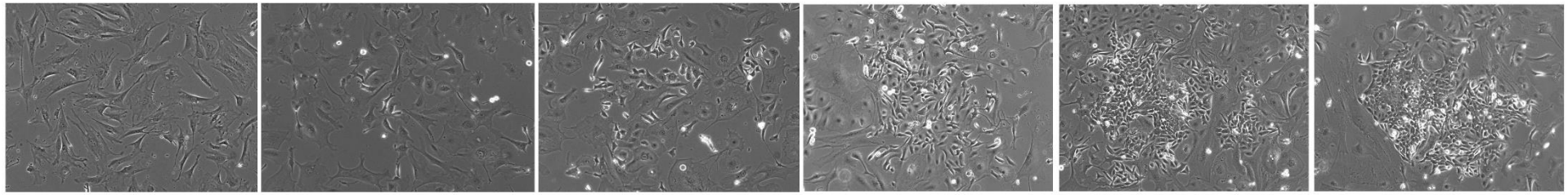
Advantage: no need for embryos!

Induced pluripotent stem cells (iPS cells)

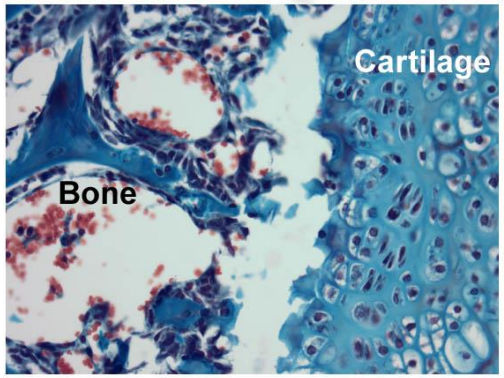
genetic reprogramming

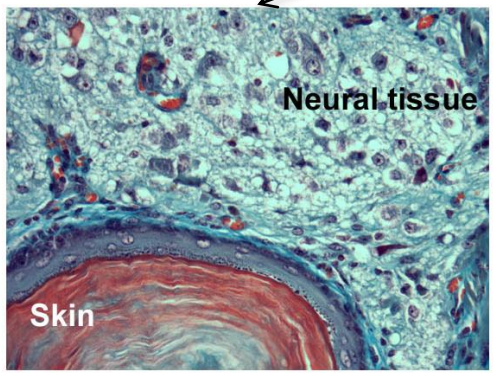
adult cell (skin)

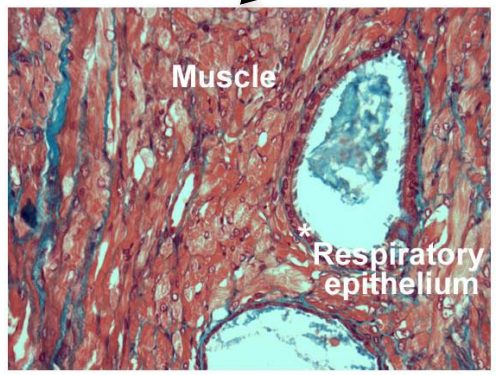
pluripotent stem cell (iPS)



differentiation







Stem cell jargon

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Potency	A measure of how many types of specialized cell a stem cell can make
Pluripotent	Can make all types of specialized cells in the body Embryonic stem cells are pluripotent
Multipotent	Can make multiple types of specialized cells, but not all types Tissue stem cells are multipotent

Tissue stem cells: Neural stem cells (NSCs)

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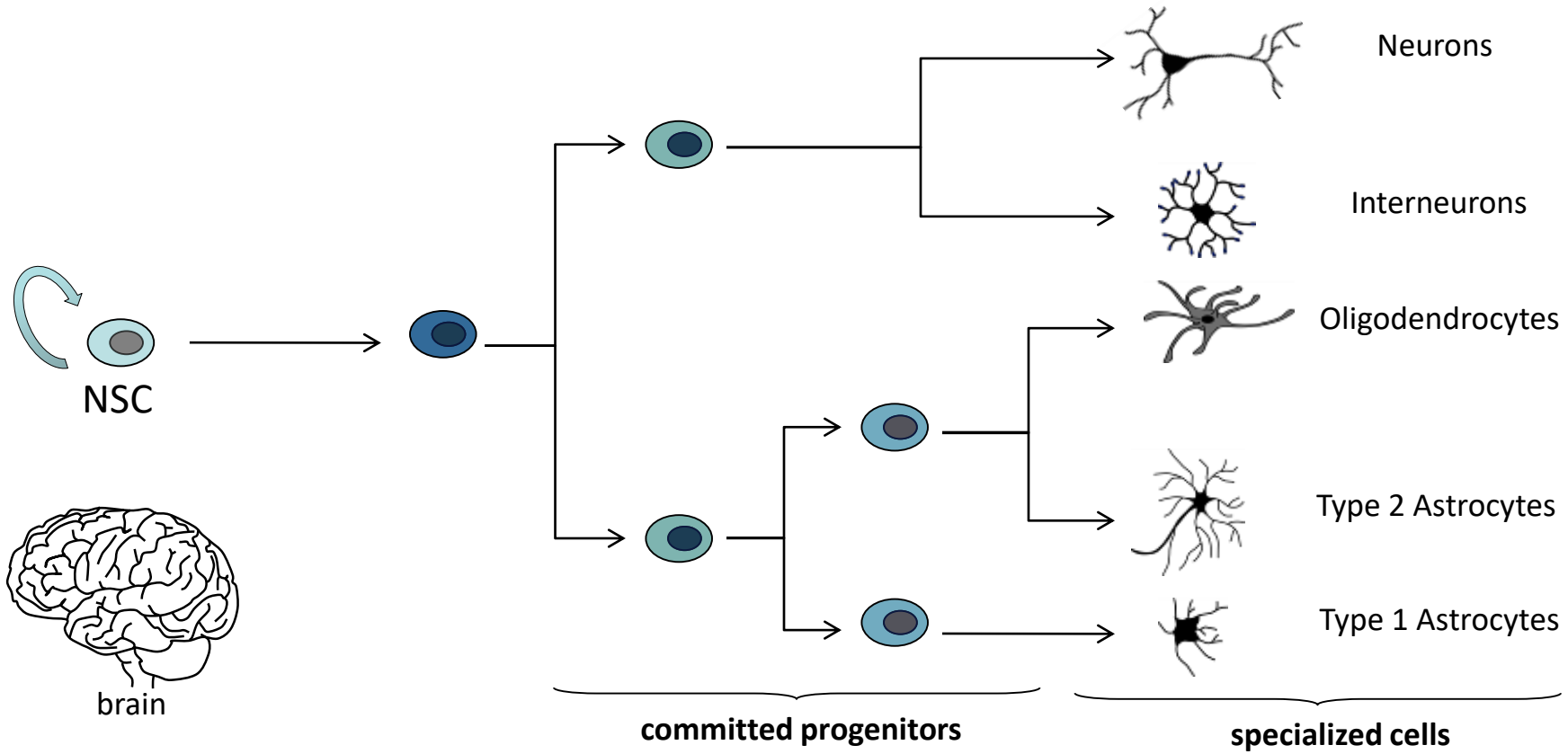
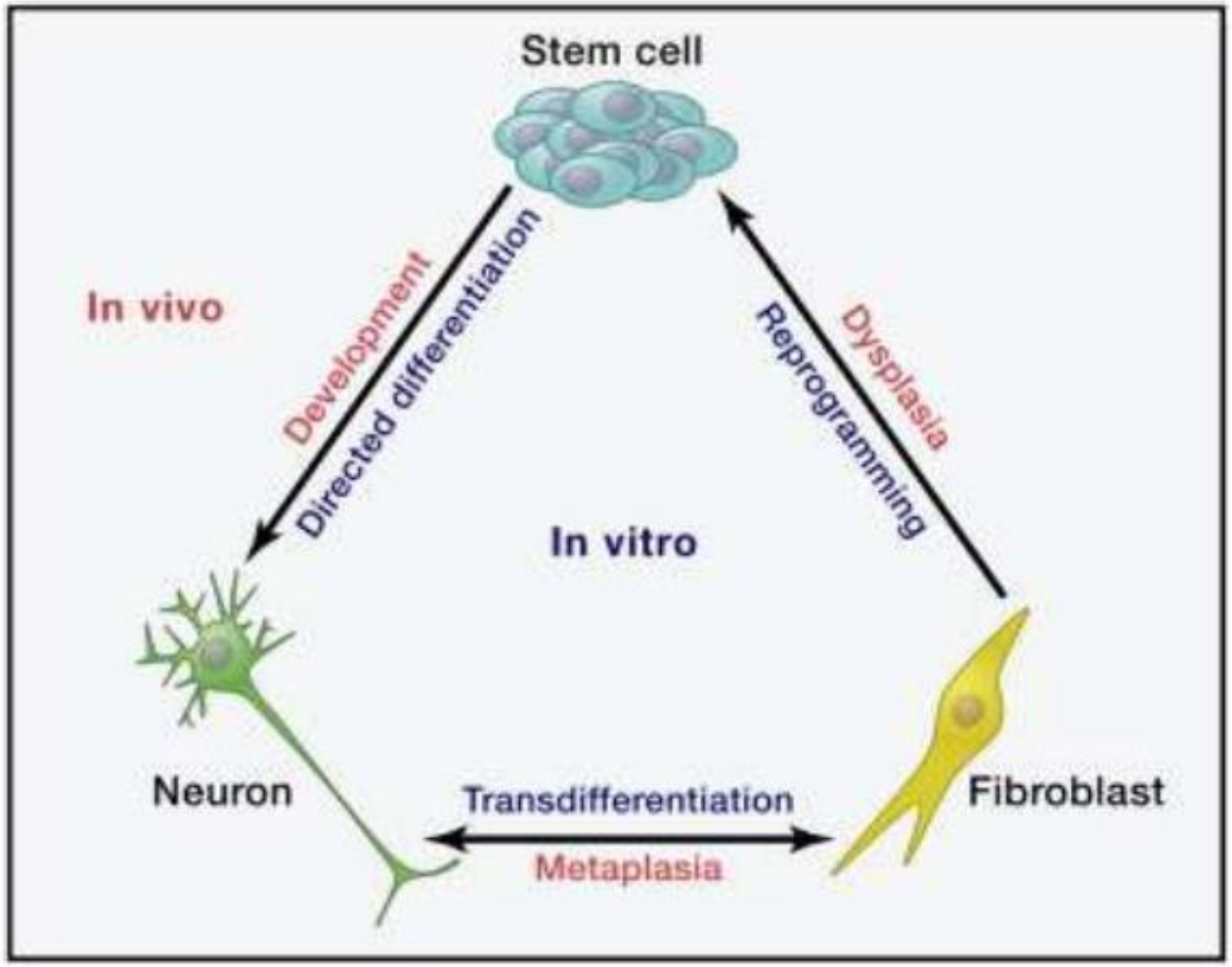


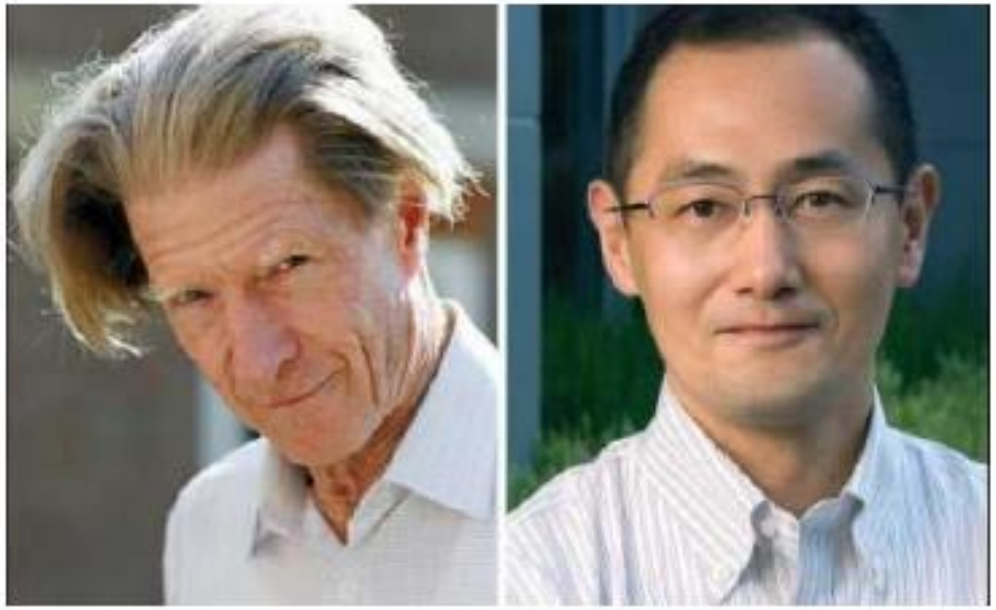
Stem cells



Cell 2012, *Cherry et al*

iPSC

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

Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors.

Takahashi K, Yamanaka S.

Cell. 2006.

Induction of Pluripotent Stem Cells from Adult Human Fibroblasts by Defined Factors

Kazutoshi Takahashi,[1] Koji Tanabe,[1] Mari Ohnuki,[1] Megumi Narita,[1,2] Tomoko Ichisaka,[1,2] Kiichiro Tomoda,[3] and Shinya Yamanaka[1,2,3,4,*]

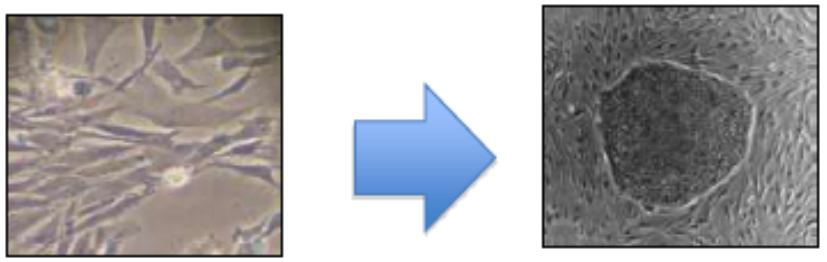
Cell, 2007

Induced Pluripotent Stem Cell Lines Derived from Human Somatic Cells

Junying Yu,[1,2*] Maxim A. Vodyanik,[2] Kim Smuga-Otto,[1,2] Jessica Antosiewicz-Bourget,[1,2] Jennifer L. Frane,[1] Shulan Tian,[3] Jeff Nie,[3] Gudrun A. Jonsdottir,[3] Victor Ruotti,[3] Ron Stewart,[3] Igor I. Slukvin,[2,4] James A. Thomson[1,2,5*]

Science, 2007

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)

Stable Karyotype
Methylation of Nanog/Oct4 promoters
Transgene expression silencing
Expression of endogenous pluripotent associated markers
In vitro/In vivo differentiation

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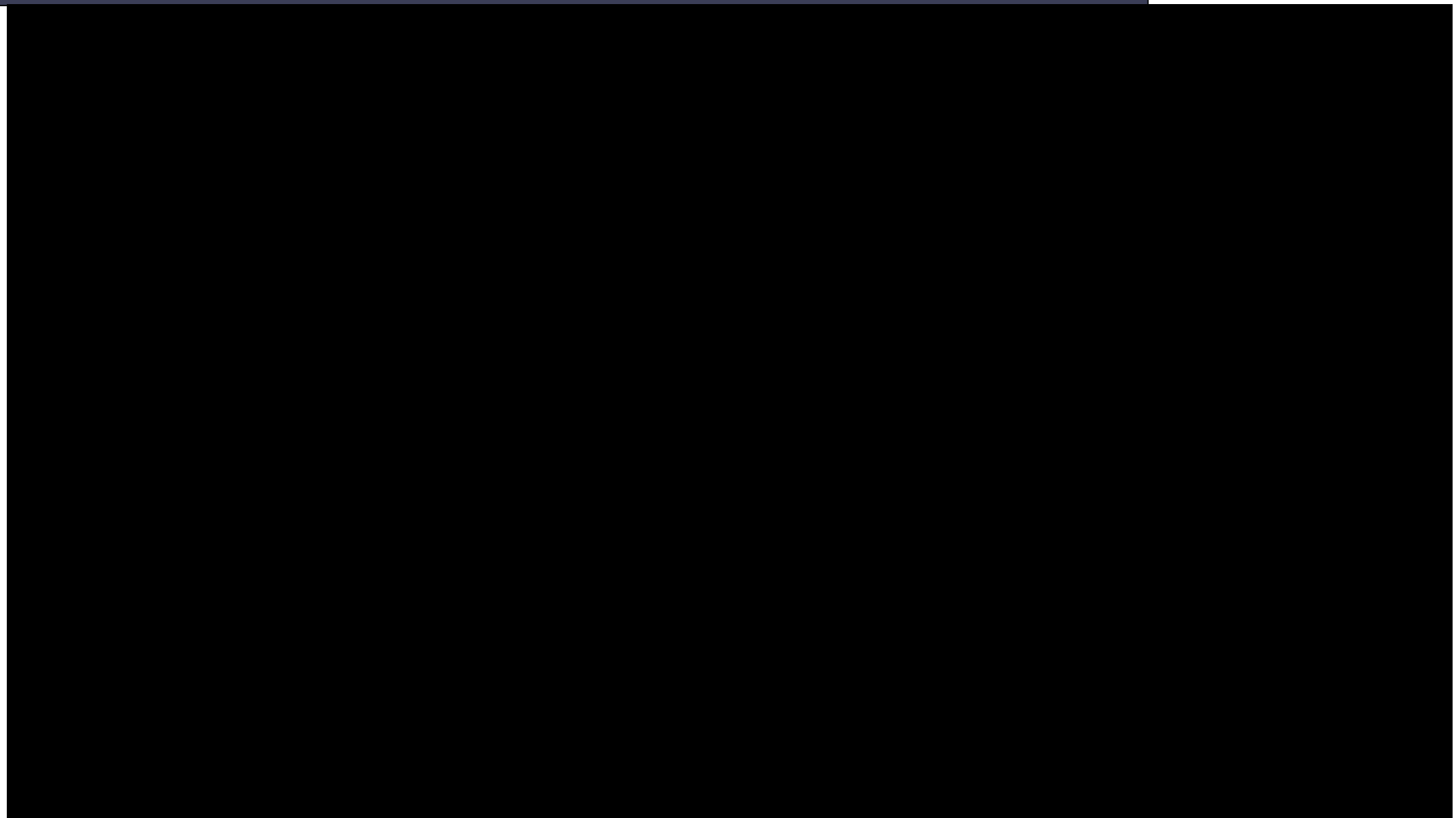

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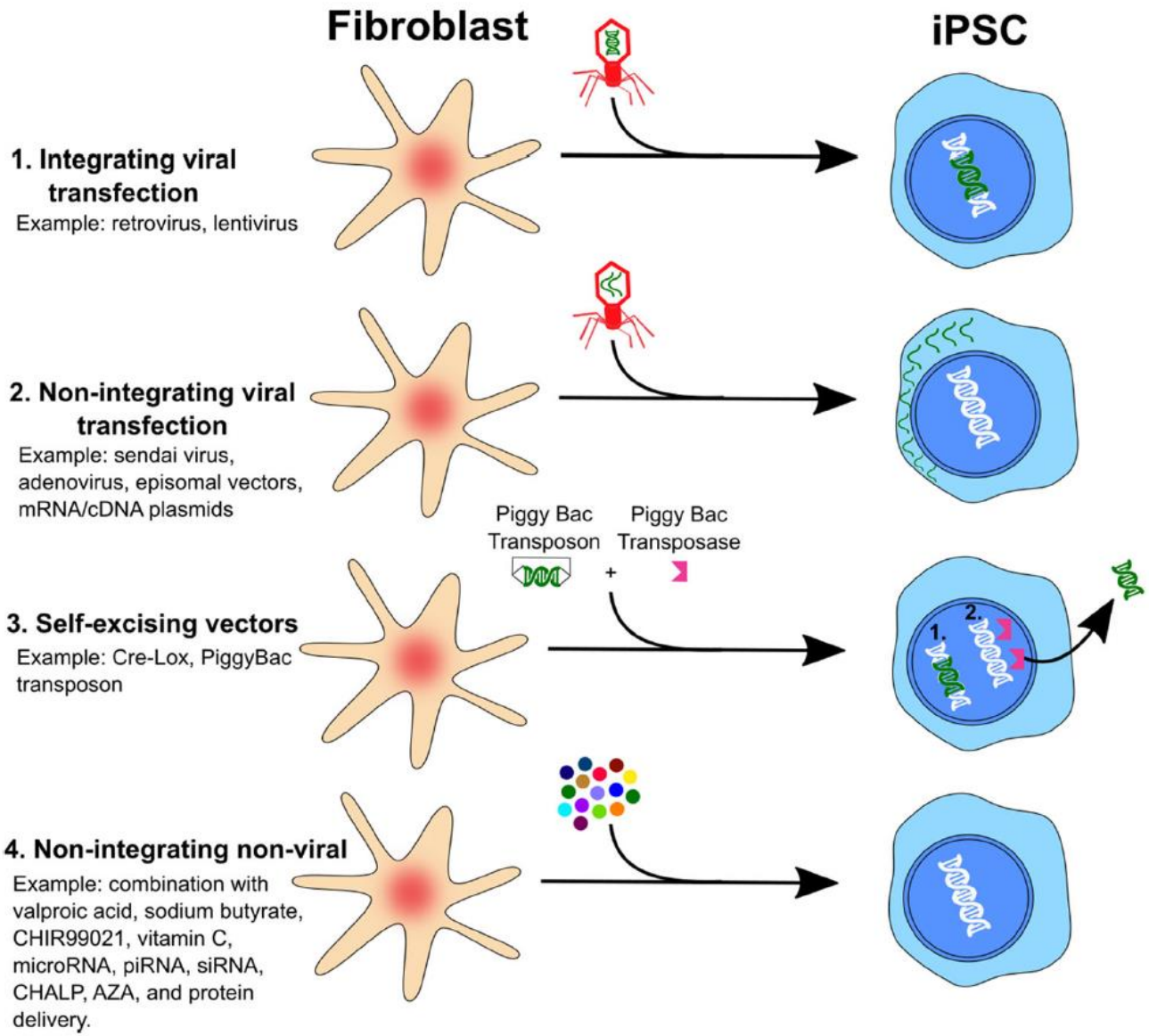
Fibroblast
iPSC
1. Integrating viral transfection
Example: retrovirus, lentivirus
2. Non-integrating viral transfection
Example: sendai virus, adenovirus, episomal vectors, mRNA/cDNA plasmids
Piggy Bac Transposon
Piggy Bac Transposase
+
3. Self-excising vectors
Example: Cre-Lox, PiggyBac transposon
1.
2.
4. Non-integrating non-viral
Example: combination with valproic acid, sodium butyrate, CHIR99021, vitamin C, microRNA, piRNA, siRNA, CHALP, AZA, and protein delivery.

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molecules

MDPI

Review

Induced Pluripotent Stem Cell (iPSC)-Based Neurodegenerative Disease Models for Phenotype Recapitulation and Drug Screening

Chia-Yu Chang [1,2,3], Hsiao-Chien Ting [1], Ching-Ann Liu [1,2,3], Hong-Lin Su [1,4], Tzyy-Wen Chiou [1,5], Shinn-Zong Lin [1,6], Horng-Jyh Harn [1,7,*] and Tsung-Jung Ho [8,9,10,*]

Why iPSC ?

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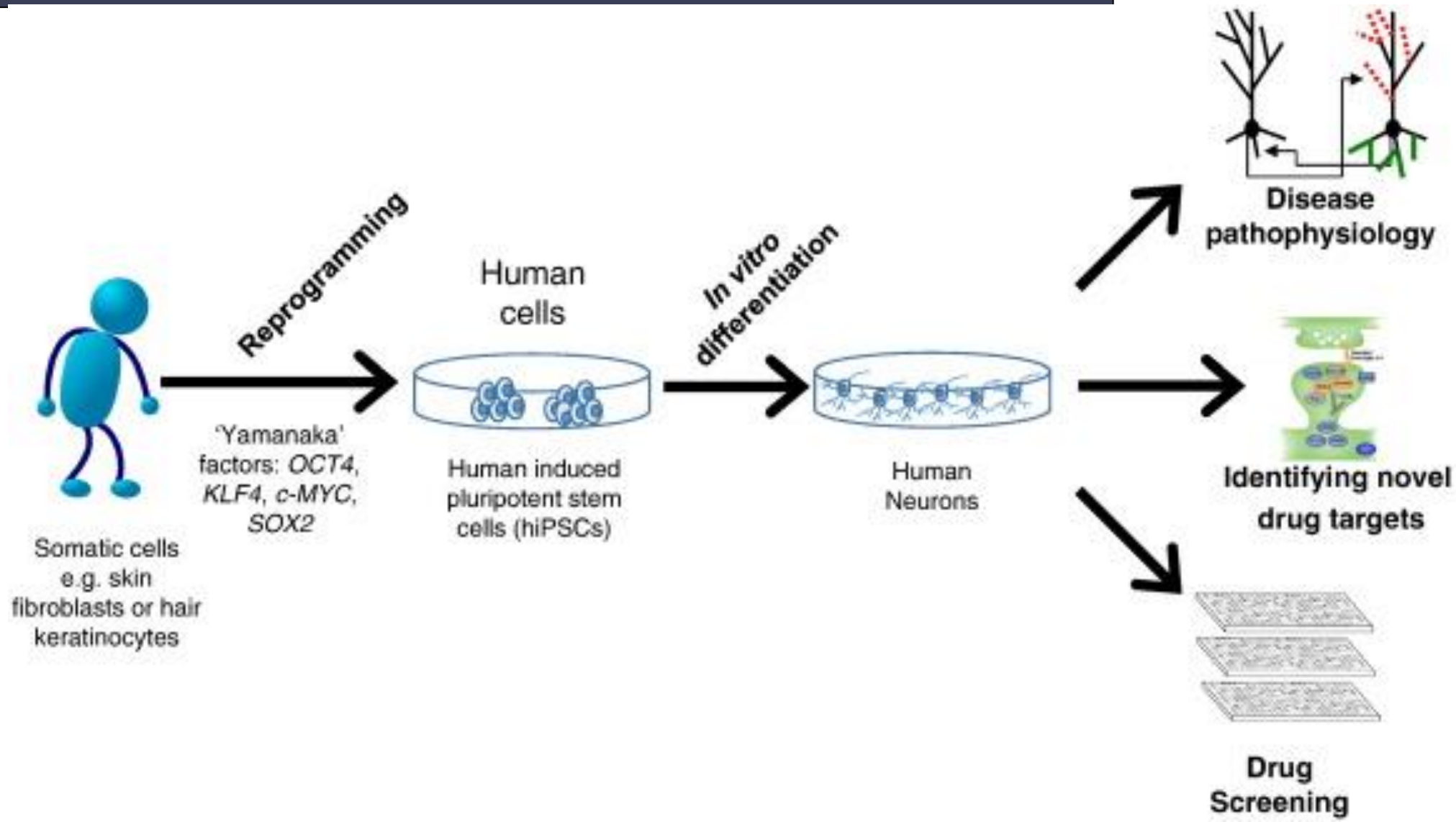
- Genetically modified human neuronal cell lines and primary animal neuronal cells are typically the first target for drug screening; animal models are typically used for documenting pre-clinical efficacy.
- The pathogenic mechanisms underlying neurodegenerative diseases are complex and still largely unknown.
- Most human cell lines and animal models were established with artificial methods and/or genetic overexpression strategies that may not fully represent human disease pathology.


Reprogramming
'Yamanaka' factors: OCT4, KLF4, c-MYC, SOX2
Somatic cells e.g. skin fibroblasts or hair keratinocytes
Human cells
Human induced pluripotent stem cells (hiPSCs)
In vitro differentiation
Human Neurons
Disease pathophysiology
Identifying novel drug targets
Drug Screening

iPSC-Derived Neurons and Glia as in Vitro Models of Neurodegenerative Diseases

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- For neurodegenerative disease modeling, iPSCs were sequentially differentiated into various kinds of neurons and glia with an effort made to mimic the process of central nervous system (CNS) development.
- iPSC-derived differentiated neurons and glia were evaluated for their capacity to model several challenging neurodegenerative diseases, including AD, HD, PD, ALS, SCA, and SMA, among others.

iPSC-Derived Neurons and Glia as in Vitro Models of Neurodegenerative Diseases

Although the iPSC technology is a powerful tool, there are various challenges ahead (Figure 2). Among these challenges, collecting samples from patients, establishing iPSC cultures, and inducing specific neuron and glial differentiation is extremely time-consuming (>30 d) and effort-intensive, which are two points that reduce enthusiasm and feasibility for drug screening.

The progression of sporadic neurodegenerative diseases is extremely complex, making it difficult to identify causes and discover ideal disease markers.

iPSC-Derived Neurons and Glia as in Vitro Models of Neurodegenerative Diseases

The generation of iPSCs erases the epigenetic markers from somatic cells and reverts the cells to the “fetal” stage. iPSC-derived neurons do not exhibit aging-related features such as genomic instability, telomere degeneration, or mitochondria function decay.

Another major barrier to recapitulating neurodegenerative disease phenotypes is the establishment of interactions among neurons, glial cells, and immune cells in in vitro models. Until recently, most iPSC-based neurodegenerative models were relatively primitive in nature

iPSC

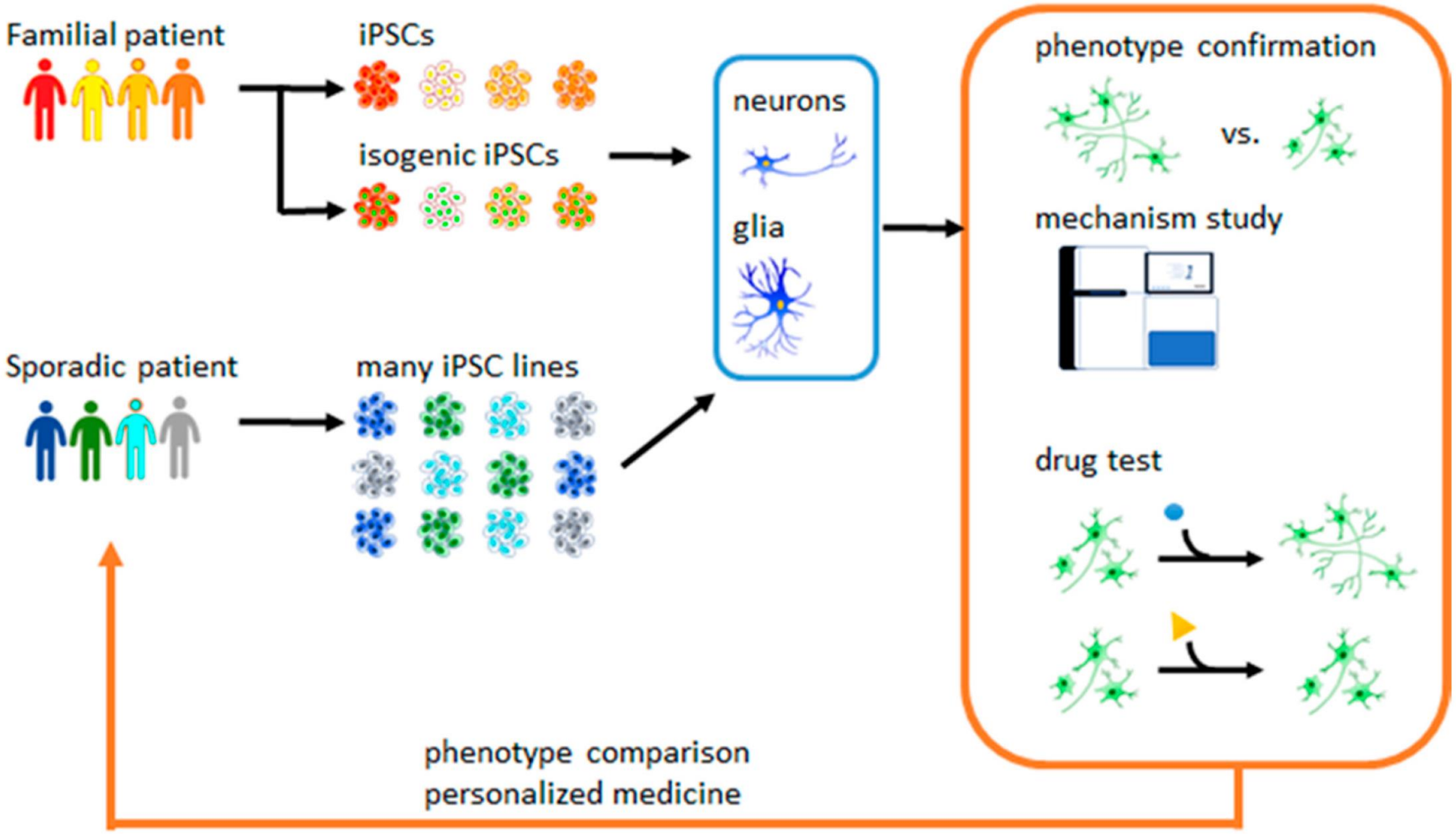

Figure 1. Apply induced pluripotent stem cells (iPSC)-derived neurons/glia for neurological disease phenotype confirmation, mechanism study, and drug test.

iPSC

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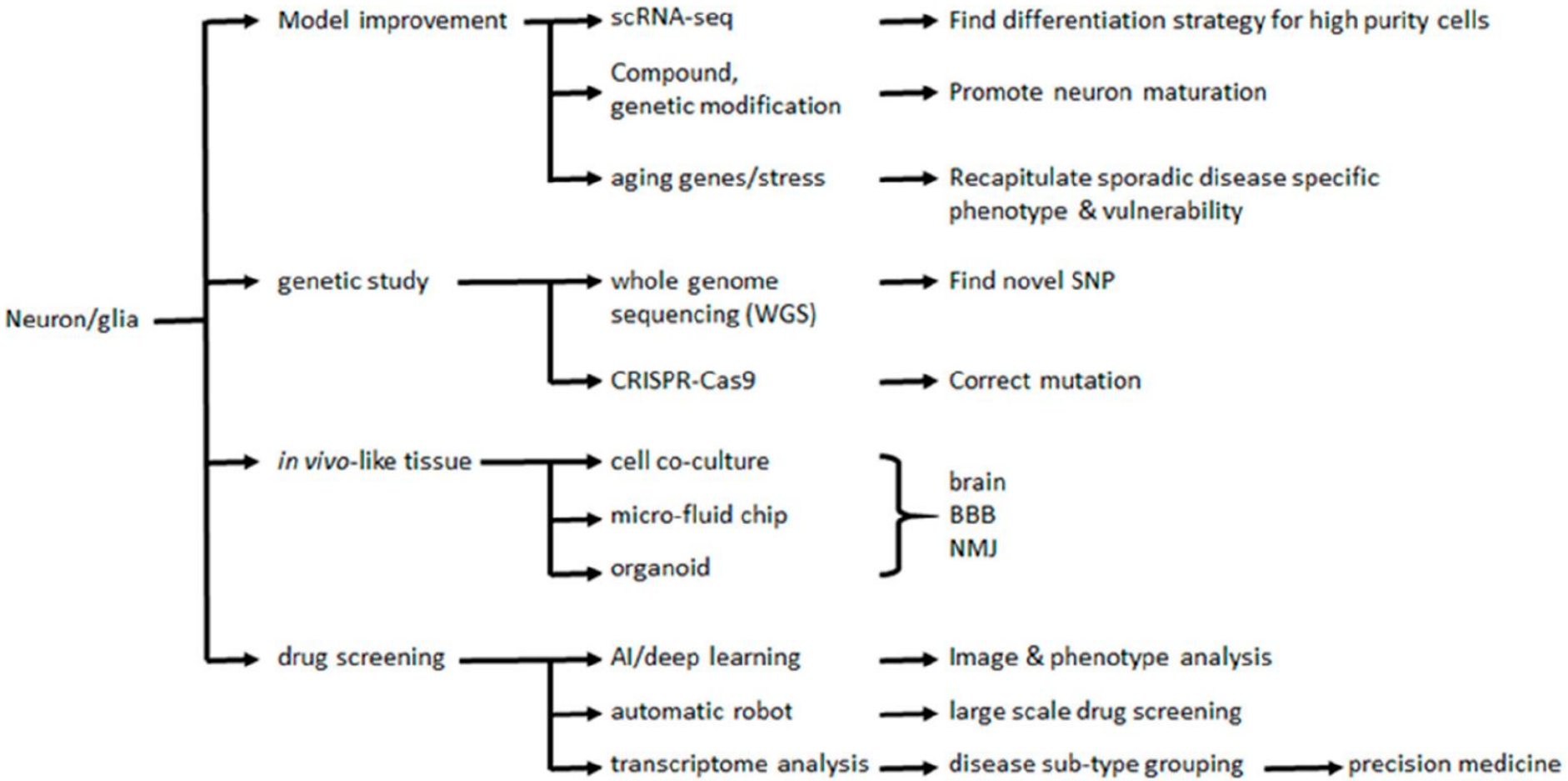

Figure 2. Combine novel technologies and iPSCs for disease model improvement, genetic studies, make complex neuronal organoids, and large-scale drug screening. scRNA: single cell RNA, SNP: single nucleotide polymorphism, BBB: blood–brain barrier, NMJ: neuromuscular junction.

iPSC

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frontiers in
CELLULAR NEUROSCIENCE

REVIEW ARTICLE
published: 11 April 2014
doi: 10.3389/fncel.2014.00109

Optimizing neuronal differentiation from induced pluripotent stem cells to model ASD

Dae-Sung Kim[1], P. Joel Ross[1], Kirill Zaslavsky[1,2] and James Ellis[1,2]*

[1] Program in Developmental and Stem Cell Biology, The Hospital for Sick Children, Toronto, ON, Canada
[2] Department of Molecular Genetics, University of Toronto, Toronto, ON, Canada

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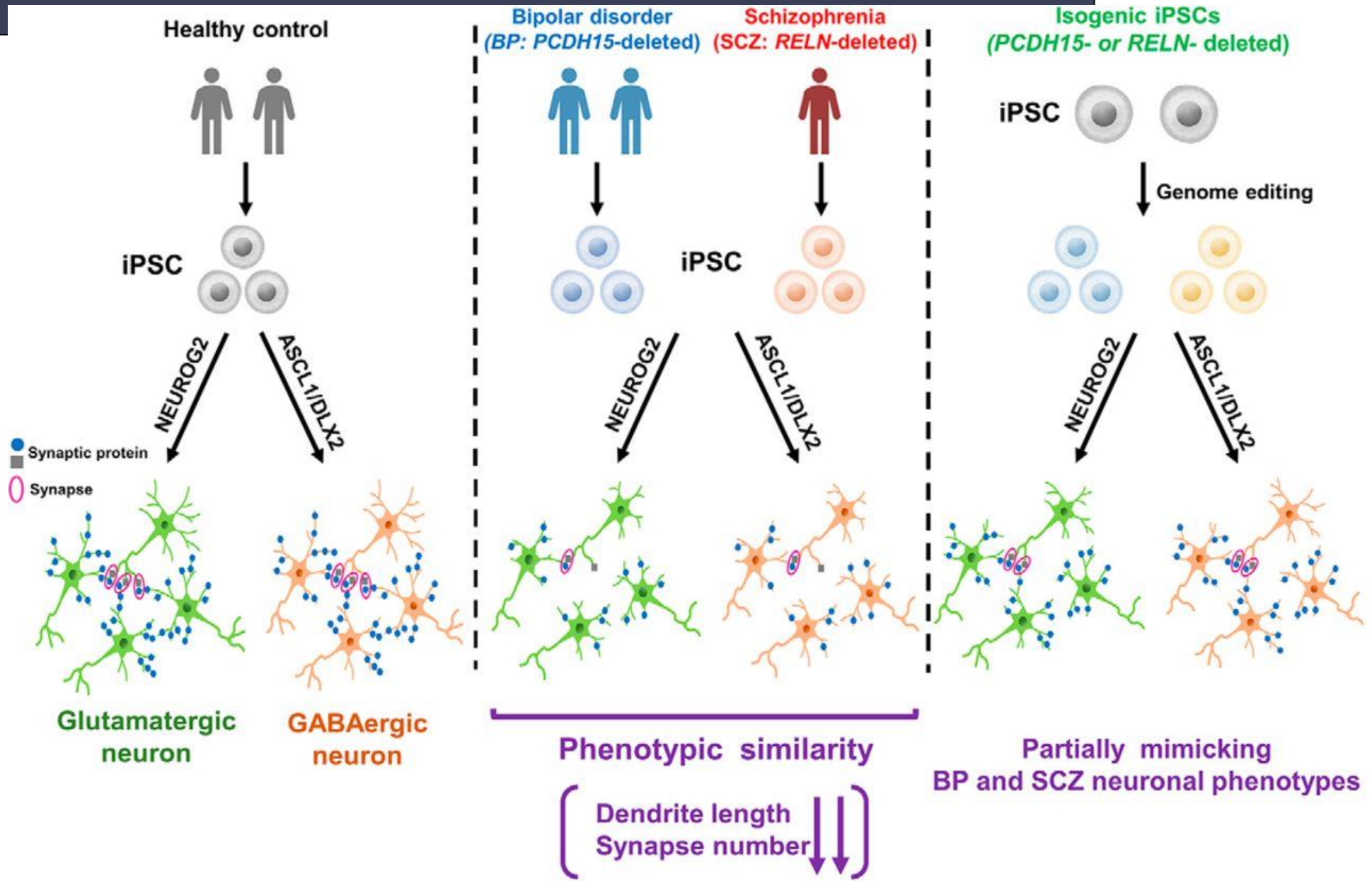
Healthy control
Bipolar disorder
(BP: PCDH15-deleted)
Schizophrenia
(SCZ: RELN-deleted)
Isogenic iPSCs
(PCDH15- or RELN- deleted)
iPSC
iPSC
iPSC
Genome editing
NEUROG2
ASCL1/DLX2
NEUROG2
ASCL1/DLX2
NEUROG2
ASCL1/DLX2
Synaptic protein
Synapse
Glutamatergic neuron
GABAergic neuron
Phenotypic similarity
Dendrite length
Synapse number
Partially mimicking
BP and SCZ neuronal phenotypes

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