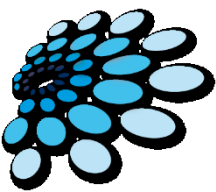


“ Techniques in Cellular and Molecular Neurobiology ”

International Master's Degree in Neuroscience

Lesson 4



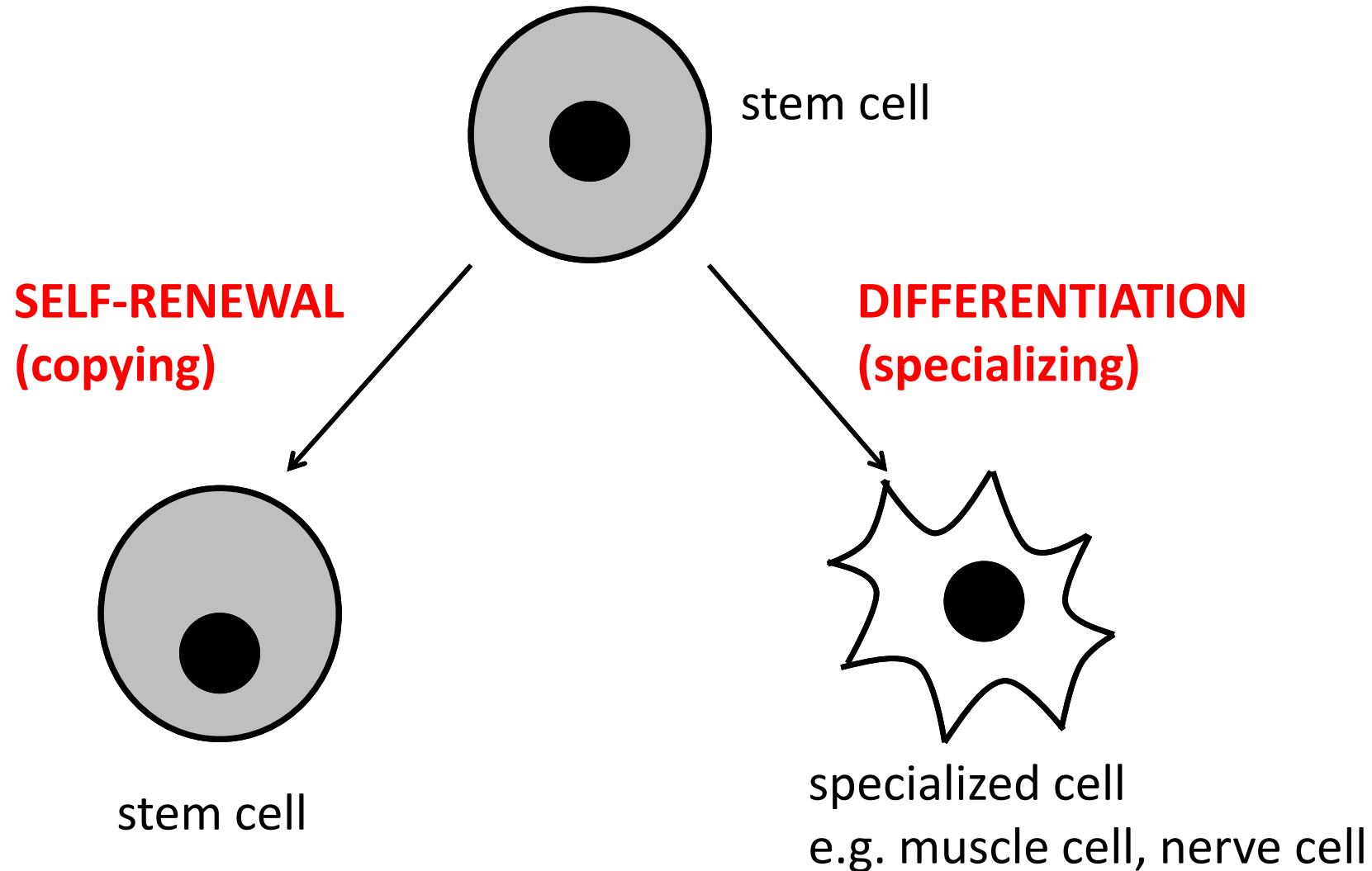
DIPARTIMENTO DI
SCIENZE DELLA VITA

Gabriele Baj
gbaj@units.it



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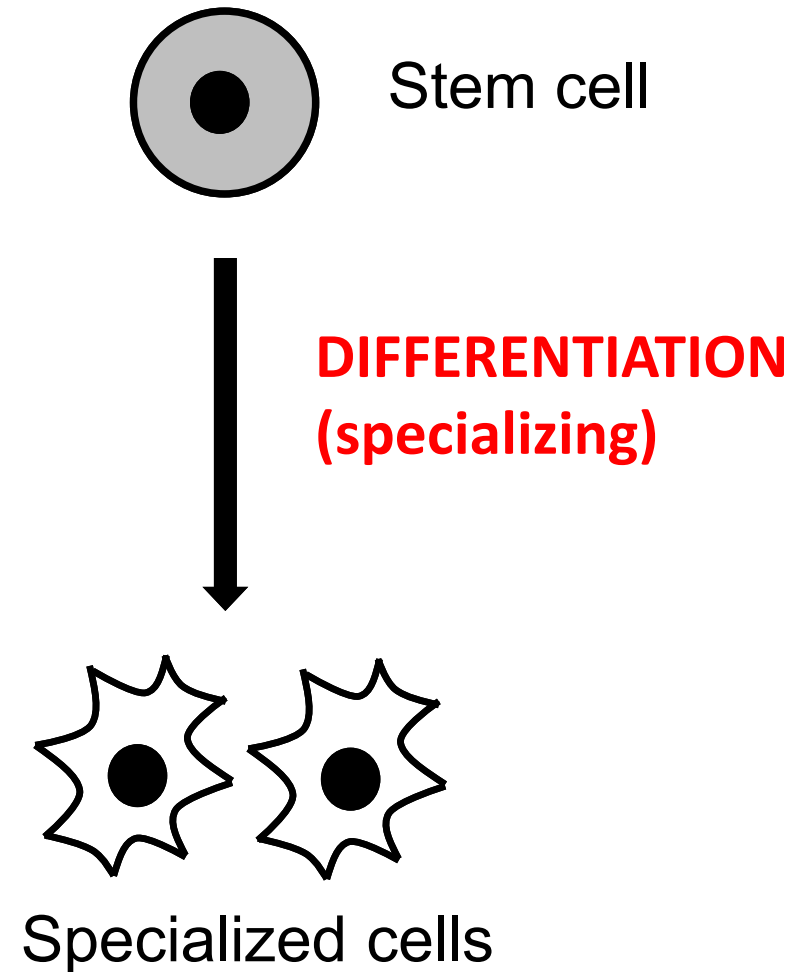
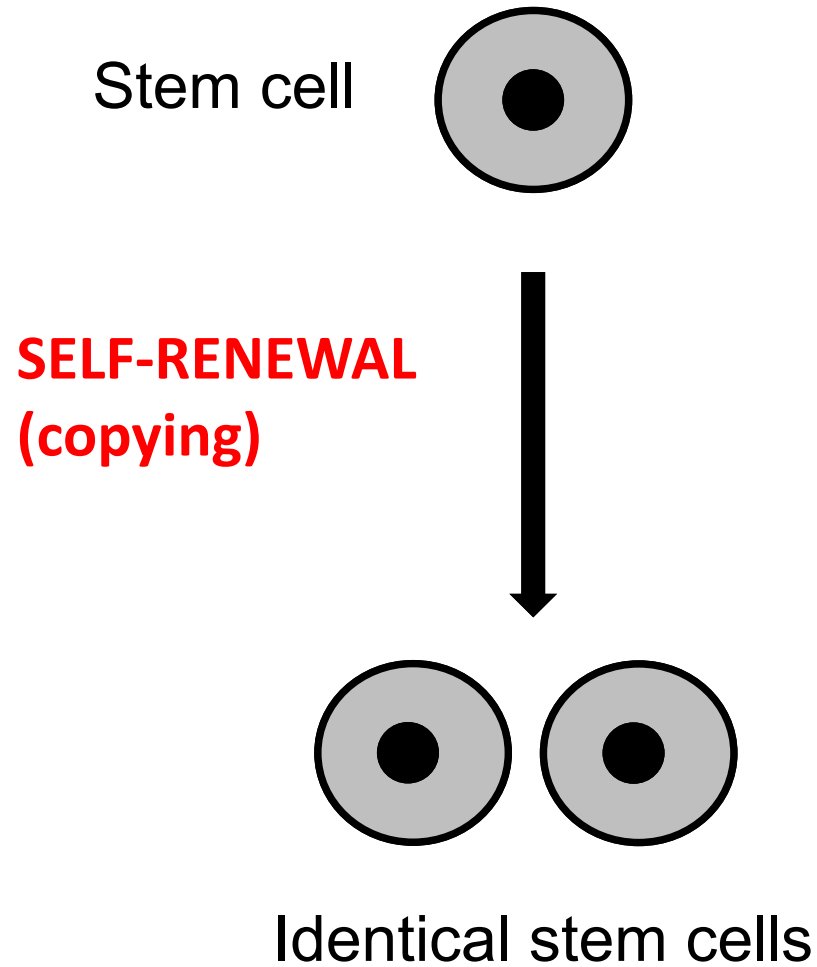
What is a stem cell?



What is a stem cell?



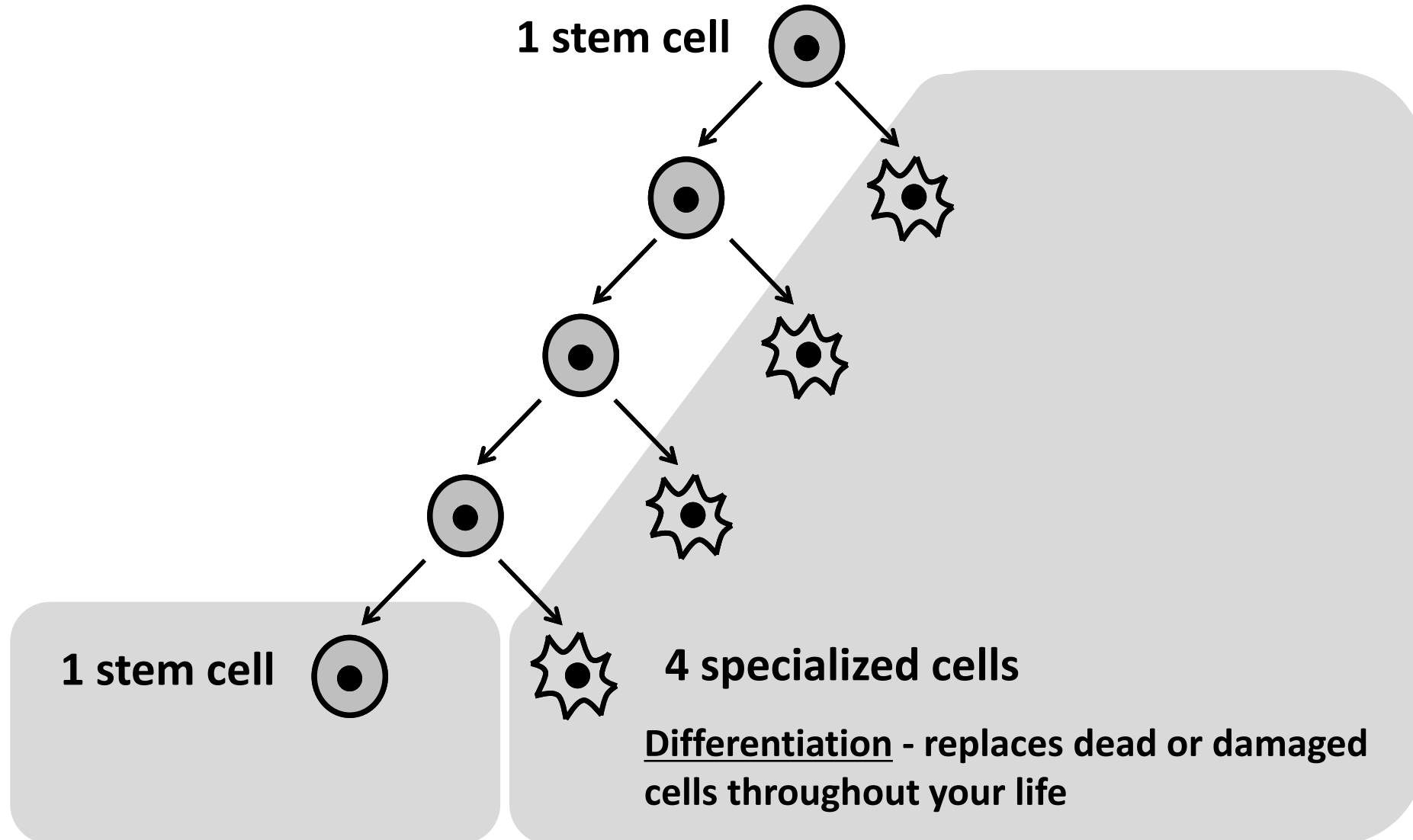
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Why self-renew AND differentiate?



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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 Liu¹ • Brian T. David¹ • Matthew Trawczynski¹ • Richard G. Fessler¹

Published online: 23 November 2019



Embryonic stem cells (ESCs)

Very Small Embryonic-Like Stem Cells (VSELs)

1

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

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.

Reprogramming Stem Cells (RSCs)

3

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.

Adult stem cells (ASCs)

2

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:

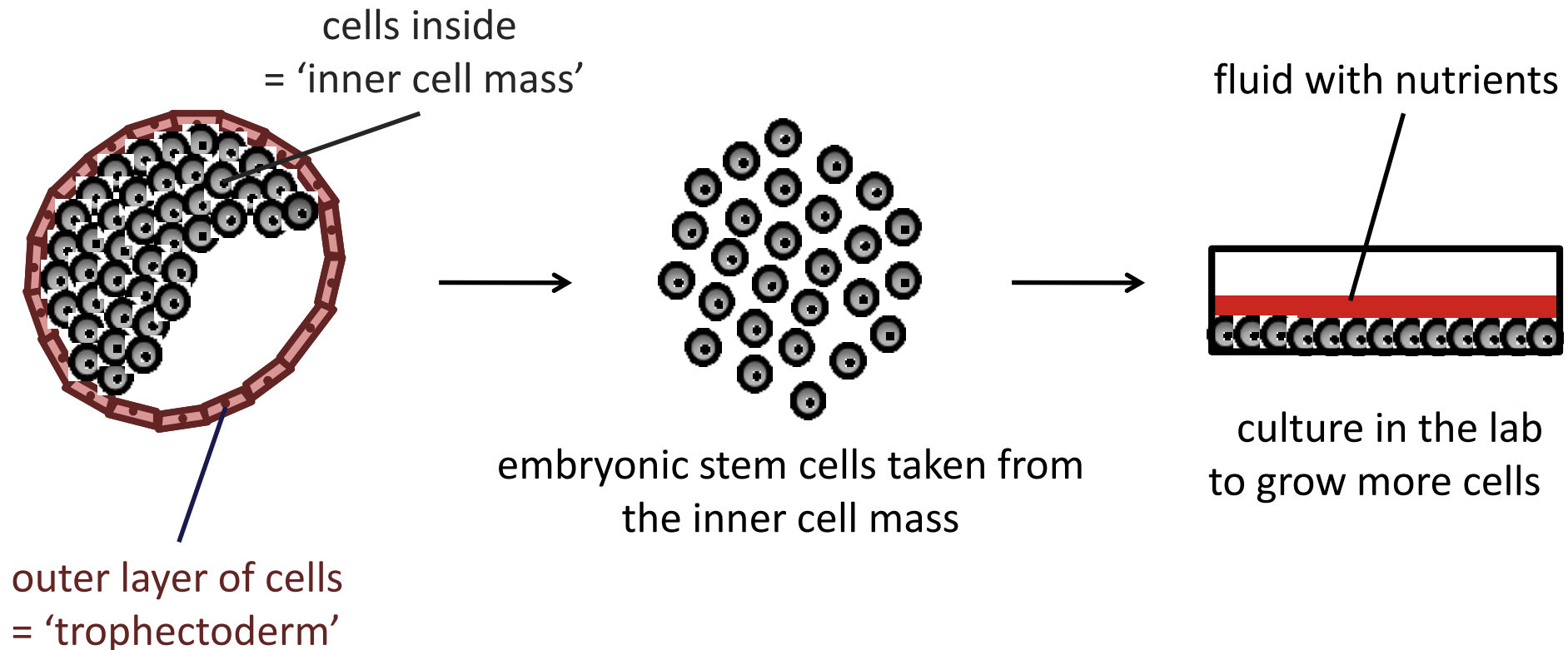
1) Embryonic stem cells

Embryonic stem (ES) cells: Where we find them



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blastocyst



Embryonic stem (ES) cells: What they can do



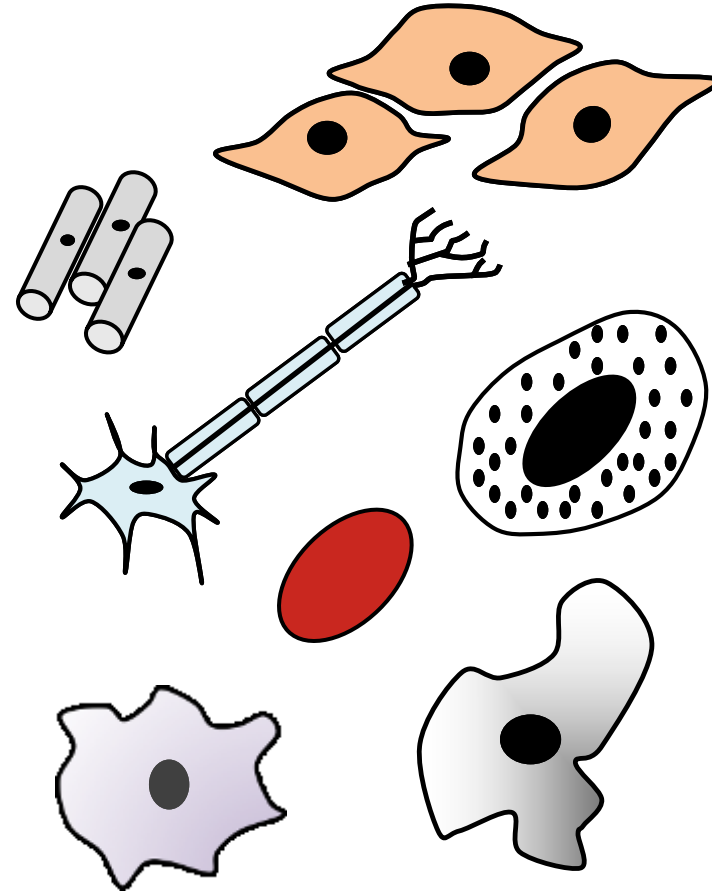
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embryonic stem cells

PLURIPOTENT

differentiation →



all possible types of specialized cells

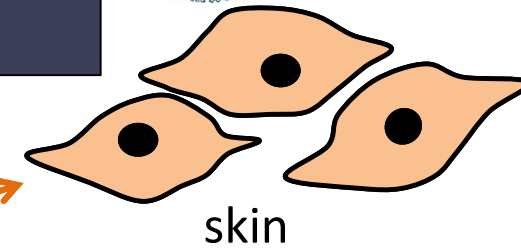
Embryonic stem (ES) cells: Challenges



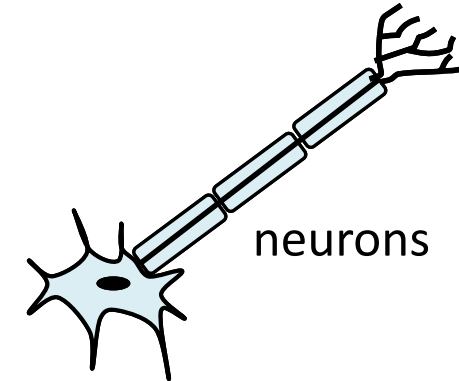
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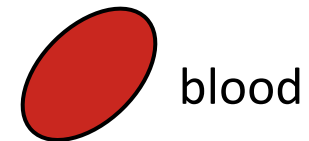
grow under conditions A



grow under conditions B



grow under conditions C



grow under conditions D



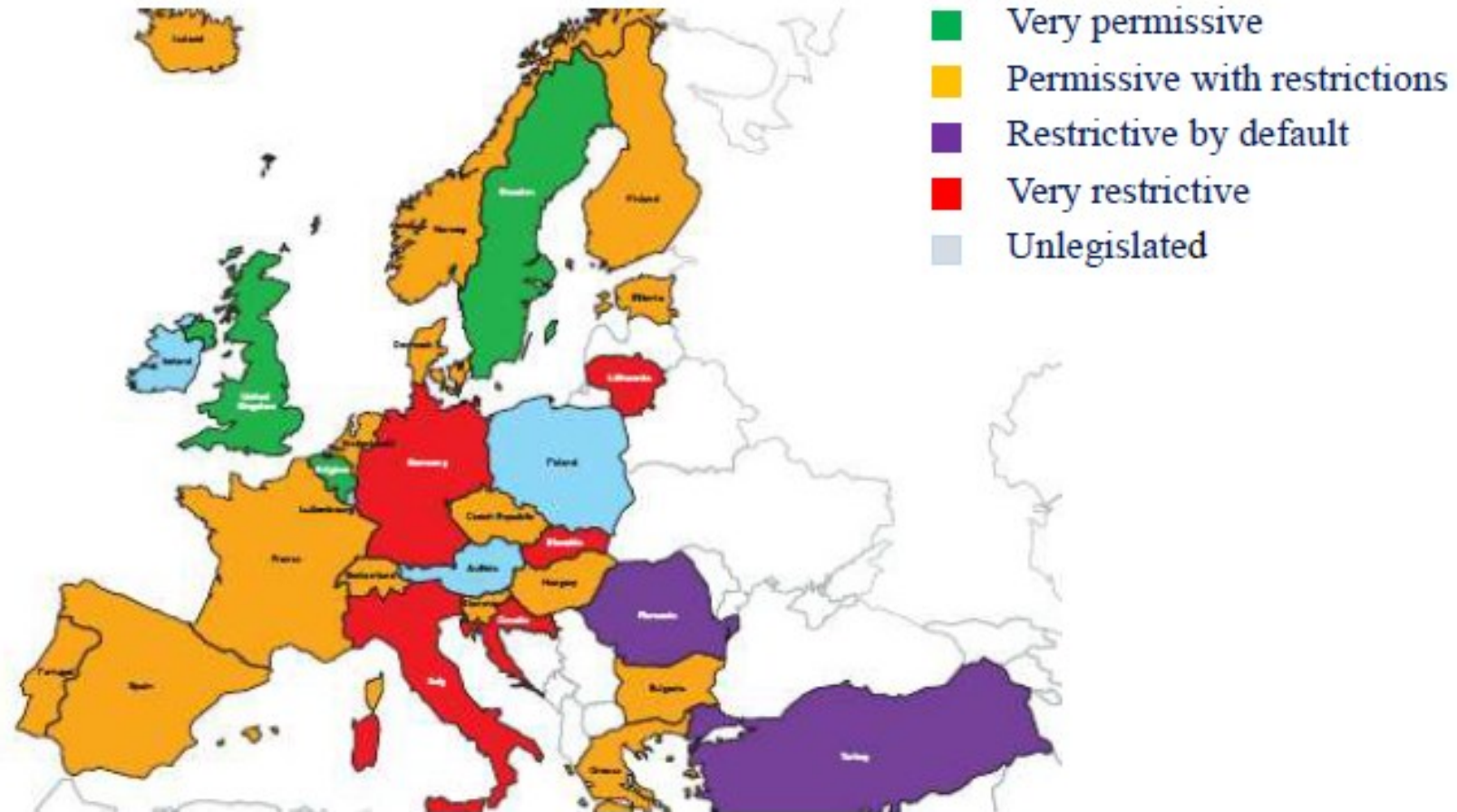
?

Human Stem Cell Research and Regenerative Medicine

Focus on European policy and scientific contributions

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



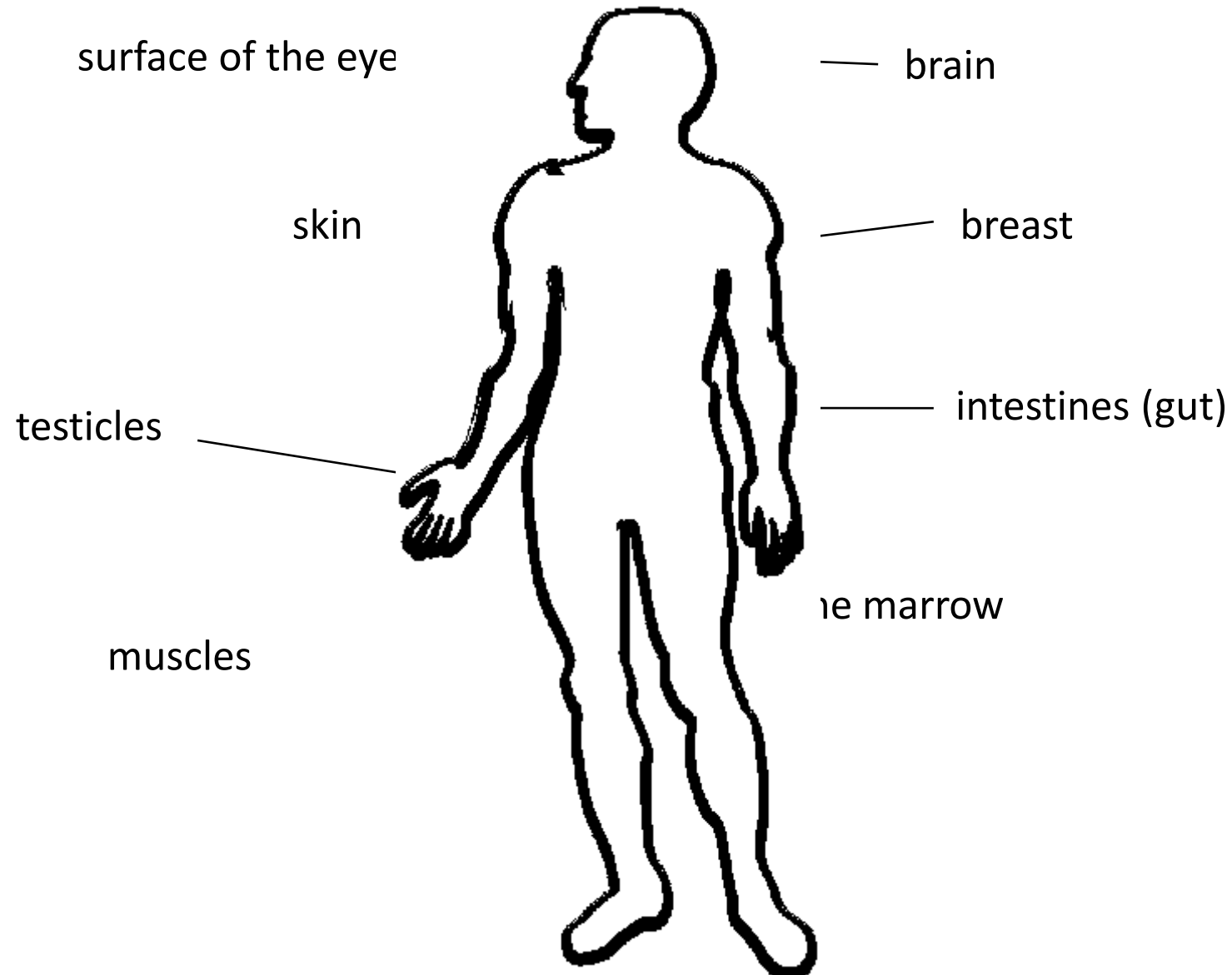
Types of stem cell:

2) Tissue stem cells

Tissue stem cells: Where we find them



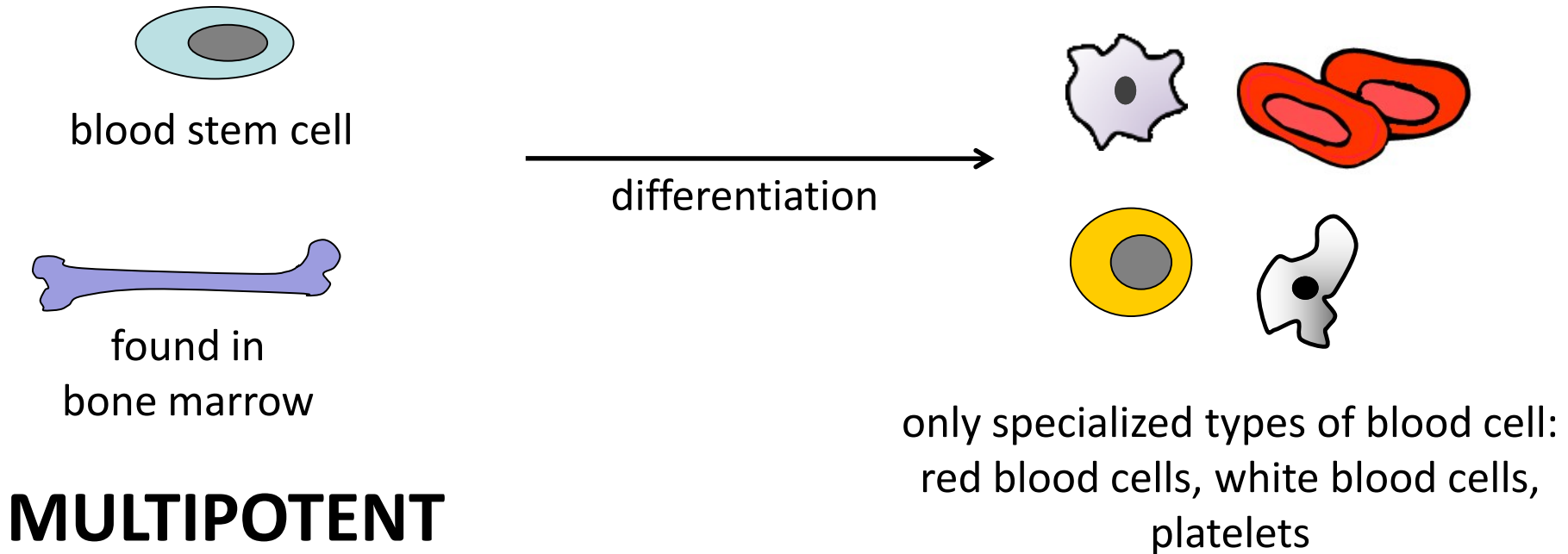
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Tissue stem cells: What they can do



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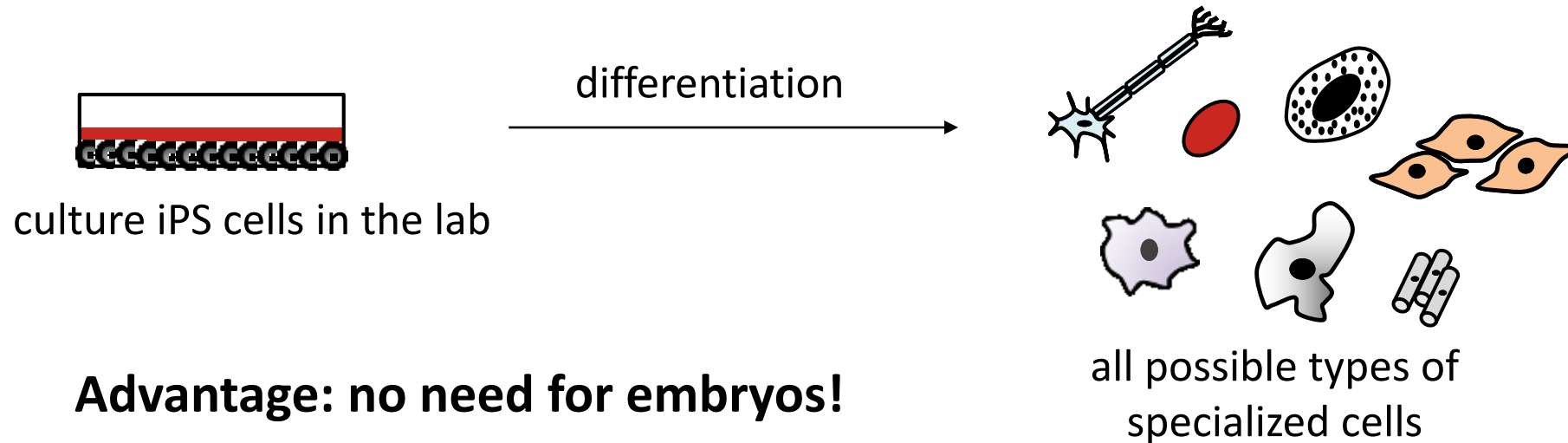
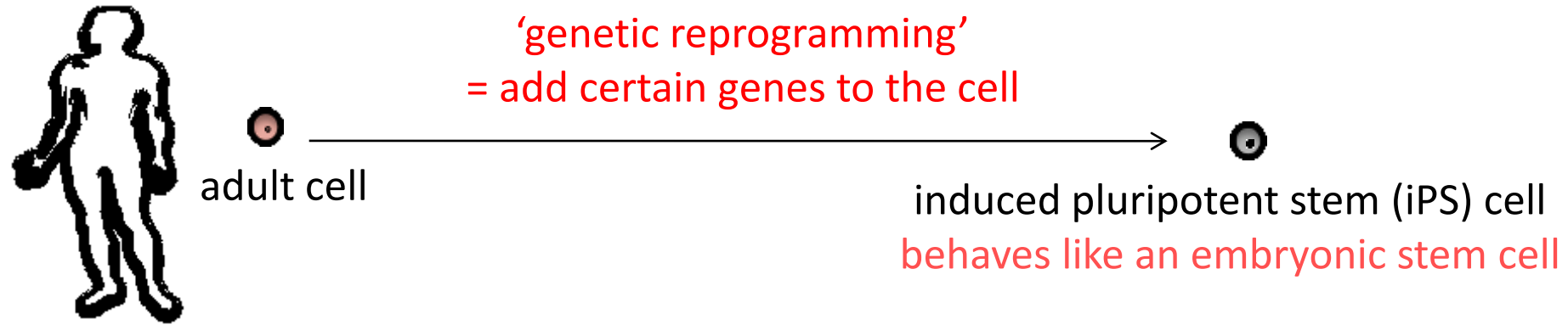


Types of stem cell:
3) Induced pluripotent (iPS)
stem cells

Induced pluripotent stem cells (iPS cells)



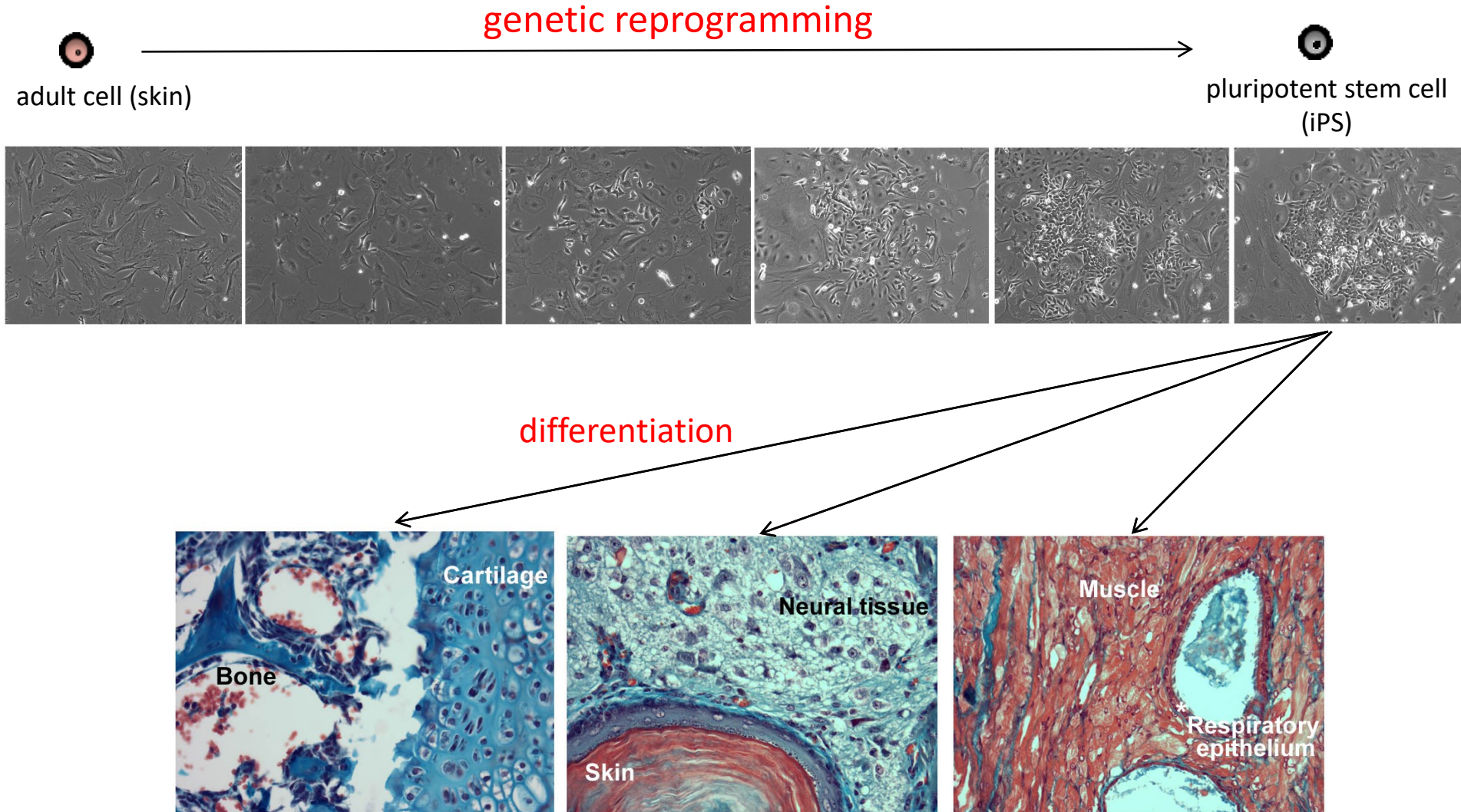
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Induced pluripotent stem cells (iPS cells)



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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

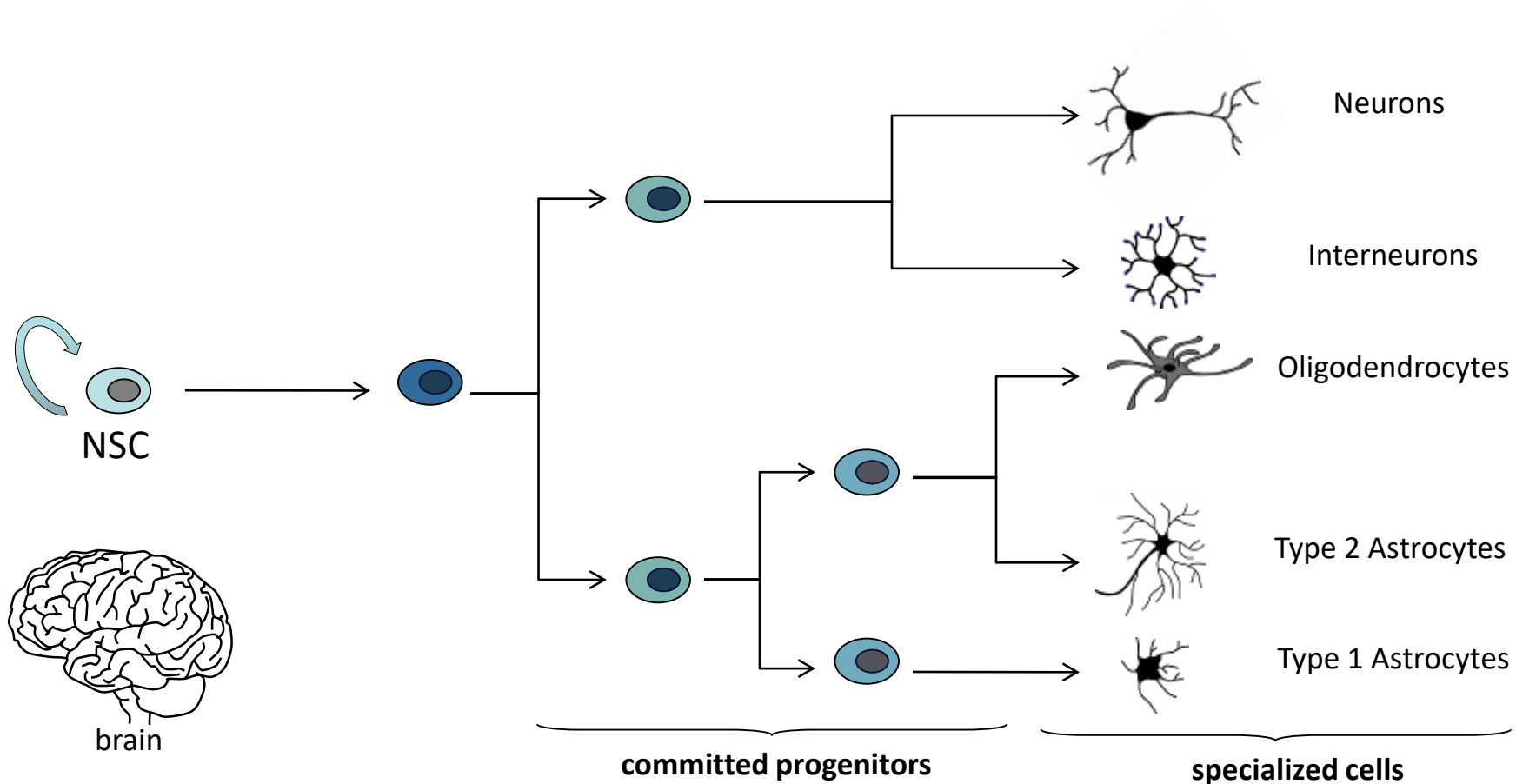
Multipotent

Can make **multiple** types of specialized cells, but not all types
Tissue stem cells are multipotent

Tissue stem cells: Neural stem cells (NSCs)



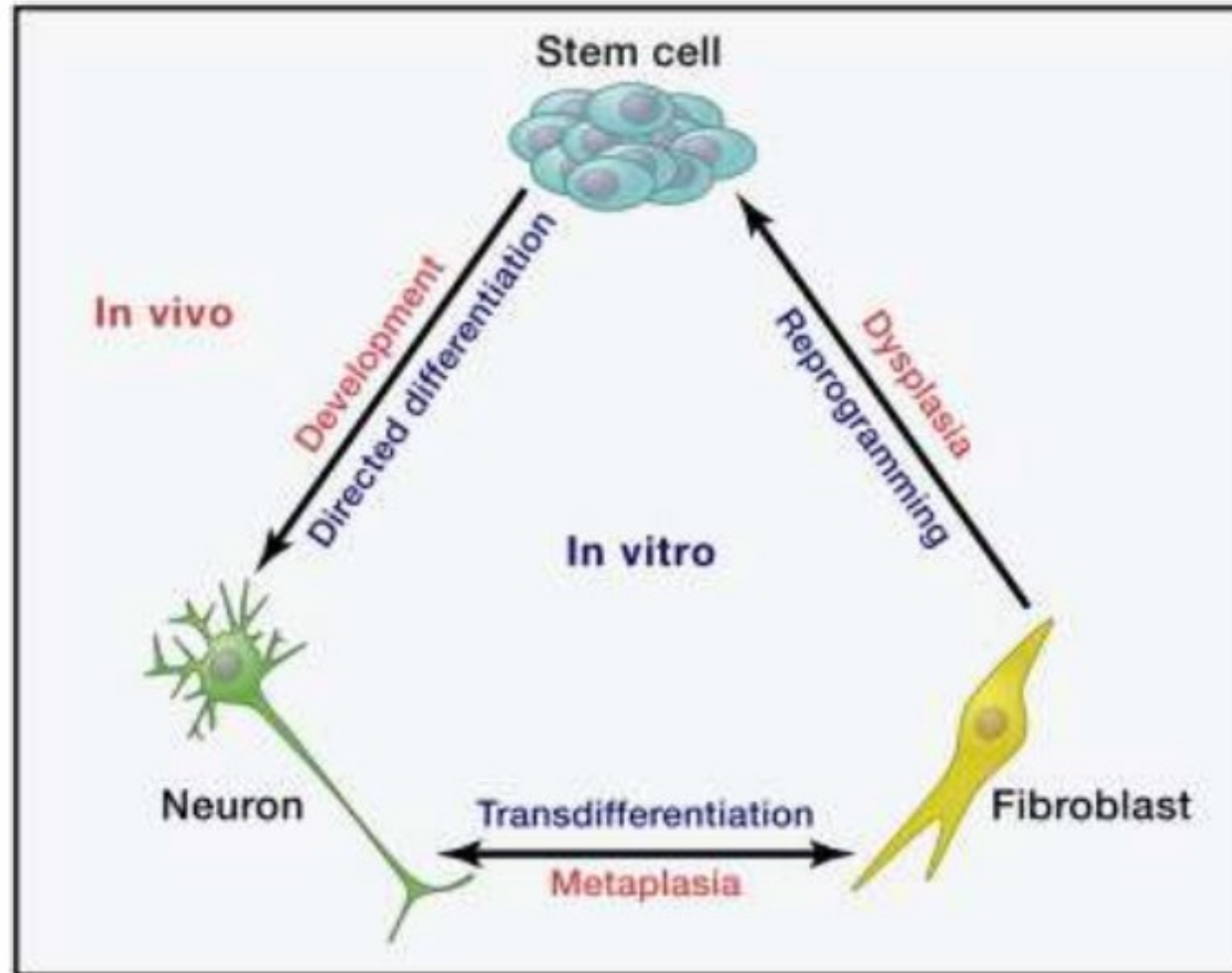
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Stem cells



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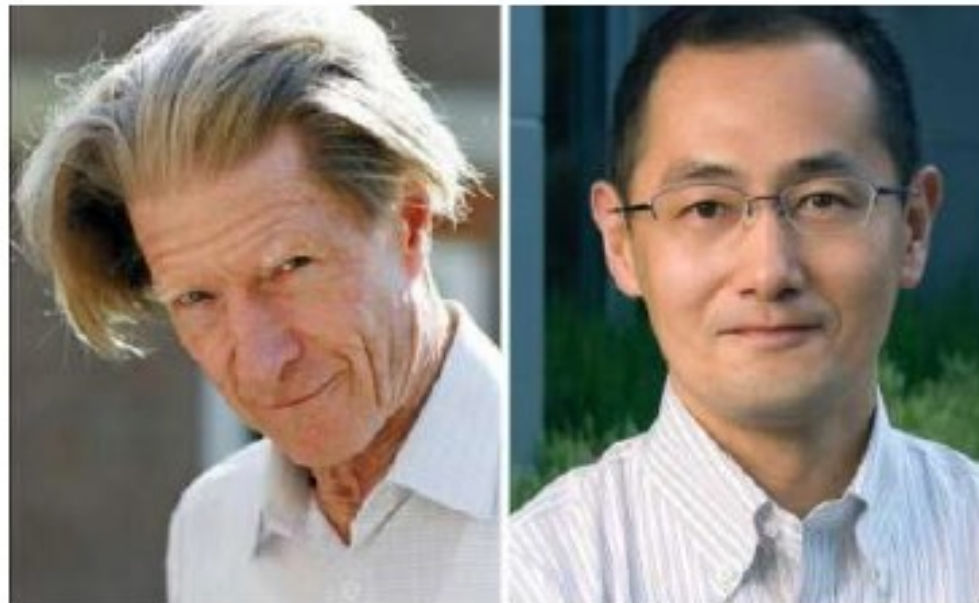


iPSC



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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,² and Shin'ya 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, 2007 Park 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

iPSC

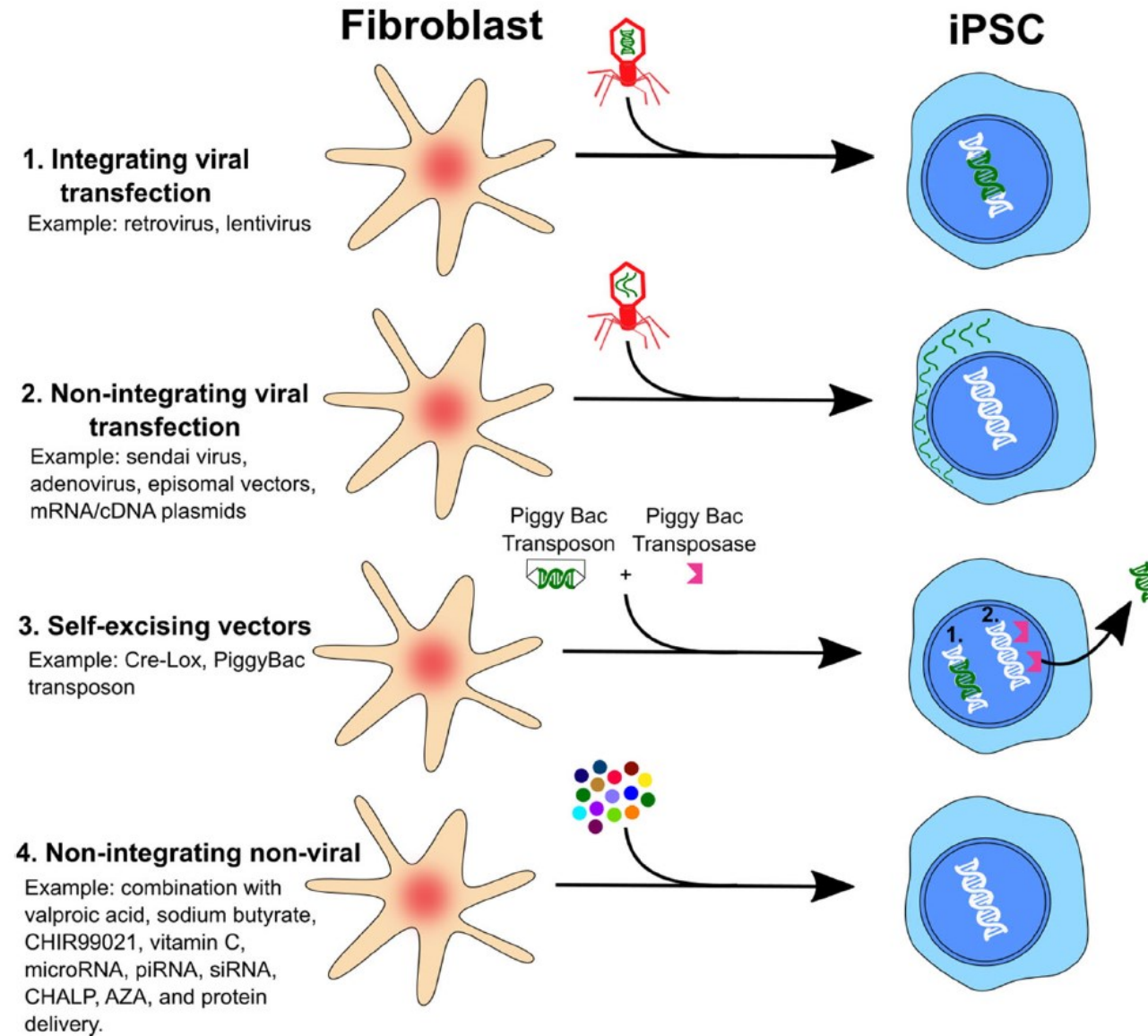


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Review

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

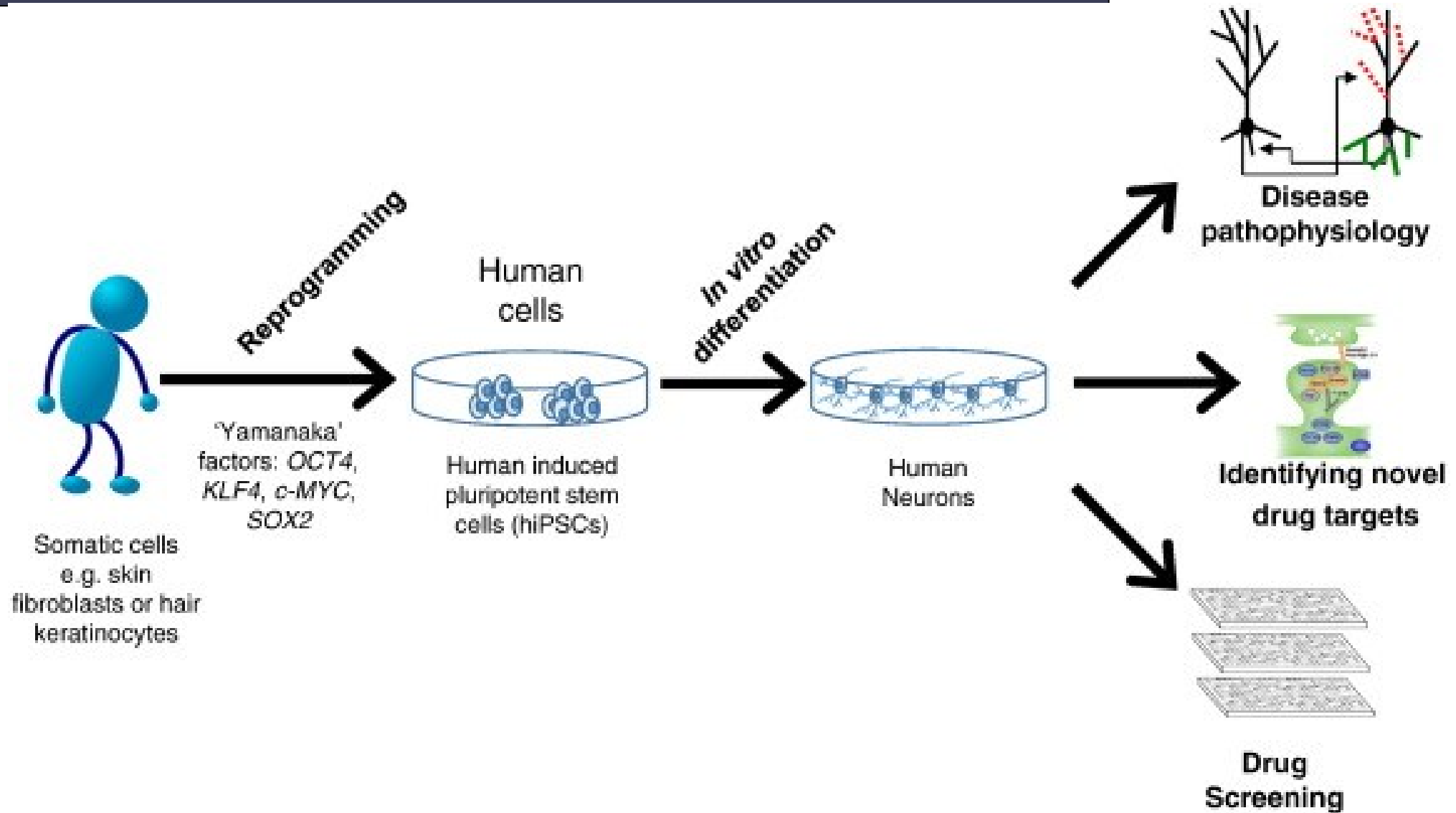
Chia-Yu Chang ^{1,2,3} , Hsiao-Chien Ting ¹, Ching-Ann Liu ^{1,2,3}, Hong-Lin Su ^{1,4},
Tzyy-Wen Chiou ^{1,5}, Shinn-Zong Lin ^{1,6}, Horng-Jyh Harn ^{1,7,*} and Tsung-Jung Ho ^{8,9,10,*}

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.



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



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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



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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



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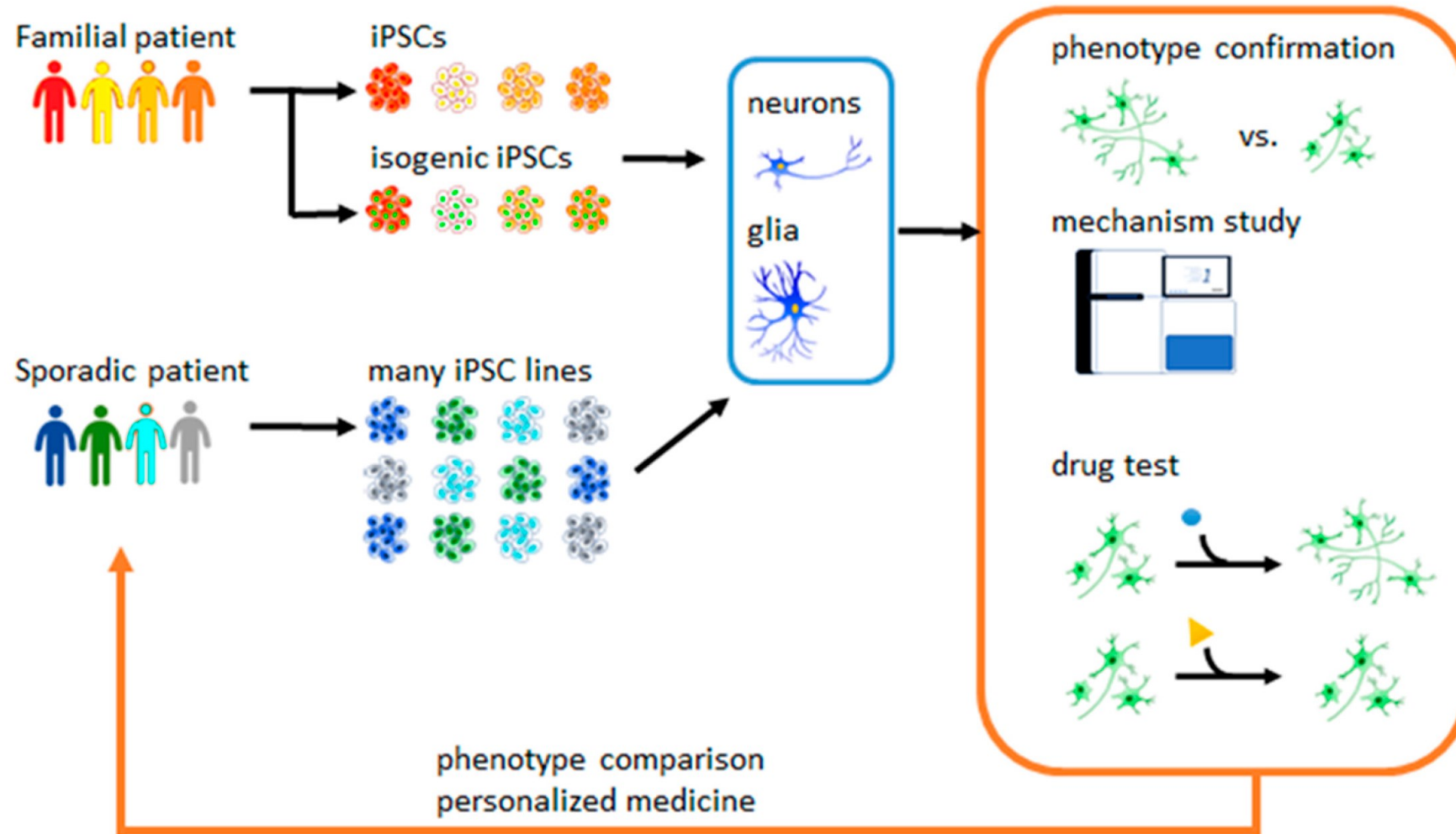


Figure 1. Apply induced pluripotent stem cells (iPSC)-derived neurons/glia for neurological disease phenotype confirmation, mechanism study, and drug test.

iPSC

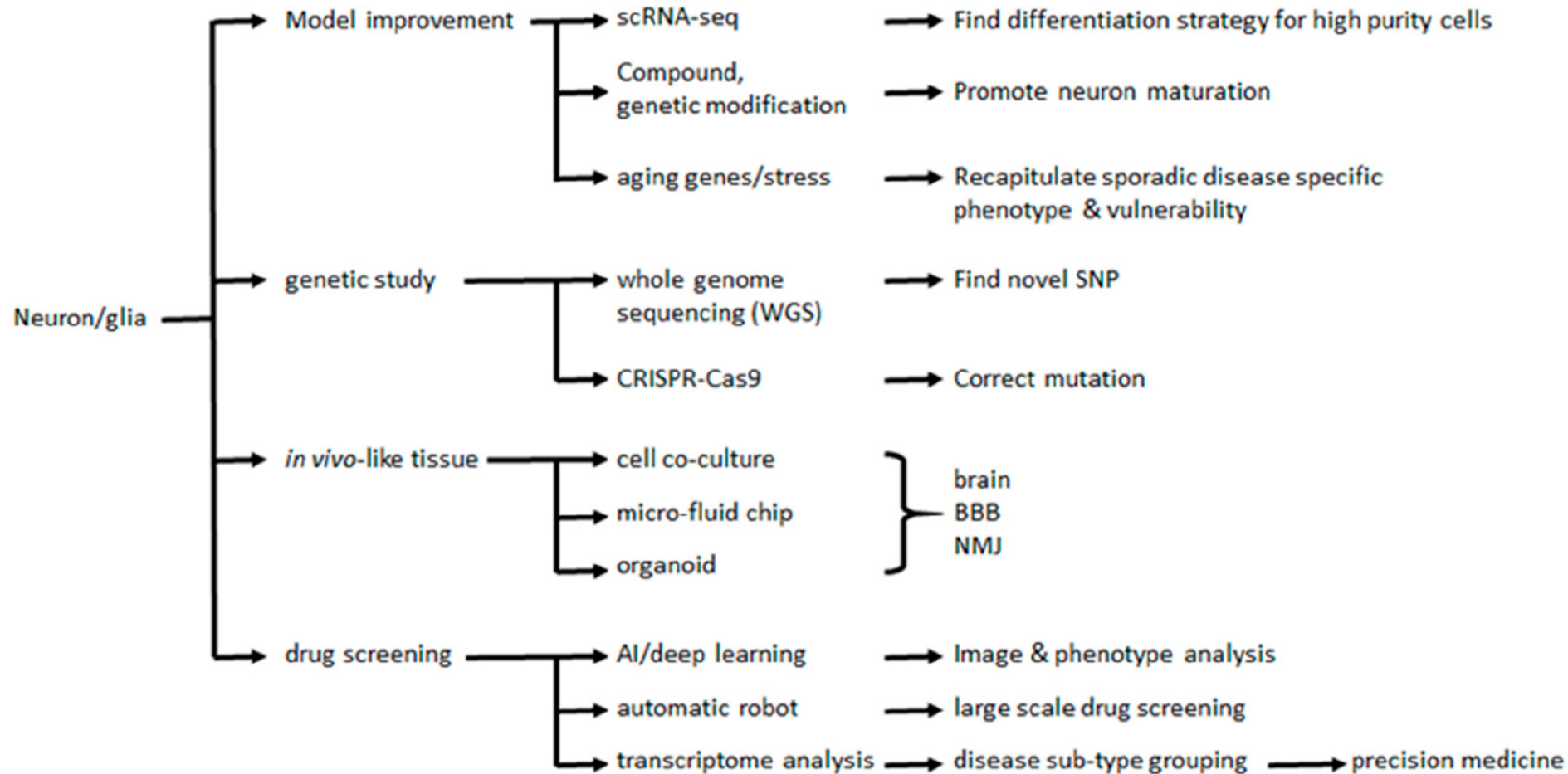


Figure 2. Combine novel technologies and iPSCs for disease model improvement, genetic studies, make complex neuronal organoids, and large-scale drug screening. scRNA: single cell RNA, SNP: single nucleotide polymorphism, BBB: blood-brain barrier, NMJ: neuromuscular junction.



Optimizing neuronal differentiation from induced pluripotent stem cells to model ASD

Dae-Sung Kim¹, P. Joel Ross¹, Kirill Zaslavsky^{1,2} and James Ellis^{1,2*}

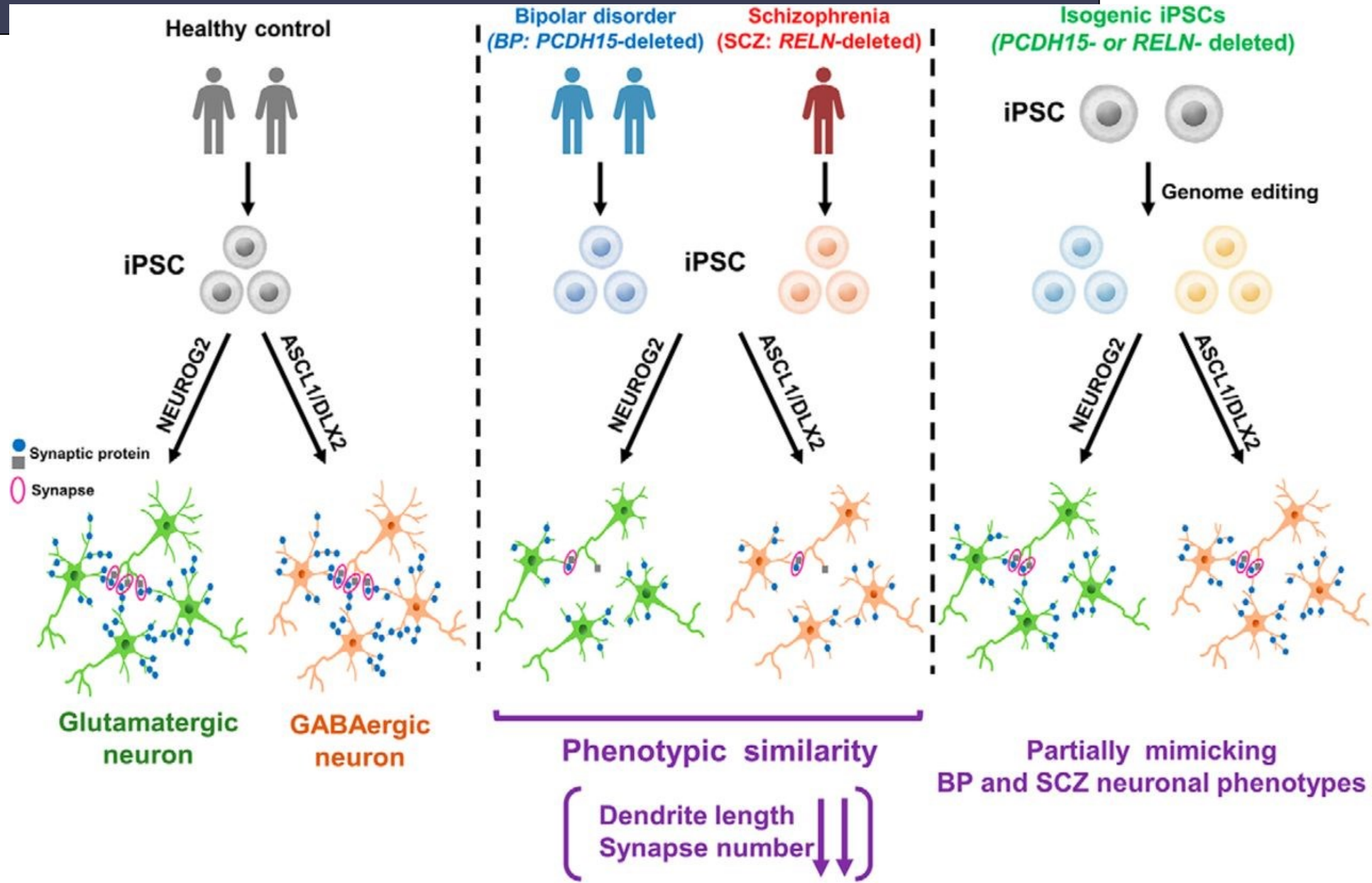
¹ Program in Developmental and Stem Cell Biology, The Hospital for Sick Children, Toronto, ON, Canada

² Department of Molecular Genetics, University of Toronto, Toronto, ON, Canada

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