Studying and Treating Neurodegeneration with iPSC models

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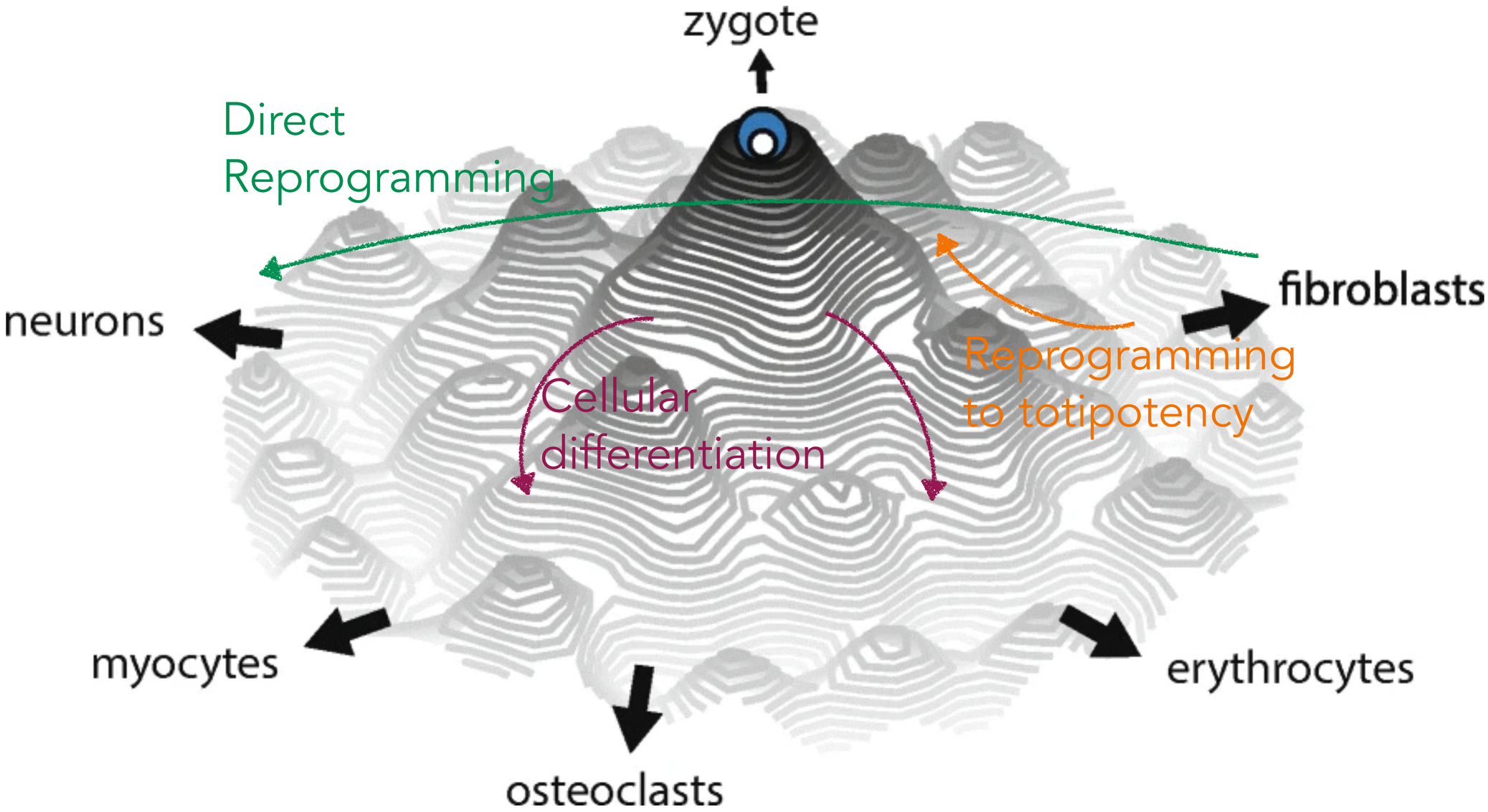
Overview

Reprogramming and Generation of pluripotent cells
Cellular Differentiation and 2D vs. 3D models
Hallmarks of ageing
Developing relevant *in vitro* models
Somatic Reprogramming vs. Direct Reprogramming
iPSC based therapies

How do cells differentiate?



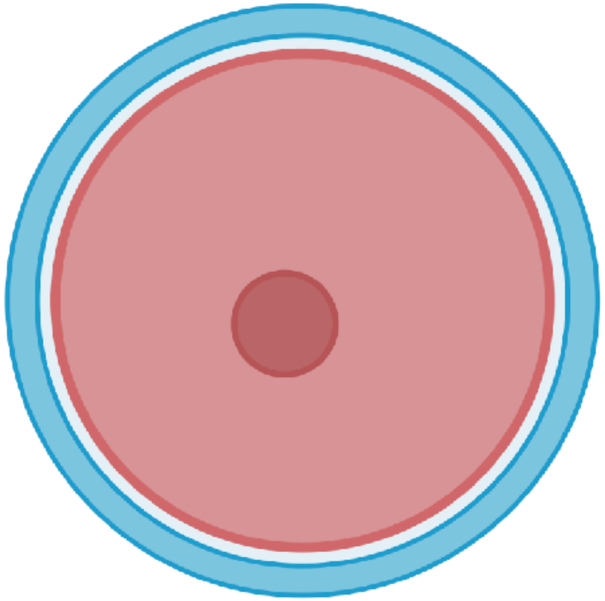
Camillo & Quinlan (2021) GeroScience

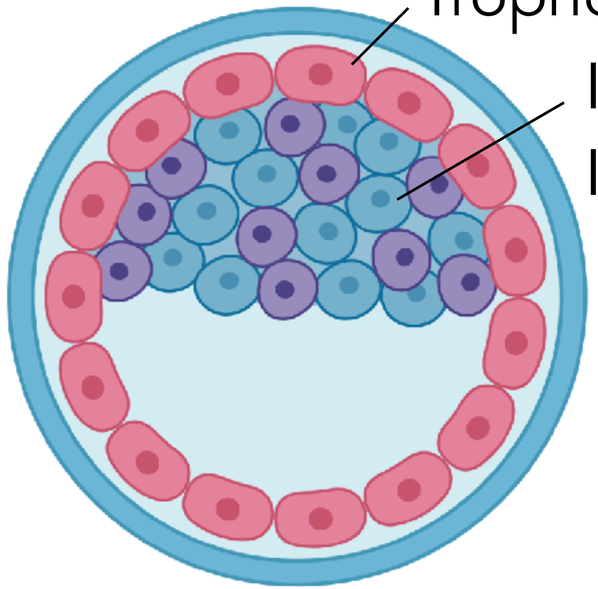
What totipotency/pluripotency?

A totipotent stem cell can give rise to all (toti) cell types of the body

A pluripotent stem cell can give rise to many (pluri) cell types of the body

These cells exist in early embryos

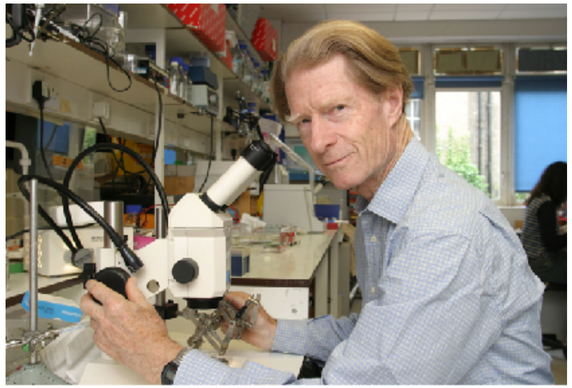


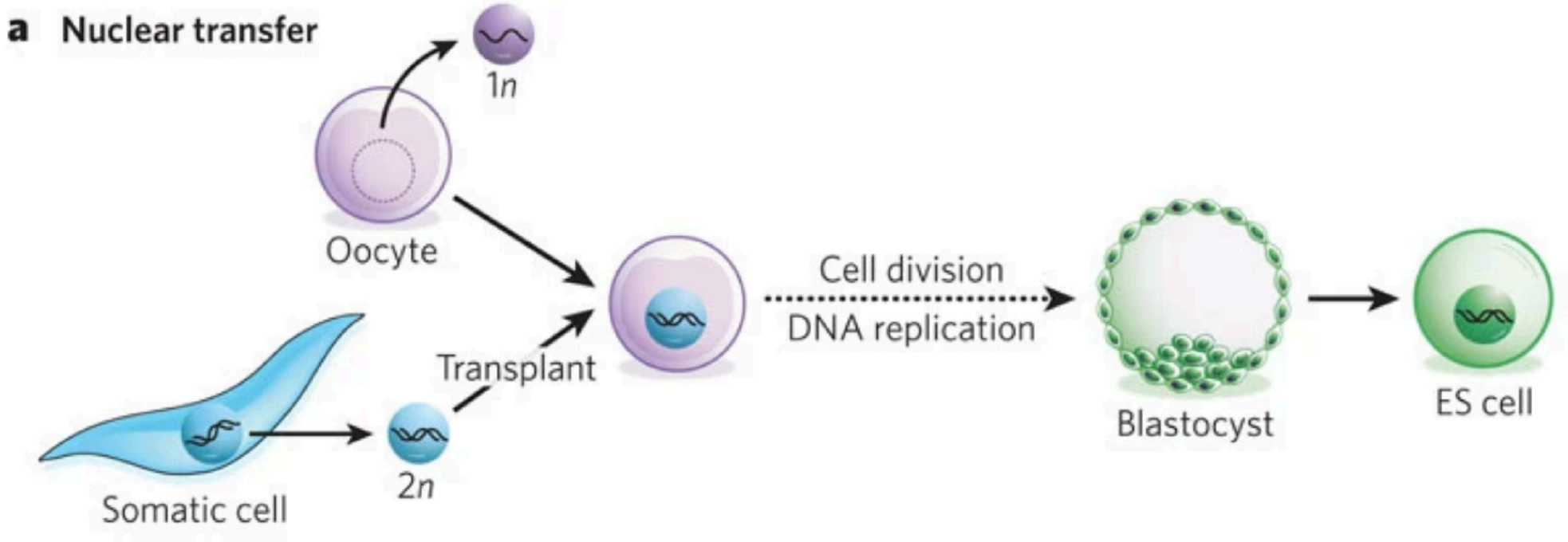


Source of embryonic stem cells

E3.5 Blastocyst

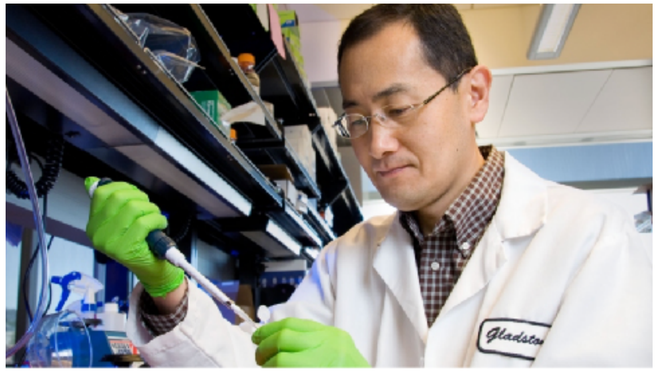
How can cells be reprogrammed to earlier Developmental stages?



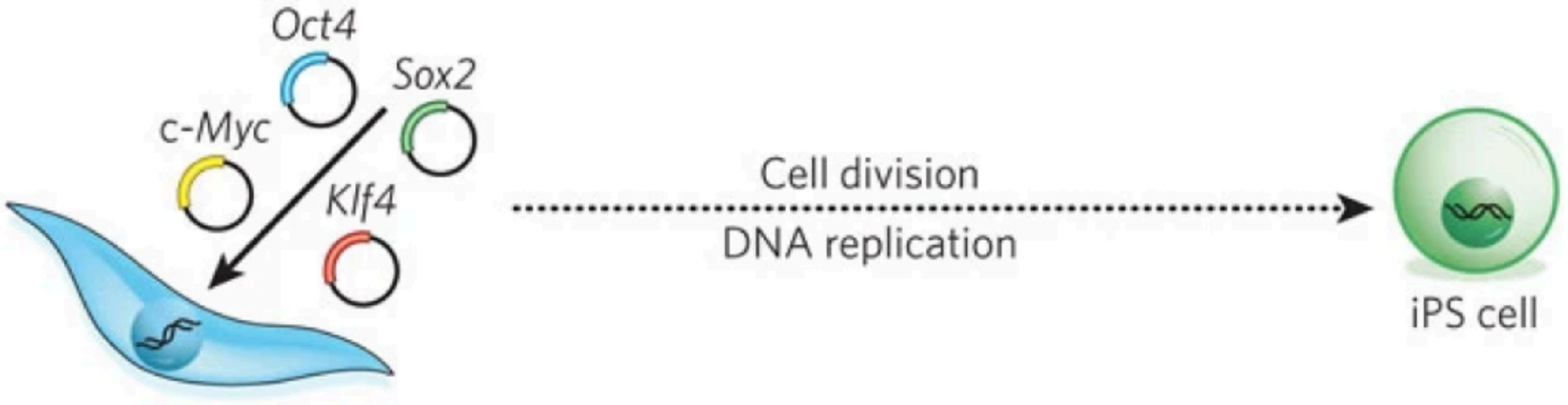


Somatic Cell Nuclear transfer into an enucleated oocyte

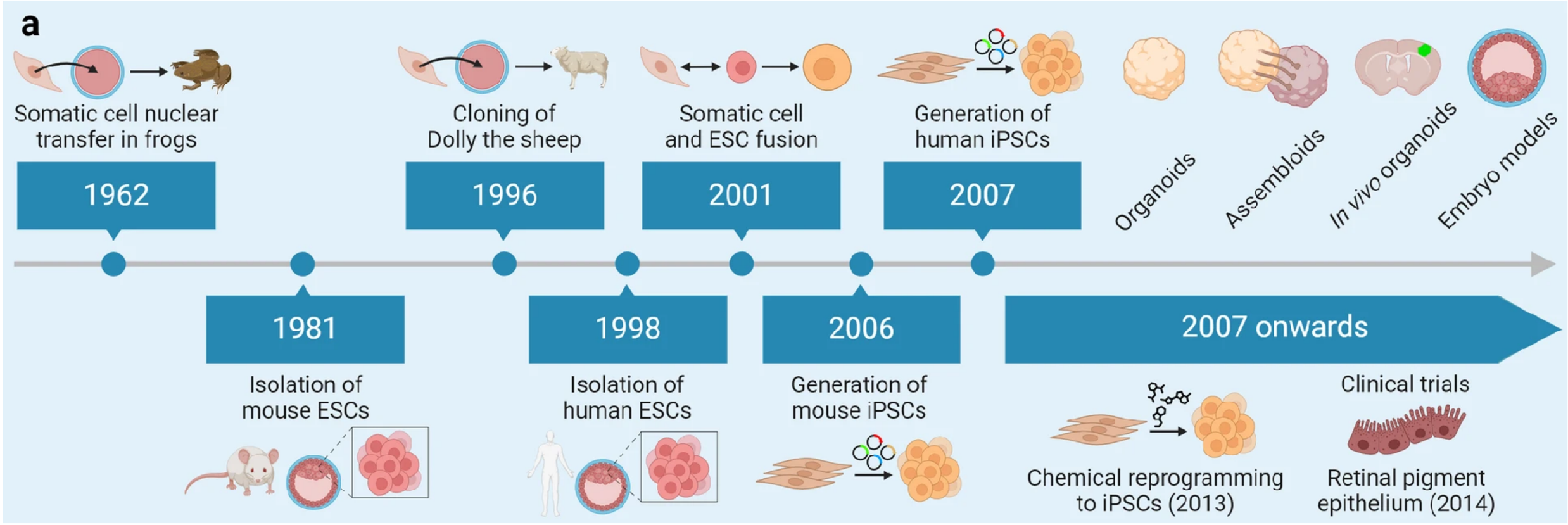
How can cells be reprogrammed to earlier Developmental stages?



c **Transcription-factor transduction**

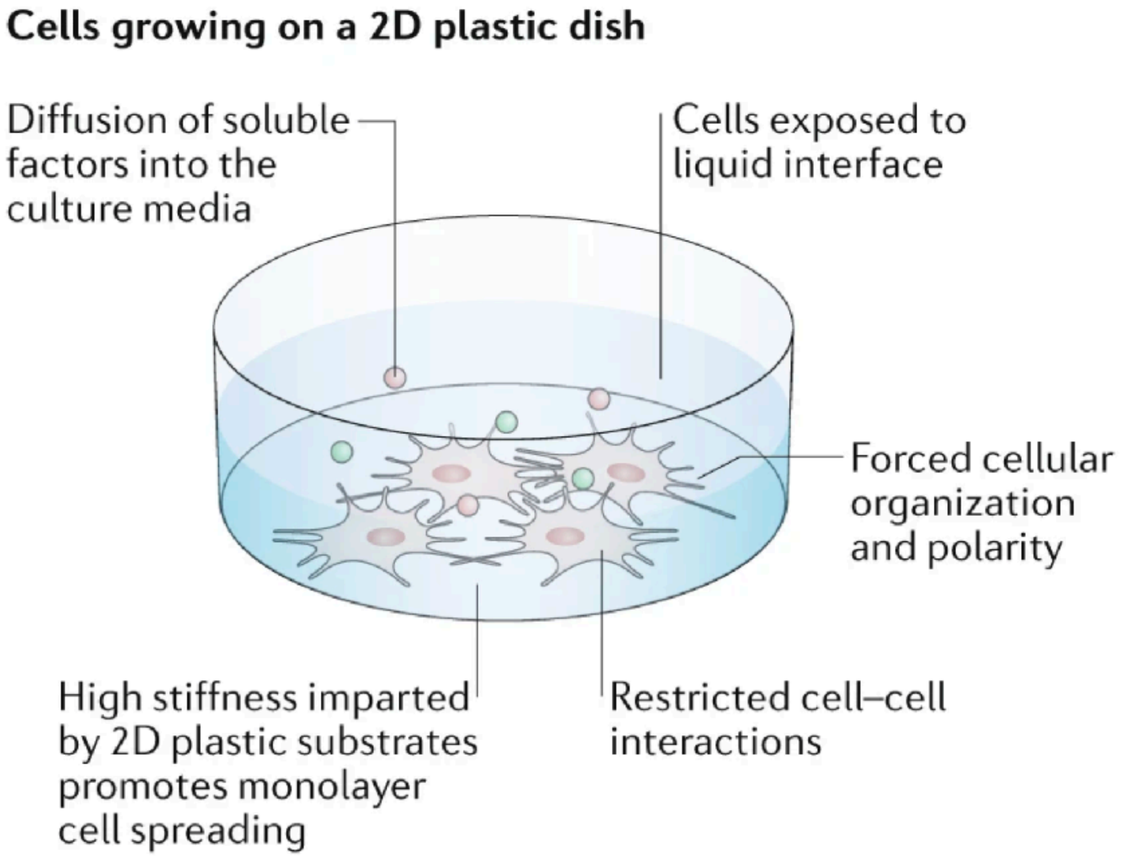


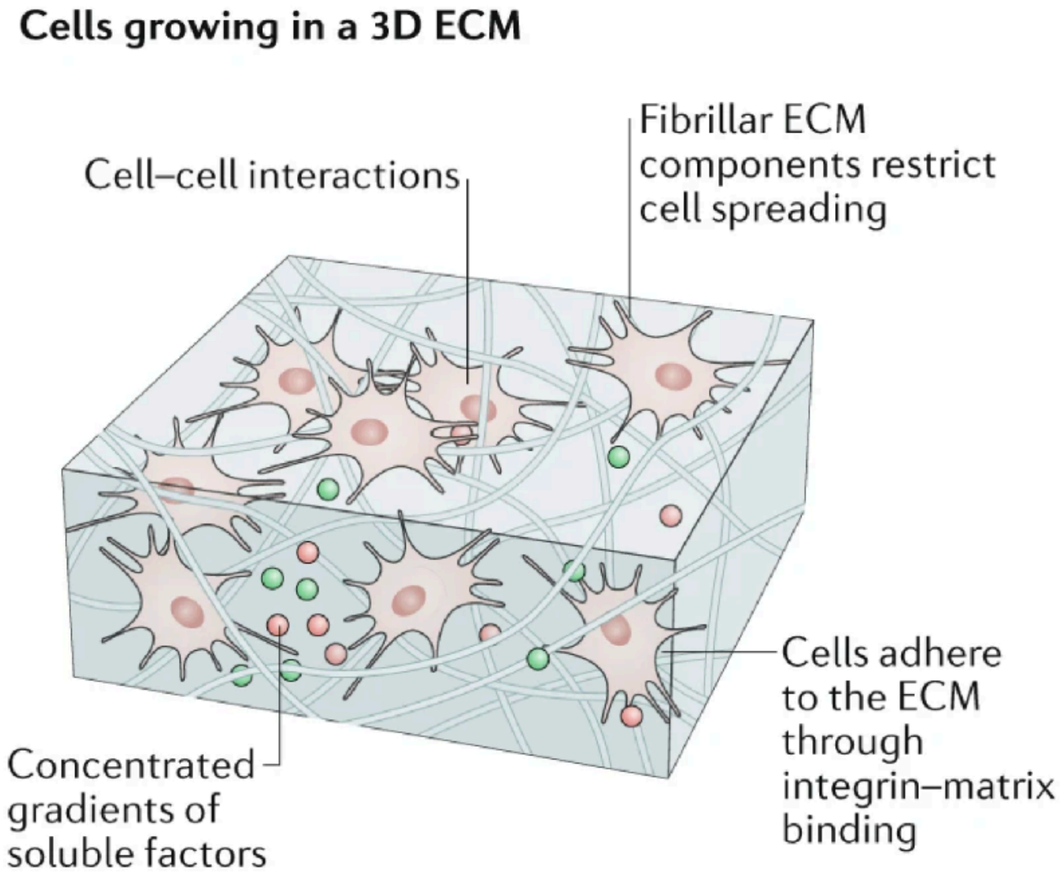
Timeline and application of iPSCs in research



Cerneckis,…, Shi (2024) Signal Transduction and Targeted Therapy

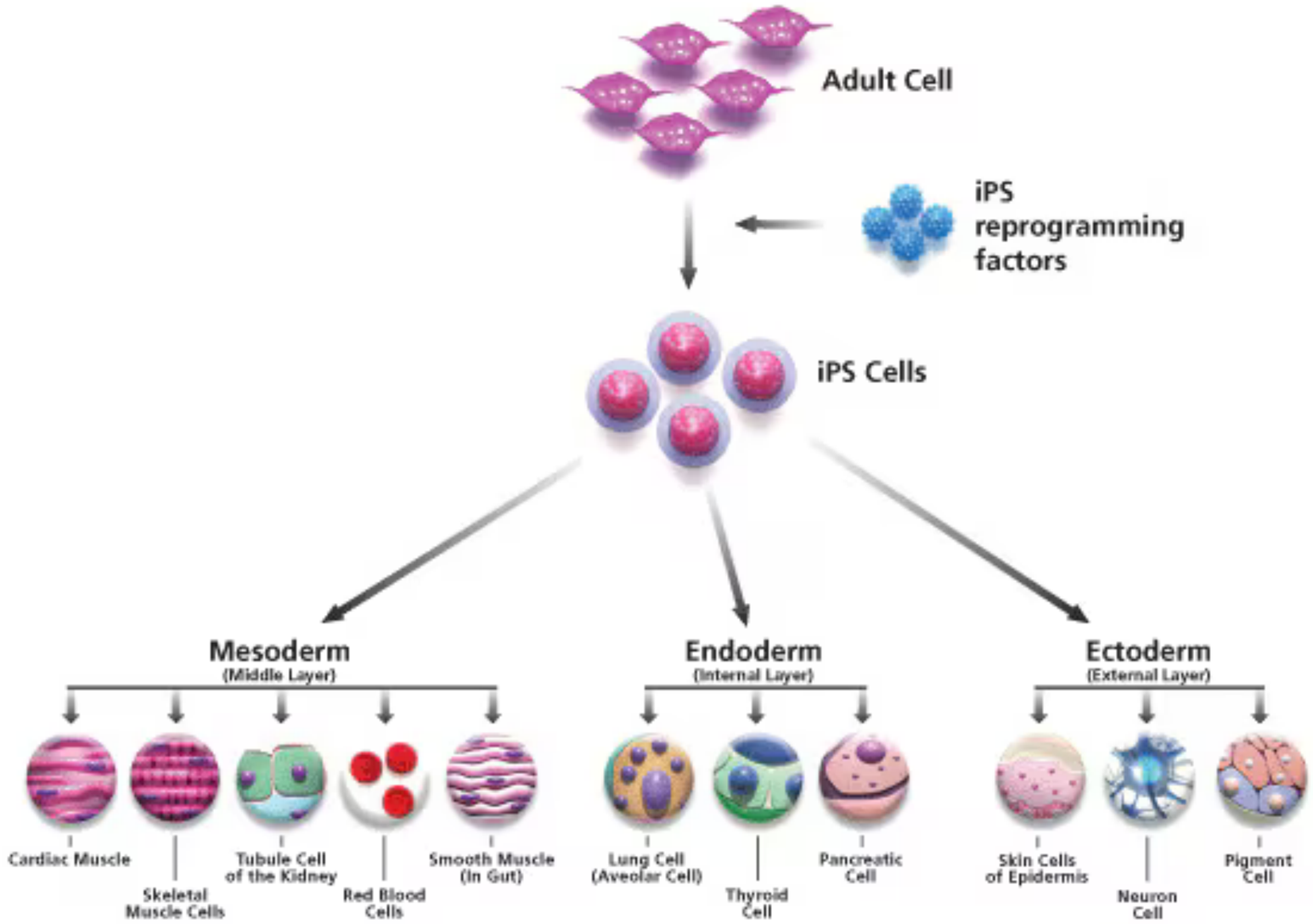
2D vs 3D cell culture





Hussei,…, Badylak (2018) Nature Reviews Materials

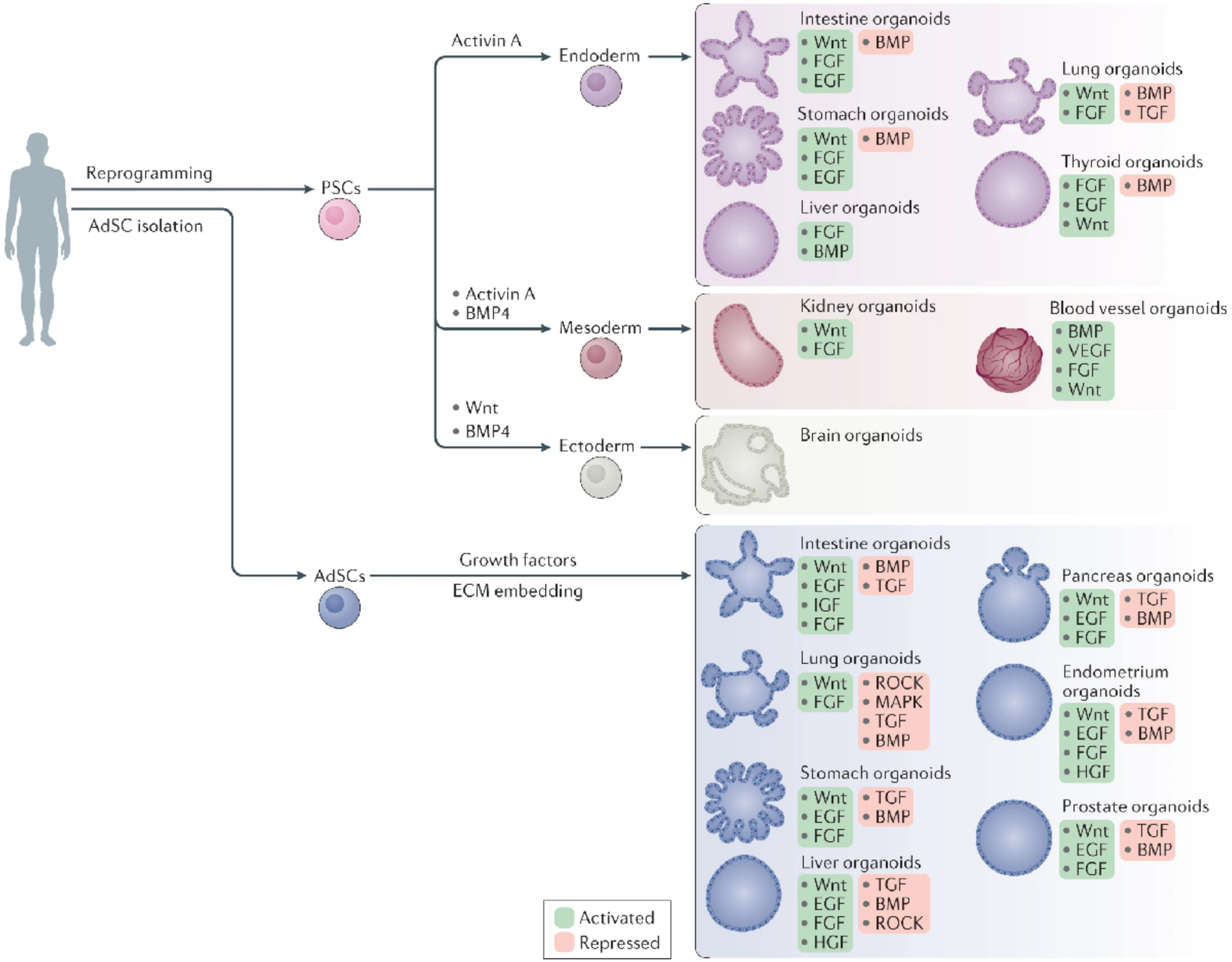
iPSCs can give rise to all cell types



Very pure individual cell types can be derived in 2D

https://www.sigmaaldrich.com/CH/de/technical-documents/technical-article/cell-culture-and-cell-culture-analysis/stem-cell-culture/ipsc-differentiation

iPSCs can be used to derive organoids



Organoids are 3D aggregates of cells that recapitulate the organotypic functions and morphology

Kim,…, Knoblich (2020) Nature Reviews Molecular Cell Biology

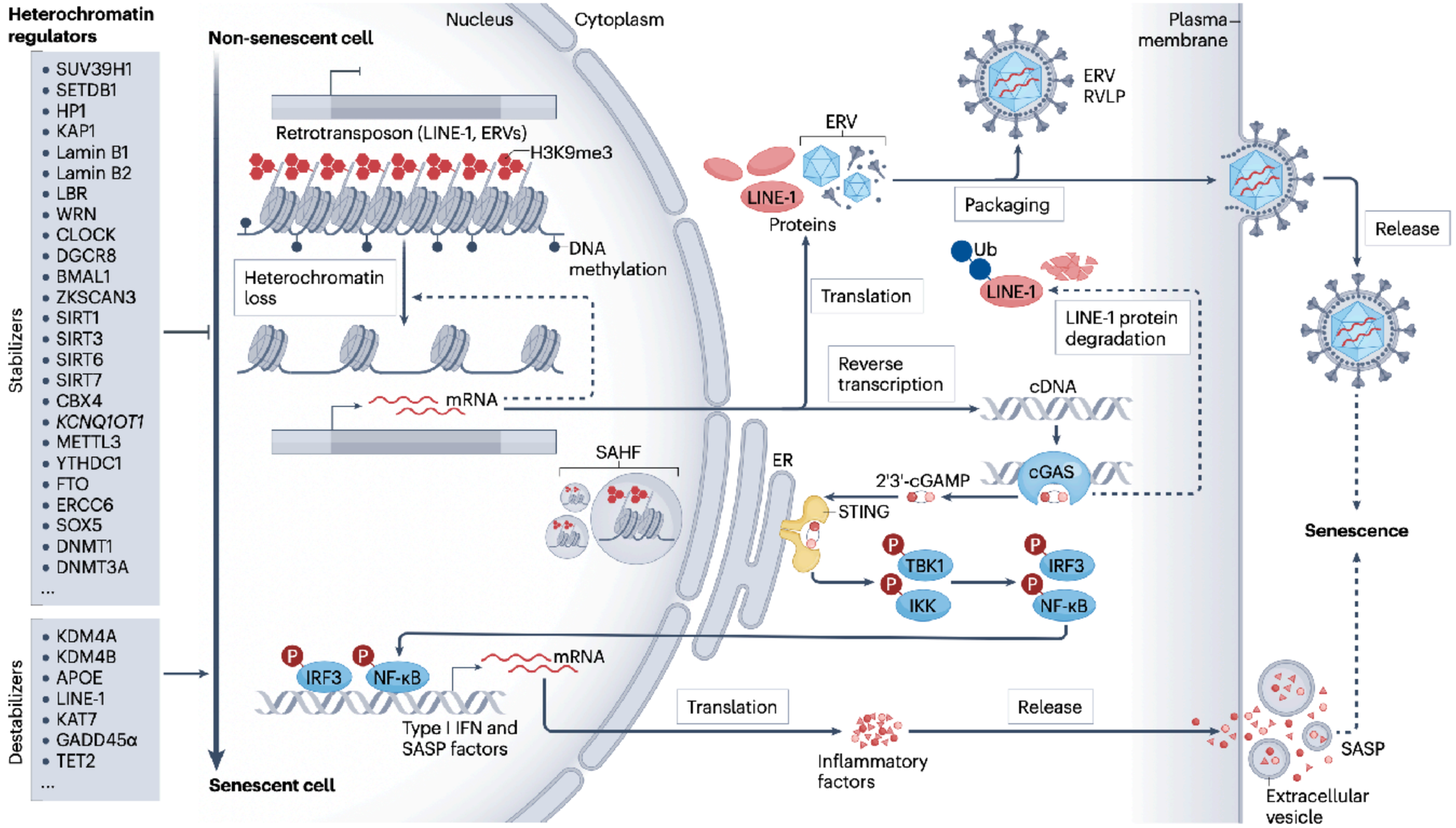
Ageing is the biggest risk factor for neurodegeneration

How can we study ageing and neurodegeneration?

What are relevant models?

How can we identify molecular markers of ageing and neurodegeneration and develop models?

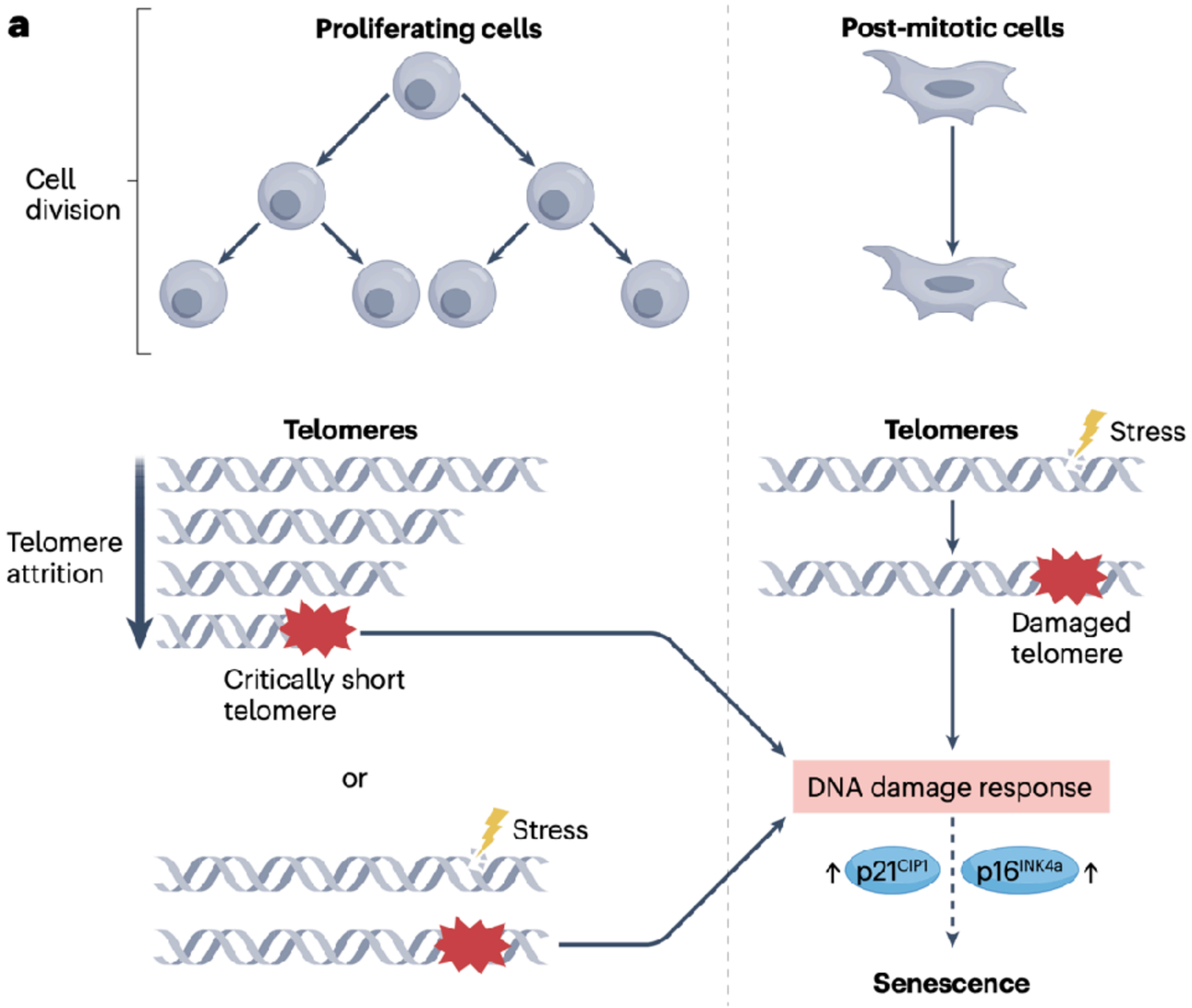
What happens to an ageing cell?



- Loss of heterochromatin
- Activation of Retroelements
- Inflammatory signalling through cGAS
- Nuclear membrane/ scaffold and nuclear pore change properties
- Causes senescence associated secretory phenotype (SASP)

Wu,…, Liu (2024) Nature Reviews Molecular Cell Biology

What happens to an ageing cell?



DNA damage and Telomere shortening

Radiation, chemical reagents, ROS (reactive oxygen species) and infection cause damage to DNA

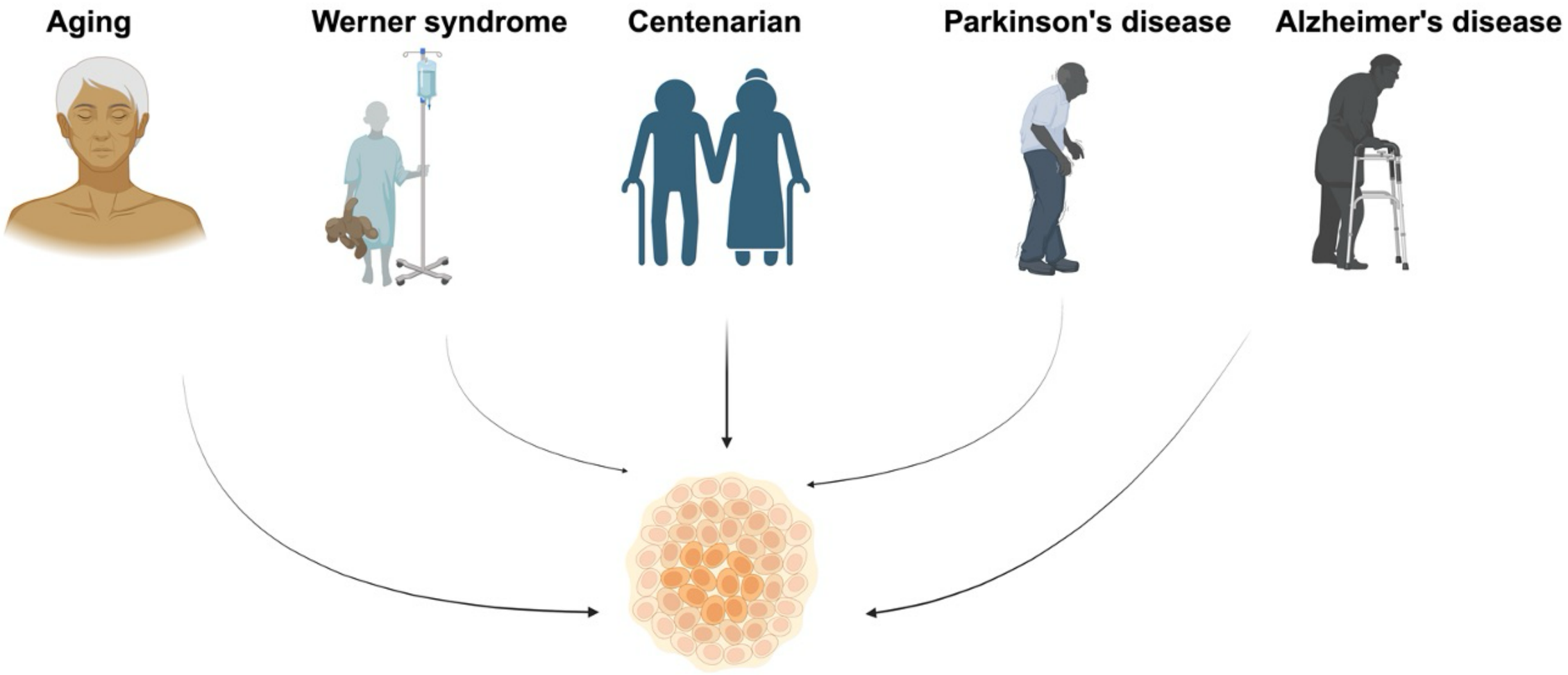
Wu,…, Liu (2024) Nature Reviews Molecular Cell Biology

How to identify a relevant model?

The maximum projected human life span is approximately 115 years!

Mice often do not develop ageing related diseases (without artificially generating human like mutations)

How to identify a relevant model?



Jothi, Kulka (2024) Ageing

General iPSC models of ageing

Modeling strategies

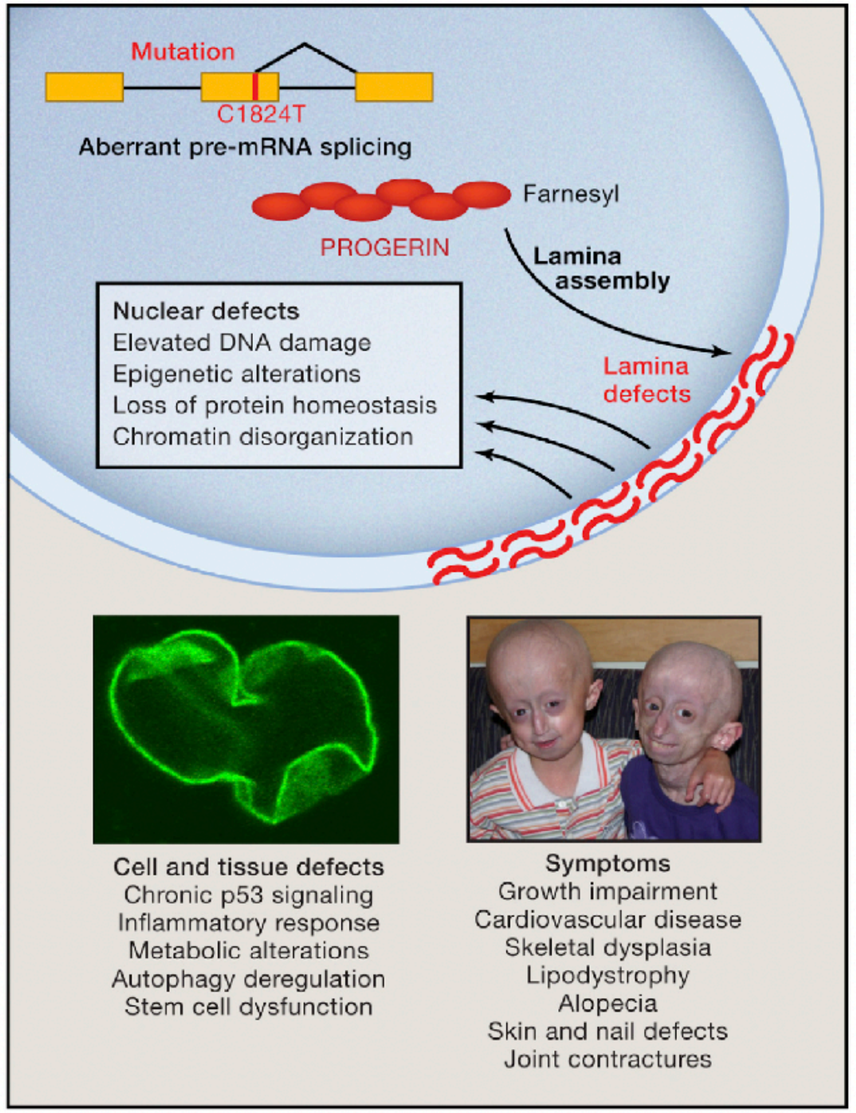
Aging

Progerin overexpression
Long-term culture
3D tissue models (with aged ECM/exposure to hypoxia/ exposure to ionizing radiation)
Telomere manipulation

Aging/disease phenotype

DNA damage ↑
Mito-ROS ↑
Telomere length ↓
% short telomeres ↑
Senescence/p21/p53 ↑
TH+ cells ↓
lipofuscin accumulation
Inclusion bodies
Dendrite degeneration
cardiac beat velocity ↓
BBB dysfunction
secretion of cytokines ↑

Jothi, Kulka (2024) Ageing



Gordon, .., Misteli (2014) Cell

General iPSC models of ageing

WRN is a RecQ recombinase that contains an exonuclease domain

It is important for DNA repair, recombination, replication and transcription. WRN plays also a role in telomere maintenance.

Oshima, Monnat (2017) Ageing Research Reviews

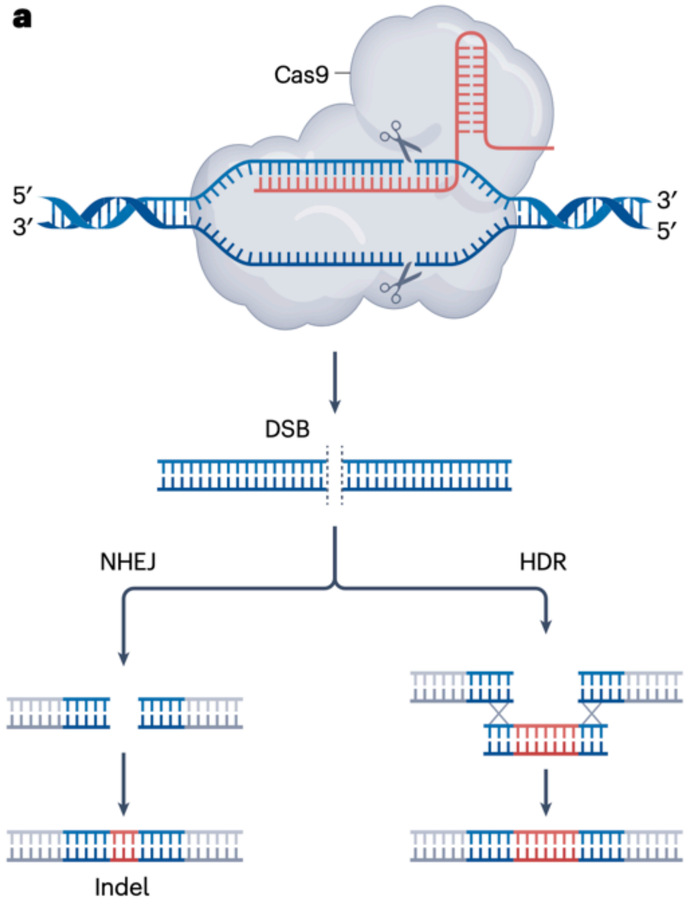
	Werner syndrome
Modeling strategies	Reprogramming by OSKM +HDACi+TGFβ RI kinase i hTERT overexpression and p53 knockdown in WS-MSC Gene correction of WRN in WS IPSC clones
Aging/disease phenotype	Senescence ↑ Telomere length ↓ p53 ↑ Rescues premature senescence (upon hTERT knock in & WRN gene correction) Increased telomere length (upon hTERT knock in) Normal karyotype (upon WRN gene correction)

Jothi, Kulka (2024) Ageing

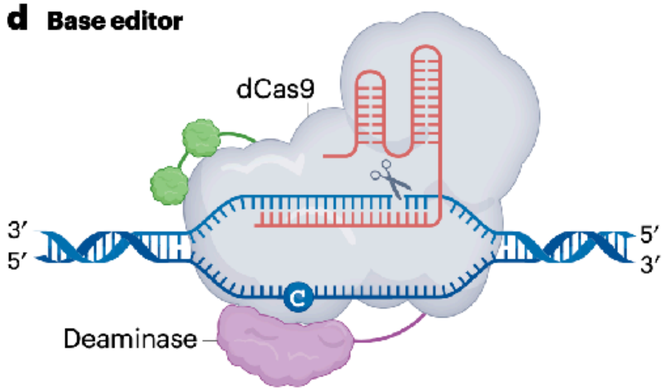
Mutations is WRN cause premature ageing

Using CRISPR to generate iPSC models

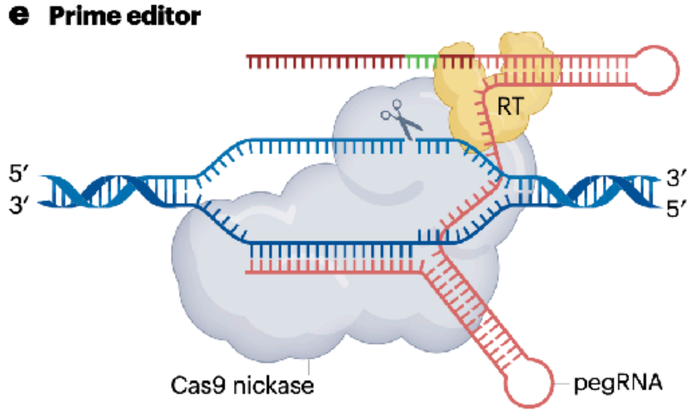
Generation of random mutations



C-T and A-G mutations

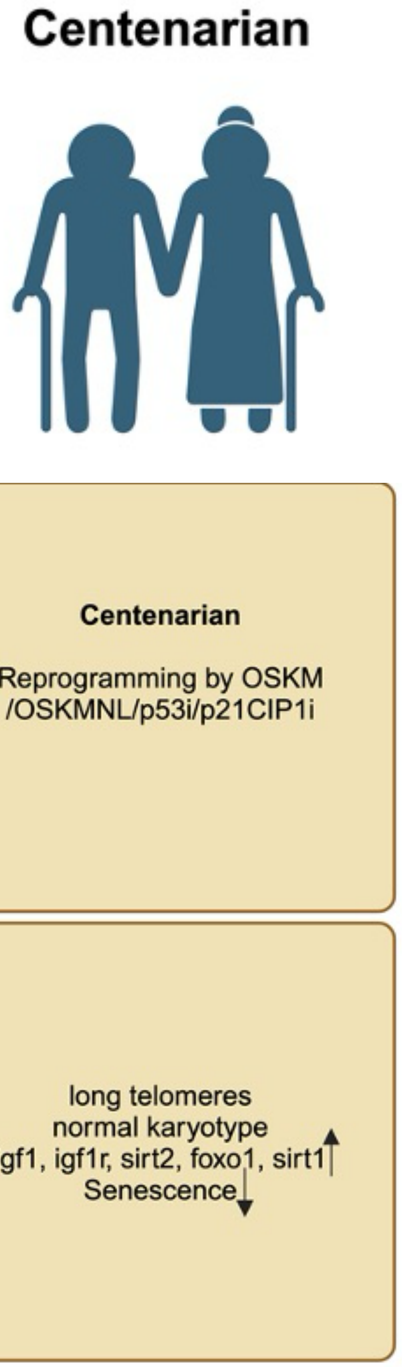


Any mutation



Smith,…, Choudhury (2024) nature reviews urology

General iPSC models of ageing



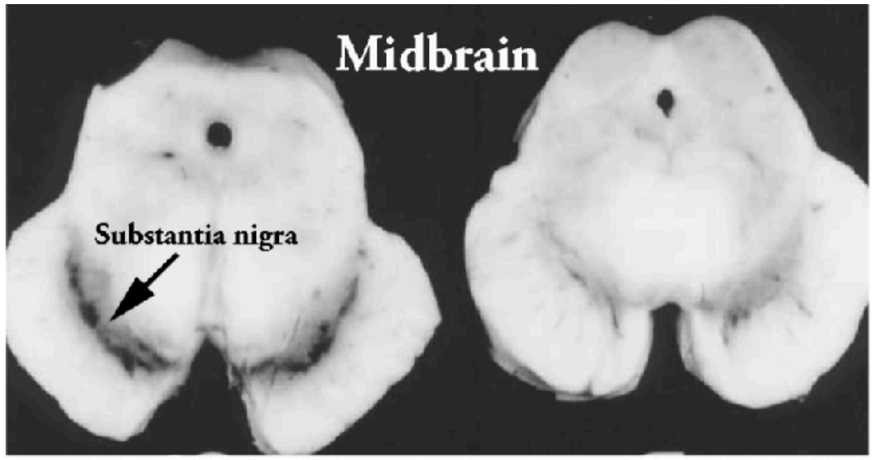
Jothi, Kulka (2024) Ageing

Reprogramming fibroblast of members of the world's oldest populations (e.g. Okinawa) to iPSCs results in cells with high SIRT1 expression and little senescence

These factors could potentially play protective roles in the donors

Drawback: -No isogenic control!
-Loss of ageing features

iPSC models of Parkinson's Disease (PD)



Selective loss of dopaminergic neurons in the midbrain (causes motor defects)

Use patient cells for reprogramming and repair mutations or induce patient mutations in commercial iPSCs

Common mutations: -Triplication of SNCA
-LRRK2
-PARK2
-PINK1

Modeling strategies

Parkinson's disease

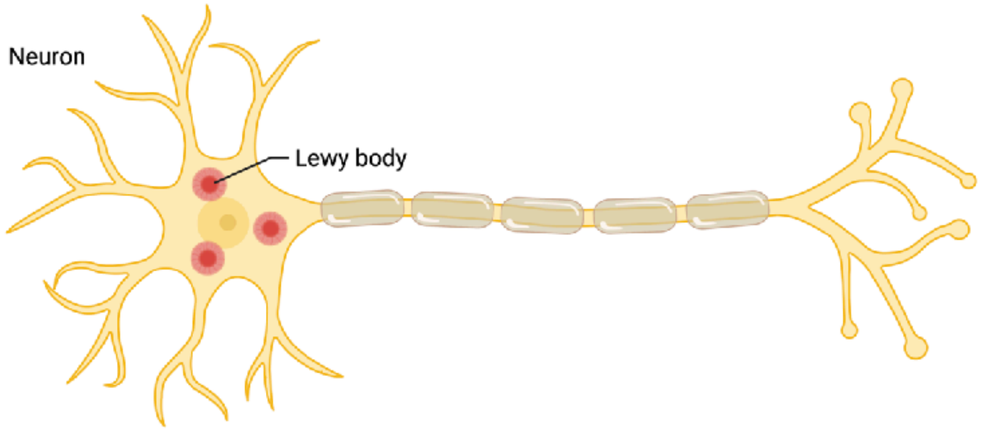
Reprogramming by OSKM/OSK and differentiation by dual SMAD inhibition + Dorsomorphin/protocol by Kirks et al., 2011, Byers et al., 2011, Nyugen et al., 2011, Cooper et al., 2012, Chambers et al., 2009, Shaltouki et al., 2015, Hanss et al., 2021, Doi et al., 2014

Aging/disease phenotype

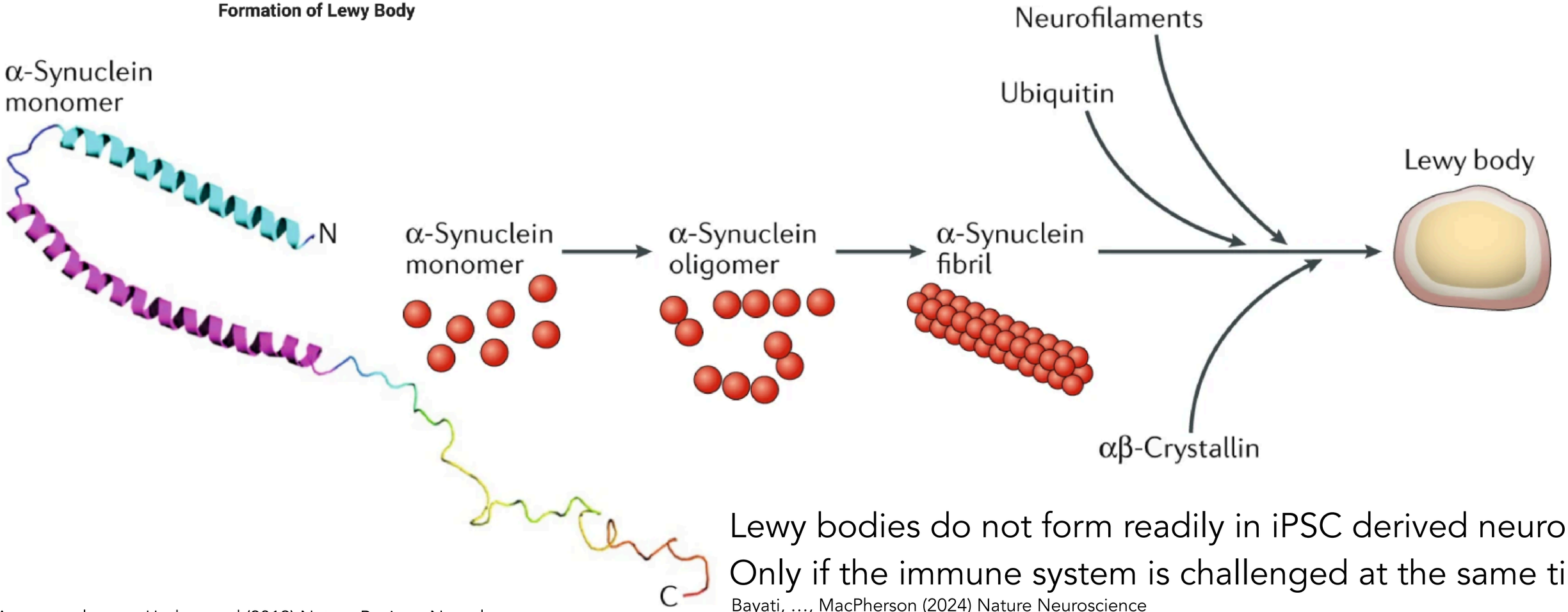
α-synuclein ↑
oxidative stress ↑
mitochondrial dysfunction
ROS ↑
synaptic dysfunction
Endosomal dysfunction
neuroinflammation
compromised BBB function

Jothi, Kulka (2024) Ageing

iPSC models of Parkinson's Disease (PD)



Formation of Lewy Body



Lewy bodies do not form readily in iPSC derived neurons

Only if the immune system is challenged at the same time

Bayati, …, MacPherson (2024) Nature Neuroscience

Arnaoutoglou, …, Underwood (2019) Nature Reviews Neurology

iPSC models of Alzheimer’s Disease (AD)

Accumulation of amyloid-b/tau plaques, increase in phosporylated tau

Common mutations: -PSEN1
-PSEN2
-APO4

Only 5% of AD cases can be explained by genetics

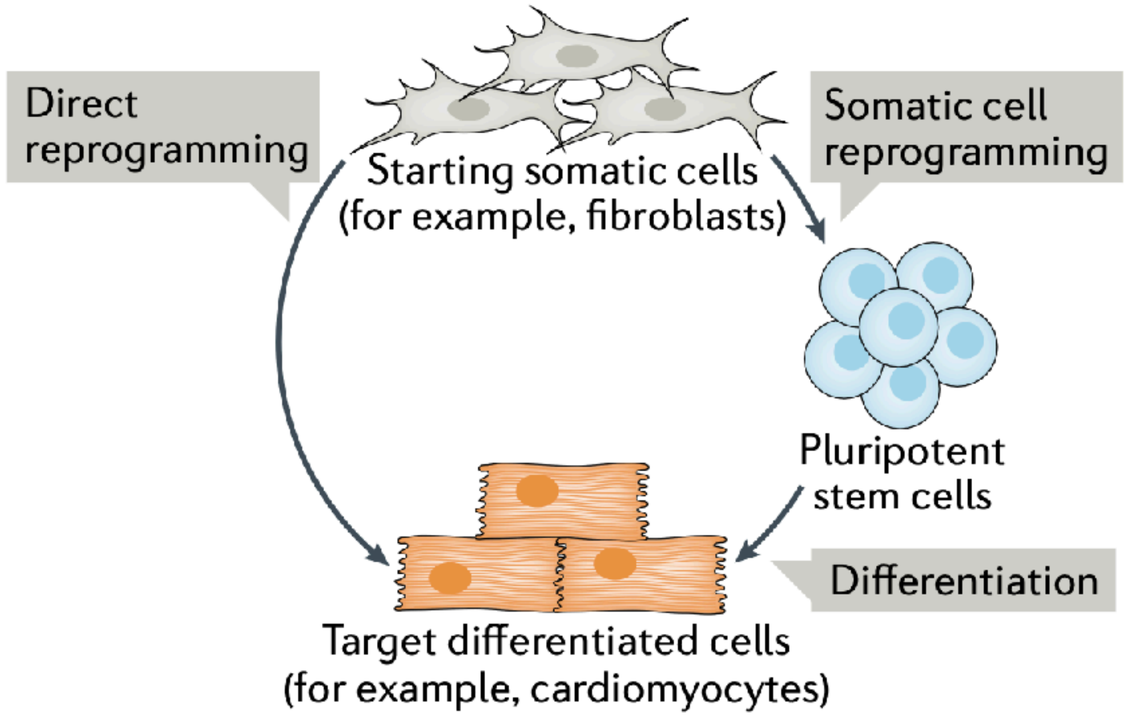
Crosstalk with pericytes, astrocytes and microglia is very important in modelling the disease

iPSC derived AD neurons show premature differentiation but no aggregate formation

Jothi, Kulka (2024) Ageing

iPSC derived neurons resemble early developmental stages

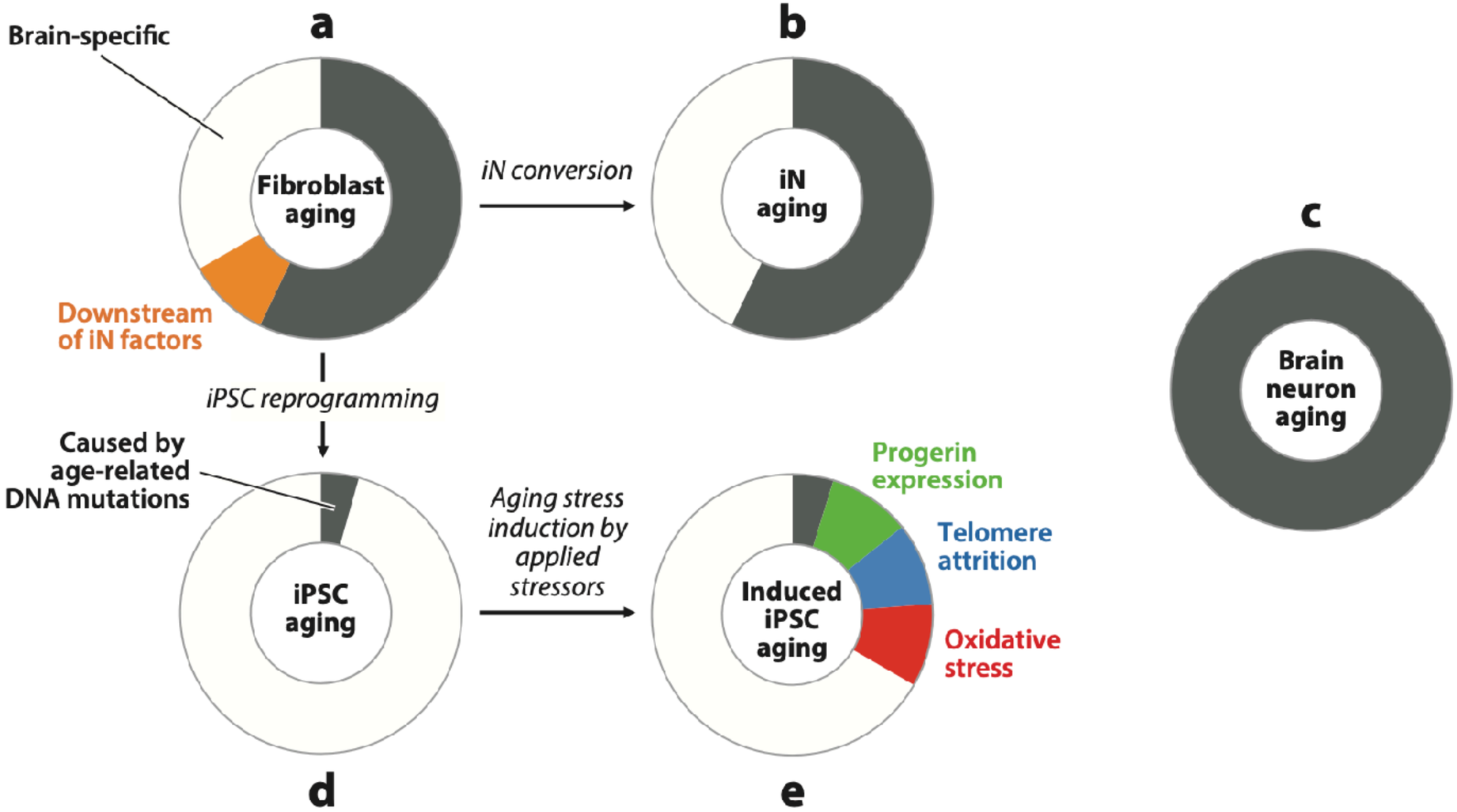
- No intermediate pluripotent/multipotent cell state
- Fast and more efficient turnaround time to acquire reprogrammed cell
- Reduced tumorigenesis risk
- Suitable for in vivo tissue repair
- Epigenetic marks (such as ageing marks) of cell of origin are maintained



- Goes through an intermediate pluripotent or multipotent cell state
- Desired target cells can be in a large scale
- Reprogramming can only be performed in vitro
- Suitable for ex vivo manipulations
- Epigenetic marks (such as ageing marks) are erased in the reprogrammed cells

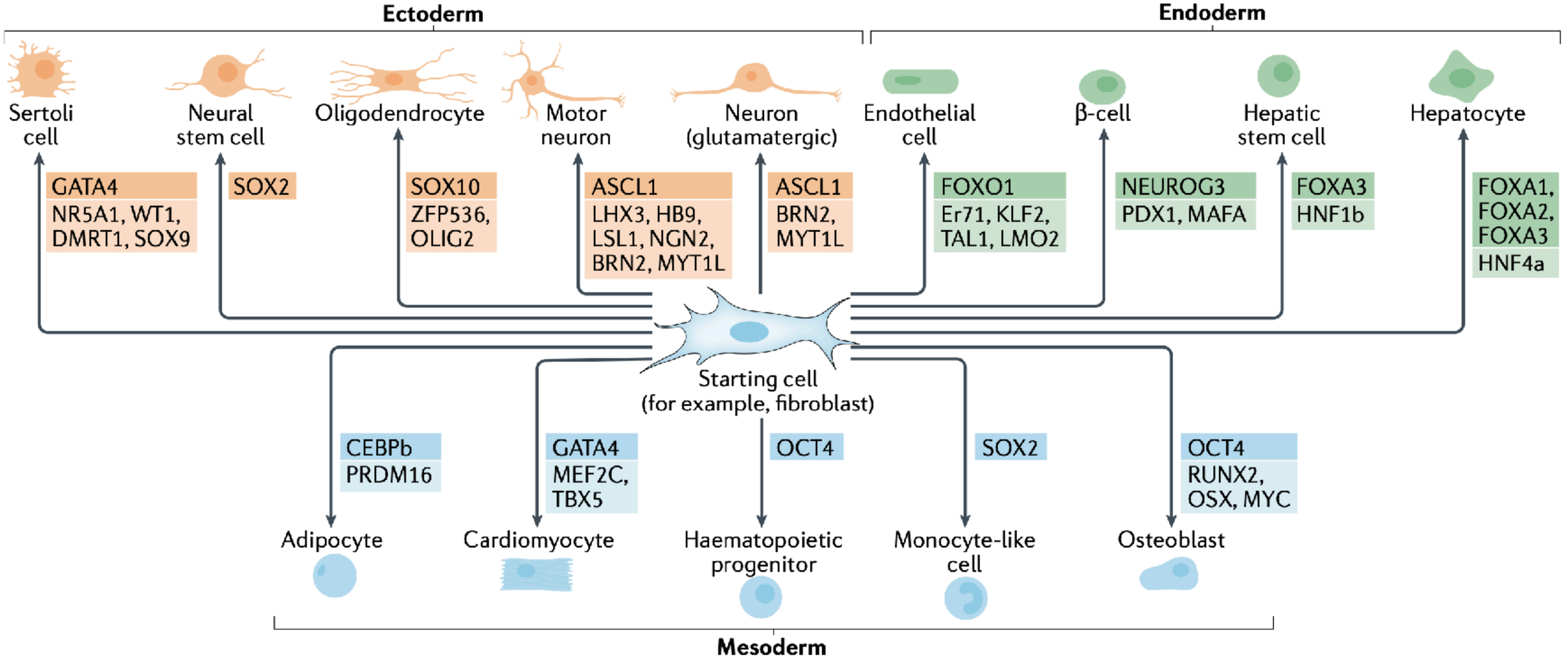
Wang,…, Qian (2021) Nature Reviews Molecular Cell Biology

Direct reprogramming maintains cellular features associated with ageing



Mertens,…, Gage (2018) Annu. Rev. Genet.

Direct reprogramming can give rise to multiple cell types

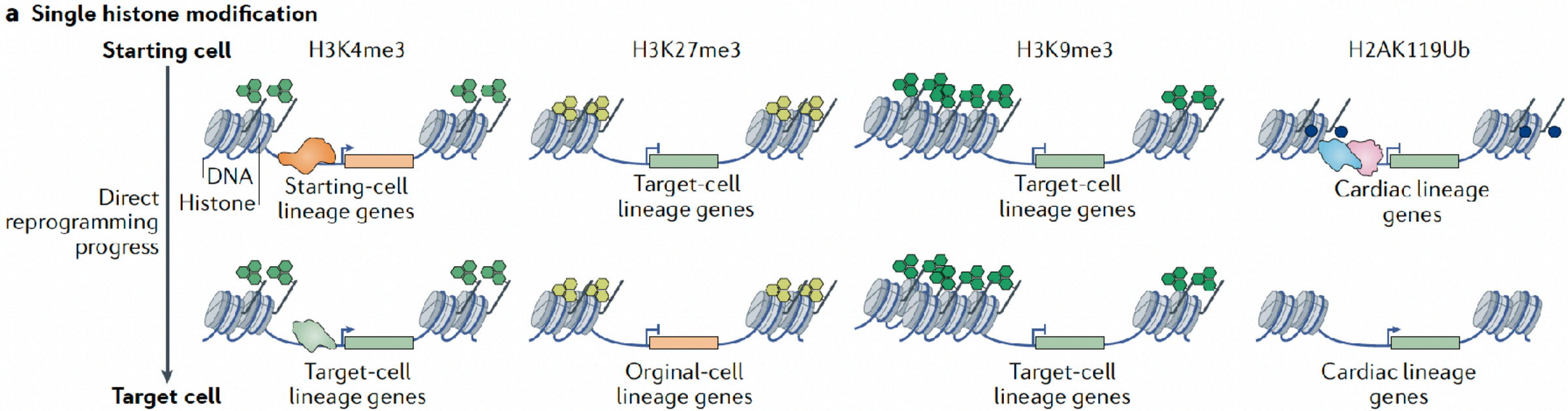


Wang,…, Qian (2021) Nature Reviews Molecular Cell Biology

Delivery of reprogramming factors

Strategy	Lentivirus	piggybac	Adenovirus	Sendai Virus	Plasmid	RNA	Protein
Integration	Integrates into genome	Integrates into genome but is excisable	No Integration	No Integration	Might integrate	No Integration	No Integration
Efficiency	Fast and efficient	Medium efficient	Low efficiency	Medium Efficiency	Medium efficiency	Relatively efficient (multiple transfections)	Low efficiency
Safety	Low	Medium	Medium	Medium	Medium	High	High

Inhibiting heterochromatin writers enhances reprogramming



Perturbation of H3K4me3 makes reprogramming less efficient

Wang,…, Qian (2021) Nature Reviews Molecular Cell Biology

Chemical repgrogramming

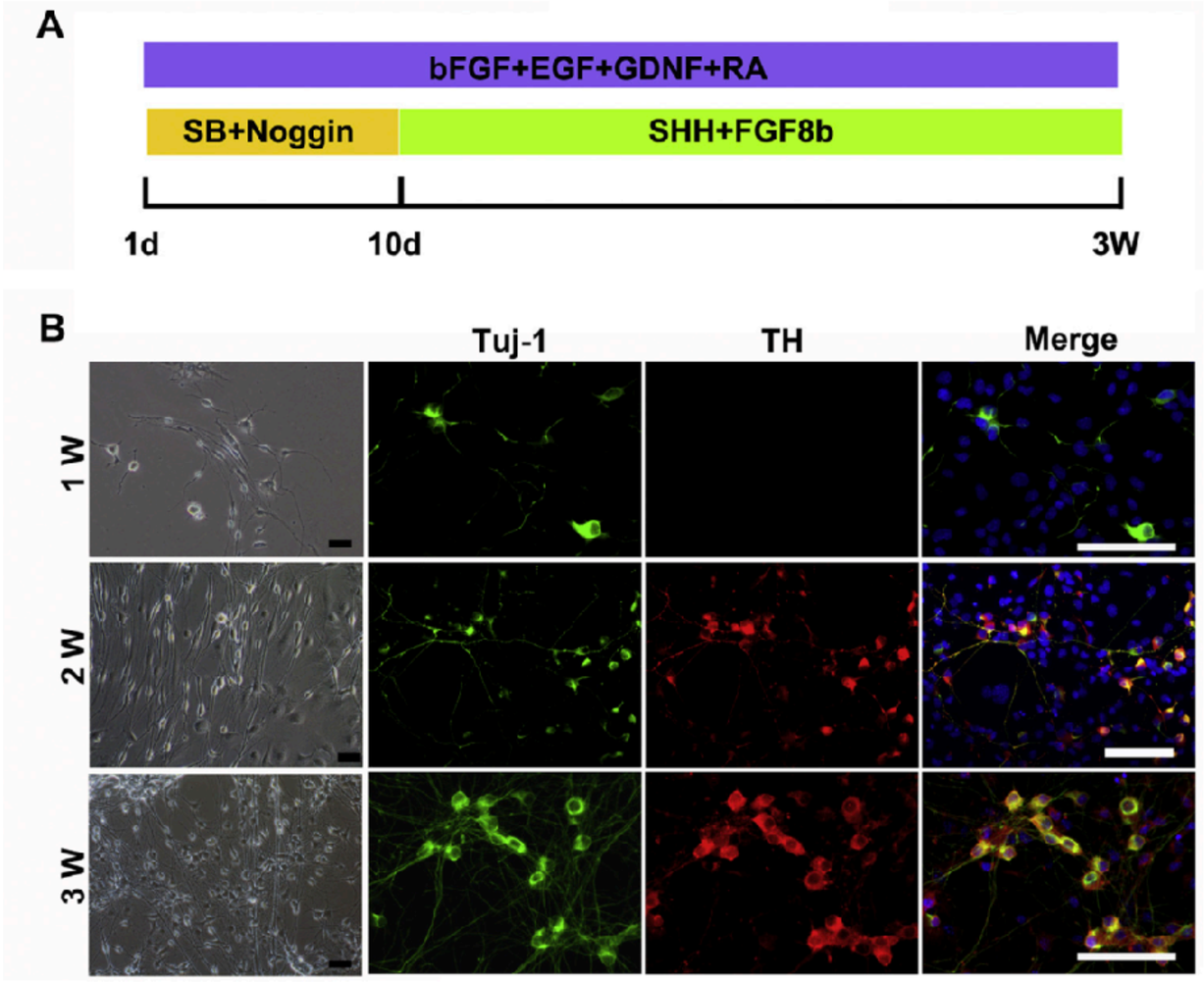
Small molecules have been used to reprogram cardiomyocytes or **fibroblasts to neurons**

Advantages:

-relatively fast

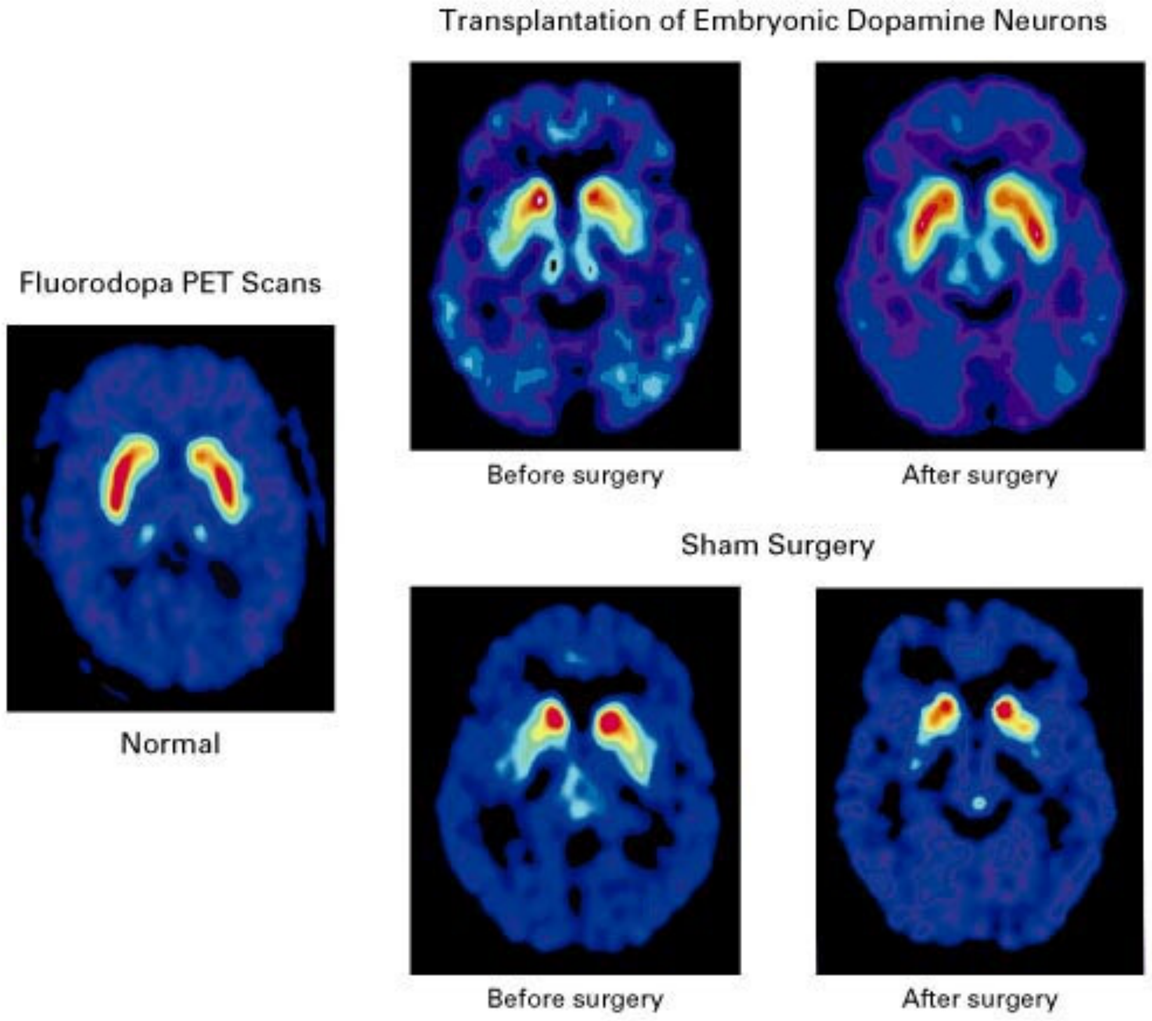
-scalable

-relatively safe for clinical application (no integration risk)



Wang, …,Gao (2016) Experimental research

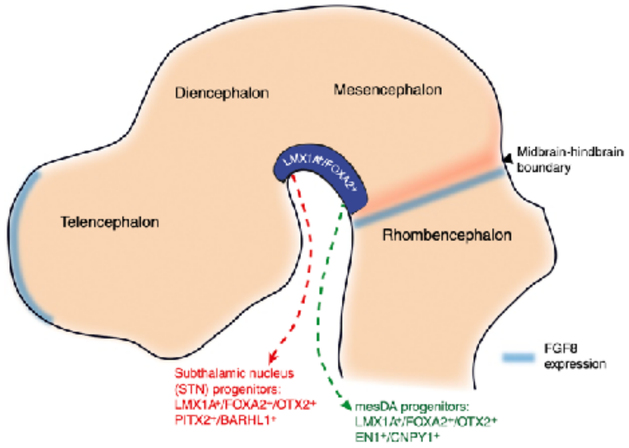
Stem Cell derived therapies

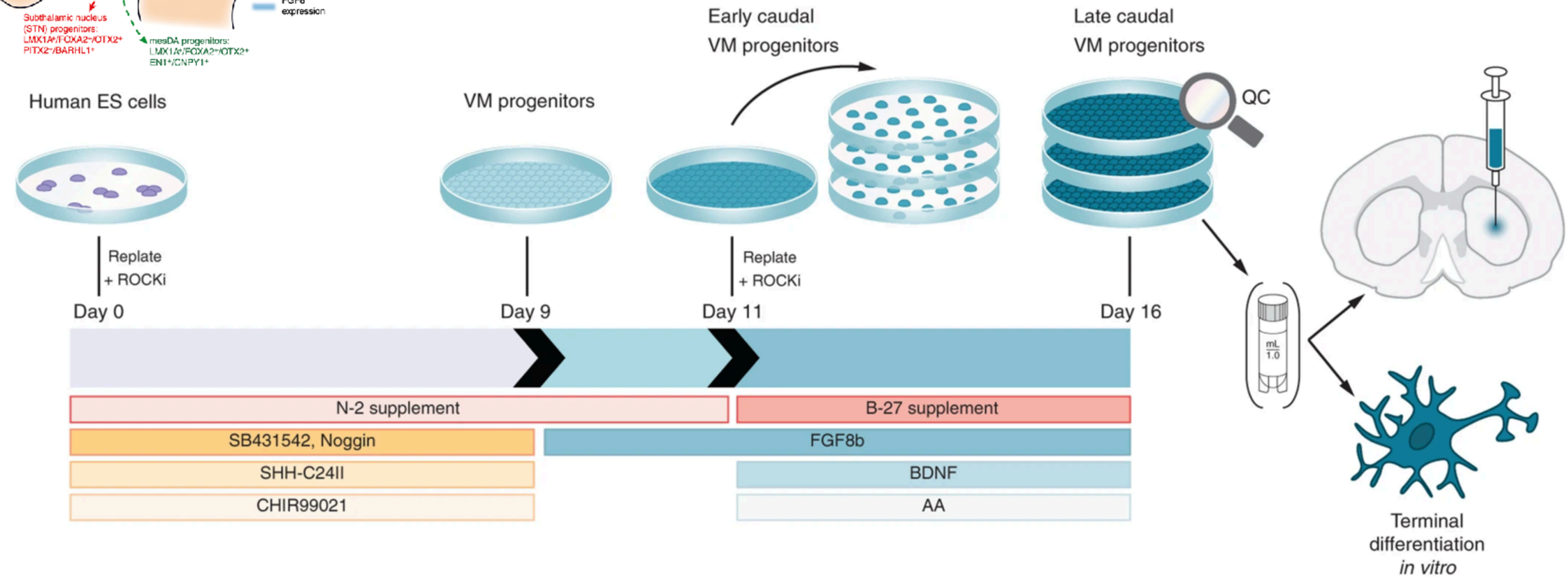


2001, first clinical trial to treat Parkinson's disease by implanting dopaminergic neurons from abortion tissue

-low efficiency of the graft
-little material
-high variability
-effect too little

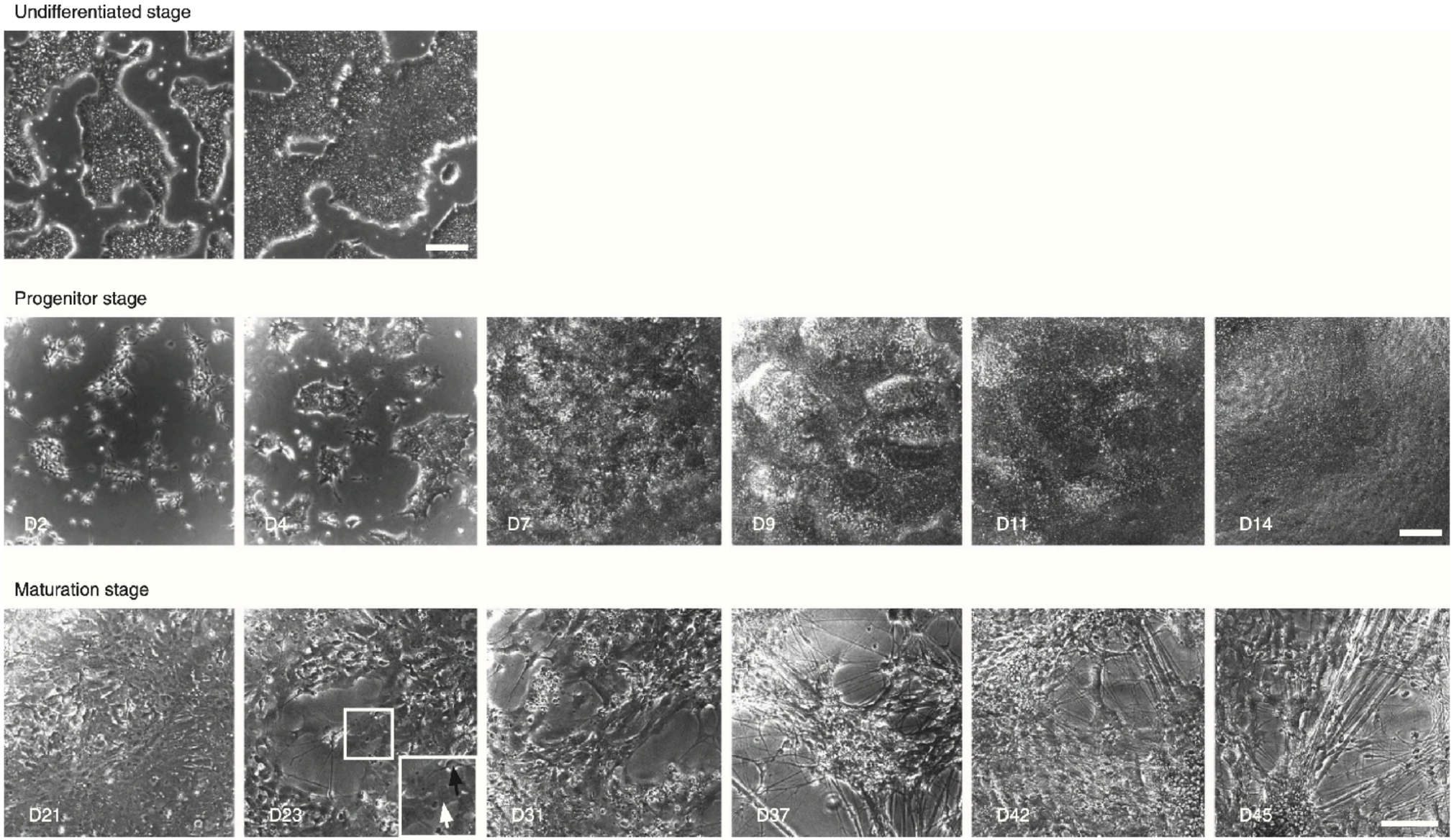
Generating mid-brain dopaminergic neurons in vitro





Nolbrant, …,Kirkeby (2017) Nature Protocols

Generating mid-brain dopaminergic neurons in vitro



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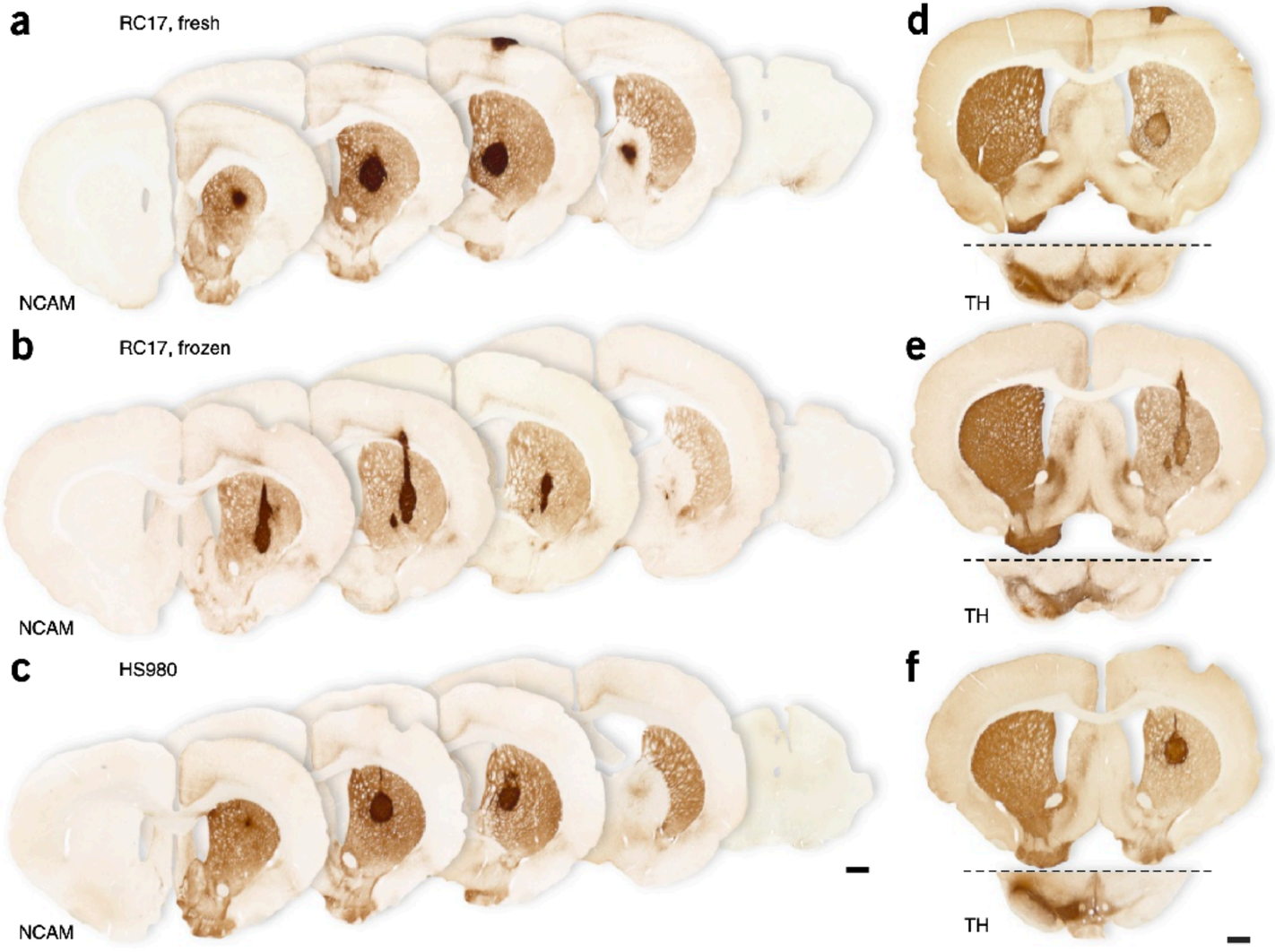
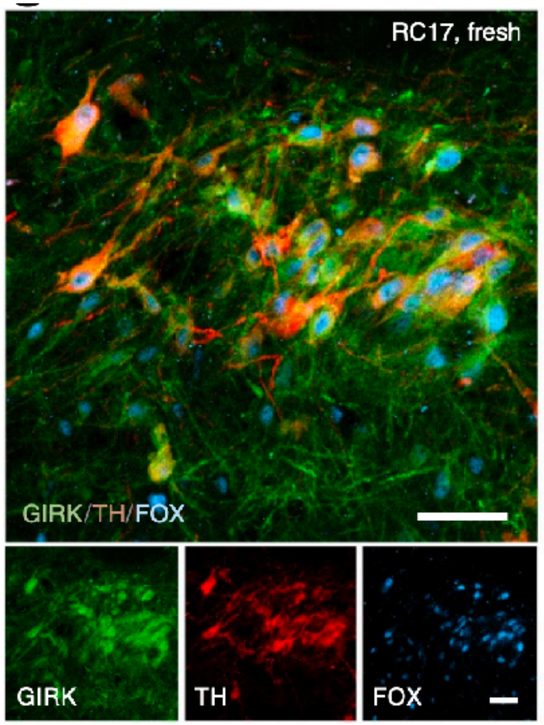
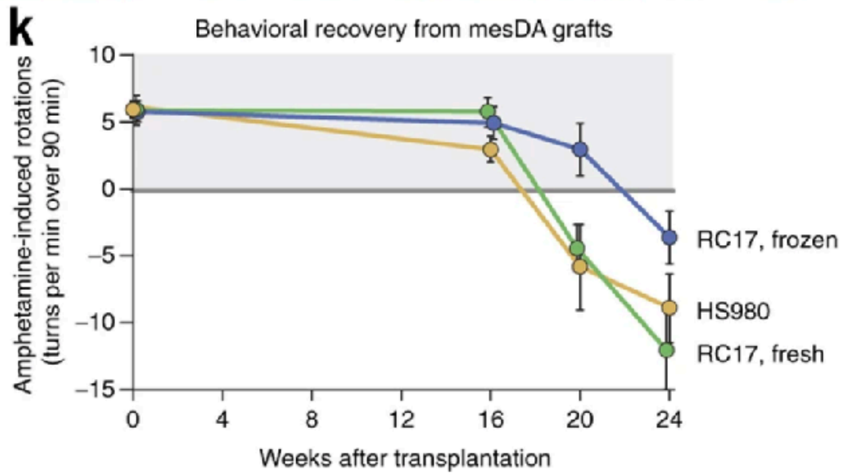
Generating mid-brain dopaminergic neurons in vitro







Nolbrant, …,Kirkeby (2017) Nature Protocols

Stem Cell derived therapies

BlueRock THERAPEUTICS

Viviane Tabar & Laurenz Studer

Bayer // United States

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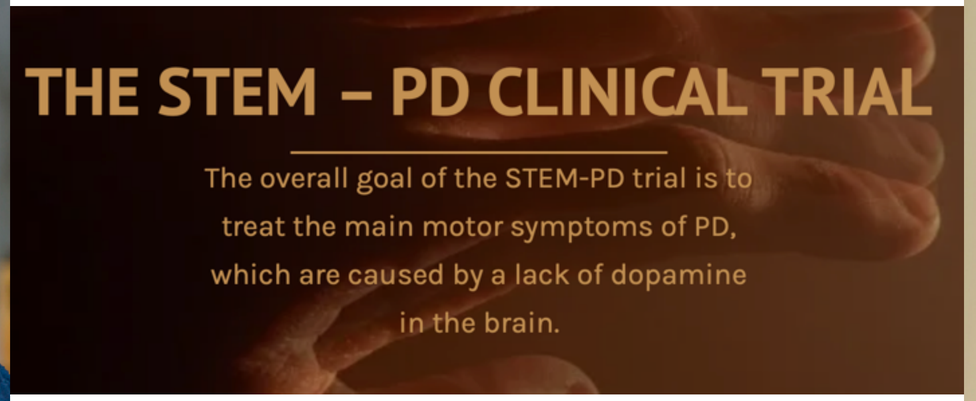
Home

Pharmaceuticals

BlueRock Therapeutics' investigational cell therapy bemdaneprocel for Parkinson's disease shows positive data at 24-months

Agnete Kirkeby



Kirkeby, …,Parmar (2023) Cell Stem Cell

Malin Parmar

Take Home - what you should know after the lecture:

What is the difference between hESC and iPSC?

How can cells be regprogrammed?

What is OKSM?

What is progerin and what is WRN and why are they used as ageing models?

What is an isogenic control line and how can it be obtained?

Choose two factors that can enhance reprogramming and explain why.

What is the difference between direct reprogramming and iPSCs?

Explain advantages and disadvantages of either and give examples how reprogramming factors can be delivered.

Exercises

1. Go through the questions of the lecture.

2. In groups, please select an aspect of ageing or a neurodegenerative disease and explain, based on your knowledge from the lecture, how iPSCs could be used to model it. What are the advantages and disadvantages?

3. Imagine you found a new and interesting phenotype in a neurodegenerative disorder.
   -How would you generate enough material to study it independently of the patient?
   -Which considerations would you take?
   -How would you try to revert the phenotype?