CHAIRE ÉPIGÉNÉTIQUE ET MÉMOIRE CELLULAIRE

Année 2013-2014 :

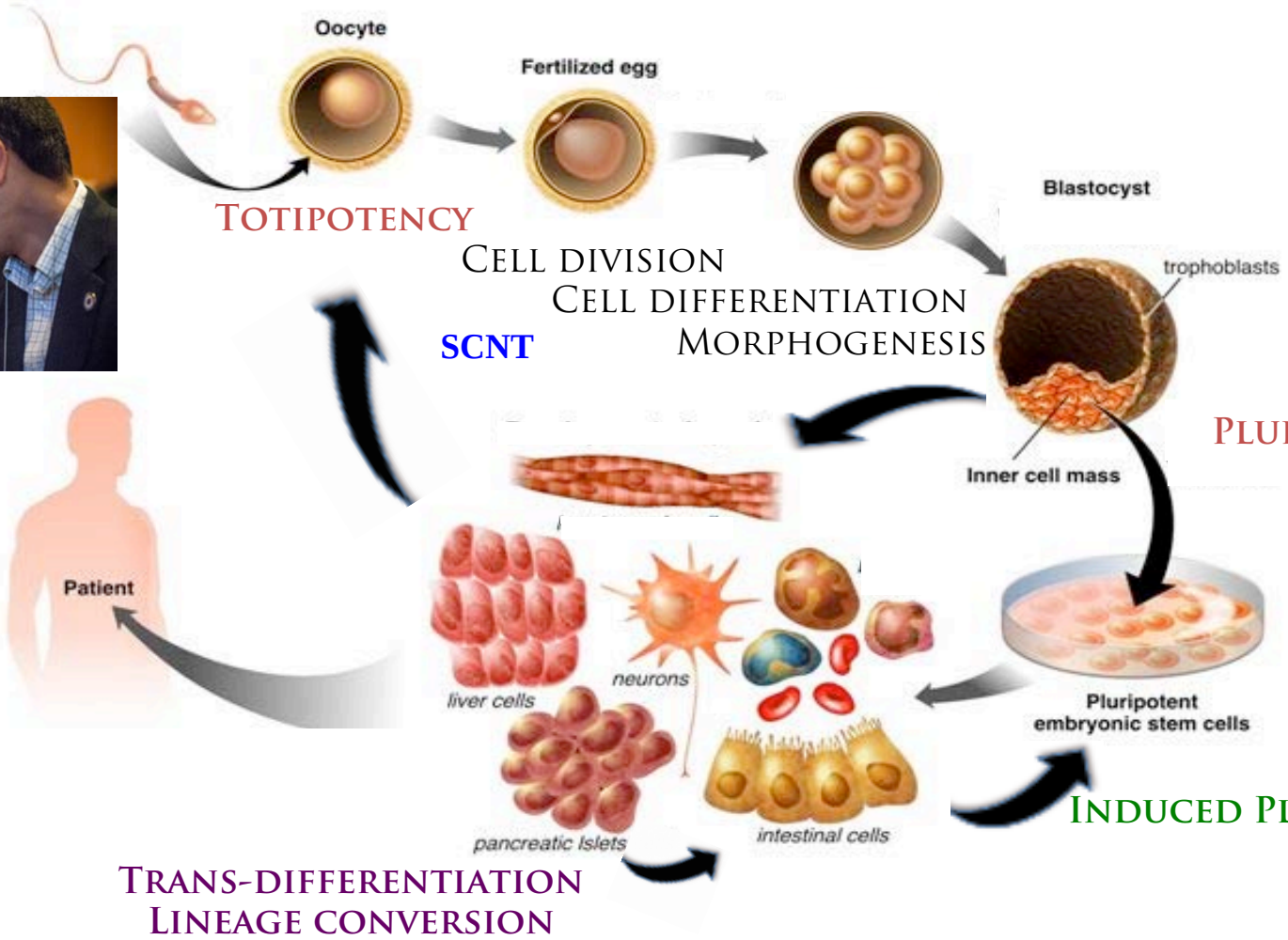
“Reprogrammations développementales, induites et pathologiques ”

Cours V

Utilisations thérapeutiques des cellules iPS et comme modèles en pathologie

7 avril 2014

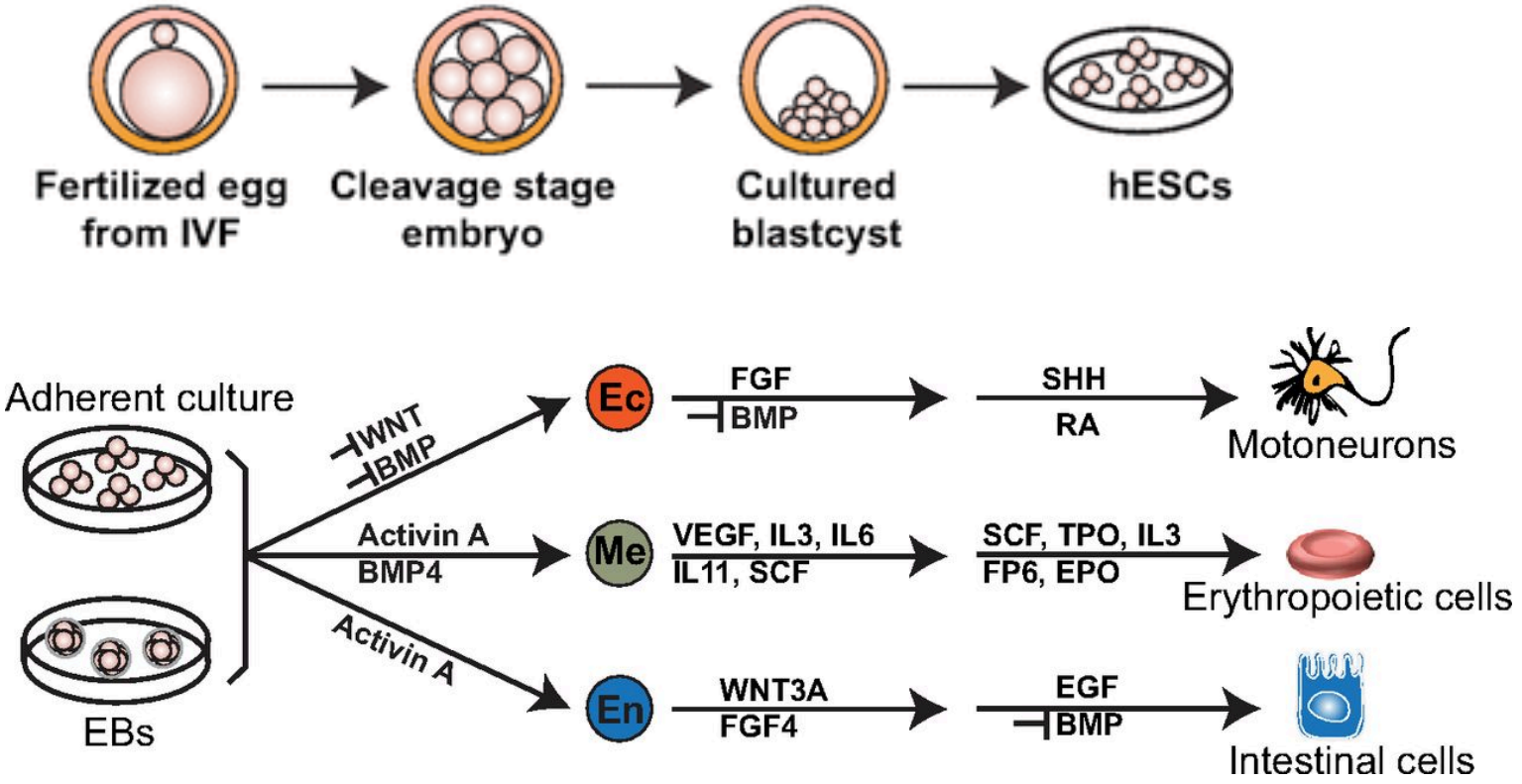
Understanding and treating disease through reprogramming



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

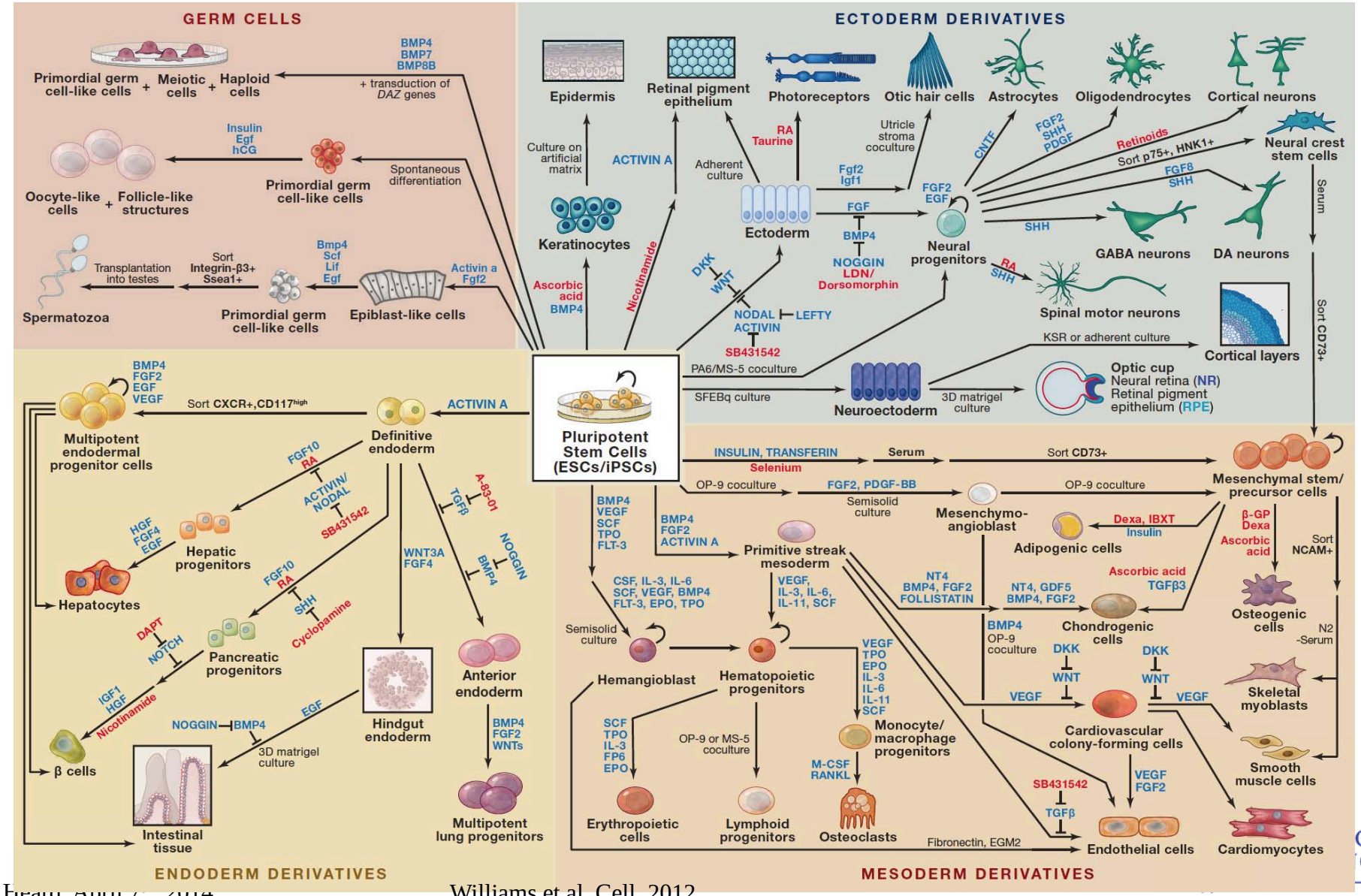
⇒ Developmental restrictions are due to reversible epigenetic modifications
rather than to permanent genetic changes

Pluripotent stem cells can be differentiated into multiple cell types



Zhu Z , and Huangfu D Development 2013;140:705-717

Pluripotent stem cells can be differentiated into multiple cell types

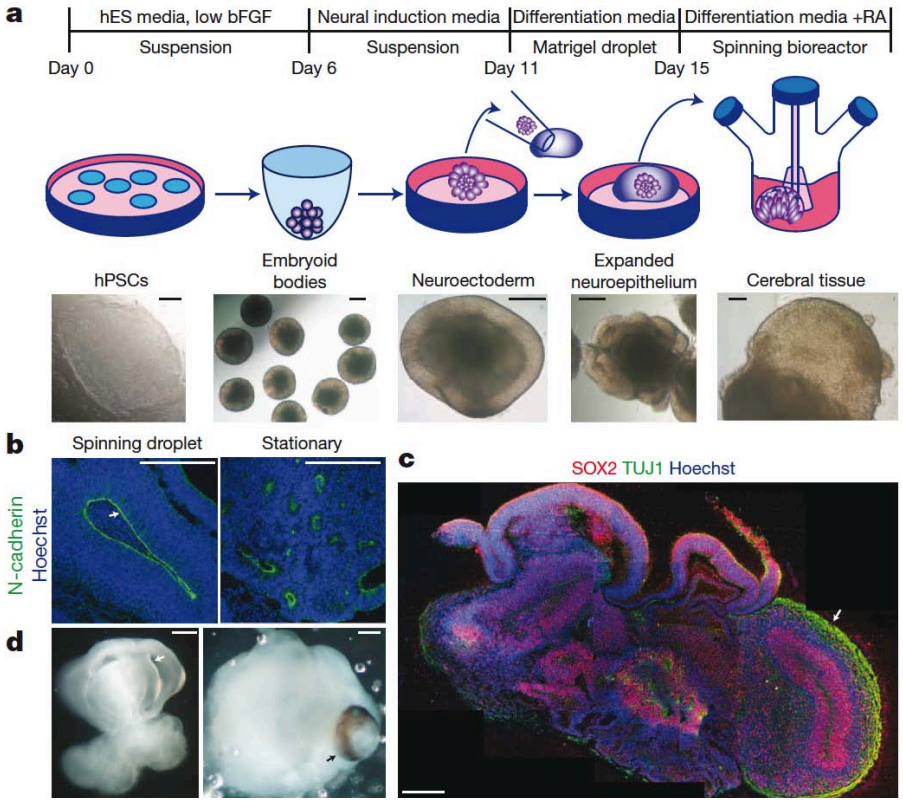


Williams et al, Cell, 2012

Pluripotent stem cells can be differentiated into organoids by 3D culture

Cerebral organoids

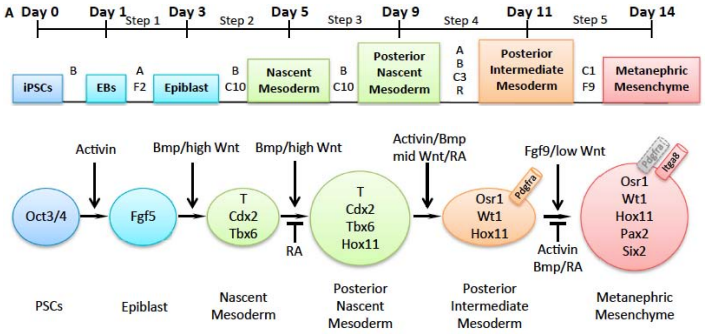
Lancaster et al, Nature, 2013

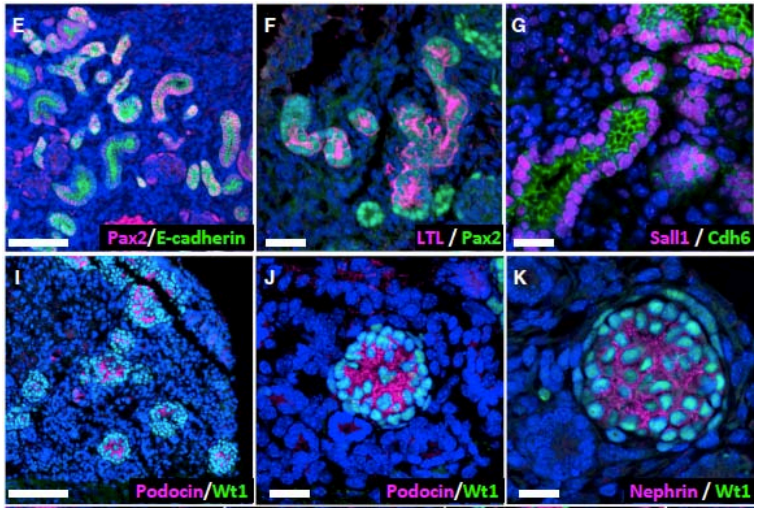


Using serum-free floating culture of embryoid-body-like aggregates with quick reaggregation) combined with the **well-timed** administration of **morphogenetic** cues (driving down one lineage and quelling others…..

Generation of Complex Kidney Structures from Pluripotent Stem Cells

Taguchi et al, Cell Stem Cell, 2013





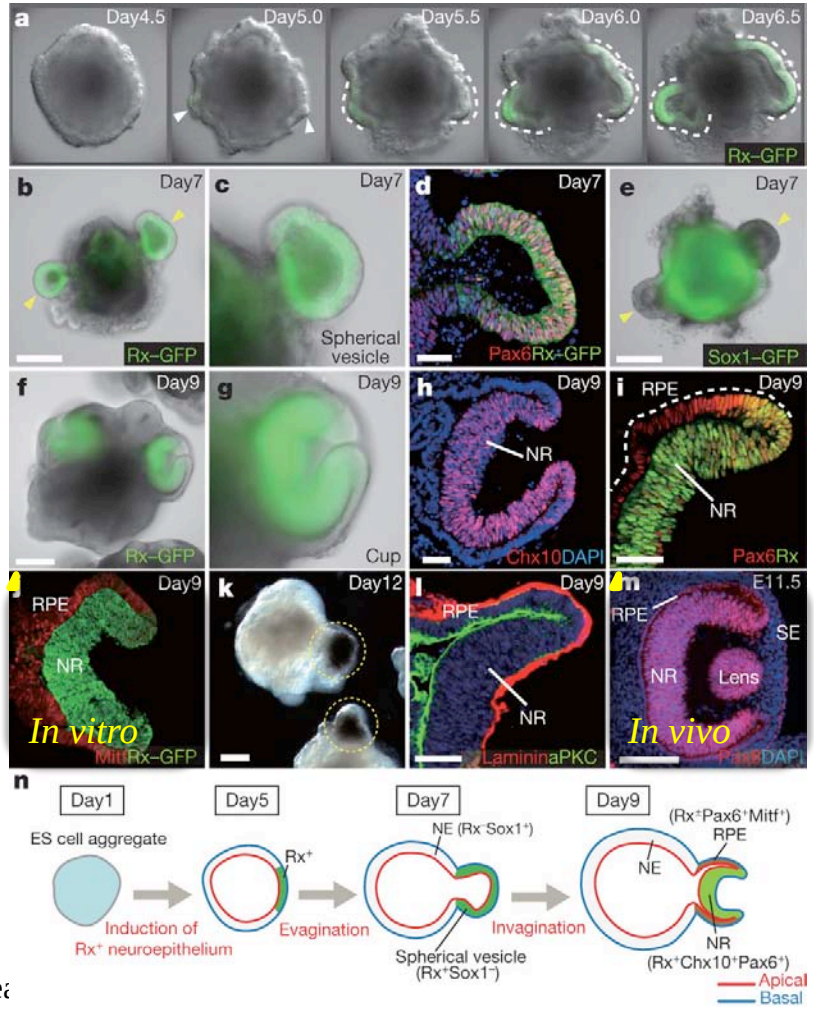
E. Hea[illegible]

COLLÈGE DE FRANCE 1530

Pluripotent stem cells can be differentiated into organoids by 3D culture

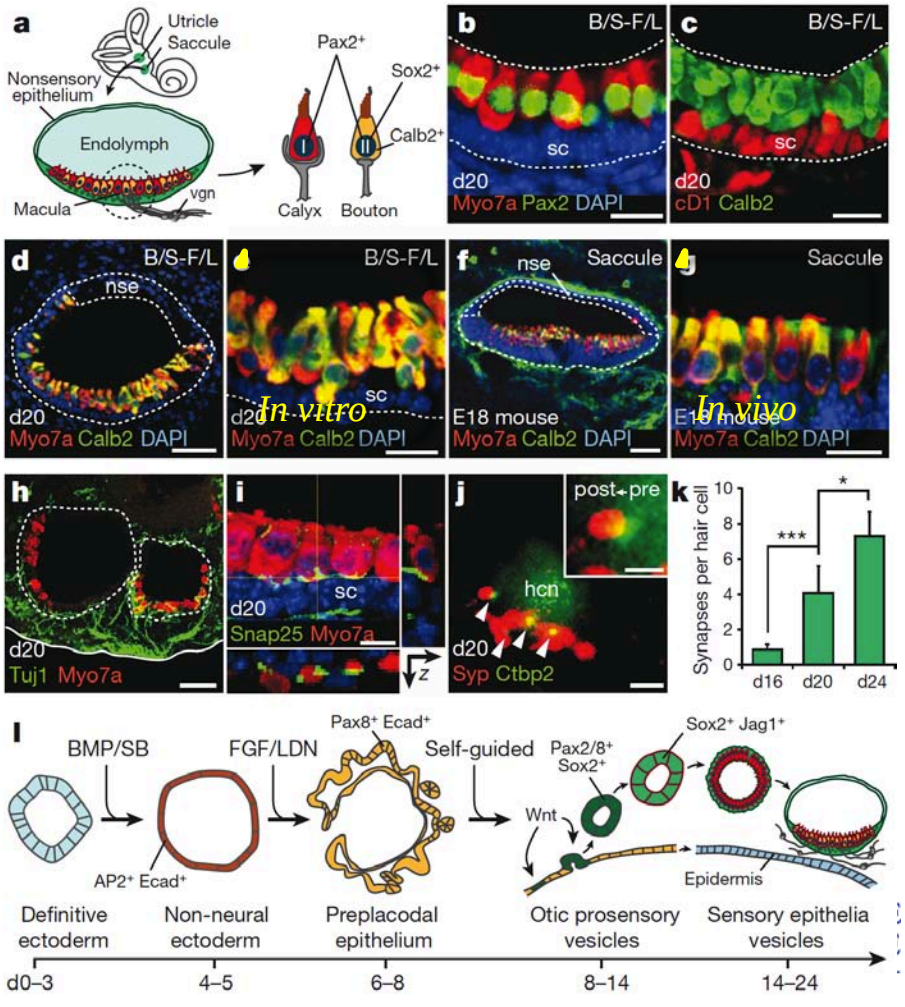
Optic cup formation by *in vitro* differentiation of ES cells & 3D culture

Eiraku and Sasai, Nature Prot., 2011

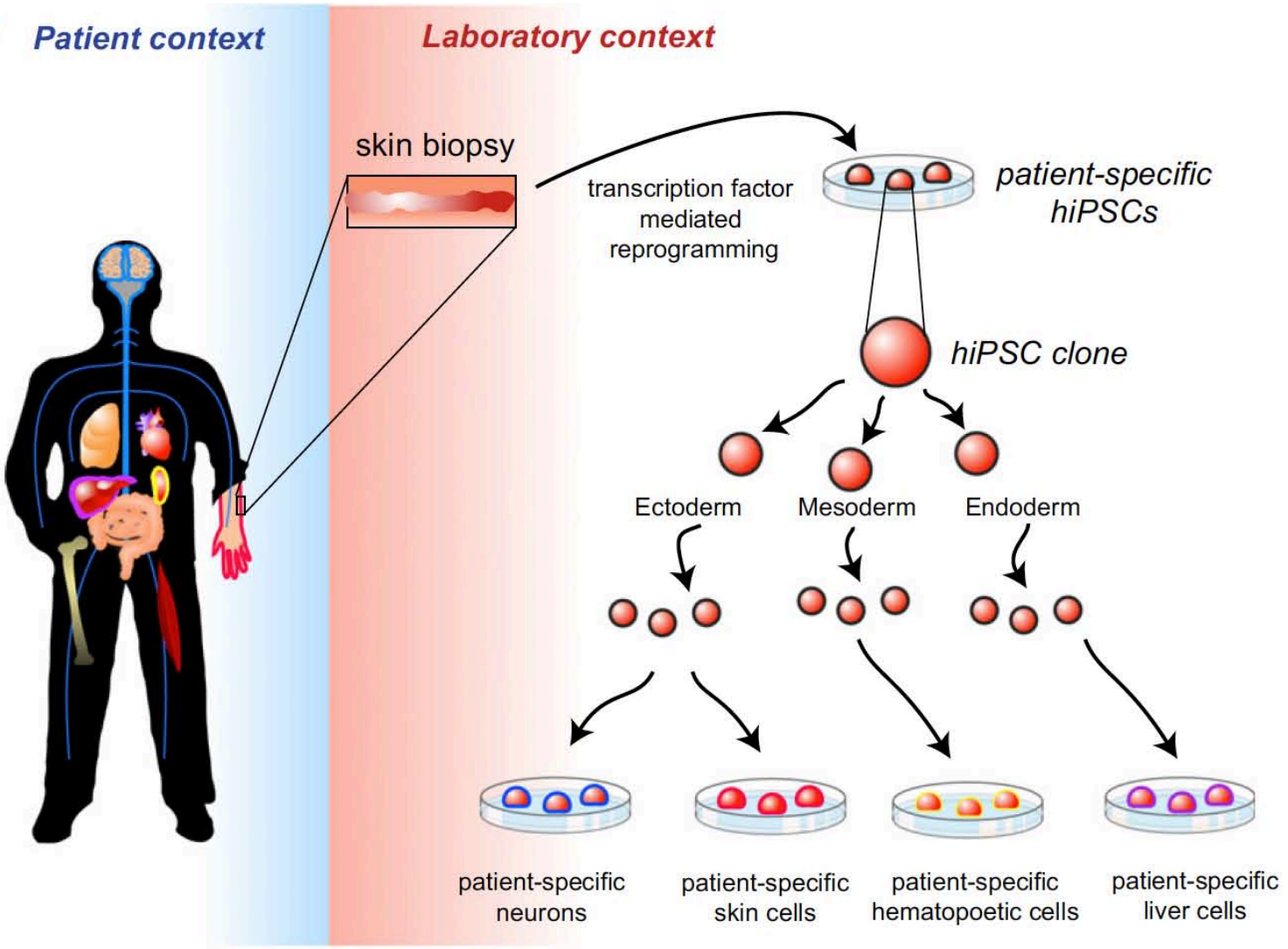


Generation of inner ear sensory epithelia from pluripotent stem cells in 3D culture

Koehler et al, Nature, 2013



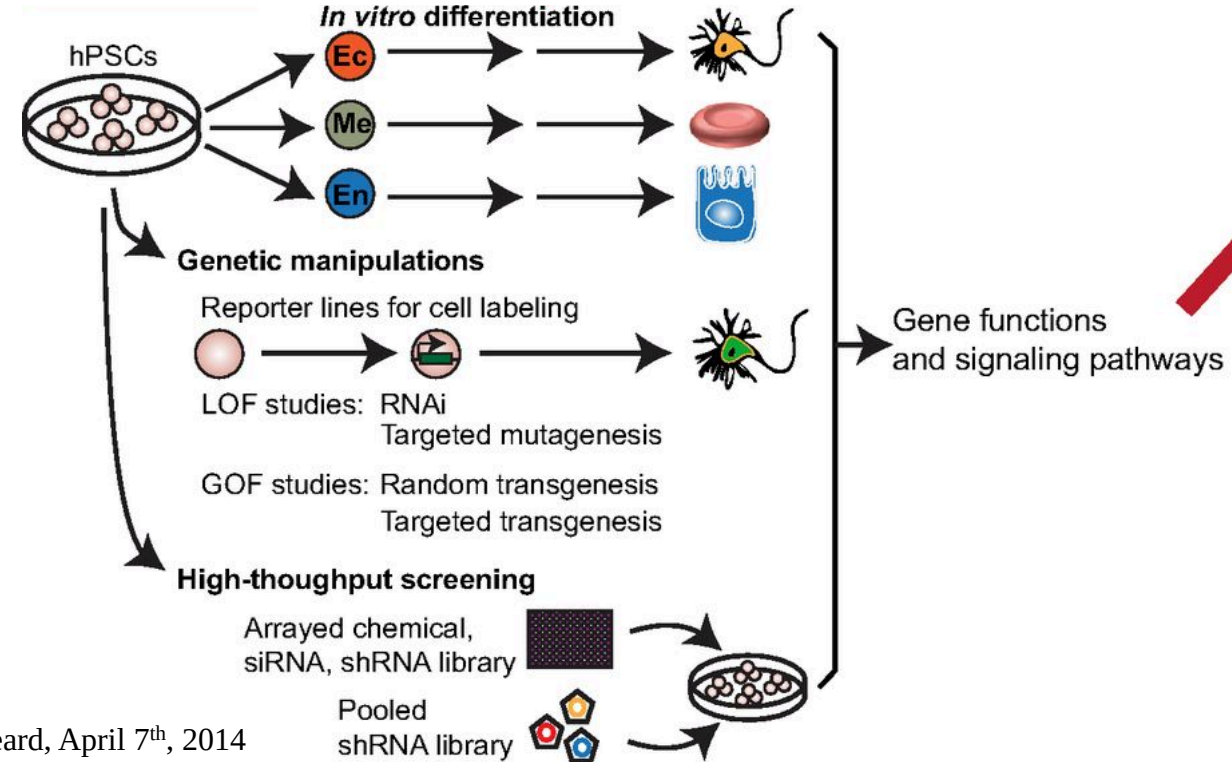
Perspectives brought by iPS cells

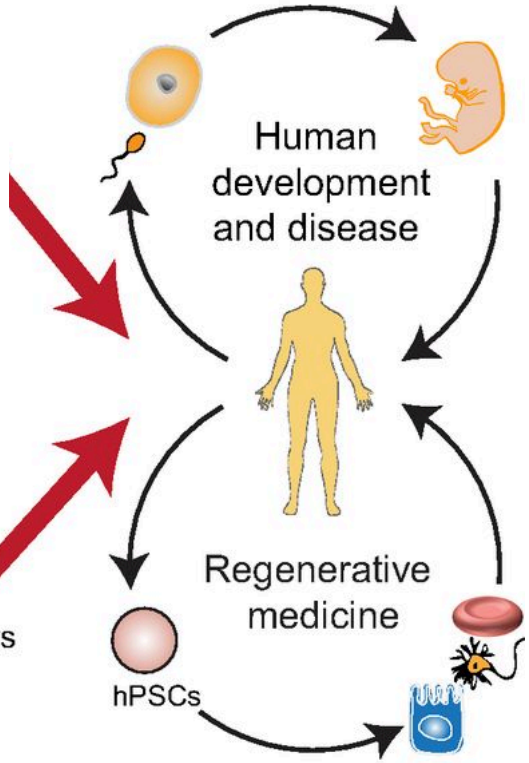


Saha and Jaenisch, Cell Stem Cell, 2009

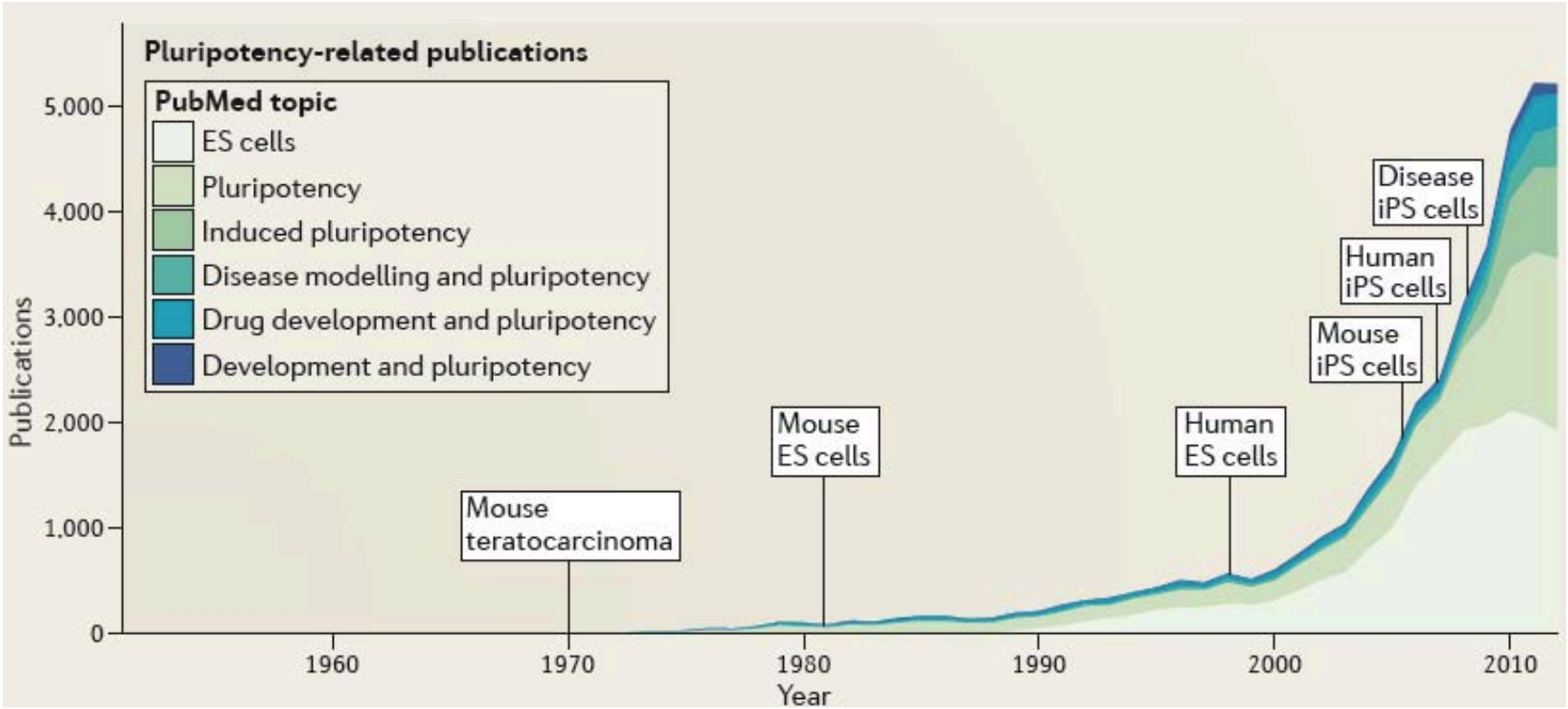
Perspectives brought by iPS cells

- Cell and Tissue therapy - *without use of human embryos* and reduces problems associated with compatibility
- Enables study of development and disease in humans
- Powerful tool for drug screening
- Permits genetic engineering for functional investigation of development and disease (eg after *in vitro* differentiation into organoids)

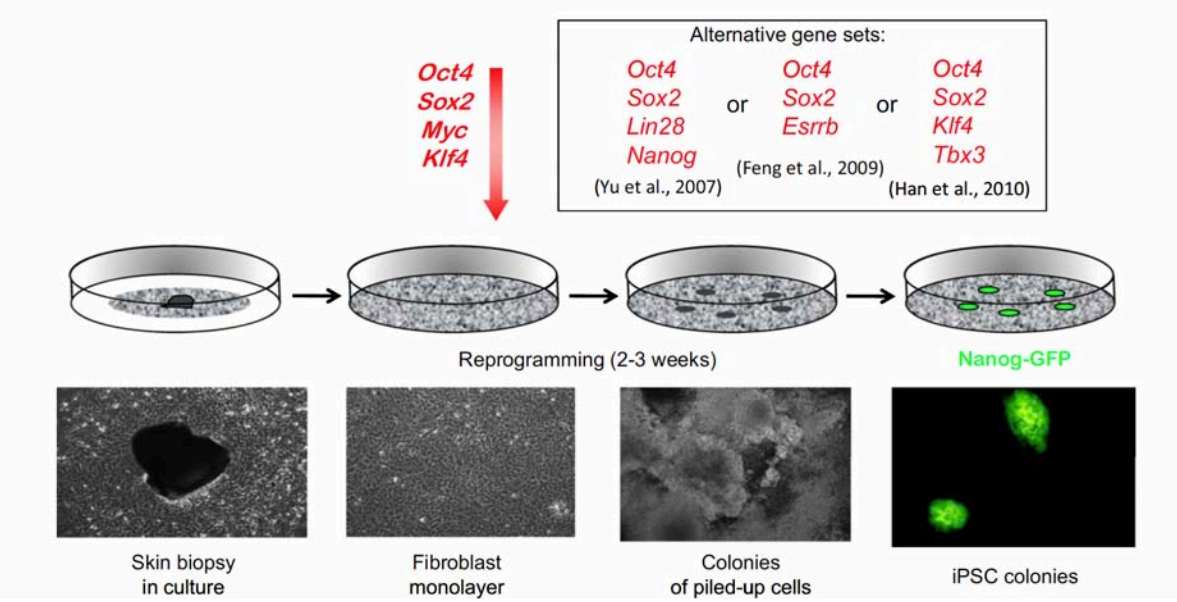




Perspectives brought by iPS cells



Issues raised by iPS cells



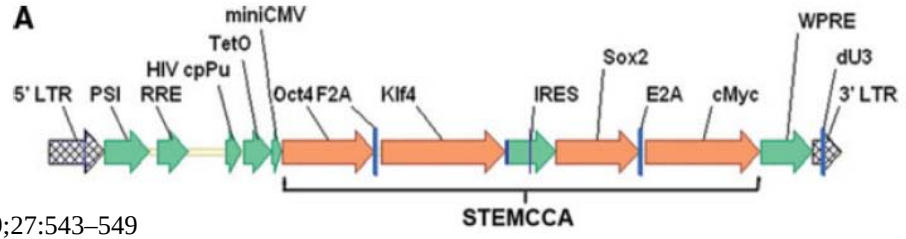
Quality control:
Exogenous transgenes silenced
Gene expression patterns
Cell surface markers
Epigenetic marks
X-reactivation in females (mouse)
Embryoid body formation
Teratomas
Germ line chimeras (mouse)

1. Efficiency and safety in reprogramming & culture conditions
2. Are iPS cells equivalent to hESCs? How pluripotent are they?
3. How efficient is differentiation?
4. How much does genetic or epigenetic variation (memory or aberrant reprogramming) interfere with differentiation?
5. Obtaining iPS cell lines swiftly and then sufficient cell numbers upon *in vitro* differentiation can be a problem (in a therapeutic context)
6. iPS brings a risk of cancer (either teratoma due to residual iPS cells or due to genetic/epigenetic modifications)

Episomal Vectors instead of Lentiviruses, Retroviruses & Plasmids to avoid integration and mutagenicity

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*]

Reprogramming differentiated human cells to induced pluripotent stem (iPS) cells has applications in basic biology, drug development, and transplantation. Human iPS cell derivation previously required vectors that integrate into the genome, which can create mutations and limit the utility of the cells in both research and clinical applications. We describe the derivation of human iPS cells with the use of nonintegrating episomal vectors. After removal of the episome, iPS cells completely free of vector and transgene sequences are derived that are similar to human embryonic stem (ES) cells in proliferative and developmental potential. These results demonstrate that reprogramming human somatic cells does not require genomic integration or the continued presence of exogenous reprogramming factors and removes one obstacle to the clinical application of human iPS cells.

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]

Induced pluripotency *without* exogenous transcription 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‡]

Stem Cell Reports
Article
ISSCR
OPEN ACCESS

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,*]

Small-molecule compounds alter cell signaling or epigenetic landscapes of somatic cells in reprogramming:

Hou et al. (2013) demonstrated that mouse fibroblasts can be induced to iPSCs solely by chemical manipulation - by seven small molecule compounds “chemical reprogramming”

The chemical combination activated upstream genes of Oct4, like Sall4 and Sox2, and also promoted Oct4 expression by epigenetic modulation.

Better option than reprogramming accomplished by either an RNA cocktail or a nonintegrating method with a single DNA vector that can be easily manufactured under current good manufacture practices (cGMP) conditions?

Factors that can improve iPS efficiency

Various growth factors and chemical compounds improve induction efficiency of iPS cells

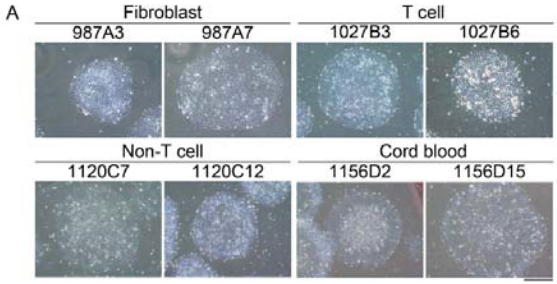
- DNA methyltransferase inhibitor (5'-azacytidine and RG108)
- Histone deacetylase (e.g., valproic acid) and methyltransferase inhibitors (BIX-01294)
- Signalling pathway inhibitors eg Wnt3A, ALK5 inhibitor etc

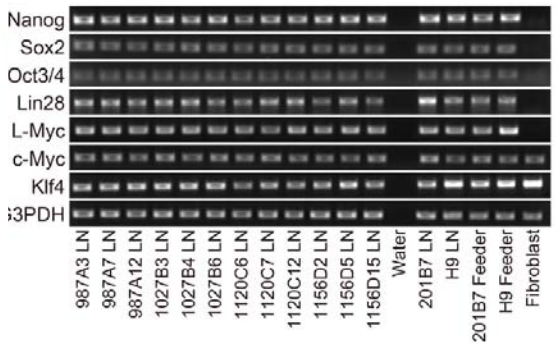
Molecule name	Target	Factors used	Cell type	Effect on reprogramming
Epigenetic modifiers				
EPZ004777	DOT1L	OS	Fibroblast	Decreases H3K79me2 levels at fibroblast EMT genes
Valproic acid	HDACs	OS	Fibroblast	20-fold efficiency increase for VPA+OSK
TSA, SAHA, NaB	HDACs	OSKM	Fibroblast	Increased efficiency over OSKM
Azacytidine	DNMT	OSKM	Fibroblast	Increases efficiency, converts partially reprogrammed cells to iPSCs
RG108	DNMT	OK	Fibroblast	OK+BIX-01294+RG108 enhances reprogramming over OK alone ~30-fold
BIX	H3K9 protein methyltransferases (PMTs)	OS KSM	Fibroblast Neural progenitor cell (NPC)	Combination eliminates the need for Oct4, NPCs express endogenous SOX2
Parnate	LSD1	OK	Keratinocyte	In combination with CHIR99021, allows keratinocyte reprogramming with OK only
Vitamin C	Histone demethylases	OSK	Fibroblast	Decreases p53 levels during reprogramming, promotes activity of the H3K36 demethylases Jhdm1a and Jhdm1b
Kinase inhibitors				
PD0325901	MEK		Fibroblast	Part of 2i cocktail
TGFβ inhibitors	ALK4, 5, 7	OK	Fibroblast	Activates Nanog in partially reprogrammed cells to facilitate transition to iPSCs
Kenpaullone	GSK3, cyclin-dependent kinases (CDKs)	OSM	Fibroblast, NPC	Identified in a high-throughput phenotypic screen to replace Klf4 in reprogramming
CHIR99021	GSK3	OK	Fibroblast	Part of 2i cocktail, increases efficiency with OSK, allows fibroblast reprogramming with OK only
BIM-0086660	Aurora kinase	OSKM	Fibroblast	Aurora A inhibition enhances Akt-mediated GSK3β inactivation
Rapamycin	Mammalian target of	OSKM	Fibroblast	Reports a correlation between longevity-promoting

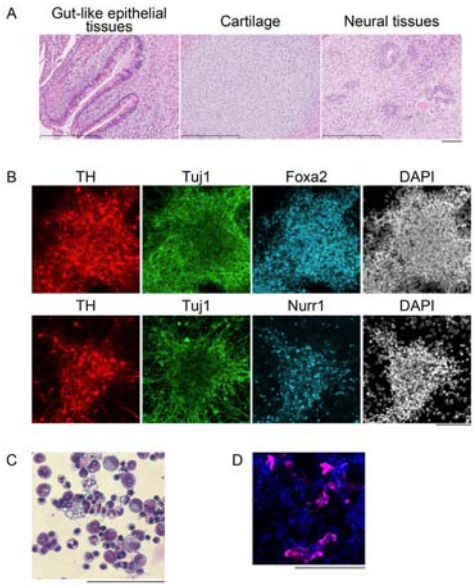
iPS culture conditions to improve safety

Novel feeder-free and xeno-free cell culture system for hESCs and hiPSCs

- Recombinant laminin-511 E8 fragments and StemFit™
- Easy to use, expandable and reproducible
- For clinical-grade hiPSCs - according to Standard Operating Procedures (SOPs) in order to meet Cell Processing Center (CPC) standards.



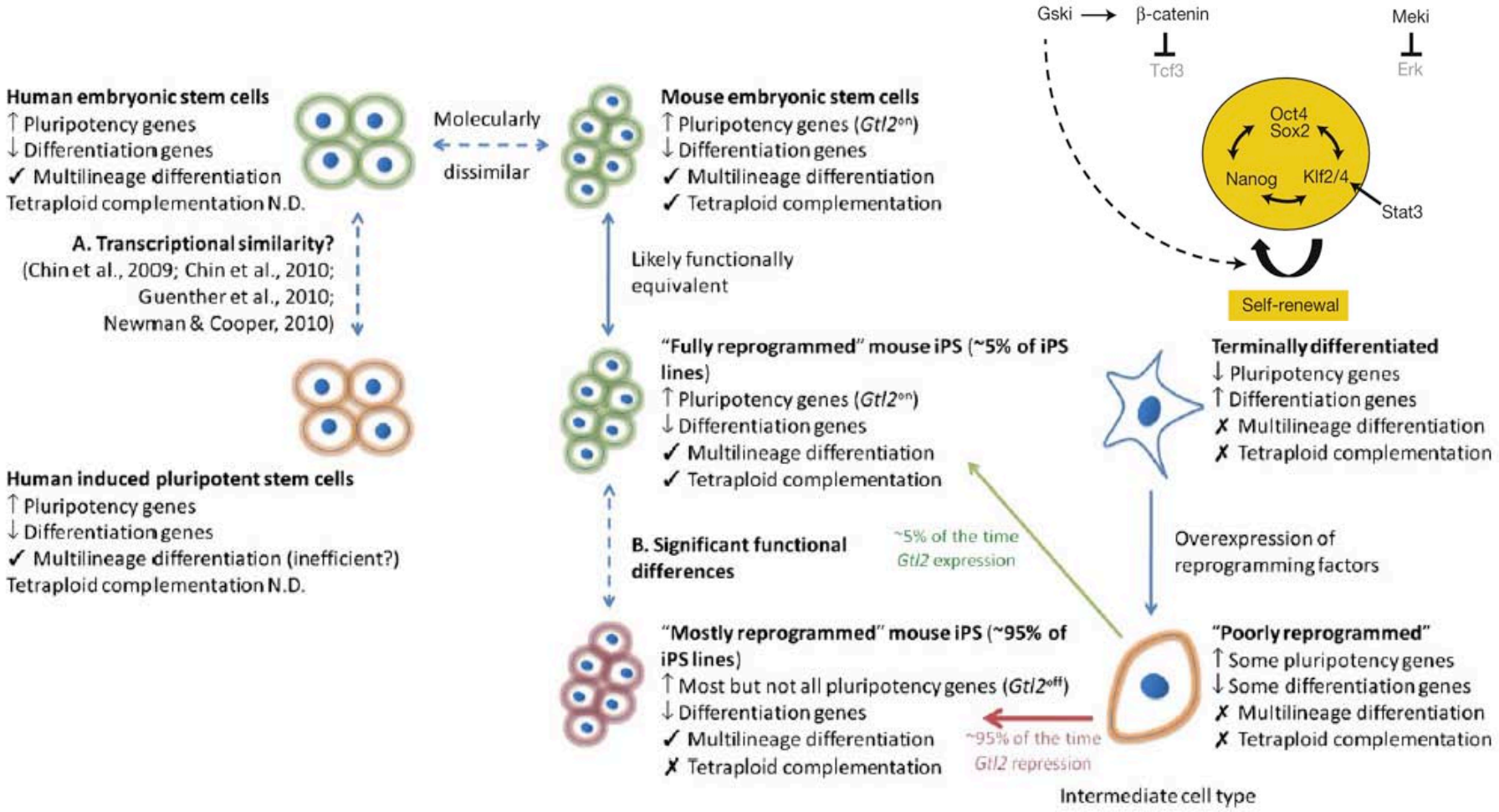




rLN511E8 matrix and StemFitTM medium (a newly developed Xf-medium for hiPSC culture from Ajinomoto Co., Inc.)

Nakagawa et al "A novel efficient feeder-free culture system for the derivation of human induced pluripotent stem cells" Scientific Reports 4:3594 (2014) DOI: 10.1038/srep03594

Are iPS cells truly equivalent to ES cells?

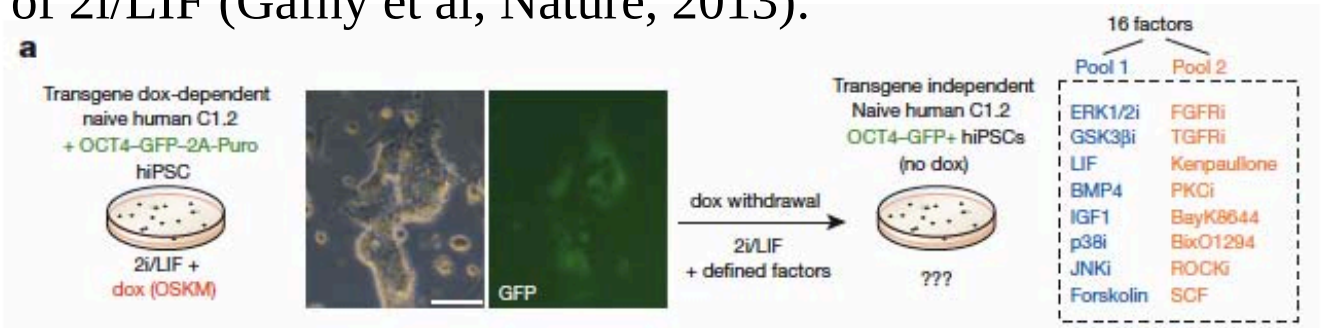


Can human iPSCs truly recapitulate differentiation if they are not in the « ground state »?

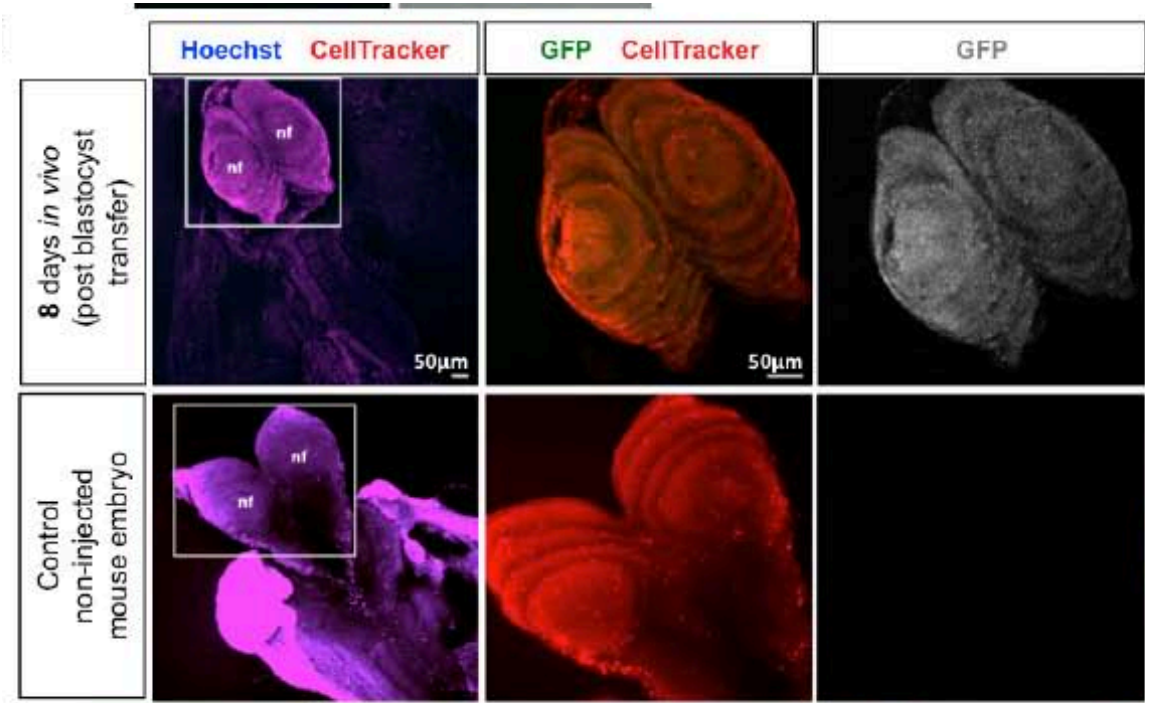
Adapted from Loh and Lim, 2010

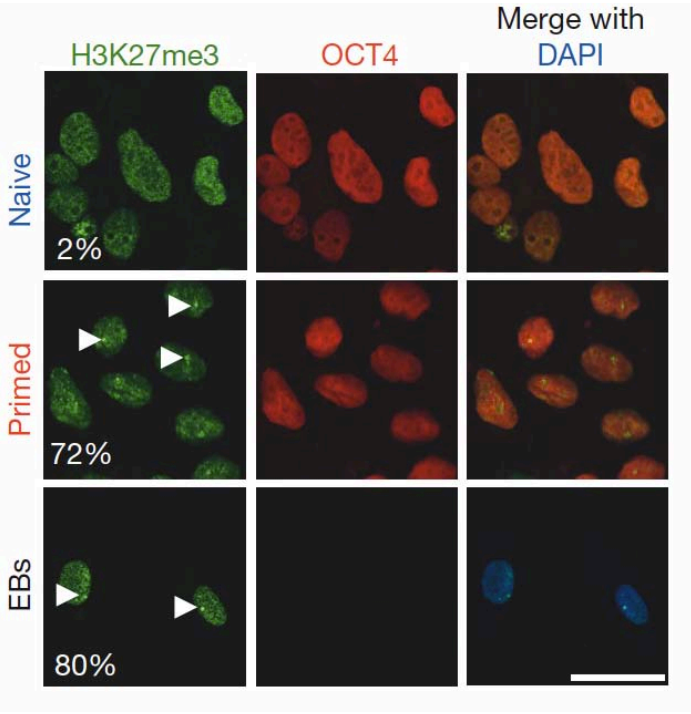
Capturing the ground state of human naïve pluripotency

Hanna lab conditions to isolate naive transgene-independent human iPS cells in presence of 2i/LIF (Gafny et al, Nature, 2013):



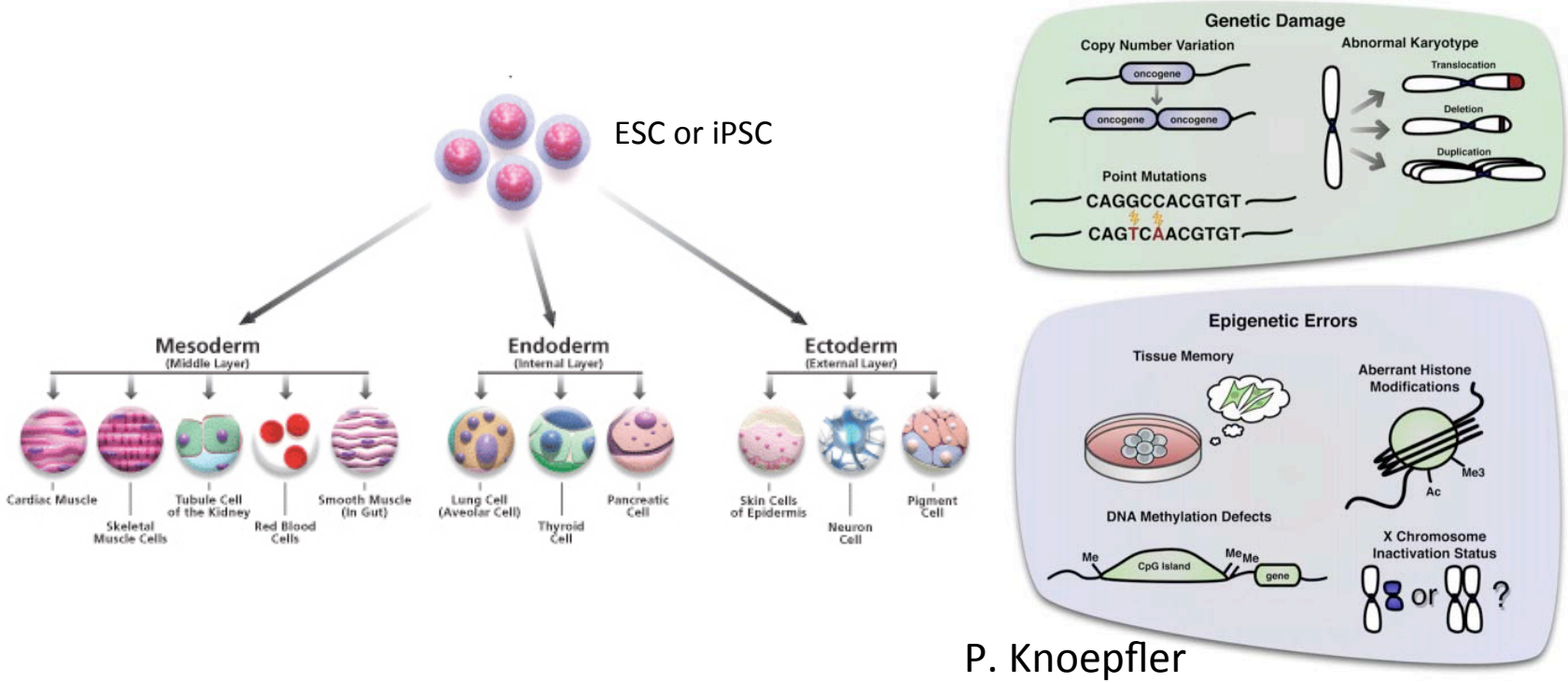
"Ground state" human iPS cells have <u>active</u> Xs, contribute efficiently to mouse embryos…





In vitro differentiation potential of hES and iPS cells?

Despite apparent functional equivalence by teratoma formation, human ESC and iPSCs have a tendency to differentiate into specific lineages *in vitro.*



P. Knoepfler

Spontaneous or directed differentiation to cardiomyocytes, pancreatic cell types and motor neuron differentiation in multiple distinct human ESC and iPSC lines –significant variation in the expression of most germ layer markers and their derivatives

Osafune, K. *et al.* Marked differences in differentiation propensity among human embryonic stem cell lines. *Nature Biotechnol.* **26**, 313–315 (2008)

Kim et al. Epigenetic memory in induced pluripotent stem cells. Nature 467, 285–290 (2010).

Kim et al. Donor cell type can influence the epigenome and differentiation potential of human induced pluripotent stem cells. Nature Biotechnol. 29, 1117–1119 (2011).

Polo et al. Cell type of origin influences the molecular and functional properties of mouse induced pluripotent stem cells. Nature Biotechnol. 28, 848–855 (2010).

Bar-Nur et al. Epigenetic memory and preferential lineage-specific differentiation in induced pluripotent stem cells derived from human pancreatic islet β-cells. Cell Stem Cell 9, 17–23 (2011).

Lister, R. et al. Hotspots of aberrant epigenomic reprogramming in human induced pluripotent stem cells. Nature 471, 68–73 (2011).

Incomplete or aberrant reprogramming of the donor cell or genetic variation/mutation?

• Differentially methylated CG regions in ES cells vs iPS cells reflect both epigenetic memory and aberrant epigenetic reprogramming events in iPS cells.

• Several 'hotspots' of aberrant epigenetic reprogramming.

• DNA methylation patterns of diverse founder cell types remain detectable in reprogrammed mouse iPS cells

• Epigenetic memory correlates with distinct *in vitro* differentiation propensities
Eg iPS cells derived from blood had a greater in vitro blood-forming capacity than fibroblast-derived iPS cells and neural progenitor-derived iPS cells.

• **Epigenetic memory can be erased by extended culture…**

Kim et al. Epigenetic memory in induced pluripotent stem cells. Nature 467, 285–290 (2010).
Kim et al. Donor cell type can influence the epigenome and differentiation potential of human induced pluripotent stem cells. Nature Biotechnol. 29, 1117–1119 (2011).
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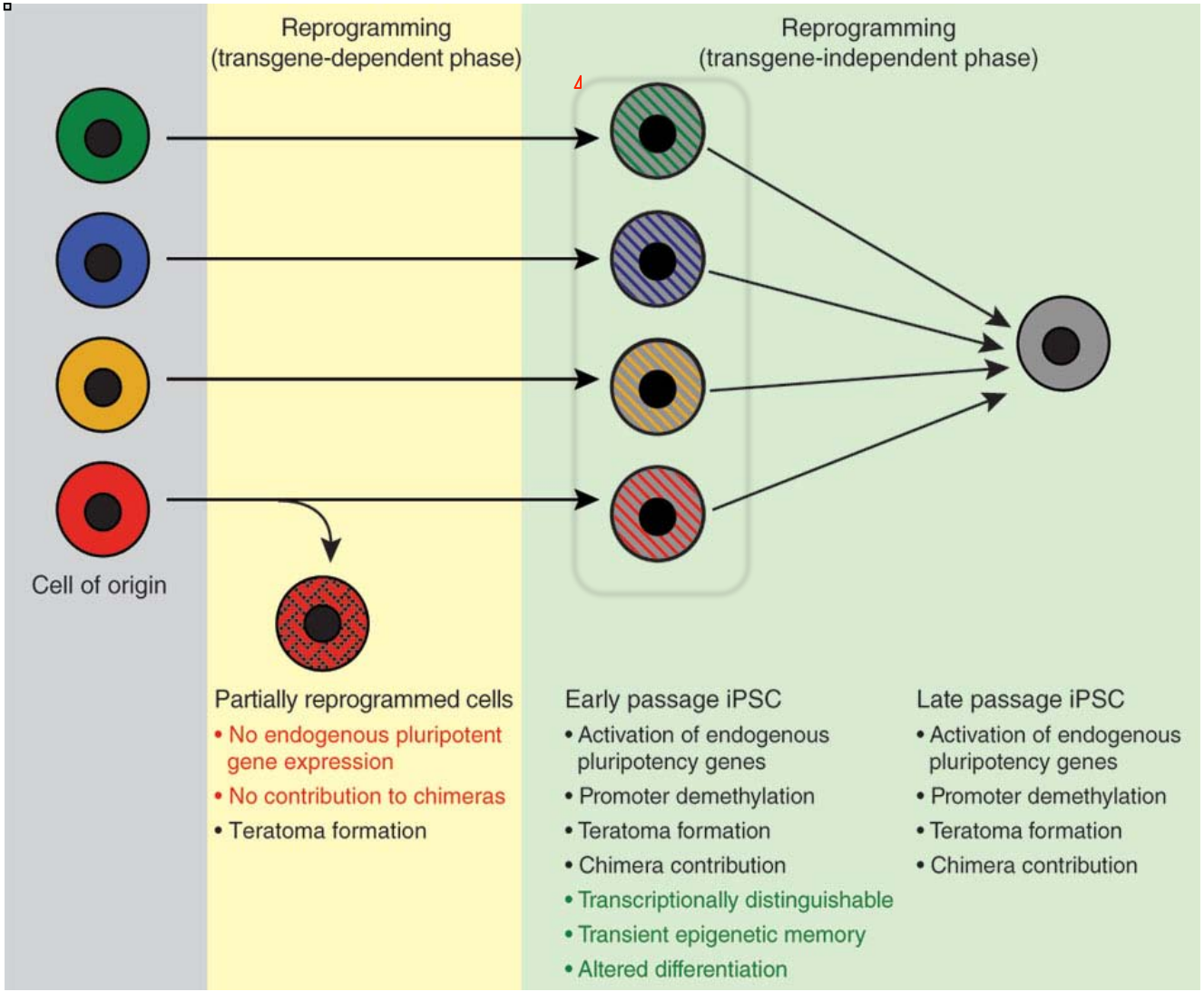
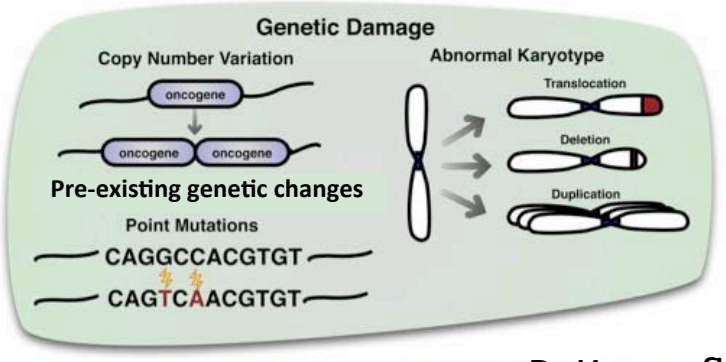
Epigenetic memory?



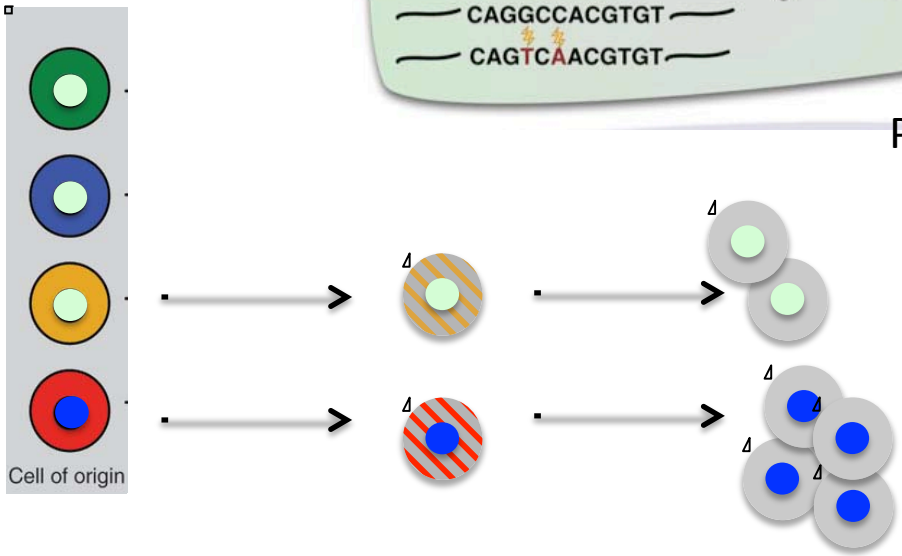
Genetic variation / mutation?

• Sequence-based mapping of DNA copy number differences between founder cells and reprogrammed human iPS cells showed that rather than being induced by reprogramming, most DNA copy number variants (CNVs) are already present as rare alleles in the founder population (Young et al, 2012; Abyzov et al, 2012).

Somatic cells with DNA sequence variants



P. Knoepfler



iPSCs with different growth and differentiation potentials?

Young, M. A. *et al.* Background mutations in parental cells account for most of the genetic heterogeneity of induced pluripotent stem cells. *Cell Stem Cell* **10**, 570–582 (2012).

Abyzov, A. *et al.* Somatic copy number mosaicism in human skin revealed by induced pluripotent stem cells. *Nature* **492**, 438–442 (2012).

Genetic variation / mutation?

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• Act of reprogramming itself does not seem to induce a high rate of mutations in common genes or pathways (*though some recurrent variations in pluripotent stem cells do occur – eg duplication of chromosome 20 - due to adaptation to TC*?)

• Find approx. 5–10 coding mutations per iPS cell genome compared with donor cells, but most of these mutations **already exist in the donor cell population** and are captured and amplified by clonal selection.

• The possibility that tumour-suppressors and oncogenes are altered in iPS cells means that any potential therapeutic use of an iPS cell line will require exhaustive DNA screening ….

• The cloning event that occurs during the reprogramming process allows analysis of the development of complex disease-harboring somatic mosaicism
⇒ useful for development of Disease Models and in vitro differentiation

Yc
St

Abyzov, A. *et al.* Somatic copy number mosaicism in human skin revealed by induced pluripotent stem cells. *Nature* **492**, 438–442 (2012).

Comparison of differentiation capacities of hES and iPS cells

iPS cells vary significantly and reproducibly in differentiation efficiency, but these differences are not correlated to the source, karyotype or residual transgene expression.

- Marked diversity in inherent differentiation propensity (Cahan and Daley, 2013 for review)
- Some of this is due to "lab-specific" differences (eg Newman and Cooper, 2010)
- Some differentiation defects can be overcome by finding appropriate culture conditions.
- Some may be due to epigenetic differences or to genetic variation…

Open questions:

- Does the isolation and propagation of pluripotent stem cells select for consistent genetic changes?
- Do such changes have oncogenic potential?
- Which mutations affect pluripotent stem cell function?

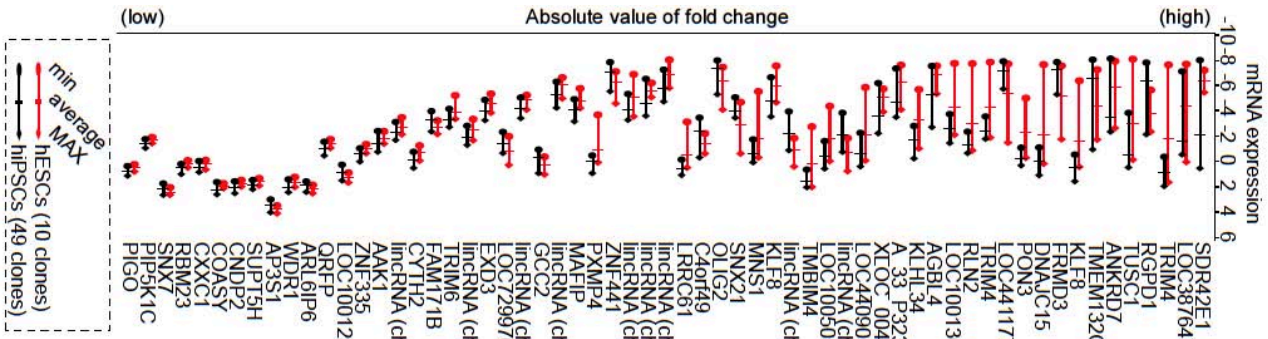
Investigations of the *in vitro* differentiation propensities of large numbers of human pluripotent stem cell lines (ES and iPS)

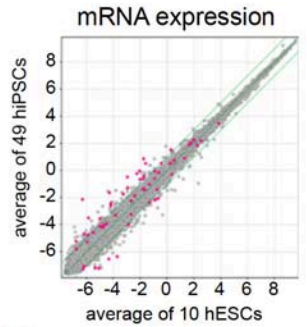
Comparison of differentiation capacities of hES and iPS cells

Gene expression and DNA methylation were examined in 49 human iPSC lines and 10 human ES cell lines:

- Overlapping variations in gene expression and DNA methylation

49 hiPSCs derived from 4 types of somatic cells (human dermal fibroblasts, dental-pulp stem cells, cord blood cells and peripheral blood mononuclear cells generated using 3 gene delivery methods, including those using retroviruses, non-integration episomal plasmids, and Sendai viruses.





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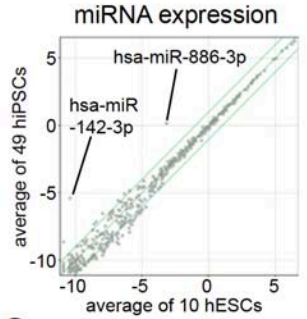

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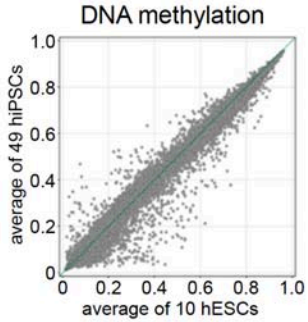


Koyanagi-Aoi et al "Differentiation-defective phenotypes revealed by large-scale analyses of human pluripotent stem cells" PNAS, 2013

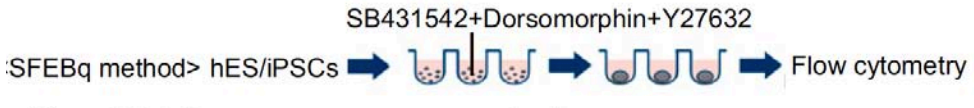
Comparison of differentiation capacities of hES and iPS cells

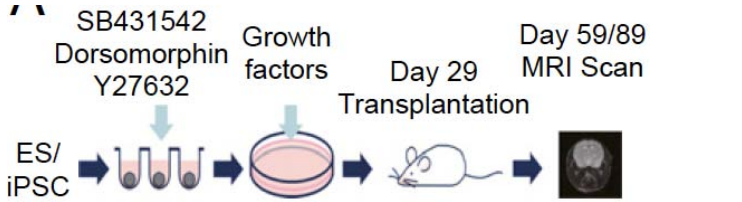
Gene expression and DNA methylation were examined in 49 human iPSC lines and 10 human ES cell lines:

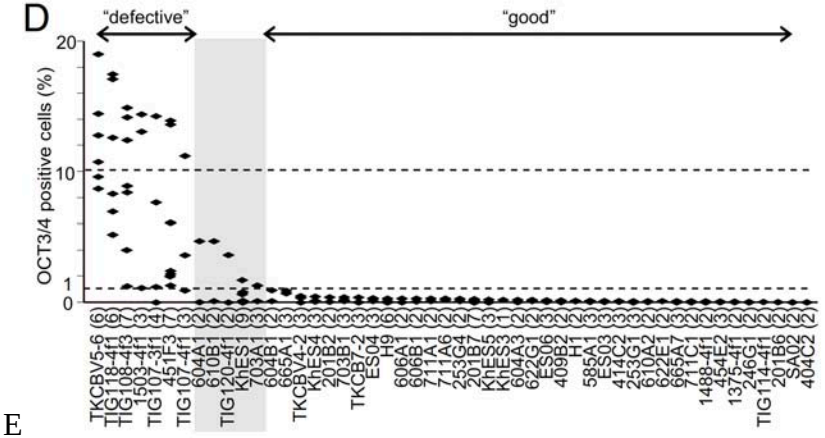
- Overlapping variations in gene expression and DNA methylation
- Comparisons of in vitro neural differentiation of 40 hiPSCs and 10 human embryonic stem cells

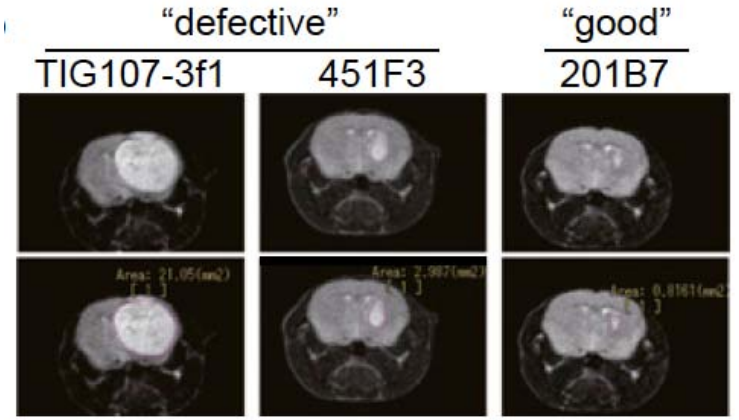
➢ 7 hiPSC clones retained a significant number of undifferentiated cells even after neural differentiation culture and formed teratomas when transplanted into mouse brains.

In vitro directed differentiation into neural stem and progenitor cells using serum-free floating culture of embryoid body-like aggregates (SFEBq) method:









E

Comparison of differentiation capacities of hES and iPS cells

Gene expression and DNA methylation were examined in 49 human iPSC lines and 10 human ES cell lines:

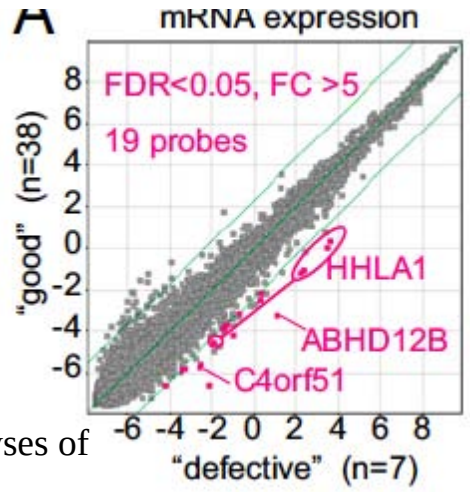
- Overlapping variations in gene expression and DNA methylation
- Comparisons of in vitro neural differentiation of 40 hiPSCs and 10 human embryonic stem cells

➢ 7 hiPSC clones retained a significant number of undifferentiated cells even after neural differentiation culture and formed teratomas when transplanted into mouse brains.

➢ The 7 differentiation-defective hiPSC clones were marked by higher expression levels of several genes, including those expressed from **LTRs of human endogenous retroviruses.**

Compared global gene expression patterns of 38 good clones and seven differentiation-defective clones in undifferentiated state:

13 genes, several of which correspond to **an aberrantly activated LTR7 (human endogenous retrovirus-H (HERV-H) adjacent to/within the gene.**



Koyanagi-Aoi et al "Differentiation-defective phenotypes revealed by large-scale analyses of human pluripotent stem cells" PNAS, 2013

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➢ The 7 differentiation-defective hiPSC clones were marked by higher expression levels of several genes, including those expressed from LTRs of human endogenous retroviruses.

⇒ Some hiPSC lines have **aberrant gene expression** and **defective potential in neural differentiation**

"A subset of hiPSCs is defective in neural differentiation and marked with activation of endogenous retroviruses. We also confirmed that some hiPSCs are different from hESCs in molecular signatures, including CpG DMRs, which has been previously reported. **It remains to be determined whether these molecular signatures specific for some hiPSCs have functional consequences**."

Koyanagi-Aoi et al "Differentiation-defective phenotypes revealed by large-scale analyses of human pluripotent stem cells" PNAS, 2013

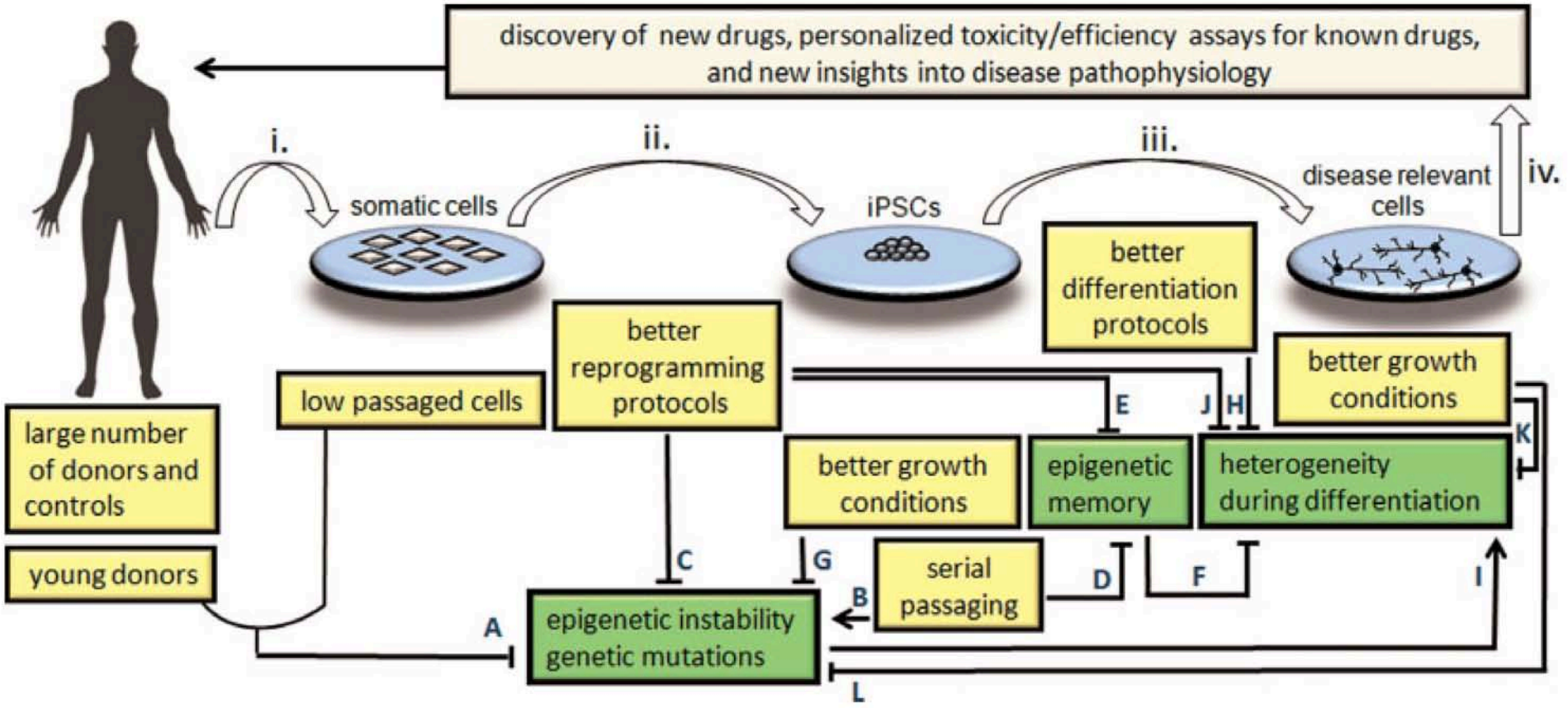
Perspectives for *in vitro* differentiation use of iPS cells

1. Identify genetic variants and molecular markers (eg epigenetic markers) indicative of the **differentiation capacity** for specific lineages.

2. Improve differentiation conditions - application of high-throughput approaches to develop directed differentiation protocols that are more robust for pluripotent stem cells.

3. Cell purification strategies (eg FACs using cell surface markers) to isolate mature cells and avoid iPSC contaminants (with risk of teratoma)

Perspectives for *in vitro* differentiation use of iPS cells

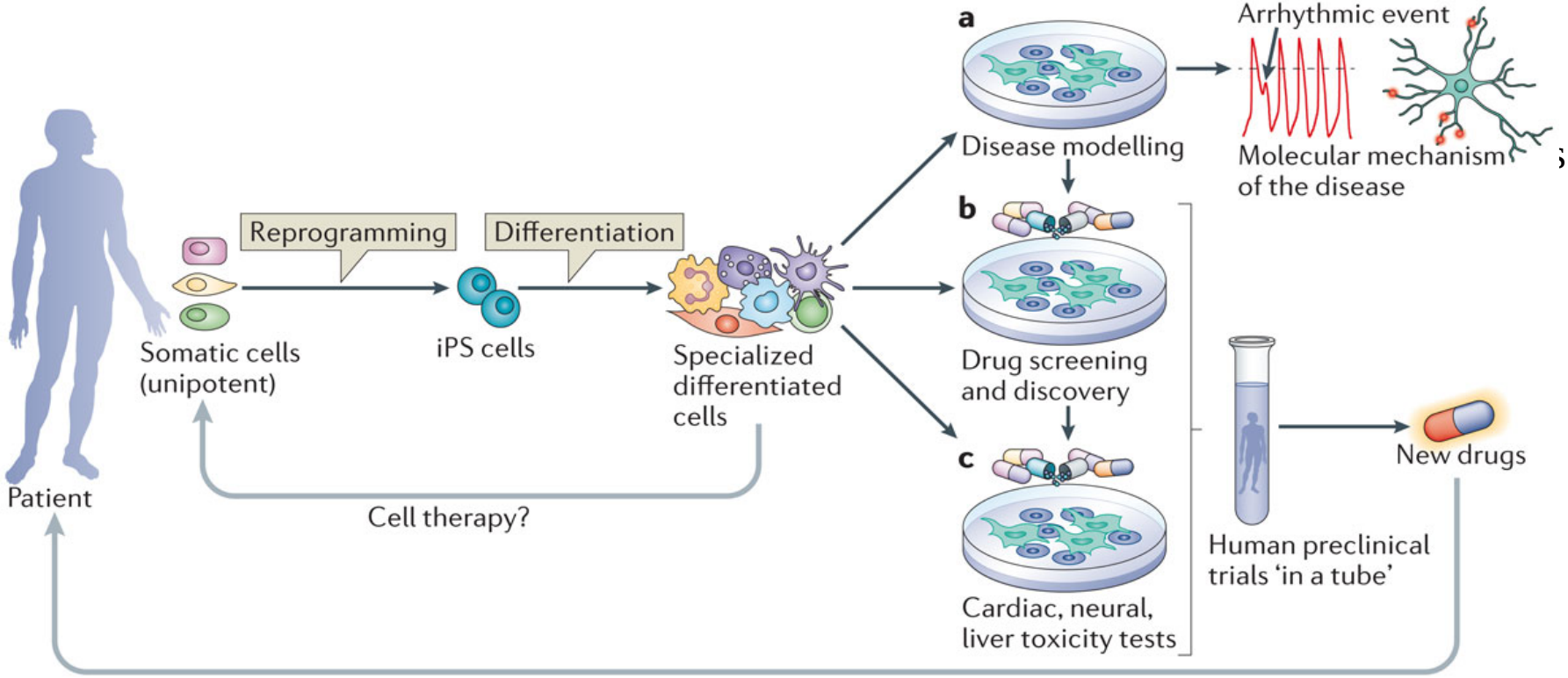


Pomp and Colman, Bioessays, 2012

Using iPS cells to model disease

First disease-specific iPSC lines (Dimos et al., 2008; Park et al., 2008)

In vitro reconstruction of the disease state eg spinal muscular atrophy (SMA) (Ebert et al., 2009; Ebert et al., 2012); several patient-specific iPSC lines now established for disease modeling, and should facilitate studies on rare diseases and drug development.



Nature Reviews | Molecular Cell Biology

Bellin et al, 2012 "Induced pluripotent stem cells: the new patient?"

Using iPS cells to model disease

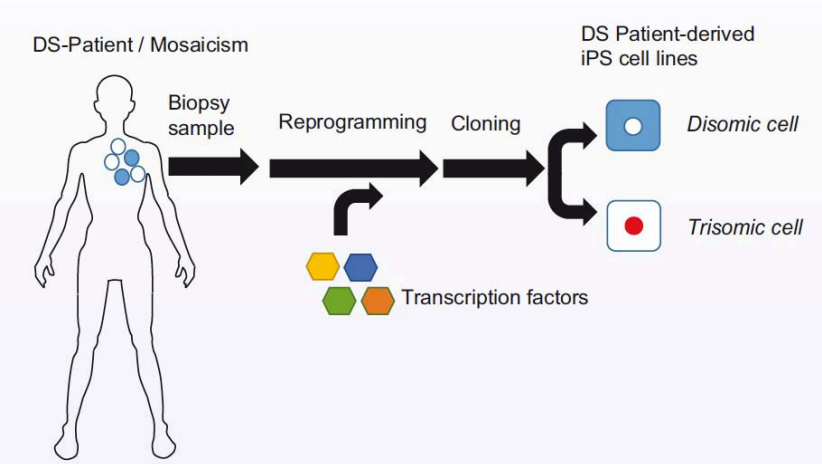
Down's Syndrome

DS = most frequent form of mental retardation due to autosomal trisomy of all or a critical portion of chromosome 21.

Patients with DS present with multiple disorders (eg, congenital heart defects, particularly atrioventricular septal defect, leukemia, and early-onset Alzheimer disease).

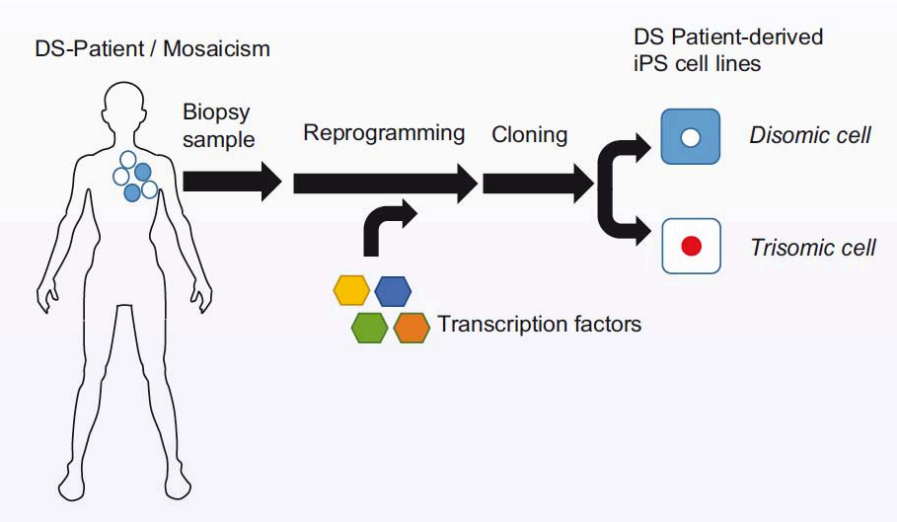
In ≈2% of cases, live-born trisomy 21 individuals display cellular mosaicism for the trisomy Shin et al, 2010).

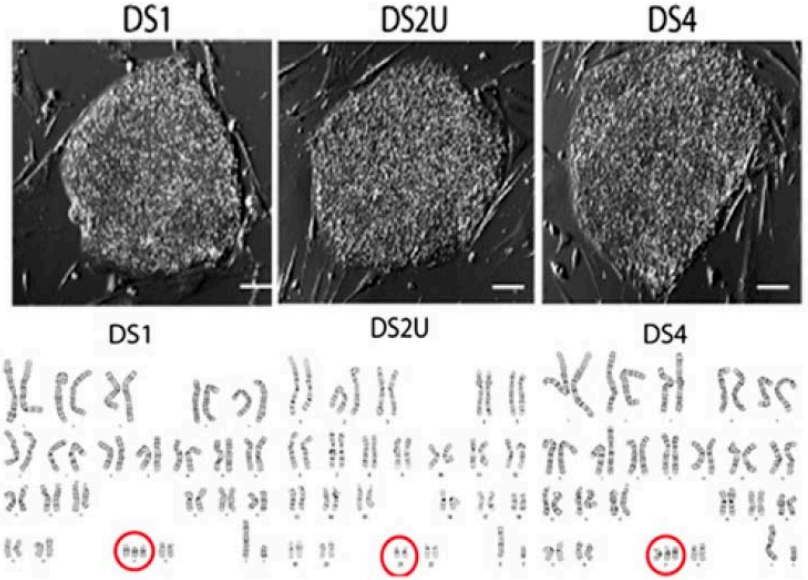
In mosaic individuals find correlation between frequency of trisomic cells and the patient phenotype (eg inverse correlation for IQ scores in individuals with mosaic trisomy 21.



- Shin M, Siffel C, Correa A. Survival of children with mosaic Down syndrome. *Am J Med Genet A.* 2010;152A:800–801.
- Papavassiliou et al. The phenotype of persons having mosaicism for trisomy 21/Down syndrome reflects the percentage of trisomic cells present in different tissues. Am J Med Genet . 2009;149A:573–583.

Using iPS cells to investigate Down's syndrome



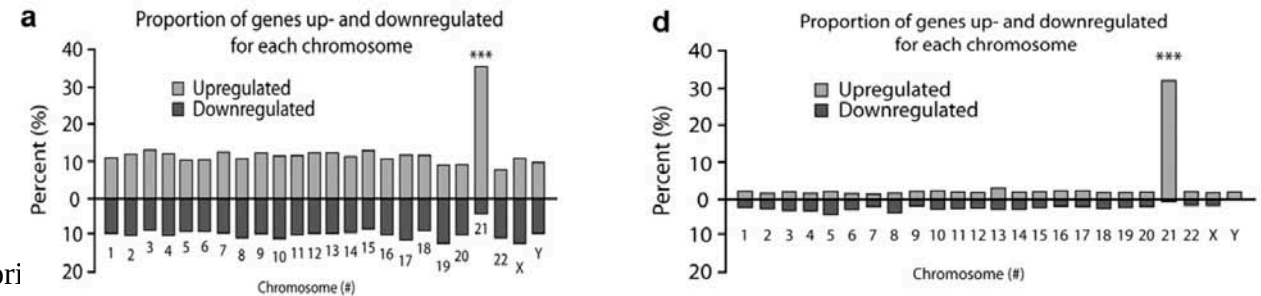


Global gene expression of 30-d-old neurons from DS1 and DS4 Ts21 iPSCs compared with those neurons from euploid DS2U iPSCs .
Ts21 neurons = preferential increase in HSA21 gene expression.

Cell line identifier	Karyotype	iPSC line name	Source fibroblasts (Coriell), gender, age	Reprogramming method
DS1	Ts21	UWWC1-DS1	AG05397, male, 1 year	retrovirus
DS2U	Euploid	UWWC1-DS2U	AG05397	retrovirus
DS4	Ts21	UWWC1-DS4	AG05397	retrovirus
2DS3	Ts21	UWWC1-2DS3	GM02504, male, 1 month	Sendai virus

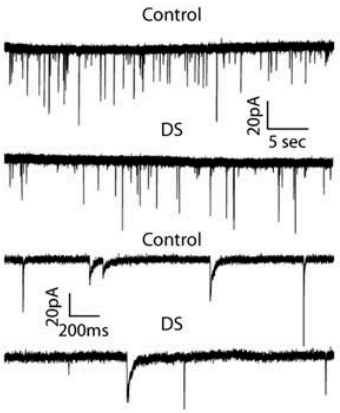
Largest gene expression changes (>5-fold) in both Ts21 iPSCs and neurons were genes (on other chromosomes) associated with <u>transcriptional regulation</u> and <u>Oxidative Stress</u>

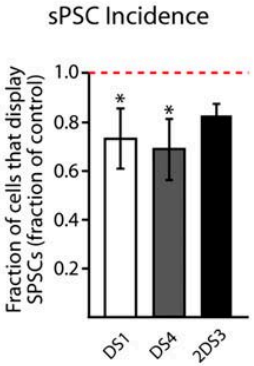
iPSCs **Neurons**

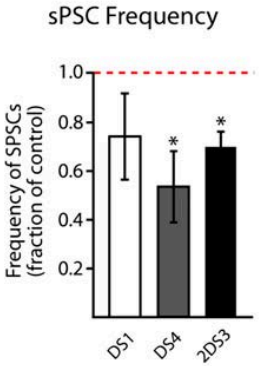


Using iPS cells to model disease

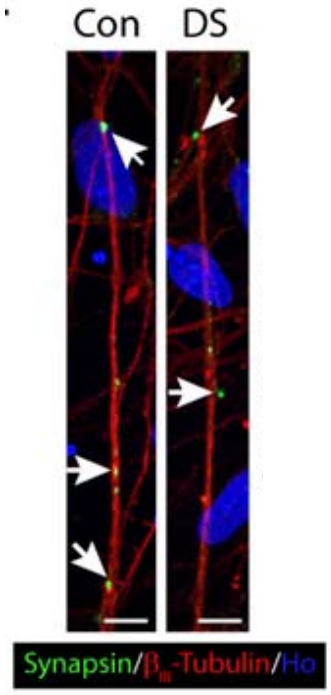
Changes in neuronal excitability and synaptic efficacy have been shown to contribute to cognitive impairment in DS mouse models. Whole-cell patch clamp recordings performed on Ts21 iPSC-derived neurons between 5 and 6 wk, a time when human PSC-derived neurons have substantial synaptic activity:



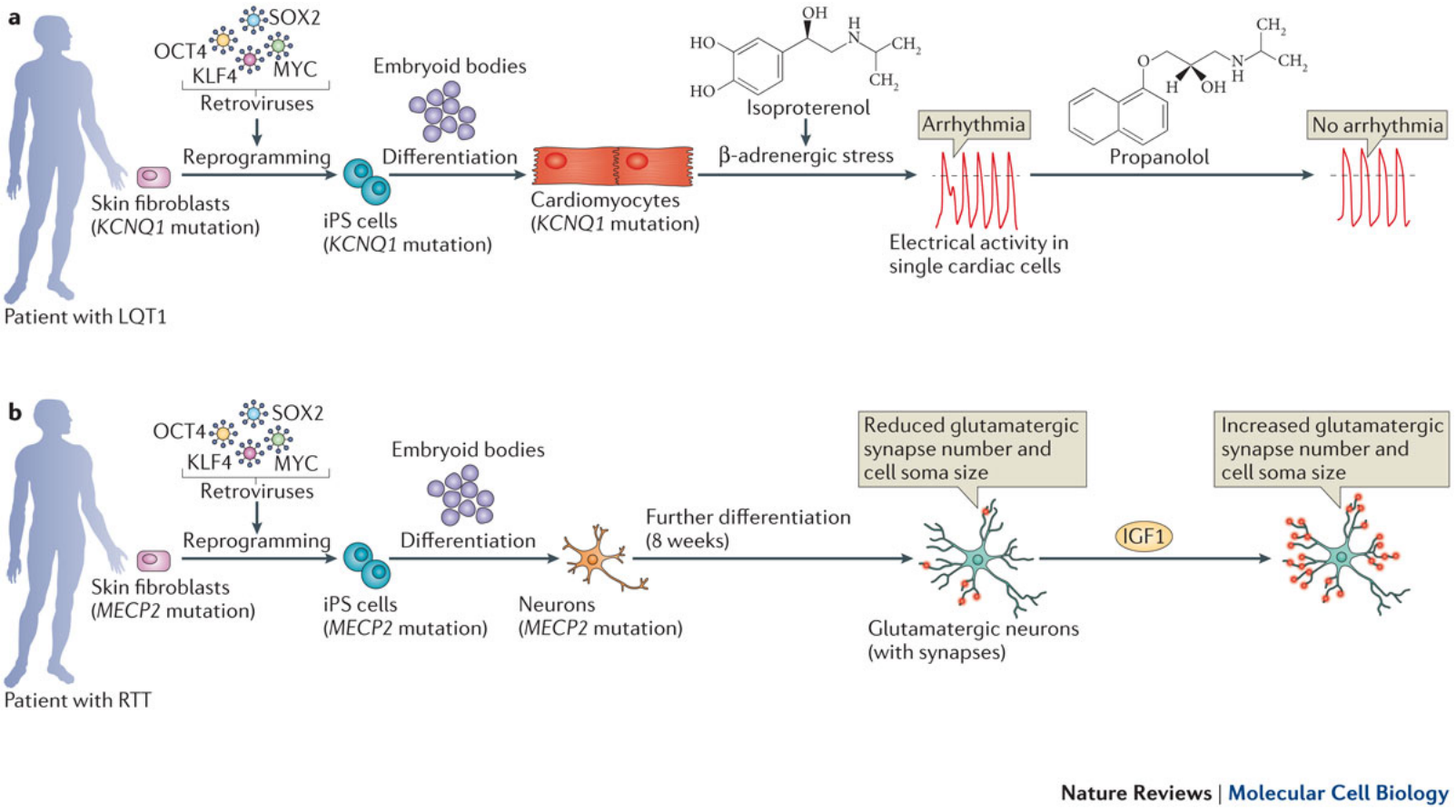




Basic physiological properties are unchanged in Ts21 iPSC-derived neurons at early stages. However, trends in all groups and significant reductions in most groups in the fraction of Ts21 iPSC-derived neurons that displayed spontaneous postsynaptic currents (sPSCs). **Significantly fewer DS neurons display synaptic activity relative to controls** (DS2U: 86 ± 3.9%; IMR90: 81 ± 3.2%). This reduction was mirrored by a decrease in the number of synapsin+ punctae on Ts21 neurites



Using iPS cells to model disease



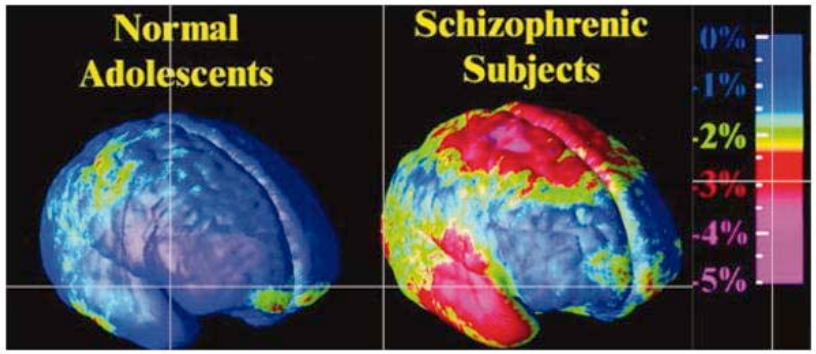
- Dimos et al. Induced pluripotent stem cells generated from patients with ALS can be differentiated into motor neurons. *Science*. 2008;321:1218–1221.
- Park et al. Disease-specific induced pluripotent stem cells. *Cell*. 2008;134:877–886.
- Poduri et al. Somatic mutation, genomic variation, and neurological disease. *Science*. 2013;341:1237758.
- Trounson et al. Human disease modeling with induced pluripotent stem cells. *Curr Opin Genet Dev*. 2012;22:509–516.

Modeling Schizophrenia

Schizophrenia: multifactorial disease, *DISC1* has been implicated

• Hallucinations, delusions, disorganized speech, aberrant neurotransmitter signalling, dendritic arborization, impaired myelination.

• Decreased connectivity in neurons, reduced neurites, decreased PSD95 protein level, reduced glutamate receptor expression, increased extra-mitochondrial oxygen consumption, increased ROS.



High-resolution magnetic resonance images (MRI scans) showing gray matter loss in adolescents with schizophrenia. Severe loss is observed (red and pink; up to 5% annually) in parietal, motor, and temporal cortices; inferior frontal cortices remain stable (blue; 0–1% loss).
Adapted from Thompson et al (2001).

Brennand and Gage, Stem Cells. 2011 Dec;29(12):1915-22

Modeling Schizophrenia

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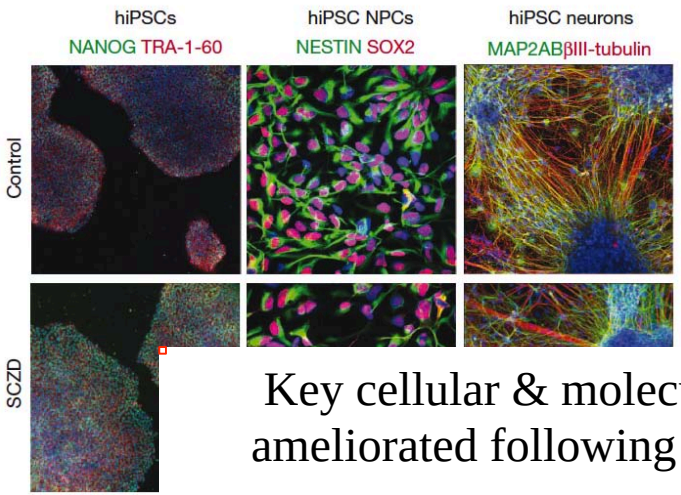
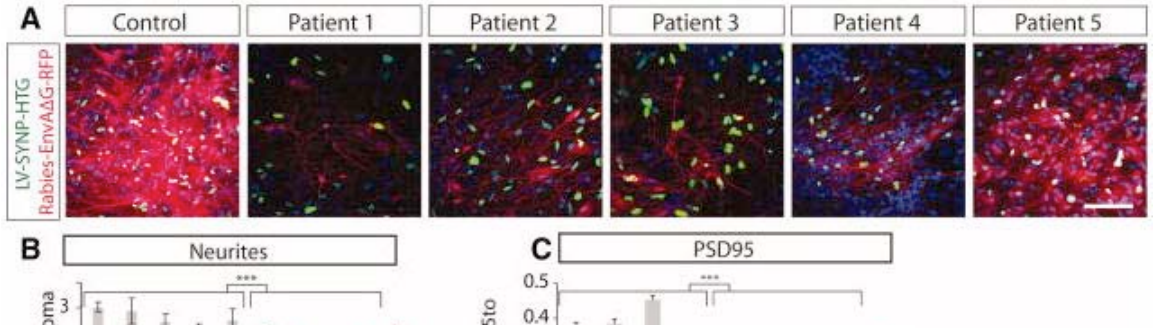
Brennand et al derived iPS cells from schizophrenic patients and differentiated these cells into neurons (2D-diff) revealing phenotypic and gene expression changes that were ameliorated upon anti-psychotic drug application.



Decreased neuronal connectivity in SCZD hiPSC neurons.



Key cellular & molecular elements of the SCZD phenotype were ameliorated following treatment of SCZD hiPSC neurons with the antipsychotic loxapine….

Brennand, K. J. *et al.* Modelling schizophrenia using human induced pluripotent stem cells. *Nature* **473**, 221–225 (2011).

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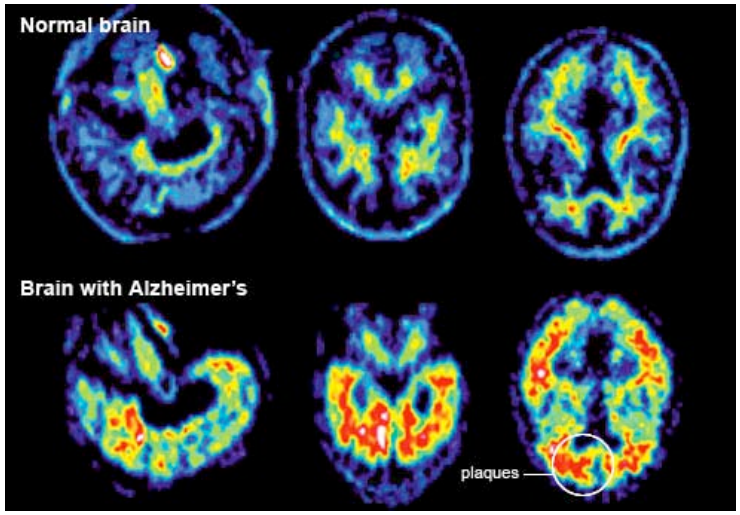
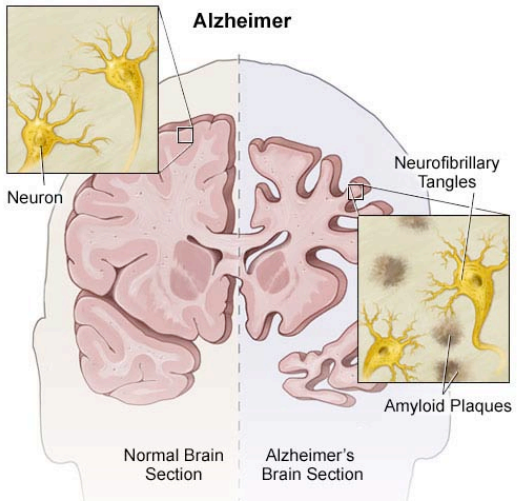
Modeling Alzheimer's Disease

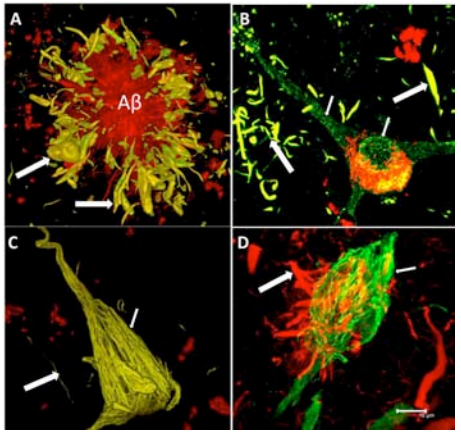
Alzheimer's disease (AD) is the most prevalent neurodegenerative disorder.

• One of the pathological features of AD is the oligomerization and aggregation and accumulation of Amyloid β peptide (Aβ), forming amyloid plaques in the brain.

• Cognitive impairment observed in clinical AD is inversely well correlated with the amount of Aβ oligomers in the soluble fraction rather than the amount of Ab fibrils (amyloid plaques) constituting the oligomers (Haass and Selkoe, 2007; Krafft and Klein, 2010).







A classical neuritic plaque in which an amyloid fibrillar plaque (Aβ), is associated with dystrophic neurites (arrows). B). Pre-tangle cells are characterized by diffuse granular deposits throughout the perinuclear area (small arrow)
From Luna-Munoz et al

Our understanding of the etiology and the treatment of this devastating disease remain limited

Modeling Alzheimer's Disease

A Human Stem Cell Model of Early Alzheimer's Disease Pathology in Down Syndrome

Shi et al, 2012.

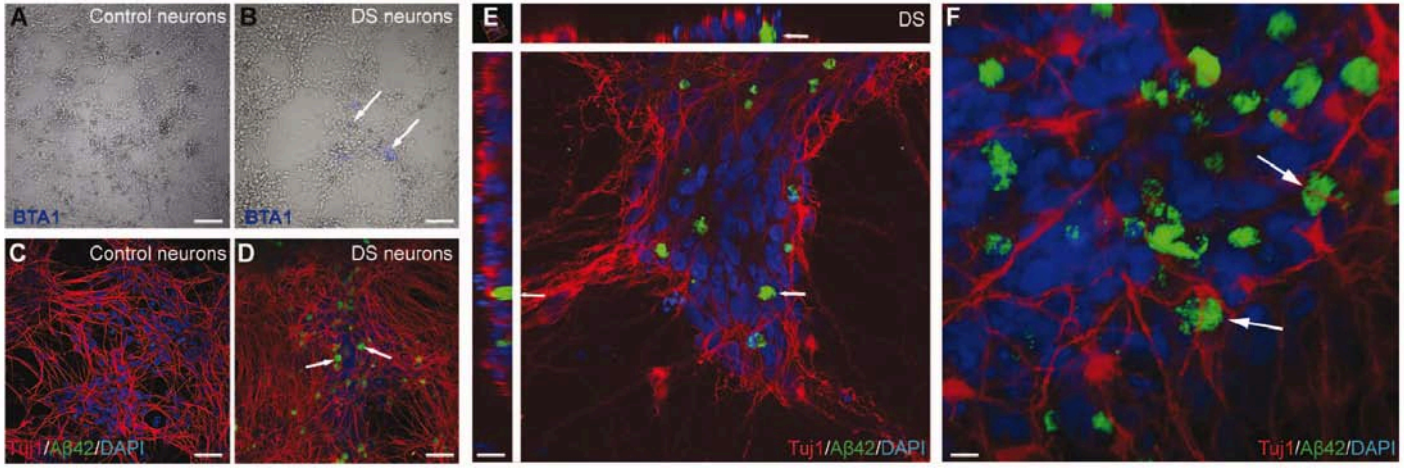
Adults with Down syndrome (trisomy chromosome 21) develop early-onset AD, probably due to increased expression of a gene on chrom. 21 that encodes amyloid precursor protein (APP).

• Cortical neurons were generated from human induced pluripotent stem (iPS) cells derived from patients with Down syndrome developed AD pathologies over months in culture, rather than years *in vivo*.

• Cortical neurons processed the transmembrane APP protein, resulting in secretion of the pathogenic peptide fragment amyloid-b42 (Ab42), forming insoluble intracellular and extracellular amyloid aggregates.

• Hyperphosphorylated tau protein, a pathological hallmark of AD, found localized to cell bodies and dendrites in iPS cell–derived cortical neurons from Down syndrome patients, recapitulating later stages of the AD pathogenic process.

Formation of amyloid aggregates by Down's Syndrome-iPS cell–derived cortical neurons after 60-90 days of culture:

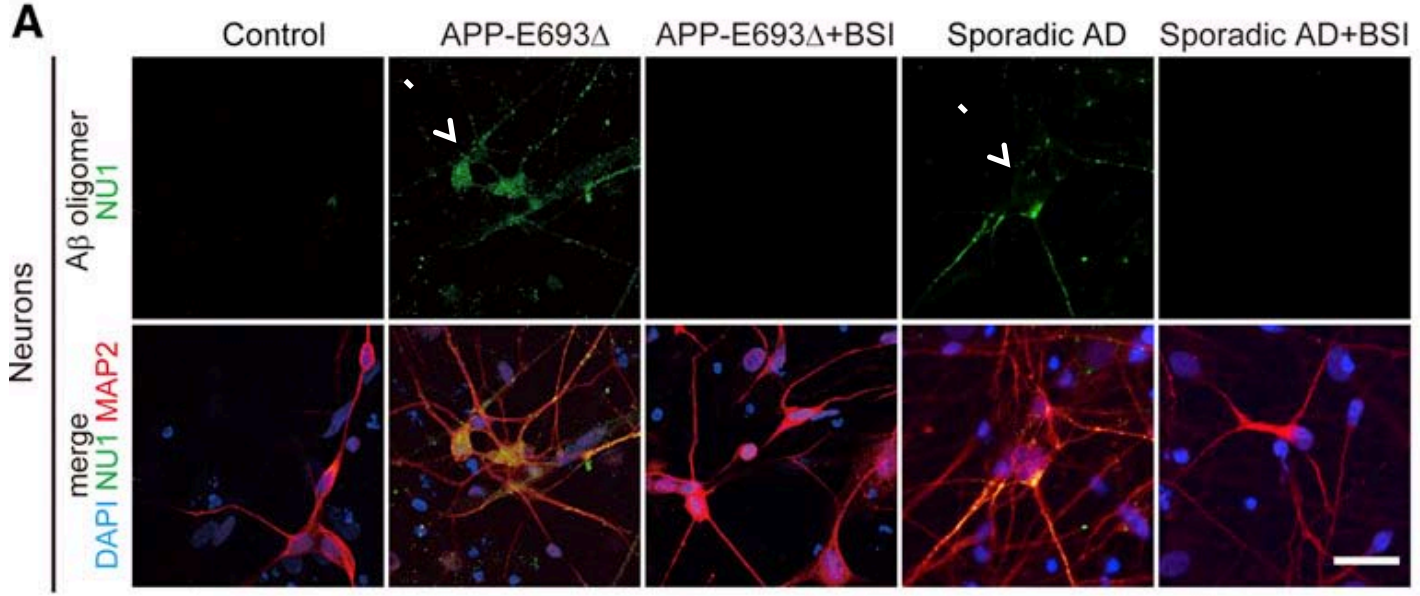


Modeling Alzheimer's Disease

Modeling Alzheimer's Disease with iPSCs Reveals Stress Phenotypes Associated with Intracellular Ab and Differential Drug Responsiveness:

Kondo et al, 2013

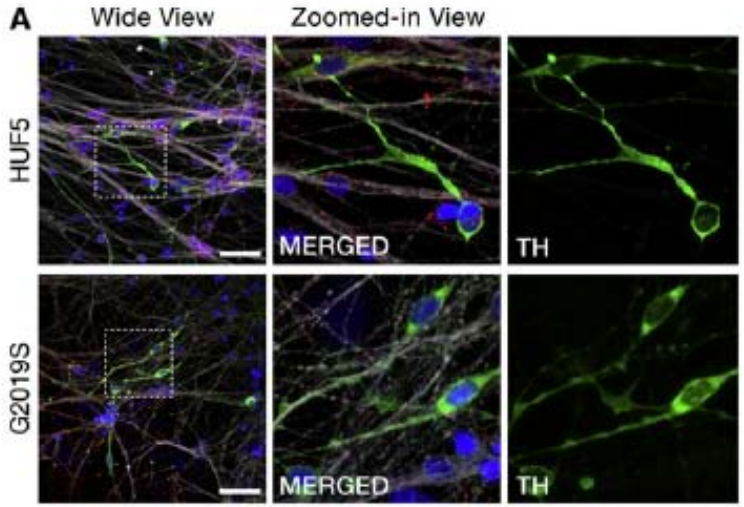
Generated iPSCs from familial and sporadic AD patients and differentiated them into neural cells. Ab oligomers accumulated in iPSC-derived neurons and astrocytes in cells from patients with a familial amyloid precursor protein (APP)- E693D mutation and sporadic AD, leading to endoplasmic reticulum (ER) and oxidative stress.

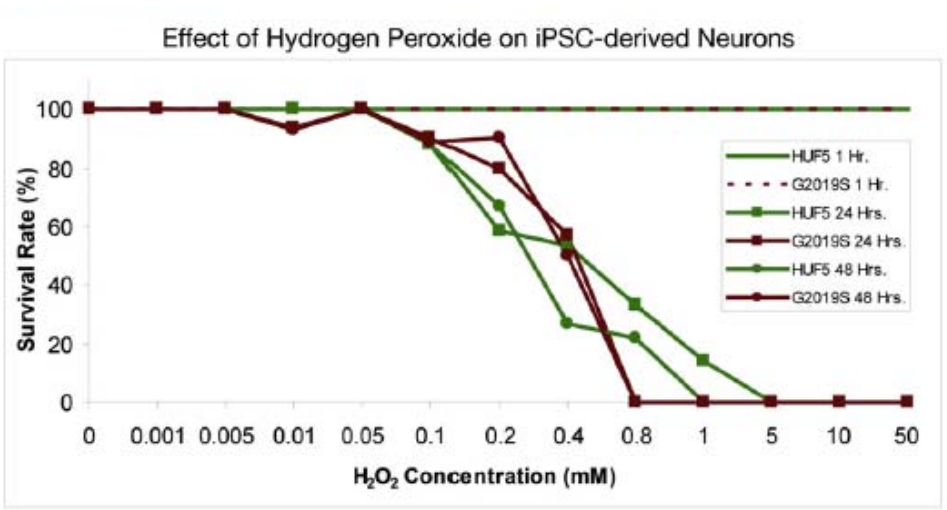


Intracellular Ab oligomer accumulation in iPSC-derived neurons (red, MAP2-positive cells) was detected by the Ab-oligomer-specific monoclonal antibody NU1 (green) with a punctate pattern. Ab oligomer accumulation was massive in AD(APP-E693D) and sporadic AD(AD8K213) neurons but only faint in control neurons. Treatment with 1 mM BSI decreased Ab oligomer accumulation.

Modeling Parkinson's Disease

- PD, the second most common late age onset neurodegenerative disorder
- Characterized by major loss of nigrostriatal Dopaminergic neurons & presence of inclusion bodies (Lewy bodies) in affected cells.
- LRRK2 mutant iPSC-derived Dopaminergic neurons demonstrate increased susceptibility to oxidative stress (Nguyen et al, 2011):





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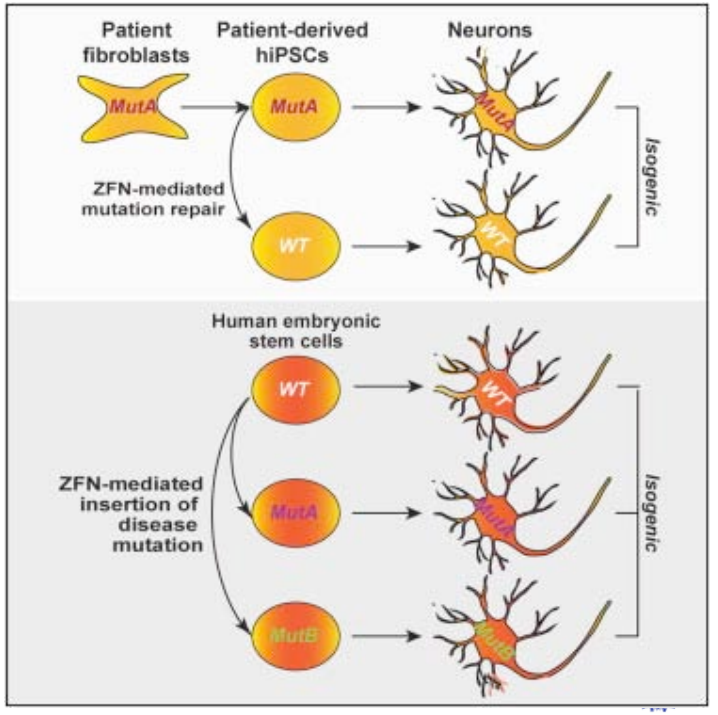
Modeling Parkinson's Disease

- PD, the second most common late age onset neurodegenerative disorder
- Characterized by major loss of nigrostriatal Dopaminergic neurons & presence of inclusion bodies (Lewy bodies) in affected cells.
- Dominant mutations in α-synuclein (A53T, E46K, A30P), the major component of Lewy bodies, found in rare familial, forms of the disease (Lees et al., 2009; Schulz, 2008).

Creation of PD models in hiPS cells:
Generation of Isogenic Pluripotent Stem Cells Differing Exclusively at Two Early Onset Parkinson Point Mutations (Soldner et al, 2011)

Zinc finger nuclease (ZFN)-mediated genome editing to create sets of isogenic disease and control human iPS cells that differ exclusively at either of two susceptibility variants for Parkinson's disease by modifying the underlying point mutations in the α-synuclein gene.



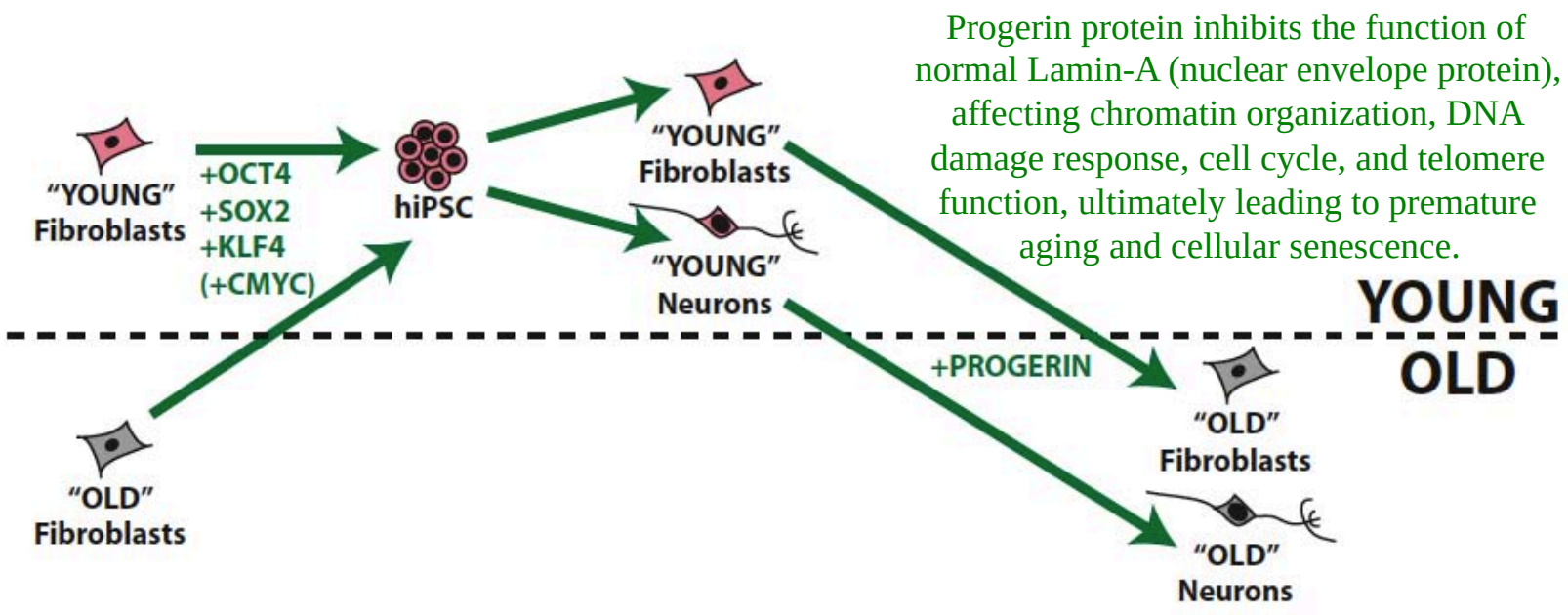
Just how good a model can *in vitro* differentiation of hiPSCs (months) be for an ageing-related disease (years)?

Modeling Aging-Related Disease

Immaturity of neurons differentiated from human iPSCs presents difficulties for modeling late-onset neurodegenerative disorders such as Parkinson's disease.

Strategy for inducing aging-related phenotypes in hiPSC-derived neurons, enabling *in vitro* study of late-onset neurodegenerative diseases

Transient expression of Progerin induces aging related phenotypes *in vitro*

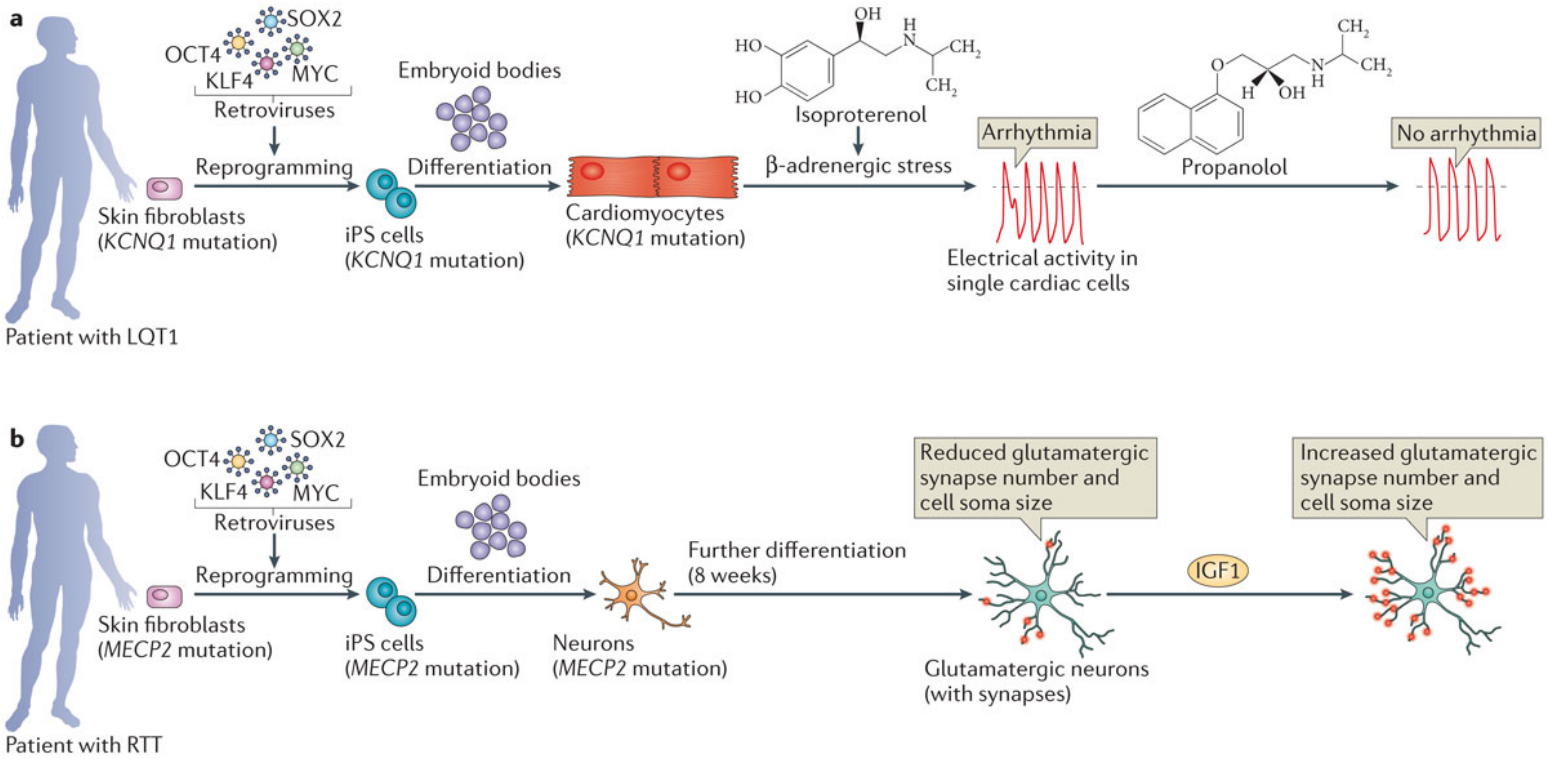


Progerin protein inhibits the function of normal Lamin-A (nuclear envelope protein), affecting chromatin organization, DNA damage response, cell cycle, and telomere function, ultimately leading to premature aging and cellular senescence.

iPS cells: a revolution in our capacity to model human disease

"Induced pluripotent stem cells: the new patient?"

Bellin et al, 2012



Nature Reviews | Molecular Cell Biology

Neurological diseases including Alzheimer's, Parkinson's, Huntington's, Rett's syndrome ALS, schizophrenia…
Cardivascular diseases including LQT1, 2, 3, Stress induced ventricular arrhythmia, DCM, ARVC…

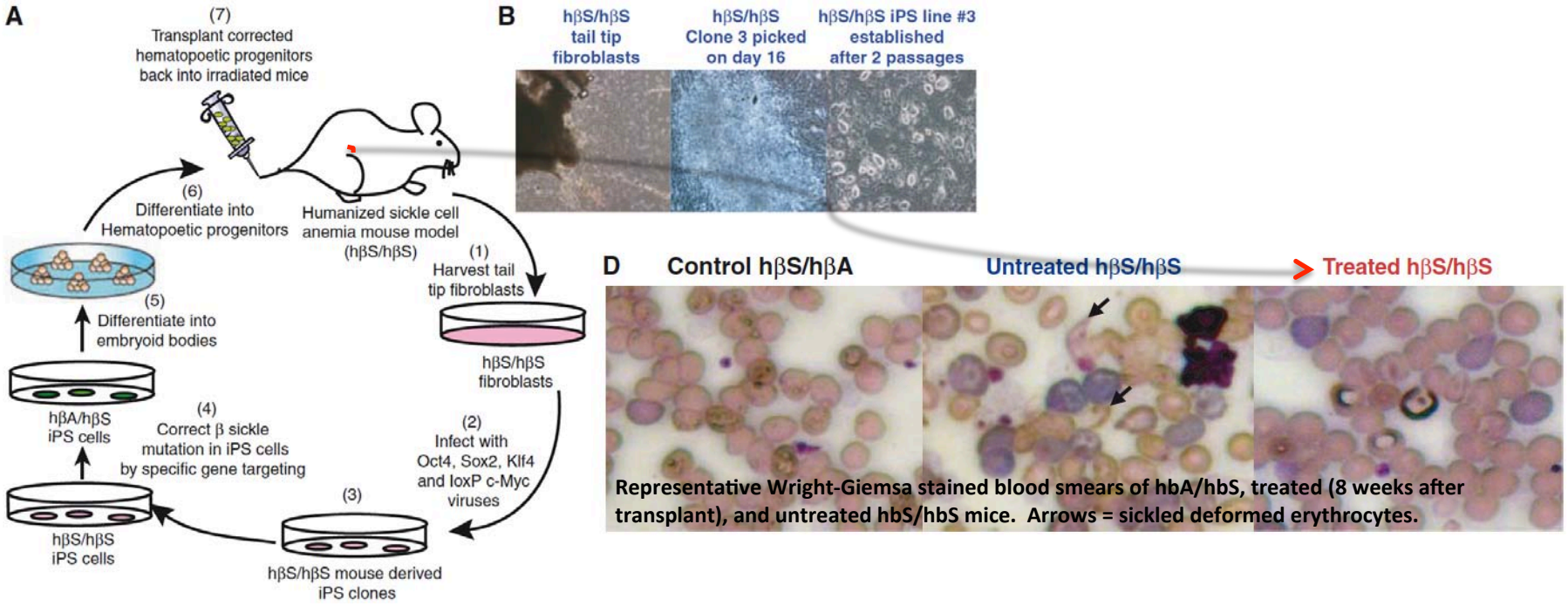
Use of iPS cells for Therapeutic Purposes

Can diseases caused by single gene defects can be treated by made-to-order gene replacement in autologous cells?

Use of iPS cells for Therapeutic Purposes

Can diseases caused by single gene defects can be treated by made-to-order gene replacement in autologous cells?

Proof of concept of the therapeutic use of iPSCs: a mouse model of sickle-cell anemia, a genetic blood disorder caused by a defect in the β-globin gene. Gene correction by homologous recombination in a mutant iPSC line, followed by transplantation into mutant mice, cured the disease.

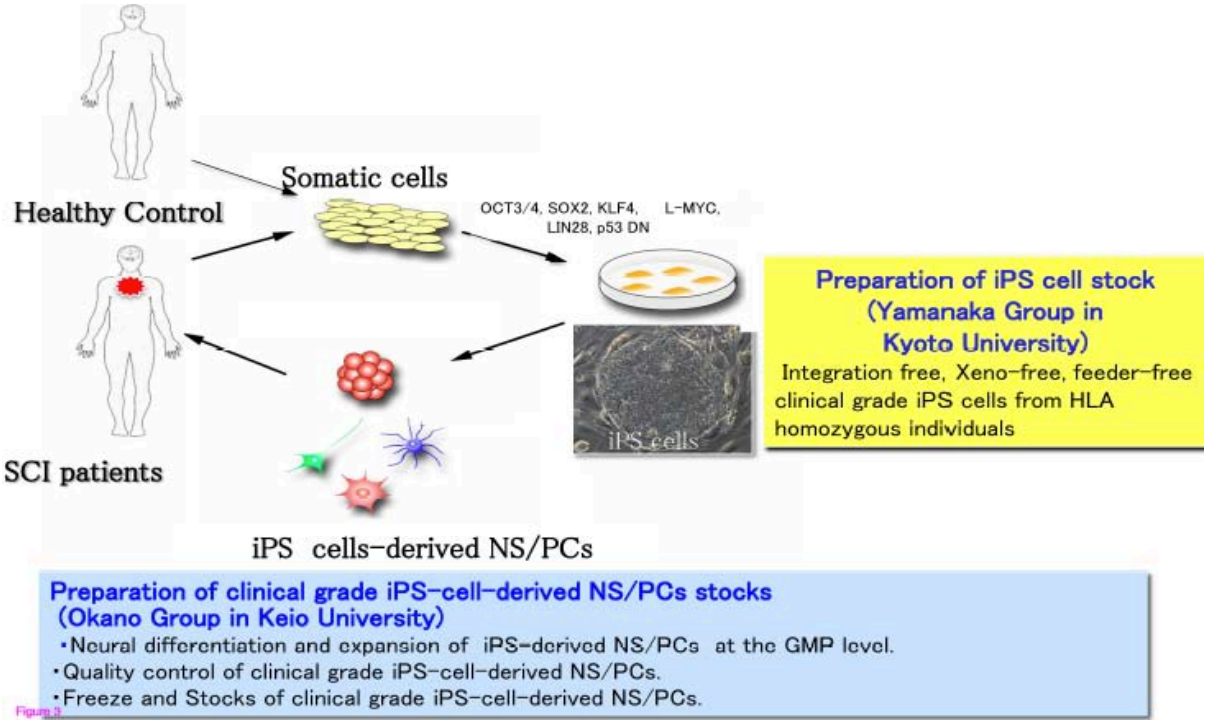


Hanna et al, (2007) Generated from Autologous Skin Treatment of Sickle Cell Anemia Mouse Model with iPS Cells, *Science* 318, 1920-5.

Use of iPS cells for Therapeutic Purposes

- Autologous iPSCs from each individual minimizes immune rejection but high medical costs.
- >3 months needed to generate iPSCs using current methods.

⇒ Effective treatment (eg for spinal cord injury), cannot be achieved within a short enough time frame..



Instead, generate clinical-grade iPSCs under Good Manufacturing Practice (GMP) compliance:

- Diversity of donor candidates, check health status and genetic variants (SNPs, CNVs..)
  Diversity of human leukocyte antigen (HLA) types => choose best match for HLA type to minimise immune rejection

Okano and Yamanaka *Molecular Brain* 2014, 7:22

Use of iPS cells for Therapeutic Purposes

Table 1 Planned clinical trials of iPS cell-based therapies

Principal Investigator (Institute/Location)	*Cell Type to transplant*	*Target Disorders*
Masayo Takahashi, (RIKEN)	Retinal Pigment Epithelium (sheet)	Age-related macular degeneration (wet type)
Alfred Lane, Anthony Oro, Marius Wernig (Stanford University)	Keratinocytes	Recessive dystrophic epidermolysis bullosa (RDEB)
Mahendra Rao (NIH)	DA neurons	Parkinson's disease
Koji Eto (Kyoto University)	Megakaryocyte	Thrombocytopenia
Jun Takahashi (Kyoto University)	DA neurons	Parkinson's disease
Steve Goldman, (University of Rochester)	Oligodendrocyte precursor cell	Multiple Sclerosis
Hideyuki Okano, Masaya Nakamura (Keio University)	Neural stem/progenitor cells	Spinal Cord Injury
Shigeto Shimmura (Keio University)	Corneal endothelial cells	Corneal endothelial dysfunction
Koji Nishida (Osaka University)	Corneal epithelial cells (sheet)	Corneal epithelial dysfunction and trauma (e.g. Stevens–Johnson syndrome)
Yoshiki Sawa (Osaka University)	Cardiomyocytes (sheet)	Heart Failure
Keiichi Fukuda (Keio University)	Cardiomyocytes (sphere)	Heart Failure
Yoshiki Sasai and Masayo Takahashi (RIKEN)	Neuroretinal sheet including photoreceptor cells	Retinitis pigmentosa
Advanced Cell Technology	Megakaryocytes	Refractory thrombocytopenia

Representative studies of iPS-based cell therapy with planned clinical trials are listed.
References: [17,19-29].

Okano and Yamanaka "iPS cell technologies: significance and applications to CNS regeneration and Disease" *Molecular Brain* 2014, 7:22

Advantages and Disadvantages of iPSCs for Therapy

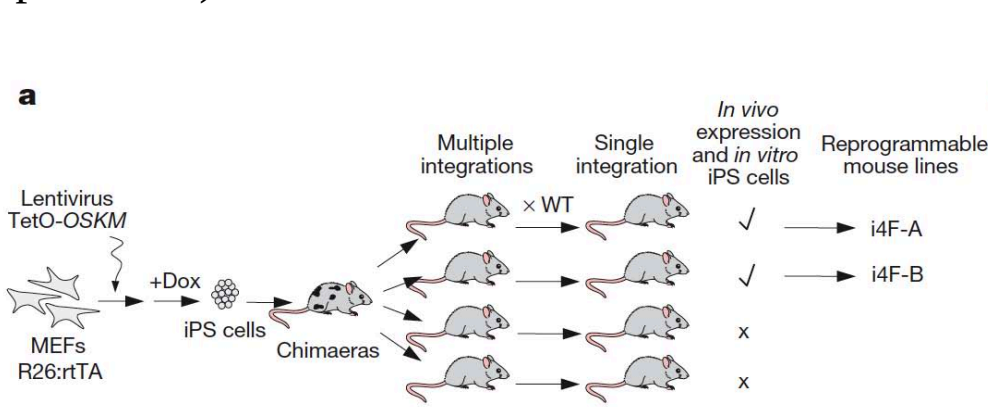
1. Reprogramming-mediated rejuvenation for T lymphocytes derived from iPSCs. (Nishimura et al., 2013; Vizcardo et al., 2013).
2. Culture media and reprogramming factor delivery can be controlled carefully
3. iPS efficiency remains low and is still slow
4. Selected iPSC will have to be sequenced and epigenomically profiled prior to use (to exclude genetic and epigenetic changes)
5. Recapitulating differentiation *in vitro* can be challenging due to inadequate understanding of molecular mechanisms
6. Directed differentiation is time-consuming
7. Production of adult human cell types might require the full length of the typical gestational period, or even longer.
8. Resulting cells may often represent embryonic cell types
9. Residual iPS cells can cause teratomas…

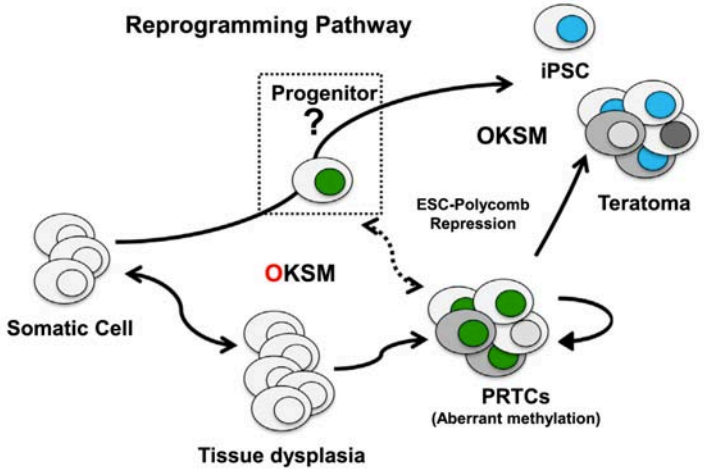
Induced pluripotency *in vivo* can lead to dysplasia and cancer

Parallels between iPS and tumorigenesis (last week's lecture).

• Recent studies (Abad et al., 2013; Ohnishi et al. 2013) demonstrate that OKSM factors can drive tumor initiation *in vivo*.

• Transient combined induction of OKSM is sufficient to induce a stably transformed state through epigenetic rather than genetic mechanisms, in some cell types (kidney in particular).



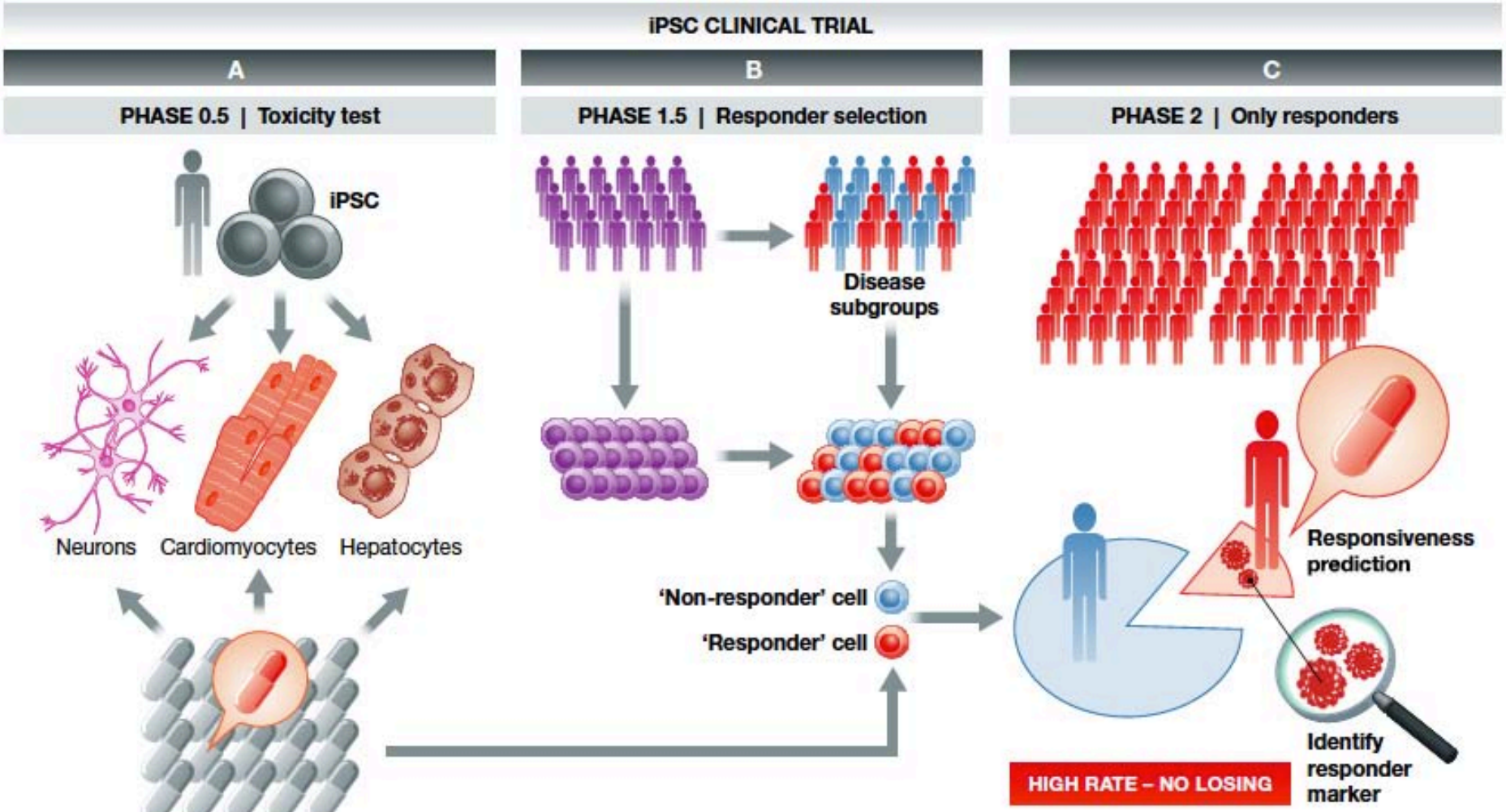


In vivo *Hobbs and Polo, 2014*

• Reprogrammable mice induce teratomas and present circulating iPS cells in the blood.
• At the transcriptome level, these *in vivo* generated iPS cells are closer to embryonic stem cells (ES cells) than standard *in vitro* generated iPS cells.
• *In vivo* iPS cells efficiently contribute to the trophectoderm lineage, suggesting that they achieve a more plastic or primitive state than ES cells!

=> **Powerful tools – but disasterous for therapy….**

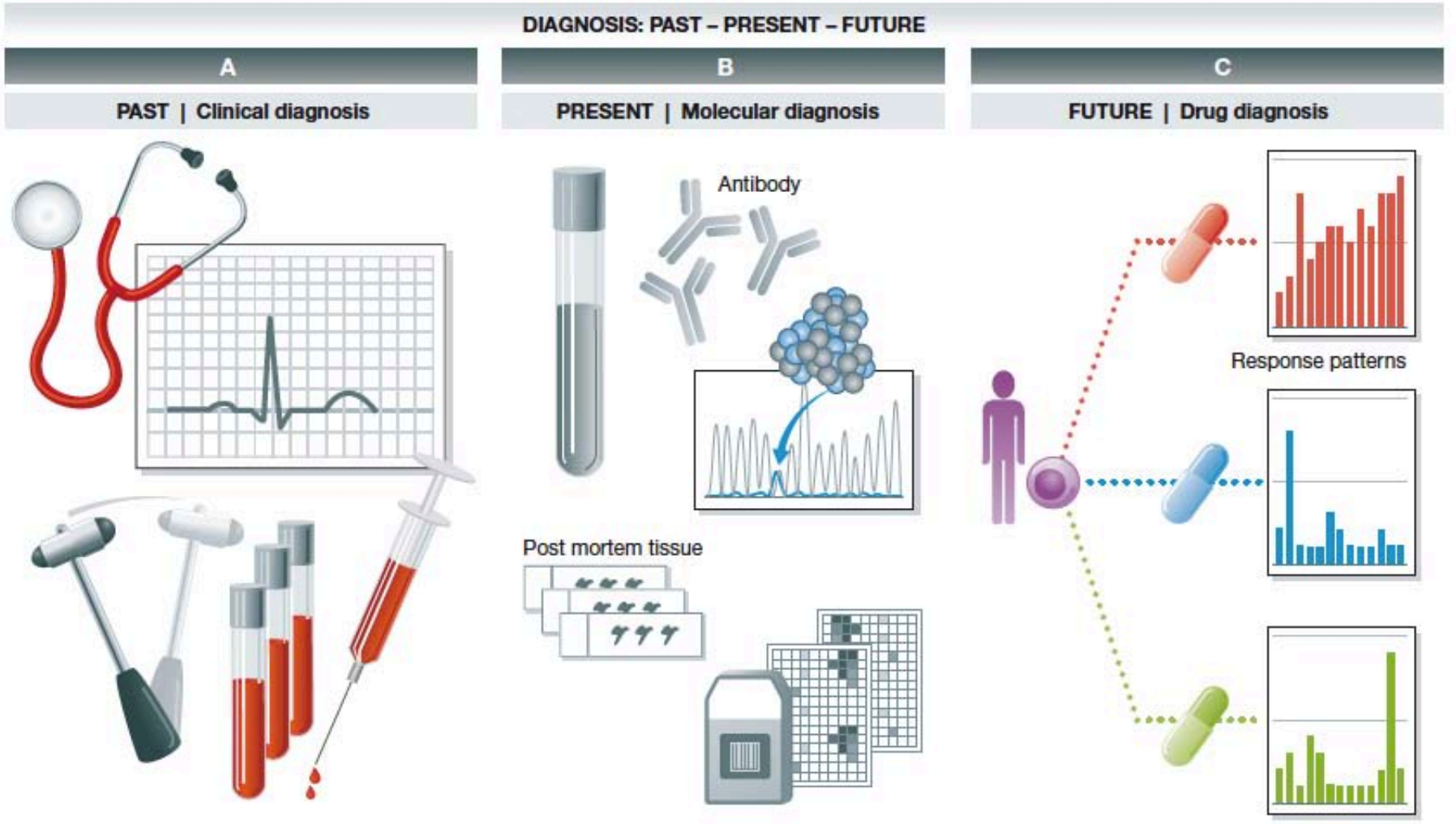
iPSC as powerful models for Drug development



Eg The drug responsiveness of neuronal cells derived from responder iPSCs and non-responder iPSCs to VPA was compatible with the results of clinical trials (Garbes et al, 2013).

iPS cells: a game changer for future medicine
Inoue et al, 2014.

Personalised Medicine via Personalised iPSCs?

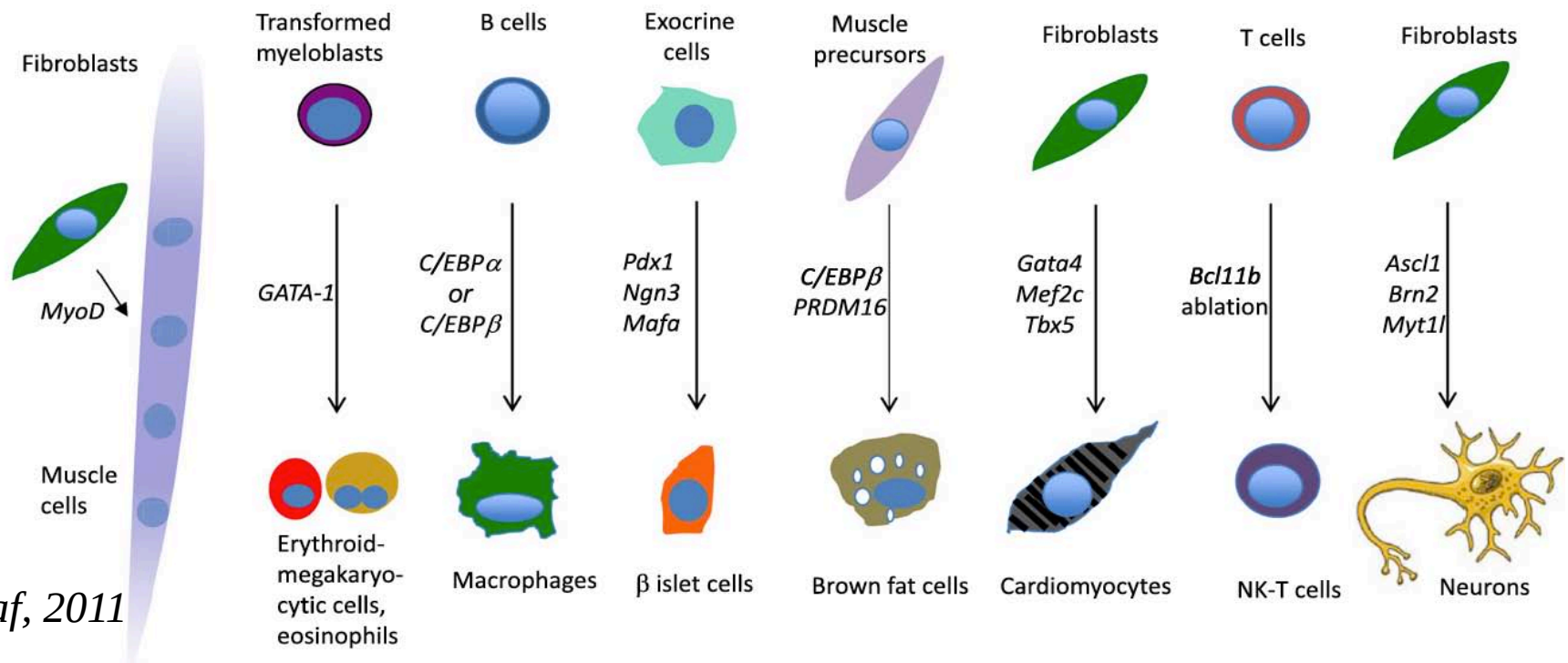


iPS cells: a game changer for future medicine
Inoue et al, 2014.

Alternative Therapeutic Strategies

Direct Conversion / Transdifferentiation

(Cours III et IV)

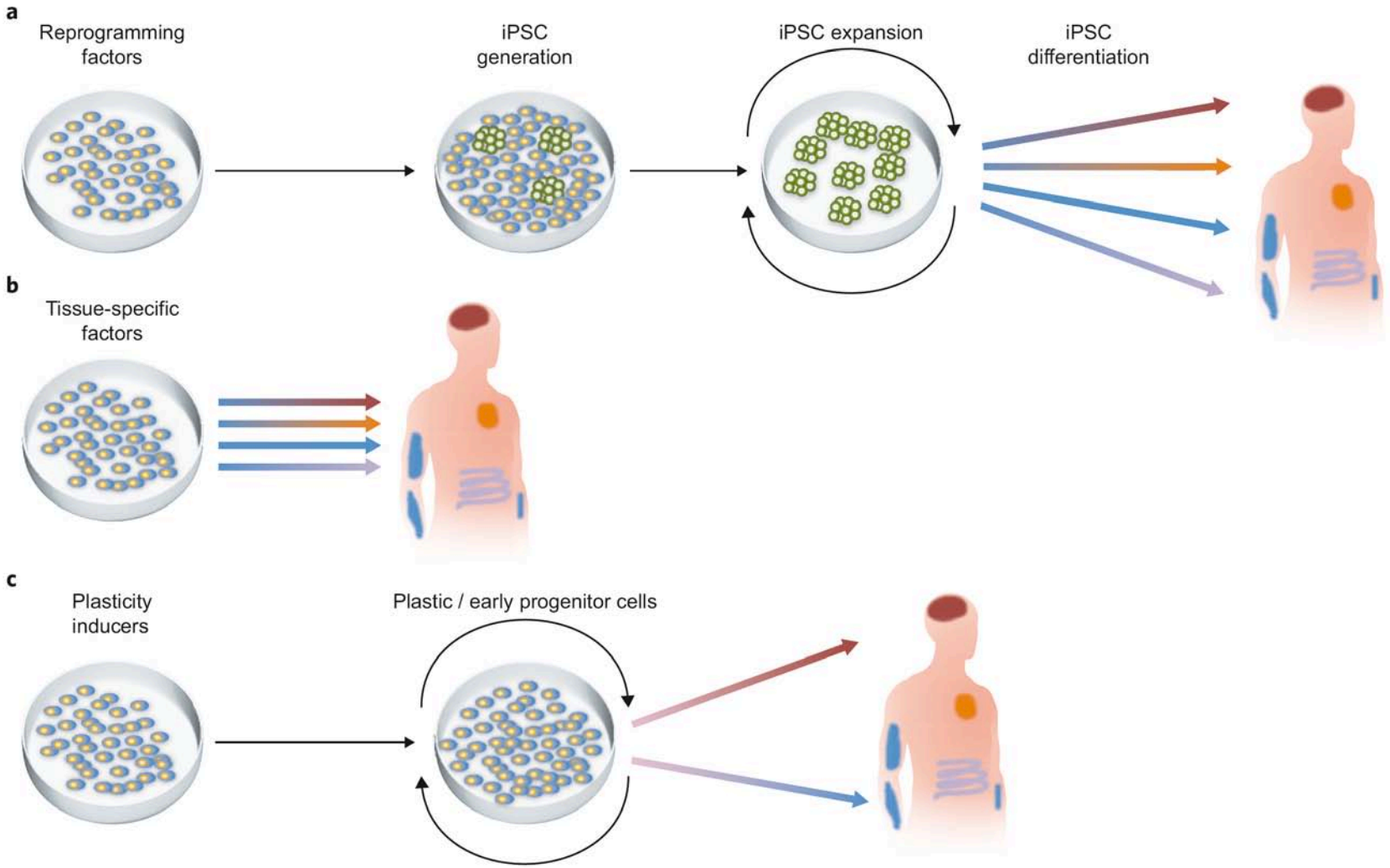


Graf, 2011

⇒ Directed lineage conversion methods to induce another lineage while bypassing pluripotency

- rapidly generate mature differentiated cells for therapy
- may also better recapitulate late-onset disease phenotypes.

Different conversion methodologies to generate cells of a given fate

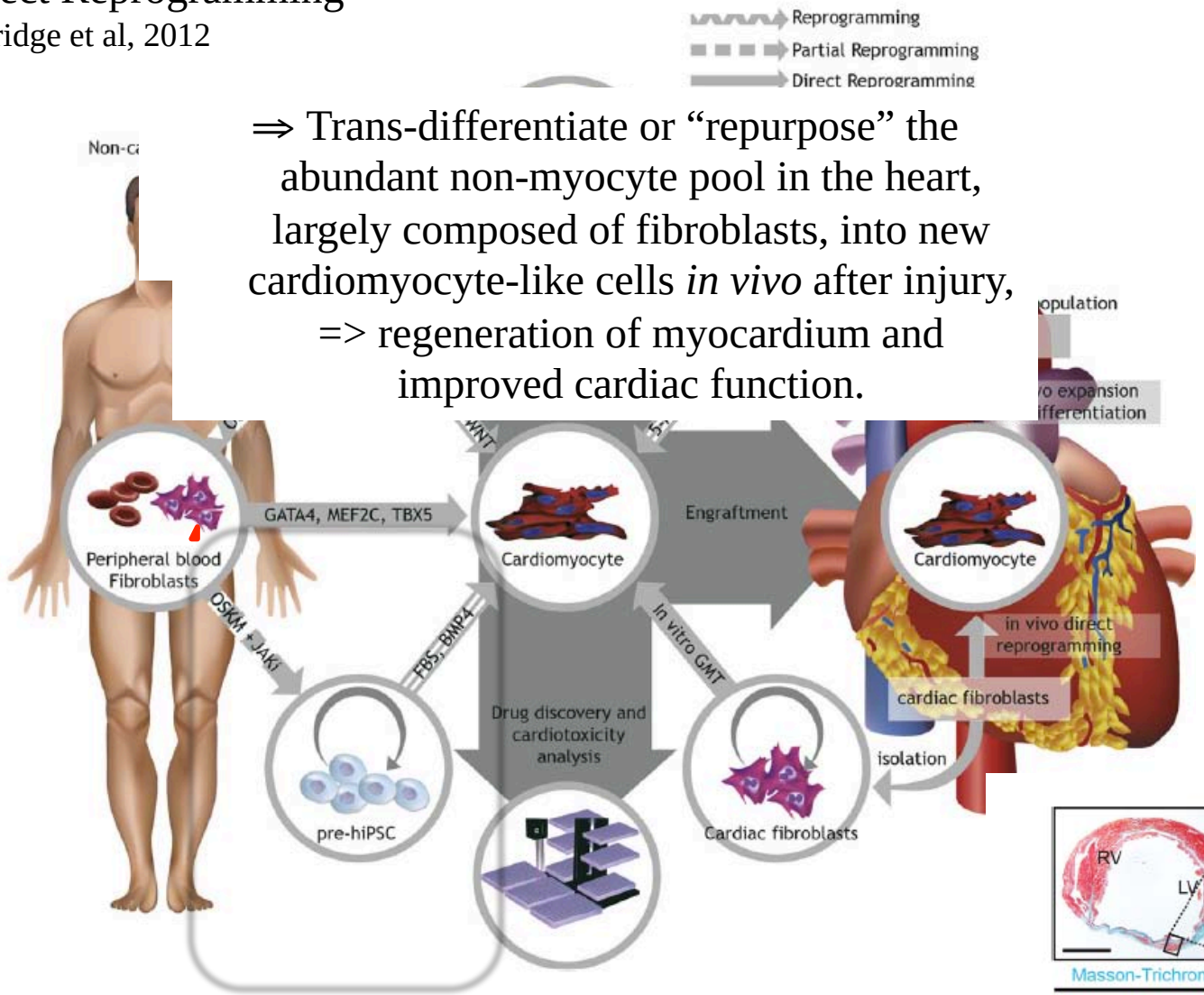


From Sancho-Martinez et al, Nat. Cell Biol. 2012

The Future: *in vitro* and *in vivo* direct reprogramming?

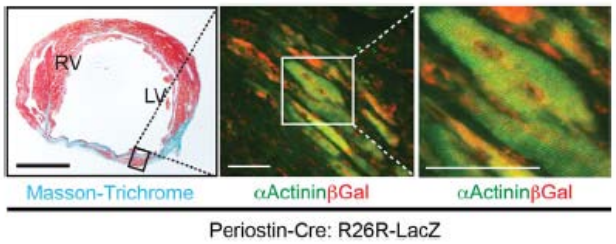
Production of De Novo Cardiomyocytes: Human Pluripotent Stem Cell Differentiation and Direct Reprogramming

Burridge et al, 2012

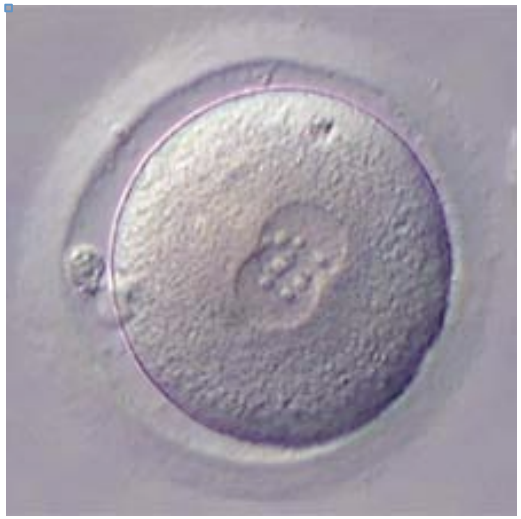


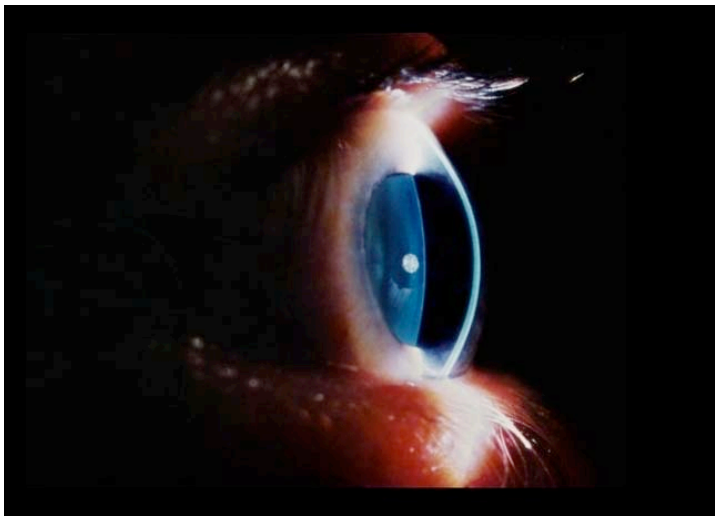
⇒ Trans-differentiate or "repurpose" the abundant non-myocyte pool in the heart, largely composed of fibroblasts, into new cardiomyocyte-like cells *in vivo* after injury, => regeneration of myocardium and improved cardiac function.

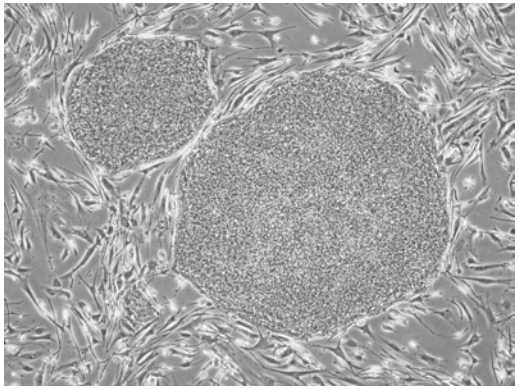
Direct conversion of cardiac fibroblasts into cardiomyocyte-like cells in vivo. Gata4, Mef2c, and Tbx5-injected periostin-Cre:R26R-lacZ mouse heart 4 weeks after coronary ligation.

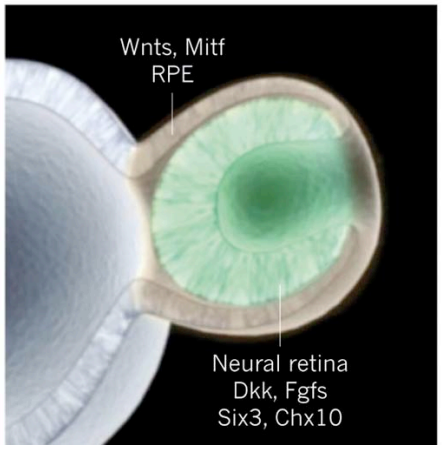


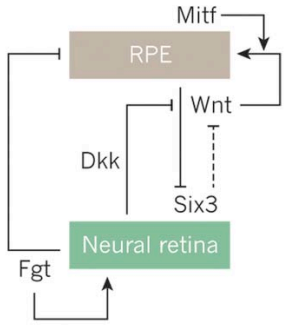
"*And thus the wonderful truth became manifest that a single cell may contain within its microscopic compass the sum total of the heritage of the species*".
EB Wilson, 1900











1 nerves

Colloque organisé par Edith Heard et Azim Surani

« Reprogrammations développementales, induites et pathologiques »
de 9h à 18h, amphithéâtre Marguerite de Navarre

Nicole Le Douarin, ***Collège de France, Paris***
Nathalie Beaujean, ***INRA, Jouy-en-Josas, France***
Helen Blau, ***Stanford University, California, USA***
Jacob Hanna, ***Weizmann Institute of Science, Israel***
Rick Livesey, ***Gurdon Institute, University of Cambridge, UK***
Rob Martienssen, ***CSHL, New York, USA***
Azim Surani, ***Gurdon Institute, University of Cambridge, UK***