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Institute of Reconstructive Neurobiology

University of Bonn Medical Center

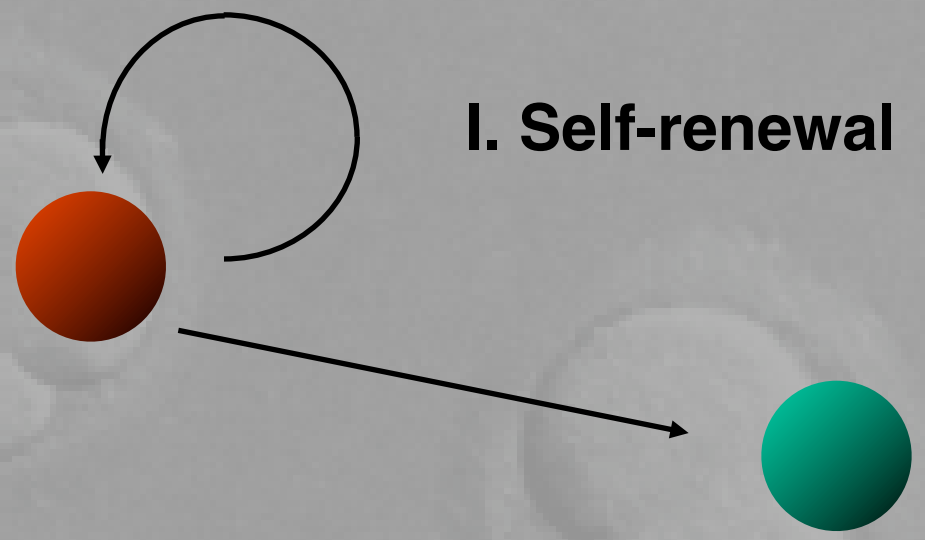
Unleashing the Genome: Maintaining, Reinstating and Exploiting Pluripotency

Oliver Brüstle

Fifth World Conference on The Future of Science
The DNA Revolution
Fondazione Cini, Venice, Italy, September 20-22, 2009

Laconic kylix with Prometheus and Atlas, 550 B.C.; Vatican Museums

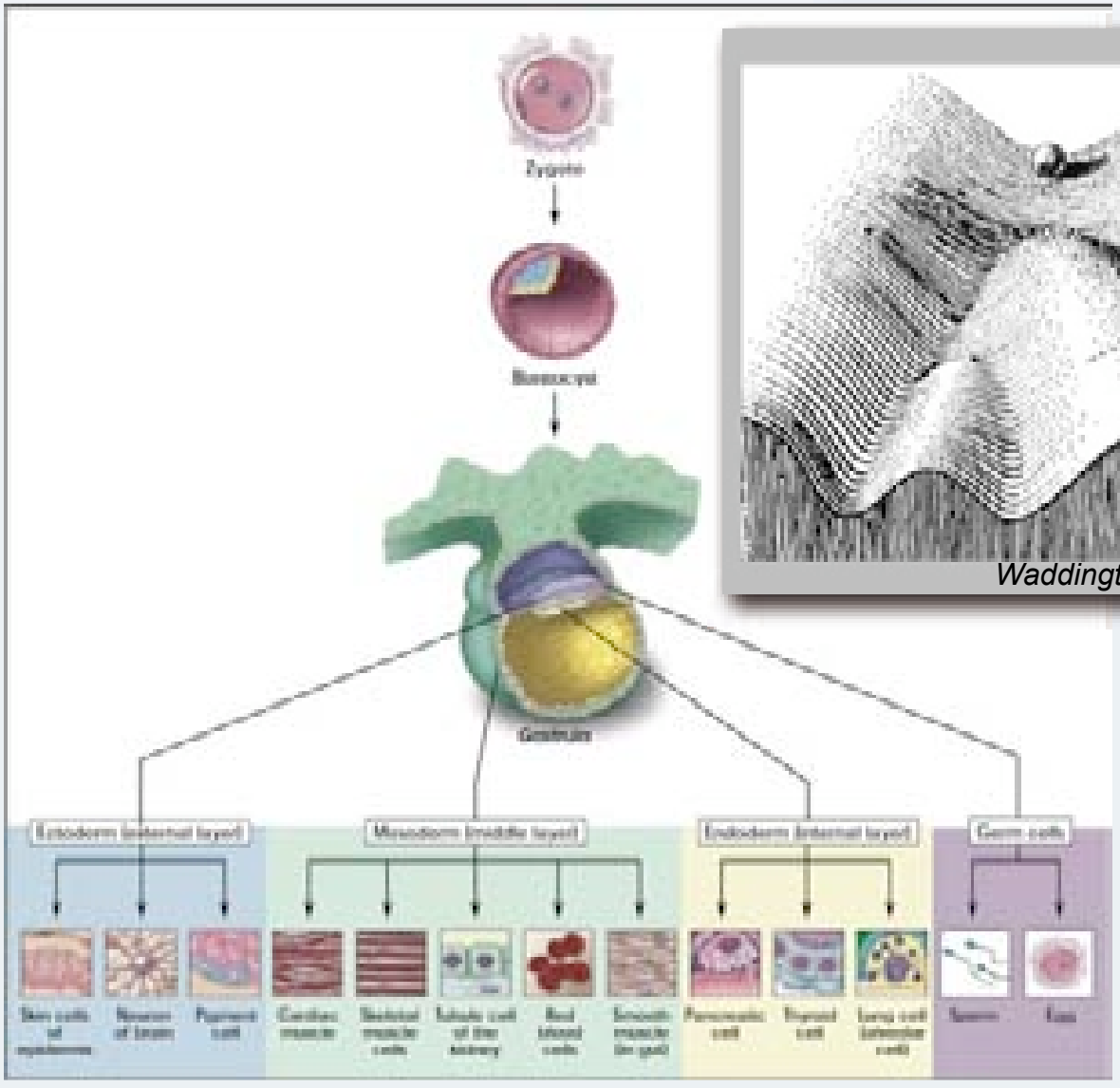
The two key properties of stem cells

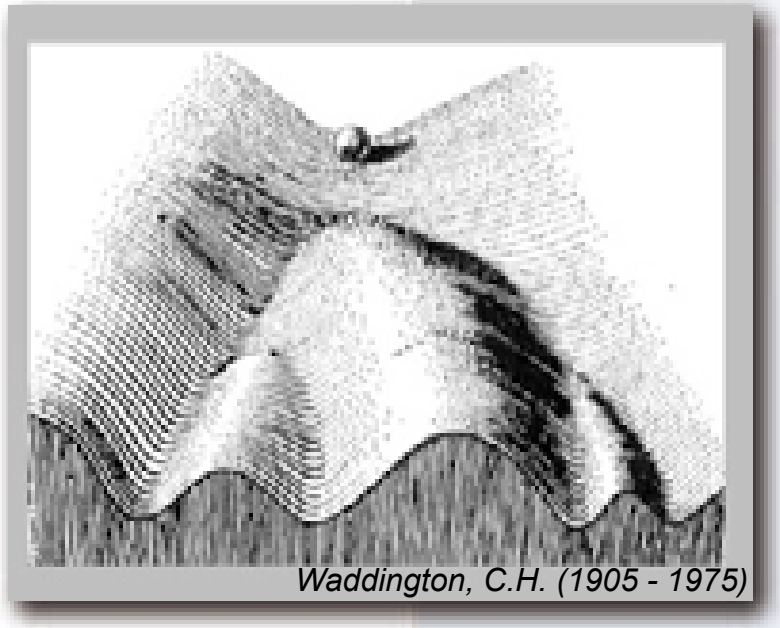


I. Self-renewal

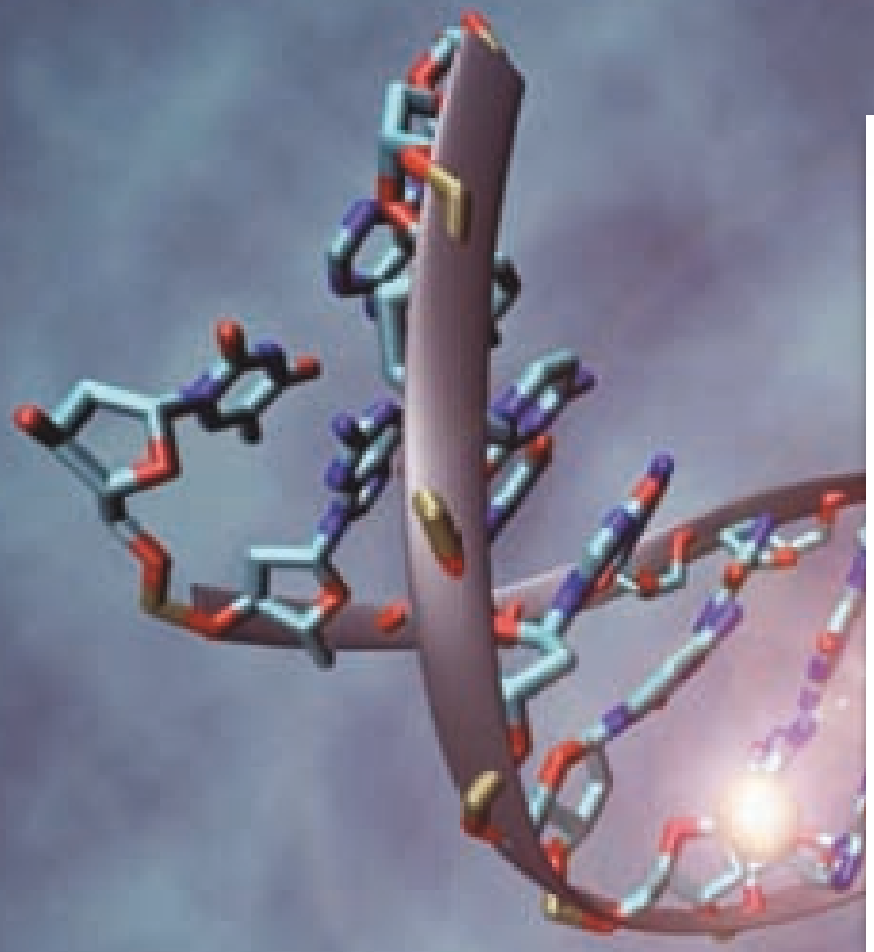
II. Differentiation into more mature daughter cells

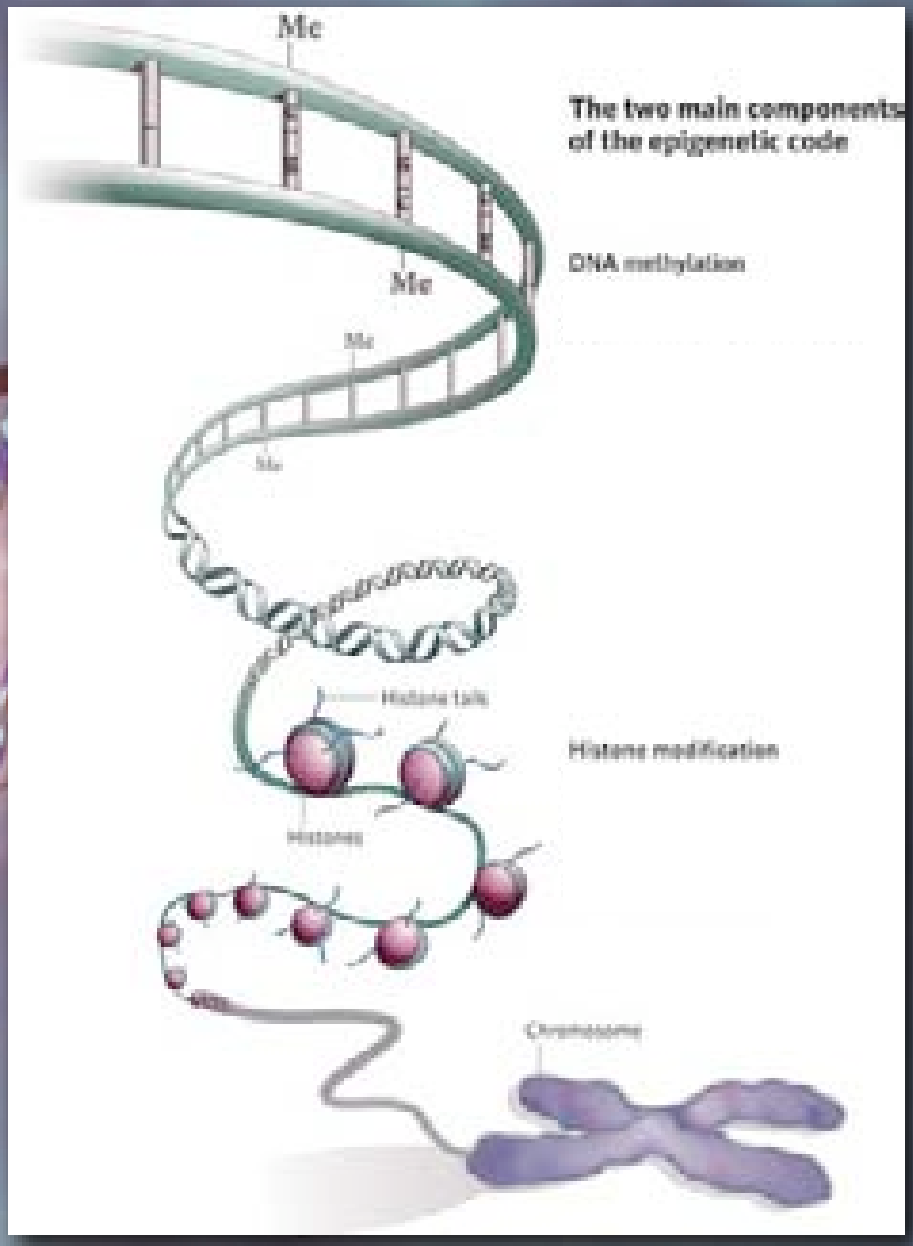
Lineage Decisions in the Early Embryo

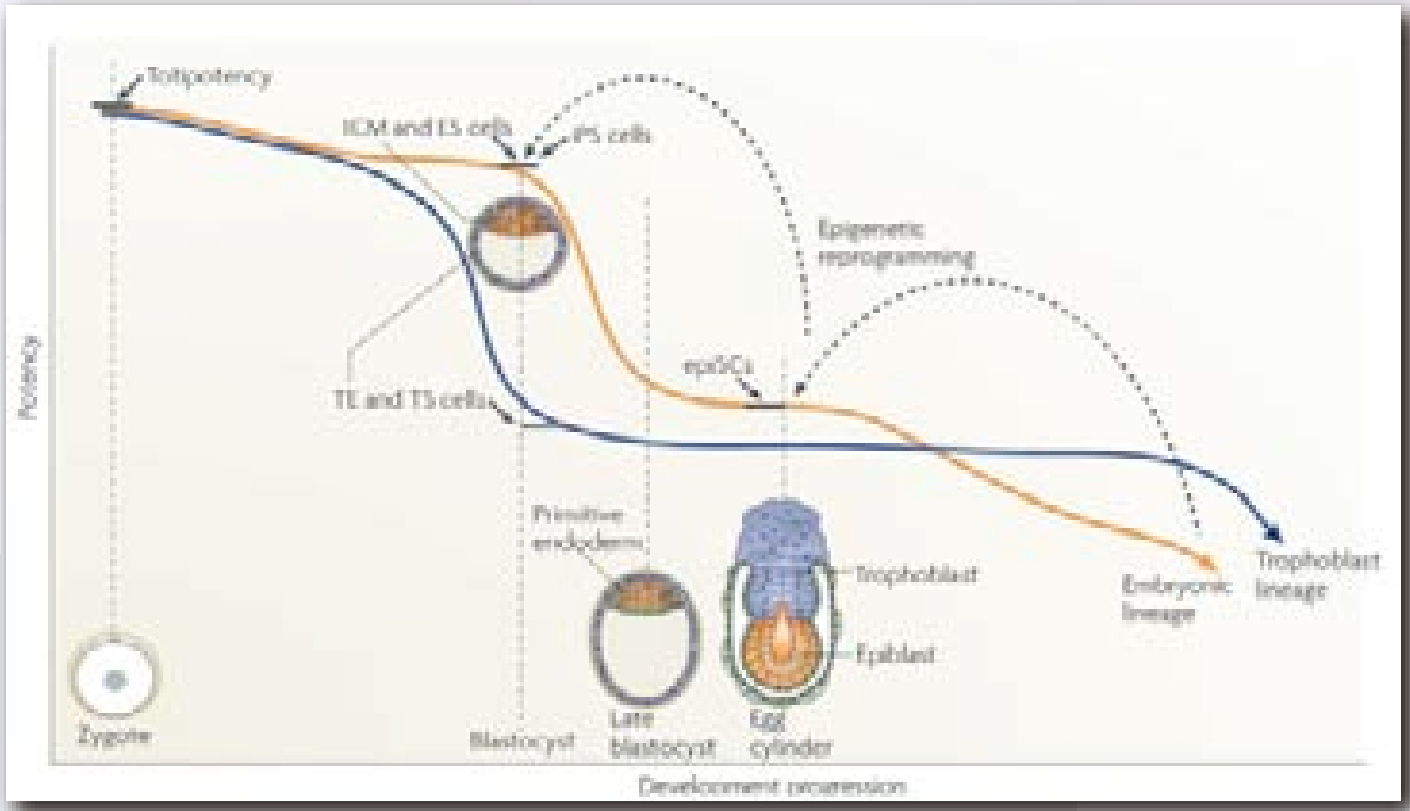




Waddington, C.H. (1905 - 1975)

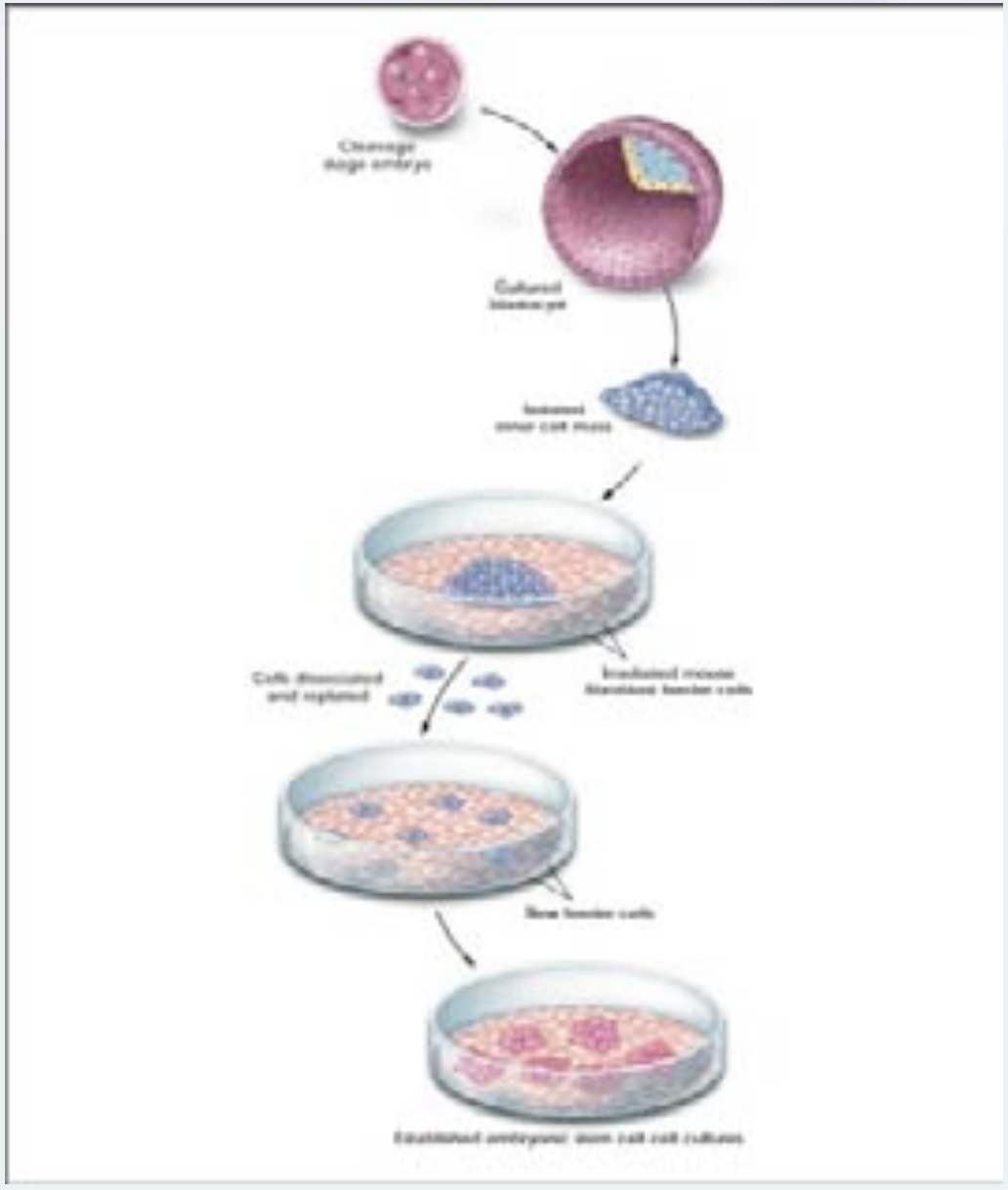


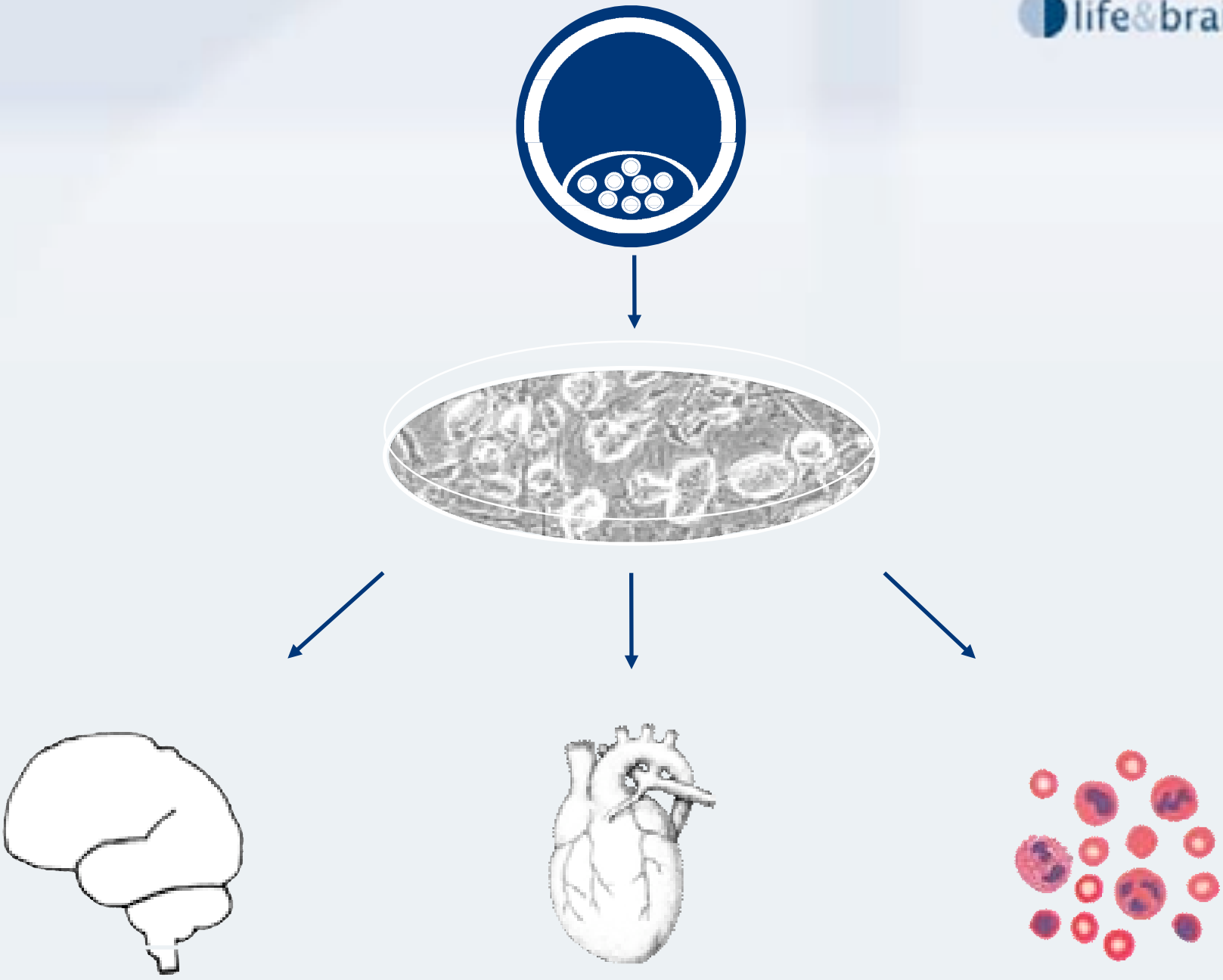

Me
The two main components of the epigenetic code
DNA methylation
Me
Me
Me
Histone tails
Histone modification
Histones
Chromosome



Hemberger M et al., Nature Rev Moll Cell Biol 2009

Derivation and Cultivation of Embryonic Stem Cells

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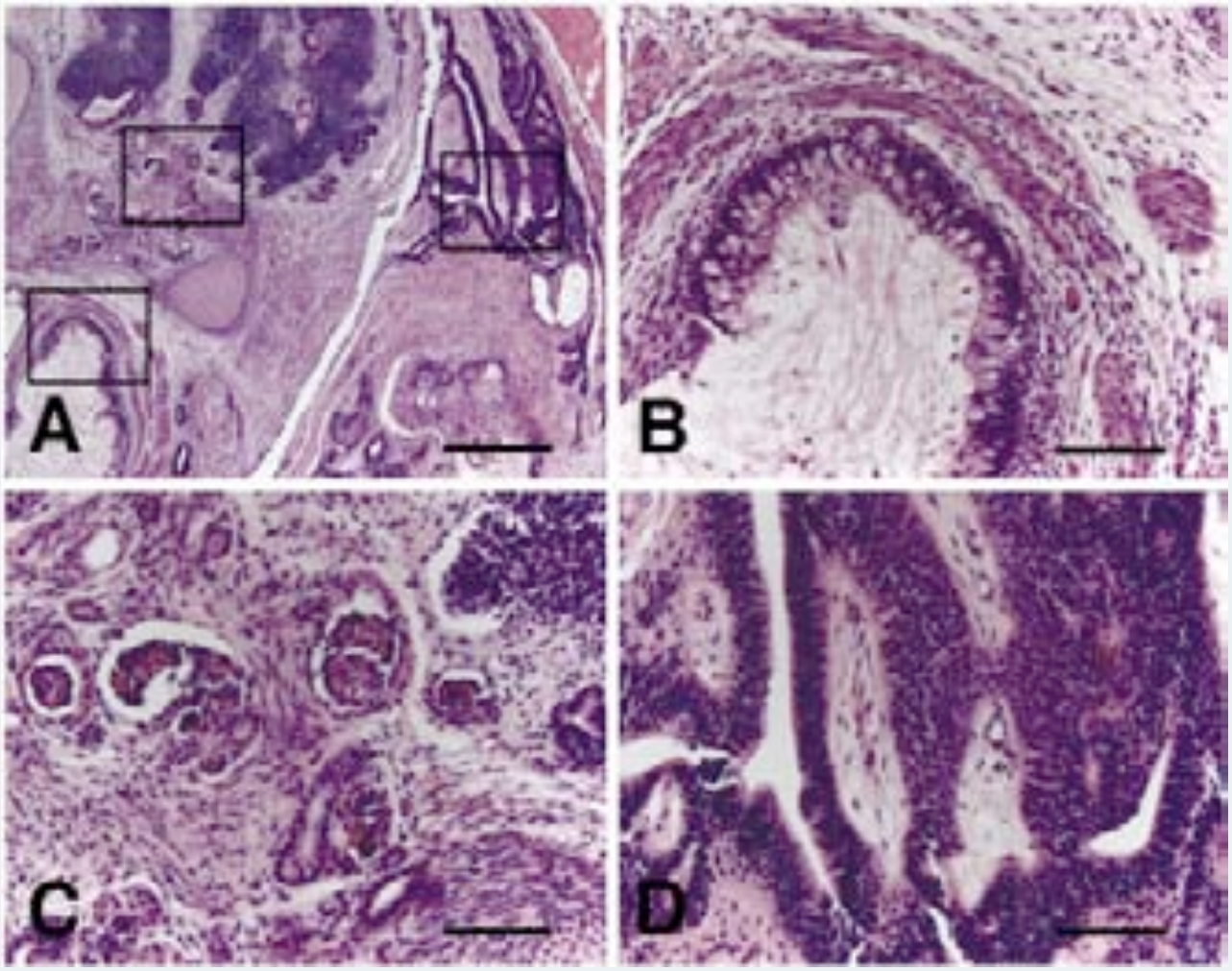
Uncontrolled differentiation of ES cells leads to tumor formation (teratomas and teratocarcinomas)



From: Amit et al. Developmental Biology **227,** 271–278 (2000)

From ES Cells to Purified Human Neurons: Biotechnological Challenges

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Stable proliferation

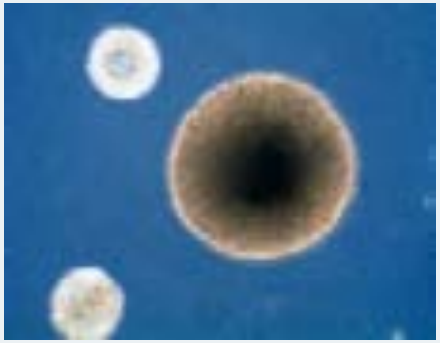
Minimized batch-to-batch variation

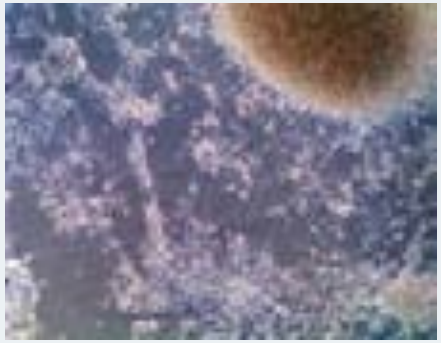
Colony selection

Neural Differentiation of Human ES Cells

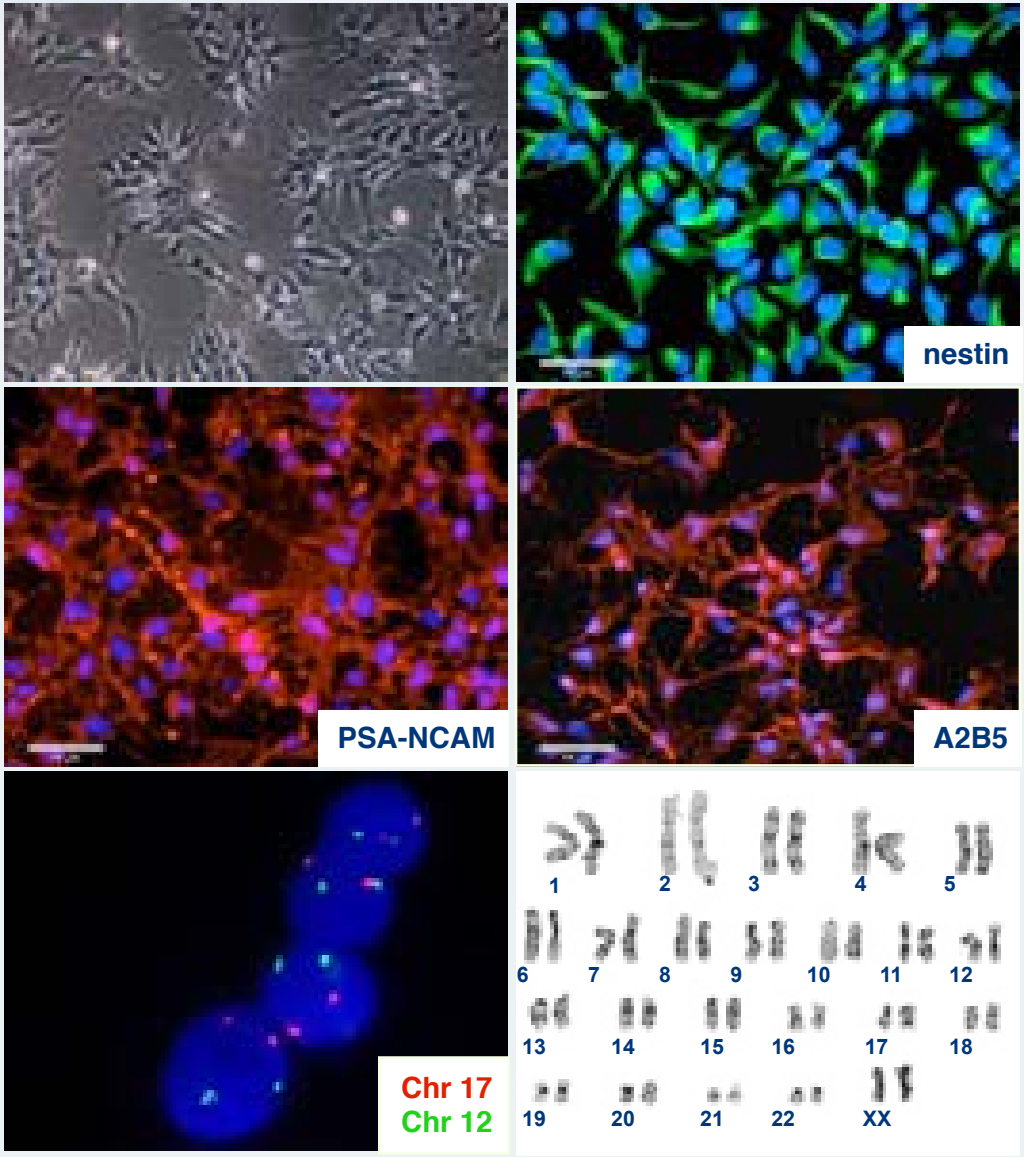
ES Cells → EB Formation → Isolation of Neural Islands in Plated EB Outgrowth

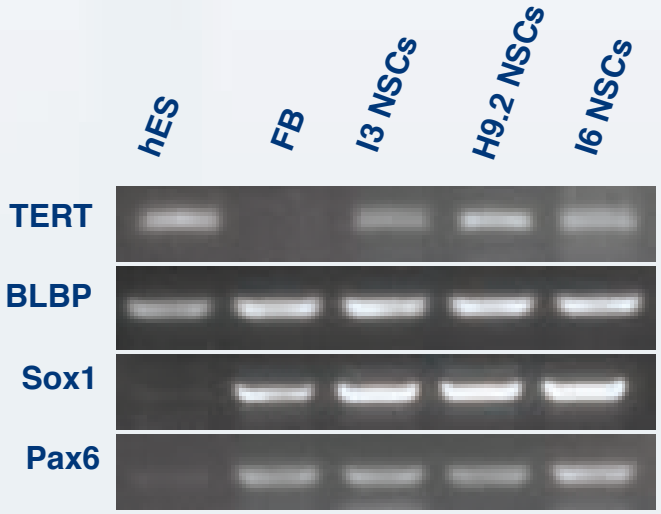
Neurosphere Cultivation → Growth Factor Withdrawal and Differentiation





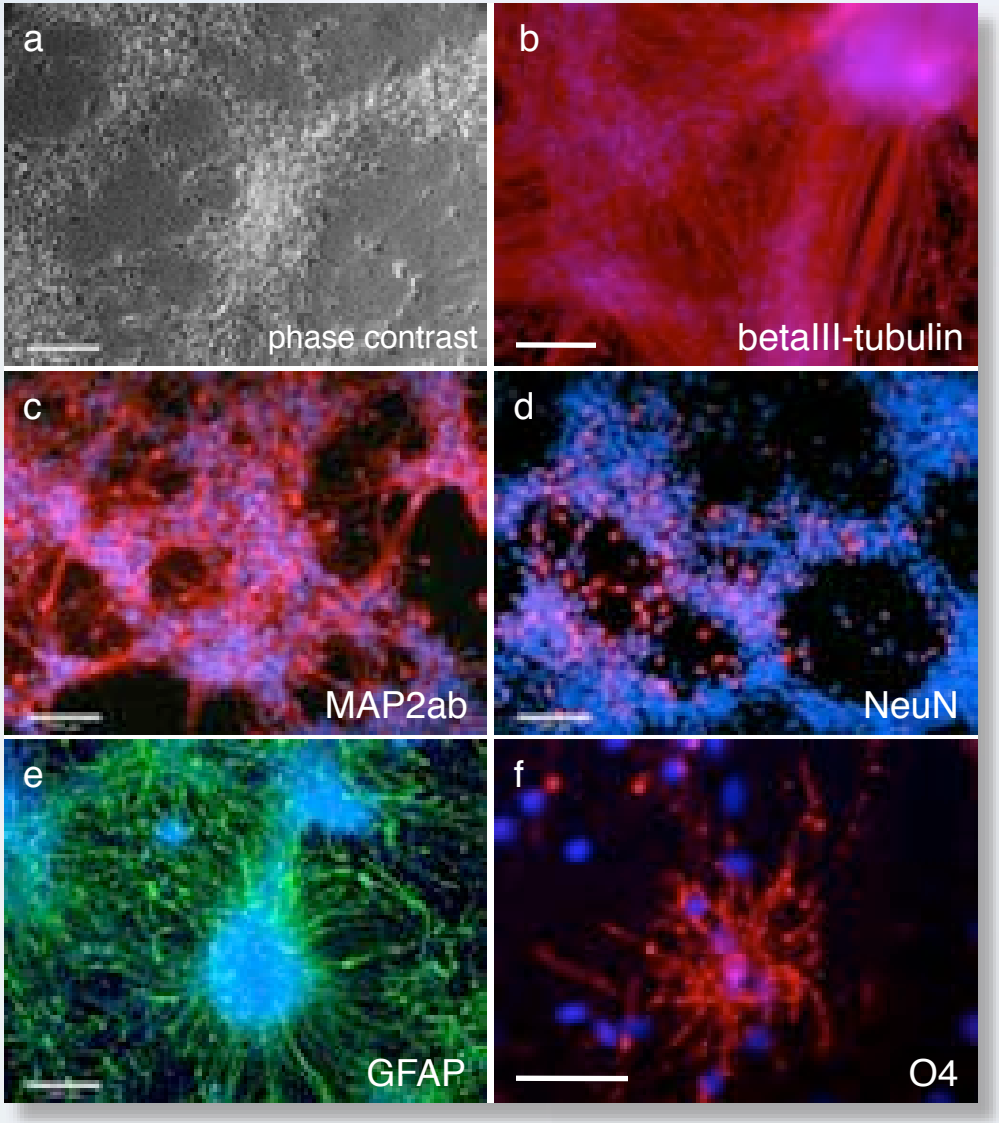
Stably Proliferating Neural Stem Cells From Human Embryonic Stem Cells (hES-NSCs)

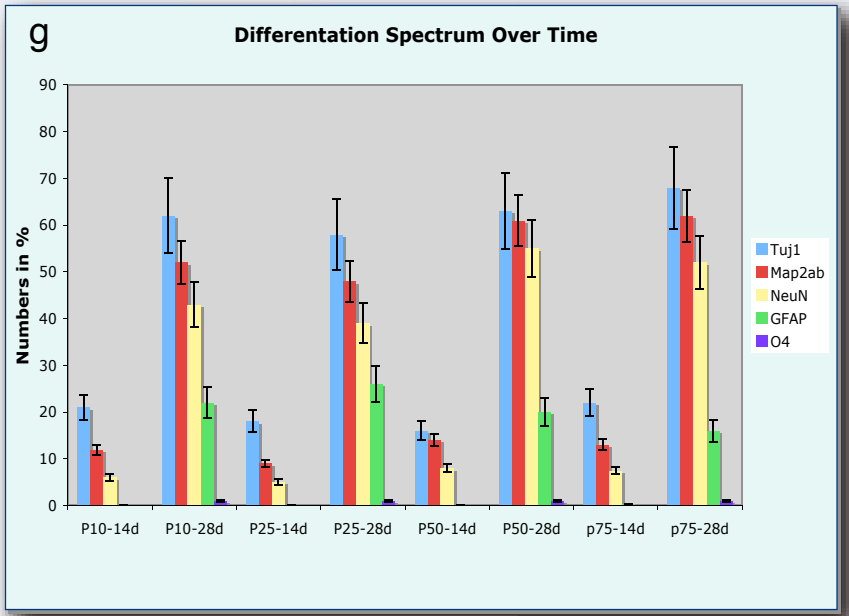




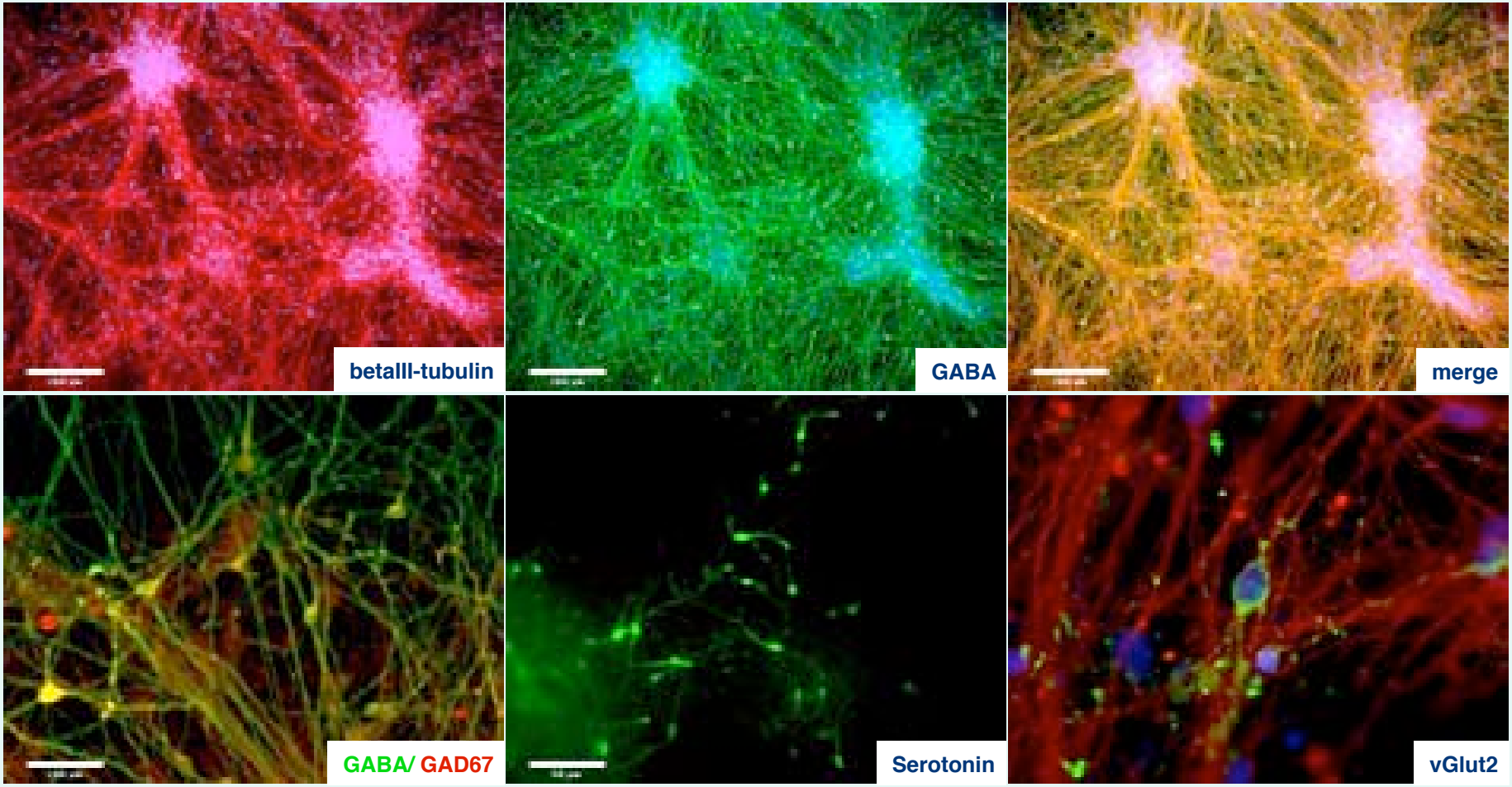
Koch et al.
PNAS 2009

ES cell-derived neural stem cells give rise to human neurons and glia



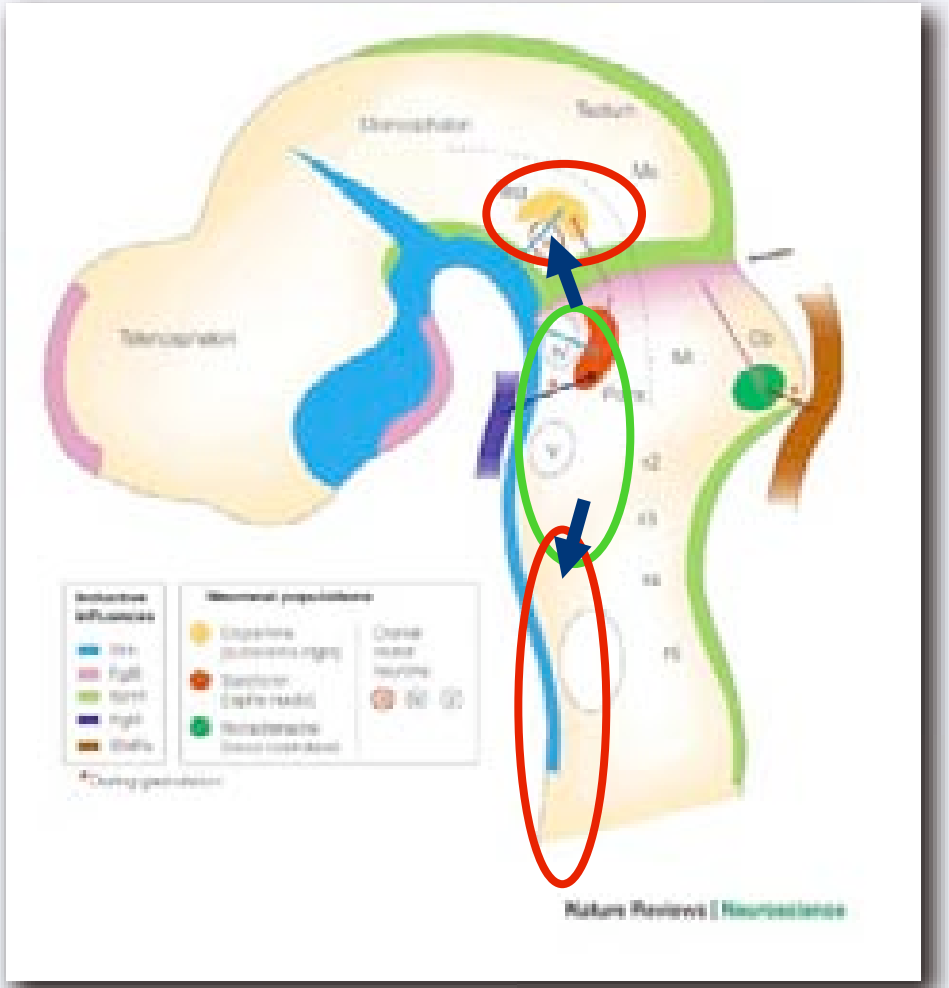


ES cell-derived neural stem cells generate different classes of nerve cells

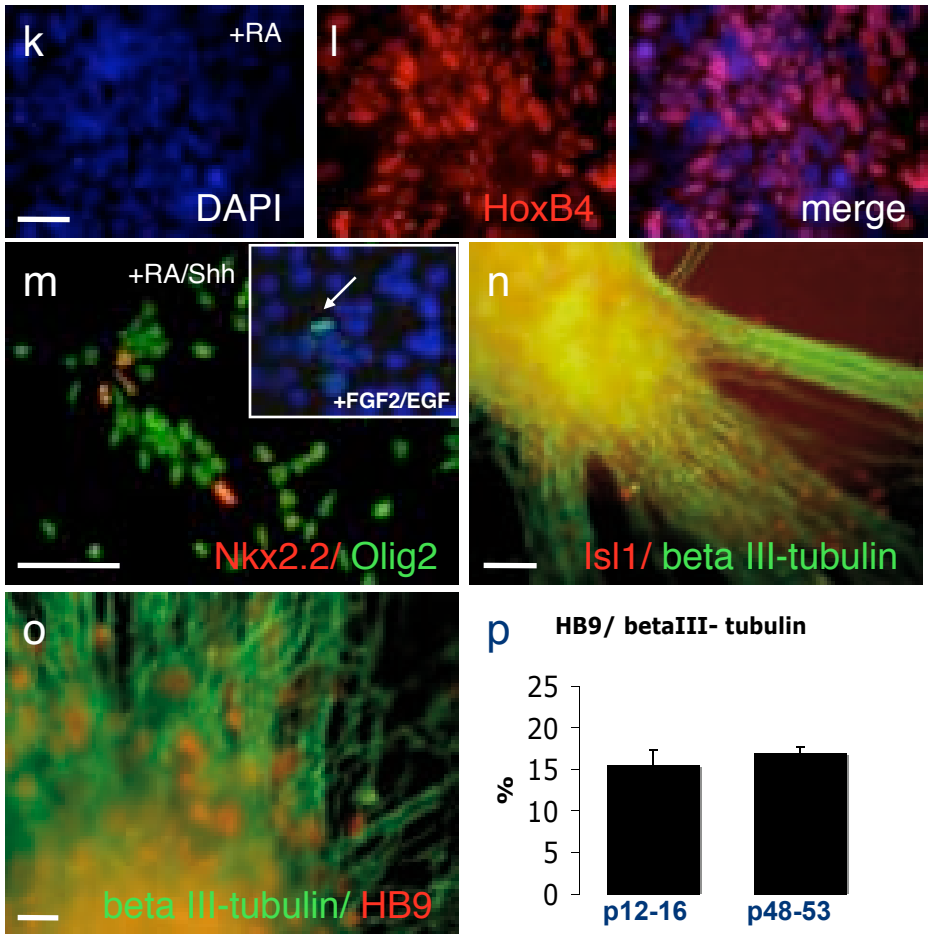


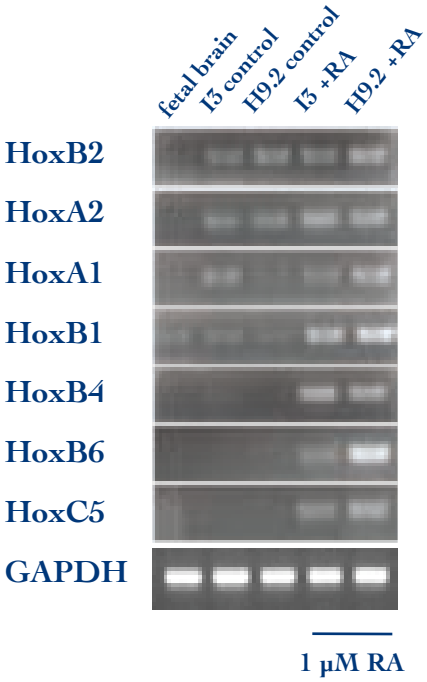
28-60 days of differentiation

Developmental signals can be used to determine the regional phenotype of neural stem cells

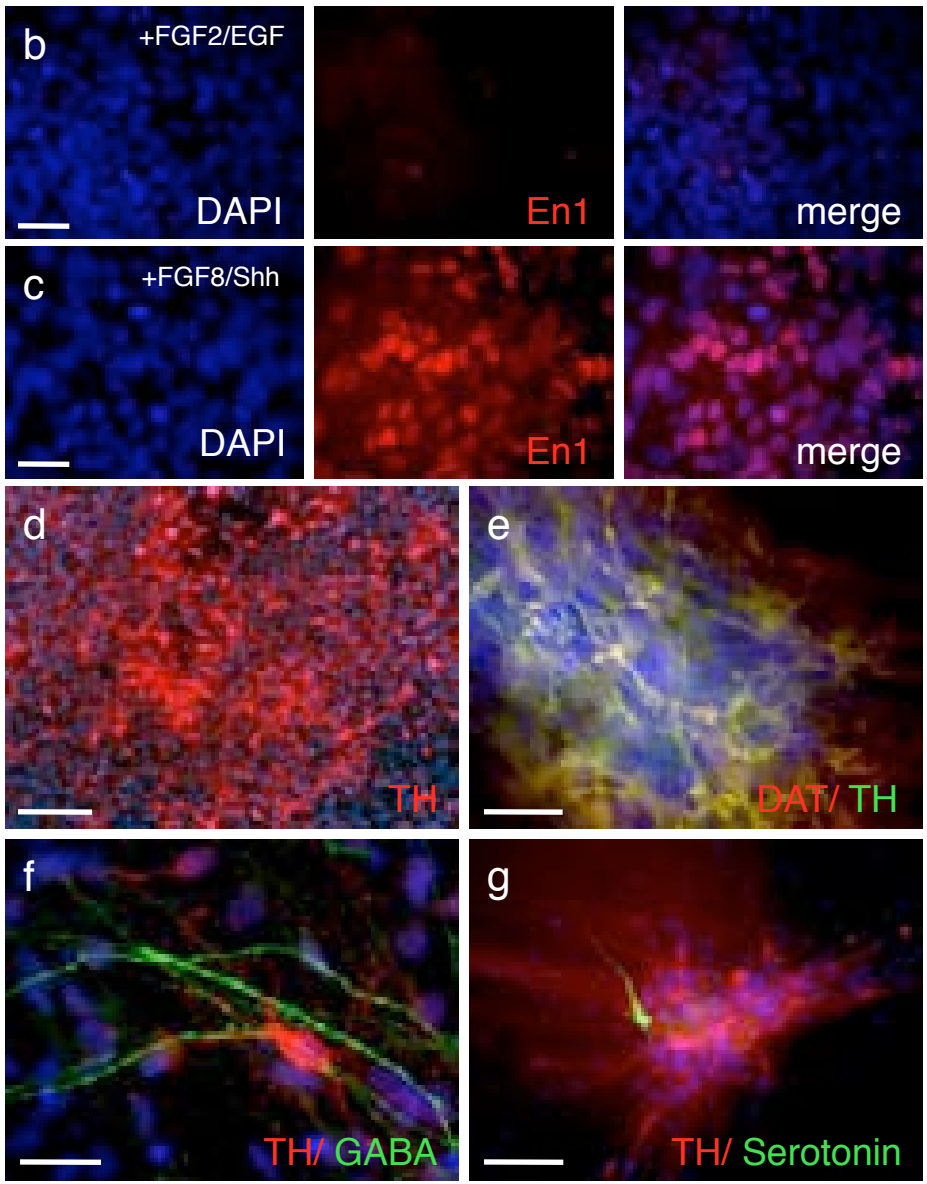


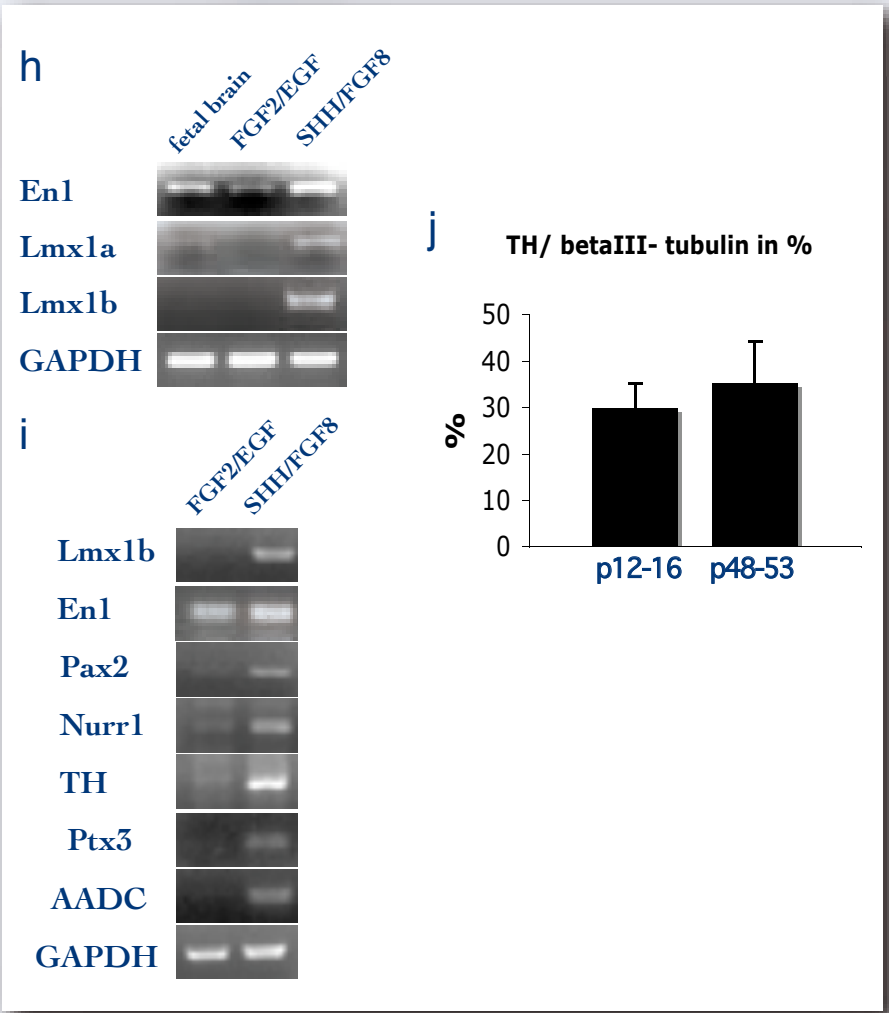
Example 1: Generation of human spinal cord motoneurons



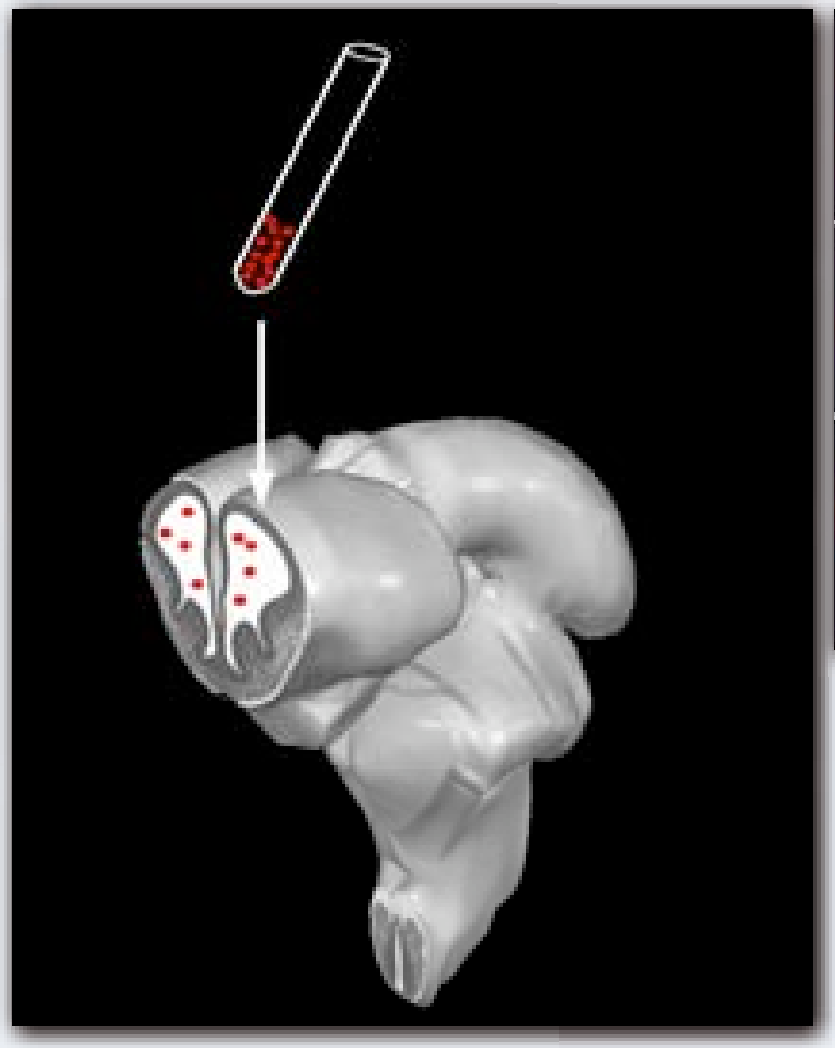


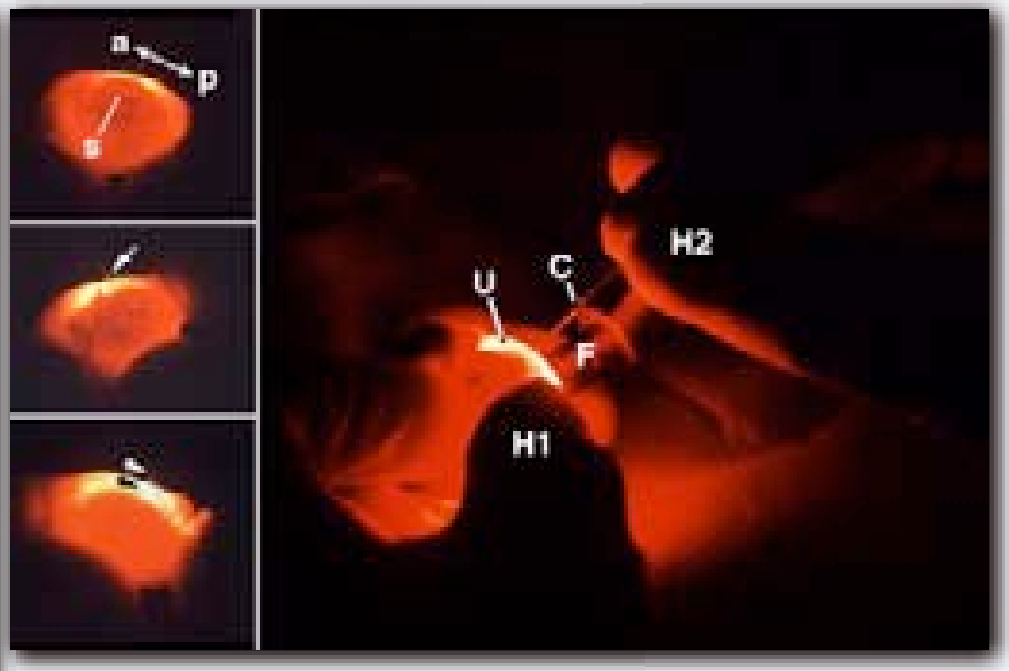
Example 2: Generation of human midbrain dopamine neurons





Generation of neural chimeras to assess functionality of stem cell-derived neurons





Generation of neural chimeras to assess functionality of stem cell-derived neurons

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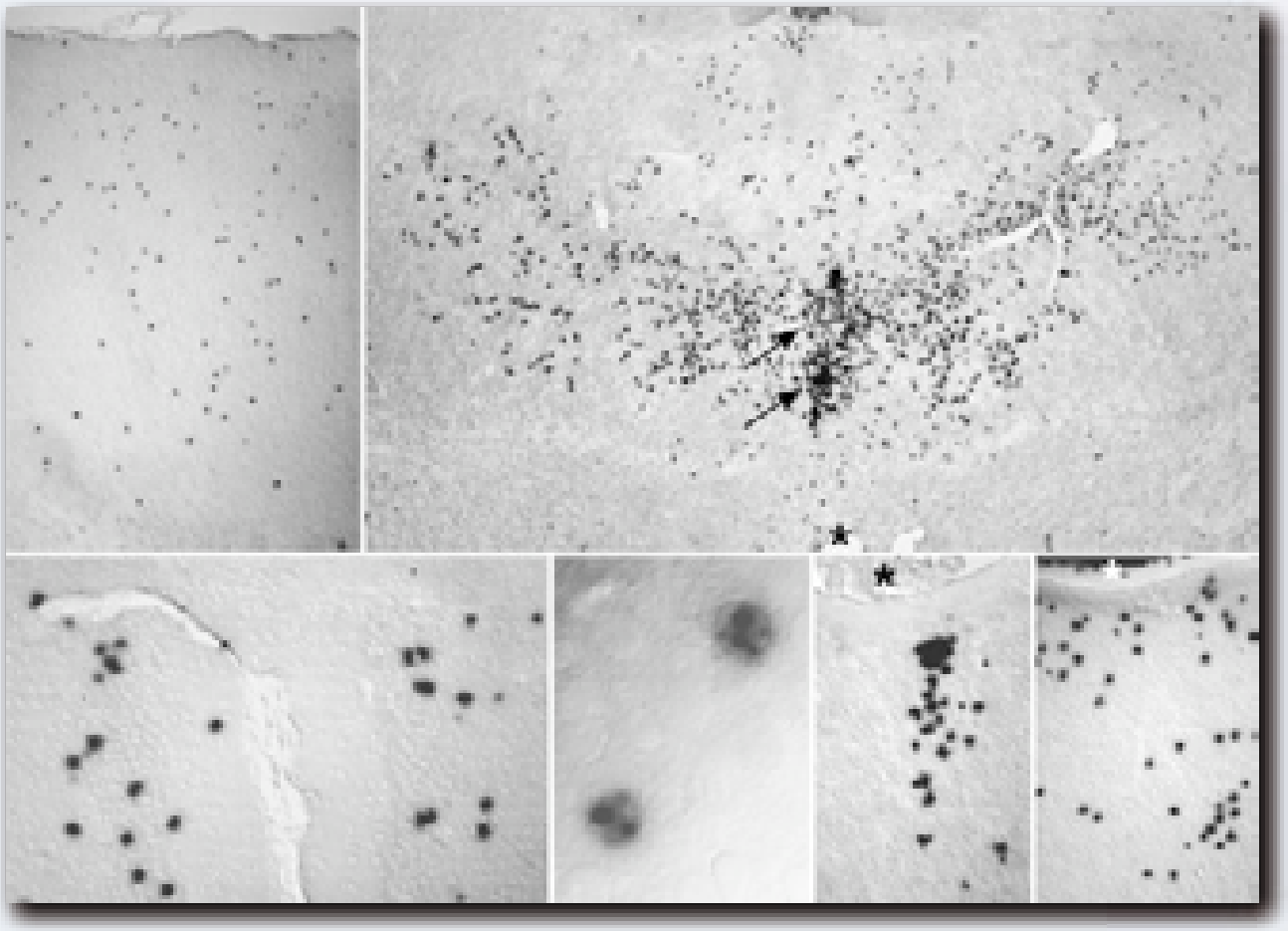
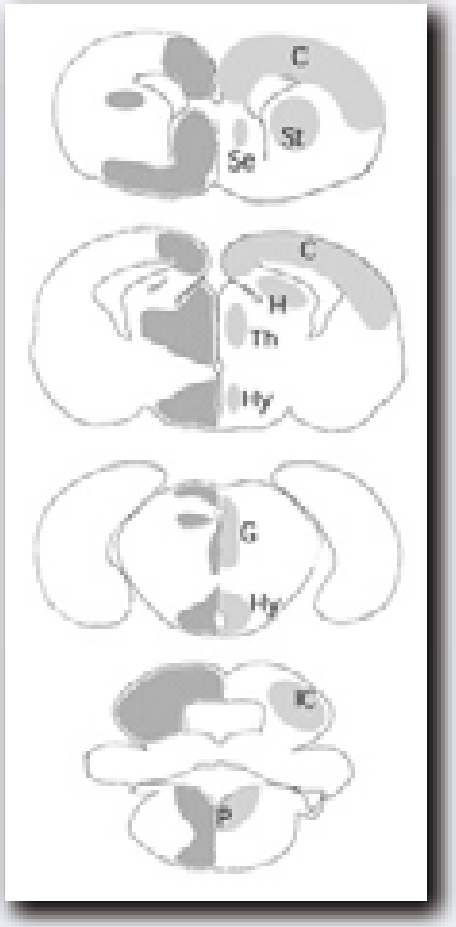
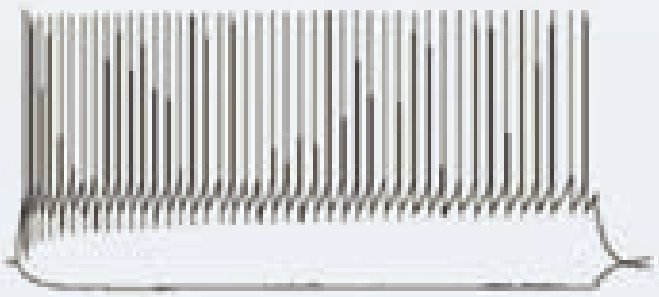


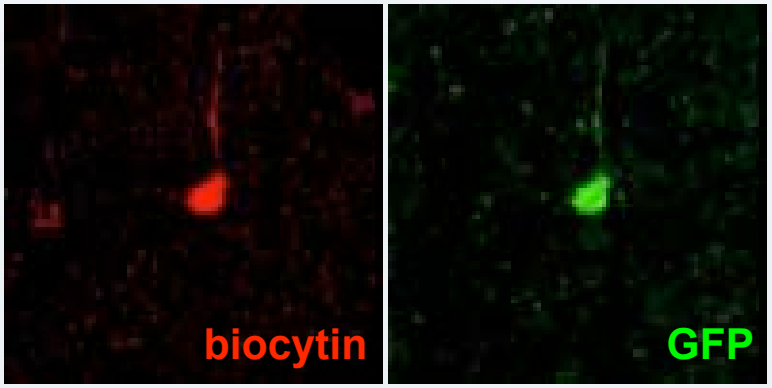


Recording from living brain slices

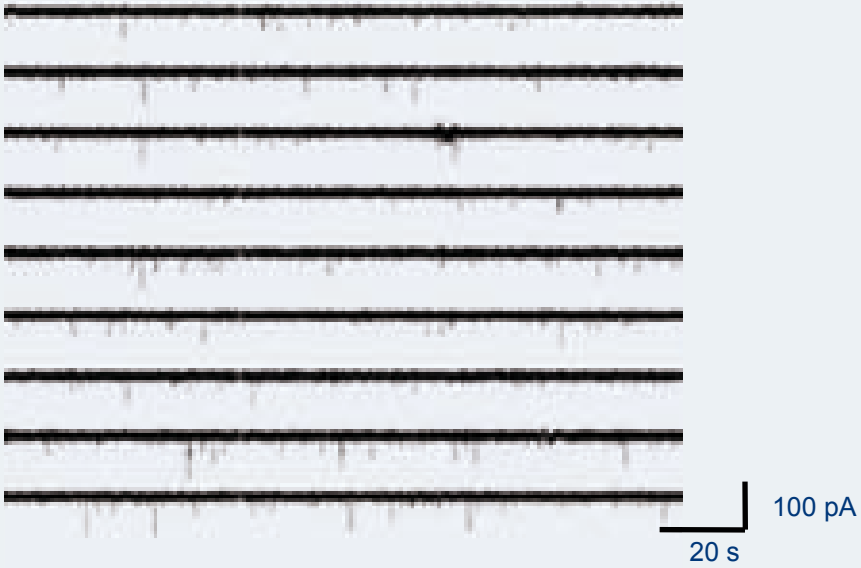
hESNP-derived neuron in striatum 138 days after transplantation

Spike pattern

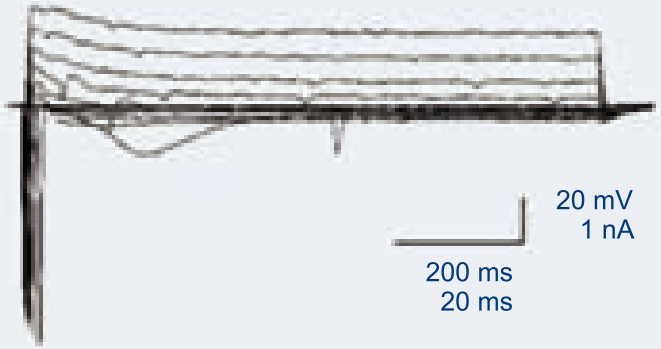




Spontaneous postsynaptic currents

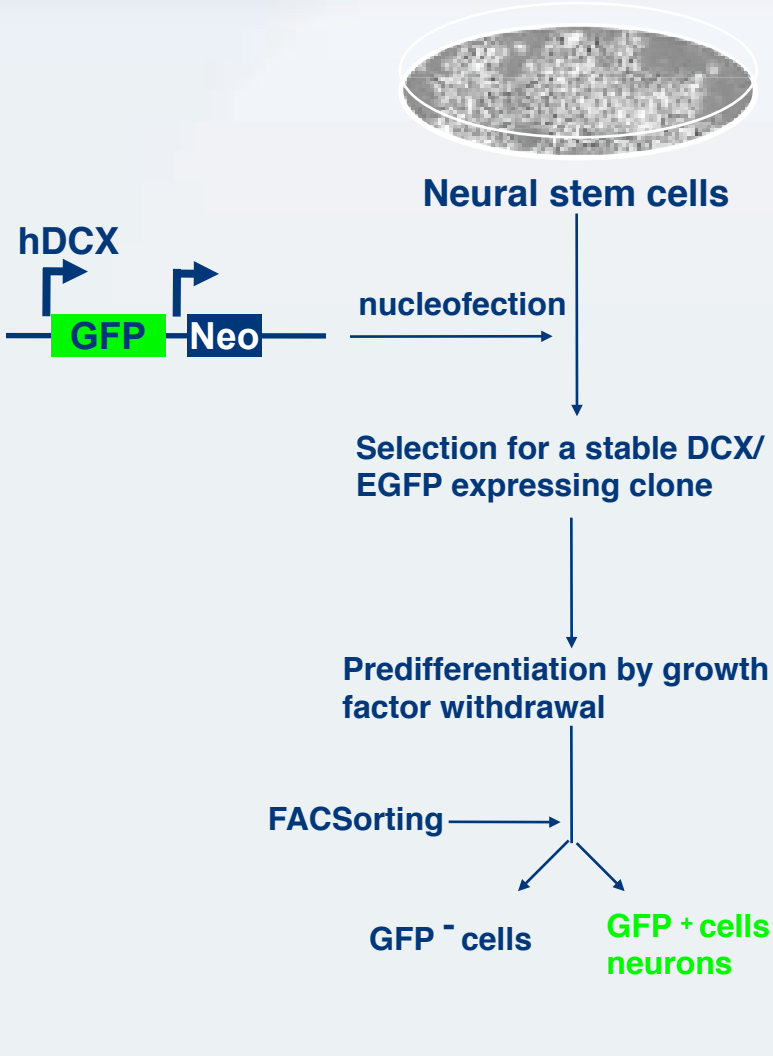


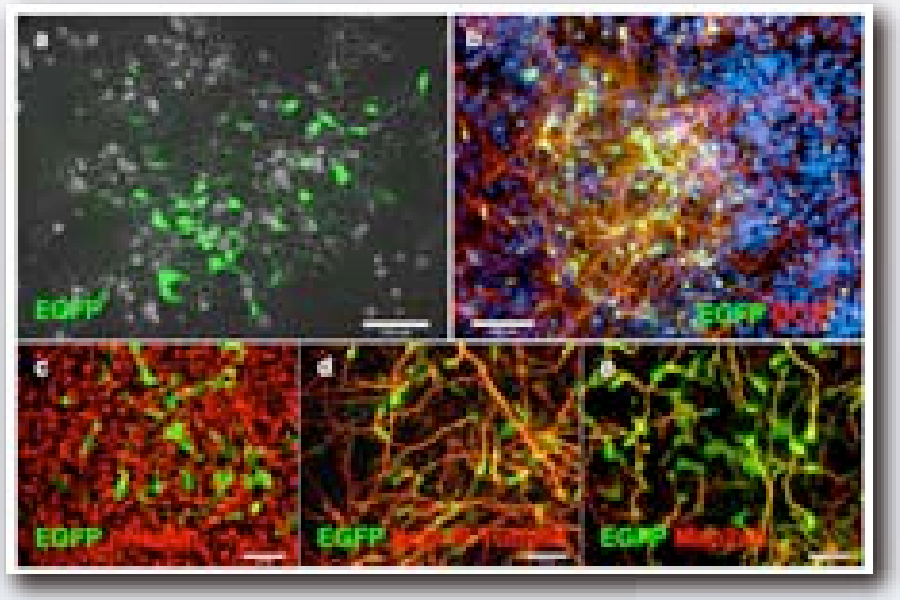
Whole cell current



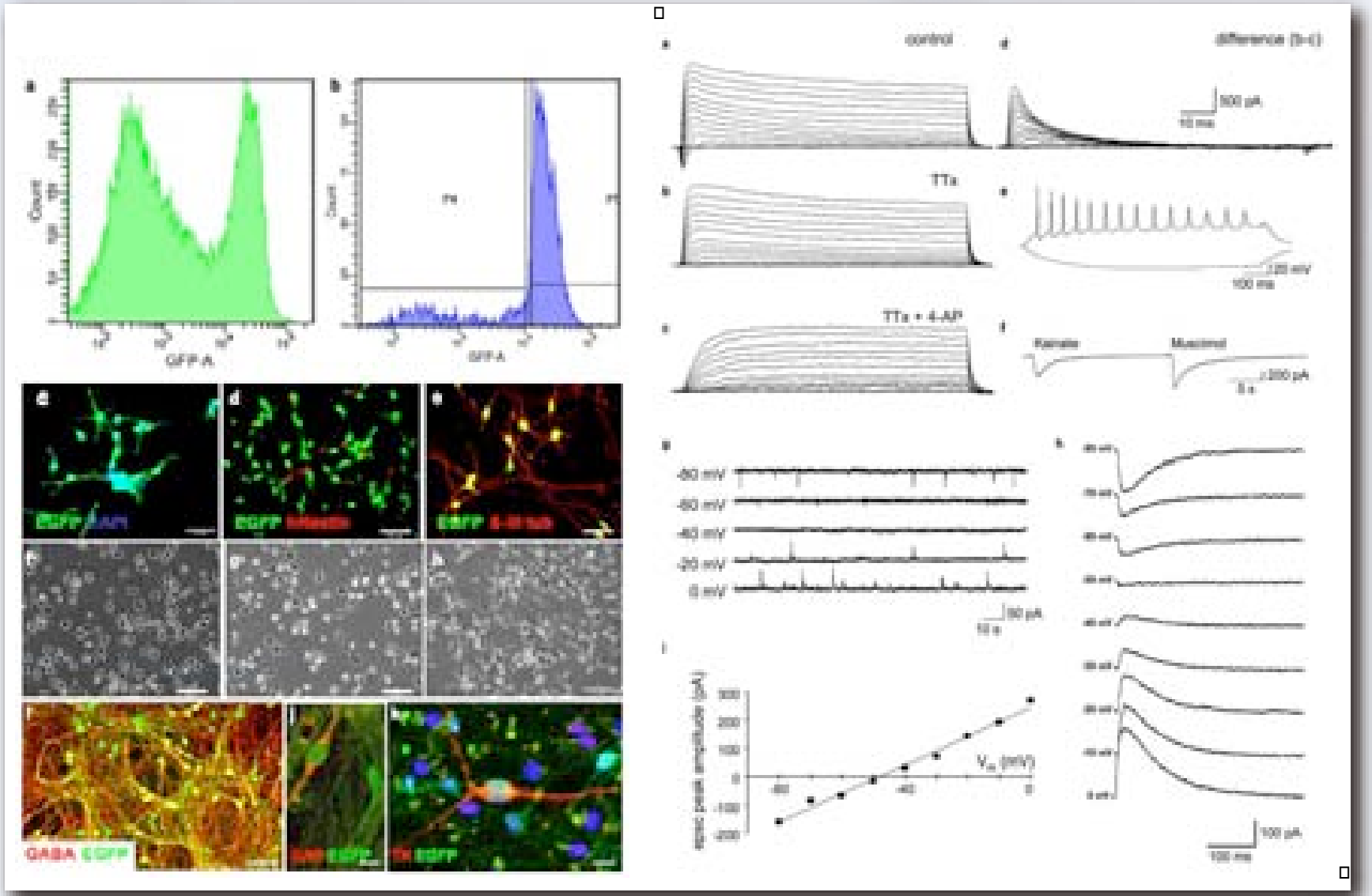
Koch et al., PNAS 2009; Opitz et al., Nature Protocols 2007

Lineage selection of young neurons





Purified human neurons can be obtained by lineage selection



Lineage Selection & Transgene Removal

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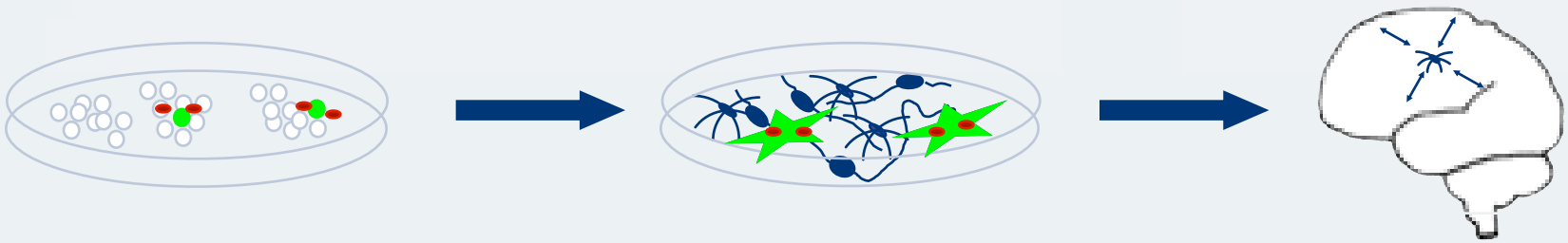
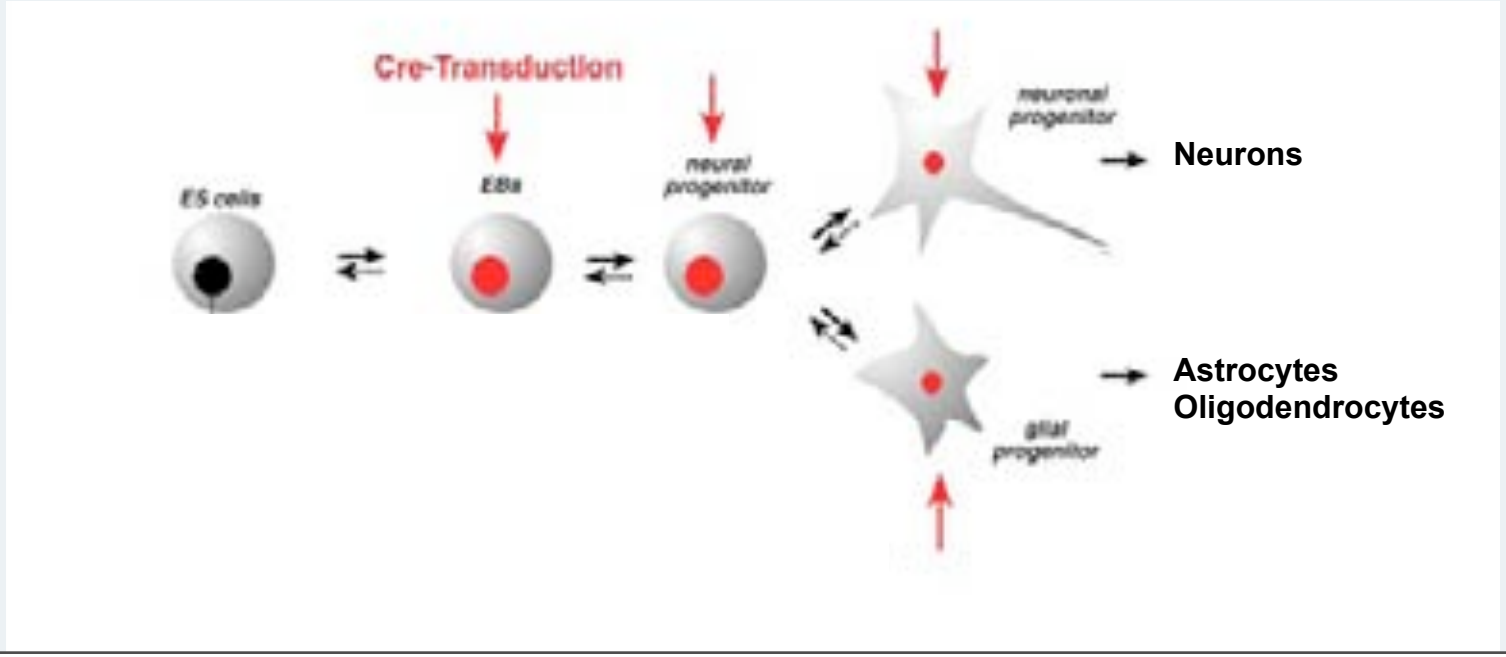
ES Cells **Controlled Differentiation** **Functional Integration**





From ES Cells to Purified Human Neurons: Biotechnological Challenges

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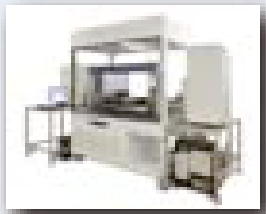

hESC — hNP — hNSC — hN

hESC

- Stable proliferation
- Minimized batch-to-batch variation
- Colony selection

hNP

- Removal of un-differentiated hESC

hNSC

- Stable proliferation
- Phenotypic stability
- Amenability to instructive cues
- Functionality

hN

- Max. purity
- Amenability to cryoconservation
- (Reversible) genetic modification

Major Biomedical Applications of Pluripotent Stem Cells

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Cellular disease models

Cell-based repair strategies

Desirable:
Patient-specific
(autologous) cells

Germline stem cells

LETTERS

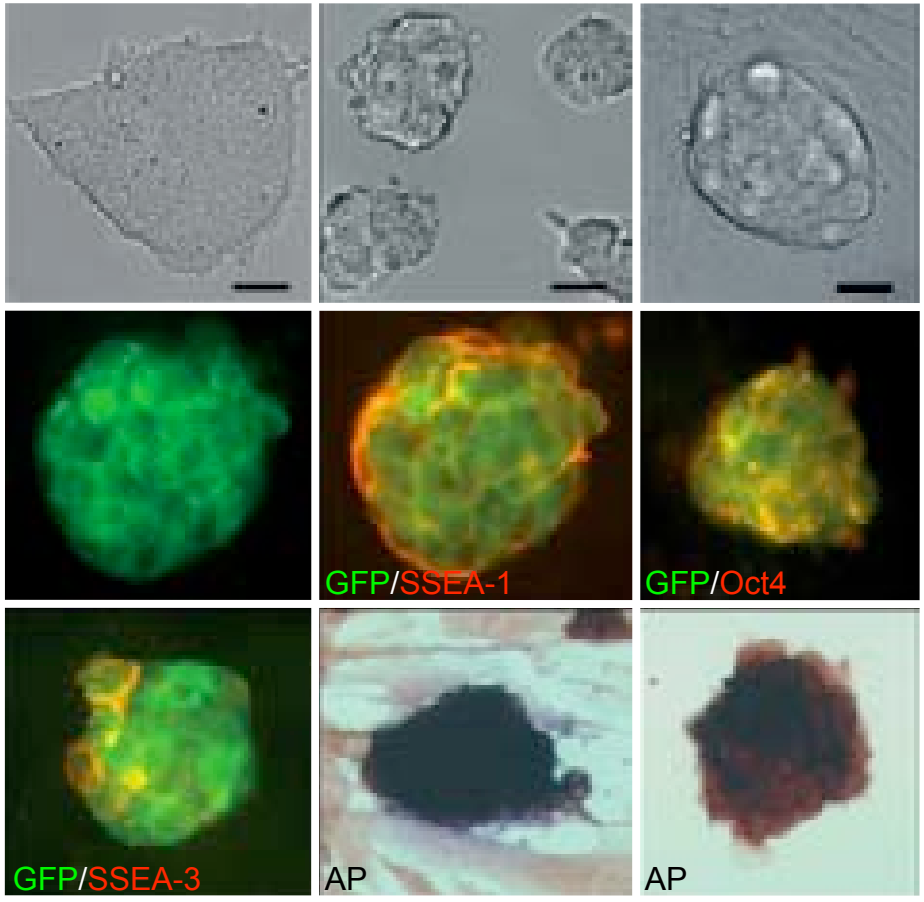
Pluripotency of spermatogonial stem cells from adult mouse testis

ARTICLES

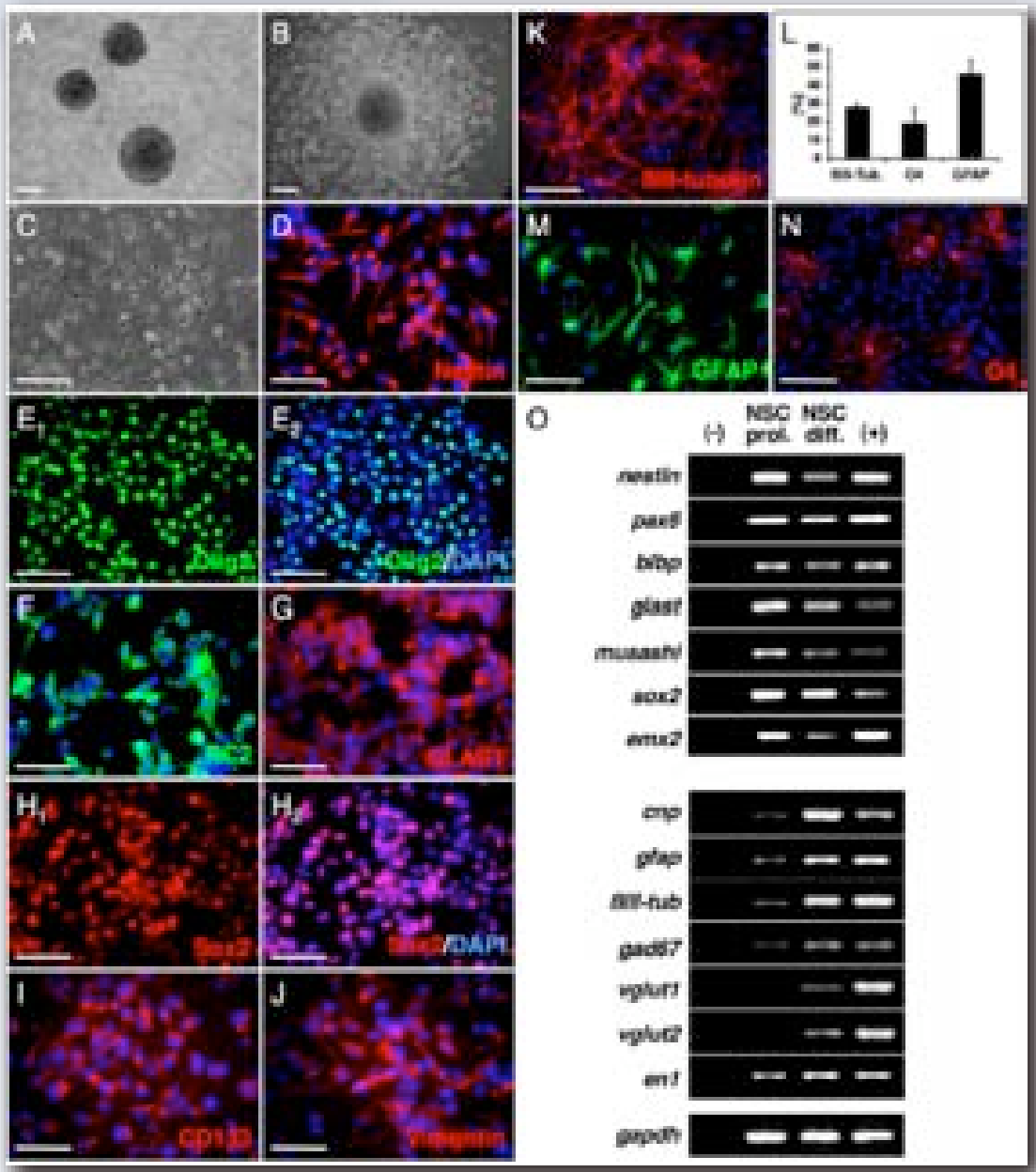
Generation of pluripotent stem cells from adult human testis

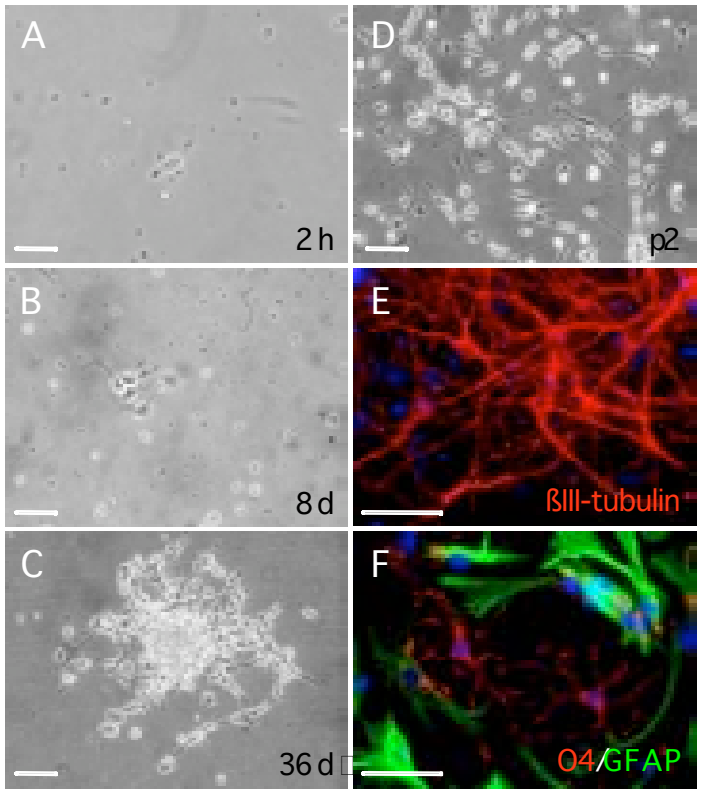
Cell Stem Cell
Resource

Induction of Pluripotency in Adult Unipotent Germline Stem Cells



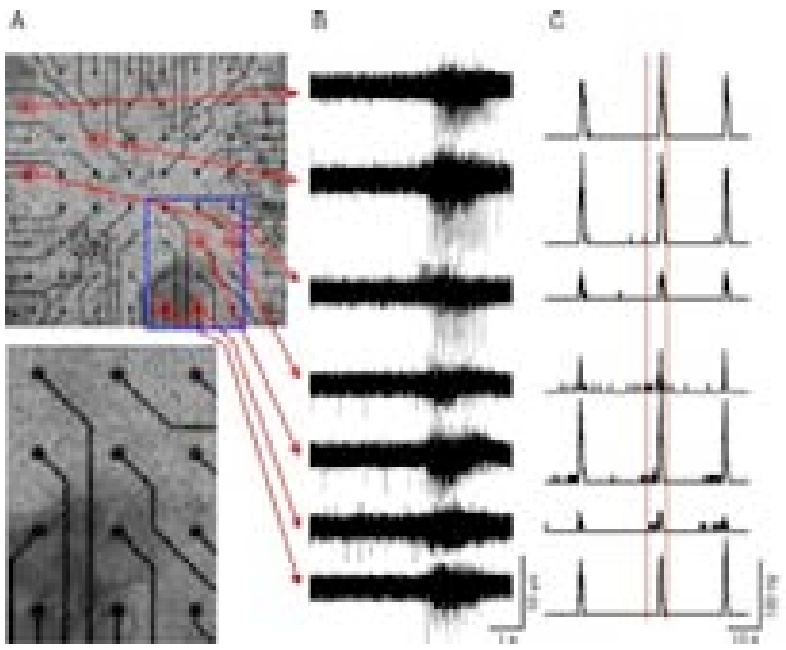
Generation and tripotential differentiation of NSC from adult testis-derived stem cells

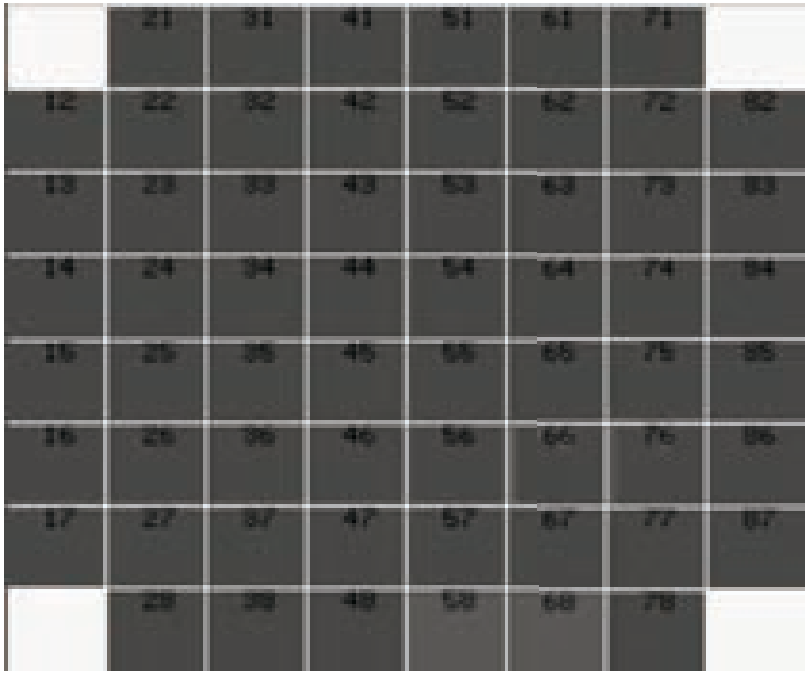




Glaser et al., Stem Cells 2008

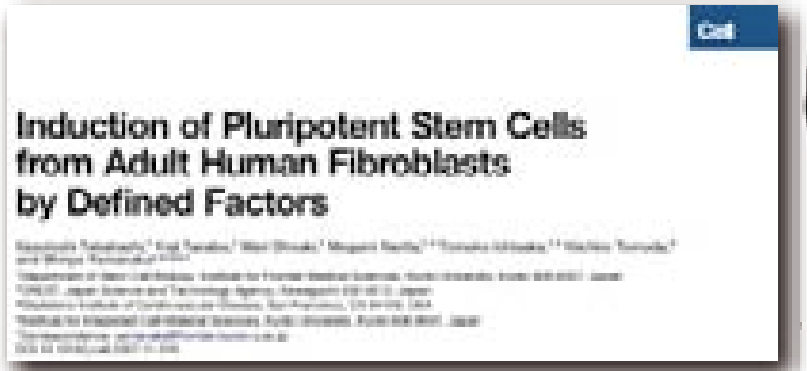
Adult testis-derived neurons develop into functional networks

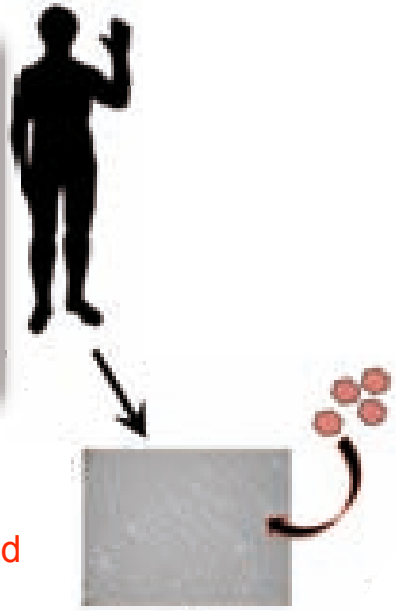




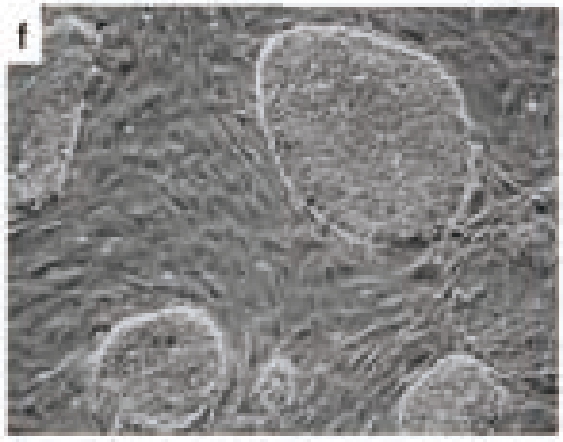
Cooperation with Philipp Sasse & Bernd Fleischmann, Department of Physiology, University of Bonn

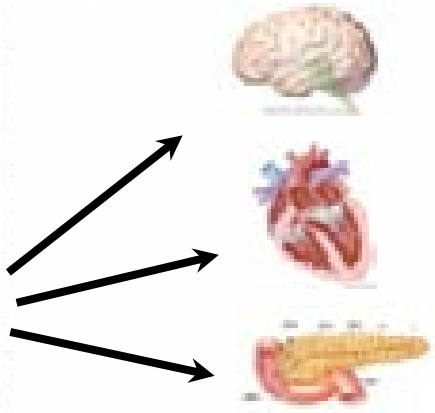
Sources of pluripotent stem cells





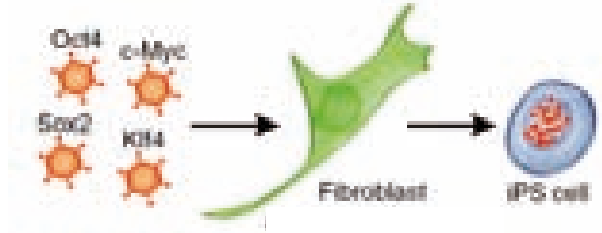
➡ The combined expression of the four transcription factors **c-myc, Klf4, Sox2 and Oct3/4** induces pluripotency in embryonic and adult fibroblasts

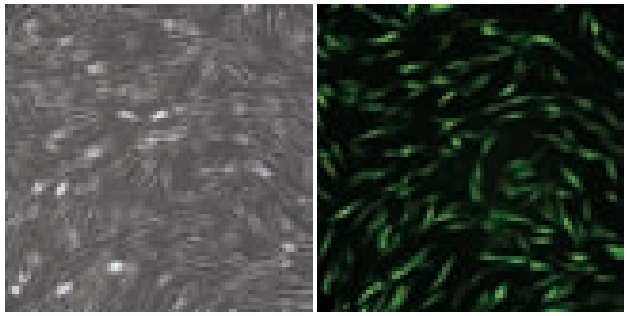




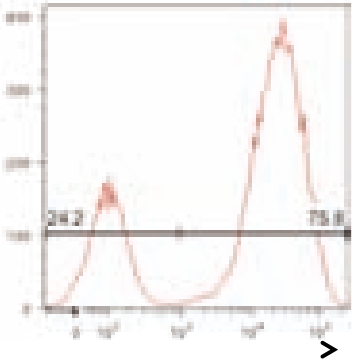
Generation of human iPS cells from patient skin biopsies

Fibroblast culture from skin biopsy



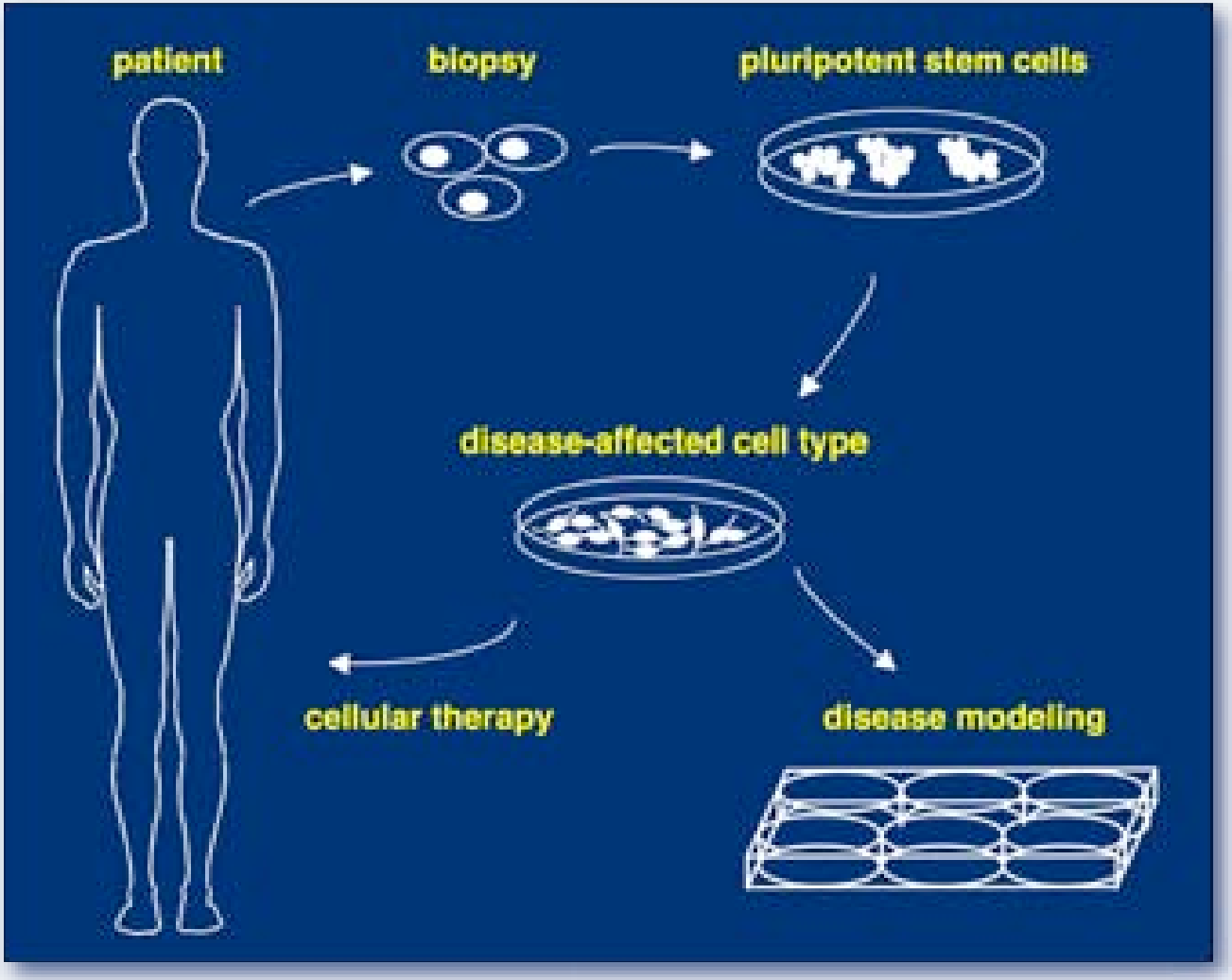


Retroviral transduction

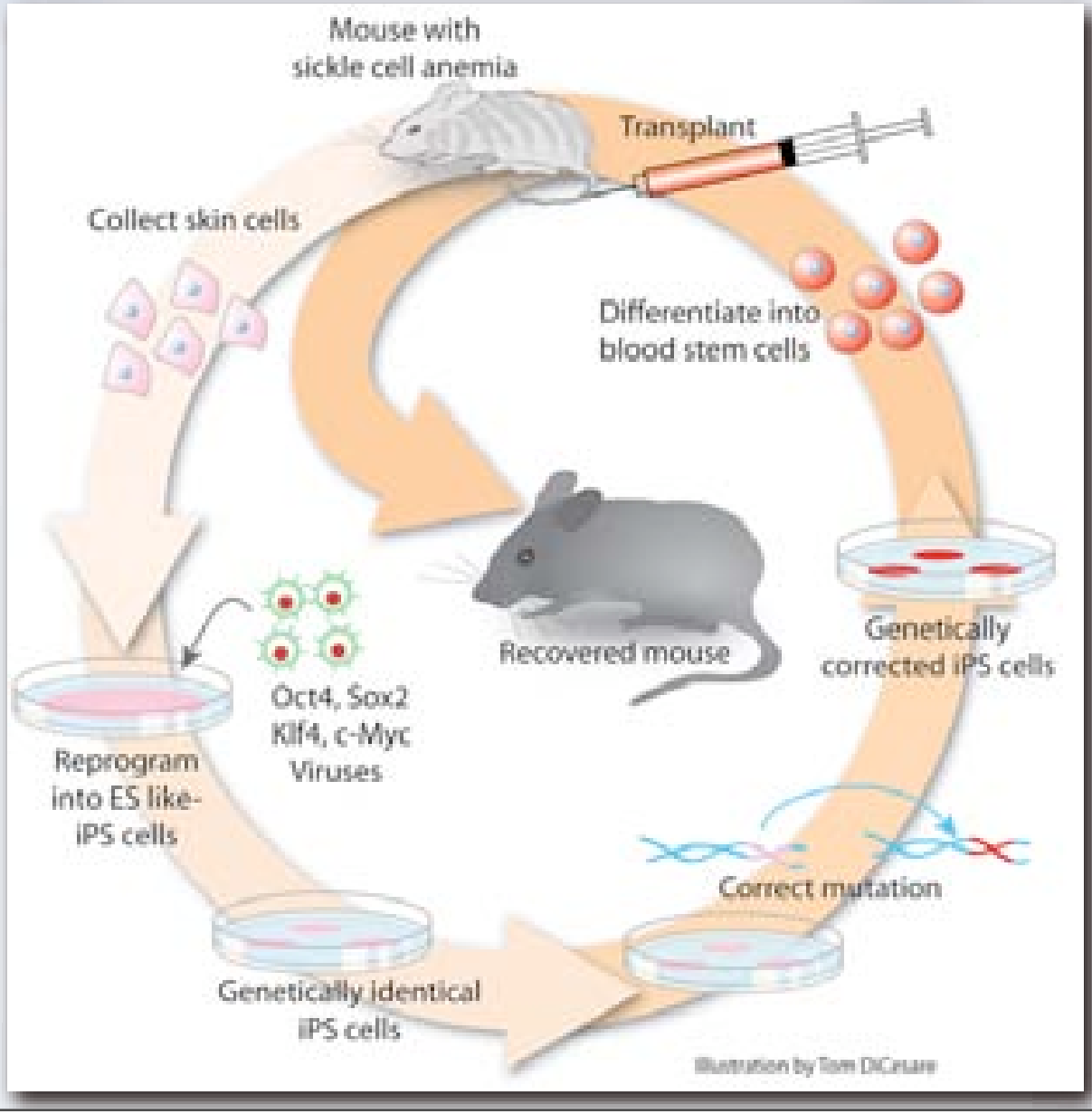


eGFP

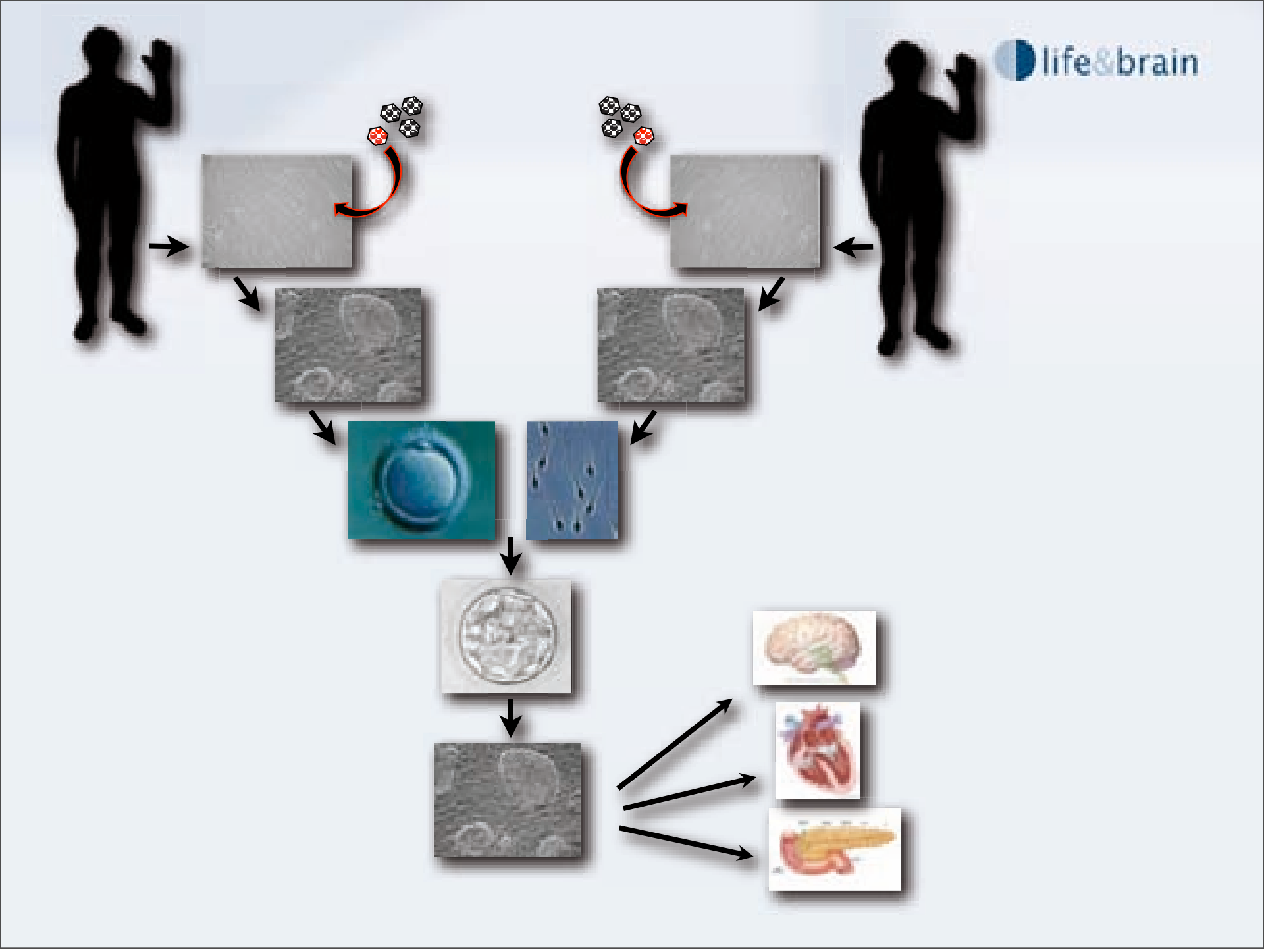
Time scheme of Reprogramming - Jaenisch & Young, Cell 2008


patient
biopsy
pluripotent stem cells
disease-affected cell type
cellular therapy
disease modeling

iPS cells can be used for therapy



Hanna et al., Science 2007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ES cell-based myelin repair

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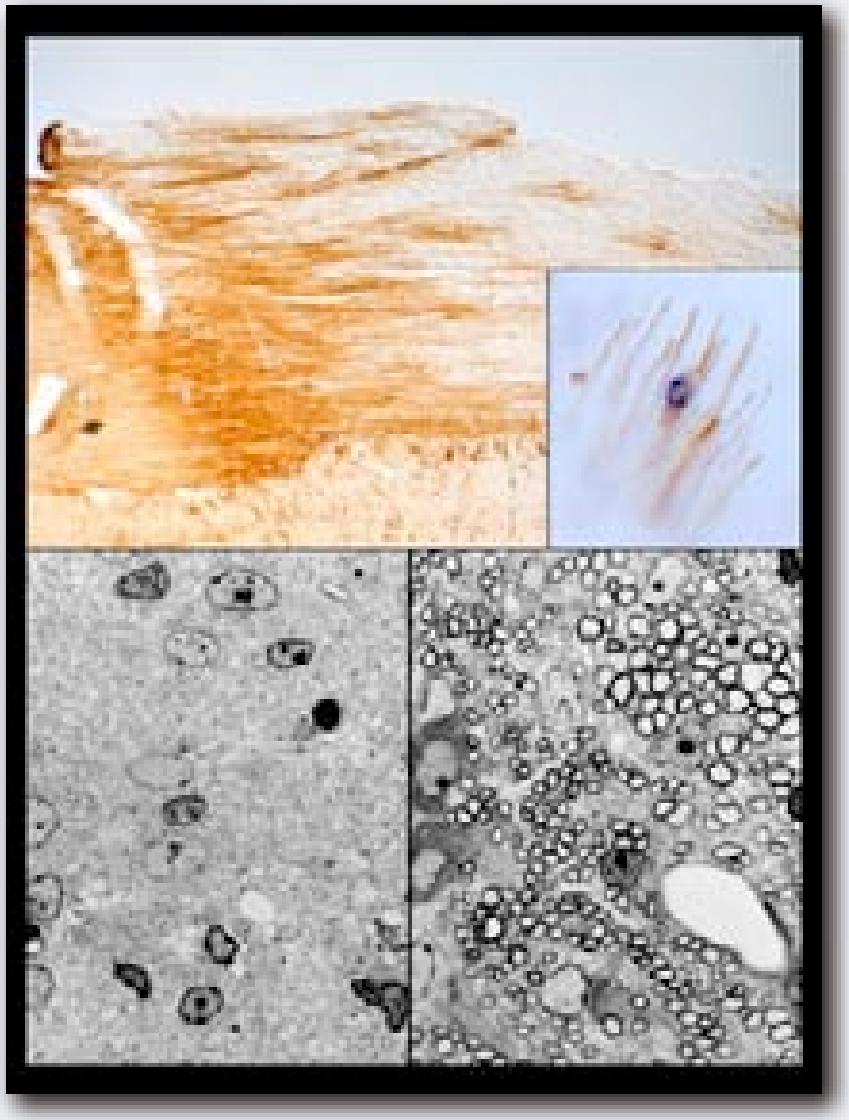
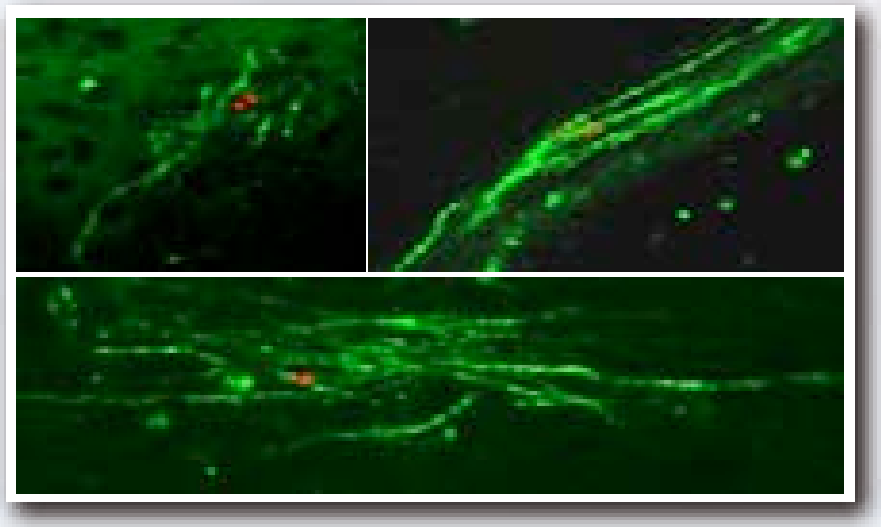
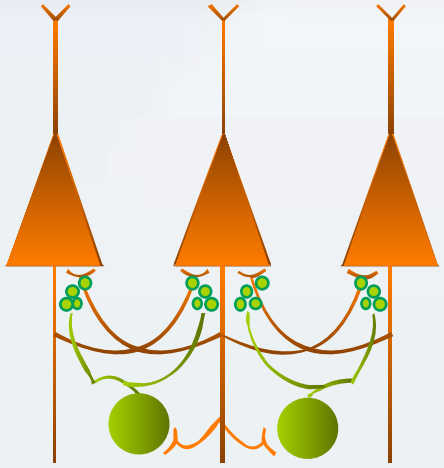
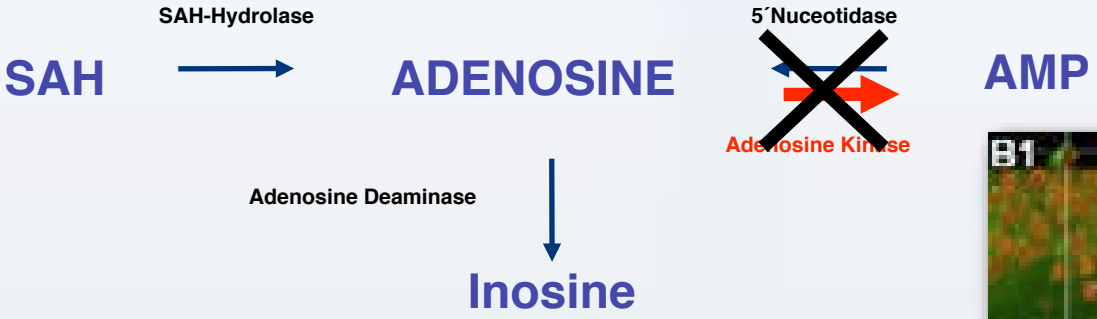


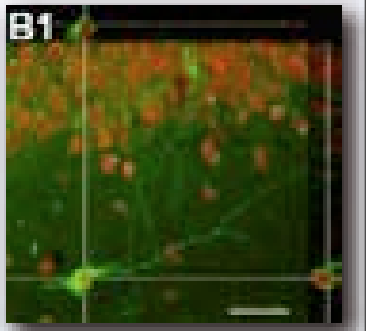


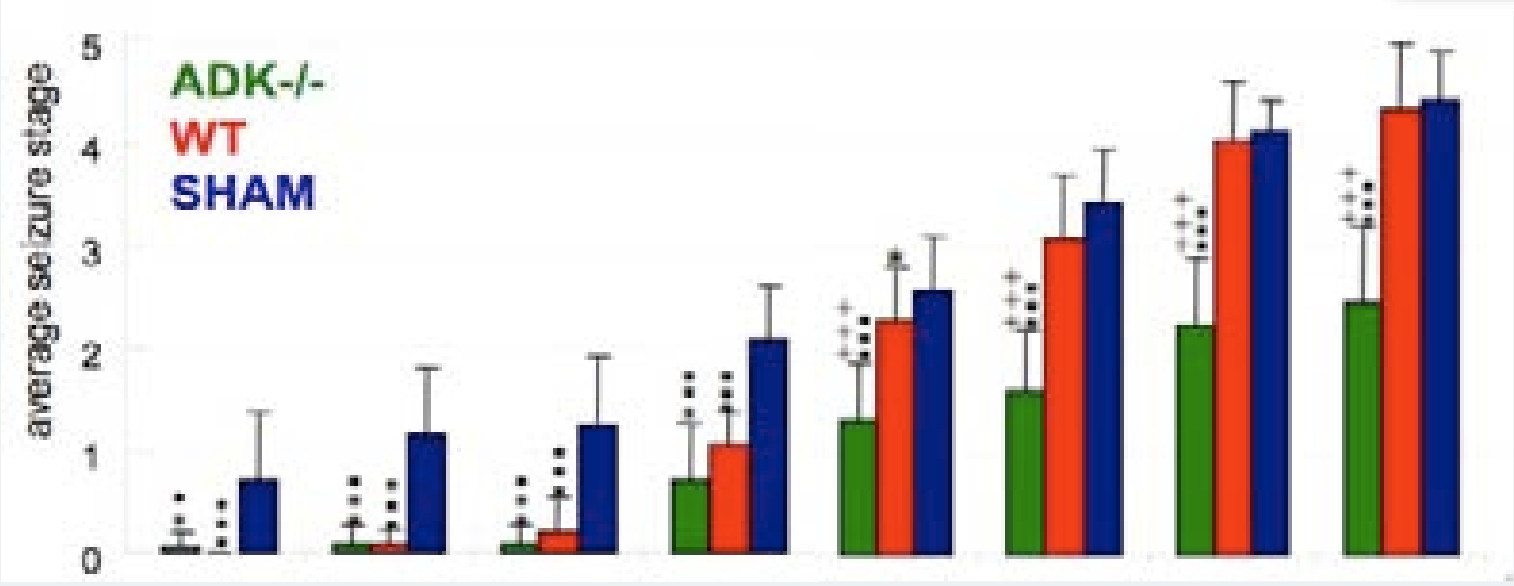
Brüstle et al., Science 1999

Neuromodulatory transplants
Example: Epilepsy







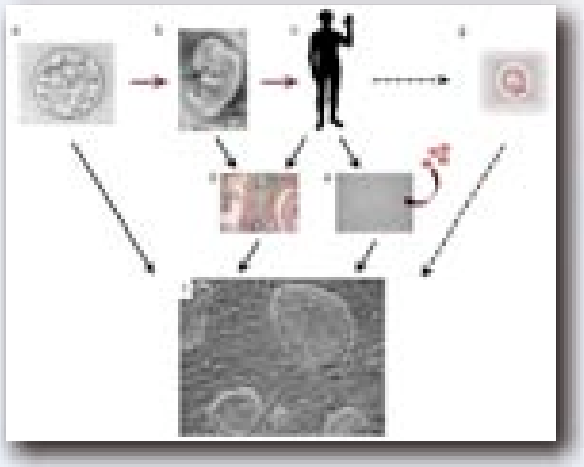


days after TX 9 12 14 16 19 21 23 26

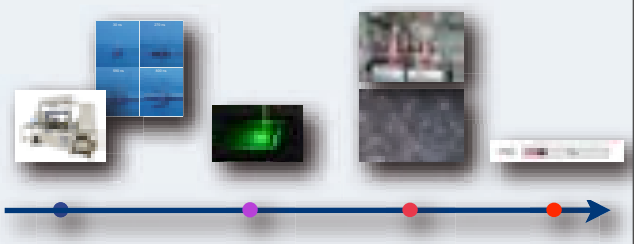
Li T*, Steinbeck JA*, et al., Brain 2007

Conclusions - I

1. ES cells, germ cells and iPS cells provide sources of pluripotent stem cells.

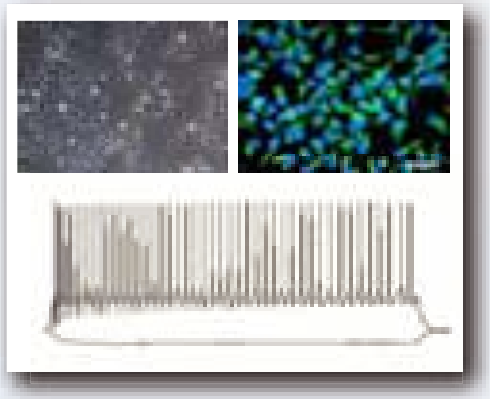


2. Combination of industrial cell purification technologies and differentiation protocols yielding stable somatic stem cells permits the generation of functional and non-tumorigenic neural precursors from pluripotent stem cells.

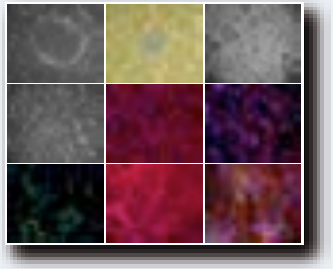


Conclusions - II

3. Stable neural stem cells can be derived from pluripotent human stem cells and give rise to functional neurons in vivo.



4. Neural differentiation paradigms might be translatable from ESC to iPS and germline stem cells.



5. Application of stem cell grafts as functional modulators may precede classic neuronal cell replacement.

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