Stem Cells

Weimin Zhong, Ph.D.
weimin.zhong@yale.edu

Department of Molecular, Cellular & Developmental Biology
Yale University

Learning Goals for This Lecture

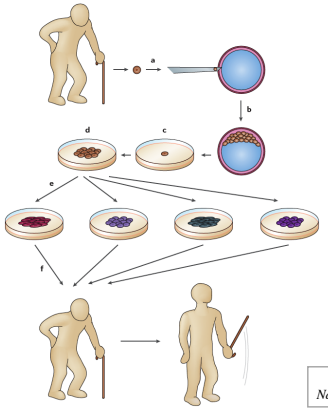
- To recognize that embryonic cells with different potency can be cultured *in vitro* and retain the ability to generate other cell types.

- To recognize that cellular differentiation can be reversed using multiple experimental approaches.

- To appreciate both the promises and the challenges of using embryonic and adult stem cells for regenerative medicine.

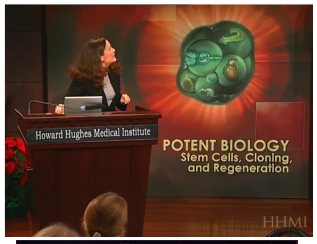
- To appreciate that knowledge gained from studying development can guide stem cell differentiation for tissue repair.

Looking for the Fountain of Youth
(stem cells give us hope)



Solter. (2006)
Nat. Rev. Genet. 7:319-327

Some Animals Have Amazing Abilities to Regenerate
(many of our tissues can repair normal wear and tear)



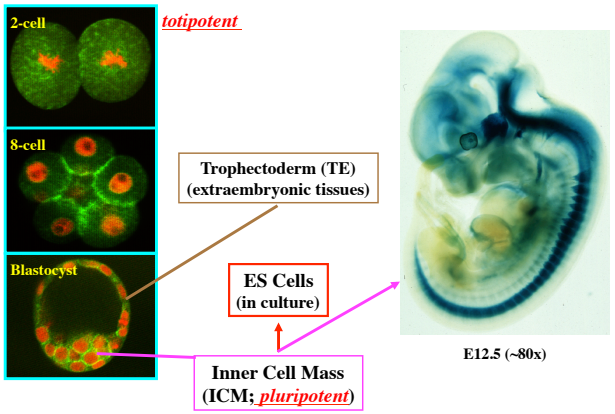
Regenerative medicine seeks to repair damages that go beyond the normal wear and tear to regenerate in ways we cannot normally do!

Stem Cells

1. Embryonic Stem (ES) Cells
2. Adult (Somatic) Stem Cells
   (including germ line stem cells)

The ability to produce more of themselves (*self-renewal*) & also cells different from themselves (*differentiation*)!

Establishing Embryonic Stem Cells: Source



Teratoma Formation & Stem Cells

(early embryonic cells form tumors when transplanted to certain adult tissues)

Extrauterine Growth of Mouse Egg-cylinders results in Malignant Teratoma

WHEN Stevens transplanted mouse ova at the one and two cell stage into the testis of a 129/Sv mouse, teratomas consisting of several types of differentiated and undifferentiated tissues resulted[1]. Tumours developed only when transplantation was into the 129/Sv-S1^{J}CP strain, known for developing frequent spontaneous testicular teratomas.

Egg-cylinders were isolated from pregnant uteri of C3H/H mice in the morning of the eighth day after the appearance of the vaginal plug. Embryonic material was isolated and cleaned of membranes under a dissecting microscope. The embryos proper were transferred with a braking pipette under the kidney capsule of each of twenty-one 3 month old male mice of the same strain (approximately 35 g). The mice were killed between 2 and 8 months later. Several mice with exceptionally large

Solter *et al.* (1970) *Nature* 227:503-504

Establishing Embryonic Stem Cells

Nature Vol. 292 9 July 1981

Establishment in culture of pluripotential cells from mouse embryos

M. J. Evans* & M. H. Kaufman†
Departments of Genetics* and Anatomy†, University of Cambridge, Downing Street, Cambridge CB2 3EH, UK

Proc. Natl. Acad. Sci. USA
Vol. 78, No. 12, pp. 7634–7638, December 1981
Developmental Biology

Isolation of a pluripotent cell line from early mouse embryos cultured in medium conditioned by teratocarcinoma stem cells

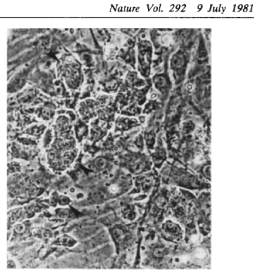
(embryonic stem cells/inner cell masses/differentiation *in vitro*/embryonal carcinoma cells/growth factors)

GAIL R. MARTIN
Department of Anatomy, University of California, San Francisco, California 94143

Establishing Embryonic Stem Cells

A feeder layer of fibroblasts

- promote self-renewal (proliferation)
- inhibit differentiation

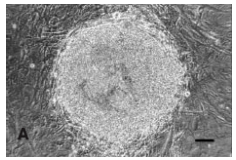


Nature Vol. 292 9 July 1981

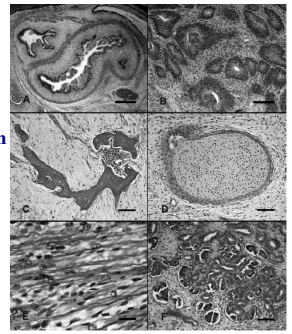
Fig. 1 Groups of pluripotential embryo cells (arrowed) growing in monolayer culture on a background of mitomycin C-inhibited STO cells. The isolation of a definite cell line from a blastocyst takes only ~ 3 weeks and the pluripotential cell colonies are visible within 5 days of passage. We have had 30% yield of lines from blastocysts in one experiment. Two of the lines have been rigorously cloned by single-cell isolation but most were only colony-picked—this makes no difference.

Establishing Human Embryonic Stem Cells
(it took nearly another 20 years after the success in mice)

hESCs

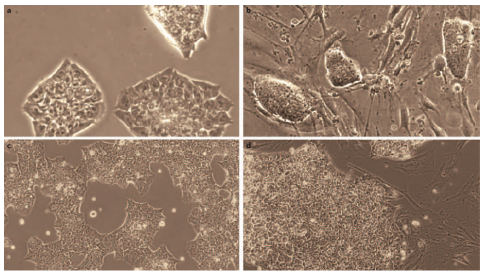


differentiation



Thomson *et al*. (1998) *Science* 282:1145-1147

Embryonic Carcinoma (EC) Cells & Embryonic Stem (ES) Cells



Mouse

Human

EC Cells **ES Cells**

Establishing Embryonic Stem Cells: The Key Technical Issues

- The exact stage at which pluripotent cells capable of growth in tissue culture exist in the embryo
- Explantation of sufficiently large number of these cells from each embryo
- Tissue culture in conditions most conductive to proliferation rather than differentiation

Not to use enzymes to dissociate cells during early passages, but to do so mechanically, turned out to the key for establishing hESCs!

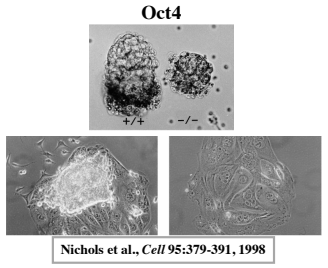
The Biology of Embryonic Stem Cells

1. Maintaining the pluripotency
2. Differentiation into distinct cell types
3. How reversible is differentiation

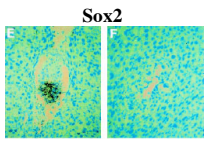
Individually tailored cells for tissue repair:

- Therapeutic cloning (somatic nuclear transfer)
- Induced pluripotent stem (iPS) cells

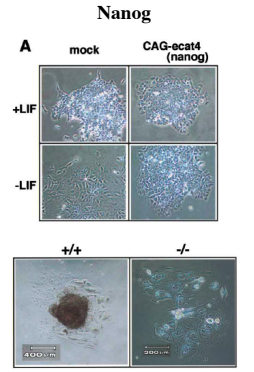
Genes Maintain ES Cell Pluripotency



Nichols et al., *Cell* 95:379-391, 1998

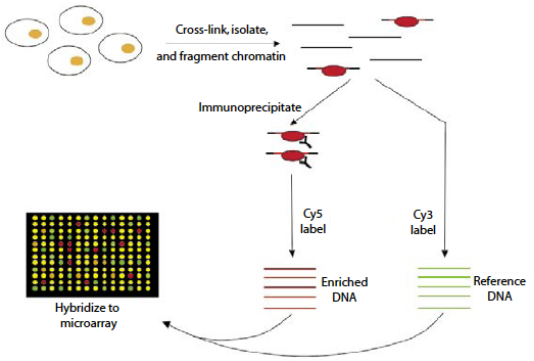


Avilion *et al*. (2003) *Genes Dev* 17:126-140



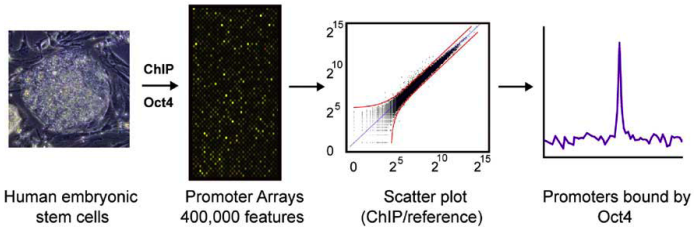
Mitsui *et al*. (2003) *Cell* 113:631-642

Chromatin Immunoprecipitation Analyzed by Microarray Hybridization (ChIP-chip)



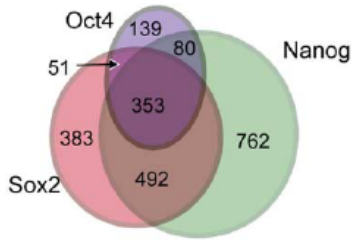
Identifying transcription factor-DNA interactions
(Hudson & Snyder. *BioTechniques* 41:673-689, 2006)

Identifying the Regulatory Circuitry for Maintaining ES Cell Pluripotency



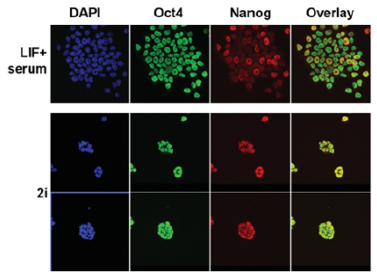
Identifying the target genes of Sox2, Oct4 and Nanog
(Boyer et al., *Cell* 122:947-956, 2005)

Genes for Maintaining ES Cell Pluripotency

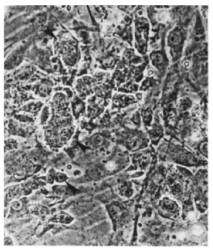


Target genes of Sox2, Oct4 and Nanog
(Boyer *et al*. *Cell* 122:947-956, 2005)

Establishing Embryonic Stem Cells: Inhibition of Differentiation



Nature Vol. 292 9 July 1981

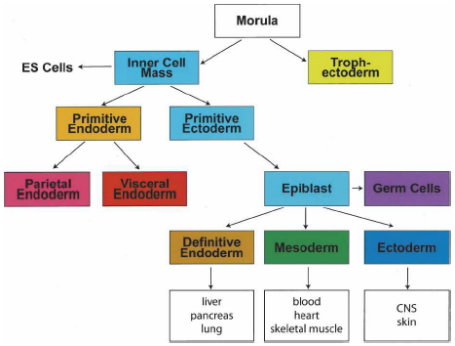


Self-renewal is the ground state!
(2i: inhibition of two kinases, MEK and GSK3)

Wray *et al*. (2010) *Biochem. Soc. Trans*. 38:1027–1032

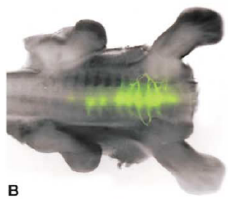
A feeder layer of fibroblasts (subsequently LIF + serum)
↑Self-renewal? ↓Differentiation?

Differentiation of ES Cells into Distinct Cell Types
(follow development or trial-and-error?)



Keller. *Genes Dev*. (2005) 19:1129-1155

Differentiation of ES Cells into Motor Neurons

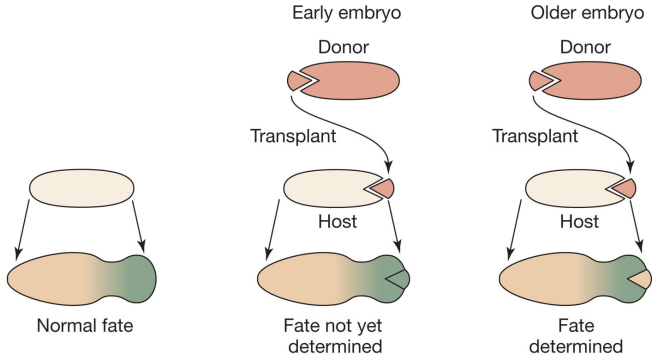


Inductive signals and transcription factors involved in motor neuron generation have been identified, raising the question of whether these developmental insights can be used to direct stem cells to a motor neuron fate. We show that developmentally relevant signaling factors can induce mouse embryonic stem (ES) cells to differentiate into spinal progenitor cells, and subsequently into motor neurons, through a pathway recapitulating that used in vivo. ES cell-derived motor neurons can populate the embryonic spinal cord, extend axons, and form synapses with target muscles. Thus, inductive signals involved in normal pathways of neurogenesis can direct ES cells to form specific classes of CNS neurons.

Witchterle et al. (2002) *Cell* 110:385-397

Cells Lose Flexibility during Development

(become more and more specialized as development proceeds)



LIFE 10e, Figure 19.2
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Is Differentiation Reversible?

(Waddington's Valleys or Captain Cook's Islands)

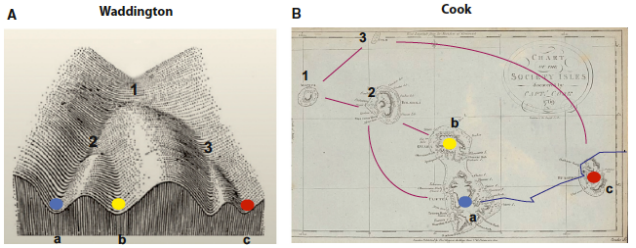


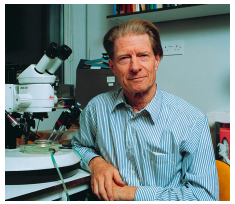
Figure 1. Waddington's and Cook's Reprogramming Landscapes
Alternative models of Waddington's (A) and Cook's (B) reprogramming landscapes showing mature cell types (a, b, and c) and intermediate states (1, 2, 3) of cellular development. Lines in (B) retrace a hypothetical Polynesian settlement of French Polynesia from west to east (red) and Captain Cook's route from east to west (blue) as models of development and reprogramming, respectively.

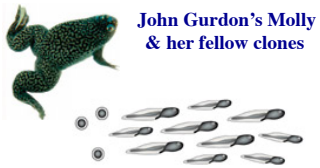
Sieweke. (2015) *Cell Stem Cell* 16:7-8

Different views of epigenetic landscape change during development
(relative importance of intrinsic changes v. environmental changes)

Cloning: Somatic Nuclear Transfer

(reversible or irreversible change of genetic materials during embryogenesis?)

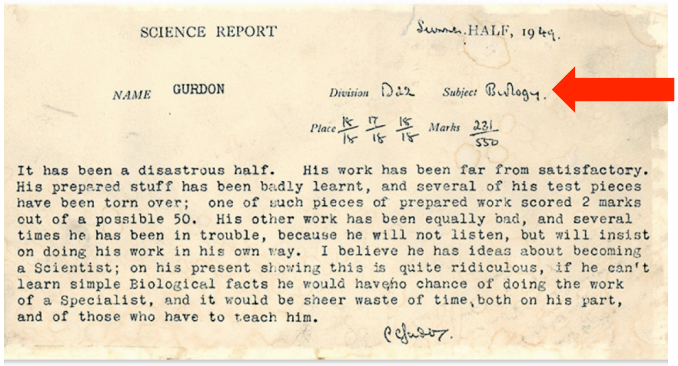




John Gurdon's Molly & her fellow clones

In 1952, Robert Briggs and Thomas King, working on a frog, *Rana pipiens*, became the first to successfully transplant living nuclei in multicellular organisms by transplanting undifferentiated blastula nuclei into enucleated eggs that developed into normal embryos.

In the late 1950s and early 1960s, John Gurdon transplanted intestinal epithelium-cell nuclei, which are differentiated, from *Xenopus* tadpoles into enucleated frog eggs and managed to produce 10 normal tadpoles. He later produced normal fertile adult animals from eggs with genetically marked nuclei transplanted from differentiated tadpole cells.



SCIENCE REPORT

Summer HALF, 1944.

NAME GURDON Division D22 Subject Biology.

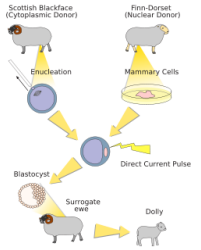
Place 15/15 17/18 15/18 Marks 221/550

It has been a disastrous half. His work has been far from satisfactory. His prepared stuff has been badly learnt, and several of his test pieces have been torn over; one of such pieces of prepared work scored 2 marks out of a possible 50. His other work has been equally bad, and several times he has been in trouble, because he will not listen, but will insist on doing his work in his own way. I believe he has ideas about becoming a Scientist; on his present showing this is quite ridiculous, if he can't learn simple Biological facts he would have no chance of doing the work of a Specialist, and it would be sheer waste of time, both on his part, and of those who have to teach him.

P. Chadwick.

Gurdon's school report from Eton, when he was 16 years old.
http://www.thetimes.co.uk/tto/science/article3561800.ece

Cloning: Somatic Nuclear Transfer



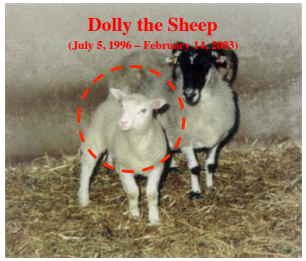


Table 1 Development of embryos reconstructed with three different cell types

Cell type	No. of fused couplets (%)*	No. recovered from oviduct (%)	No. cultured	No. of morula/ blastocyst (%)	No. of morula or blastocysts transferred†	No. of pregnancies/ no. of recipients (%)	No. of live lambs (%)‡
Mammary epithelium	277 (63.8)[a]	247 (89.2)	-	29 (11.7)[a]	29	1/13 (7.7)	1 (3.4%)
Fetal fibroblast	172 (84.7)[b]	124 (86.7)	-	34 (27.4)[b]	34	4/10 (40.0)	2 (5.9%)
			24	13 (54.2)[b]	6	1/6 (16.6)	1 (16.6%)§
Embryo-derived	385 (82.8)[b]	231 (85.3)	-	90 (39.0)[b]	72	14/27 (51.8)	4 (5.6%)
			92	36 (39.0)[b]	15	1/5 (20.0)	0

Cloning is extremely inefficient

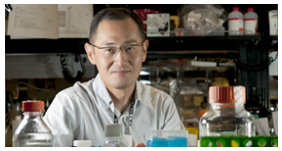
Cloning: Somatic Nuclear Transfer

The broader legacy of cloning research:

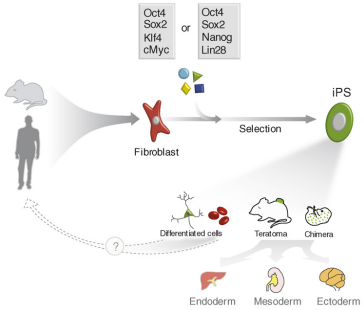
- **differentiation of a cell is not irreversible**
- **nuclei can be reprogrammed**

Therapeutic cloning only seeks to establish ES cell lines – not to make a human being – that can be manipulated and used for tissue repair or replacement!

Induced Pluripotent (iPS) Cells



Shinya Yamanaka
(2012 Nobel Prize with John Gurdon)



Takahashi & Yamanaka. (2006) *Cell* 126:663-676

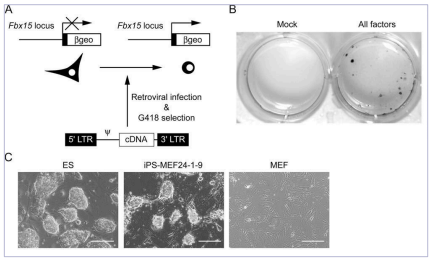
Welstead *et al.* (2008) *Curr. Opin. Genet. Dev.* 28:1-7, 2008)

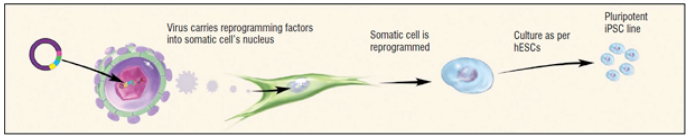
Using a defined set of factors to directly reprogram a differentiated somatic cell back to the pluripotent state

How Were the First iPS Cells Made?

(a *tour de force* but also through "educated" guess)

- **The *Fbx15* gene is active in ESCs but not in non-ESCs.**
- **Expression of a genetically engineered *Fbx15*-reporter results in resistance to the drug G418 (for cells to live).**
- **Selected 24 different candidate genes and tested various combinations.**





<http://stemcells.nih.gov/info/Regenerative_Medicine/pages/2006chapter10.aspx>

Generation of germline-competent induced pluripotent stem cells

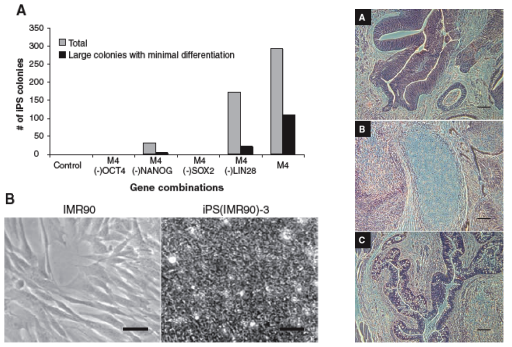
Keisuke Okita[1], Tomoko Ichisaka[1,2] & Shinya Yamanaka[1,2]

- First iPSCs were able to contribute to all germ layers during embryogenesis but unable to produce offspring.
- Improvements in technology quickly overcame this hurdle.

… 20% of the offspring developed tumors …

Okita *et al*. (2007) *Nature* 448:313-3187

Human Induced Pluripotent (iPS) Cells



A set of transcription factors can directly reprogram a differentiated human somatic cell back to the pluripotent state
(Takahashi *et al*. *Cell* 131:861-972, 2007; Yu *et al*. *Science* 318:1917-1920, 2007)

Research on Human ESCs and iPSCs

- Creating individually tailored cells/tissues for repairing or replacing damaged organs (personalized medicine)
- Modeling human development to understand developmental and disease mechanisms
- Screening drugs to develop new therapeutic reagents

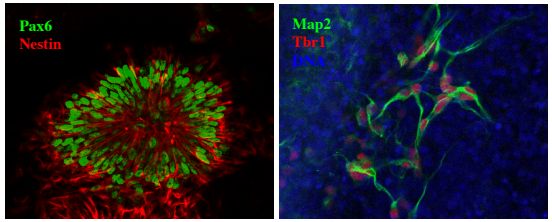
(and many more …)

Whether ESCs and iPSCs are the same remain to be determined (so therapeutic cloning may still be necessary)

Modeling Human Development & Disease Using ESCs and iPSCs

- Expression pattern
- Loss-of-function (necessary)
- Gain-of-function (sufficient)

How to take advantage of RNAi?



The Definition of Adult (Somatic) Stem Cells

1. Can generate a particular tissue or are derived from this tissue
2. Have some capacity of self-renewal (multipotent)
3. Can generate cells other than themselves
4. Self-renewal capacity lasts a lifetime (more strict definition)

Some of Our Tissues Have Amazing Regenerative Capacity

Donate your bone marrow! (hematopoietic stem cells)

hair, skin, blood, gut, muscle, liver, female breast, etc.

Name a few tissues with stem cells that continuously replenish differentiated cells

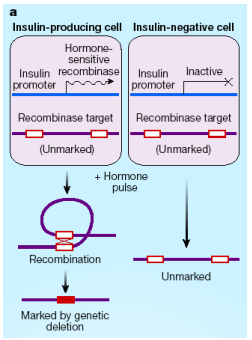
Is There A Need for Adult Stem Cells to Maintain Tissue Function?

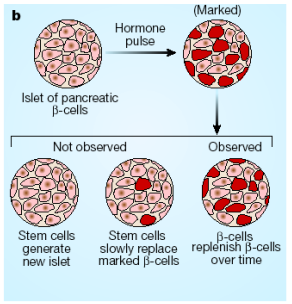
1. Tissues can be maintained without turnover (the central nervous system - neocortex?)
2. Tissue turnover may NOT need stem cells
3. Stem cells may only play a limited role

- Regenerative medicine seeks to repair tissue damages that go well beyond the normal wear and tear.
- Thus we need to start with fundamental questions.

Tissue Turnover May NOT Need Stem Cells

(differentiated cells in liver and pancreas can divide and replenish themselves)





Dor *et al.* (2004) *Nature* 429:41-46

Do Pancreatic Stem Cells Exist?

β Cells Can Be Generated from Endogenous Progenitors in Injured Adult Mouse Pancreas

Xiaobo Xu,[1,5] Joke D'Hoker,[1,5] Geert Stangé,[1,5] Stefan Bonné,[1,5] Nico De Leu,[1,5] Xiangwei Xiao,[1,5] Mark Van De Casteele,[1,5] Georg Mellitzer,[2,5] Zhidong Ling,[1,5] Danny Pipeleers,[1,5] Luc Bouwens,[1,5] Raphael Scharfmann,[3,4,5] Gerard Gradwohl,[2,4,5] and Harry Heimberg[1,4,5,*]
[1]Diabetes Research Center, Vrije Universiteit Brussel, Laarbeeklaan 103, B1090 Brussels, Belgium
[2]INSERM, U682, Development and Physiopathology of the Intestine and Pancreas, 67200 Strasbourg, France
[3]INSERM, U845, Faculty of Medicine Necker, 75730 Paris, France
[4]Beta Cell Biology Consortium, 2213 Garland Avenue, 9465 MRB IV, Nashville, TN 37323-0494, USA
[5]JDRF Center for Beta Cell Therapy in Diabetes, Laarbeeklaan 103, B1090 Brussels, Belgium
*Correspondence: harry.heimberg@vub.ac.be
DOI 10.1016/j.cell.2007.12.015

under physiological or pathological conditions

The Dogma until 1980s: No New Neurons in the Adult Central Nervous System (CNS) of Mammals

Fish: sustained growth of the CNS
Song birds: replacement of neurons
Mammals: no or very limited in the brain
- **rodents**
- **primates**
  - **old world - rhesus monkey (Africa & Asia)**
  - **new world - marmoset (Central & South America)**
  - **humans**

Song Birds: Adult Canary & Zebra Finch

(replacement of neurons in the song learning system)

Male canaries develop complex, learned song repertoires
Females sing very little, and the song is relatively simple,
but after testosterone treatment
- **can sing like males**
- **the song nuclei (such as HVC) expand**

HVC is involved in song acquisition and production
The HVC of adult male canaries shows strong seasonal oscillations in volume (larger in spring and smaller after the end of mating season)

Goldman & Nottebohm. (1983) *PNAS* 80:2390-2394

Joseph Altman: Adult Neurogenesis in Mammals

(a classical example of discovery made ahead of time)

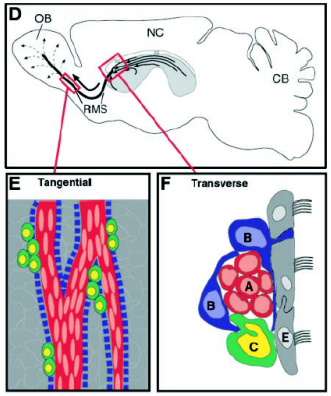
Are new neurons formed in the brains of adult mammals?
(Altman, *Science* 135:1127, 1962)

"Contrary to older views, … undifferentiated cells multiply at a high rate after birth in various germinal regions of the growing brain, and a large proportion of these cells become differentiated short axoned neurons …"
(Altman, 1967)

"… the addition of these cells does not have a growth but a renewal function (replacement of dying cells in the olfactory bulb?)"
(Altman, 1969)

The standard of proof is proportional to the importance and novelty of a claim, and others raised legitimate concerns regarding the interpretation of his data because of technological limitations at the time

Neurogenesis in the Adult Olfactory Bulb

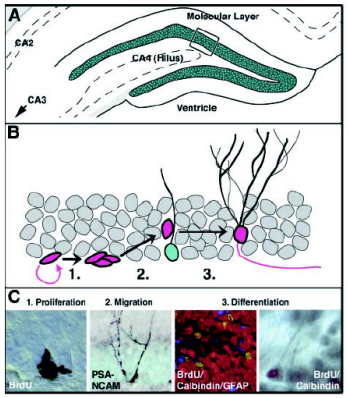


Stem cells and lineage-determined progenitor cells reside in the SVZ (subventricular zone) of the lateral ventricles.

Neuronal precursor cells migrate through the RMS (rostral migratory stream) like a chain to the olfactory bulb to become certain types of neurons.

Temple & Alvarez-Buylla. (1999) *Curr. Opin. Neurobiol.* 9:135-141
Zhao *et al.* (2008) *Cell* 132:645-650

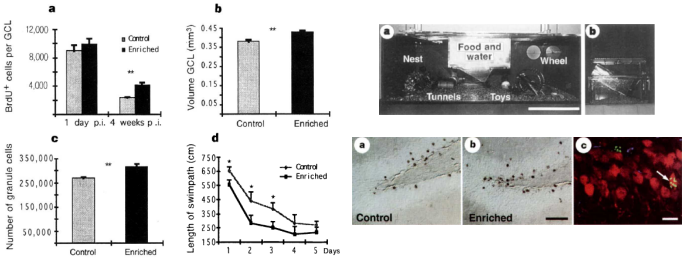
Adult Neurogenesis in the Hippocampus



Stem cells and lineage-determined progenitor cells reside in the SGZ (subgranular zone)

Gage. (2000) *Science* 287:1433-1438

Adult Neurogenesis in the Hippocampus: Enriched Envrionment and Learning

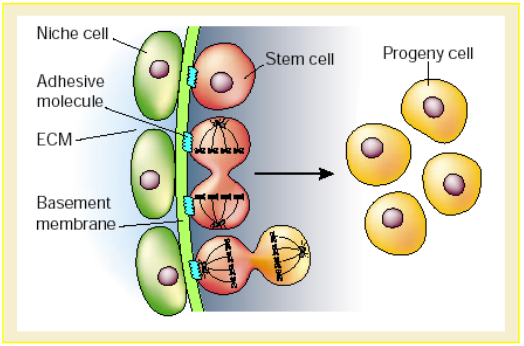


Kempermann *et al.* (1997) *Nature* 386:493-495

increase in neurogenesis v. survival promoting enrichment v. novelty

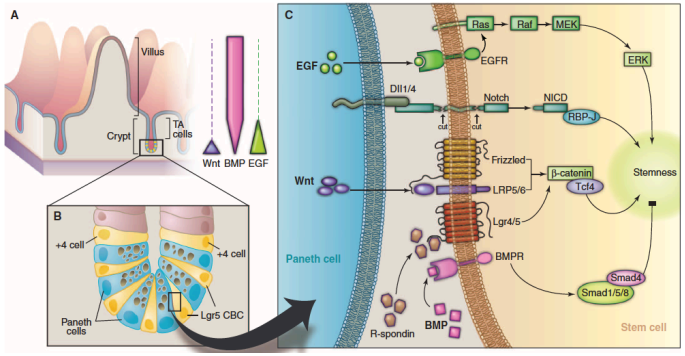
Maintaining Stem Cells: The Niche Hypothesis

(adult stem cells reside in the specialized region of a tissue)



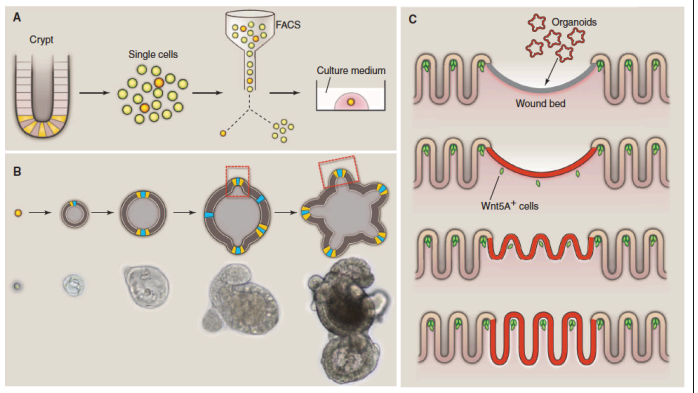
Ohlstein *et al.* (2004) *Curr. Opin. Cell Biol.* 16:693-699

Maintaining Stem Cells: The Niche Hypothesis
(intestinal stem cells reside in the crypt region of our gut)



Sato & Clevers (2013) *Science* 340:1190-1194

Generating Organs in A Dish
(growing self-organizing mini-guts from a single intestinal stem cell)



Sato & Clevers (2013) *Science* 340:1190-1194

Plasticity of Mammalian Stem Cell Hierarchies
(for your information only; no details in exam)

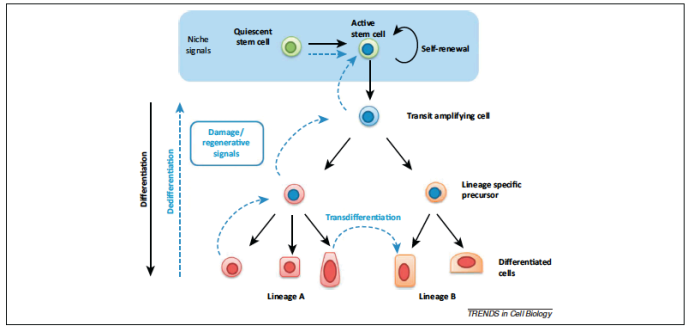
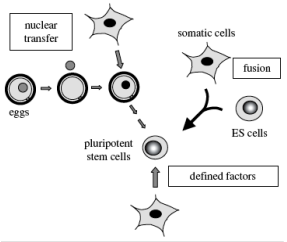


Figure 1. Stem cell hierarchy in homeostasis and regeneration. Niche signals drive stem cell self-renewal and differentiation to generate the specialized lineage populations that maintain the tissue during homeostasis. Cellular dynamics during homeostasis is indicated by black arrows. During damage and regeneration (blue arrows), cells can be replenished by mobilization of quiescent stem cells and increased proliferation of surviving stem cells. Alternatively committed cells can dedifferentiate and re-enter the cell cycle. Lost cells can also be replenished via transdifferentiation into another differentiated cell lineage. Proliferating cells are indicated with a blue nucleus, differentiated cells are represented with a red nucleus.

Tetteh *et al.* (2014) *TICB*. doi: 10.1016/j.tcb.2014.09.003

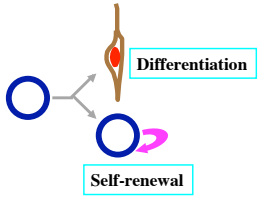
Creating Individually Tailored Stem Cells
(a bright future for regenerative medicine & personalized medicine)

Inducing Pluripotency in Somatic Cells

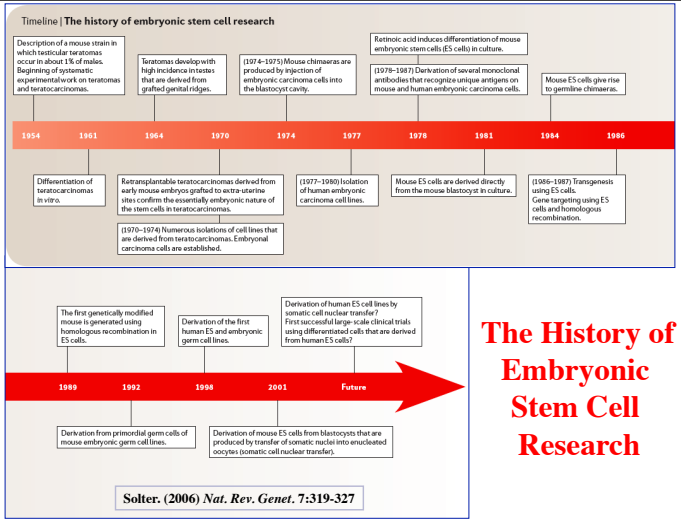


Yamanaka. (2008) *Phil. Trans. R. Soc. B* 2008

Manipulating Adult Stem Cells



Trans-differentiation of Adult Cells
(directly converting one cell type to another)



The History of Embryonic Stem Cell Research

Key Concepts from This Lecture

1. Stem cells are defined by their ability to renew themselves (self-renewal) and also generate other cell types (differentiation).
2. Embryonic stem (ES) cells are derived from embryonic cells before they generate the 3 germ layers (are pluripotent).
3. Differentiated cells can be reverted to a pluripotent state using defined factors or through somatic nuclear transfer (cloning).
4. Induced pluripotent stem (iPS) cells generated by defined factors have virtually the same characteristics as ES cells.
5. Many (but not all) tissues contain adult (somatic) stem cells that can repair damages caused by normal wear and tear.
6. Human iPS cells will facilitate studies of human disease and developmental mechanism and revolutionize medicine.