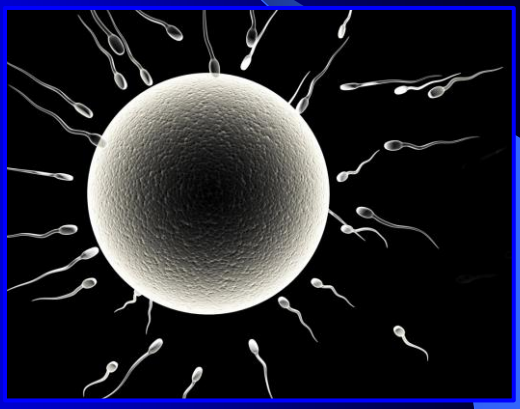
Potency of the cells

Fejlődés- és Molekuláris Genetika
2021

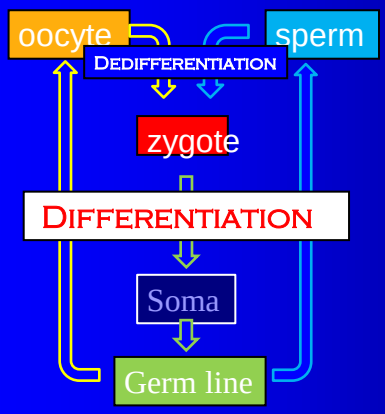
At the beginning

Oocyte: Differentiated? Totipotent?
Sperm: Differentiated? Totipotent?

Yes. Not.

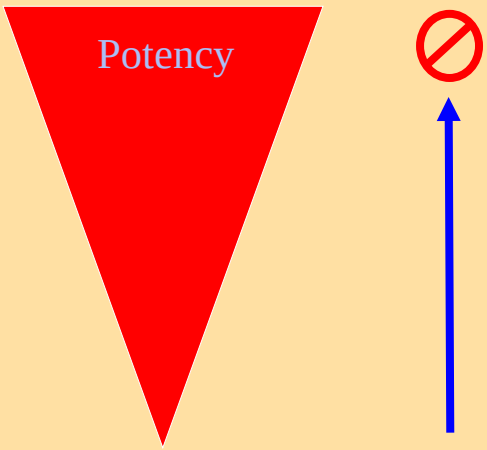


The fundamental cycle



Potency of the cells

- Totipotent
- Pluripotent
- Multipotent
- Oligopotent
- Unipotent



Potency

The range of commitment options available to a cell

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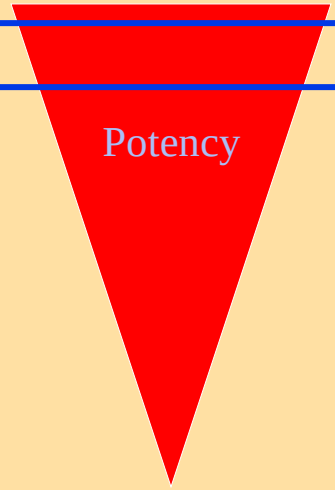
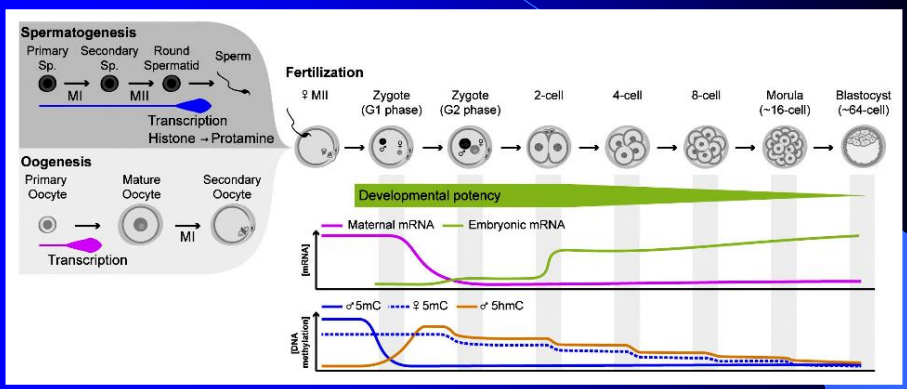


How does zygote become totipotent?

1. *via* demethylation:



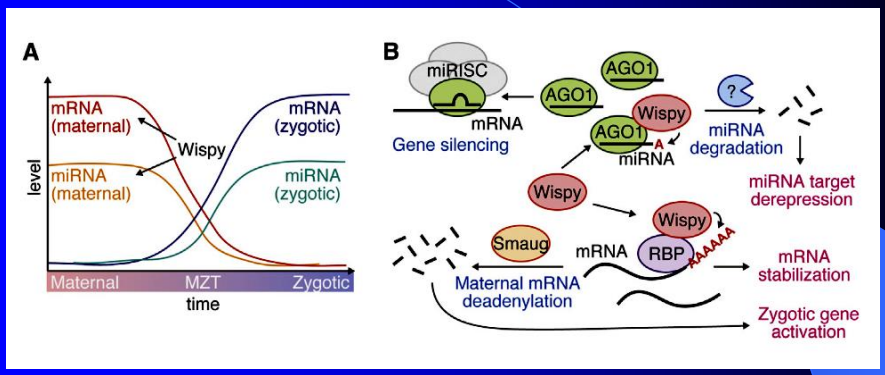
5mC: 5 methyl cytosine
5hmC: 5 hydroxymethyl cytosine

Maximal demethylation

Ladstätter and Tachibana, JBC, 2018

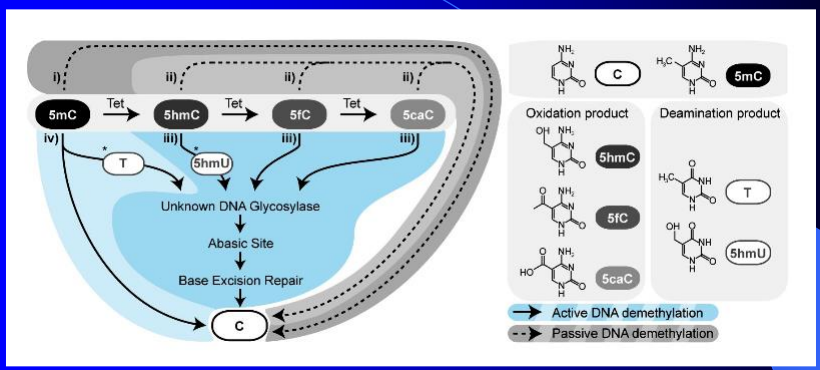
How does zygote become totipotent?

2. *via* removing maternal mRNAs:



wispy (wisp) encodes a conserved cytoplasmic poly-A polymerase of the GLD2 family
smaug (smg) encodes a sequence-specific RNA-binding proteins.

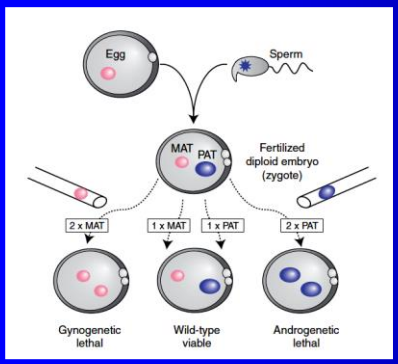
Passive and active DNA demethylation



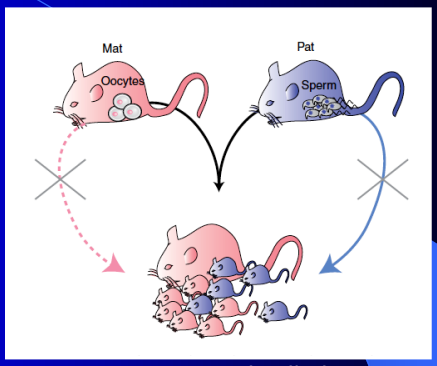
Ladstätter and Tachibana, JBC, 2018

5hmC: 5 hydroxymethyl cytosine
5fC: 5-formylcytosine
5caC: 5-carboxylcytosine
Tet: Ten-eleven translocation enzymes 1-3 (methylcytosine dioxygenases)
TdT: Terminal deoxynucleotidyl transferase

Result of the asymmetrical demethylation



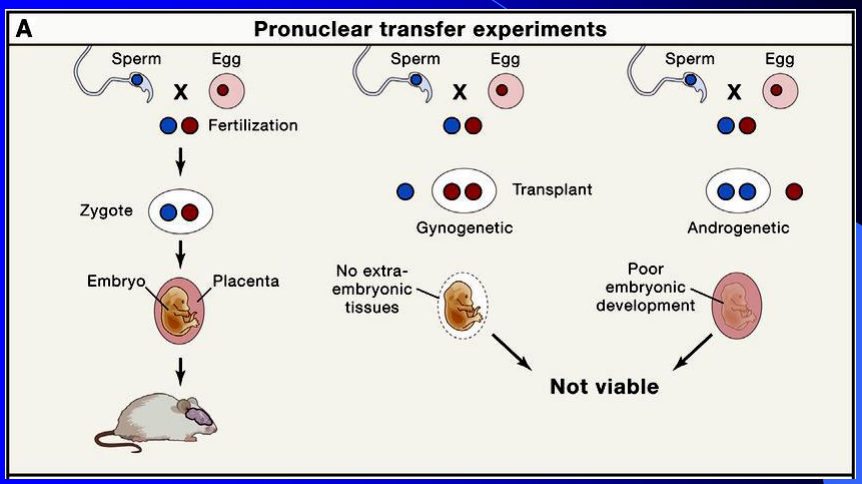
McGrath and Solter, Cell, 1984



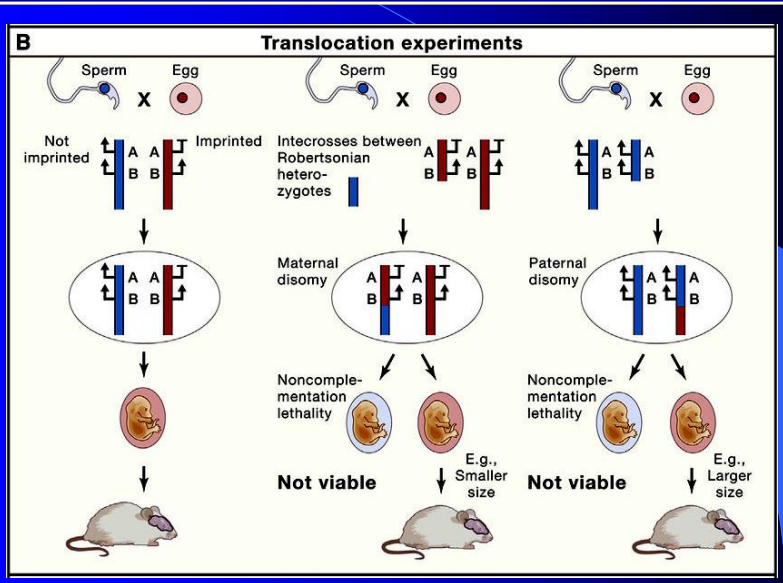
Barlow and bartolomei, CSHL, 2014

Even if the lines were inbred.

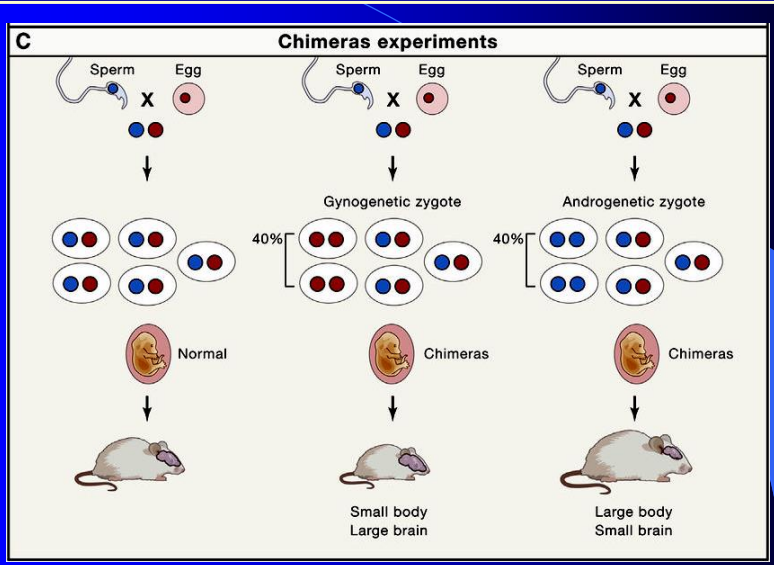
Maternal / paternal imprints



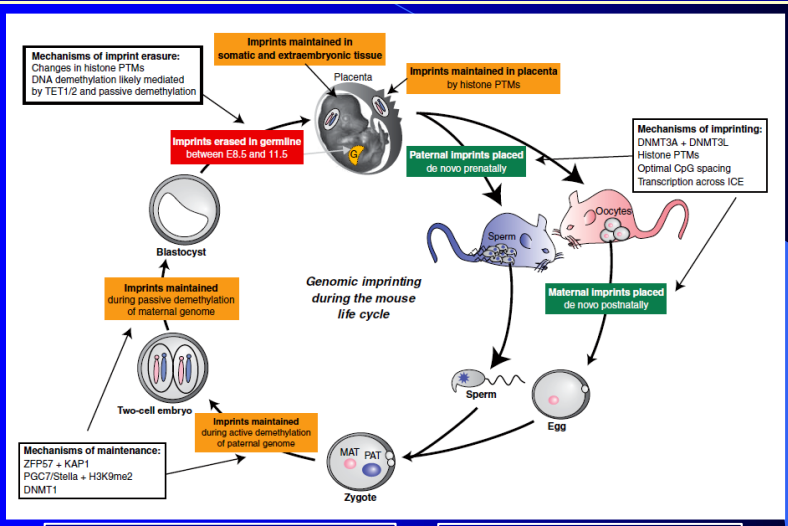
Maternal / paternal imprints



Maternal / paternal imprints



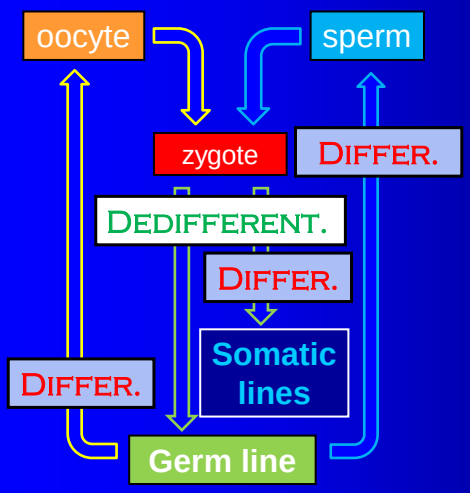
Establishment, maintenance, and erasure of genomic imprints in mouse development



PTMs: posttranslational modifications
ICE: imprinting control elements

DNMT: DNA methyltransferase
Tet: methylcytosine dioxygenase

The Fundamental Cycle - Reloaded



Potency

The range of commitment options available to a cell

- Totipotent
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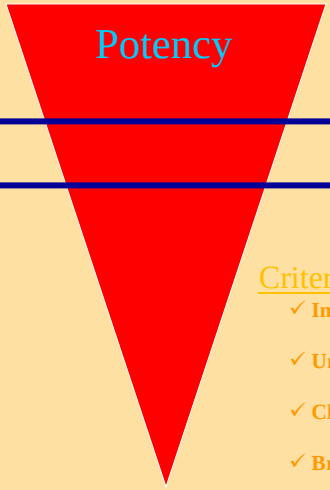
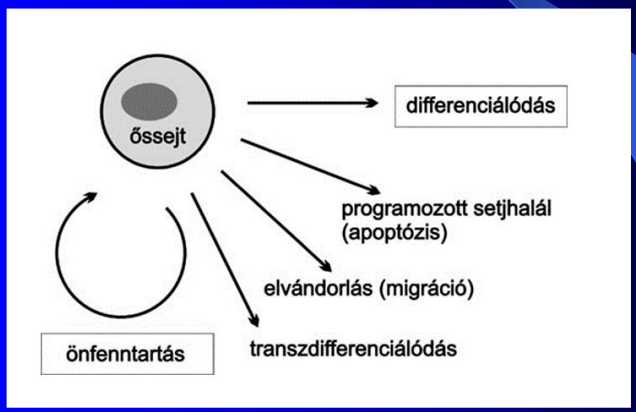


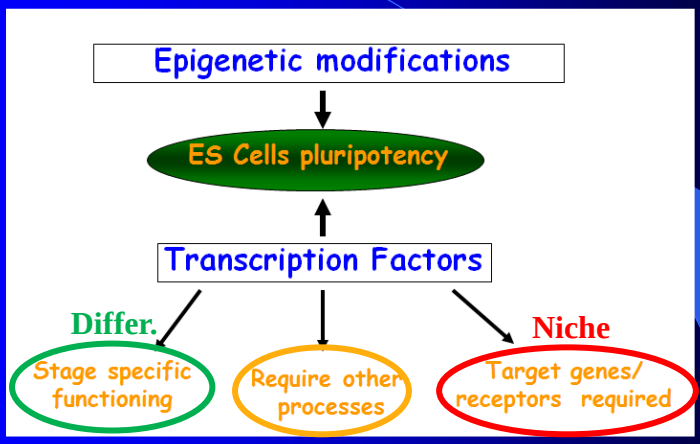
Criteria for pluripotency:

- ✓ Immortality
- ✓ Undifferentiation
- ✓ Clonality
- ✓ Broad developmental potential

- **Stem cells** are biological cells found in all multicellular organisms, that can divide (through mitosis) and differentiate into diverse specialized cell types and can self-renew to produce more stem cells.

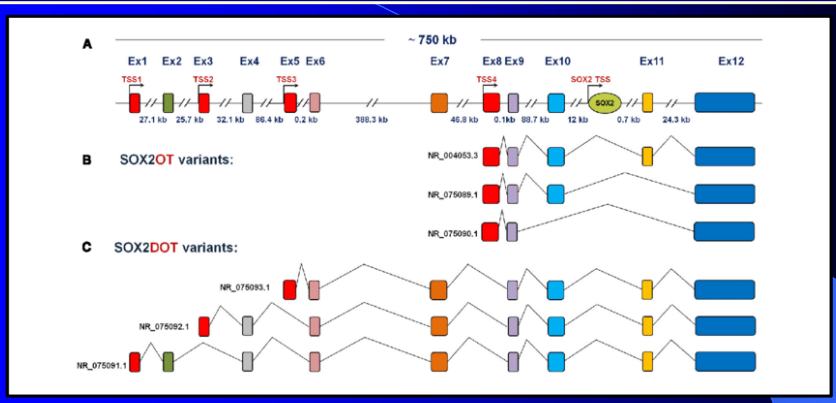


Maintenance of ESCs' pluripotency



ESCs: Embyonic stem cells

Sox2 /SRY (sex-determining region Y)-box2)

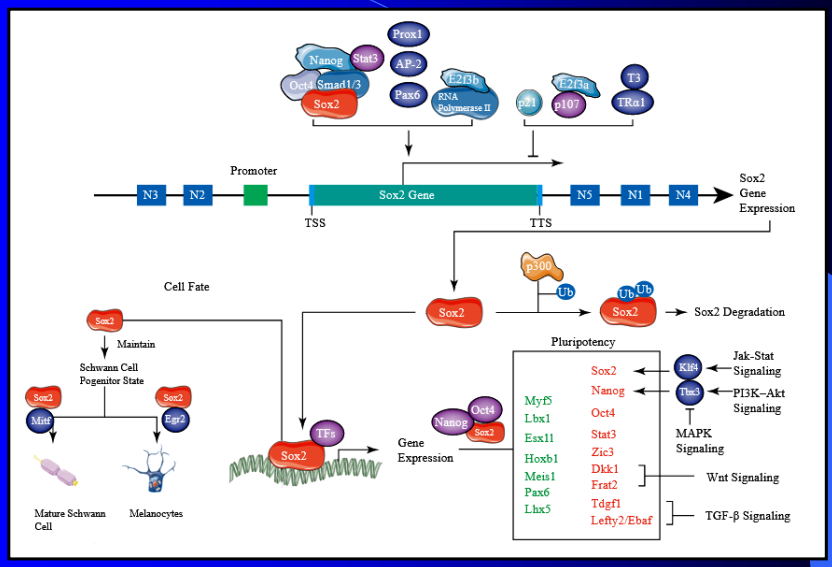


SOX2OT : SOX2 overlapping transcript
lncRNA
Tss: transcriptional start sites

Oct4: octamer (AGTCAAAT) binding tr. factor,
Homeodomain

Sox2
Intronless gene
HMG-box
Transcription factor
Binds only to non-B DNA
Maintenance of pluripotency
Trimeric complex with Oct4

Sox2



Oct4 and Nanog

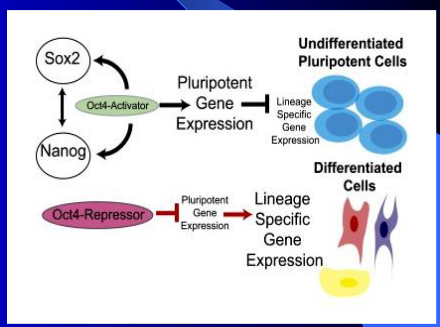
Octamer-binding tr. factor
ATTTGCAT
Maternal gene
Heterodimer with Sox2
Oct4-/- : ICM ⟶ trophoectoderm

Optimal level: maintenance of pluripotency
Low level: differentiation
2x level: primitive entoderm and mesoderm

Low level ⟶ Rex1 activation
High level ⟶ Rex1 inactivation

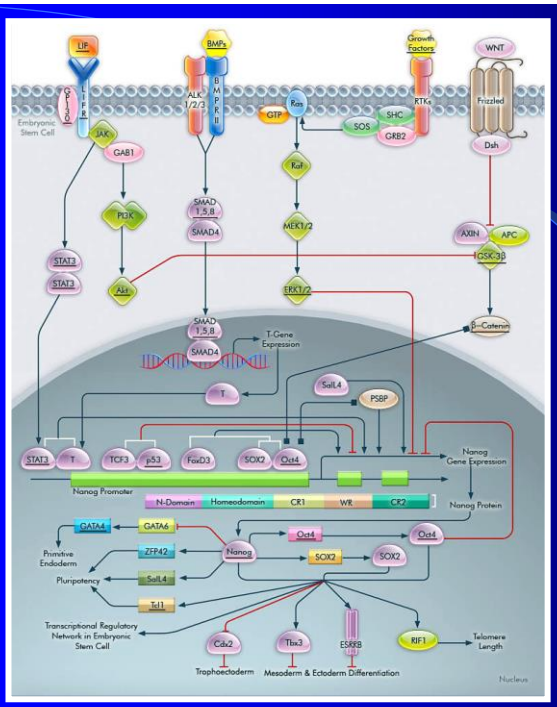
Rex1: *R*equired for *Ex*cision 1

Tír na nÓg (Nanog): homeodomain tr. factor
Nanog -/- : entoderm formation
P53 binding site on the promoter



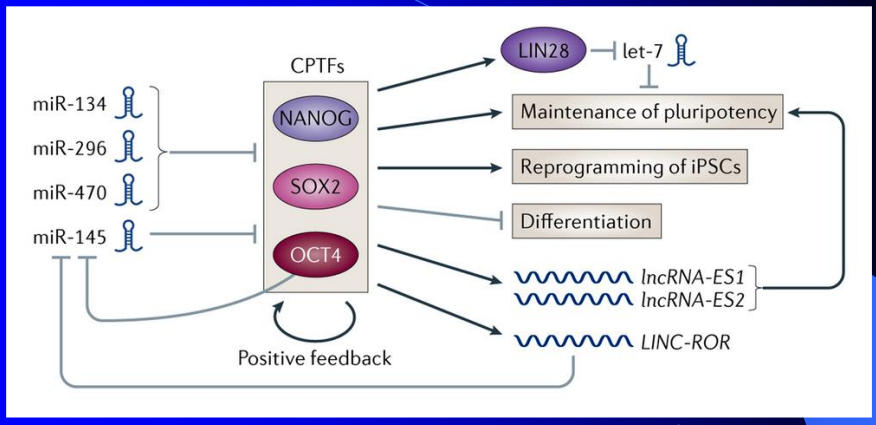
Hammachi et al., Cell Rep., 2012

Control of the core factors



www.eBioscience.com

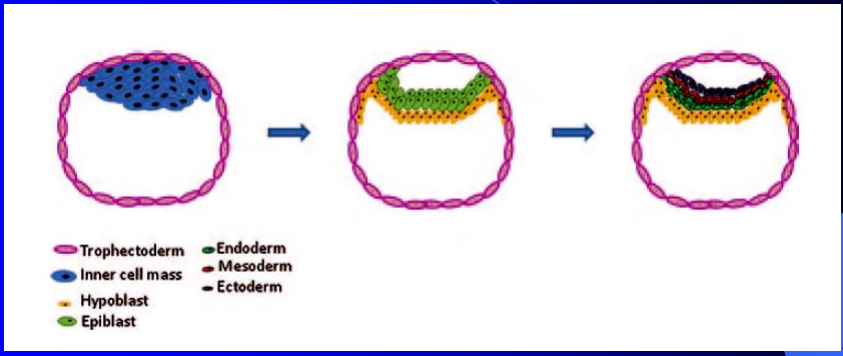
Fine regulation of core factors



Fatica and Bozzoni, Nat. Rev. Gen, 2014

CPTFs: core pluripotency transcription factors
LINC-ROR: long intergenic non-protein coding RNA, regulator of reprogramming
LIN28: micro-RNA binding protein

Formation of the germ layers



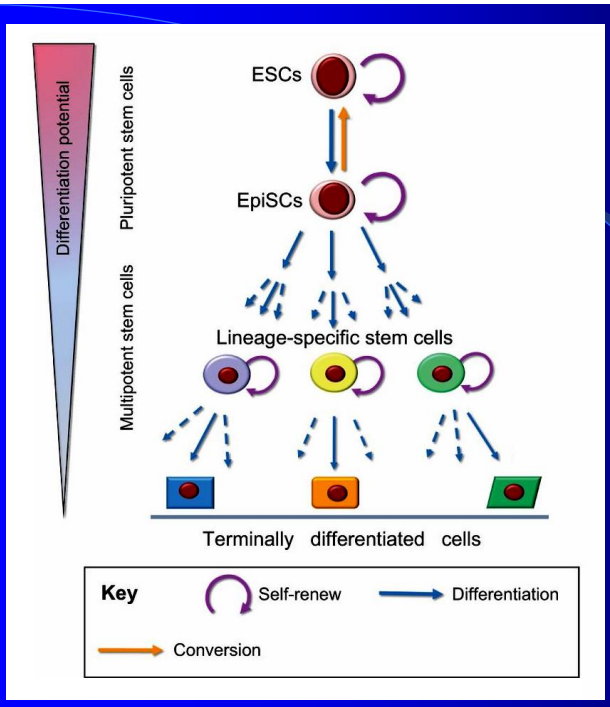
Brevini and Pennarossa: Gametogenesis, Springer, 2013

Trophectoderm (placenta formation) and inner cell mass (embryo proper)

Blastula: Epiblast and hypobast layers

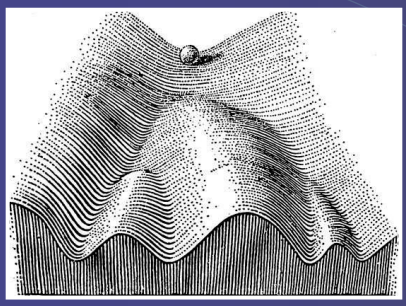
Gastrulation: Trilaminar embryo disc + hypoblast

Differentiation

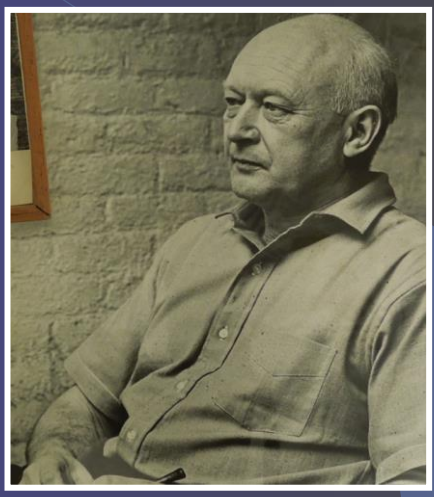


ESCs: Embryonal Stem Cells
EpiSCs: Epiblast SCs

Epigenetic landscape

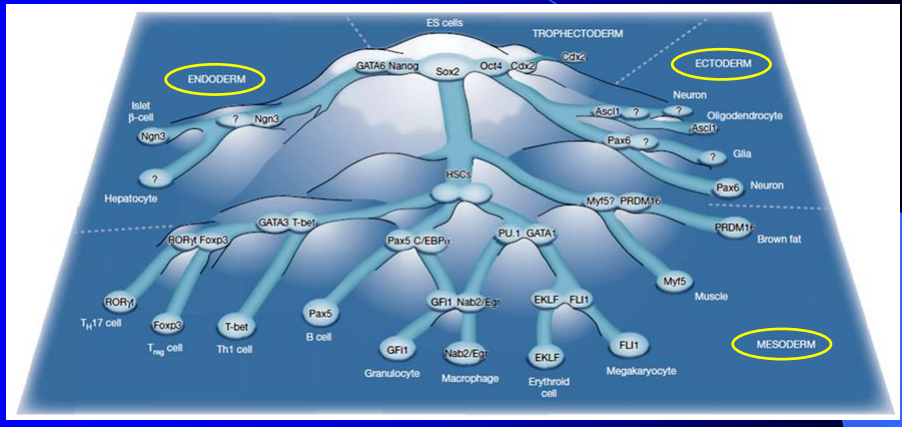


- Mutations can modulate the epigenetic landscape.
- Evolution mainly occured through mutations that affected developmental anatomy



Conrad Hal Waddington (1905–1975)

Classic epigenetic landscape 2015



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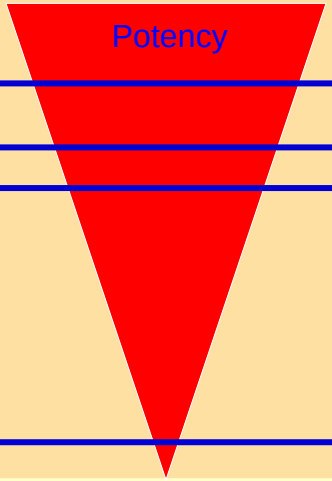
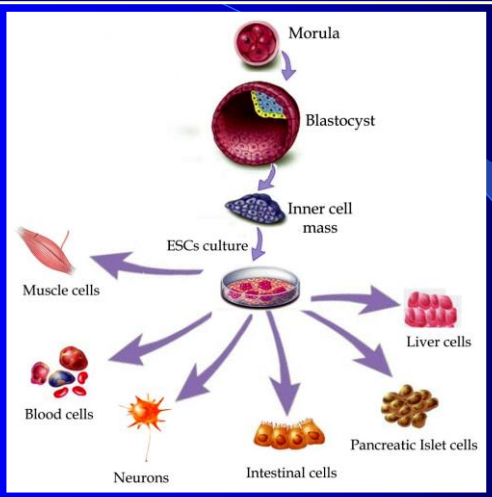


Embryonic

Amniotic

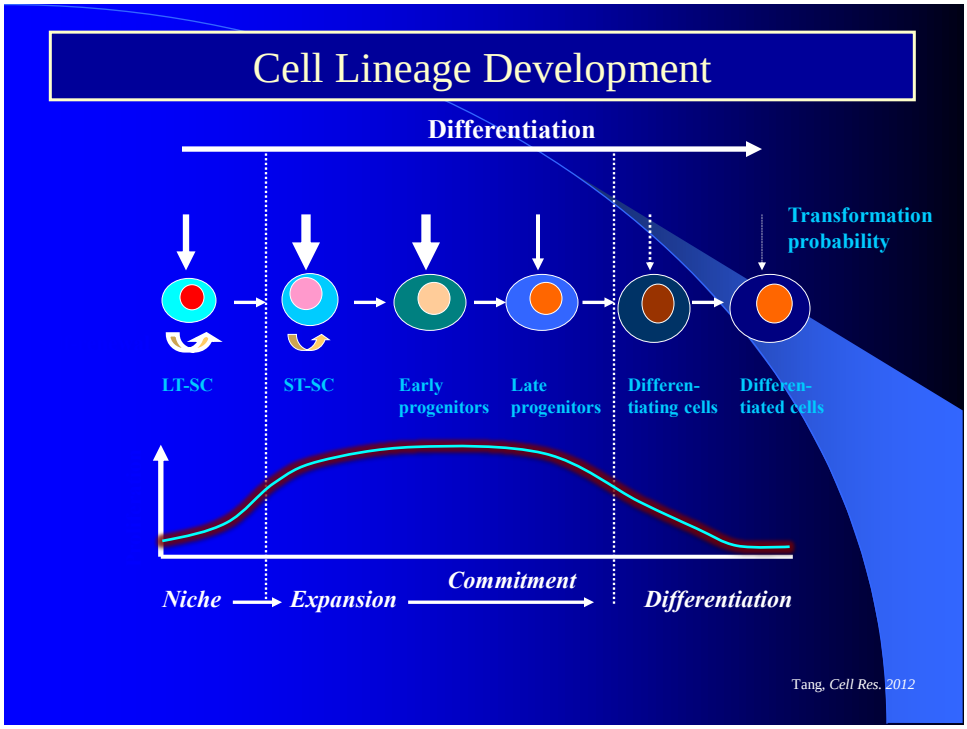
Adult

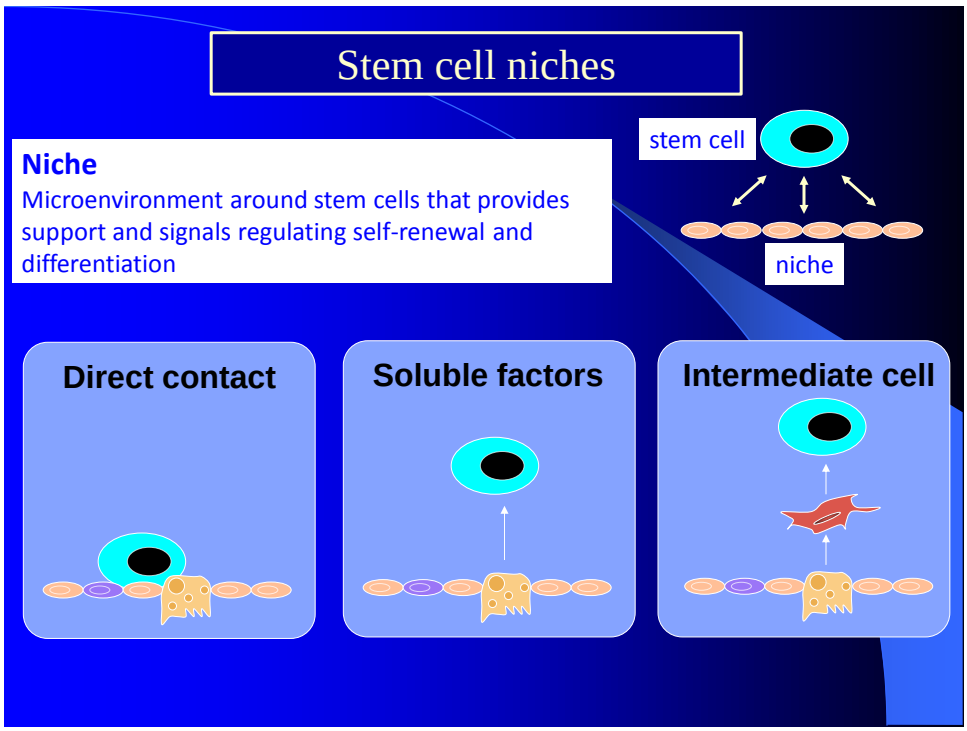
ICM or EpiSCs

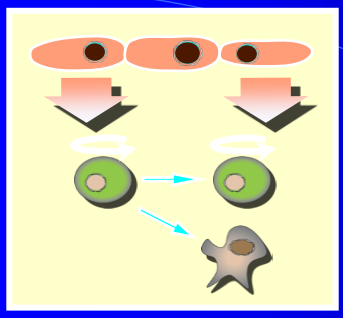


Nearly all research to date has made use of mouse embryonic stem cells (mES) or human embryonic stem cells (hES).

Without optimal culture conditions or genetic manipulation, embryonic stem cells will rapidly differentiate. For mice: LIF, for human: FGF2.


Cell Lineage Development
Differentiation
Transformation probability
LT-SC
ST-SC
Early progenitors
Late progenitors
Differen-tiating cells
Differen-tiated cells
Niche
Expansion
Commitment
Differentiation
Tang, Cell Res. 2012


Stem cell niches
Niche
Microenvironment around stem cells that provides support and signals regulating self-renewal and differentiation
stem cell
niche
Direct contact
Soluble factors
Intermediate cell

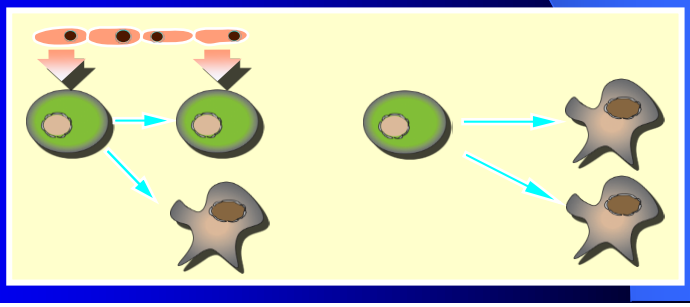


Signals from Niche

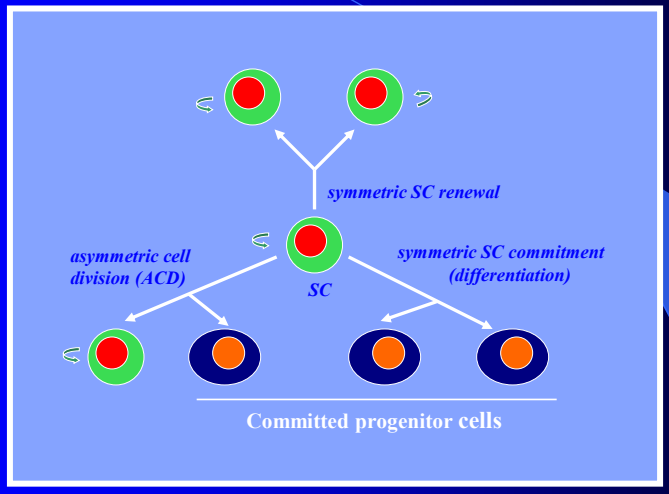
- Signals are local; niches have a limited capacity and cells compete for the signals
- The signals control tissue homeostasis, also after damage.

Signals from niches maintain adult stem cells and tissues.

In the absence of niche signals, adult stem cells will differentiate, by default.



Alternate Stem Cell Fates



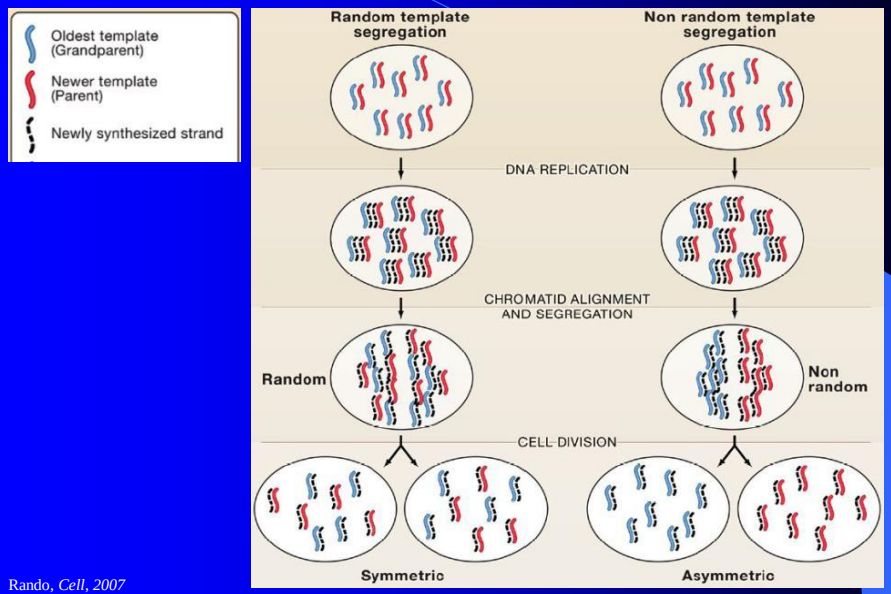
Tang, *Cell Res. 2012*

Motivation for Asymmetric Strand Segregation

- Adult rat contains $6x10^{10}$ cells
- In its small intestine, a rat sheds over 10^{13} epithelial cells during its lifetime.
- Requires 10^3 symmetric cell doublings from embryo to adult followed by 10^{13} asymmetric cell doublings during its lifetime
- How do epithelial cells minimize mutations that lead to cancer?

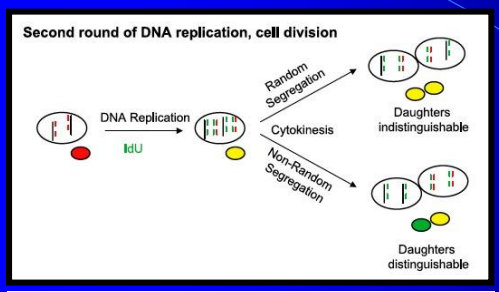
Cairns, *Nature, 1975*

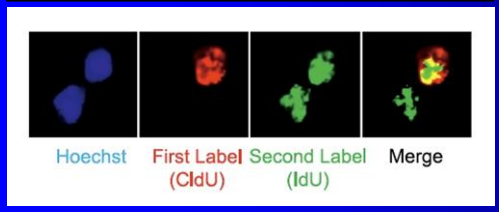
Asymmetric Segregation of Parental DNA Strands?

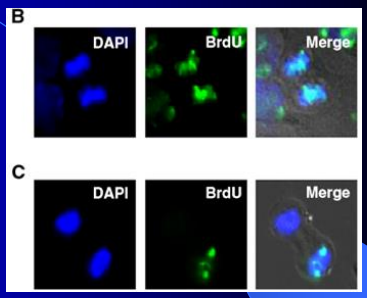


Rando, *Cell, 2007*

Asymmetric DNA segregation







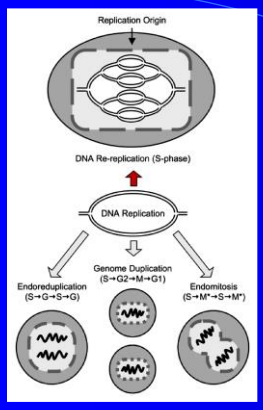
Liu et al., *Mol Cancer*, *2013*

Human breast cancer cell lines asymmetrically segregate their template DNA strands.

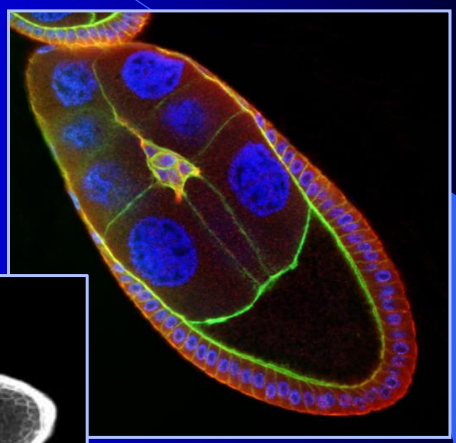
Figure 2. Evidence of Co-Segregation of DNA Template Strands during Muscle Progenitor Cell Division
(B) Cell pairs were immunostained for CldU and IdU. Shown is a representative photograph of an immunostained pair of cells, in which both daughter cells were labeled with the second label, IdU (green), but only one daughter inherited the first label, CldU (red).

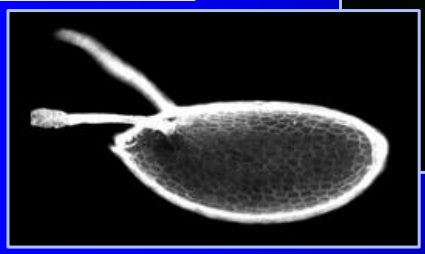
DNA re-replication

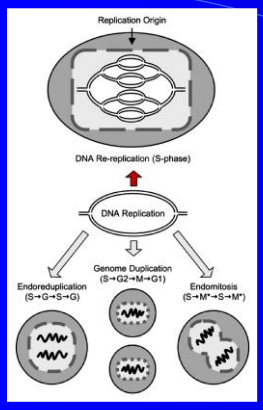
- DNA re-replication occurs when one or more of the normal controls that prevent reutilization of replication origins during S-phase is circumvented.
- Re-replicated DNA sequences: amplicons
- They are in: MA of Tetrahymena
  SG of Sciarid flies
  chorion genes on X in Drosophila
  Tumor cells



Chorion genes in the follicular cells are amplified







Re-replication of chorion genes

Despite of the follicular cells are endoreduplicated, they amplify some of their chorion genes.

Minors: diploids, majors: amplified
Minor: 15 hours long expression,
Major: 2-3 hours long expression.

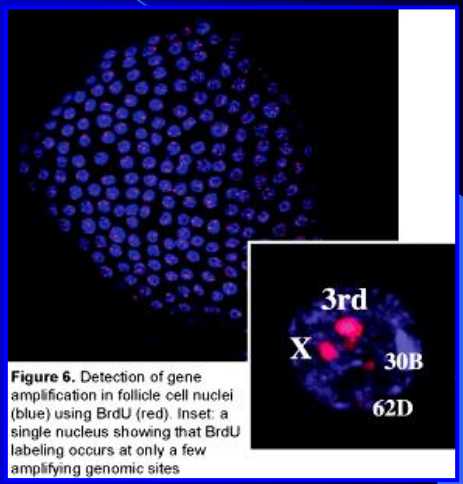
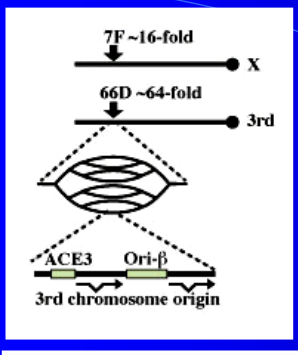
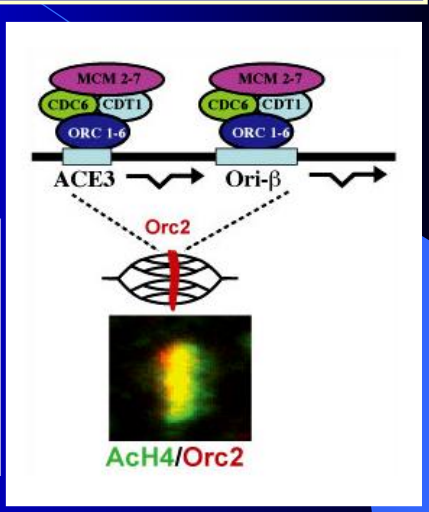


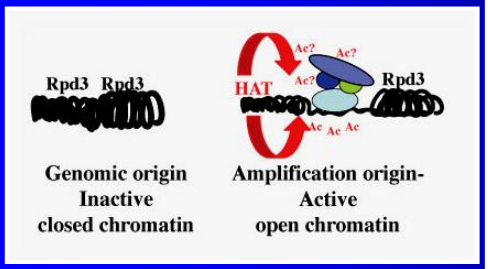
Figure 6. Detection of gene amplification in follicle cell nuclei (blue) using BrdU (red). Inset: a single nucleus showing that BrdU labeling occurs at only a few amplifying genomic sites

Amplification goes hand in hand with chromatin rearrangement



ACE3: Amplification control element

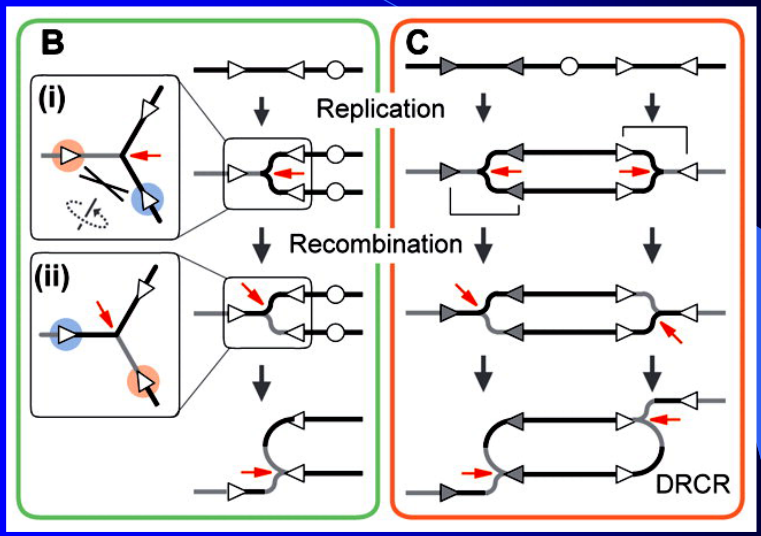




Rpd3: histone deacetylase

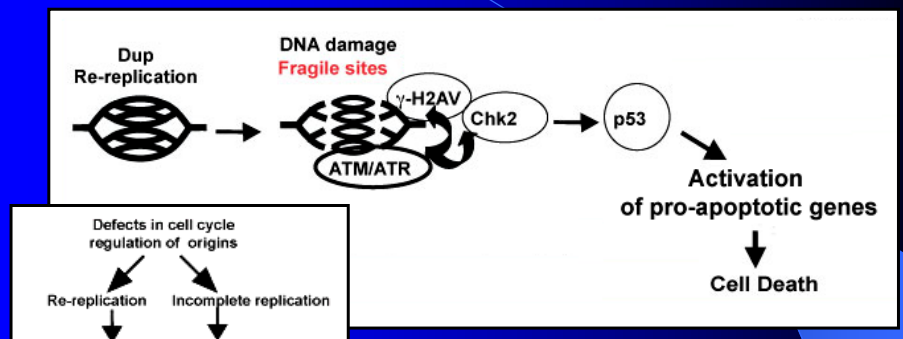
Aggrawal and Calvi, *Nature, 2004*

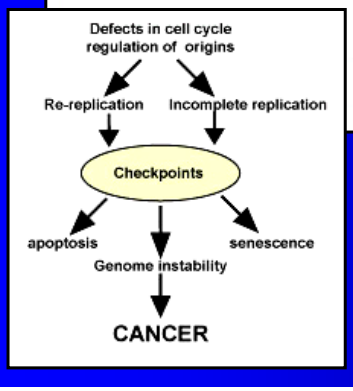
Double rolling circle replication

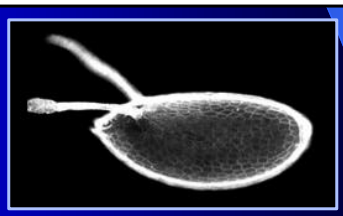


Watanabe et al, *Nucl Acid Res., 2011*

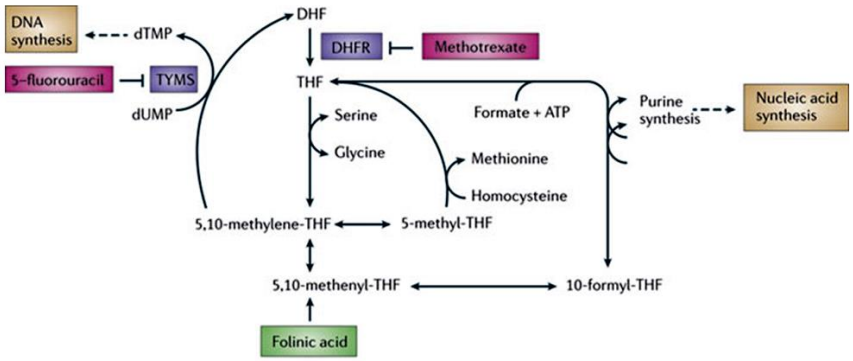
Re-replication of genes (amplification)







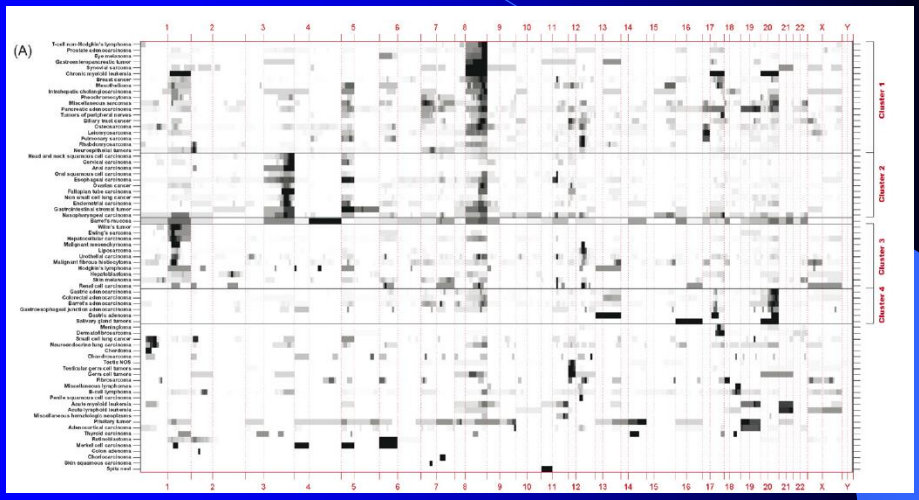
DHFR amplification in drug-resistent cancer cells



Heiden, *Nat. Rev. Drug Discovery, 2011*

TYM: thymidylate synthase, **DHFR**: dihydrofolate reductase, **THF**: tetrahydrofolate

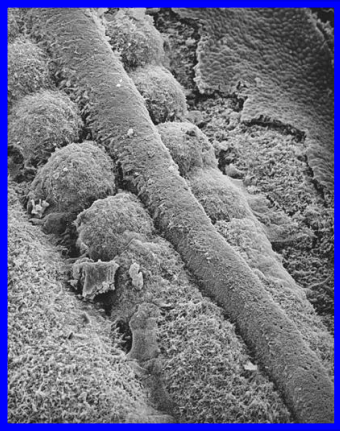
Amplification frequency in human neoplasms.

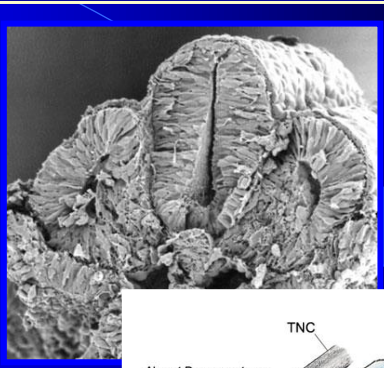


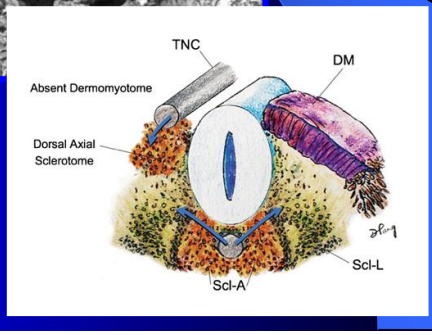
Myllykangas et al, *Sem. Cancer biol., 2007*

Skeletal Muscle Stem Cells

Somite



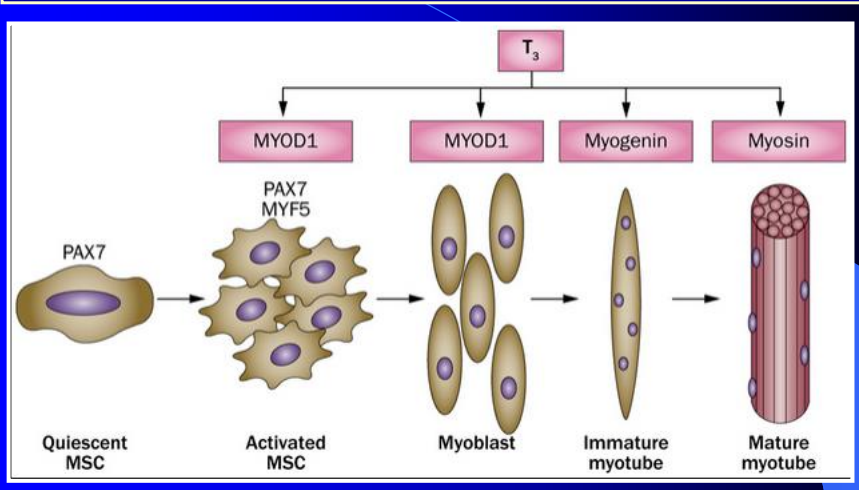




TNC: tenascin (drug)

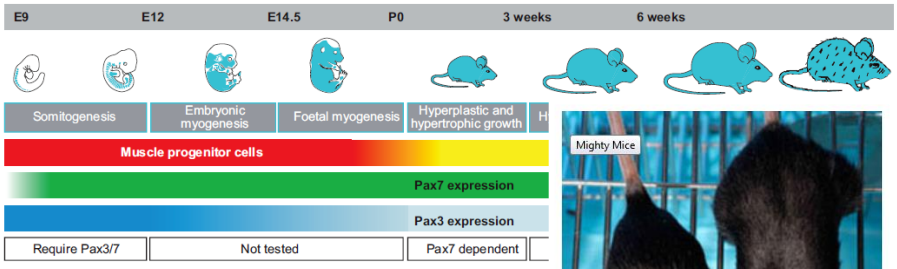
Pax3 → Pax7 → MyoD

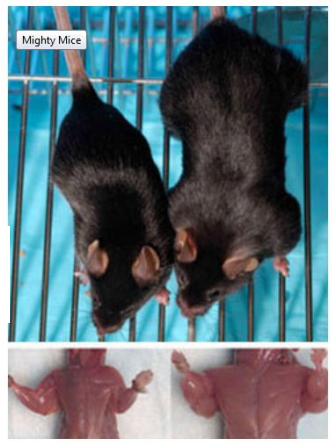
An overview of myogenesis



T3: trijodotironin
MyF5: myogenic factor 5, transcription factor
MyoD transcription factor

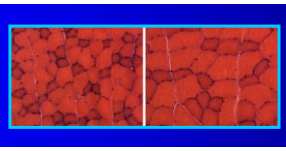
Steps of myogenesis





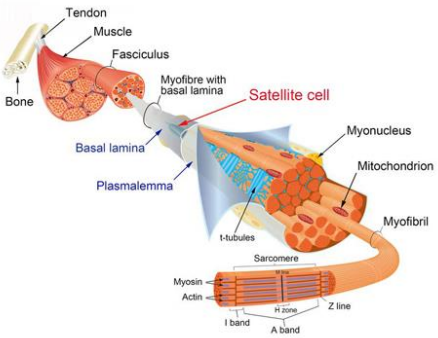
Myostatin / ActivinA ➡ Activin receptor 2B/A pathway

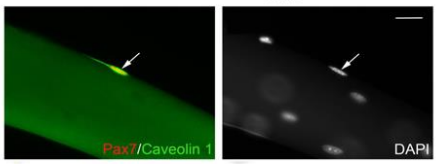
ACVR2A -/-: Mighty mice

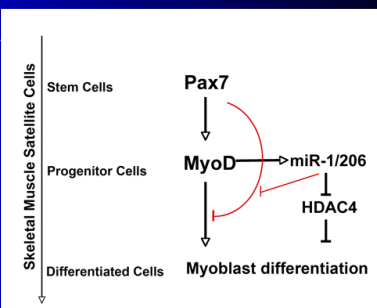


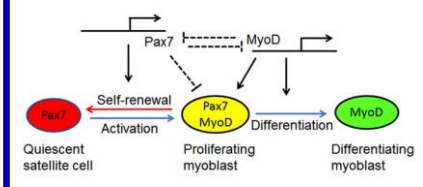
Lee et al., *PNAS, 2012*

Myosatellite cells

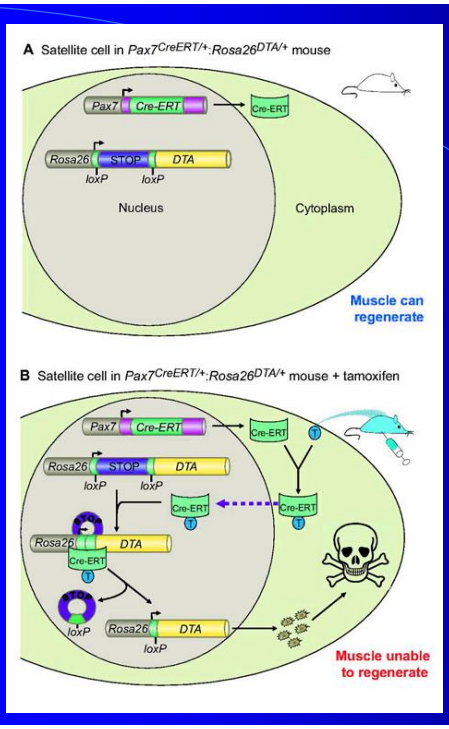








Relaix and Zammit, *Development, 2012*



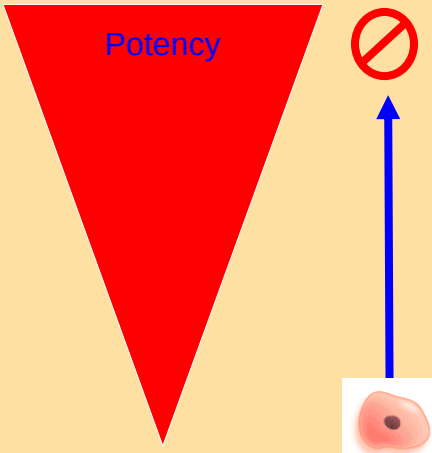
How could we kill a myosatellite and why?

Relaix and Zammit, *Development*, *2012*

Stem Cells Reprogramming

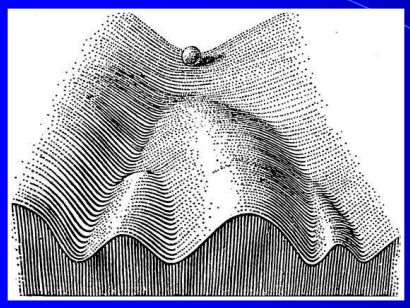
There is no way back?

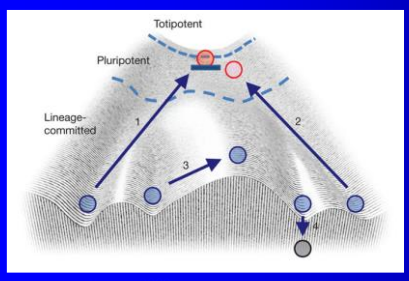
- Totipotent
- Pluripotent
- Multipotent
- Oligopotent
- Unipotent

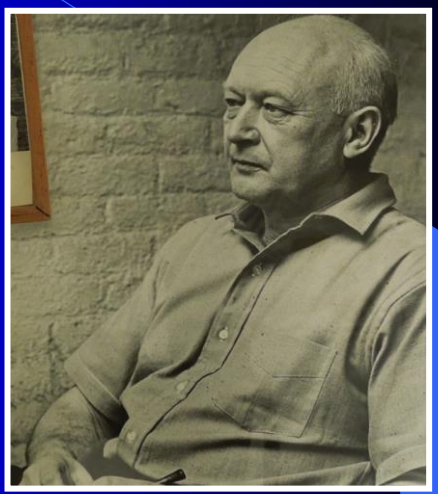


Somatic cell

Epigenetic landscape

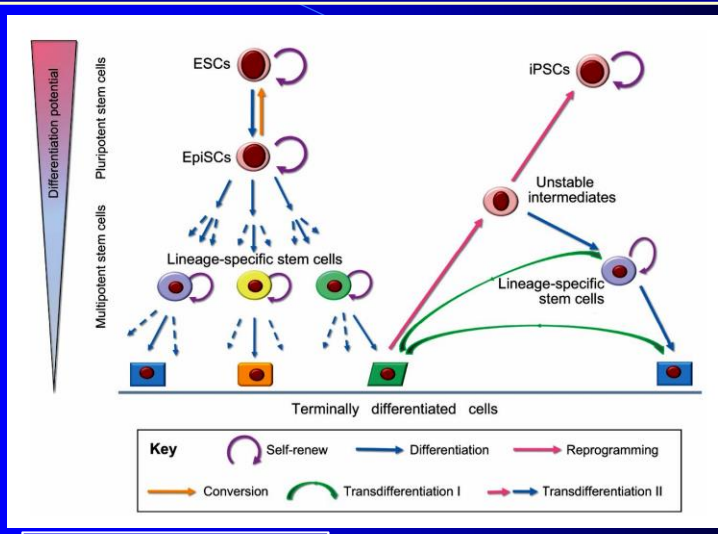






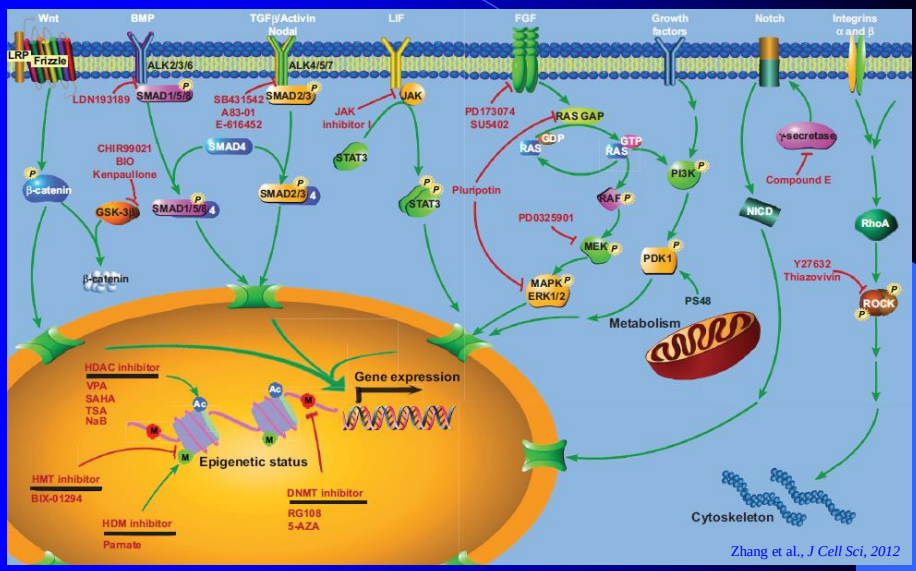
Conrad Hal Waddington (1905–1975)

Change of differentiation potencial



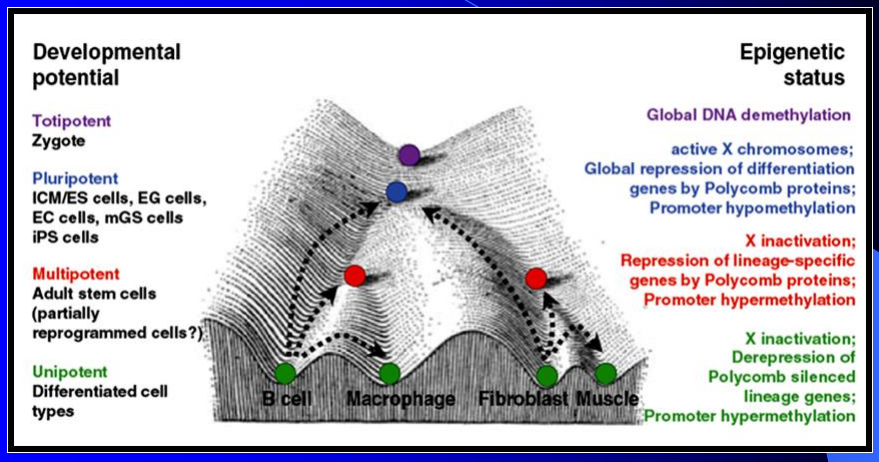
ESCs: Embryonic Stem Cells
EpiSCs: Epiblast Stem Cells
iPSC: induced Pluripotent Stem Cells

Chemical manipulation of stem cell fate



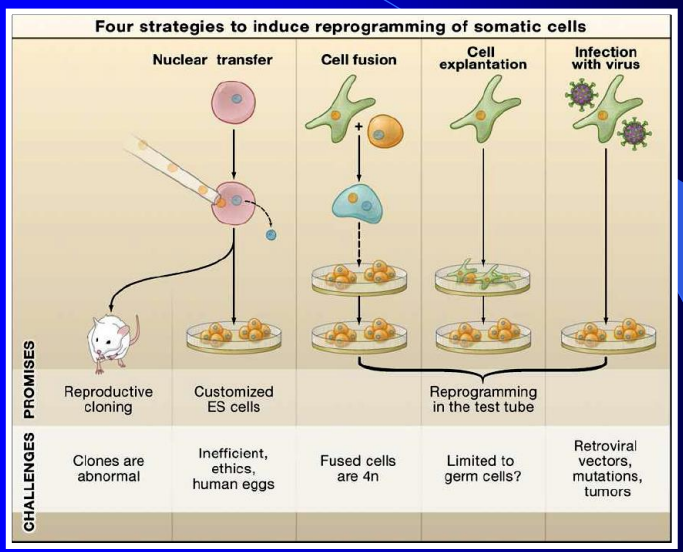
Zhang et al., *J Cell Sci, 2012*

Development and epigenetic (re)programming

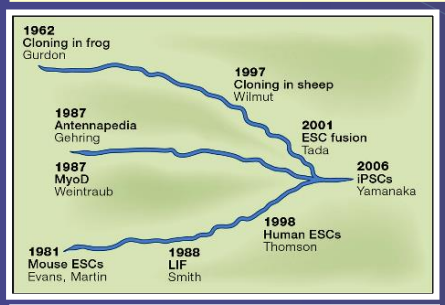


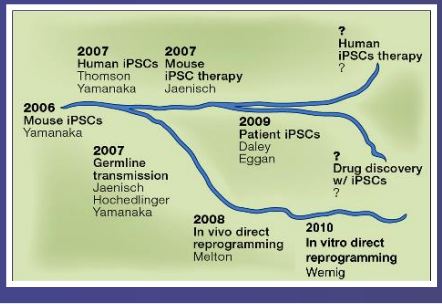
Hochedlinger, *Development*, 2009

Reprogramming strategies



Induced pluripotent stem cells (iPSCs)



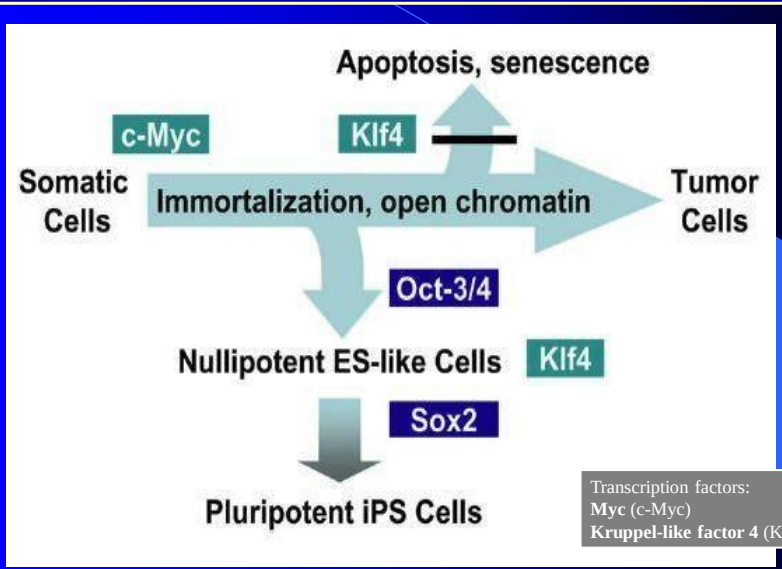


John Gurdon

Shinya Yamanaka

Nobel Prize: 2012

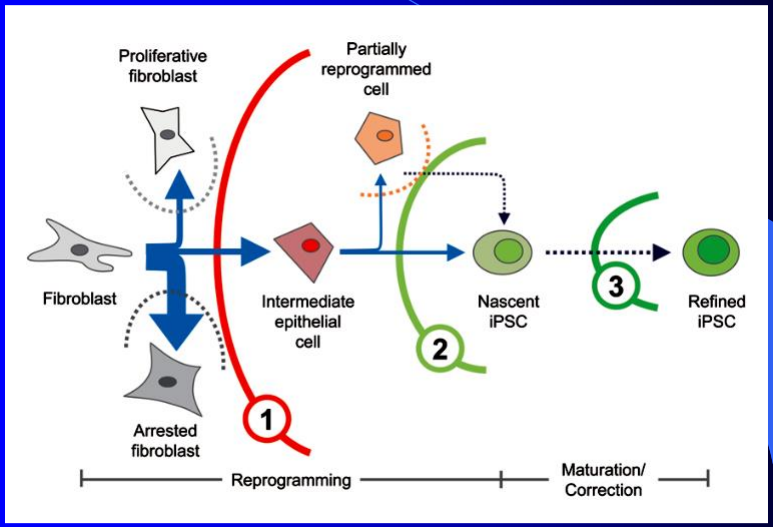
Induction of Pluripotent Stem Cells (iPS) from Somatic Stem Cells



Transcription factors:
Myc (c-Myc)
Kruppel-like factor 4 (KLF4)

Yamanaka, *Stem Cell*, *2007*

Induced pluripotent stem cells



Liang and Zhang, *Nature Cell Res, 2013*

Cancer Stem Cells

Stem Cells & Cancer

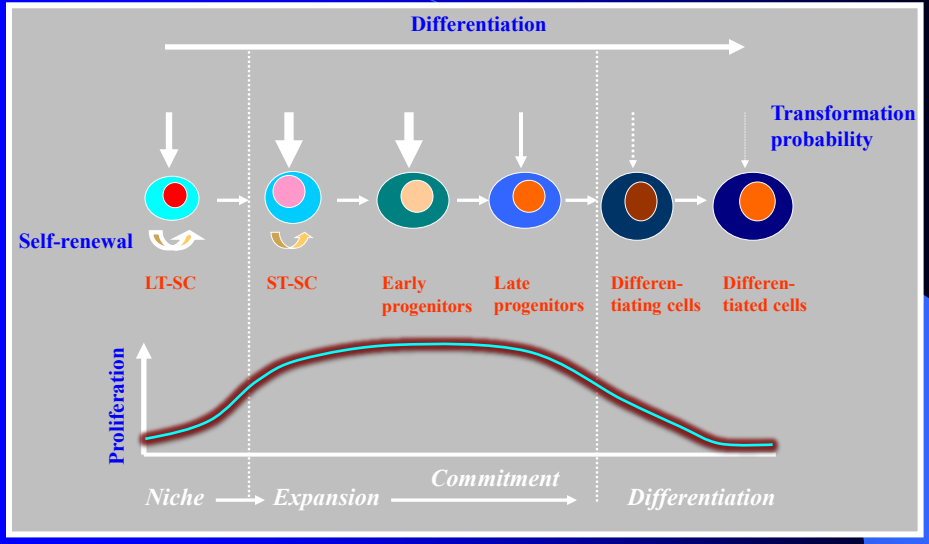
Three tumor biology puzzles:

1. Most tumors are of a clonal origin but tumor cells are heterogeneous.
2. It is very difficult to establish stable tumor cell lines from tumors.
3. Large numbers of established tumor cells have to be injected to re-initiate an orthotopic tumor in mice.

Key reviews:

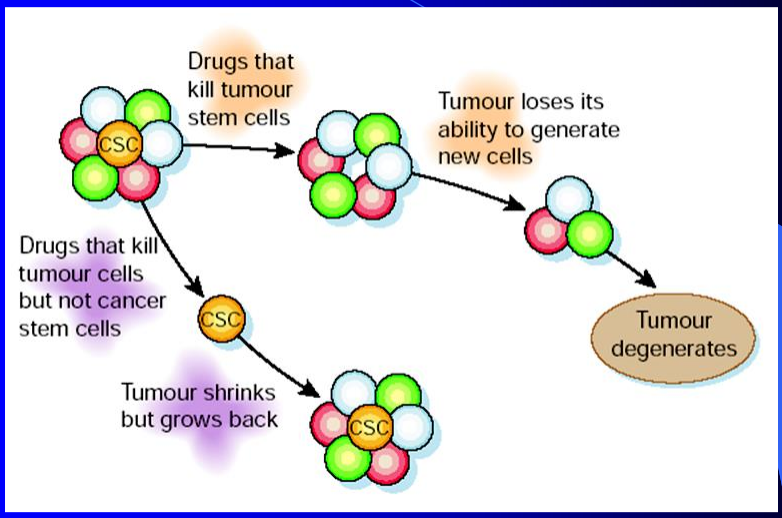
1. Reya T et al. Stem cells, cancer, and cancer stem cells. *Nature* 414, 105-111, 2001.
2. Dick JE. Stem cell concepts renew cancer research. *Blood* 112: 4793-4807, 2008.
3. Visvader JE, and Lindeman GJ. Cancer Stem Cells: Current Status and Evolving Complexicities. Cell Stem Cell 10: 717-728, 2012.
4. Tang DG. Understanding cancer stem cell heterogeneity and plasticity. Cell Res, 22(3):457-472, 2012.
5. Magee JA, Piskounova E, & Morrison SJ. Cancer Stem Cells: Impact, Heterogeneity, and Uncertainty. Cancer Cell 21: 283-296, 2012.

Where do tumor cells come from?



Tang, *Cell Res.* 2012

Cancer Stem Cells & Treatment



Weissman, *Nature*, *2001*

Medical perspectives – ethical dilemmas

Ethical dilemma

The End