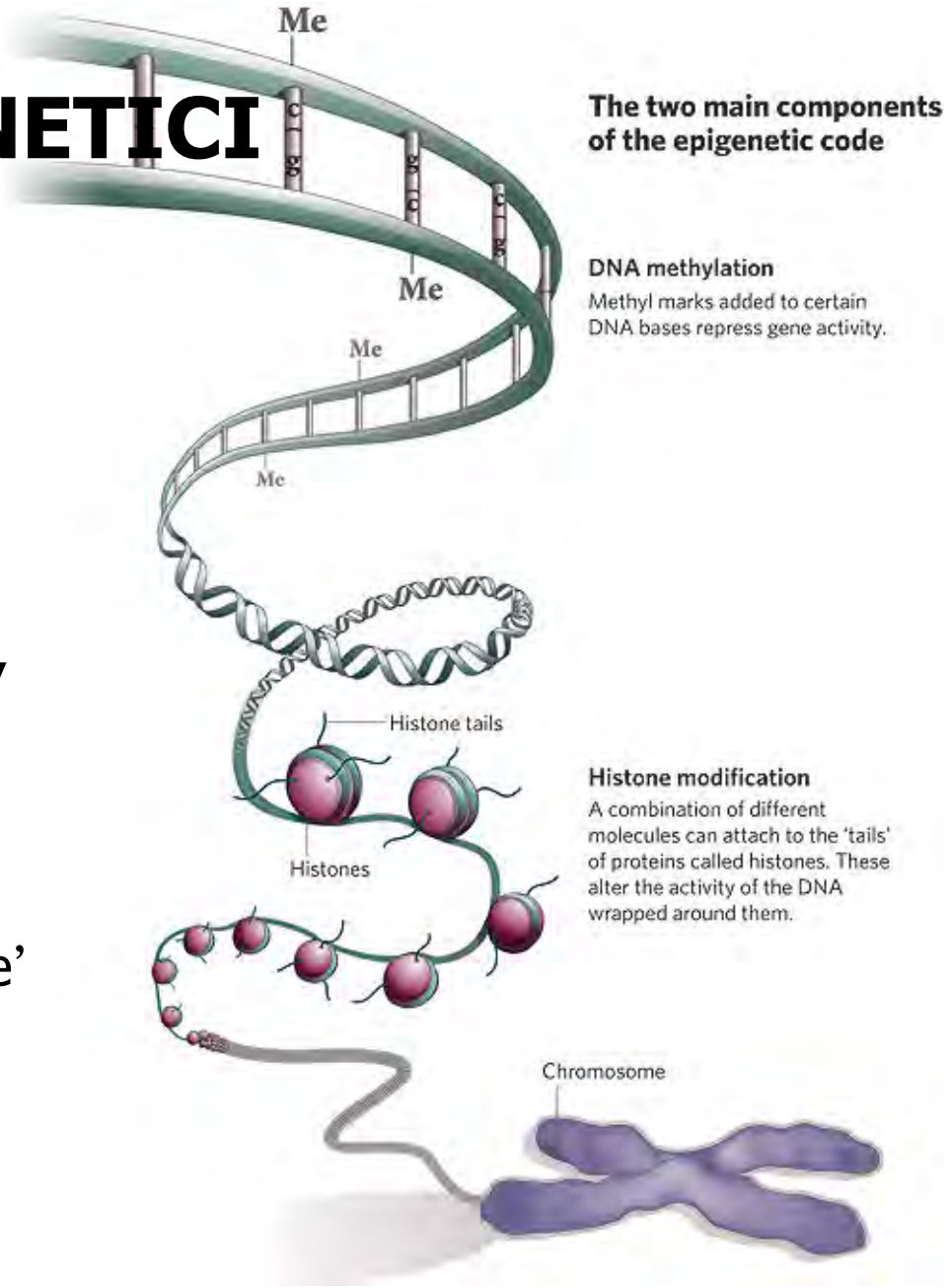
MECCANISMI EPIGENETICI

Fattori che vengono trasmessi alla progenie, ma che non sono direttamente attribuibili a sequenze di DNA.

<u>MODIFICAZIONI DEGLI ISTONI</u>
Acetilazioni, fosforilazioni e metilazioni, responsabili dei cambiamenti conformazionali della cromatina.

- <u>METILAZIONE DEL DNA</u>
Nelle cells eucariotiche la metilazione e' a carico della G. Solo il 3% delle C e' metilato; in genere e' bersaglio della metilazione la C delle doppiette CpG.



DNA methylation

is mainly associated with **transcriptional repression** and plays a major role in different processes such as X chromosome inactivation (XCI), genomic imprinting, silencing of transposons, repetitive elements and germ-line specific genes.

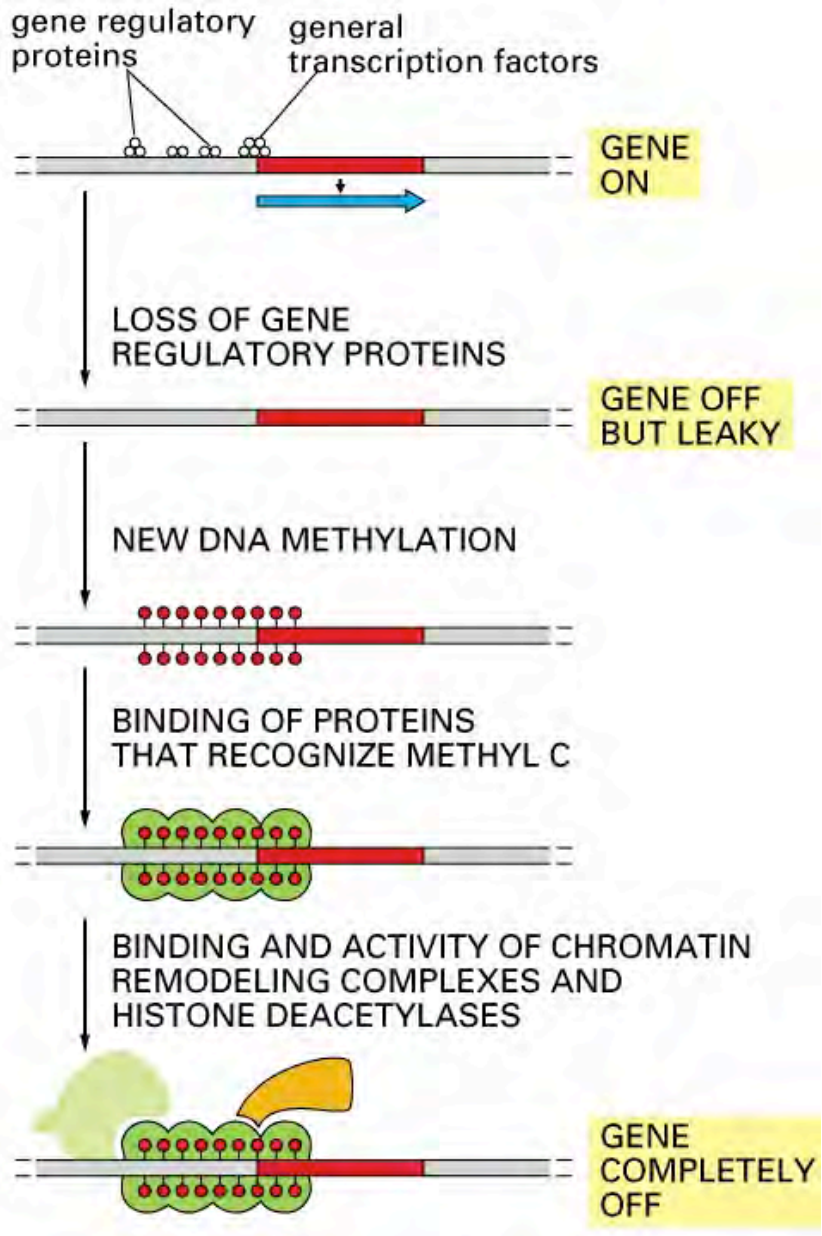
Given the robust stability of DNA methylation, ectopic promoter methylation might lead to long-term silencing of important genes. Tight regulation of the mark is of crucial importance, for proper cellular function (Greenberg, 2021).

The epigenetic memory linked to DNA methylation is robust in somatic tissues, where the levels of CpG methylation are globally stable, with 70-80% of CpG dinucleotides harboring the mark (Lee et al., 2014).

Vertebrates Use DNA Methylation to Lock Genes in a Silent State

Vertebrate cells contain a family of **proteins (MeCP2) that bind methylated DNA**.

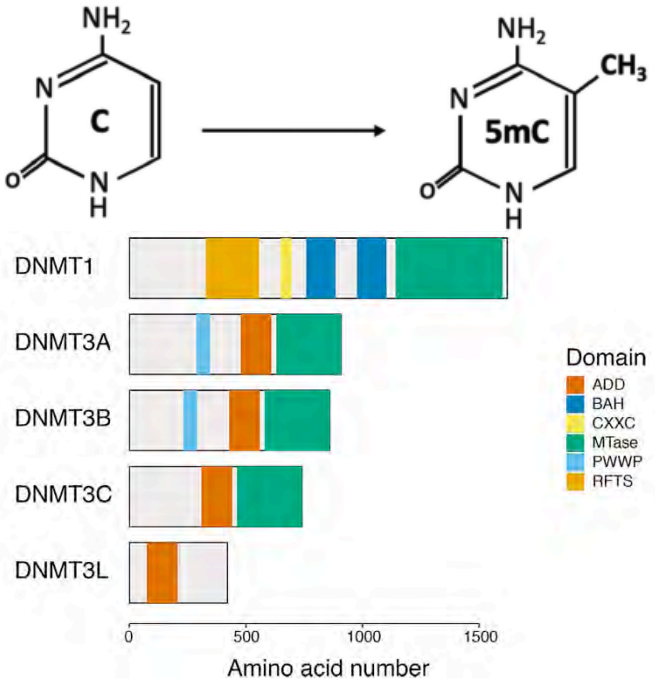
These DNA-binding proteins, in turn, interact with **chromatin remodeling complexes** and **histone deacetylases** that condense chromatin so it becomes transcriptionally inactive.



Ad ogni ciclo di duplicazione, deve essere mantenuto il profilo di metilazione (e quindi poi di espressione) del filamento parentale

DNA Methyl-transferases

Factor	Function	Mouse loss-of-function phenotype	Human diseases associated with genetic mutations
DNMT1	Maintenance DNA methyltransferase	• Low global DNA methylation • Derepression of IAP transposons • Early embryonic lethality	• Hereditary sensory autonomic neuropathy 1E (HSAN1E; OMIM 614116) • Autosomal-dominant cerebellar ataxia, deafness and narcolepsy (ADAC-DN; OMIM 604121)
UHRF1	DNMT1 cofactor	• Low global DNA methylation • Early embryonic lethality	
DNMT3A	De novo DNA methyltransferase	• Constitutive knockouts die ~4 weeks after birth[a] • Sterility in both males and females in germline-specific knockouts	• Microcephalic dwarfism • Tatton-Brown–Rahman syndrome (TBRS; OMIM 602729) • Acute myeloid leukaemia (AML; OMIM 601626)
DNMT3B	De novo DNA methyltransferase	Constitutive knockouts die mid-gestation[a]. More important for embryonic DNA methylation than for germline DNA methylation	Immunodeficiency, centromeric instability and facial anomalies syndrome (ICF; OMIM 602900)



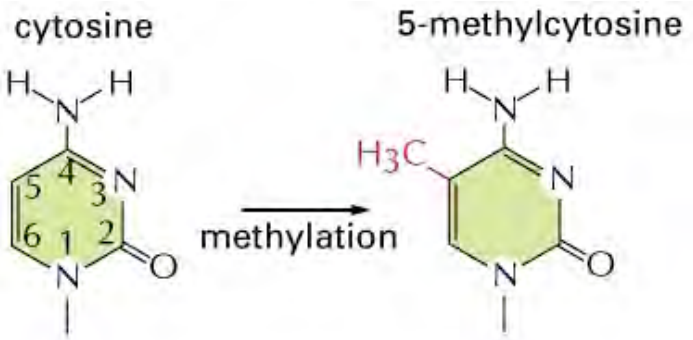
In mammals, there are 2 families of **DNA Methyl-transferases**:

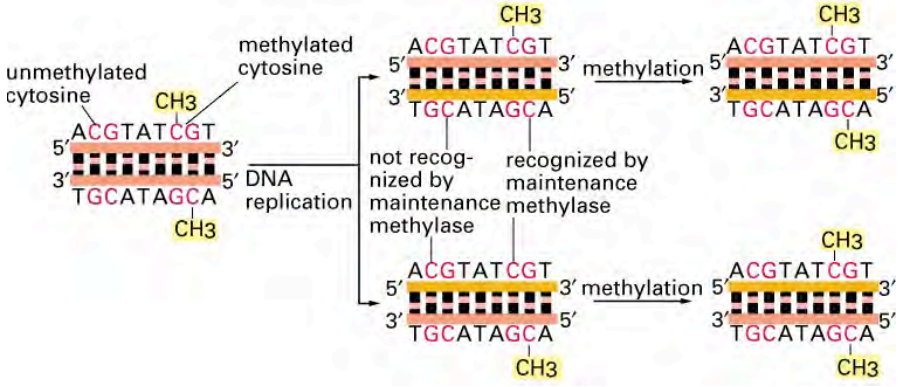
a) **DNMT1**, the maintenance methyltransferase that is responsible for the methylation of hemi-methylated CpG sites during DNA replication.

b) *de novo* methyltransferases (**DNMT3A** and **DNMT3B**) that act primarily on CpG dinucleotides during the **embryonic life**

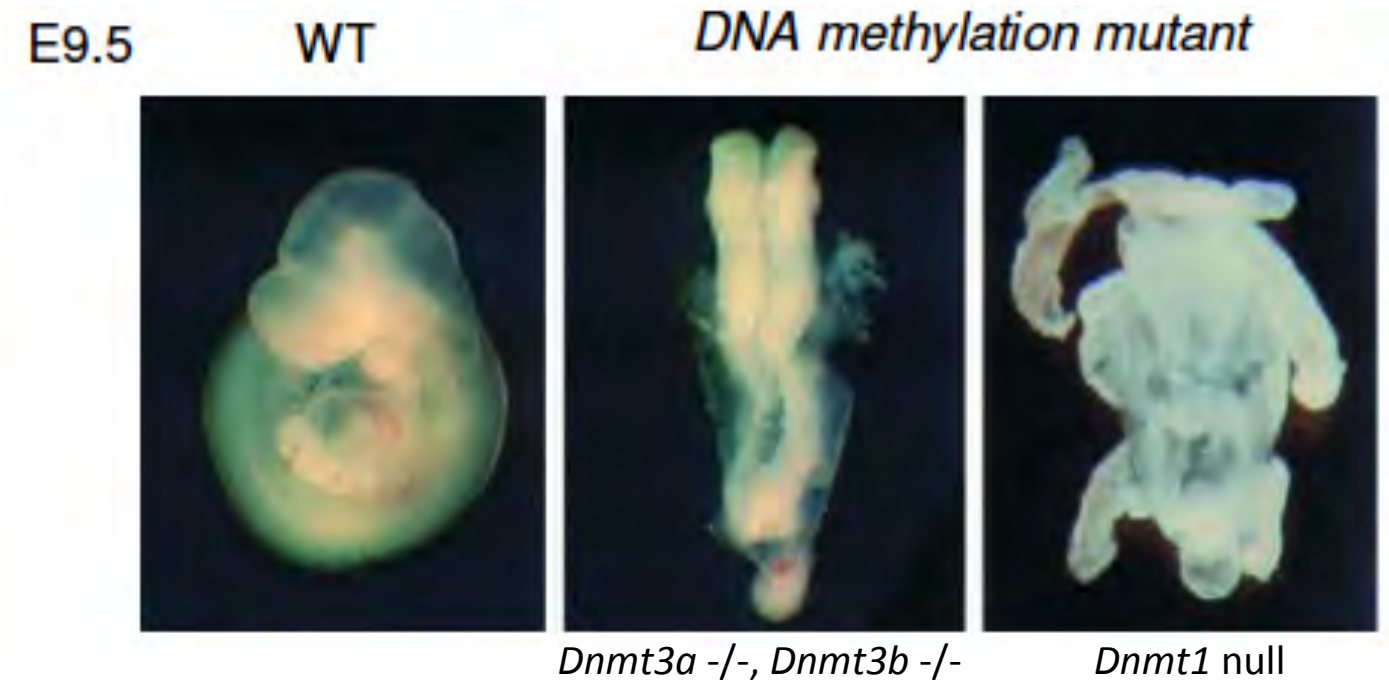
Maintenance of DNA methylation

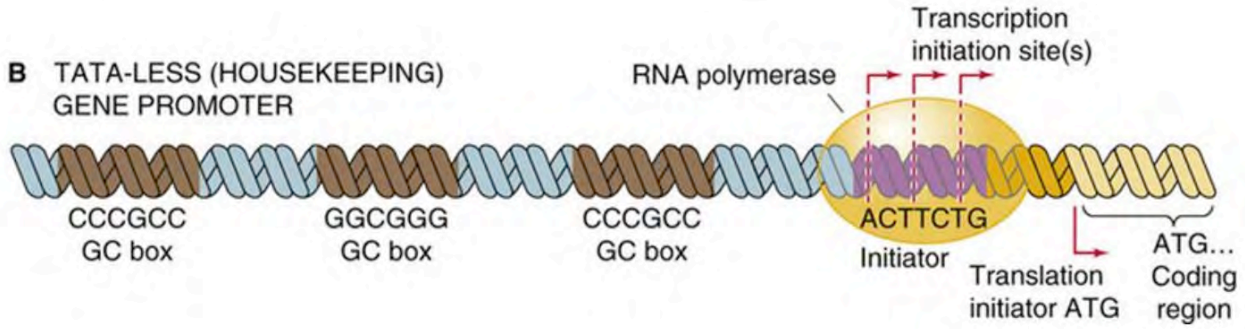
1. **Dnmt1** maintains the methyl-CpG content of both daughter DNA duplexes following replication (higher affinity for hemimethylated mCpG DNA)
2. Dnmt1 Methyltransferase is localized to the chromosomal replication complex
3. Methylation of newly synthesized DNA takes place less than one minute following replication (chromatin assembly takes 10-20 min)





The essential role of DNA methylation for a proper differentiation is supported by the severe developmental defects and embryonic lethality exhibited in DNMT-deficient mice.





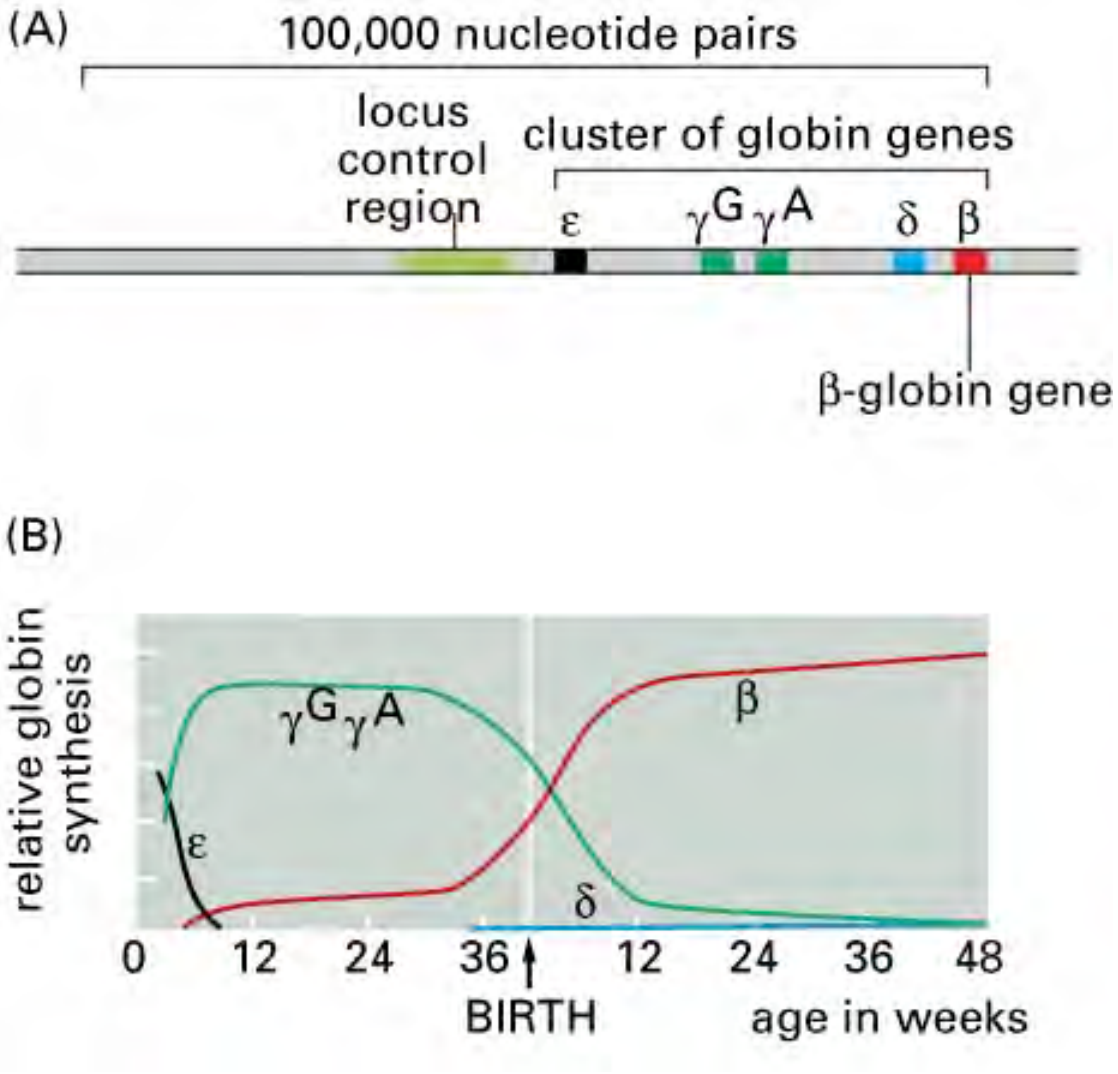
- 4% total cytosines in the genome are methylated ($3x10^7$ 5-mC residues/genome)
- All 5-mC in the dinucleotide CpG (70-80% CpG methylated)
- CpG islands: 1-2% of the total genome - consistently non methylated; all the rest (98%) all methylated

CpG island (CGI) promoters are not methylated

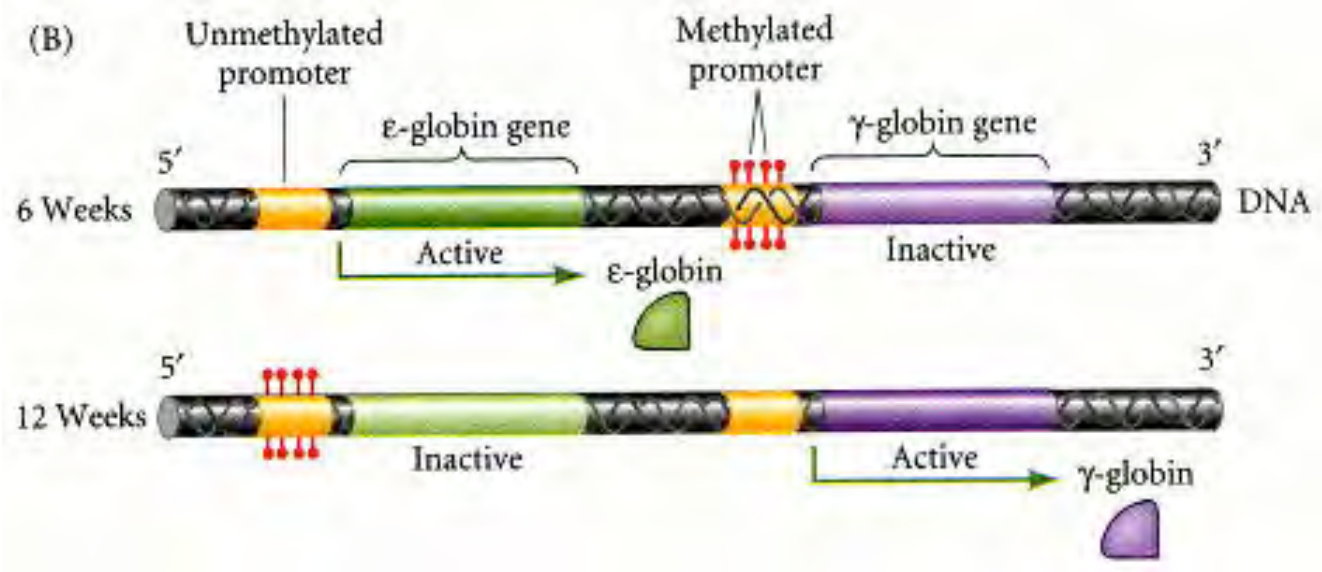
- Roughly two-thirds of promoters are CpGIs, and comprise most housekeeping and developmental genes.
- ALL CpG islands are associated with transcribed genes
- Keeping promoters free of methylation is absolutely crucial for proper cellular function.
- X-linked CpG islands become methylated upon X inactivation;

There is nothing about the sequence, per se, that should repel de novo DNA methylation.

The β-globin gene cluster



The activity of the globin genes correlates inversely with the methylation of their promoters



In developing human and chick red blood cells, the DNA of the globin promoters is almost completely **unmethylated**, whereas the same promoters are **highly methylated** in cells that do not produce globin.

Moreover, the methylation pattern changes during development. The cells that produce hemoglobin in the human embryo have unmethylated promoters for the genes encoding the ε-globins of embryonic hemoglobin. These promoters become methylated in the fetal tissue. Similarly, when the fetal globin gives way to adult globin, the γ-globin gene promoters become methylated.

DNA demethylation

Passive through DNA replication

Possible involvement of DNA-binding transcription factors (simple binding of transcription factor or even of the lac repressor can drive loss of methylation from flanking CpG dinucleotides in dividing cells)

De-methylase?? (Bhattacharya S.K. & Szyf, M. Nature 1999. Vol 397, 579)

The amazing demethylase

Howard Cedar and Gregory L. Verdine

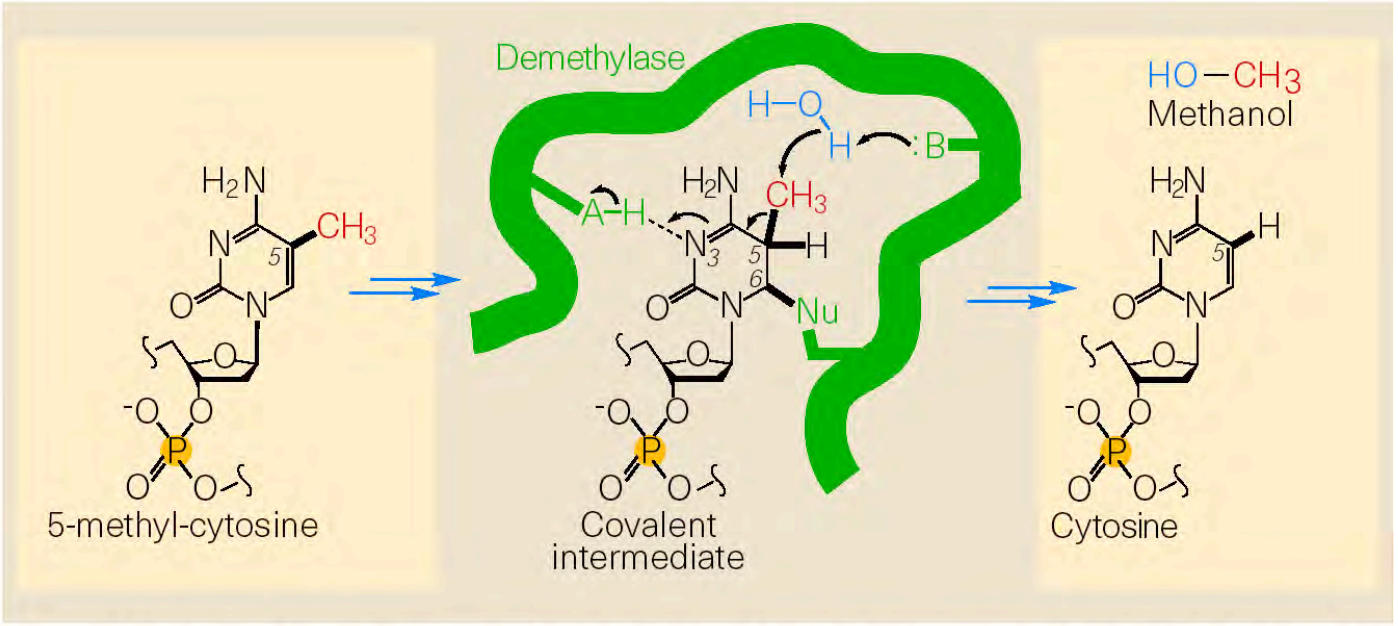
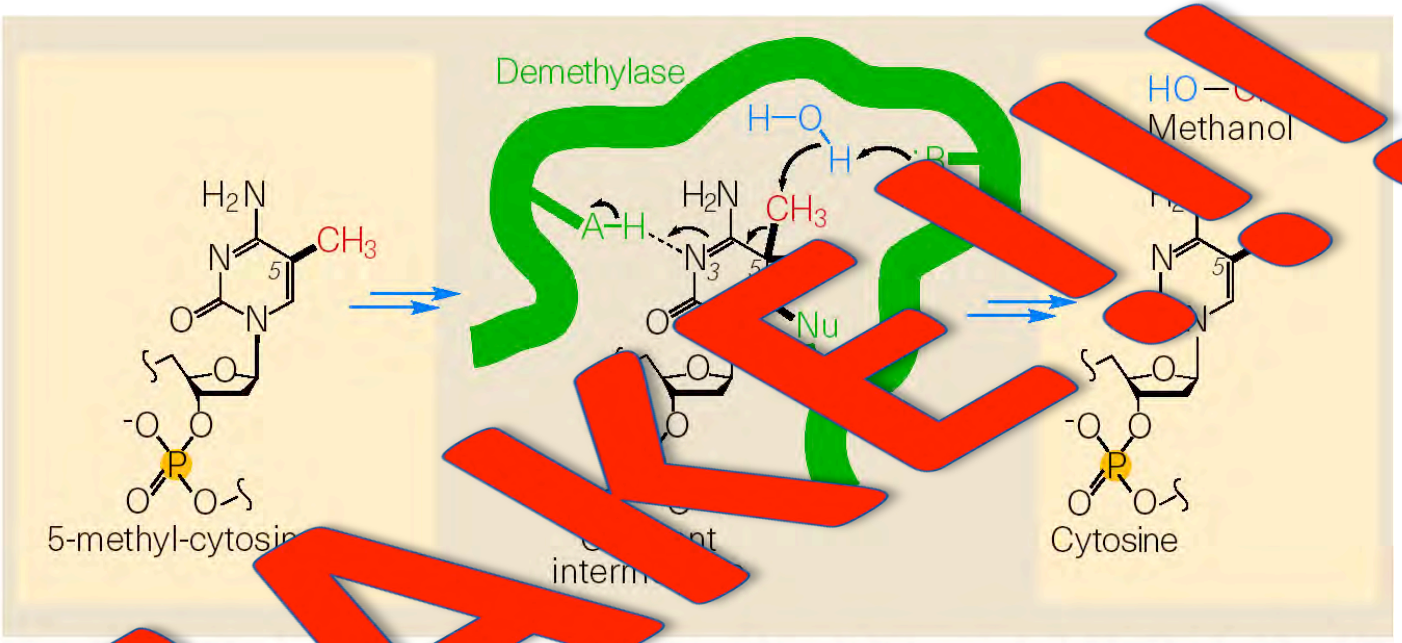


Figure 1 **Mechanism for the enzymatic demethylation of 5-methyl-cytosine. The demethylase (green) is envisaged to form a covalent intermediate by addition of an enzymatic nucleophile (Nu–H) across the 5,6 double bond, assisted by proton shuffling at N3. This intermediate is poised to attack the hydroxide ion, which is generated by *in situ* activation of water. Double arrows indicate two reaction steps, with the intermediates not shown. In the case of enzymatic methylation, an analogous covalent intermediate is formed, but is further processed by cleavage of the C5–H bond as opposed to the C5–CH_3 bond. The 3′-phosphate labelled with ^{32}P in the tracer studies of Bhattacharya *et al.*[1] is in yellow.**

Gene expression

The amazing demethylase

Howard Cedar and Gregory L. Verdine



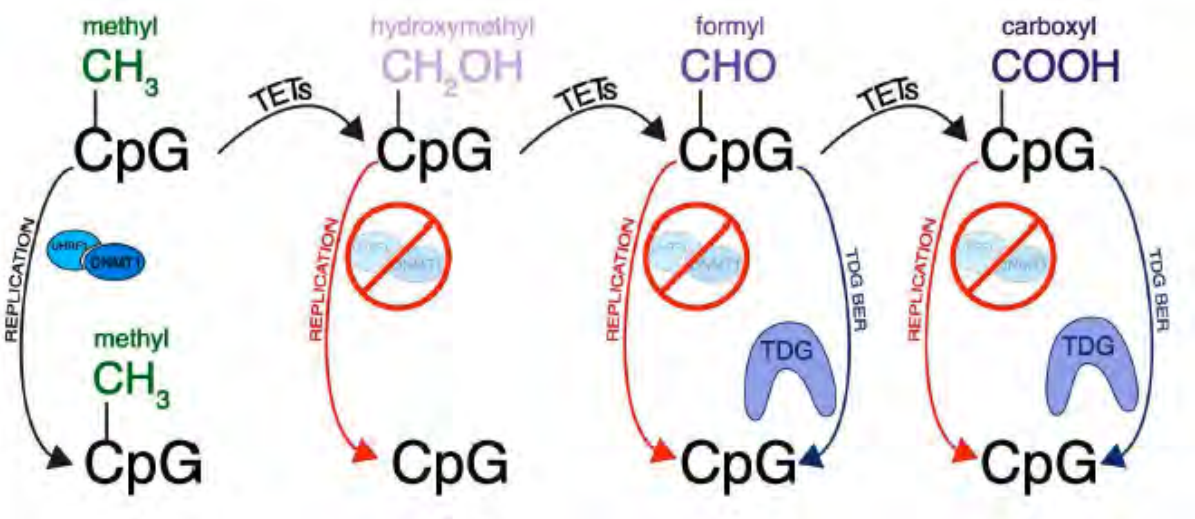
FAKE!!!

Figu… …echanism for t… …matic demethylation of 5-methyl-cytosine. The demethylase (green) is e… …d to fo… …ovalent… …mediate by addition of an enzymatic nucleophile (Nu–H) across the 5,6 dou… …, assisted by proton shuffling at N3. This intermediate is poised to attack the hydroxide ion, which is g… …ted by *in situ* activation of water. Double arrows indicate two reaction steps, with the intermediates not shown. In the case of enzymatic methylation, an analogous covalent intermediate is formed, but is further processed by cleavage of the C5–H bond as opposed to the C5–CH_3 bond. The 3′-phosphate labelled with ^{32}P in the tracer studies of Bhattacharya *et al.*[1] is in yellow.

There are different mechanisms of DNA demethylation: both **passive** and **active** processes can occur.

- Passive demethylation simply requires the impairment of maintenance DNA methylation machinery (Dnmt-1), which results in 2-fold dilution of methyl-CpGs during each round of DNA synthesis.

- Active DNA demethylation in mammals involves the action of Ten-eleven translocase (TET) family of dioxygenases.



Uncovering the role of 5-hydroxymethylcytosine in the epigenome

Miguel R. Branco, Gabriella Ficz and Wolf Reik

Abstract | Just over 2 years ago, TET1 was found to catalyse the oxidation of 5-methylcytosine, a well-known epigenetic mark, into 5-hydroxymethylcytosine in mammalian DNA. The exciting prospect of a novel epigenetic modification that may dynamically regulate DNA methylation has led to the rapid accumulation of publications from a wide array of fields, from biochemistry to stem cell biology. Although we have only started to scratch the surface, interesting clues on the role of 5-hydroxymethylcytosine are quickly emerging.

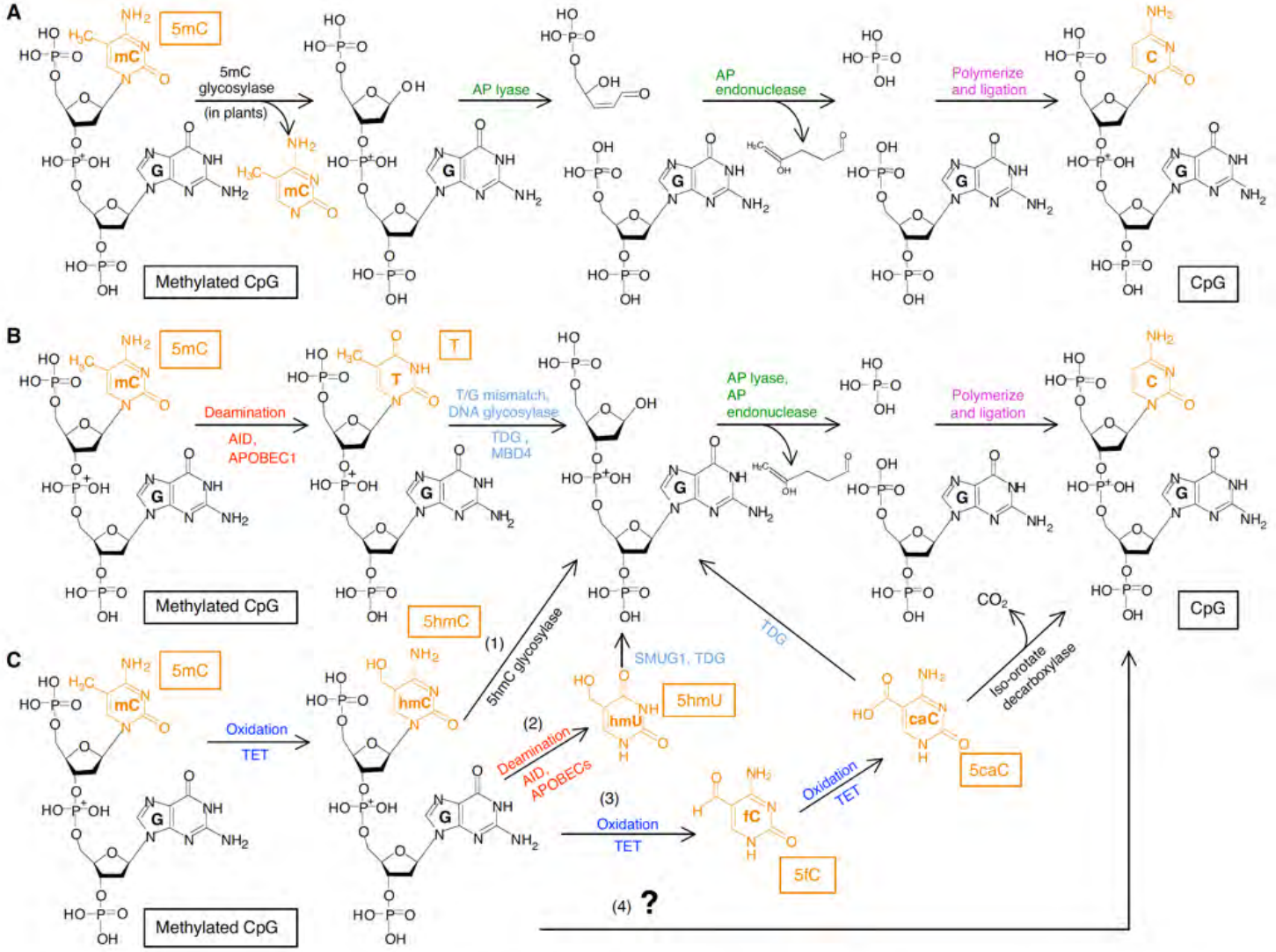
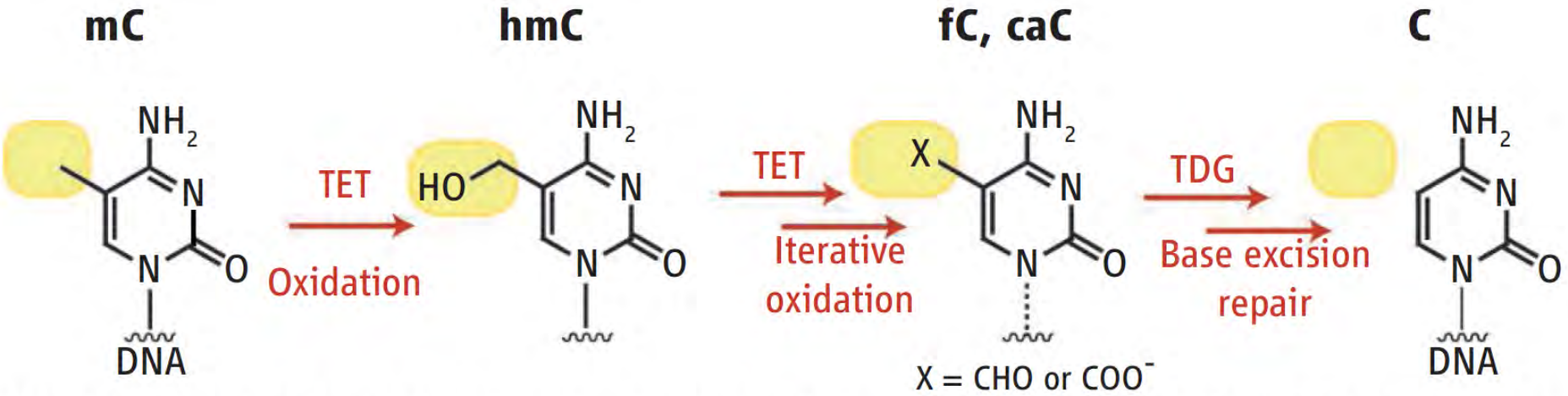


Fig. 4. Potential chemical pathways for active DNA demethylation. (**A**) Direct excision of 5mC (orange) by a 5mC glycosylase followed by repair via the base excision repair (BER) pathway (green and pink), as occurs in plants. (**B**) Cytosine deamination by AID/APOBEC1 (red), followed by base excision mismatch repair, involving the TDG/MBD4 (pale blue) and BER pathways. (**C**) Hydroxylation by TET (blue) initiates four potential pathways leading to demethylated cytosine: (1) removal of 5hmC by an unidentified 5hmC glycosylase, followed by BER; (2) deamination of 5hmC by AID or APOBECs creates 5hmU, which is removed by SMUG1 (single-strand selective monofunctional uracil DNA glycosylase) or TDG, followed by BER; (3) further oxidization of 5hmC to 5fC and then to 5caC, which then may be converted to C by a decarboxylase or by TDG followed by BER; and (4) direct conversion of 5hmC to 5mC by an unidentified enzyme (?). 5caC, 5-carboxylcytosine; 5fC, 5-formylcytosine; 5hmC, 5-hydroxymethylcytosine; 5hmU, 5-hydroxymethyluracil;5mC, 5-methylcytosine; AP, apurinic/apyrimidinic; AID, activation-induced deaminase; APOBEC1, apolipoprotein B mRNA editing enzyme, catalytic polypeptide 1; C, cytosine; G, guanine; MBD4, methyl CpG binding domain protein 4; SMUG1, single-strand selective monofunctional uracil DNA glycosylase; T, thymidine; TDG, thymine DNA glycosylase; TET, ten-eleven translocation.

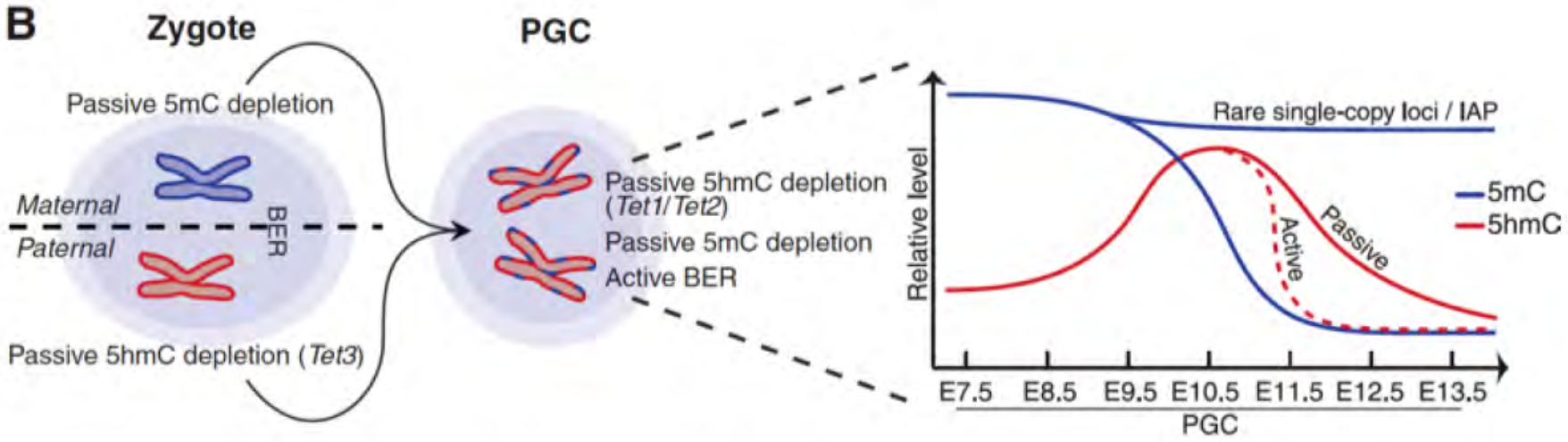


DNA demethylation. TET enzymes are proposed to oxidize 5-methylcytosine (mC) to 5-hydroxymethylcytosine (hmC) and subsequently to generate the higher oxidation substituents 5-formylcytosine (fC) and 5-carboxylcytosine (caC) (shown as the structure with the 5-X substituent). Unmodified cytosine (C) is on the far right. Base excision repair, initiated by thymine-DNA glycosylase (TDG), releases and replaces the entire modified oxidized base with unmodified C.

Germline DNA Demethylation Dynamics and Imprint Erasure Through 5-Hydroxymethylcytosine

Jamie A. Hackett,[1,2] Roopsha Sengupta,[1,2]* Jan J. Zylicz,[1,2,3]* Kazuhiro Murakami,[1,2]* Caroline Lee,[1,2] Thomas A. Down,[1] M. Azim Surani[1,2,3]†

Mouse primordial germ cells (PGCs) undergo sequential epigenetic changes and genome-wide DNA demethylation to reset the epigenome for totipotency. Here, we demonstrate that erasure of CpG methylation (5mC) in PGCs occurs via conversion to 5-hydroxymethylcytosine (5hmC), driven by high levels of TET1 and TET2. Global conversion to 5hmC initiates asynchronously among PGCs at embryonic day (E) 9.5 to E10.5 and accounts for the unique process of imprint erasure. Mechanistically, 5hmC enrichment is followed by its protracted decline thereafter at a rate consistent with replication-coupled dilution. The conversion to 5hmC is an important component of parallel redundant systems that drive comprehensive reprogramming in PGCs. Nonetheless, we identify rare regulatory elements that escape systematic DNA demethylation in PGCs, providing a potential mechanistic basis for transgenerational epigenetic inheritance.



Mammalian genome methylation

Mammals exhibit two rounds of dramatic DNA methylation reprogramming during embryonic development:

1. immediately after fertilization
2. in the germline (Seisenberger et al., 2013; Wu and Zhang, 2014).

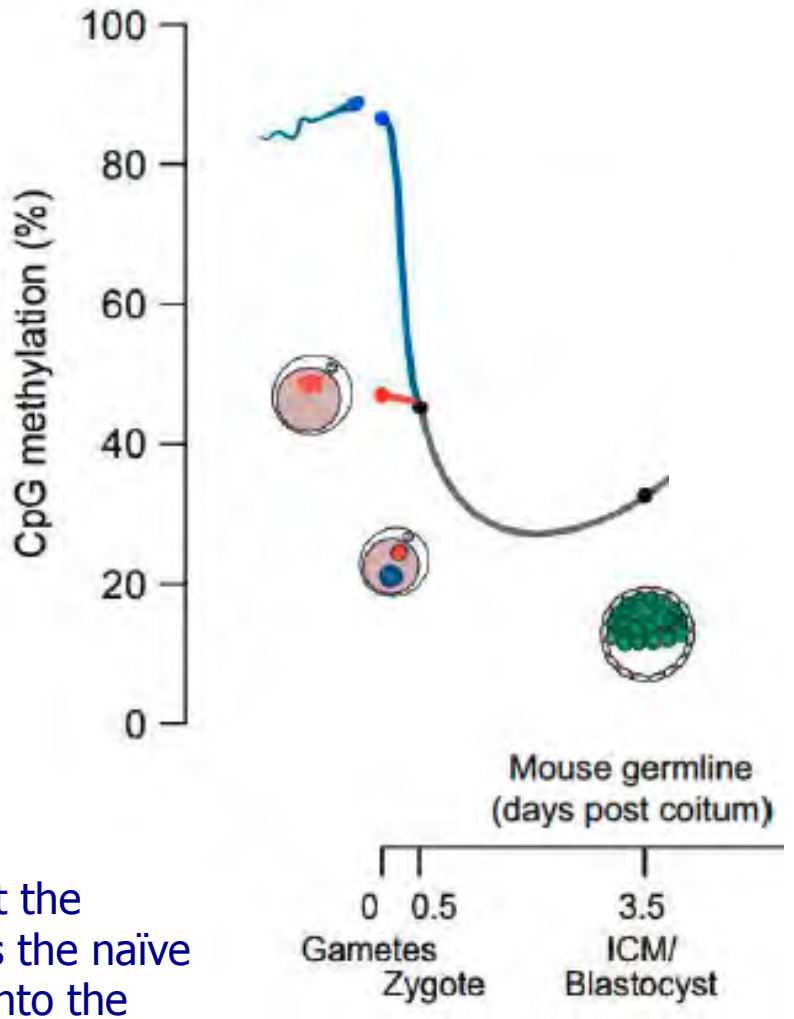
As the embryo implants in the uterus, the de novo DNA methyltransferases, DNMT3A and DNMT3B, rapidly remethylate the genome, establishing a pattern that is globally maintained in somatic tissue-types

A dramatic genome-wide reprogramming of DNA methylation occurs during **embryogenesis**

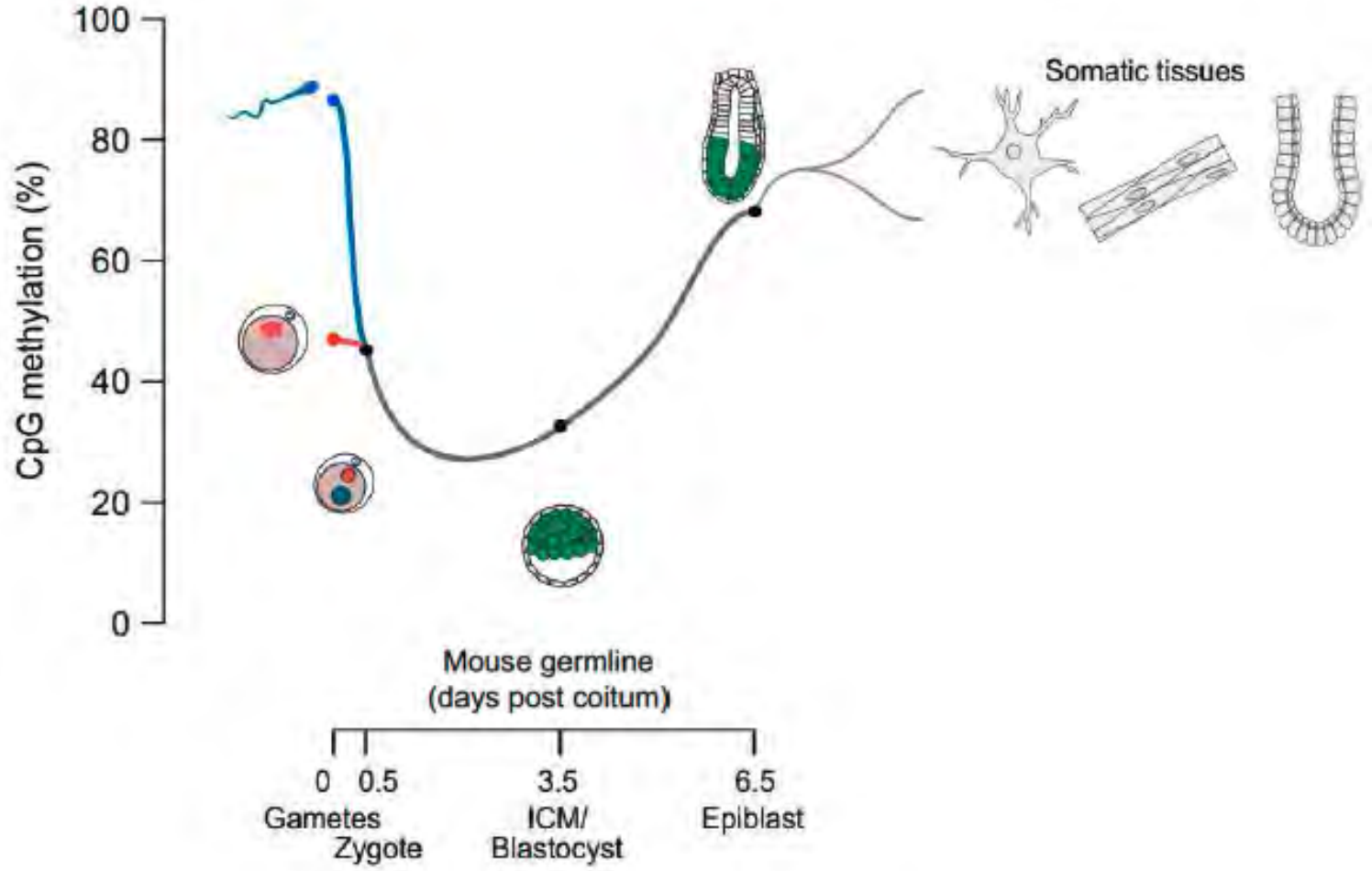
Upon fertilization, both the maternal and paternal genomes are subjected to a dramatic loss of DNA methylation

Such reprogramming is found exclusively in mammals.

A reprogramming of DNA methylation at this stage of development is needed to cellular memory towards an undifferentiated state in order to allow for naïve pluripotency

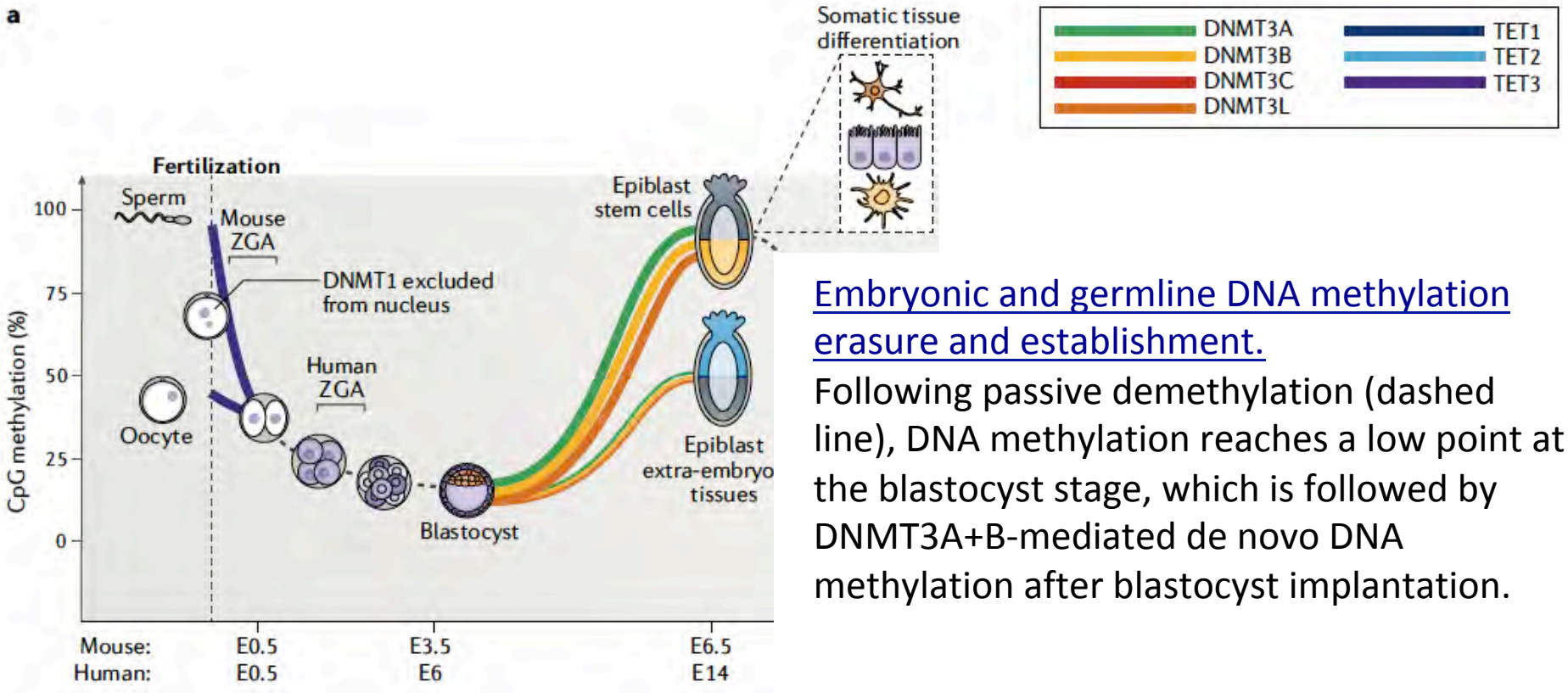


In mice, in the inner cell mass (ICM) at the blastocyst stage (E3.5), which contains the naïve pluripotent cells that will differentiate into the embryo, only 20% of CpGs remain methylated.



Following this dramatic global demethylation wave, the *de novo* methyltransferases, **DNMT3A** and **DNMT3B**, rapidly re-establish high levels of methylation.
Importantly, the wave of re-methylation coincides with the transition from naïve to primed pluripotency.
By E6.5, the primed stem cells in the epiblast will then further differentiate into the somatic lineages, which will globally maintain the pattern and levels of CpG methylation established during these early stages of development.

Methylation patterning in development



Embryonic and germline DNA methylation erasure and establishment.

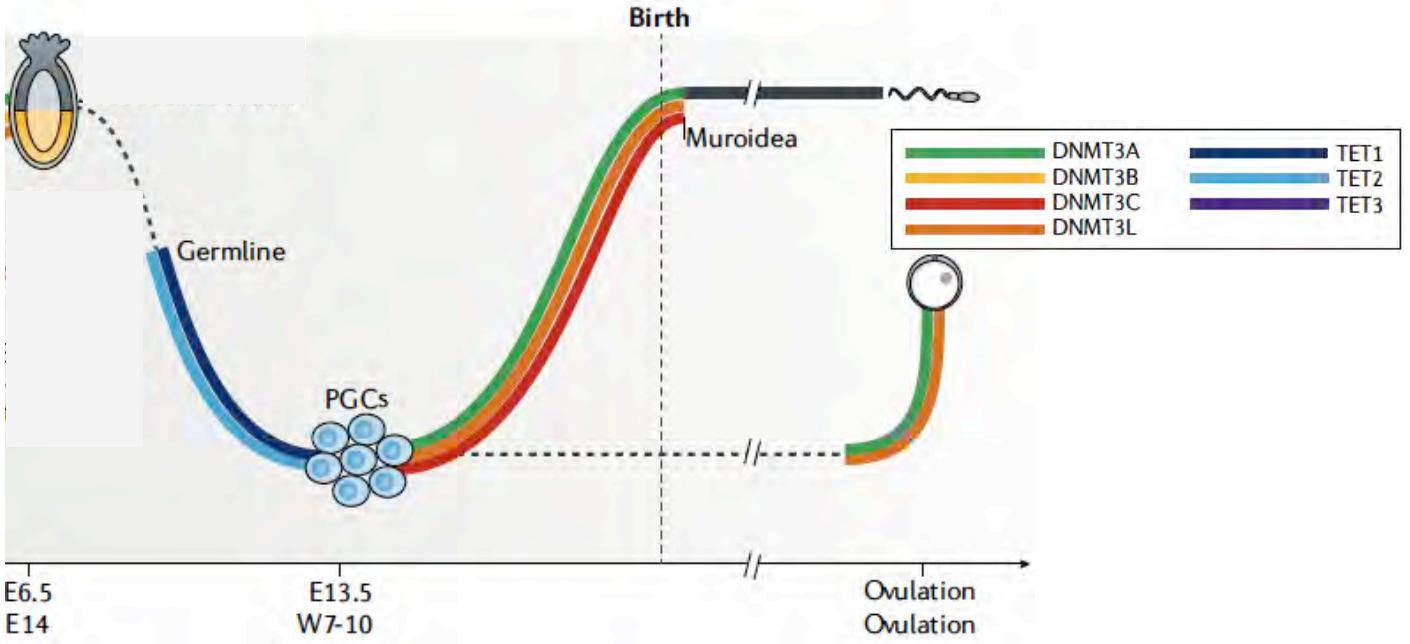
Following passive demethylation (dashed line), DNA methylation reaches a low point at the blastocyst stage, which is followed by DNMT3A+B-mediated de novo DNA methylation after blastocyst implantation.

During post- fertilization reprogramming, the embryo loses gamete- specific DNA methylation patterns inherited from the oocyte and the sperm as it progresses towards pluripotency.

The paternal genome is actively demethylated by TET3; the two parental genomes then undergo rounds of passive, DNA replication- dependent dilution of DNA methylation, as the maintenance enzyme DNMT1 provided by the oocyte is excluded from the nucleus during subsequent cell divisions.

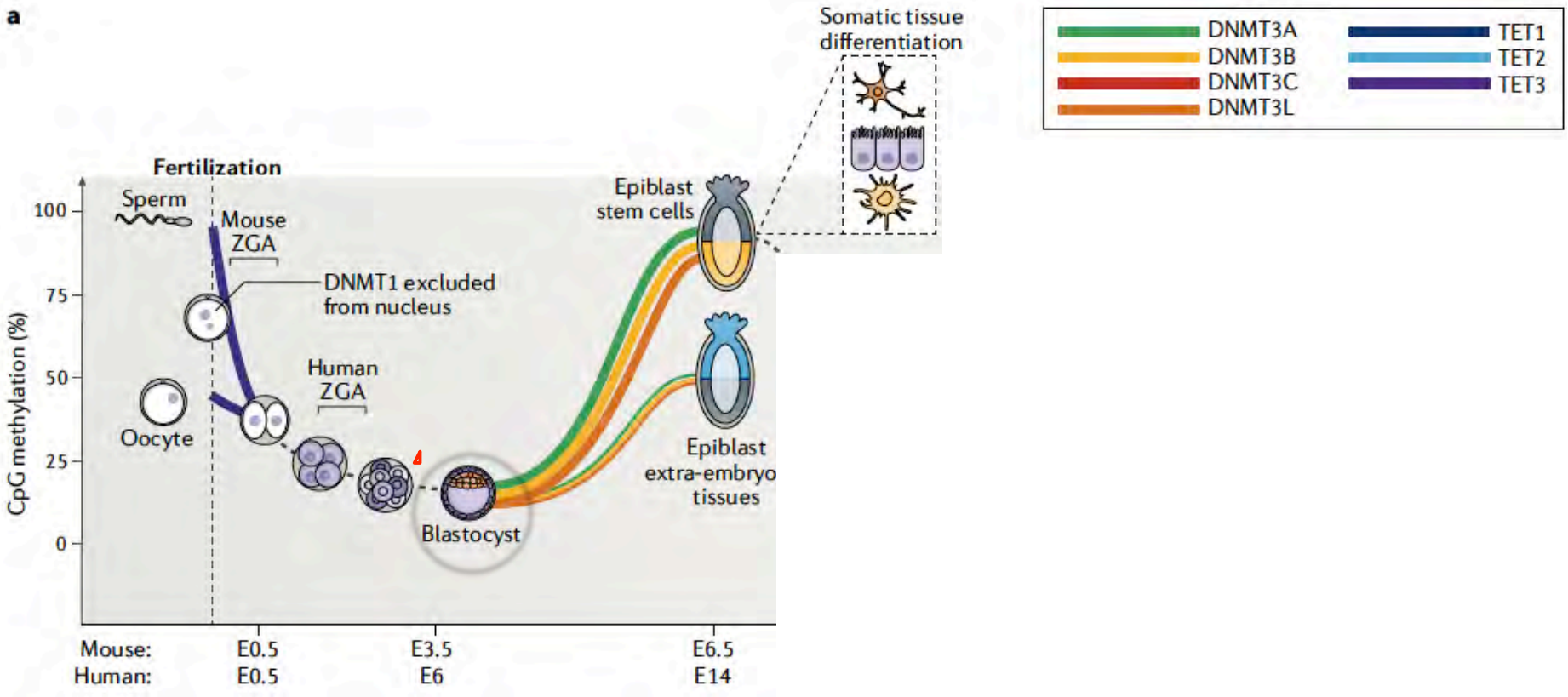
What about germ cells?

Methylation patterning in PGCs development



- Demethylation occurs in developing PGCs (primordial germ cells), as a prerequisite for subsequent acquisition of sex- specific DNA methylation patterns during male and female germline differentiation.
- Post implantation, in the epiblast, a subset of stem cells is specified for the germline, where they undergo two waves of DNA demethylation: one passive and one mediated by TET1 and TET2.
- **Male gametes** become highly methylated **before birth** through the activity of DNMT3A and DNMT3L.
- **The oocyte** gains methylation **after birth**, after meiosis and prior to ovulation through the activity of DNMT3A in humans.

Methylation patterning in development



In the inner cell mass of preimplantation embryos, approximately 20% of CpGs retain gamete- inherited methylation in both mice and humans.
These notably map to ICRs (imprinting control regions), as expected from the intergenerational nature of genomic imprinting, which is linked to the sequence-specific DNA demethylation resistance

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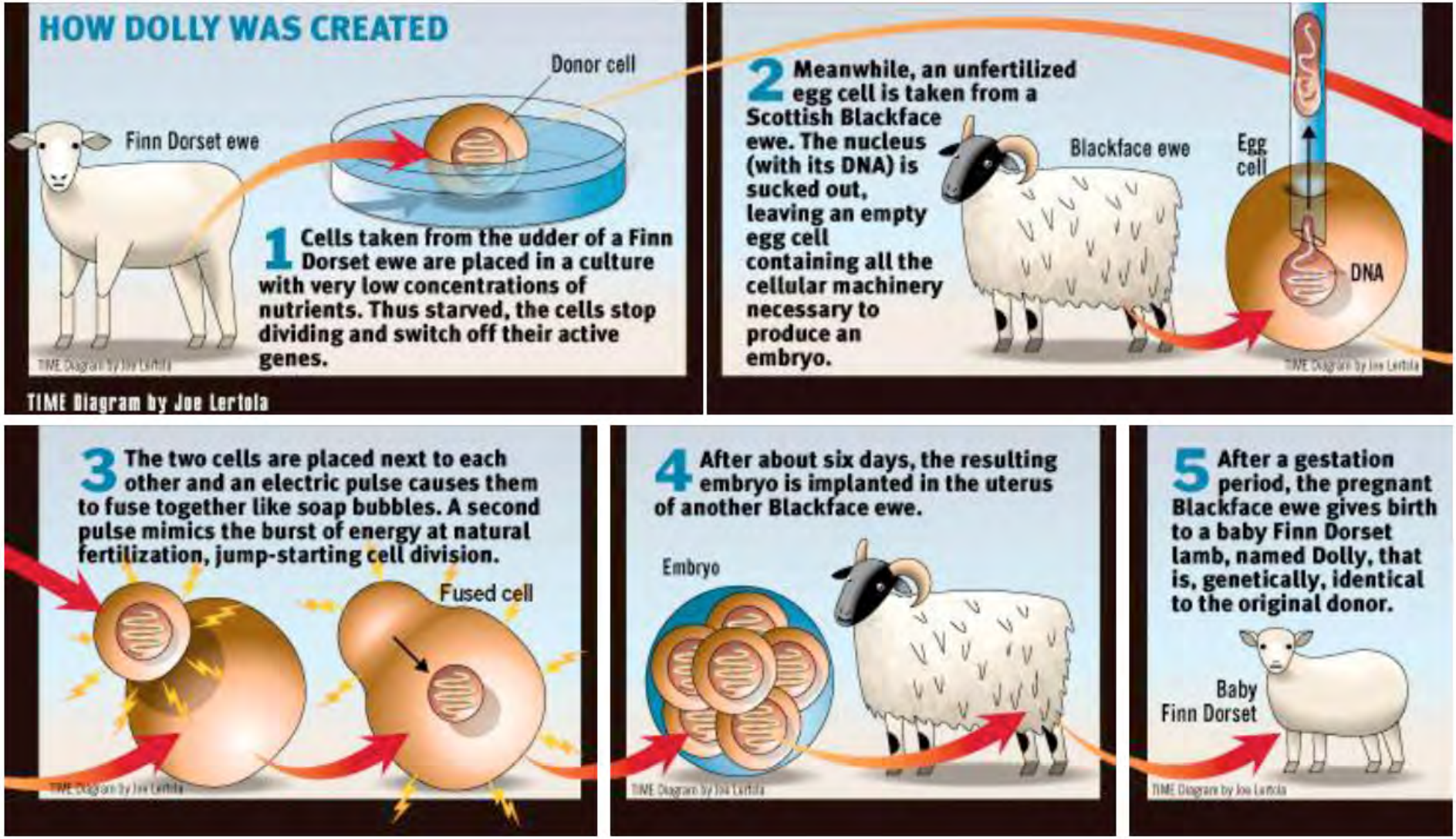
VOL. 278 • PAGES 2021–2192
$7.00

Breakthrough of the Year
CLONING

Animal cloning by nuclear transfer



Viable offspring derived from fetal and adult mammalian cells

NATURE | VOL 385 | 27 FEBRUARY 1997

I. Wilmut, A. E. Schnieke*, J. McWhir, A. J. Kind* & K. H. S. Campbell

Roslin Institute (Edinburgh), Roslin, Midlothian EH25 9PS, UK
** PPL Therapeutics, Roslin, Midlothian EH25 9PP, UK*

Nuclei donatori:

embrione di 9 giorni
un feto di 26 giorni
ghiandola mammaria di una pecora di 6 anni nell'ultimo trimestre di gravidanza.

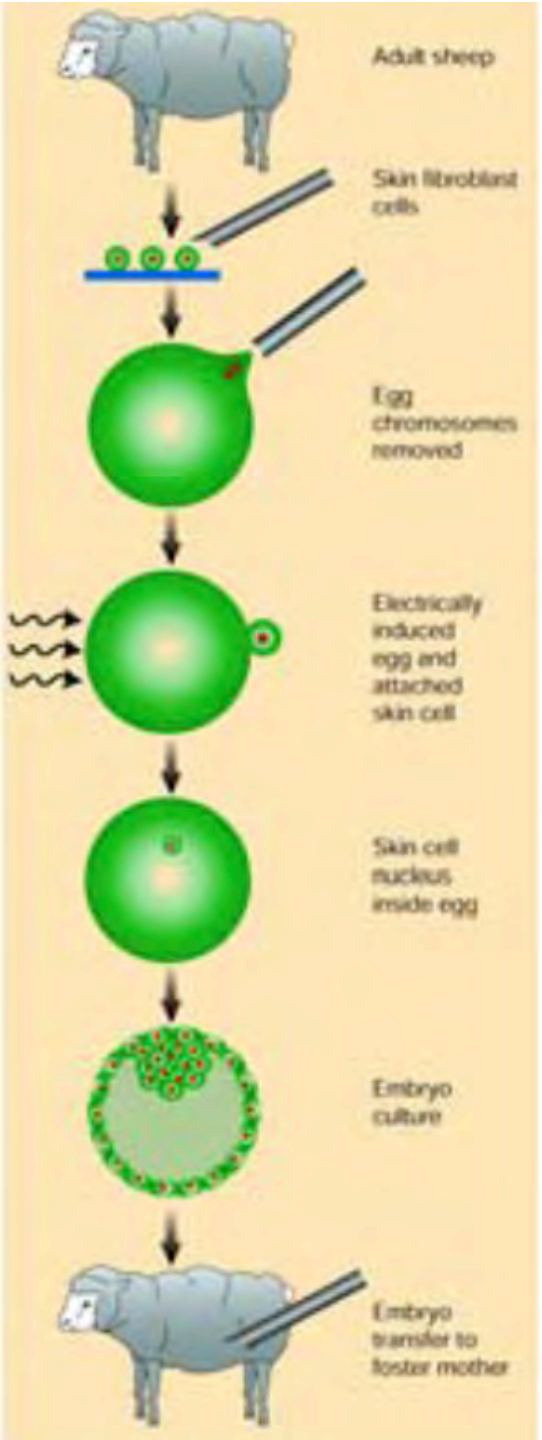
In tutti tre i casi, le cellule donatrici erano state indotte ad entrare uno stato di quiescenza replicativa (G_0) mediante riduzione della concentrazione di siero fetale bovino dal 10% a 5% per i 5 giorni precedenti il trasferimento nucleare. L'uscita dal ciclo era stata confermata mediante la ricerca dell'antigene PCNA

Procedura di trasferimento nucleare:
Oociti ovulati dopo 28-33 ore di trattamento con con GnRH
Enucleati mediante aspirazione
Nuclear transfer mediante brevi scariche elettriche

Risultati (nuclei da cellule ghiandola mammaria):

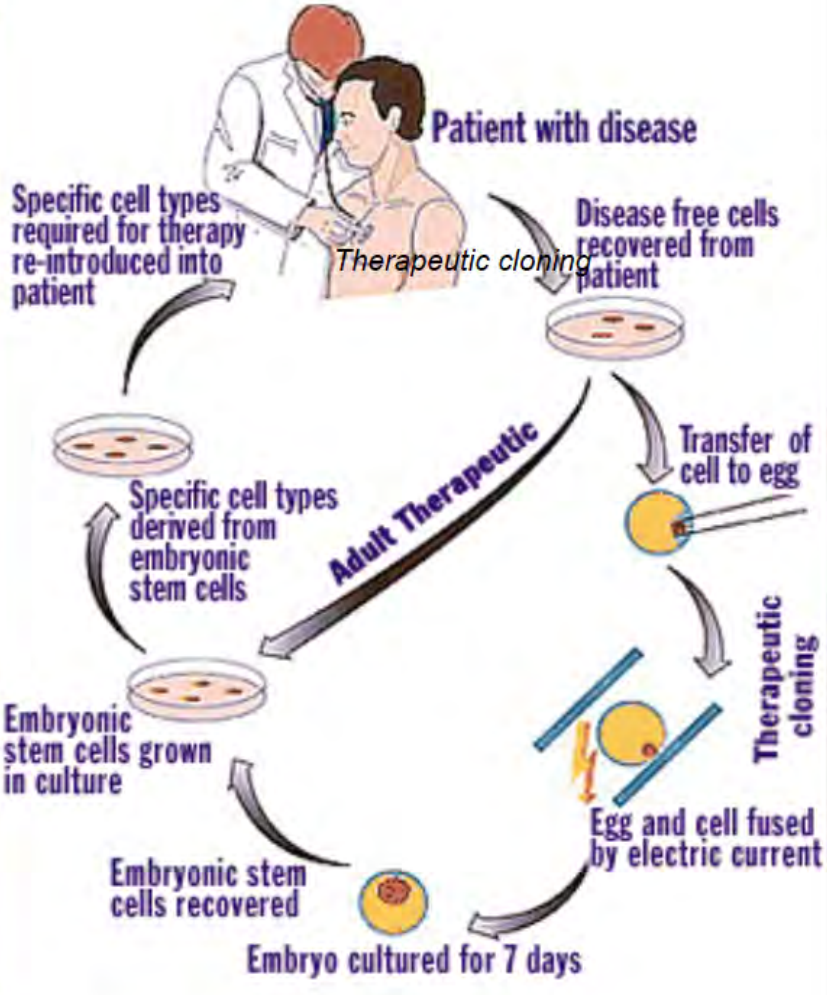
247 oociti ricostruiti coltivati all'interno delle ovidotti ligati di una pecora
29 (11.7%) progrediti allo stadio di morula/blastocisti dopo 6 giorni di coltura, e trasferiti in 13 pecore sincronizzate riceventi per lo sviluppo a termine.
1 embrione (0.4% del totale; 3.4% degli embrioni trasferiti) sviluppato allo stadio di feto; dopo 148 giorni nata una pecora dello stesso fenotipo e genotipo del nucleo donatore (Dolly).

Dolly e' il primo mammifero sviluppatosi a partire da un tessuto adulto.



Applications of cloning

- Treatment of human infertility NO!
- Transgenic animals for drug production
- Genetic rescue of endangered mammals
- Animal organs for human xenotransplantation
- Therapeutic cloning for human stem cell production for tissue and organ regeneration
- Rescue of genetic defect by ex vivo gene therapy



Please Don't Call It Cloning!

Bert Vogelstein et al., Science 2002

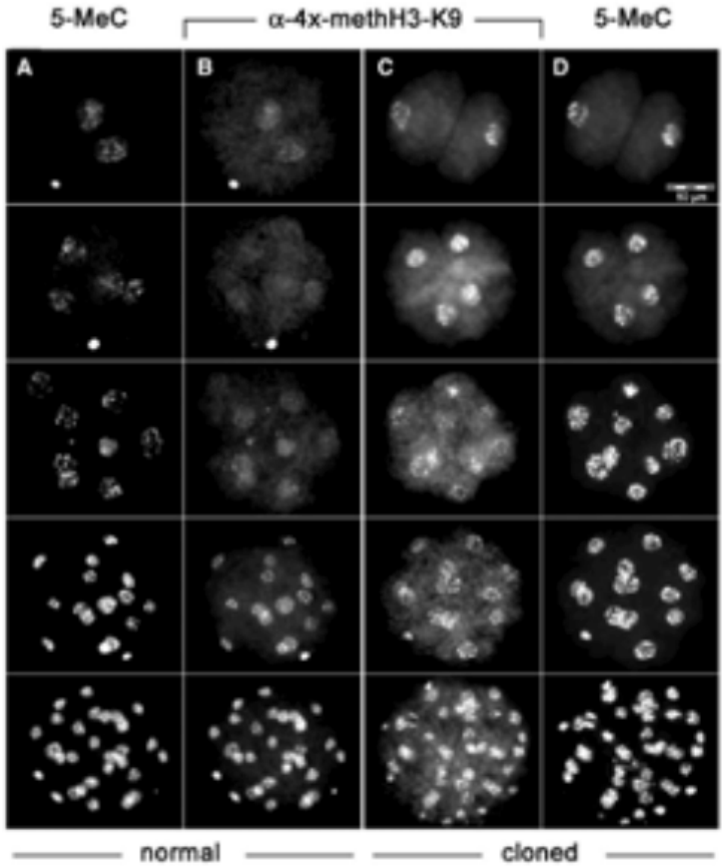
THE CRUCIAL DIFFERENCES

	Nuclear transplantation	Human reproductive cloning
End product	Cells growing in a petri dish	Human being
Purpose	To treat a specific disease of tissue degeneration	Replace or duplicate a human
Time frame	A few weeks (growth in culture)	9 months
Surrogate mother needed	No	Yes
Sentient human created	No	Yes
Ethical implications	Similar to all embryonic cell research	Highly complex issues
Medical implications	Similar to any cell-based therapy	Safety and long-term efficacy concerns

The EMBO Journal Vol. 21 No. 5 pp. 1092–1100, 2002

Limited demethylation leaves mosaic-type methylation states in cloned bovine pre-implantation embryos

Yong-Kook Kang, Jung Sun Park, Deog-Bon Koo, Young-Hee Choi, Sun-Uk Kim, Kyung-Kwang Lee and Yong-Mahn Han[1]



› Nat Genet. 2001 Jun;28(2):173-7. doi: 10.1038/88903.

Aberrant methylation of donor genome in cloned bovine embryos

Y K Kang [1], D B Koo, J S Park, Y H Choi, A S Chung, K K Lee, Y M Han

Affiliations + expand

PMID: 11381267 DOI: 10.1038/88903

Brief Communication | Published: February 2008

Aberrant DNA methylation in cloned ovine embryos

Liu Lei, Hou Jian, Lei TingHua, Bai JiaHua, Guan Hong & An XiaoRong

Conservation of methylation reprogramming in mammalian development: Aberrant reprogramming in cloned embryos

Wendy Dean*, Fátima Santos*, Miodrag Stojkovic†, Valeri Zakhartchenko†, Jörn Walter‡§, Eckhard Wolf†, and Wolf Reik*¶

*Laboratory of Developmental Genetics and Imprinting, Developmental Genetics Program, Babraham Institute, Cambridge CB2 4AT, United Kingdom; †Institute of Molecular Animal Breeding, Gene Centre, Ludwig-Maximilian University, Munich, Germany; ‡Max-Planck-Institut für Molekulare Genetik, Ihnestrasse 73, 14195 Berlin, Germany; and §Universität des Saarlandes, Genetik, 66041 Saarbrücken, Germany

13734–13738 | PNAS | November 20, 2001 | vol. 98 | no. 24

cloned embryos. Cloned, but not normal, morulae had highly methylated nuclei in all blastomeres that resembled those of the fibroblast donor cells. Our study shows that epigenetic reprogramming occurs aberrantly in most cloned embryos; incomplete reprogramming may contribute to the low efficiency of cloning.

Methylation reprogramming, cloning and imprinting

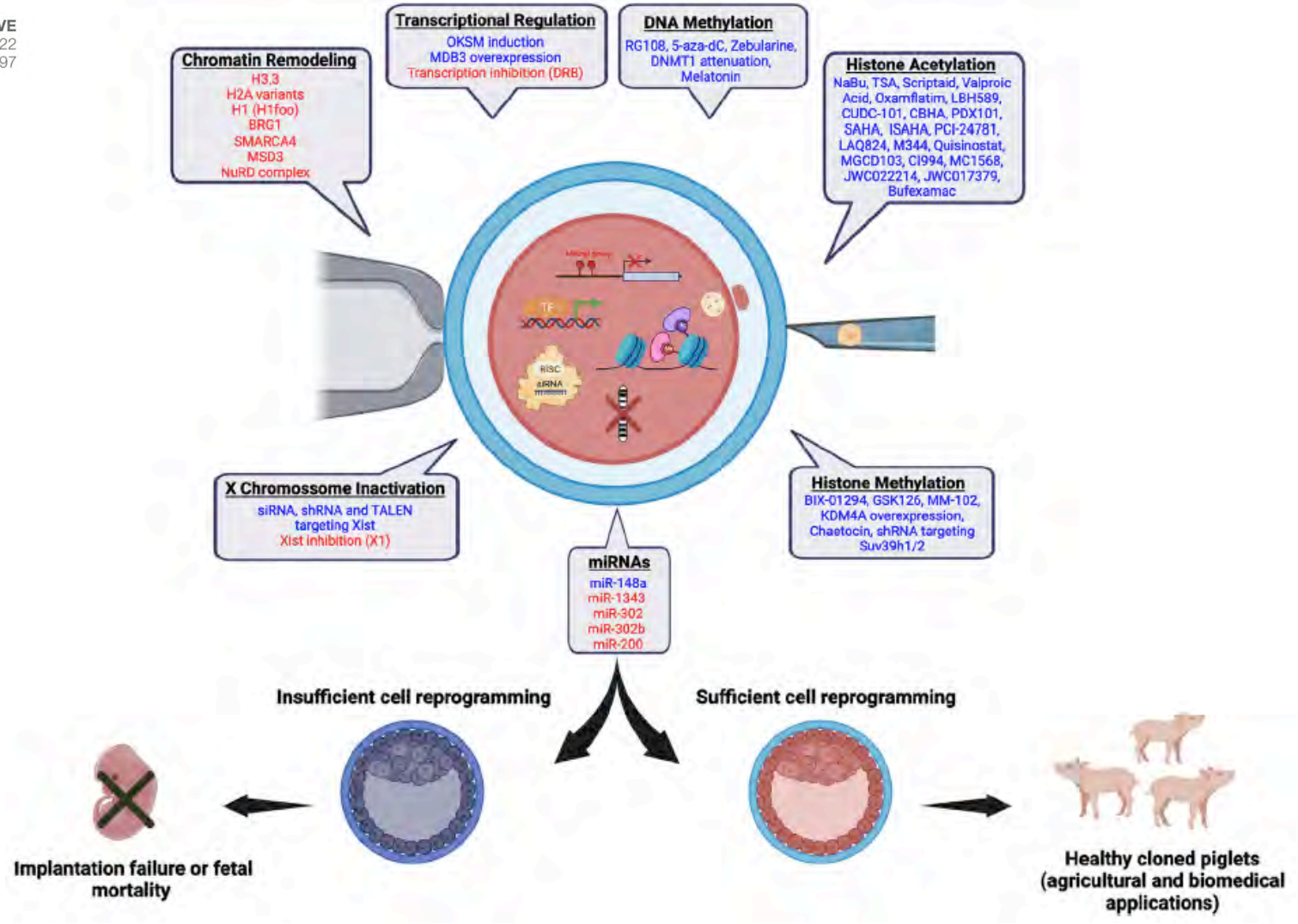
- In the mammalian embryos there are two major cycles of epigenetic reprogramming of the genome: during **pre-implantation** development and during **germ-cell** development

- Reprogramming is deficient in most **cloned** preimplantation embryos; in particular, demethylation seems to be inefficient, perhaps because the somatic nuclei contain the somatic form of Dnmt1 which, unlike the oocyte form, is capable of maintaining methylation levels
- Most cloned embryos die at preimplantation or various postimplantation stages, and even those that develop to term often have specific abnormalities, particularly of the placenta

Enhancement of Chromatin and Epigenetic Reprogramming in Porcine SCNT Embryos—Progresses and Perspectives

*Werner Giehl Glanzner, Mariana Priotto de Macedo, Karina Gutierrez and Vilceu Bordignon **

PERSPECTIVE
published: 11 July 2022
doi: 10.3389/fcell.2022.940197



“Patient tailored therapy”

5740–5741 | PNAS | April 9, 2013 | vol. 110 | no. 15

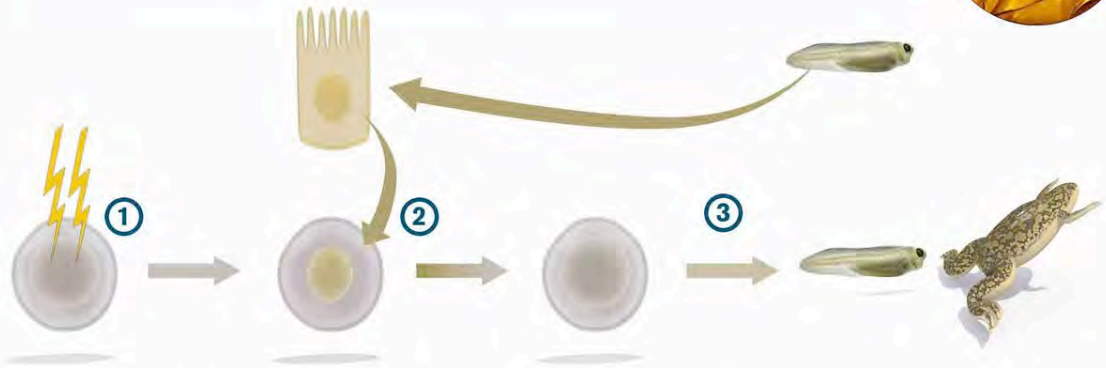
Profile of John Gurdon and Shinya Yamanaka, 2012 Nobel Laureates in Medicine or Physiology

In 1962, by inserting the nuclei of intestinal epithelial cells into enucleated eggs, Gurdon was able to create healthy swimming tadpoles. These experiments were the first successful instances of somatic cell nuclear transfer (SCNT) using genetically normal cells.

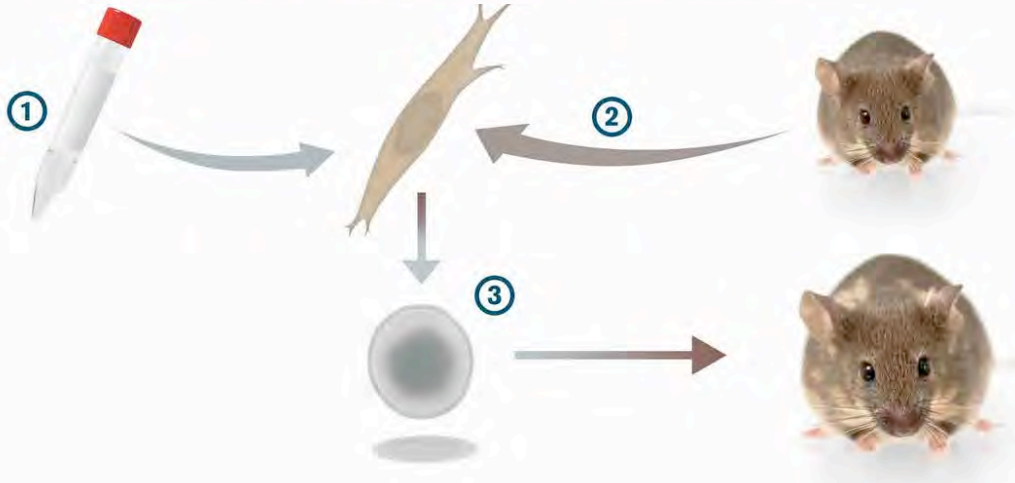
In 2006, Yamanaka with four defined transcription factors induced intact mouse somatic cells to revert to a pluripotent state without an egg or embryo as intermediary.

The Nobel Prize in Physiology or Medicine 2012

John B. Gurdon



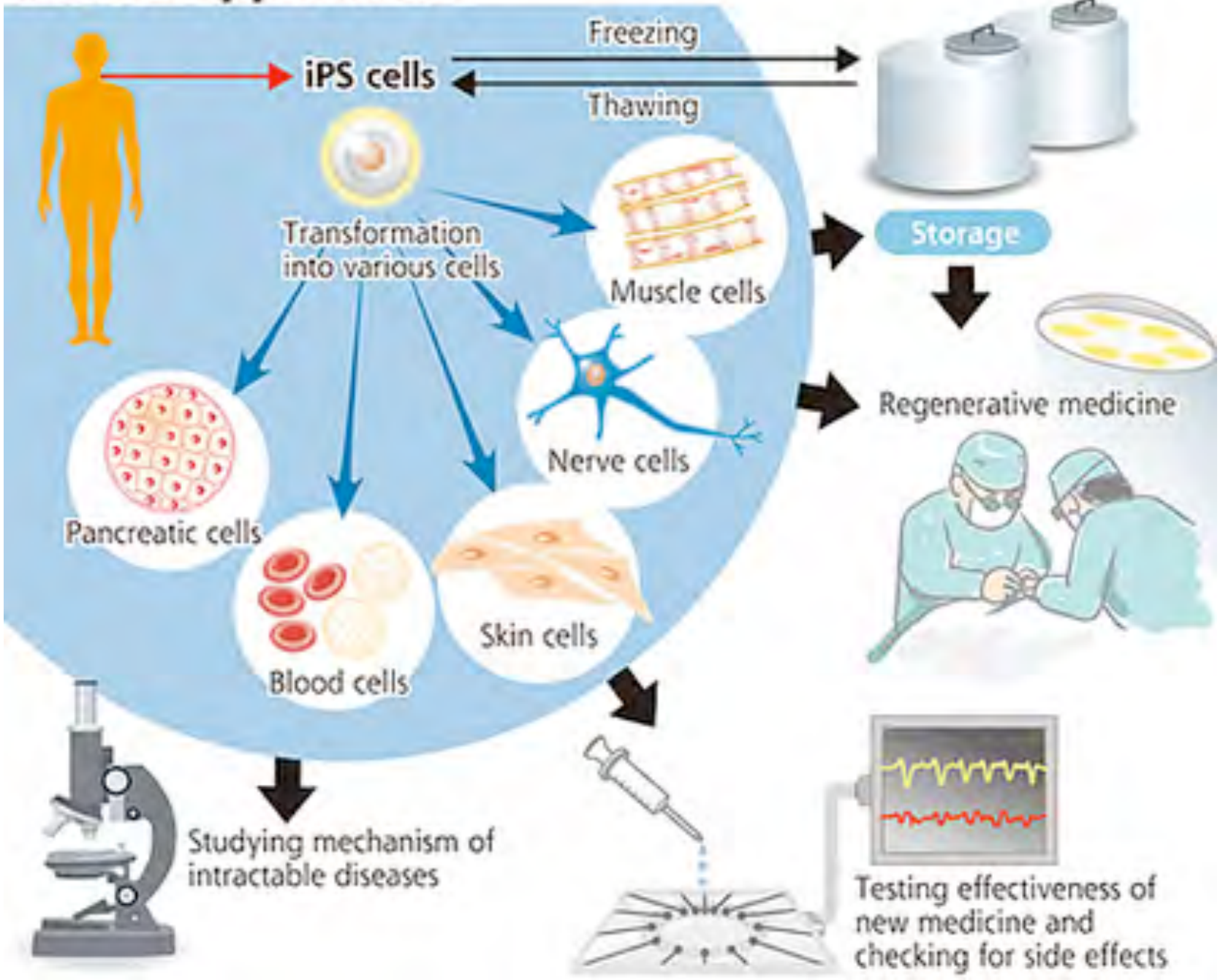
John B. Gurdon eliminated the nucleus of a frog egg cell (1) and replaced it with the nucleus from a specialised cell taken from a tadpole (2). The modified egg developed into a normal tadpole (3). Subsequent nuclear transfer experiments have generated cloned mammals (4).



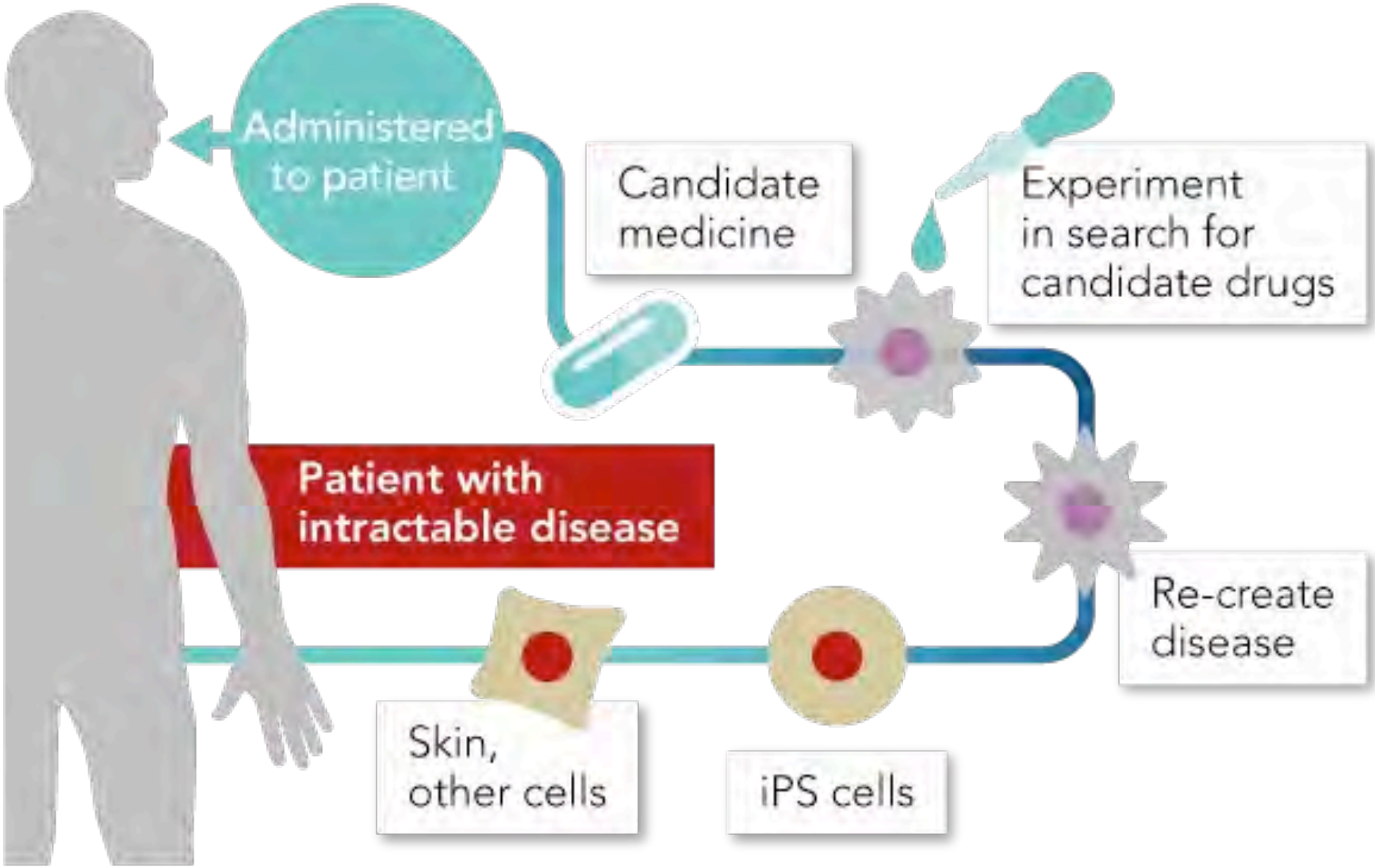
Shinya Yamanaka

Shinya Yamanaka studied genes that are important for stem cell function. When he transferred four such genes (1) into cells taken from the skin (2), they were reprogrammed into pluripotent stem cells (3) that could develop into all cell types of an adult mouse. He named these cells induced pluripotent stem (iPS) cells.

iPS cell applications



How iPS cells can help develop new drugs



Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors

Kazutoshi Takahashi[1] and Shinya Yamanaka[1,2,*]
[1]Department of Stem Cell Biology, Institute for Frontier Medical Sciences, Kyoto University, Kyoto 606-8507, Japan
[2]CREST, Japan Science and Technology Agency, Kawaguchi 332-0012, Japan
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DOI 10.1016/j.cell.2006.07.024

Cell 126, 663-676, August 25, 2006

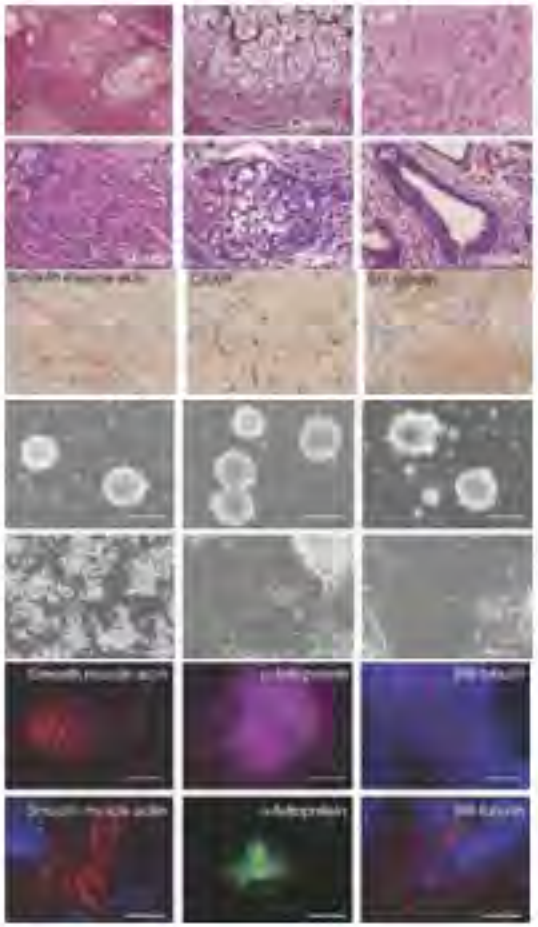
Induction of pluripotent stem cells from mouse embryonic or adult fibroblasts by introducing four factors, **Oct3/4, Sox2, c-Myc**, and **Klf4** in the FBX15 locus, under ES cell culture conditions.

Various tissues present in teratomas derived from iPS

Neural tissues and muscles in teratomas

In vitro embryoid body formation and differentiation

In vitro differentiation into all three germ layers.



These cells, which were designated iPS (induced pluripotent stem) cells, exhibit the morphology and growth properties of ES cells and express ES cell marker genes.

1- Subcutaneous transplantation of iPS cells into nude mice resulted in tumors containing a variety of tissues from all three germ layers.

2- Following injection into blastocysts, iPS cells contributed to mouse embryonic development, but embryos failed to develop beyond mid-gestation stage.

Differentiation

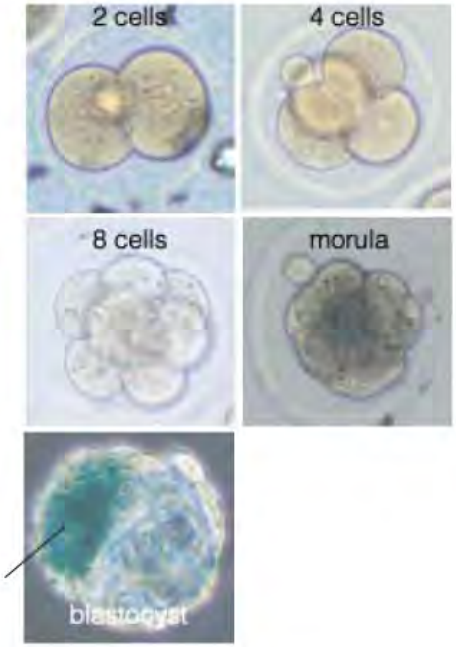
Zygote

Blastocyst

Gastrula

Zygote *totipotent*

Embryonic stem cells
pluripotent



Adult stem cells
multi- o uni-potent

Ectoderm (external layer)
Skin cells of epidermis
Neuron of brain
Pigment cell

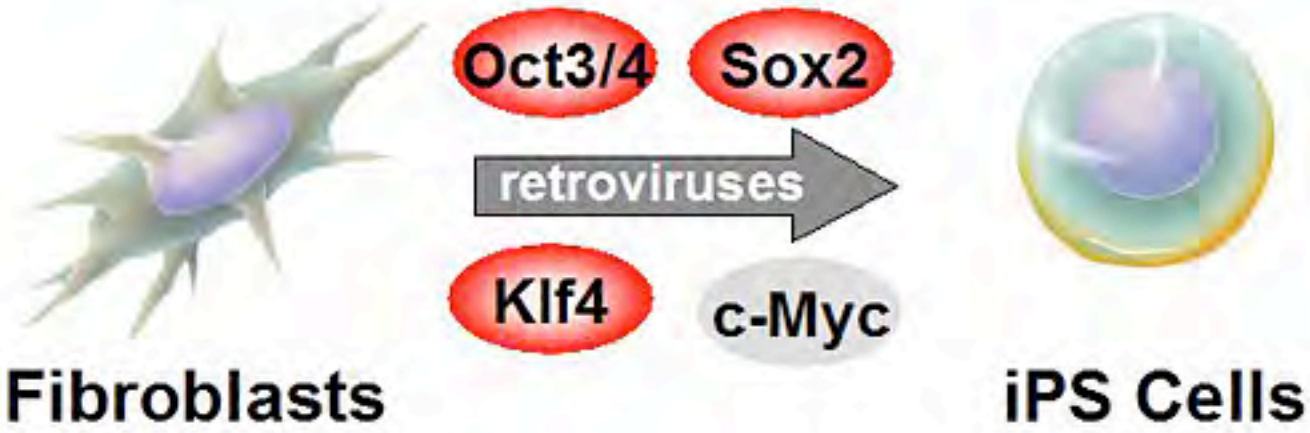
Mesoderm (middle layer)
Cardiac muscle
Skeletal muscle cells
Tubule cell of the kidney
Red blood cells
Smooth muscle (in gut)

Endoderm (internal layer)
Pancreatic cell
Thyroid cell
Lung cell (alveolar cell)

Germ cells
Sperm
Egg

Hematooietic stem cells,
neural stem cells,
epidermal stem cells,
bone marrow
mesenchymal stem cells,
amniotic stem cells, etc.,

Induced Pluripotent Stem (iPS) Cells



Mouse iPS cells reported in 2006

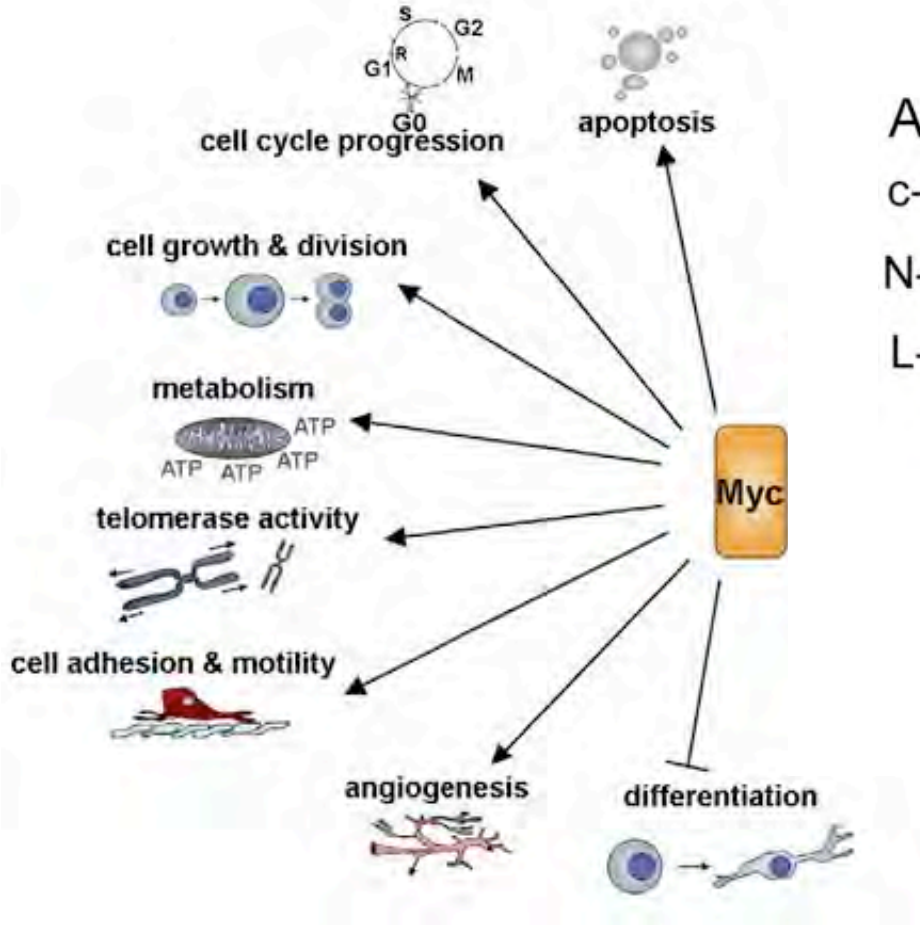
Human iPS cells reported in 2007

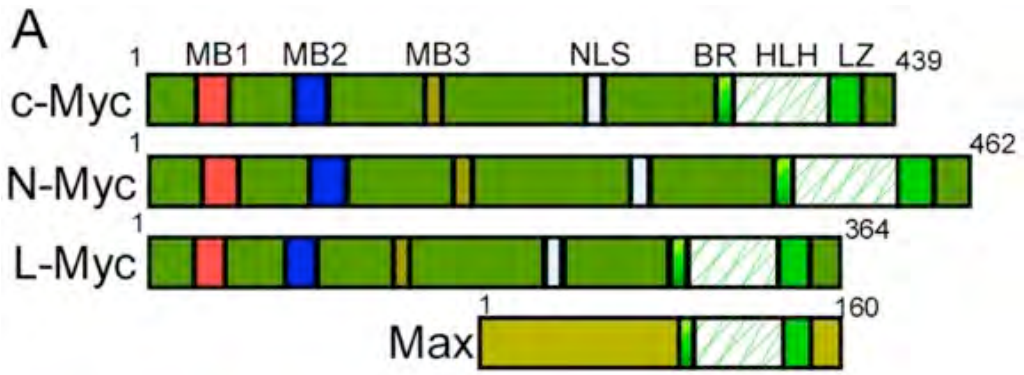
Table 2

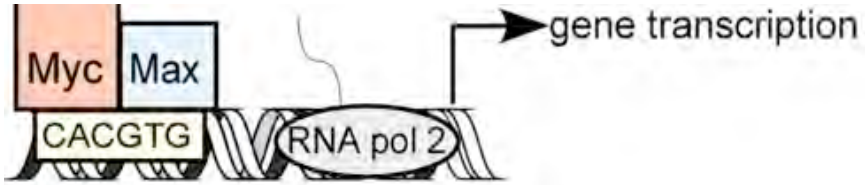
Minimum number of factors required for iPS cell generation

Transgene	Known functions in maintenance of pluripotency
Oct3/4	Oct3/4 is a tightly regulated transcription factor that is associated with a large number of target genes implicated in maintenance of pluripotency. Regulatory elements in target genes are often in close vicinity of Sox2-binding sites. Oct3/4 is likely to be a key factor in the transcriptional framework of self-renewing stem cells.
Sox2	The transcription factor Sox2 is necessary for embryonal development and to prevent ES cell differentiation. Although many ES cell pluripotency-associated genes are co-regulated by Sox2 and Oct3/4, Sox2 may also cooperate with other transcription factors, for example Nanog, to activate transcription of pluripotency markers.
c-Myc	c-Myc, a helix-loop-helix/leucine zipper transcription factor, takes part in a broad variety of cellular functions. It has been implicated in LIF receptor signalling as a downstream effector of STAT3. In Wnt signalling c-Myc is a substrate for GSK3β. In iPS cells, c-Myc may compensate anti-proliferative effects of Klf4.
Klf4	Klf4, the fourth member of the quartet, is a Krueppel-type zinc finger transciption factor. It can act as an oncogene but also as a tumor suppressor protein. Klf4 is like c-Myc a STAT3 target in the LIF pathway and its overexpression inhibits differentiation of ES cells. Klf4 upregulates, in concert with Oct3/4, Lefty1 transcription but the role as co-factor for Oct3/4 may be limited to only a few targets. Klf4 can repress p53, a negative regulator of Nanog.

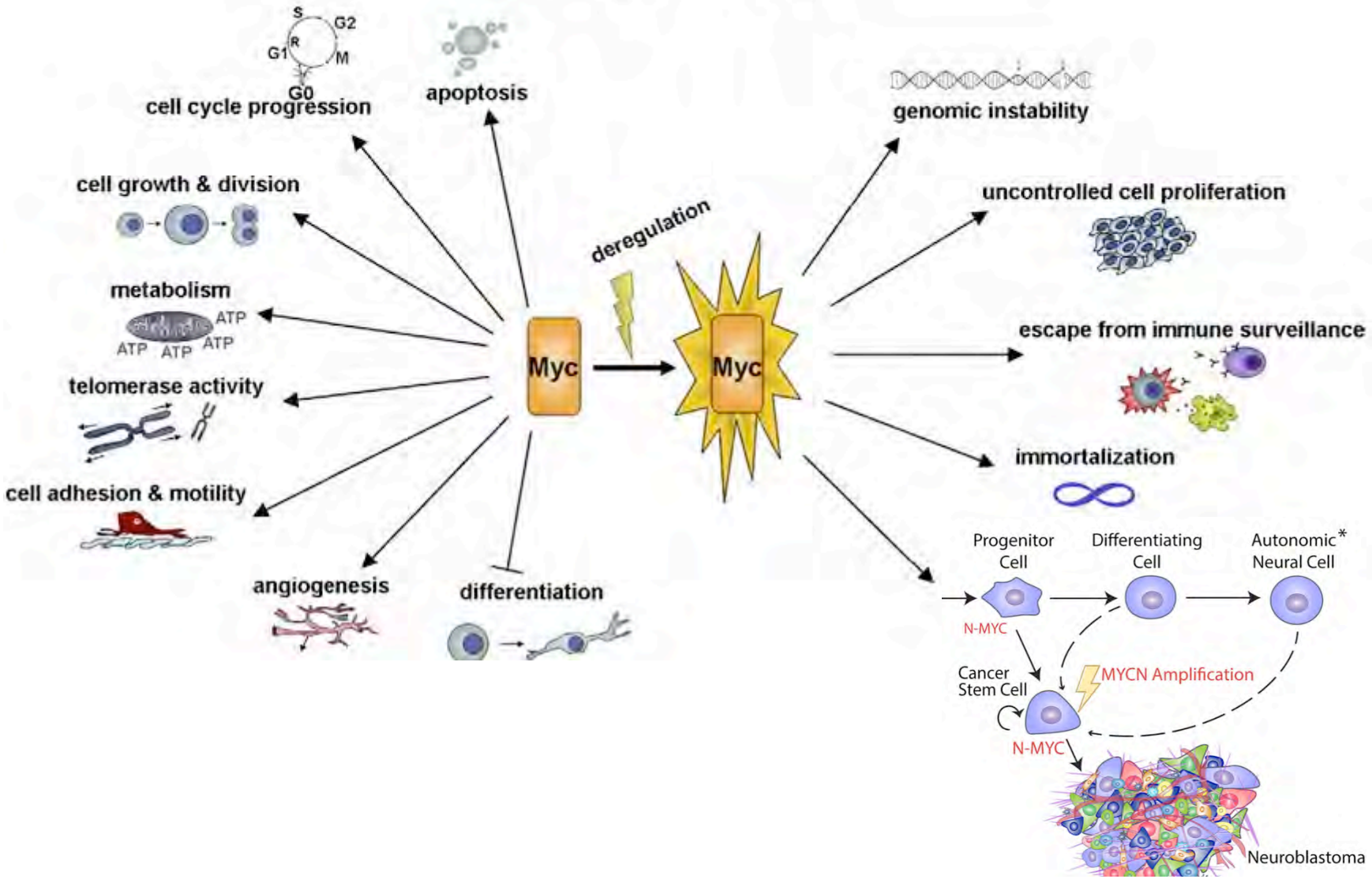
The Myc Transcription Factor







The Myc Transcription Factor



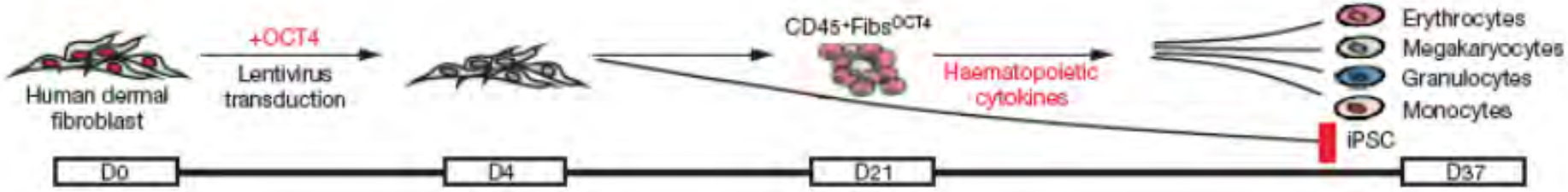
Transdifferentiation: the direct reprogramming of one somatic cell type into another without a stem cell intermediate

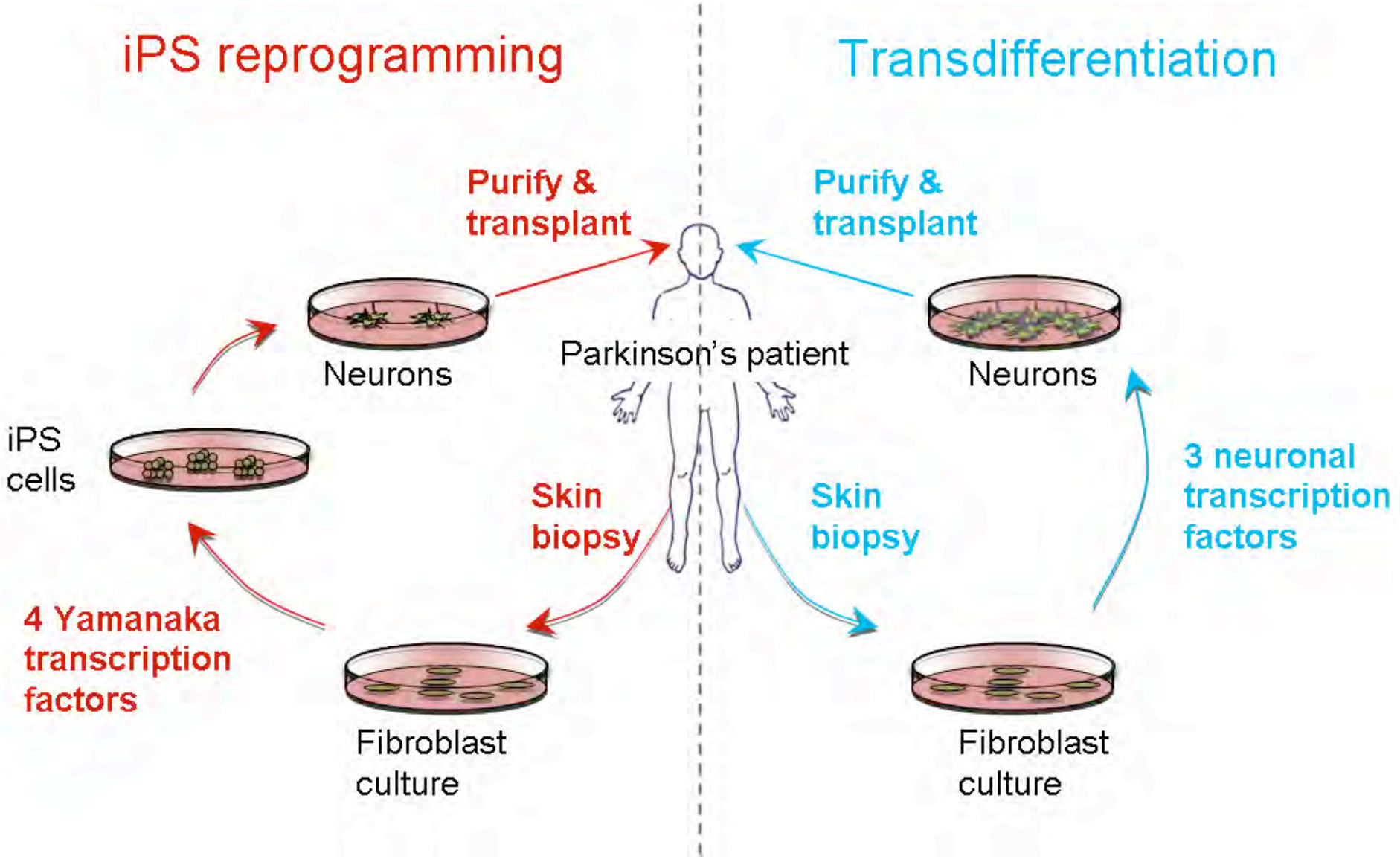
ARTICLE

doi:10.1038/nature09591

Direct conversion of human fibroblasts to multilineage blood progenitors

Eva Szabo[1], Shravanti Rampalli[1], Ruth M. Risueño[1], Angelique Schnerch[1,2], Ryan Mitchell[1,2], Aline Fiebig-Comyn[1], Marilyne Levadoux-Martin[1] & Mickie Bhatia[1,2]

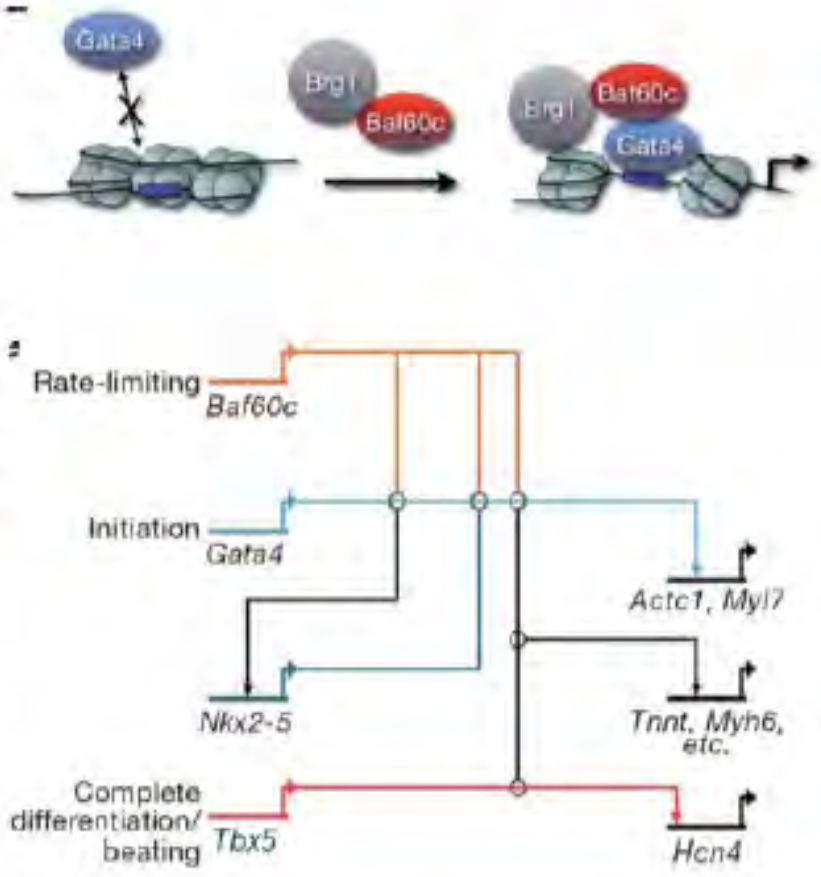



iPS reprogramming
Transdifferentiation
Purify & transplant
Purify & transplant
Neurons
Parkinson's patient
Neurons
iPS cells
Skin biopsy
Skin biopsy
3 neuronal transcription factors
4 Yamanaka transcription factors
Fibroblast culture
Fibroblast culture

Directed transdifferentiation of mouse mesoderm to heart tissue by defined factors

Jun K. Takeuchi[1,2] & Benoit G. Bruneau[1,3]

Heart disease is the leading cause of mortality and morbidity in the western world. The heart has little regenerative capacity after damage, leading to much interest in understanding the factors required to produce new cardiac myocytes. Despite a robust understanding of the molecular networks regulating cardiac differentiation[1,2], no single transcription factor or combination of factors has been shown to activate the cardiac gene program *de novo* in mammalian cells or tissues. Here we define the minimal requirements for transdifferentiation of mouse mesoderm to cardiac myocytes. We show that two cardiac transcription factors, Gata4 and Tbx5, and a cardiac-specific subunit of BAF chromatin-remodelling complexes, Baf60c (also called Smarcd3), can direct ectopic differentiation of mouse mesoderm into beating cardiomyocytes, including the normally non-cardiogenic posterior mesoderm and the extraembryonic mesoderm of the amnion. Gata4 with Baf60c initiated ectopic cardiac gene expression. Addition of Tbx5 allowed differentiation into contracting cardiomyocytes and repression of non-cardiac mesodermal genes. Baf60c was essential for the ectopic cardiogenic activity of Gata4 and Tbx5, partly by permitting binding of Gata4 to cardiac genes, indicating a novel instructive role for BAF complexes in tissue-specific regulation. The combined function of these factors establishes a robust mechanism for controlling cellular differentiation, and may allow reprogramming of new cardiomyocytes for regenerative purposes.



NATURE | Vol 459 | 4 June 2009

Cell *142*, 375–386, August 6, 2010 ©2010 Elsevier Inc. 375

Direct Reprogramming of Fibroblasts into Functional Cardiomyocytes by Defined Factors

Masaki Ieda,[1,2,3,6,*] Ji-Dong Fu,[1,2,3] Paul Delgado-Olguin,[1,2,4] Vasanth Vedantham,[1,5] Yohei Hayashi,[1] Benoit G. Bruneau,[1,2,4] and Deepak Srivastava[1,2,3,*]
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DOI 10.1016/j.cell.2010.07.002

ARTICLE

31 MAY 2012 | VOL 485 | NATURE | 599

doi:10.1038/nature11139

Heart repair by reprogramming non-myocytes with cardiac transcription factors

Kunhua Song[1], Young-Jae Nam[1,2], Xiang Luo[3], Xiaoxia Qi[1], Wei Tan[3], Guo N. Huang[3], Asha Acharya[1], Christopher L. Smith[1], Michelle D. Tallquist[1], Eric G. Neilson[3], Joseph A. Hill[1,2], Rhonda Bassel-Duby[1] & Eric N. Olson[1]

The adult mammalian heart possesses little regenerative potential following injury. Fibrosis due to activation of cardiac fibroblasts impedes cardiac regeneration and contributes to loss of contractile function, pathological remodelling and susceptibility to arrhythmias. Cardiac fibroblasts account for a majority of cells in the heart and represent a potential cellular source for restoration of cardiac function following injury through phenotypic reprogramming to a myocardial cell fate. Here we show that four transcription factors, GATA4, HAND2, MEF2C and TBX5, can cooperatively reprogram adult mouse tail-tip and cardiac fibroblasts into beating cardiac-like myocytes *in vitro*. Forced expression of these factors in dividing non-cardiomyocytes in mice reprograms these cells into functional cardiac-like myocytes, improves cardiac function and reduces adverse ventricular remodelling following myocardial infarction. Our results suggest a strategy for cardiac repair through reprogramming fibroblasts resident in the heart with cardiogenic transcription factors or other molecules.

ARTICLE

doi:10.1038/nature11044

In vivo reprogramming of murine cardiac fibroblasts into induced cardiomyocytes

Li Qian[1,2,3], Yu Huang[1,2,3], C. Ian Spencer[1,2,3], Amy Foley[1,2,3], Vasanth Vedantham[1,4,5], Lei Liu[1,2,3], Simon J. Conway[6], Ji-dong Fu[1,2,3] & Deepak Srivastava[1,2,3]

The reprogramming of adult cells into pluripotent cells or directly into alternative adult cell types holds great promise for regenerative medicine. We reported previously that cardiac fibroblasts, which represent 50% of the cells in the mammalian heart, can be directly reprogrammed to adult cardiomyocyte-like cells *in vitro* by the addition of Gata4, Mef2c and Tbx5 (GMT). Here we use genetic lineage tracing to show that resident non-myocytes in the murine heart can be reprogrammed into cardiomyocyte-like cells *in vivo* by local delivery of GMT after coronary ligation. Induced cardiomyocytes became binucleate, assembled sarcomeres and had cardiomyocyte-like gene expression. Analysis of single cells revealed ventricular cardiomyocyte-like action potentials, beating upon electrical stimulation, and evidence of electrical coupling. *In vivo* delivery of GMT decreased infarct size and modestly attenuated cardiac dysfunction up to 3 months after coronary ligation. Delivery of the pro-angiogenic and fibroblast-activating peptide, thymosin β4, along with GMT, resulted in further improvements in scar area and cardiac function. These findings demonstrate that cardiac fibroblasts can be reprogrammed into cardiomyocyte-like cells in their native environment for potential regenerative purposes.

Circulation Research
JOURNAL OF THE AMERICAN HEART ASSOCIATION

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MicroRNA-Mediated In Vitro and In Vivo Direct Reprogramming of Cardiac Fibroblasts to Cardiomyocytes

Tilanthi M. Jayawardena, Bakytbek Egemnazarov, Elizabeth A. Finch, Lunan Zhang, J. Alan Payne, Kumar Pandya, Zhiping Zhang, Paul Rosenberg, Maria Mirotsou and Victor J. Dzau

Rationale: Repopulation of the injured heart with new, functional cardiomyocytes remains a daunting challenge for cardiac regenerative medicine. An ideal therapeutic approach would involve an effective method at achieving direct conversion of injured areas to functional tissue in situ.
Objective: The aim of this study was to develop a strategy that identified and evaluated the potential of specific micro (mi)RNAs capable of inducing reprogramming of cardiac fibroblasts directly to cardiomyocytes in vitro and in vivo.
Methods and Results: Using a combinatorial strategy, we identified a combination of miRNAs 1, 133, 208, and 499 capable of inducing direct cellular reprogramming of fibroblasts to cardiomyocyte-like cells in vitro. Detailed studies of the reprogrammed cells demonstrated that a single transient transfection of the miRNAs can direct a switch in cell fate as documented by expression of mature cardiomyocyte markers, sarcomeric organization, and exhibition of spontaneous calcium flux characteristic of a cardiomyocyte-like phenotype. Interestingly, we also found that miRNA-mediated reprogramming was enhanced 10-fold on JAK inhibitor I treatment. Importantly, administration of miRNAs into ischemic mouse myocardium resulted in evidence of direct conversion of cardiac fibroblasts to cardiomyocytes in situ. Genetic tracing analysis using Fsp1Cre-traced fibroblasts from both cardiac and noncardiac cell sources strongly suggests that induced cells are most likely of fibroblastic origin.
Conclusions: The findings from this study provide proof-of-concept that miRNAs have the capability of directly converting fibroblasts to a cardiomyocyte-like phenotype in vitro. Also of significance is that this is the first report of direct cardiac reprogramming in vivo. Our approach may have broad and important implications for therapeutic tissue regeneration in general. (*Circ Res.* 2012;110:00-00.)

Key Words: direct reprogramming ■ cardiac differentiation ■ microRNAs ■ tissue regeneration

Direct conversion of human fibroblasts to dopaminergic neurons

Ulrich Pfisterer[1], Agnete Kirkeby[1], Olof Torper[1], James Wood, Jenny Nelander, Audrey Dufour, Anders Björklund, Olle Lindvall, Johan Jakobsson, and Malin Parmar[2]

Departments of Experimental Medical Science and Clinical Sciences, Wallenberg Neuroscience Center, and Lund Stem Cell Center, Lund University, SE-; Lund, Sweden

Edited* by Fred H. Gage, The Salk Institute, San Diego, CA, and approved May 13, 2011 (received for review March 31, 2011)

PNAS | June 21, 2011 | vol. 108 | no. 25 | 10343–10348

Recent reports demonstrate that somatic mouse cells can be directly converted to other mature cell types by using combined expression of defined factors. Here we show that the same strategy can be applied to human embryonic and postnatal fibroblasts. By overexpression of the transcription factors Ascl1, Brn2, and Myt1l, human fibroblasts were efficiently converted to functional neurons. We also demonstrate that the converted neurons can be directed toward distinct functional neurotransmitter phenotypes when the appropriate transcriptional cues are provided together with the three conversion factors. By combining expression of the three conversion factors with expression of two genes involved in dopamine neuron generation, *Lmx1a* and *FoxA2*, we could direct the phenotype of the converted cells toward dopaminergic neurons. Such subtype-specific induced neurons derived from human somatic cells could be valuable for disease modeling and cell replacement therapy.

Direct reprogramming of mouse fibroblasts to neural progenitors

Janghwan Kim[a,b], Jem A. Efe[a], Saiyong Zhu[a], Maria Talantova[c], Xu Yuan[a], Shufen Wang[d,e], Stuart A. Lipton[c], Kang Zhang[d,e], and Sheng Ding[a,f,1]

[a]Department of Chemistry, The Scripps Research Institute, La Jolla, CA 92037; [b]Development and Differentiation Research Center, Korea Research Institute of Bioscience and Biotechnology, Yuseong-gu, Daejeon, 305-806, Republic of Korea; [c]Del E. Webb Center for Neuroscience, Aging, and Stem Cell Research, Sanford-Burnham Medical Research Institute, La Jolla, CA 92037; [d]Institute for Genomic Medicine and Shiley Eye Center, University of California at San Diego, La Jolla, CA 92093; [e]Molecular Medicine Research Center and Department of Ophthalmology, West China Hospital, Sichuan University, Chengdu 610065, China; and [f]Gladstone Institute of Cardiovascular Disease, Department of Pharmaceutical Chemistry, University of California, San Francisco, CA 94158

PNAS | May 10, 2011 | vol. 108 | no. 19 | 7839

The simple yet powerful technique of induced pluripotency may eventually supply a wide range of differentiated cells for cell therapy and drug development. However, making the appropriate cells via induced pluripotent stem cells (iPSCs) requires reprogramming of somatic cells and subsequent redifferentiation. Given how arduous and lengthy this process can be, we sought to determine whether it might be possible to convert somatic cells into lineage-specific stem/progenitor cells of another germ layer in one step, bypassing the intermediate pluripotent stage. Here we show that transient induction of the four reprogramming factors (Oct4, Sox2, Klf4, and c-Myc) can efficiently transdifferentiate fibroblasts into functional neural stem/progenitor cells (NPCs) with appropriate signaling inputs. Compared with induced neurons (or iN cells, which are directly converted from fibroblasts), transdifferentiated NPCs have the distinct advantage of being expandable in vitro and retaining the ability to give rise to multiple neuronal subtypes and glial cells. Our results provide a unique paradigm for iPSC-factor–based reprogramming by demonstrating that it can be readily modified to serve as a general platform for transdifferentiation.

Direct conversion of fibroblasts to functional neurons by defined factors

Thomas Vierbuchen[1,2], Austin Ostermeier[1,2], Zhiping P. Pang[3], Yuko Kokubu[1], Thomas C. Südhof[3,4] & Marius Wernig[1,2]

Cellular differentiation and lineage commitment are considered to be robust and irreversible processes during development. Recent work has shown that mouse and human fibroblasts can be reprogrammed to a pluripotent state with a combination of four transcription factors. This raised the question of whether transcription factors could directly induce other defined somatic cell fates, and not only an undifferentiated state. We hypothesized that combinatorial expression of neural-lineage-specific transcription factors could directly convert fibroblasts into neurons. Starting from a pool of nineteen candidate genes, we identified a combination of only three factors, *Ascl1*, *Brn2* (also called *Pou3f2*) and *Myt1l*, that suffice to rapidly and efficiently convert mouse embryonic and postnatal fibroblasts into functional neurons *in vitro*. These induced neuronal (iN) cells express multiple neuron-specific proteins, generate action potentials and form functional synapses. Generation of iN cells from non-neural lineages could have important implications for studies of neural development, neurological disease modelling and regenerative medicine.

Vol 463|25 February 2010|doi:10.1038/nature08797

Forcing cells to change lineages

Thomas Graf[1] & Tariq Enver[2]

The ability to produce stem cells by induced pluripotency (iPS reprogramming) has rekindled an interest in earlier studies showing that transcription factors can directly convert specialized cells from one lineage to another. Lineage reprogramming has become a powerful tool to study cell fate choice during differentiation, akin to inducing mutations for the discovery of gene functions. The lessons learnt provide a rubric for how cells may be manipulated for therapeutic purposes.

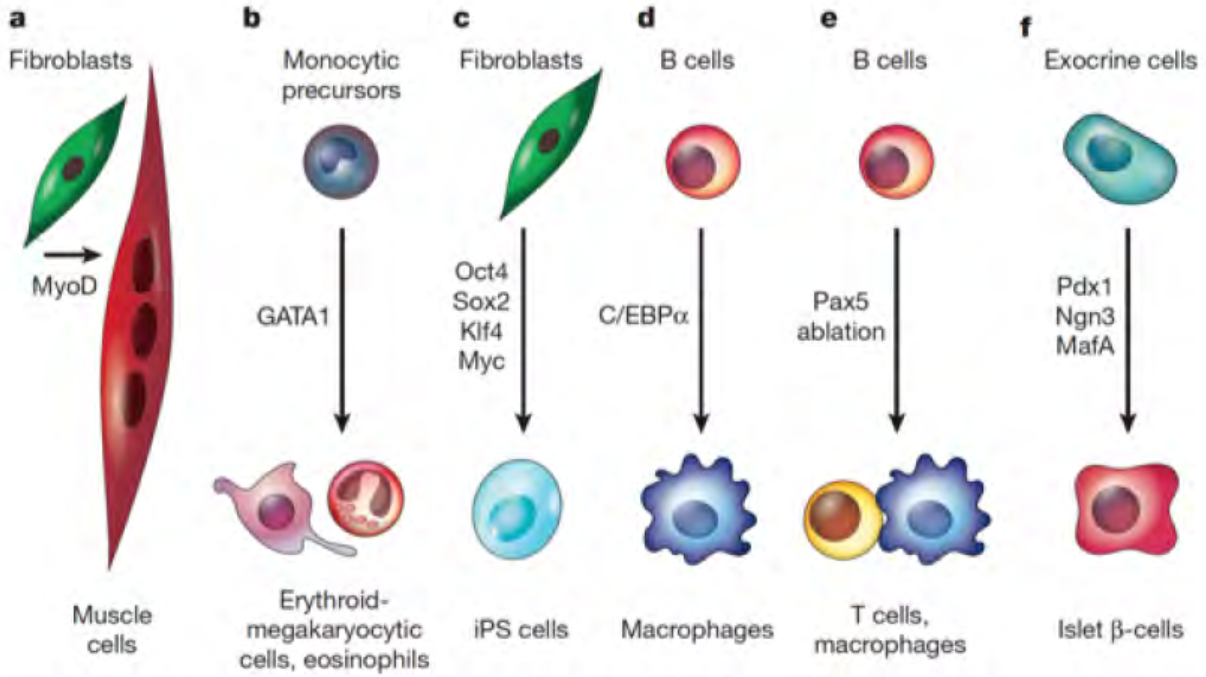

Figure 1 | Examples of transcription factor overexpression or ablation experiments that result in cell fate changes. For explanation of panels **a–f** see text.

[1]Center for Genomic Regulation and ICREA, 08003 Barcelona, Spain. [2]MRC Molecular Haematology Unit, Weatherall Institute of Molecular Medicine, John Radcliffe Hospital, Headington, Oxford OX3 9DS, UK.

Pre-natal epigenetic modifications

- Children born to mothers who were in the early stages of pregnancy during the Dutch famine of 1944–1945 were at significantly increased risk of cardiometabolic disorders in adulthood.
- Relationship between low birth weight and increased risk for type 2 diabetes in a British cohort.
- The “thrifty phenotype” hypothesis (1992), which posits that malnutrition during pregnancy results in structural and functional changes in the developing fetus

Epigenetic understanding of gene-environment interactions in psychiatric disorders: a new concept of clinical genetics

Takeo Kubota*, Kunio Miyake and Takae Hirasawa

Abstract

Epigenetics is a mechanism that regulates gene expression independently of the underlying DNA sequence, relying instead on the chemical modification of DNA and histone proteins. Although environmental and genetic factors were thought to be independently associated with disorders, several recent lines of evidence suggest that epigenetics bridges these two factors. Epigenetic gene regulation is essential for normal development, thus defects in epigenetics cause various rare congenital diseases. Because epigenetics is a reversible system that can be affected by various environmental factors, such as drugs, nutrition, and mental stress, the epigenetic disorders also include common diseases induced by environmental factors. In this review, we discuss the nature of epigenetic disorders, particularly psychiatric disorders, on the basis of recent findings: 1) susceptibility of the conditions to environmental factors, 2) treatment by taking advantage of their reversible nature, and 3) transgenerational inheritance of epigenetic changes, that is, acquired adaptive epigenetic changes that are passed on to offspring. These recently discovered aspects of epigenetics provide a new concept of clinical genetics.

Featured Research

from universities, journals, and other organizations

Listening to classical music modulates genes that are responsible for brain functions

Date: March 13, 2015

Source: Helsingin yliopisto (University of Helsinki)

Summary: Although listening to music is common in all societies, the biological determinants of listening to music are largely unknown. According to a new study, listening to classical music enhanced the activity of genes involved in dopamine secretion and transport, synaptic neurotransmission, learning and memory, and down-regulated the genes mediating neurodegeneration. Several of the up-regulated genes were known to be responsible for song learning and singing in songbirds, suggesting a common evolutionary background of sound perception across species.

Seminars in Cell & Developmental Biology xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Seminars in Cell & Developmental Biology

journal homepage: www.elsevier.com/locate/semcdb

Review

Developmental windows of susceptibility for epigenetic inheritance through the male germline

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ARTICLE INFO

Article history:
Received 15 June 2015
Accepted 20 July 2015
Available online xxx

Keywords:
Sperm
Epigenetics
Paternal effects
Intergenerational inheritance
Male germ cells
Epigenetic reprogramming

ABSTRACT

Exposure of developing male germ cells to environmental insults has been linked to adverse effects in the offspring. One mechanism by which germ cell defects may be passed intergenerationally is through perturbations in the epigenome at the level(s) of DNA methylation, histone post-translational modifications and/or small non-coding RNAs. Epigenetic programs are particularly dynamic in germ cells undergoing erasure, re-establishment and maintenance of patterns, events potentially susceptible to prenatal and/or postnatal exposures. In this review, we focus on the epigenetic events occurring at each phase of male germ cell development including the prenatal period covering primordial germ cells and prospermatogonia and the postnatal period covering mitotic spermatogonia, meiotic spermatocytes and post-meiotic haploid spermatids and spermatozoa. Strong barriers to the passage of abnormal epigenetic patterns between generations are erected at two times of genome-wide epigenomic reprogramming, first in the germline in primordial germ cells and second, post-fertilization, during preimplantation development. Evidence from high resolution profiling studies that not all epigenetic marks are erased during germ cell and embryonic reprogramming provides a potential explanation for the intergenerational inheritance of abnormal epigenetic marks that may affect offspring health.

THE *SINS* OF THE FATHER

The roots of inheritance may extend beyond the genome, but the mechanisms remain a puzzle.

- In Svezia, i nipoti di uomini che avevano sperimentato la fame prima della pubertà avevano meno probabilità di sviluppare malattie cardiache di quelli dei nonni cresciuti in condizioni di benessere.
- In Gran Bretagna, i padri che avevano iniziato a fumare prima degli 11 anni avevano figli con peso superiore alla norma.
- In Cambogia, gli individui che erano stati traumatizzati durante il genocidio dei Khmer Rossi avevano figli con depressione e ansia.
- In Australia, i figli dei veterani della guerra del Vietnam diventavano più frequentemente suicidi.

EPIGENETICS

Sperm RNA carries marks of trauma

Stress alters the expression of small RNAs in male mice and leads to depressive behaviours in later generations.

BY VIRGINIA HUGHES

Trauma is insidious. It not only increases a person's risk for psychiatric disorders, but can also spill over into the next generation. People who were traumatized during the Khmer Rouge genocide in Cambodia tended to have children with depression and anxiety, for example, and children of Australian veterans of the Vietnam War have higher rates of suicide than the general population.

Trauma's impact comes partly from social factors, such as its influence on how parents interact with their children. But stress also leaves 'epigenetic marks' — chemical changes that affect how DNA is expressed without altering its sequence. A study published this week in *Nature Neuroscience* finds that stress in early life alters the production of small RNAs, called microRNAs, in the sperm of mice (K. Gapp *et al. Nature Neurosci.* http://dx.doi.org/10.1038/nn.3695; 2014). The mice show depressive behaviours that persist in their progeny, which also show glitches in metabolism.

The study is notable for showing that sperm responds to the environment, says Stephen Krawetz, a geneticist at Wayne State University School of Medicine in Detroit, Michigan, who studies microRNAs in human sperm. (He was not involved in the latest study.) "Dad is having a much larger role in the whole process, rather than just delivering his genome and being done with it," he says. He adds that this is one of a growing number of studies to show that subtle changes in sperm microRNAs "set the stage for a huge plethora of other effects".

In the new study, Isabelle Mansuy, a neuroscientist at the University of Zurich, Switzerland, and her colleagues periodically separated mother mice from their young pups and exposed the mothers to stressful situations — either by placing them in cold water or physically restraining them. These separations

- Gli spermatozoi dei topi sottoposti a stress trasmettono la propensione allo stress fino ad almeno la terza generazione.
- Gli spermatozoi di topi nutriti con una dieta ricca di grassi generano figli con tendenza al diabete
- Ratti nati da madri trattate con pesticidi producono spermatozoi con alterazioni che si mantengono sino alla quarta generazione.

Sperm RNAs Transmit Stress

Stressed male mice can pass on an abnormal stress response to their offspring via microRNAs found in sperm, a study shows.

By Kate Yandell | October 19, 2015

FLICKR, BERIT WATKIN

In the past several years, it has become clear that parents' life experiences can alter germ cells epigenetically, and that events in parents' lives can influence the health and behavior of their children and even grandchildren. But it can be difficult to establish a causal connection between epigenetic changes and changes in behavior and health. Researchers at the University of Pennsylvania led by Tracy Bale have now demonstrated that an increase in a group of microRNAs (miRNAs) in sperm from stressed mice can lead to altered stress response in adult offspring. The work, published today (October 19) in *PNAS*, shows that simultaneously injecting nine miRNAs into mouse zygotes recapitulates the changes found in the offspring of stressed mice.

"I think it's a fine paper [and a] well-designed study," said Michael Skinner, who studies epigenetic inheritance at Washington State University and was not involved in the study. "It shows a very nice role for noncoding RNA at the early embryonic stage for transmission of the transgenerational phenotype."

NEUROSCIENZE

Si può ringiovanire grazie all'(epi) genetica? La strada è aperta

L'idea è quella di riprogrammare le cellule 'vecchie' in modo che recuperino le sane caratteristiche giovanili. Così i ricercatori hanno ridato la vista ad animali di laboratorio

di **Adriana Bazzi**

Ringiovanire si potrà, forse. Ecco perché suscita curiosità (e speranze per il futuro) una ricerca appena pubblicata sulla rivista *Nature* firmata, con il suo team, dal biologo molecolare David Sinclair, della Harvard Medical School a Boston, da tempo alla caccia di strategie anti-invecchiamento. Sinclair si è chiesto: cellule vecchie e malandate possono ricordare come erano da giovani e comportarsi di conseguenza? Da qui è partito lo studio in cui i ricercatori hanno dimostrato, in estrema sintesi, quanto segue: neuroni, cioè cellule nervose, della retina dell'occhio, opportunamente "riprogrammate", possono comportarsi come quando erano giovani e recuperare le funzioni perse, cioè quelle di permettere la visione. Al momento questi studi sono stati condotti su animali da esperimento, topi per la precisione. Ma fanno ben sperare.

3 DICEMBRE 2020

Reprogramming to recover youthful epigenetic information and restore vision

https://doi.org/10.1038/s41586-020-2975-4
Received: 31 July 2019
Accepted: 22 October 2020
Published online: 2 December 2020
Check for updates

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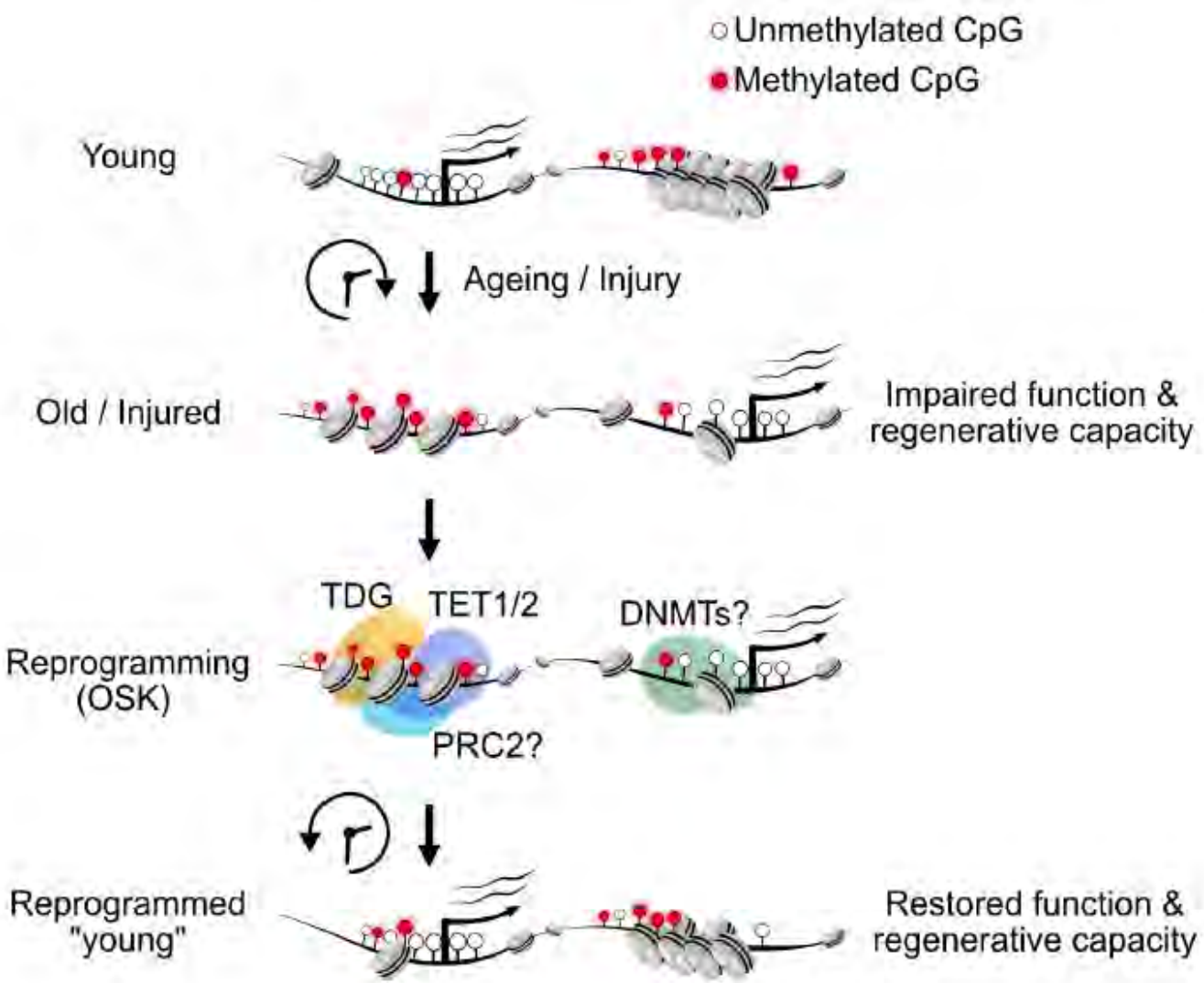
Ageing is a degenerative process that leads to tissue dysfunction and death. A proposed cause of ageing is the accumulation of epigenetic noise that disrupts gene expression patterns, leading to decreases in tissue function and regenerative capacity[1–3]. Changes to DNA methylation patterns over time form the basis of ageing clocks[4], but whether older individuals retain the information needed to restore these patterns—and, if so, whether this could improve tissue function—is not known. Over time, the central nervous system (CNS) loses function and regenerative capacity[5–7]. Using the eye as a model CNS tissue, here we show that ectopic expression of *Oct4* (also known as *Pou5f1*), *Sox2* and *Klf4* genes (OSK) in mouse retinal ganglion cells restores youthful DNA methylation patterns and transcriptomes, promotes axon regeneration after injury, and reverses vision loss in a mouse model of glaucoma and in aged mice. The beneficial effects of OSK-induced reprogramming in axon regeneration and vision require the DNA demethylases TET1 and TET2. These data indicate that mammalian tissues retain a record of youthful epigenetic information—encoded in part by DNA methylation—that can be accessed to improve tissue function and promote regeneration in vivo.



- The loss of youthful epigenetic information during ageing and injury causes a decline in tissue function and regenerative capacity.
- OSK-mediated reprogramming recovers youthful epigenetic information, reverses the DNA methylation clock, restores youthful gene expression patterns, and improves tissue function and regenerative capacity, a process that requires active DNA demethylation by TET1/TET2 and TDG.