EPIGENETICA

EPIGENETICA

L’epigenetica è quel ramo della genetica che studia le modificazioni ereditabili (ovvero trasmissibili alle successive generazioni) **che portano a variazioni dell’espressione genica senza però alterare la sequenza del DNA.**

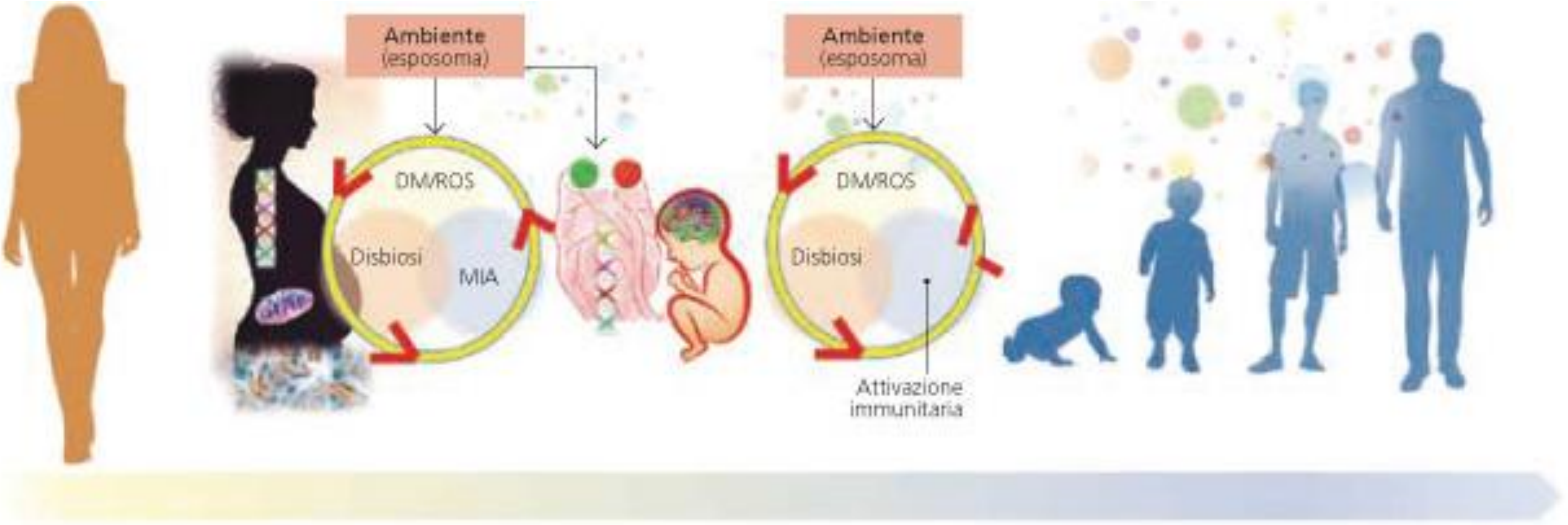
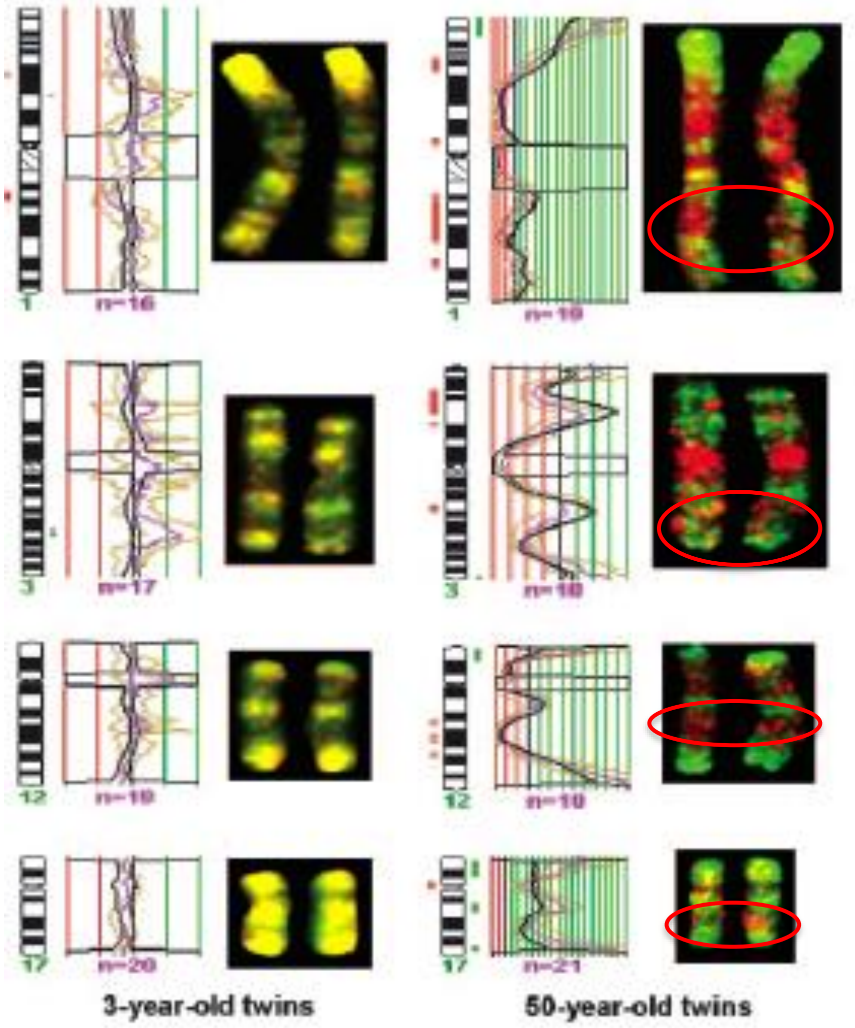


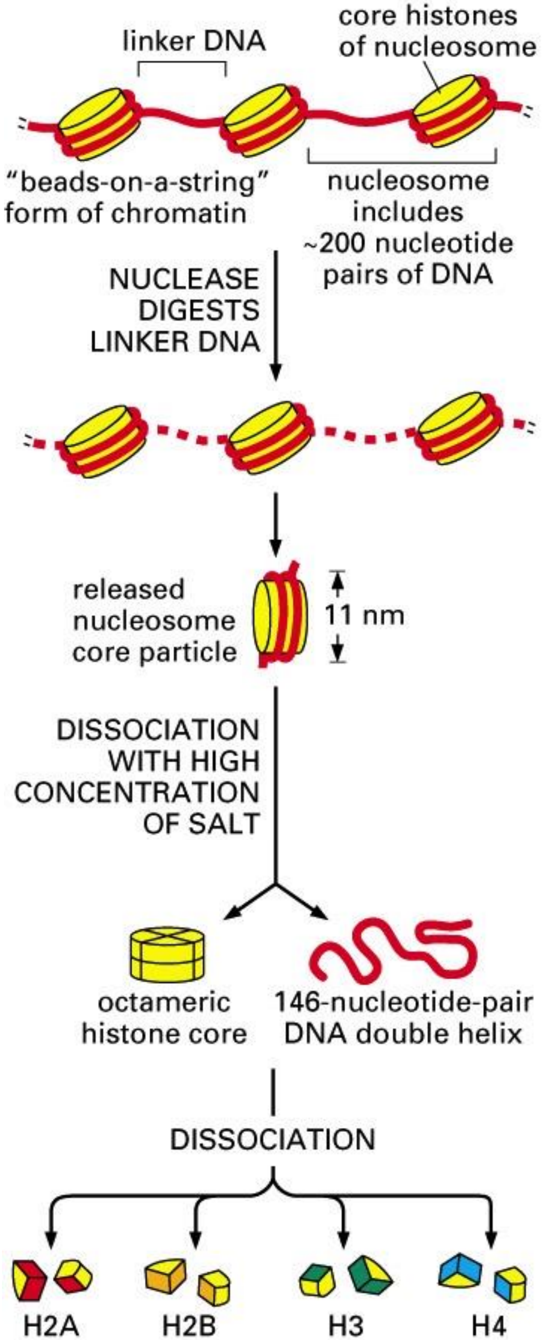
Figura 2. **Durante l’ontogenesi, i fattori ambientali (esposoma) influiscono sulla programmazione epigenetica embriofetale, direttamente o attraverso i meccanismi del “trio cattivo” costituito da disbiosi/attivazione immunitaria materna (MIA)/disfunzione mitocondriale (DM)-stress ossidativo (ROS). Dopo la nascita, i medesimi meccanismi (fattori ambientali e “trio”) possono incidere sulla salute della persona con ASD. Il periodo embriofetale e i primi due anni di vita sono la finestra temporale di massimo impatto sulla formazione del connettoma cerebrale.** (Modificata da Panisi C, *et al.*).

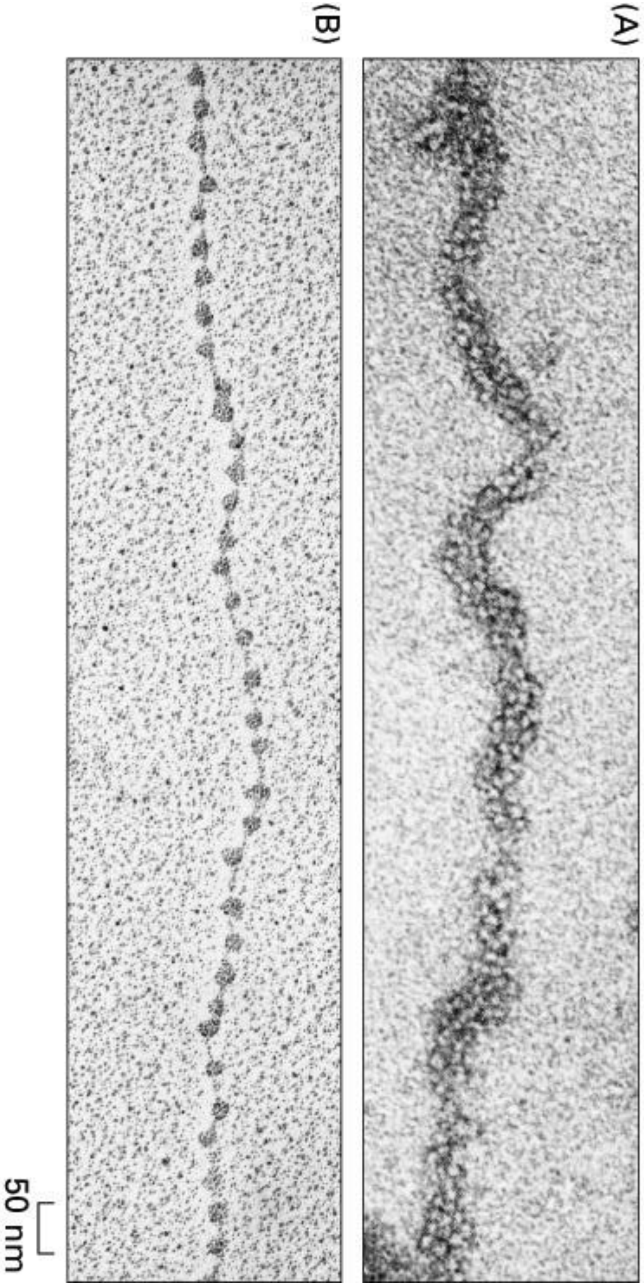
Epigenetic differences arise during the lifetime of monozygotic twins

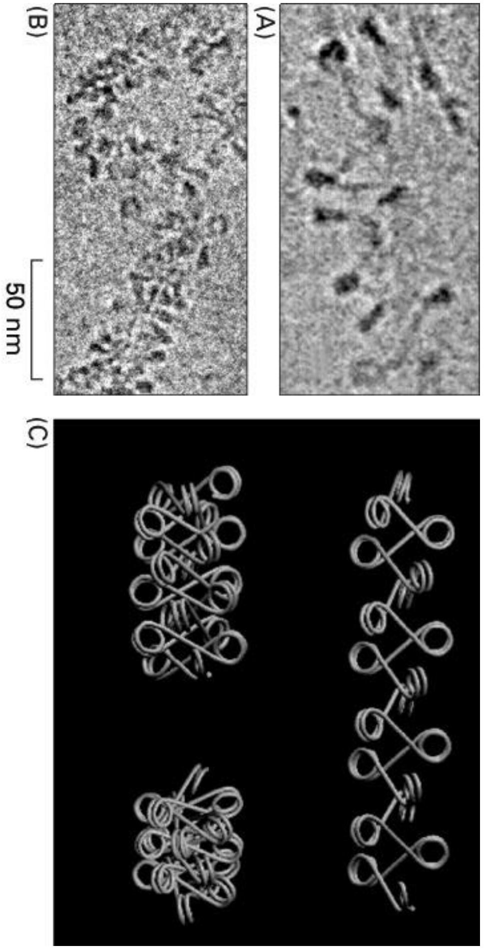
Mario F. Fraga[*], Esteban Ballestar[*], Maria F. Paz[*], Santiago Ropero[*], Fernando Setien[*], Maria L. Ballestar[†],
Damia Heine-Suñer[‡], Juan C. Cigudosa[§], Miguel Urioste[¶], Javier Benitez[¶], Manuel Boix-Chornet[†],
Abel Sanchez-Aguilera[†], Charlotte Ling[||], Emma Carlsson[||], Pernille Poulsen[**], Allan Vaag[**],
Zarko Stephan[††], Tim D. Spector[††], Yue-Zhong Wu[‡‡], Christoph Plass[‡‡], and Manel Esteller[*,§§]

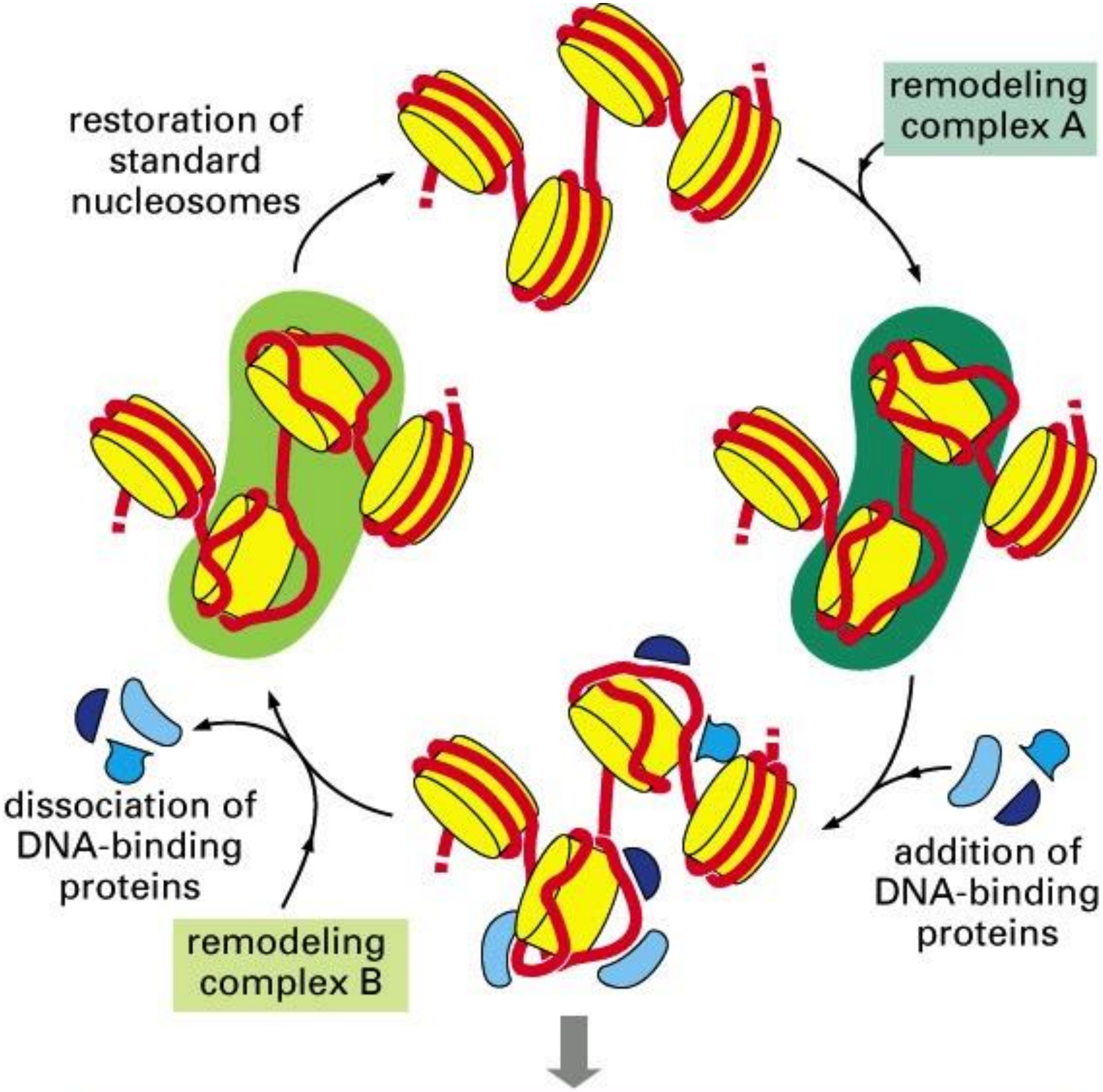
> "Epigenomics is where genomics was 30 years ago, when everyone was working on part of the puzzle."
> — Peter Jones




linker DNA
core histones of nucleosome
"beads-on-a-string" form of chromatin
nucleosome includes ~200 nucleotide pairs of DNA
NUCLEASE DIGESTS LINKER DNA
released nucleosome core particle
11 nm
DISSOCIATION WITH HIGH CONCENTRATION OF SALT
octameric histone core
146-nucleotide-pair DNA double helix
DISSOCIATION
H2A
H2B
H3
H4


(A)
(B)
50 nm


(A)
(B)
(C)
50 nm



restoration of standard nucleosomes

remodeling complex A

addition of DNA-binding proteins

dissociation of DNA-binding proteins

remodeling complex B

GENE EXPRESSION, DNA REPLICATION,
AND OTHER PROCESSES THAT REQUIRE ACCESS TO
DNA PACKAGED IN NUCLEOSOMES

MECCANISMI EPIGENETICI

Fattori che influenzano l'espressione genica, trasmessi alla progenie, ma che non sono direttamente attribuibili a sequenze di DNA.

MODIFICAZIONI DEGLI ISTONI
Acetilazioni, fosforilazioni e metilazioni, responsabili dei cambiamenti *conformazionali* della cromatina.

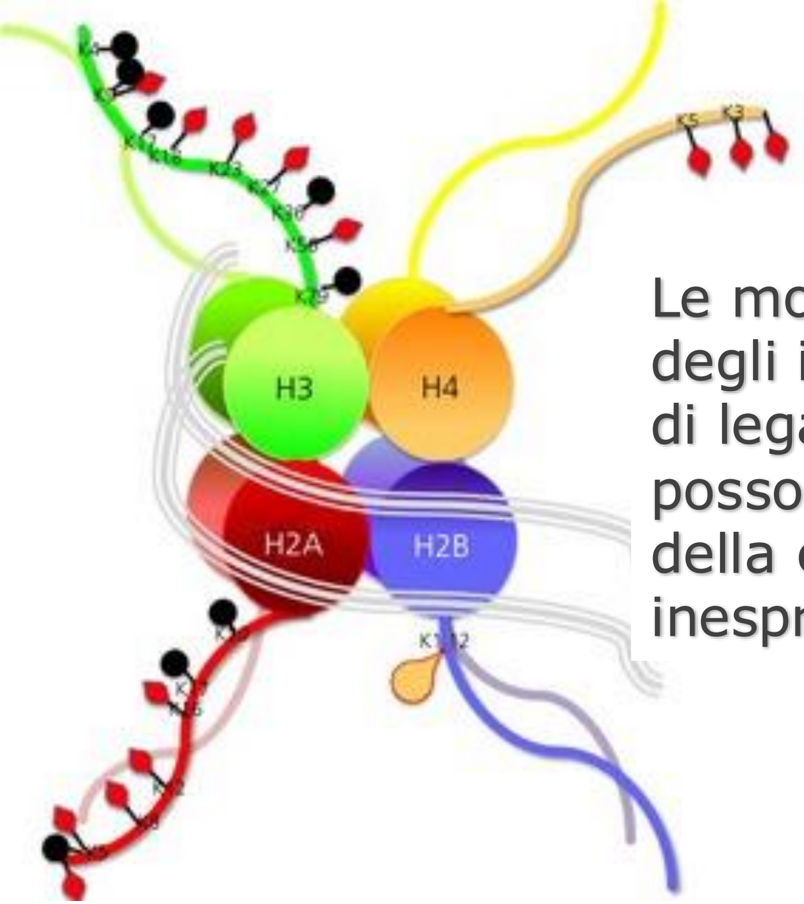
METILAZIONE DEL DNA

The two main components of the epigenetic code

DNA methylation
Methyl marks added to certain DNA bases repress gene activity.

Histone modification
A combination of different molecules can attach to the 'tails' of proteins called histones. These alter the activity of the DNA wrapped around them.

Le **code N-terminali** degli istoni sporgono dal nucleo dell'ottamero

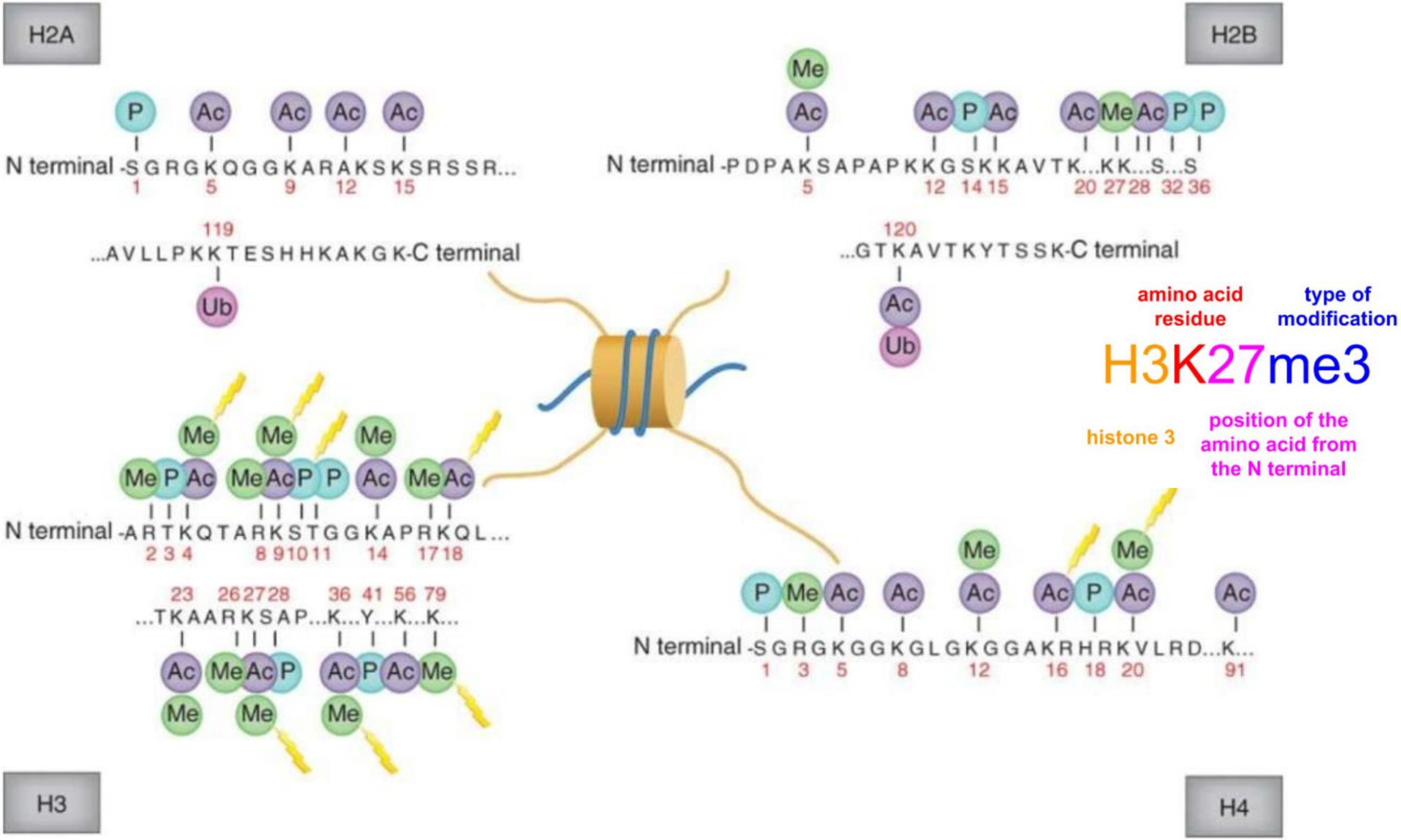


Le modificazioni chimiche degli istoni forniscono siti di legame per proteine che possono cambiare lo stato della cromatina in attivo o inespresso

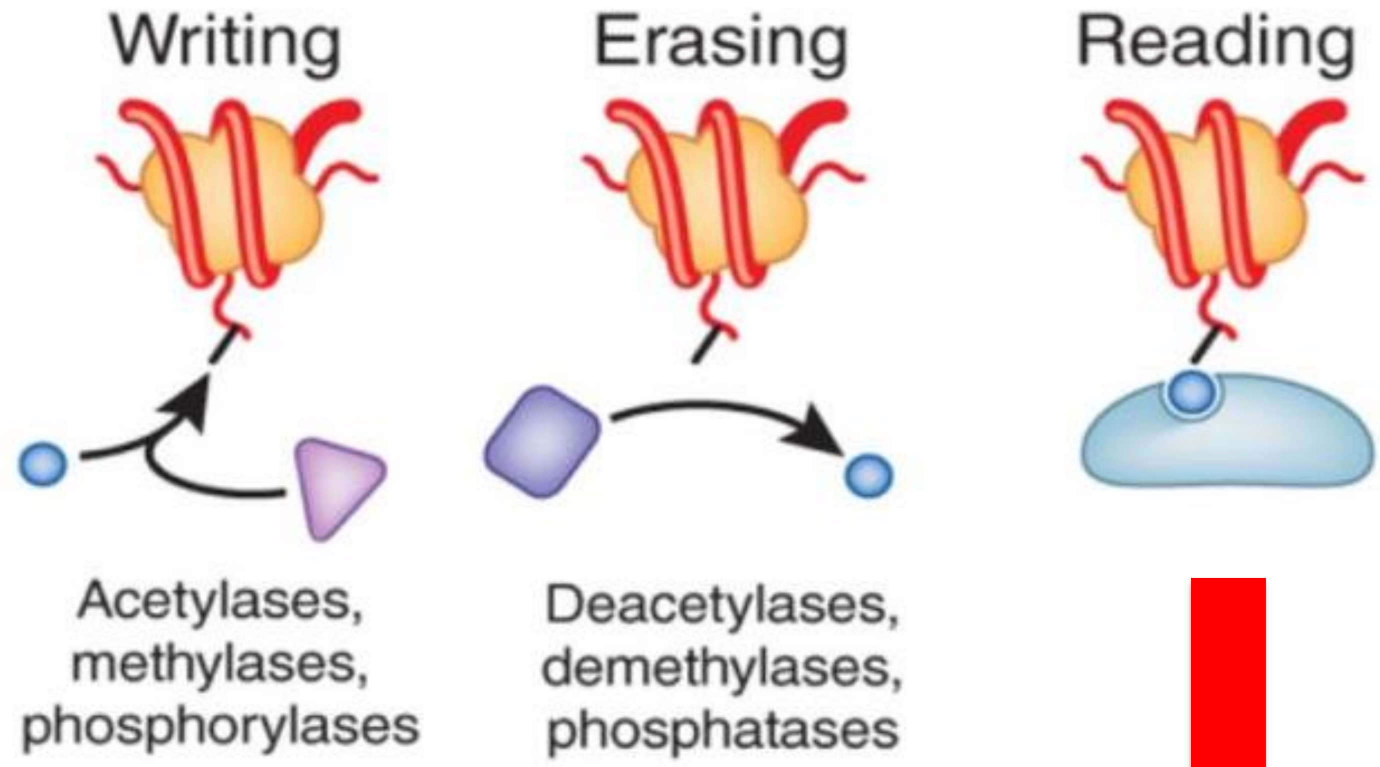
Una particolare combinazione di tali modificazioni ha un significato biologico (CODICE ISTONICO)

Modificazioni possibili:
A = Acetilazione di lisine (K)
M = Metilazione di lisine (K) e arginine (R)
P = Fosforilazione di serine e treonine (S/T)
U = Ubiquitinazione di lisine (K)

The histone code

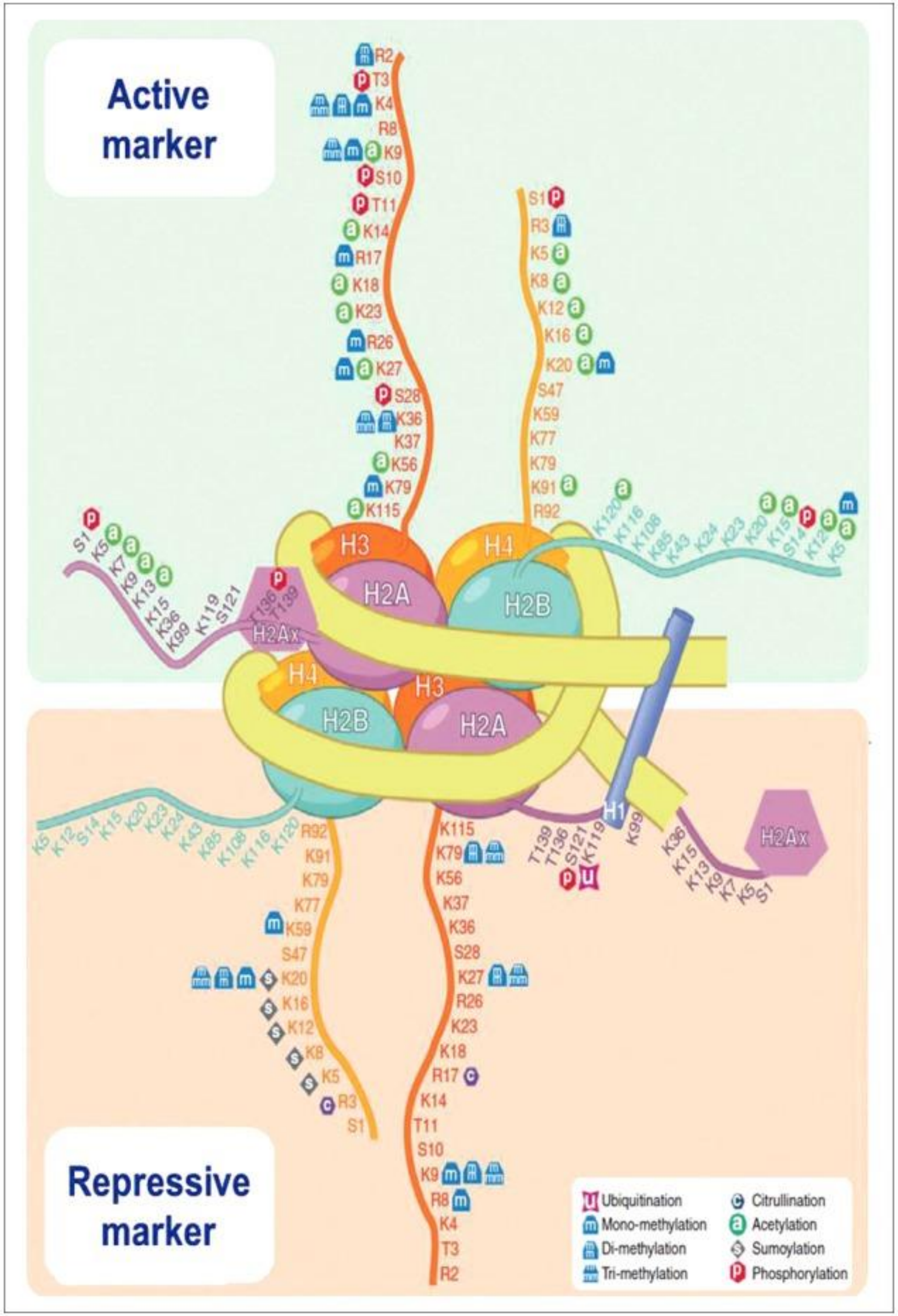


L'ipotesi del **codice istonico** propone che modificazioni covalenti post-traduzionali delle code degli istoni vengano "lette" dalla cellula portando ad un risultato trascrizionale combinatorio complesso



Modificazioni post-traduzionali
DINAMICHE

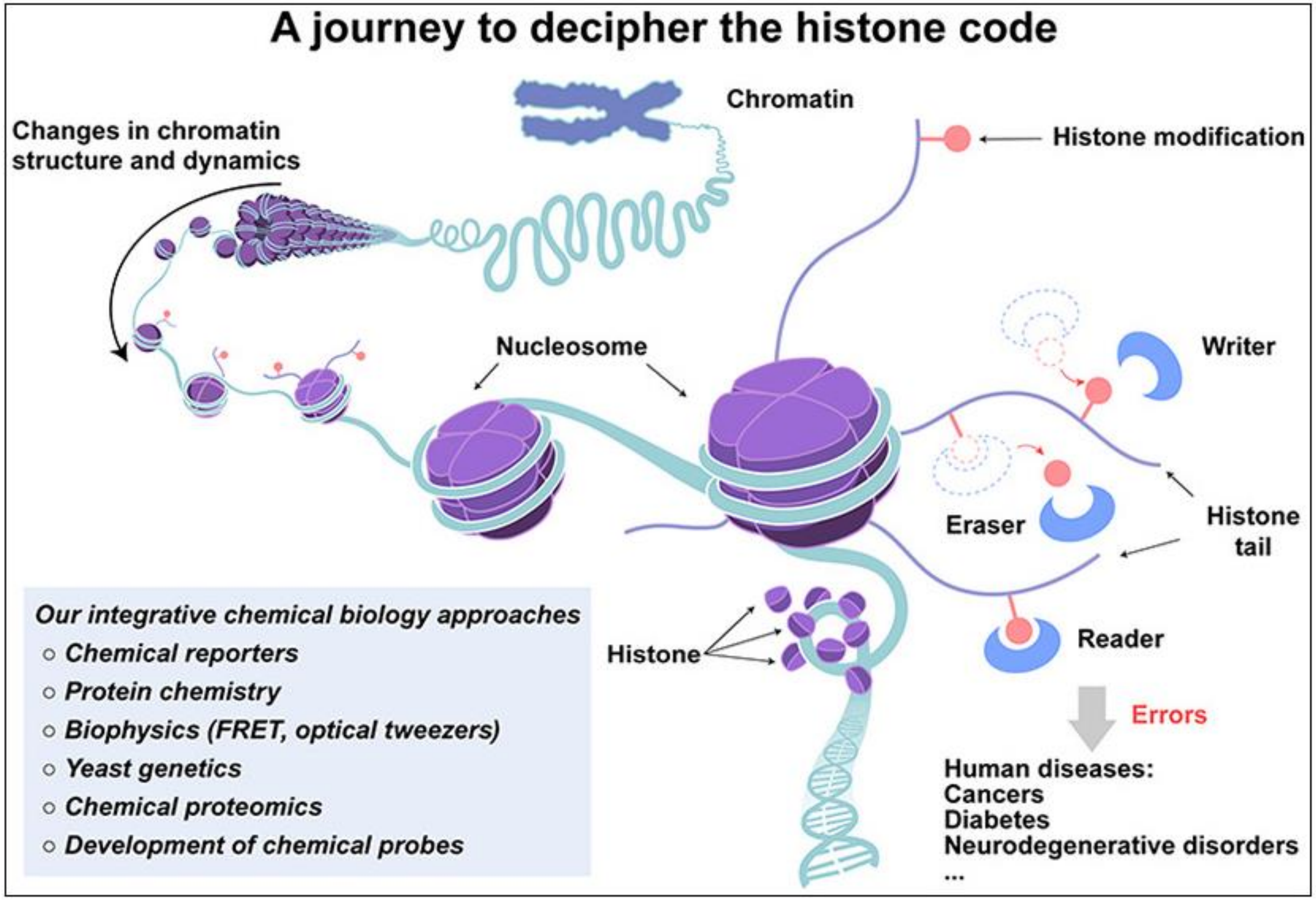
Risposta
trascrizionale
specifica



modification state	"meaning"
unmodified	gene silencing?
acetylated	gene expression
acetylated	histone deposition
methylated	gene silencing/ heterochromatin
phosphorylated	mitosis/meiosis
phosphorylated/ acetylated	gene expression
higher-order combinations	?

unmodified	gene silencing?
acetylated	histone deposition
acetylated	gene expression

Qual è la funzione del codice istonico?



Our integrative chemical biology approaches

- *Chemical reporters*
- *Protein chemistry*
- *Biophysics (FRET, optical tweezers)*
- *Yeast genetics*
- *Chemical proteomics*
- *Development of chemical probes*

Signalling pathway model postulates that histone modifications serve as signalling platforms to facilitate binding of enzymes for their function on chromatin

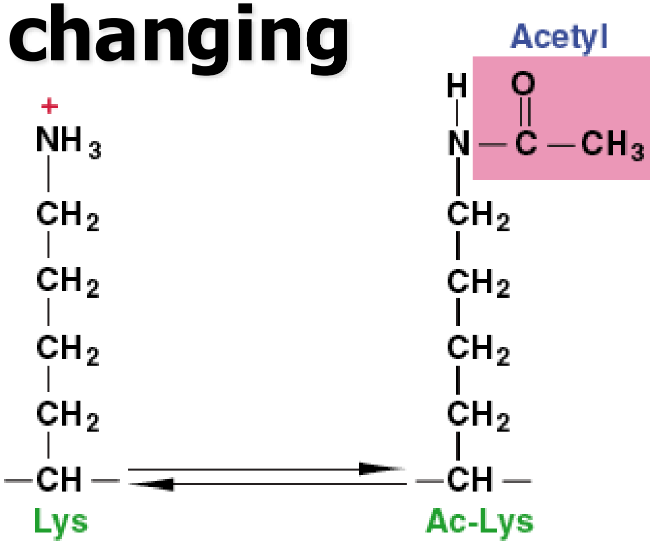
Modificazioni possibili:
A = Acetilazione di lisine (K)
M = Metilazione di lisine (K) e arginine (R)
P = Fosforilazione di serine e treonine (S/T)
U = Ubiquitinazione di lisine (U)

CHI AGISCE?

COMPLESSI DI MODIFICAZIONE DELLA CROMATINA:

HAT, HDAC
ISTONE METILTRANSFERASI (HMT) E DEMETILASI
CHINASI
ENZIMI CHE CONIUGANO UBIQUITINA

Acetylation is very dynamic and rapidly changing

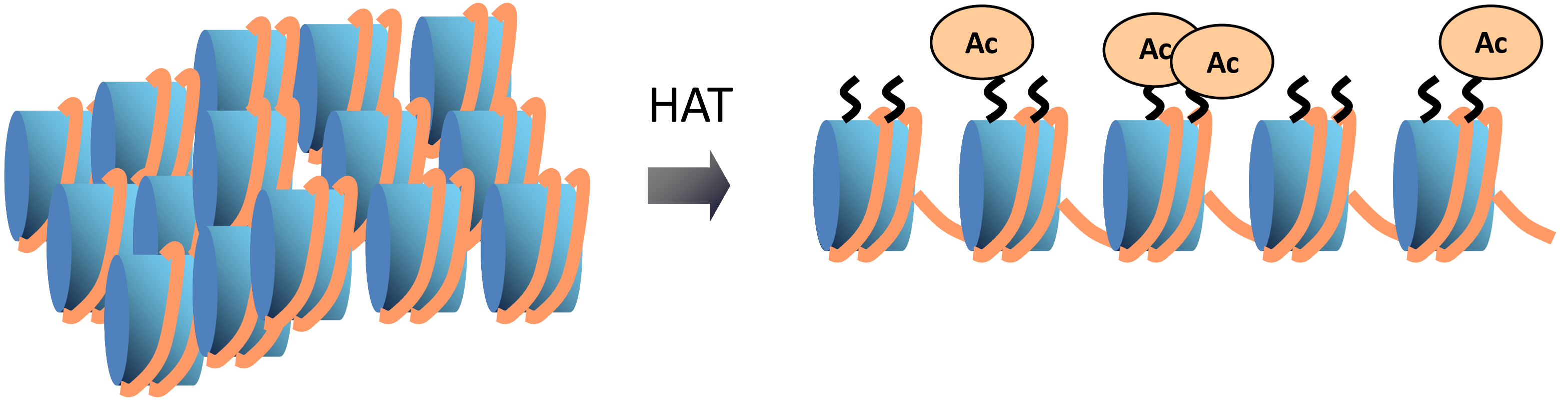


HAT catalyzes the transfer of an acetyl group from AcCoA to the ∑ amino group of the lysine residue, releasing its positive charge and therefore lowering its affinity for DNA

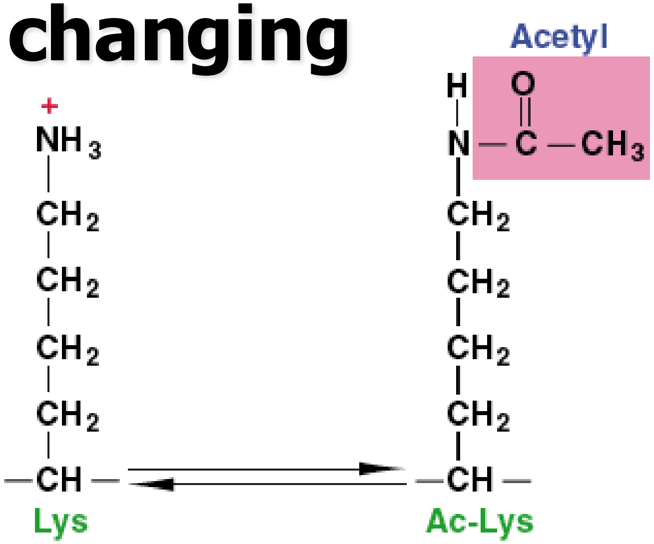
HDAC promotes the removal of the acetyl group from the acetyl-lysine regenerating the ∑ amino group and releasing the acetate molecule

heterochromatin
(transcriptionally inactive/condensed)

euchromatin
(transcriptionally active/accessible)

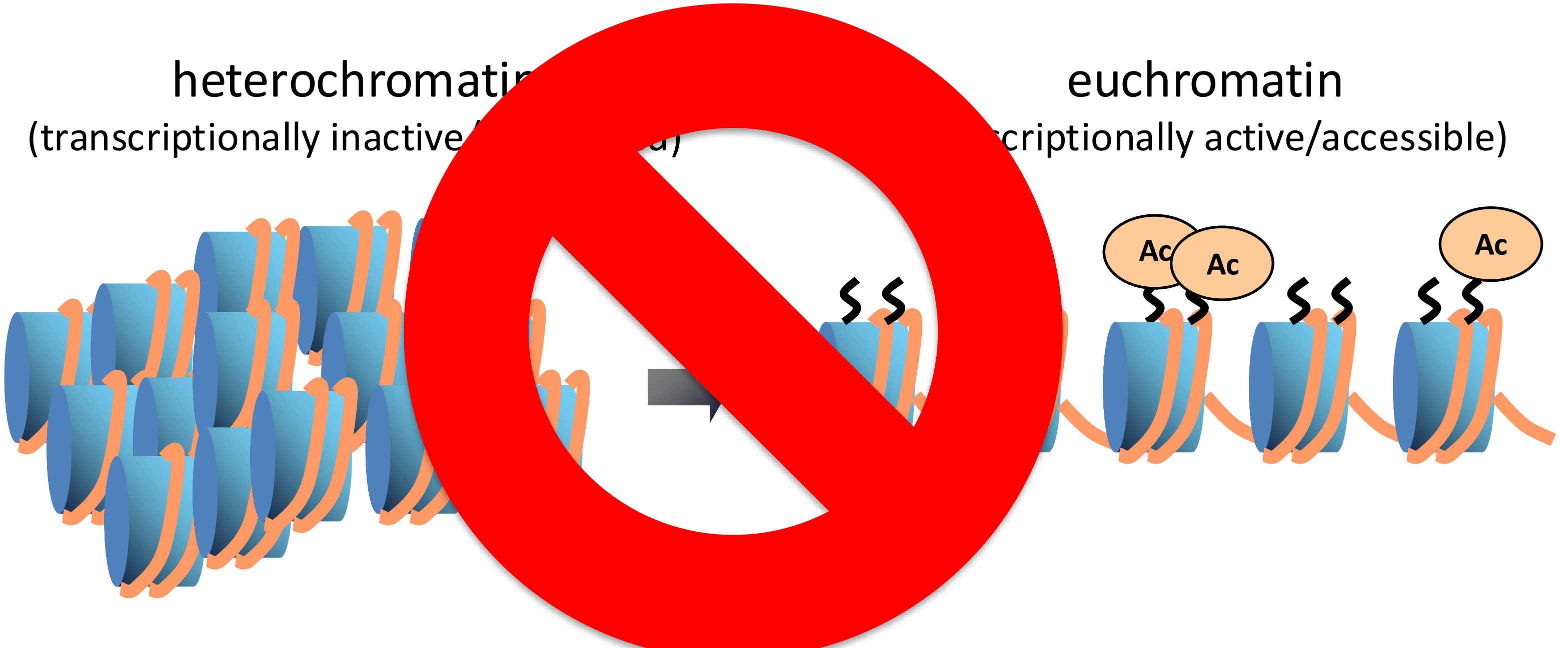


Acetylation is very dynamic and rapidly changing



HAT catalyzes the transfer of an acetyl group from AcCoA to the ∑ amino group of the lysine residue, releasing its positive charge and therefore lowering its affinity for DNA

HDAC promotes the removal of the acetyl group from the acetyl-lysine regenerating the ∑ amino group and releasing the acetate molecule



heterochromati

(transcriptionally inactive/ d)

euchromatin

criptionally active/accessible)

Cell *146*, August 19, 2011 ©2011 Elsevier Inc.

Mechanisms for the Inheritance of Chromatin States

Danesh Moazed[1],*
[1]Howard Hughes Medical Institute, Department of Cell Biology, Harvard Medical School, Boston, MA 02115, USA
*Correspondence: danesh@hms.harvard.edu

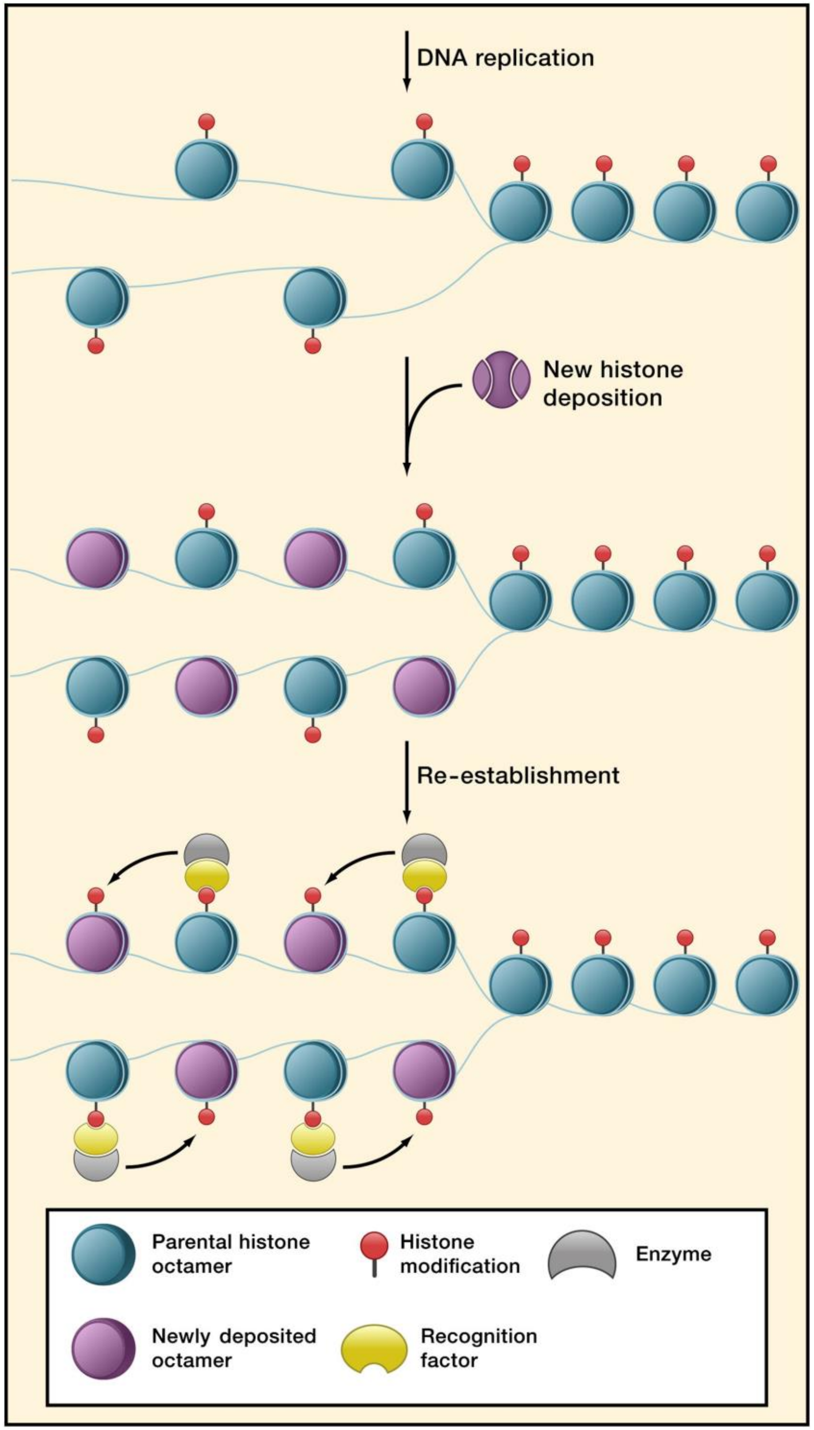
DOI 10.1016/j.cell.2011.07.013

Parental histones and their posttranslational modifications are retained and randomly associate with the newly synthesized daughter DNA strands.

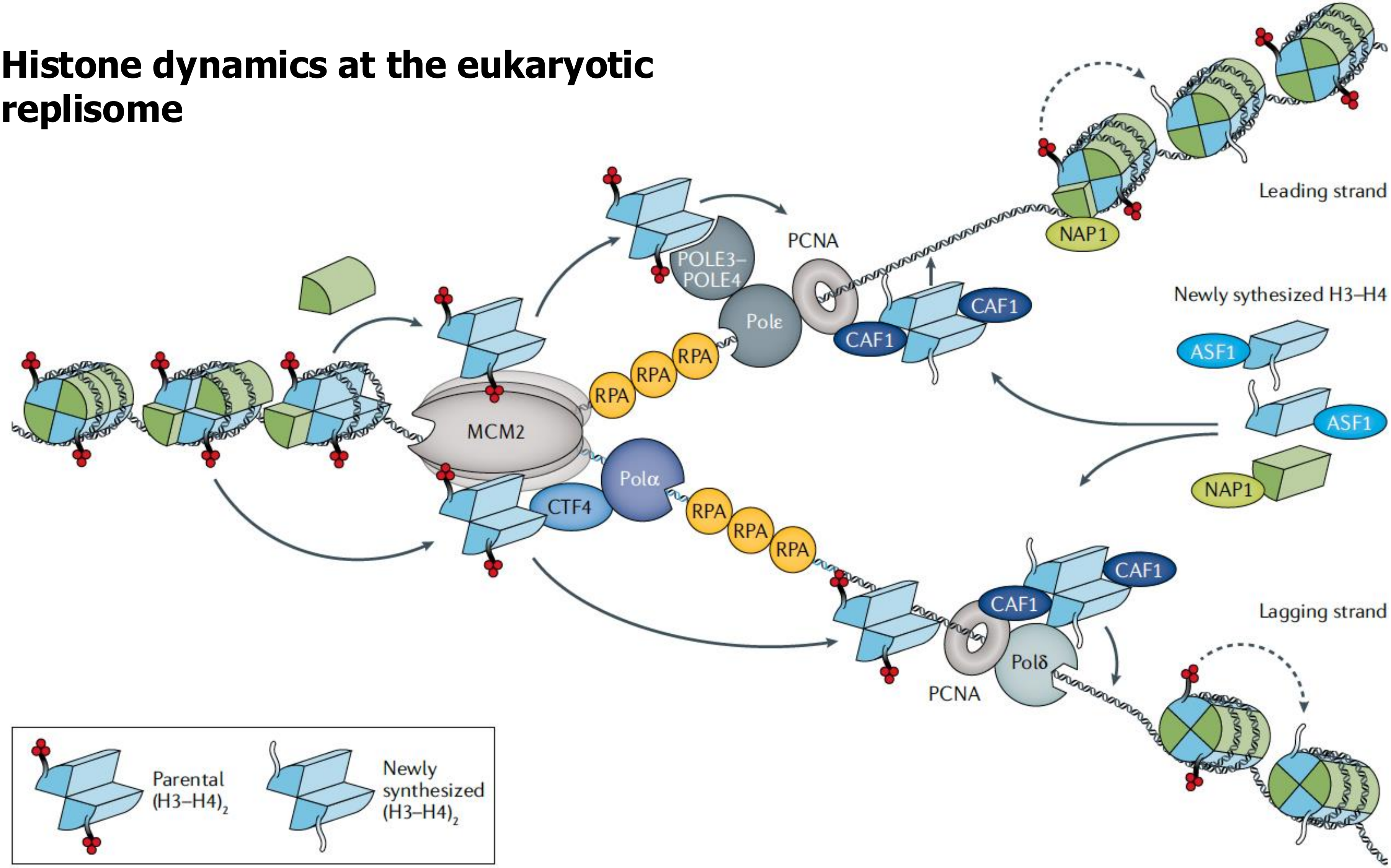
The modifications of parental histones are copied onto newly deposited histones by *chromatin modification complexes:*

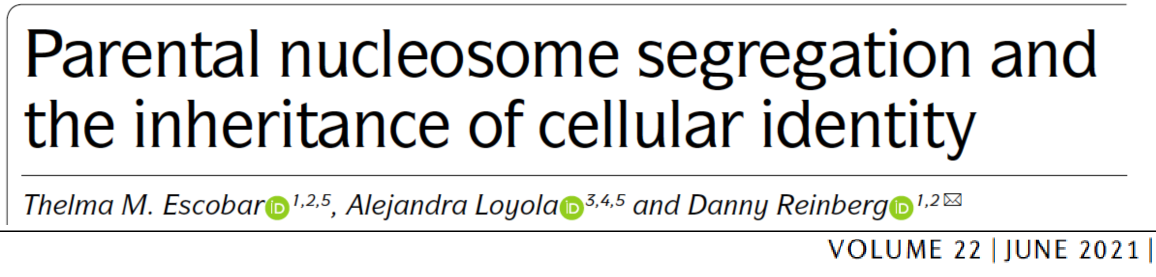
- a subunit recognizes the modification on the parental histone
- another subunit catalyzes the same modification on an adjacent nucleosome.

Note that distribution of histones to daughter DNA strands is **random**.



Histone dynamics at the eukaryotic replisome





Parental nucleosome segregation and the inheritance of cellular identity

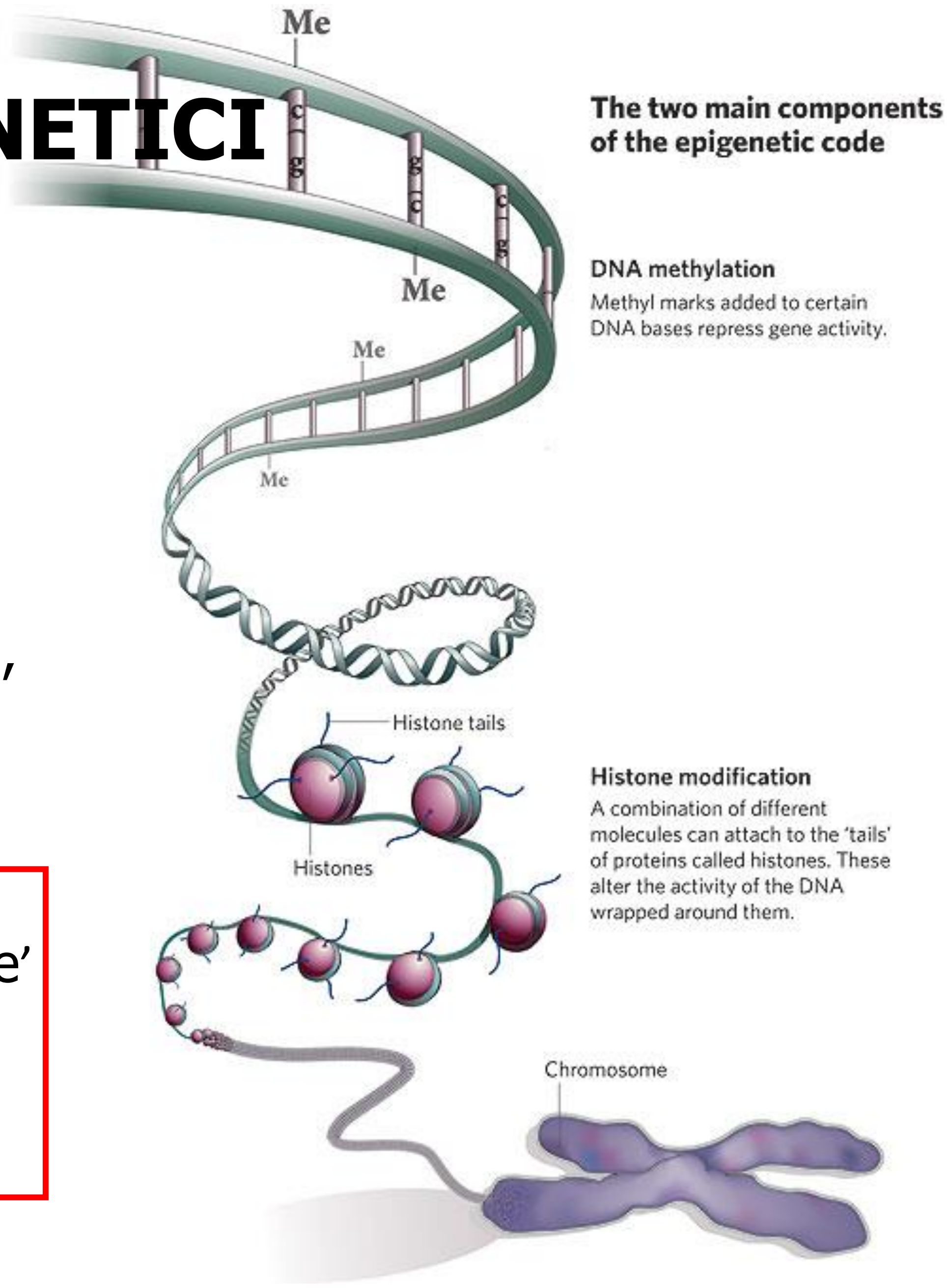
Thelma M. Escobar[1,2,5], *Alejandra Loyola*[3,4,5] *and Danny Reinberg*[1,2] ✉

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.



The two main components of the epigenetic code

DNA methylation
Methyl marks added to certain DNA bases repress gene activity.

Histone modification
A combination of different molecules can attach to the 'tails' of proteins called histones. These alter the activity of the DNA wrapped around them.

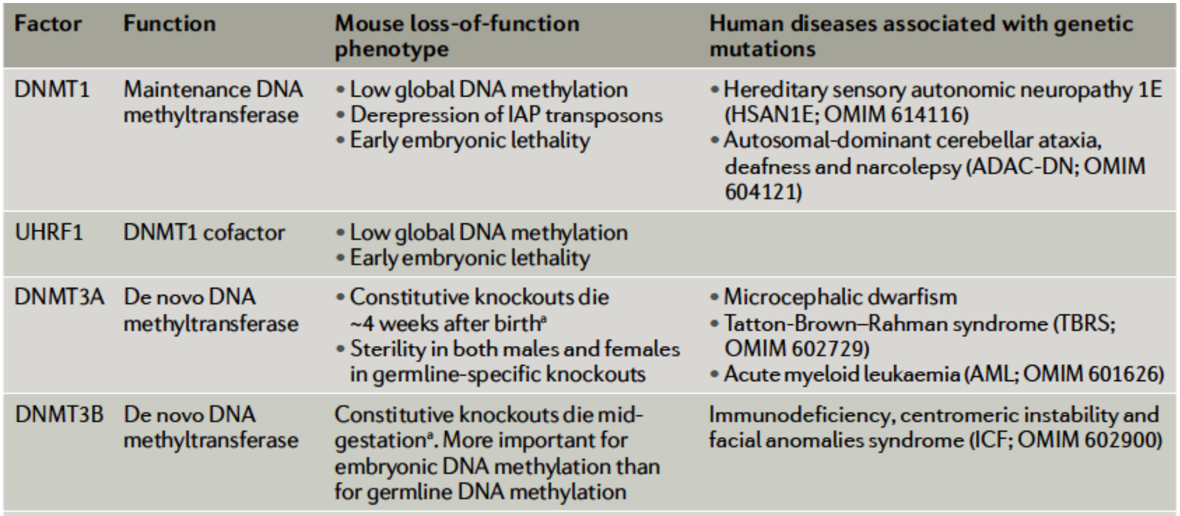
DNA methylation

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.

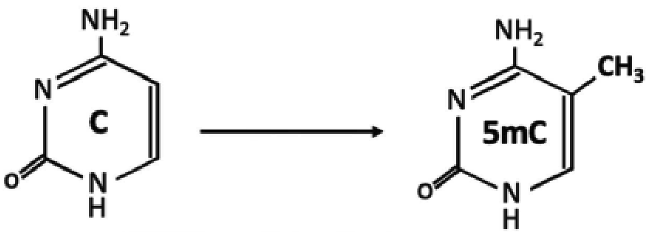
Given the robust stability of DNA methylation, tight regulation is of crucial importance, for proper cellular function (Greenberg, 2021).

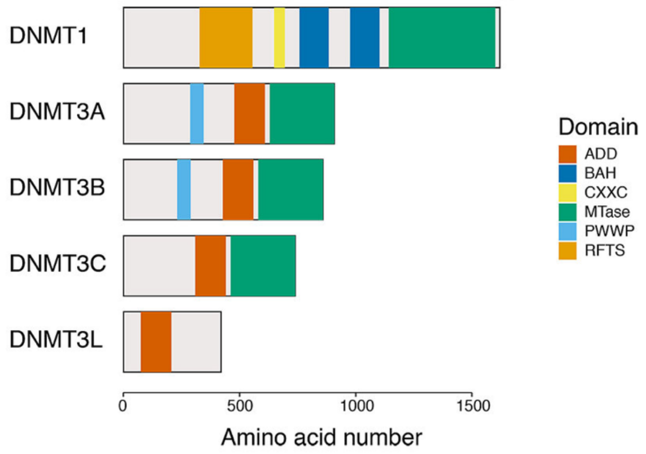
The epigenetic memory linked to DNA methylation is robust in somatic tissues, where the levels of CpG methylation are globally stable, with 70-80% of CpG dinucleotides harboring the mark (Lee et al., 2014).

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)



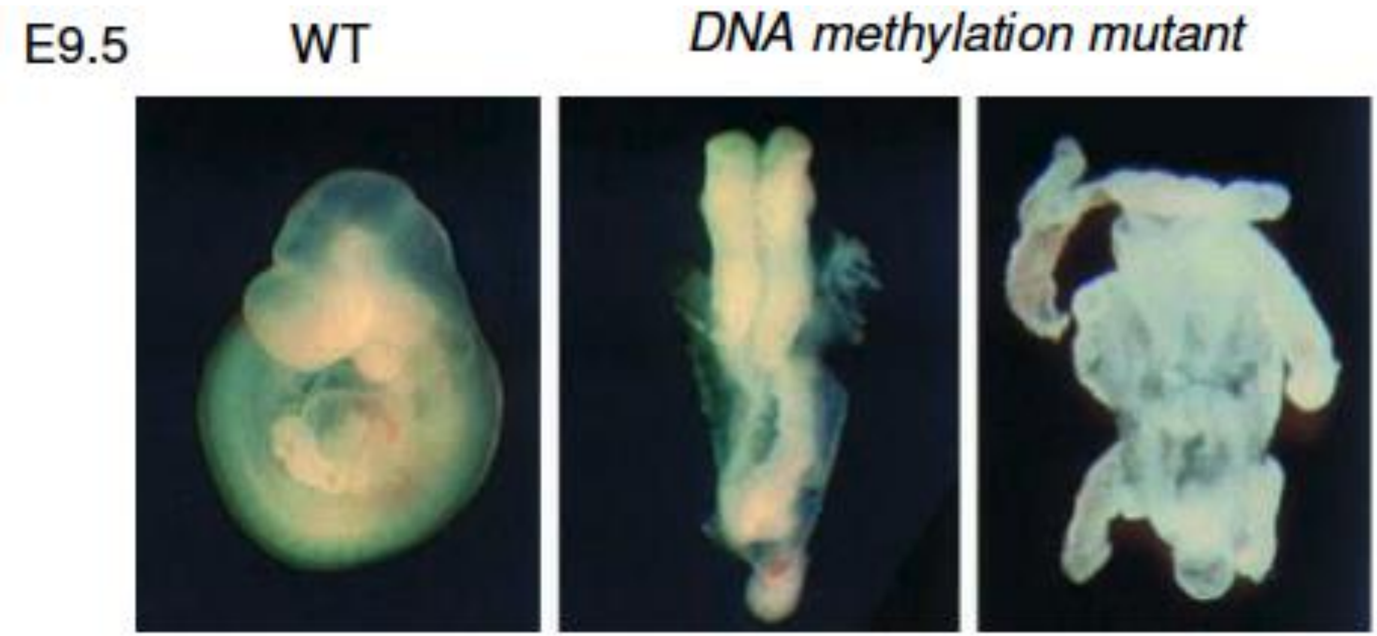


In mammals, there are 2 families of **DNA Methyl-transferases**:

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

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

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.

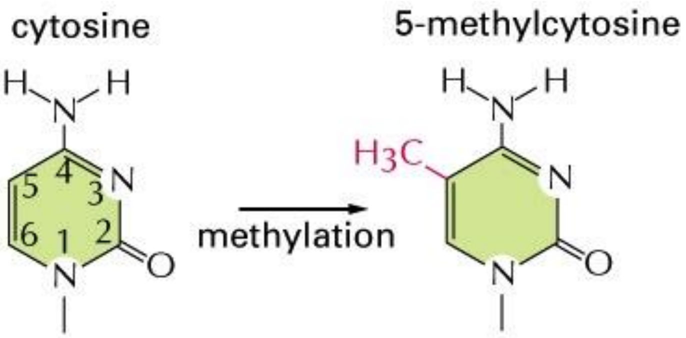


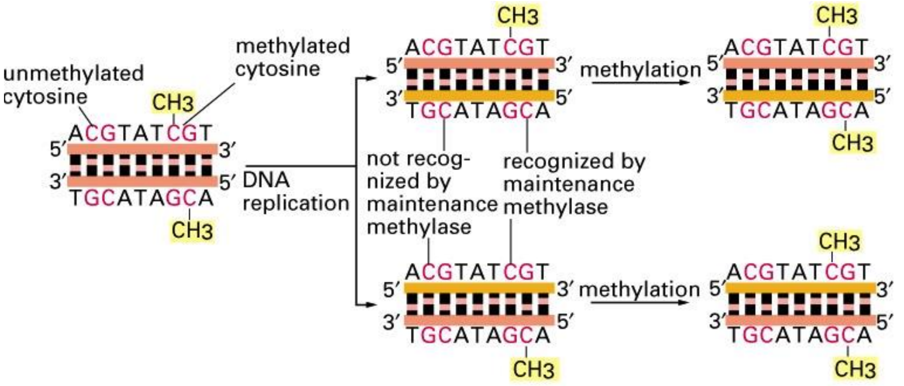
Dnmt3a -/-, Dnmt3b -/- *Dnmt1* null

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

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)

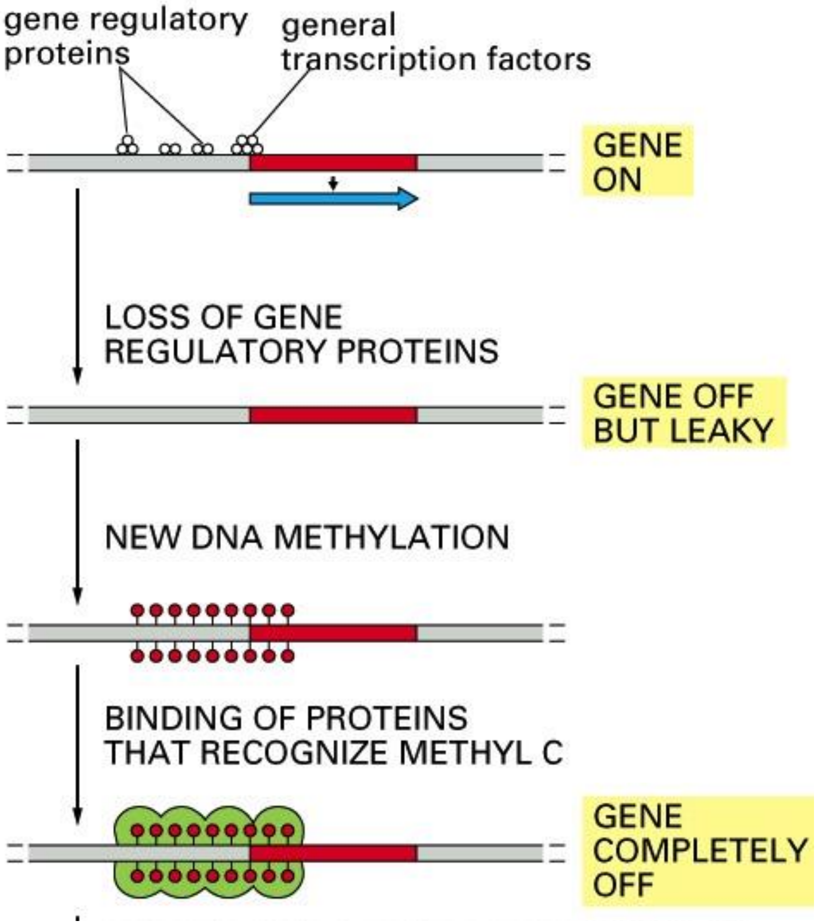


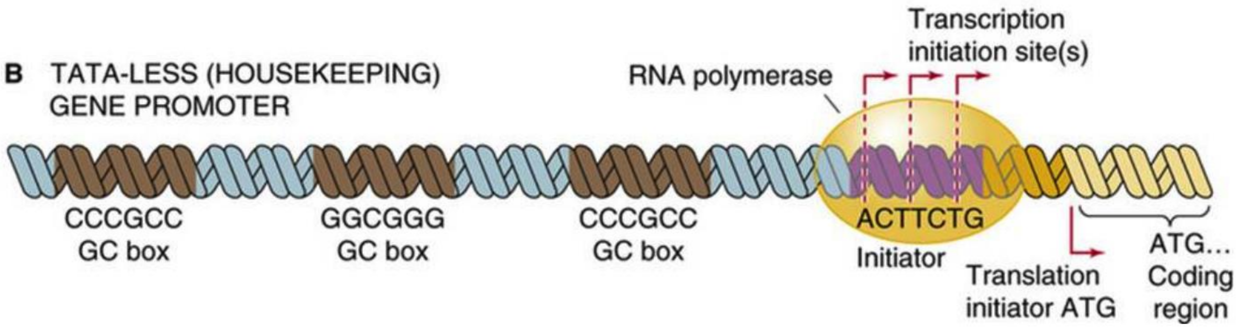


Vertebrates Use DNA Methylation to Lock Genes in a Silent State

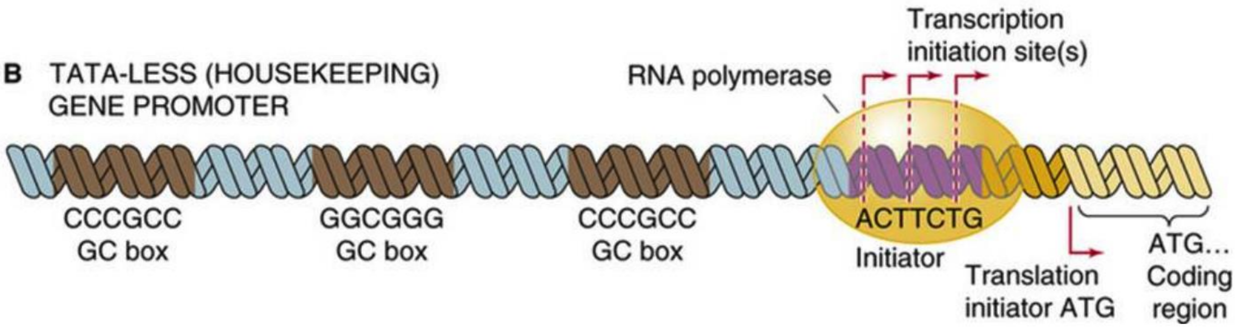
Vertebrate cells contain a family of **proteins (MeCP2) that bind methylated DNA**.

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





- 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



- 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

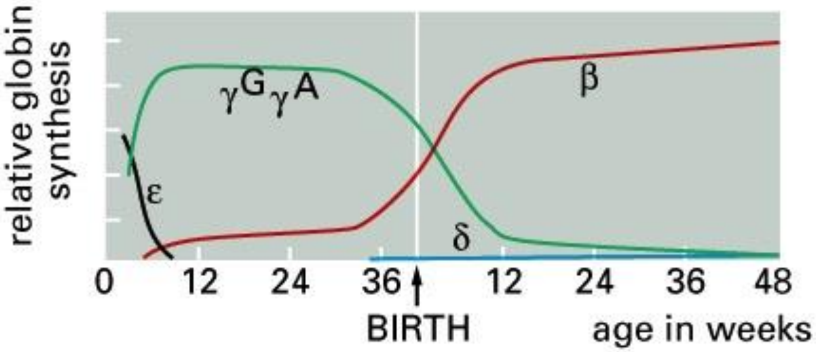
CpG island (CGI) promoters are not methylated

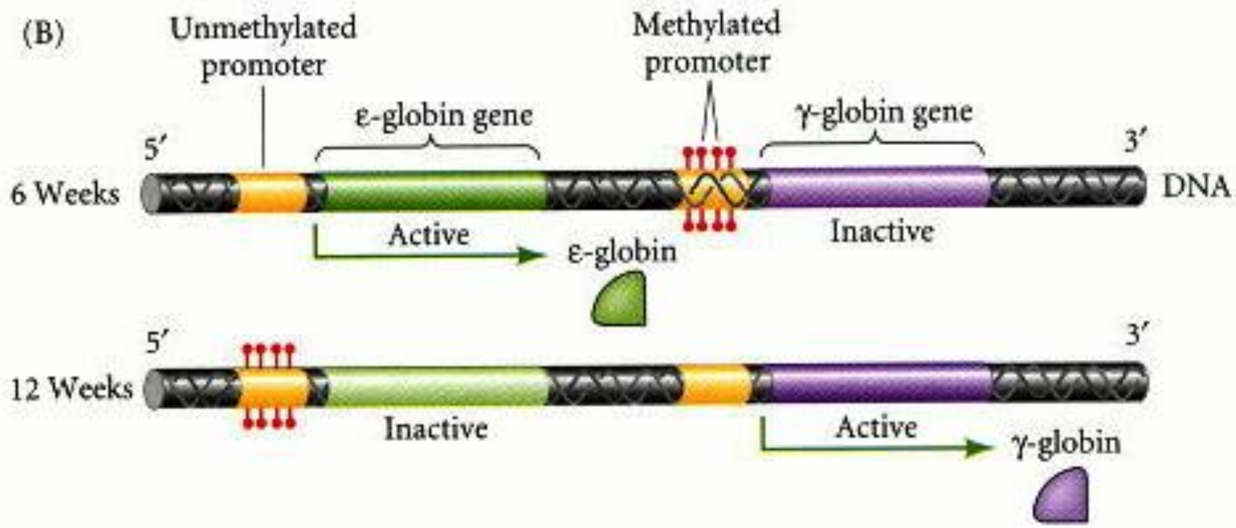
- Roughly two-thirds of promoters are CpG-Islands, and comprise most housekeeping and developmental genes.
- ALL CpG islands are associated with transcribed genes
- Keeping promoters free of methylation is absolutely crucial for proper cellular function.
- X-linked CpG islands become methylated upon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

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

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

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)

Gene expression

The amazing demethylase

Howard Cedar and Gregory L. Verdine

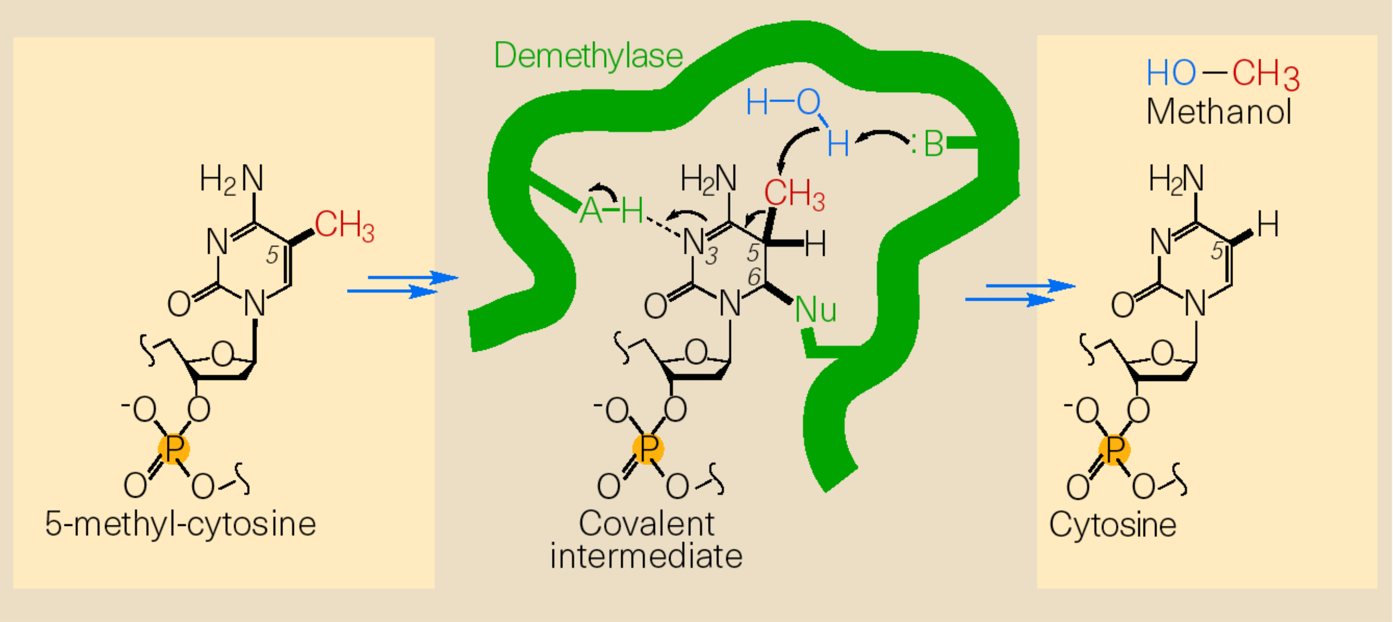
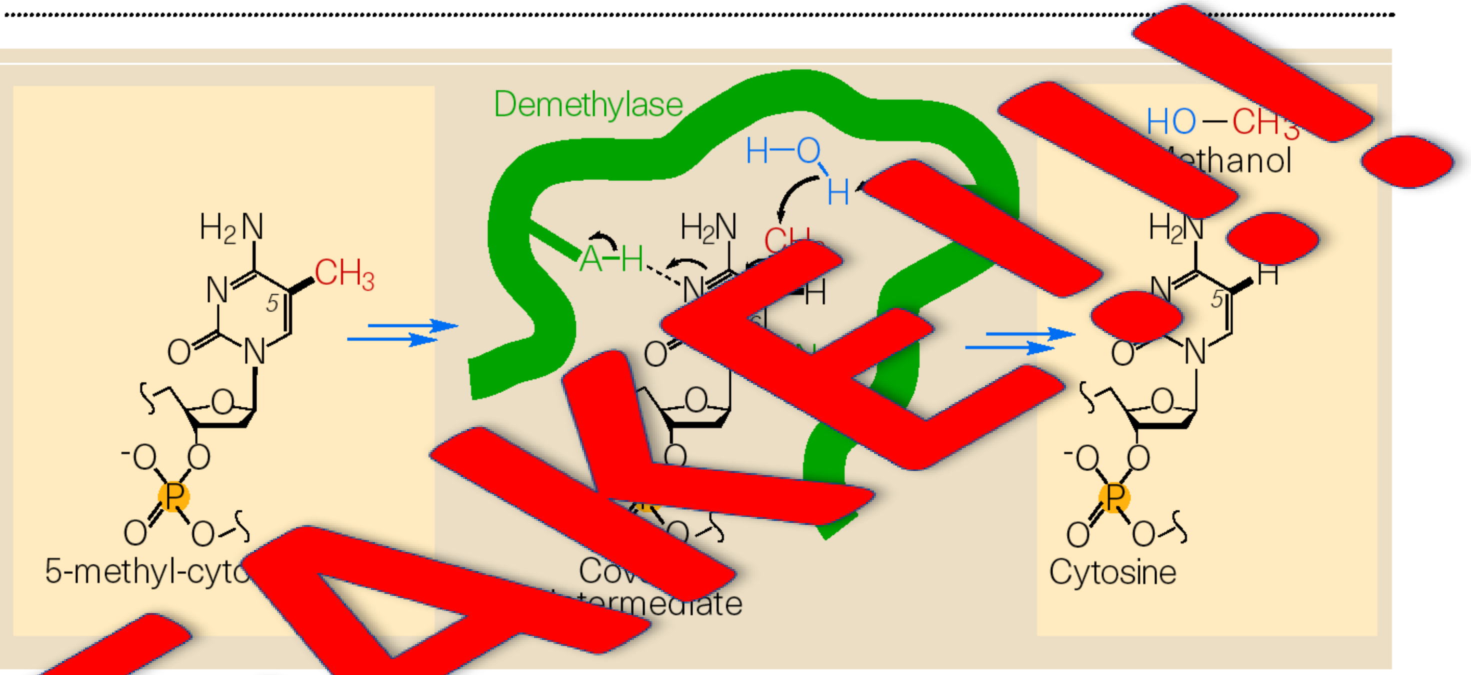


Figure 1 **Mechanism for the enzymatic demethylation of 5-methyl-cytosine. The demethylase (green) is envisaged to form a covalent intermediate by addition of an enzymatic nucleophile (Nu–H) across the 5,6 double bond, assisted by proton shuffling at N3. This intermediate is poised to attack the hydroxide 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

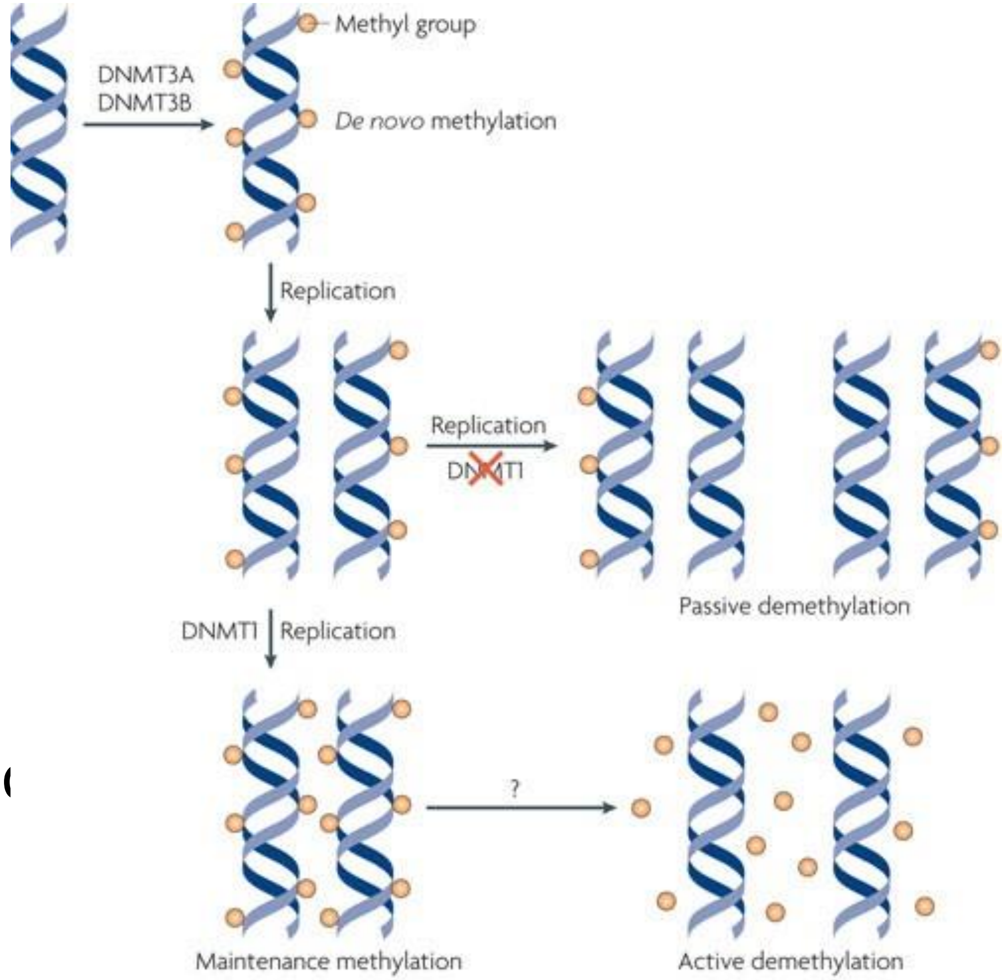


Fig[illegible]Mechan[illegible]or the[illegible]matic demethylation of 5-methyl-cytosine. The demethylase (green) is envisage[illegible]n a covalent [illegible]ermediate by addition of an enzymatic nucleophile (Nu–H) across the 5,6 double bo[illegible]ted by proton shuffling at N3. This intermediate 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.

NATURE | VOL 397 | 18 FEBRUARY 1999

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

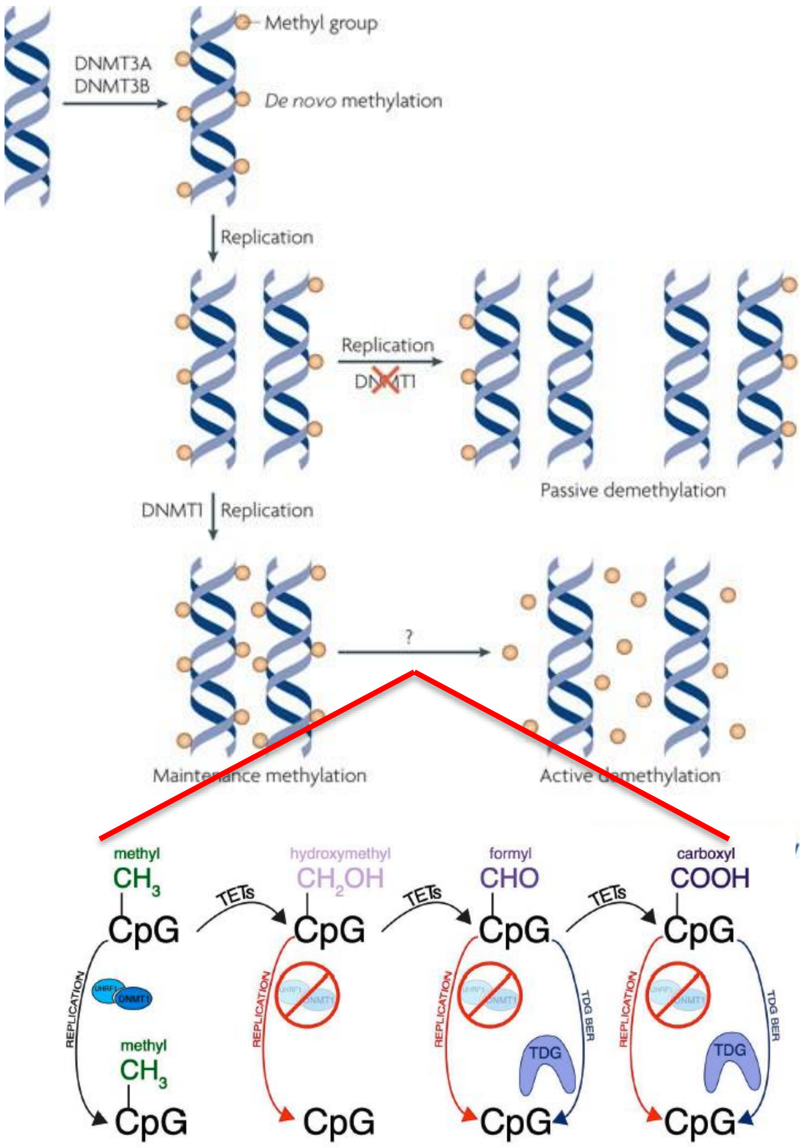
- Passive demethylation simply requires the impairment of maintenance DNA methylation machinery (Dnmt-1), which results in 2-fold dilution of methyl-CpGs during each roun of DNA synthesis.



Nature Reviews | Molecular Cell Biology

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

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



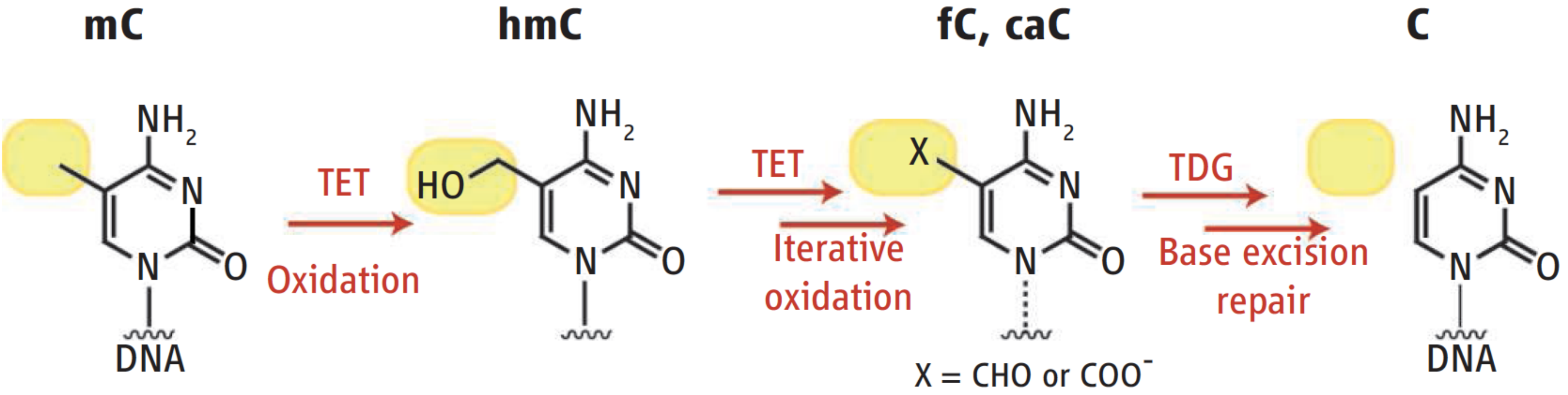
NATURE REVIEWS | GENETICS
VOLUME 13 | JANUARY 2012 | 7

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.



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.

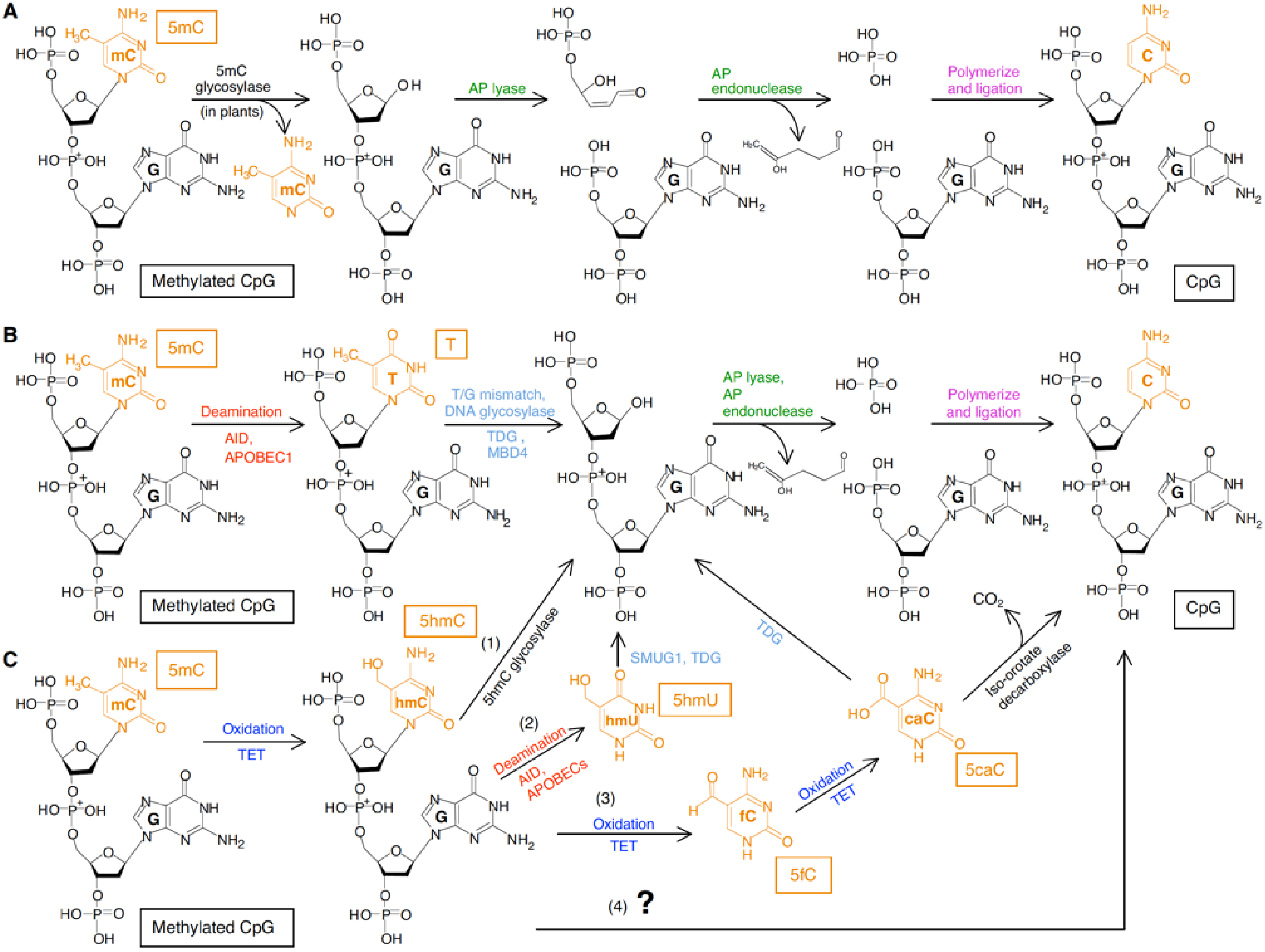
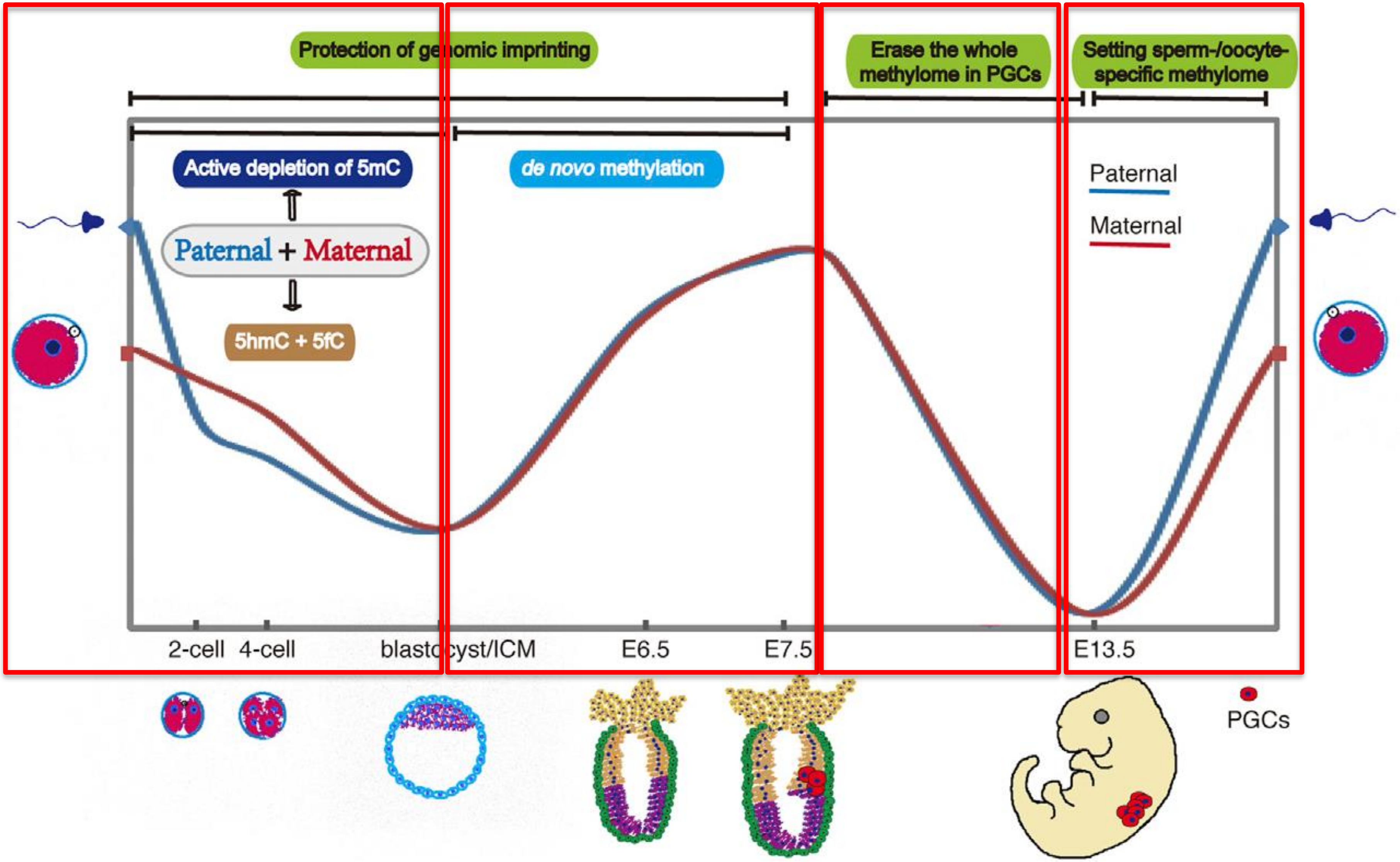


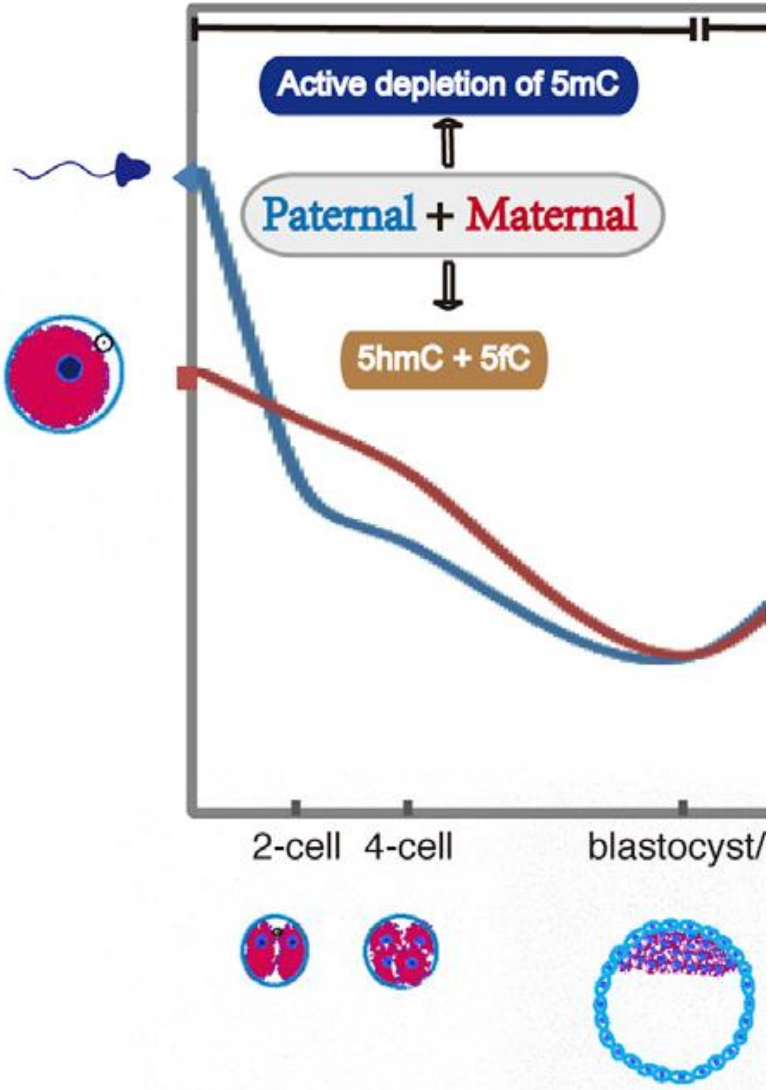
Fig. 4. Potential chemical pathways for active DNA demethylation. (**A**) Direct excision of 5mC (orange) by a 5mC glycosylase followed by repair via the base excision repair (BER) pathway (green and pink), as occurs in plants. (**B**) Cytosine deamination by AID/APOBEC1 (red), followed by base excision 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.

Mammalian genome methylation

Such reprogramming is found exclusively in mammals.



Methylation patterning in development



In mammals, paternal and maternal genomes undergo parent-specific epigenetic reprogramming.

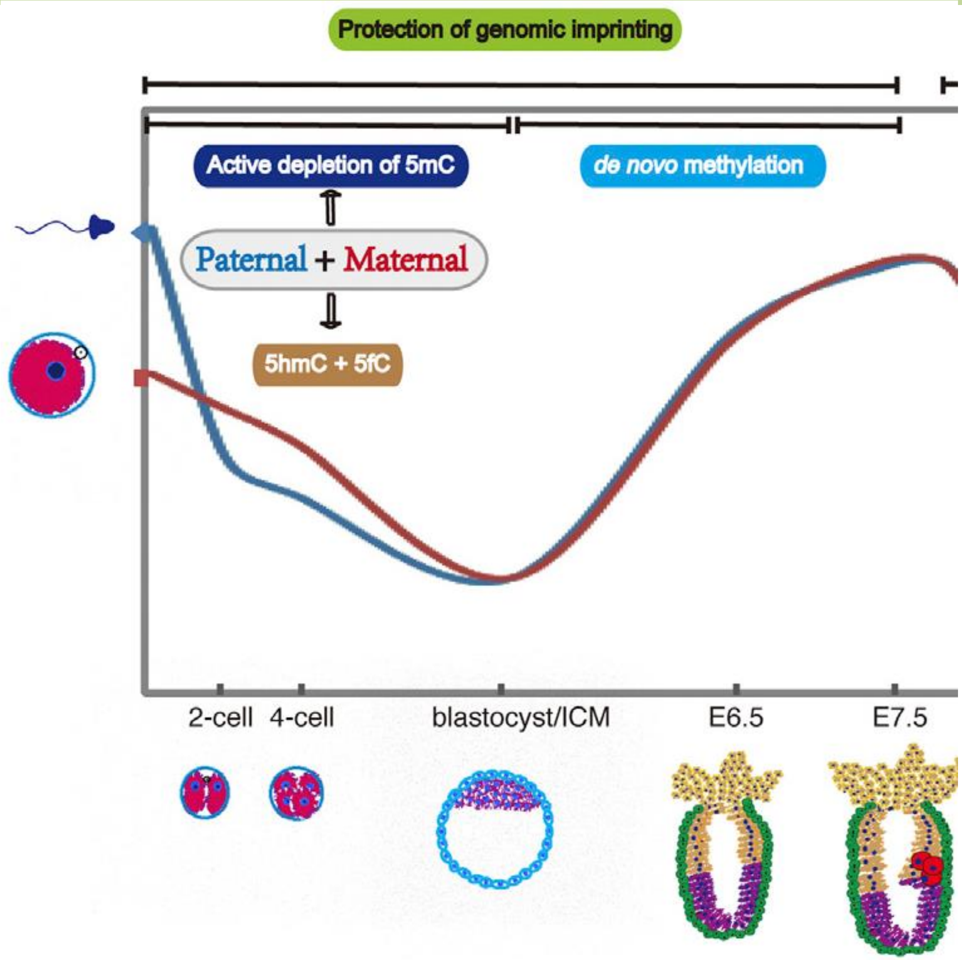
During post- fertilization reprogramming, the embryo loses gamete- specific DNA methylation patterns inherited from the oocyte and the sperm.

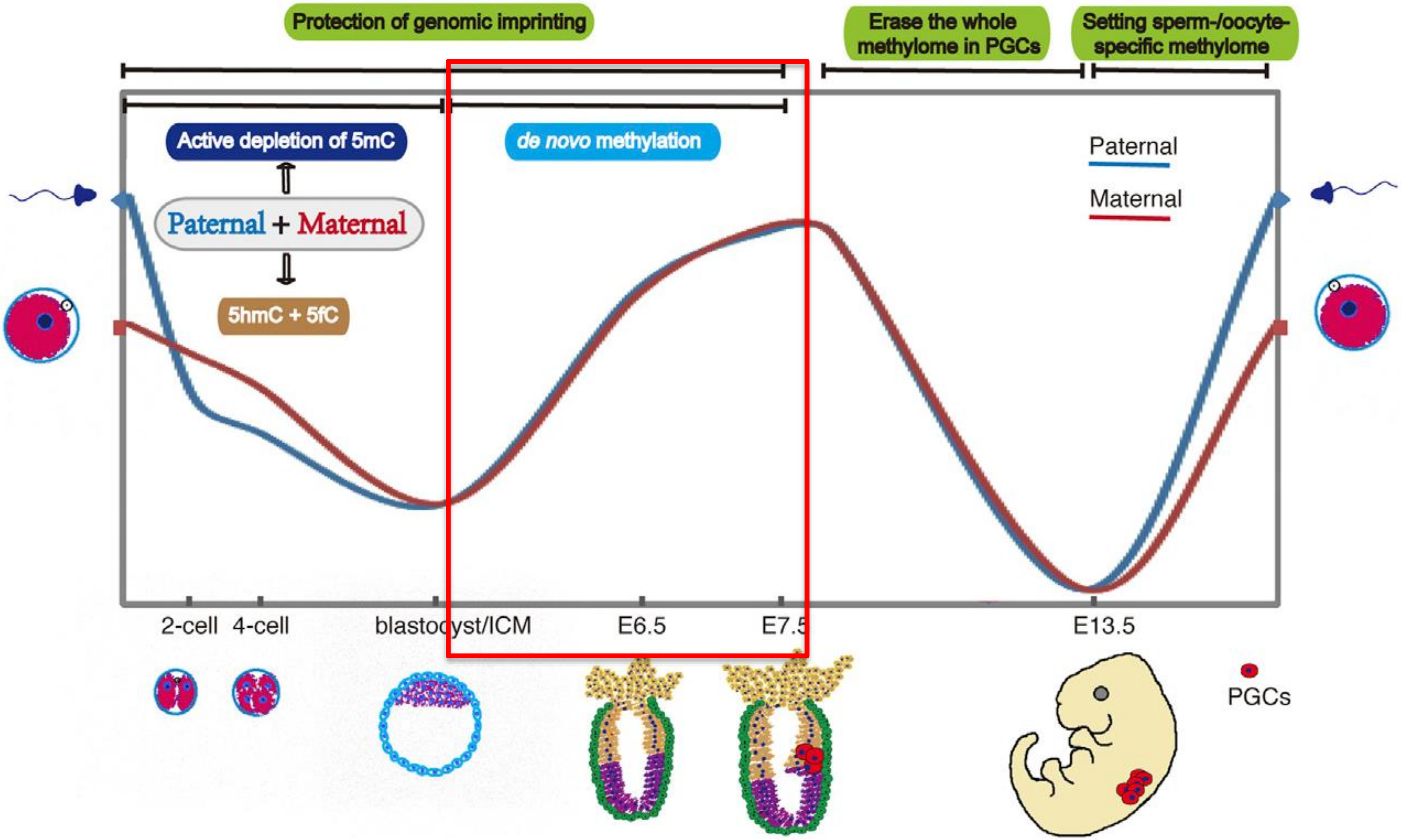
The paternal genome is actively demethylated within a few hours after fertilization.

Maternal genome is passively demethylated by a replication-dependent mechanism after the two-cell embryo stage, as the maintenance enzyme DNMT1 provided by the oocyte is excluded from the nucleus during subsequent cell divisions.

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





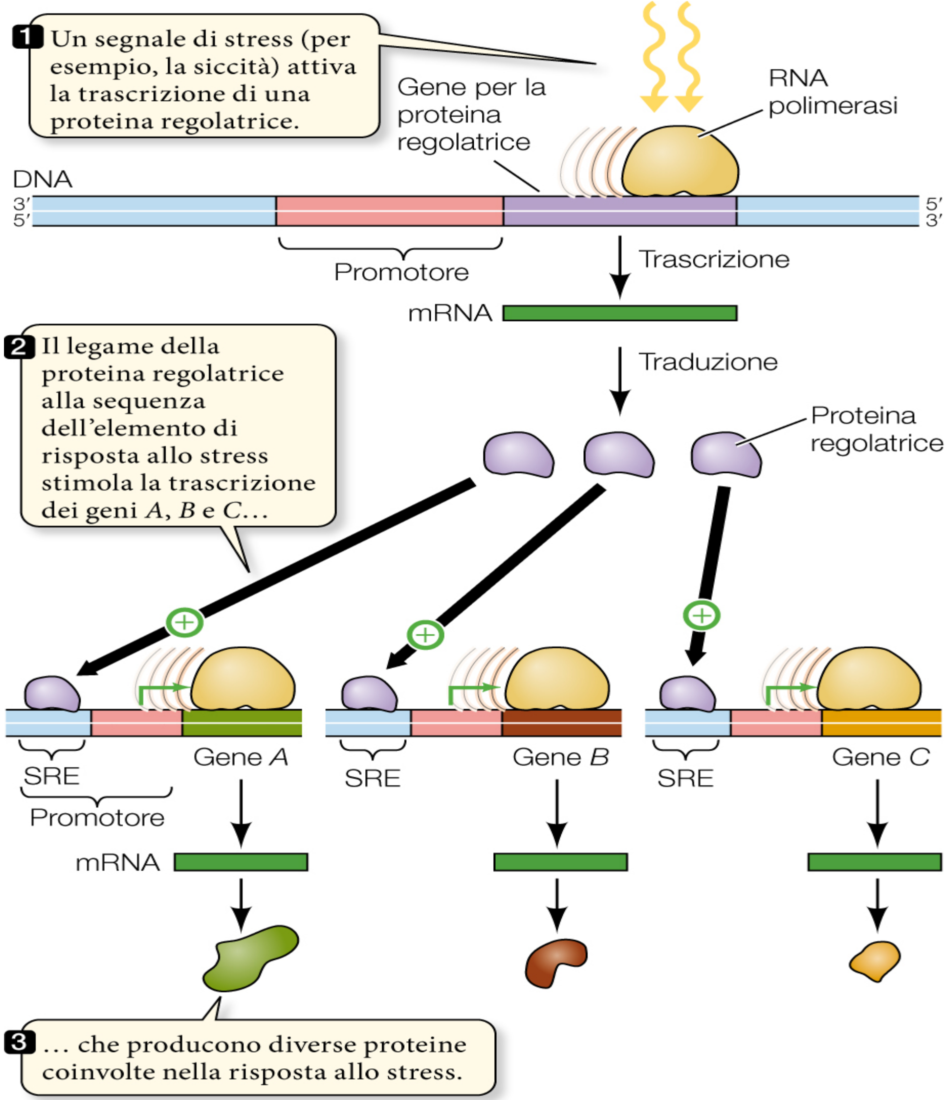
Following this dramatic global demethylation wave, the *de novo* methyltransferases, **DNMT3A** and **DNMT3B**, rapidly re-establish high levels of methylation.
By E6.5, the primed stem cells in the epiblast will then further differentiate into the somatic lineages, which will globally maintain the pattern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

- A parita’ di sequenza del DNA in tutte le cellule di un organismo, come si spiega la tessuto specificita’ di espressione di alcuni geni?

- In altre parole……

- Come l’insulina viene espressa solo e soltanto dalle cellule beta del pancreas e non in altre cellule?

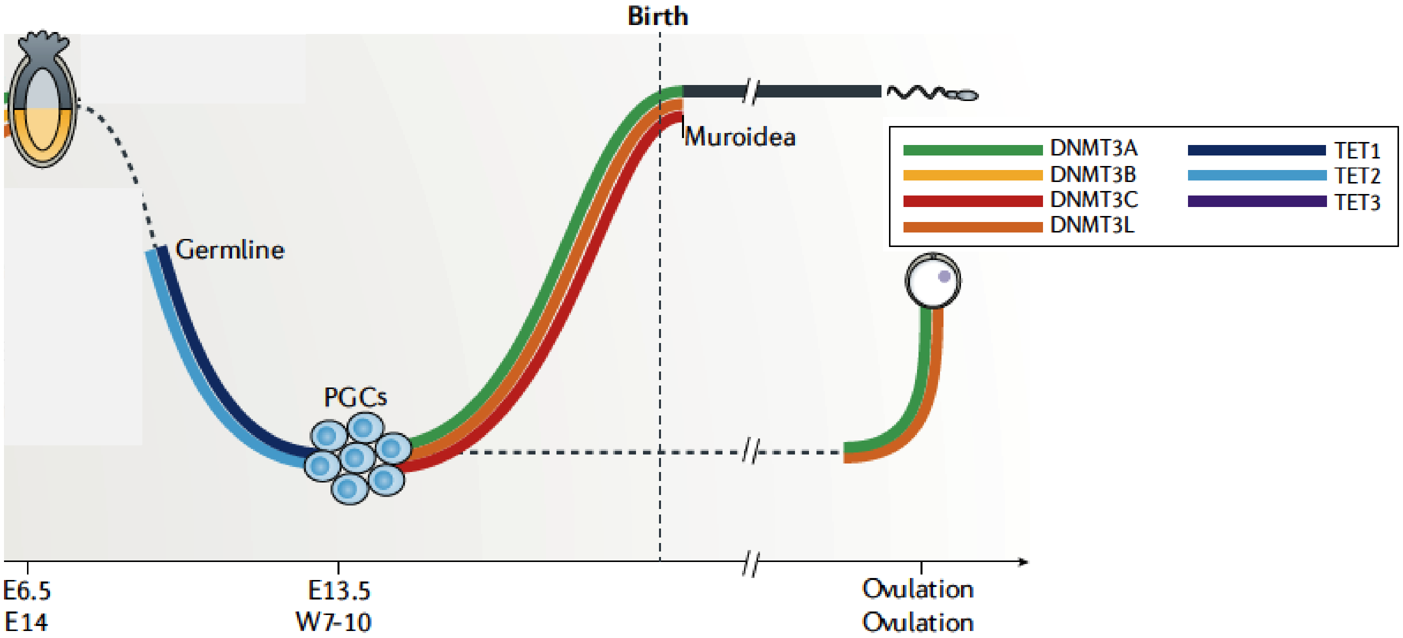
La regolazione durante la trascrizione: coordinazione fra geni



La coordinazione dell'espressione di più geni avviene grazie a un singolo segnale ambientale, che induce la sintesi di una proteina regolatrice della trascrizione.

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 PASSIVE DNA demethylation, 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.

American Association for the Advancement of Science

SCIENCE

19 December 1997 $7.00
Vol. 278 • Pages 2021–2192

Breakthrough of the Year
CLONING

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.

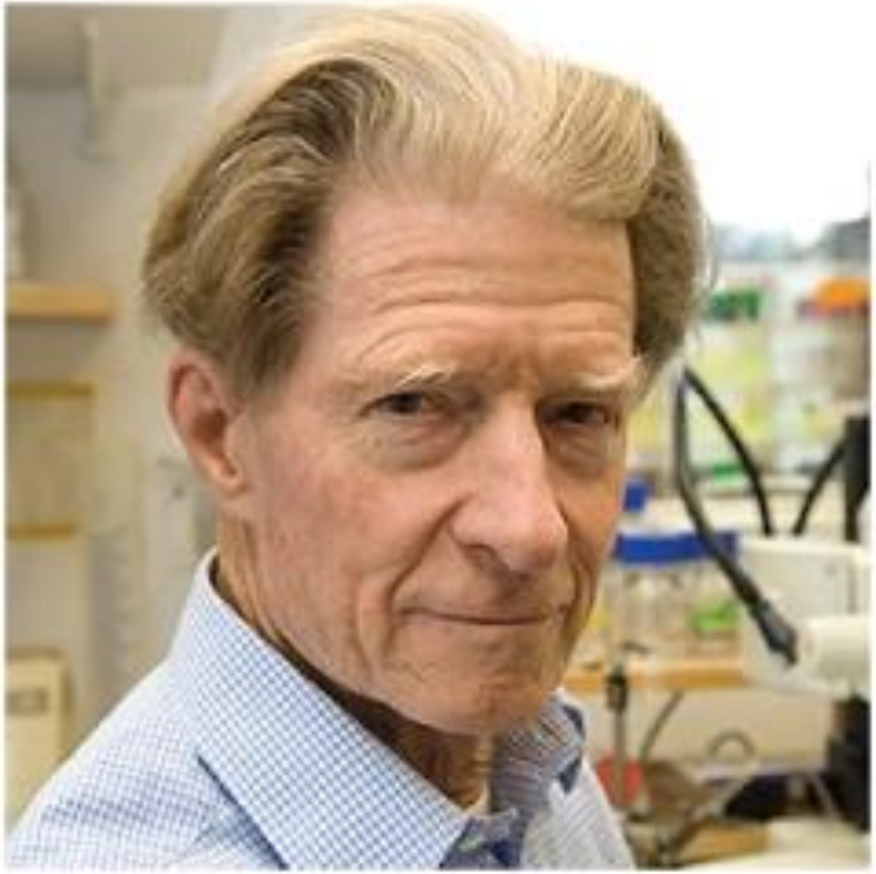
In 2006, Yamanaka with four defined transcription factors induced intact mouse somatic cells to revert to a pluripotent state without an egg or embryo as intermediary.


Shinya
Yamanaka
Turku, Finland,
2016

True, living people!!!

2012 Nobel Prize in Physiology or Medicine

Shinya Yamanaka
University of Kyoto, Japan
Photo Credit:
Center for iPS cell Research and Application, Kyoto University



John B. Gurdon
Gurdon Institute in Cambridge, UK

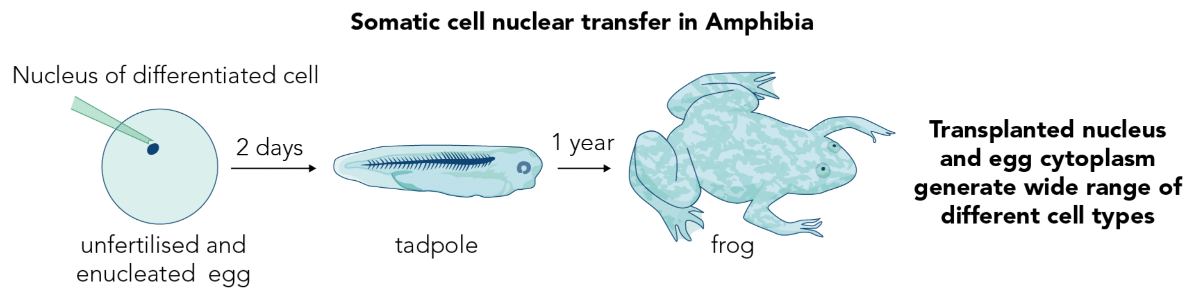
John Gurdon

Distinguished group leader

Research summary

Nuclear reprogramming by oocytes and eggs

Can we make cell reprogramming more efficient? Our group focuses on somatic cell nuclear transfer to amphibian eggs and oocytes from two complementary points of view. One aims to identify the molecules and mechanisms by which the cytoplasm of an egg or oocyte can reprogramme the nucleus of a differentiated somatic cell to behave like that of an embryo. From this state, many different kinds of cells for replacement can be generated.



Somatic cell nuclear transfer in Amphibia

Transplanted nucleus and egg cytoplasm generate wide range of different cell types

October 7th, 2025

Nobel Laureate Professor Sir John Gurdon dies aged 92

It is with great sadness that the University shares the news of the death of Professor Sir John Gurdon, founder of the Gurdon Institute.

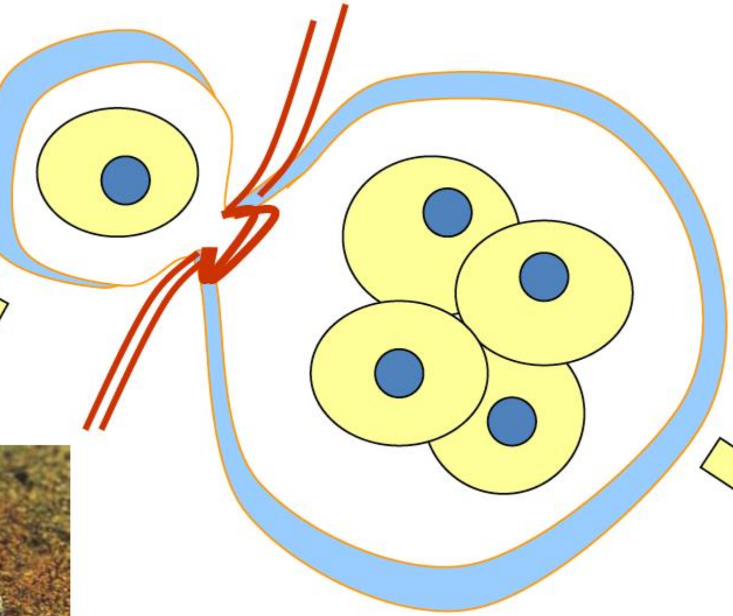
“Ho questo rapporto scolastico, piuttosto sorprendente, che grosso modo dice che ero il peggior studente a cui l’insegnante di biologia avesse mai insegnato”

JOHN GURTON

FARMACEUTICAYOUNGER.SCIENCE

A cloning history

Hans Spemann's experiment (1928)



Blastomeri prima della
Attivazione genoma embrionario
Sono totipotenti

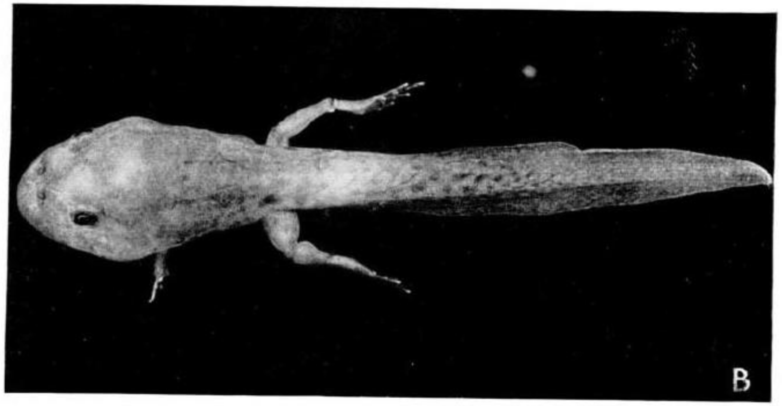
L’esperimento “fantastico” realizzato nel 1953 da Briggs & King

Transplantation of Living Nuclei of Late Gastrulae
into Enucleated Eggs of Rana pipiens
by THOMAS j . KING and ROBERT BRIGGS
1953

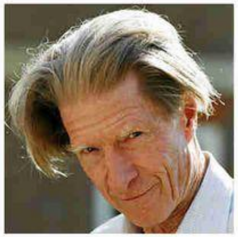
Robert Briggs (1911-1983)


Tadpole (girini) derivati da
trapianto nucleare di cellule
Embrionali in oociti enucleati

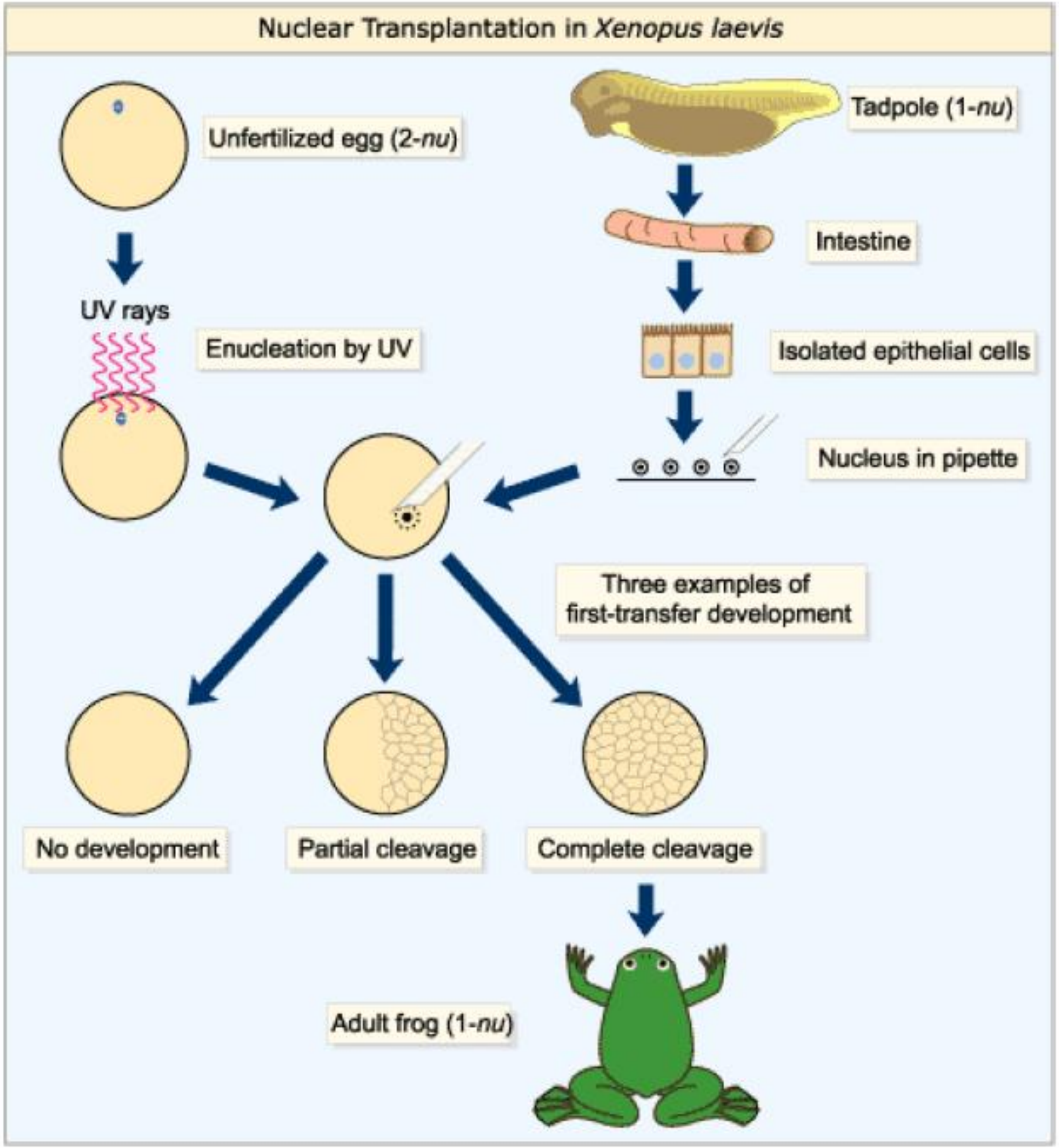
Thomas King 1921-2000



The Nobel Prize in Physiology or Medicine 2012



John B. Gurdon



Nuclear Transplantation in *Xenopus laevis*

Unfertilized egg (2-*nu*)

UV rays

Enucleation by UV

Tadpole (1-*nu*)

Intestine

Isolated epithelial cells

Nucleus in pipette

Three examples of
first-transfer development

No development

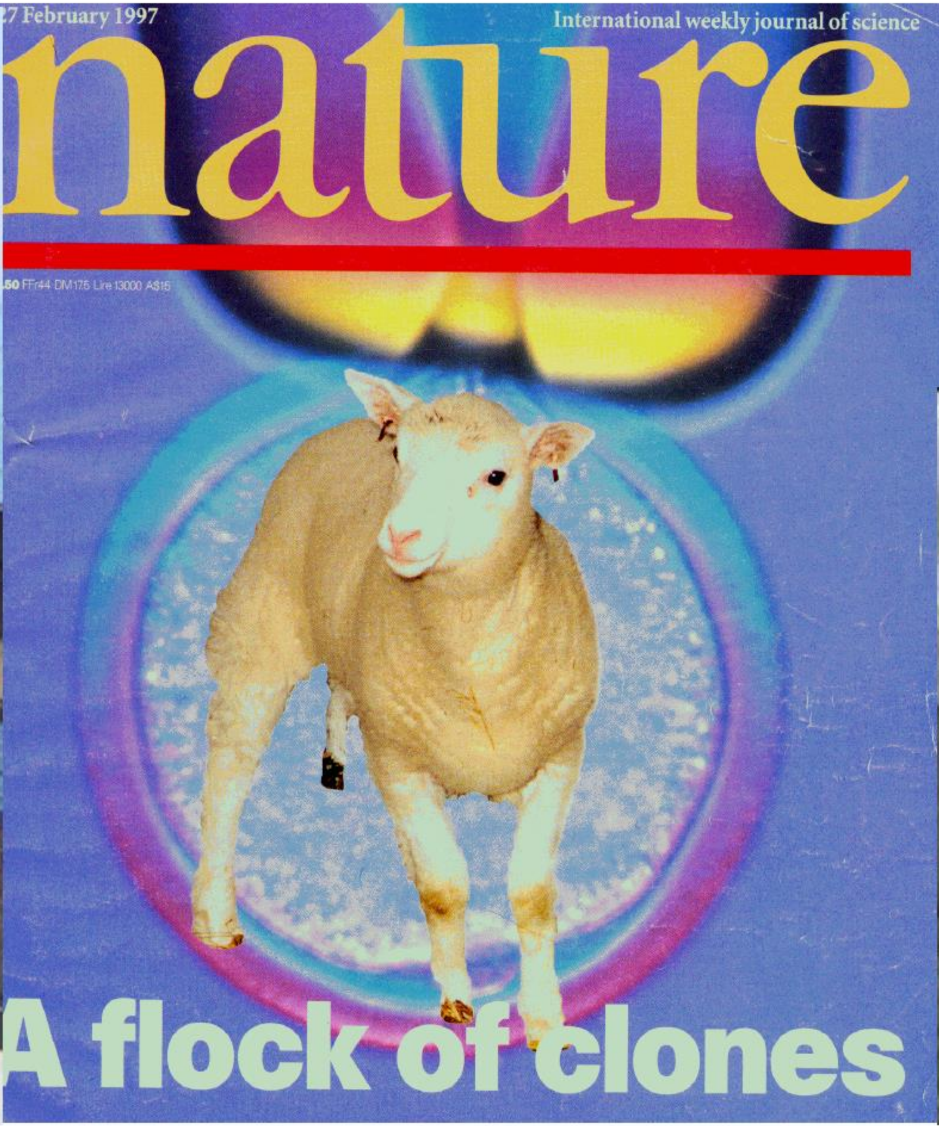
Partial cleavage

Complete cleavage

Adult frog (1-*nu*)

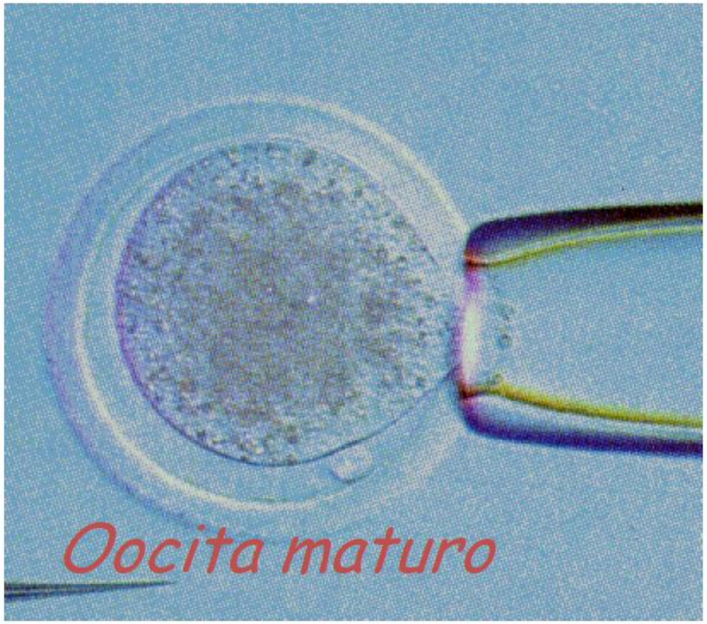
L'esperimento di
John Gurdon
Ha stabilito la
"totipotenza"
del genoma in
tutte le cellule

Keith Campbell, 1954-2012

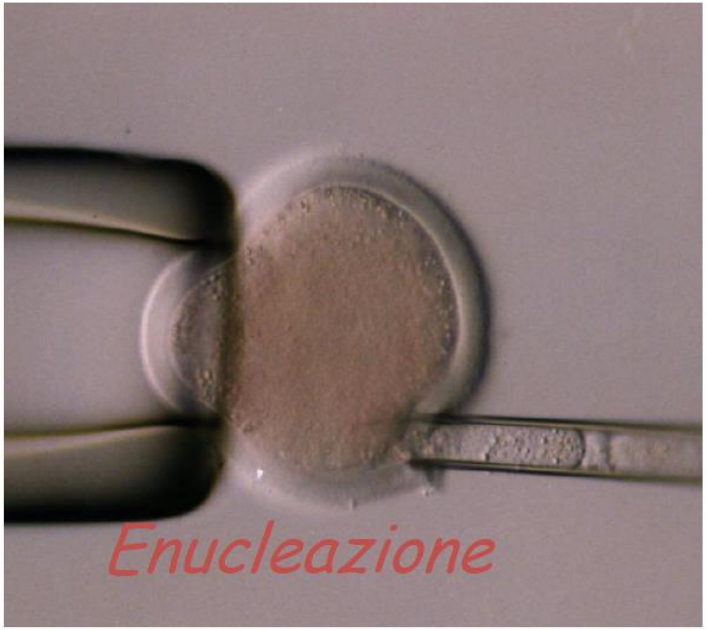


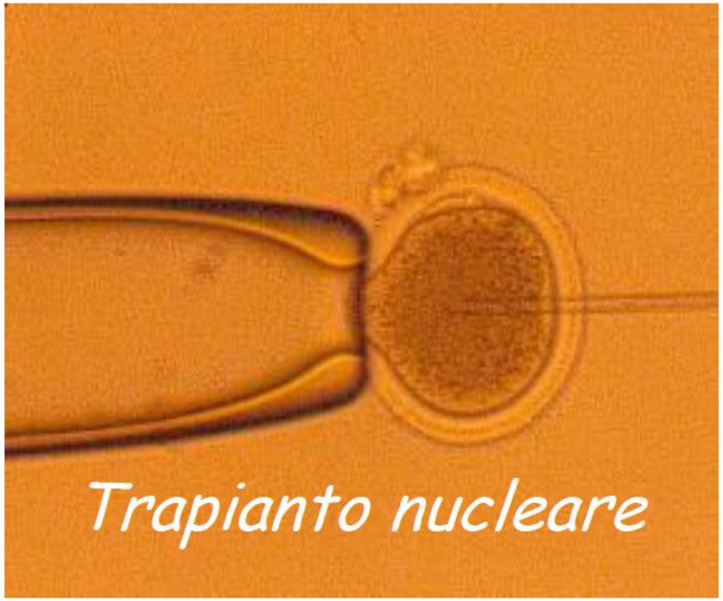
Ian Wilmut, 1943-

Clonazione con cellule somatiche


Oocita maturo


Conferma enucleazione


Enucleazione


Trapianto nucleare

BIOLOGY OF REPRODUCTION **50**, 1385–1393 (1994)

Improved Development to Blastocyst of Ovine Nuclear Transfer Embryos Reconstructed during the Presumptive S-Phase of Enucleated Activated Oocytes

K.H.S. CAMPBELL,[1] P. LOI,[2] P. CAPPAI,[2] and I. WILMUT

AFRC Roslin Institute (Edinburgh), Roslin, Midlothian EH25 9PS, United Kingdom

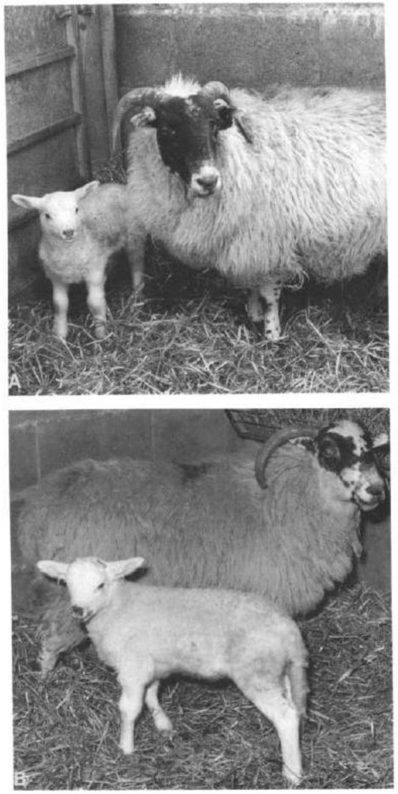


FIG. 4. Lambs born after transfer to final recipients of blastocysts derived from nuclear transfer reconstructed embryos. A) Lamb derived from the control group (0 hpa); B) lamb derived from an embryo reconstructed during the late S-phase (16–18 hpa) of enucleated activated MII oocytes.

Cloni di 7-15 animali normali ottenibili da un singolo embrione……
(Loi, et al. Biology of Reproduction 1998)

La pecora Dolly

Dolly Patton, American folk singer……..

Morto il «padre» della pecora Dolly: Ian Wilmut, il primo a clonare un mammifero

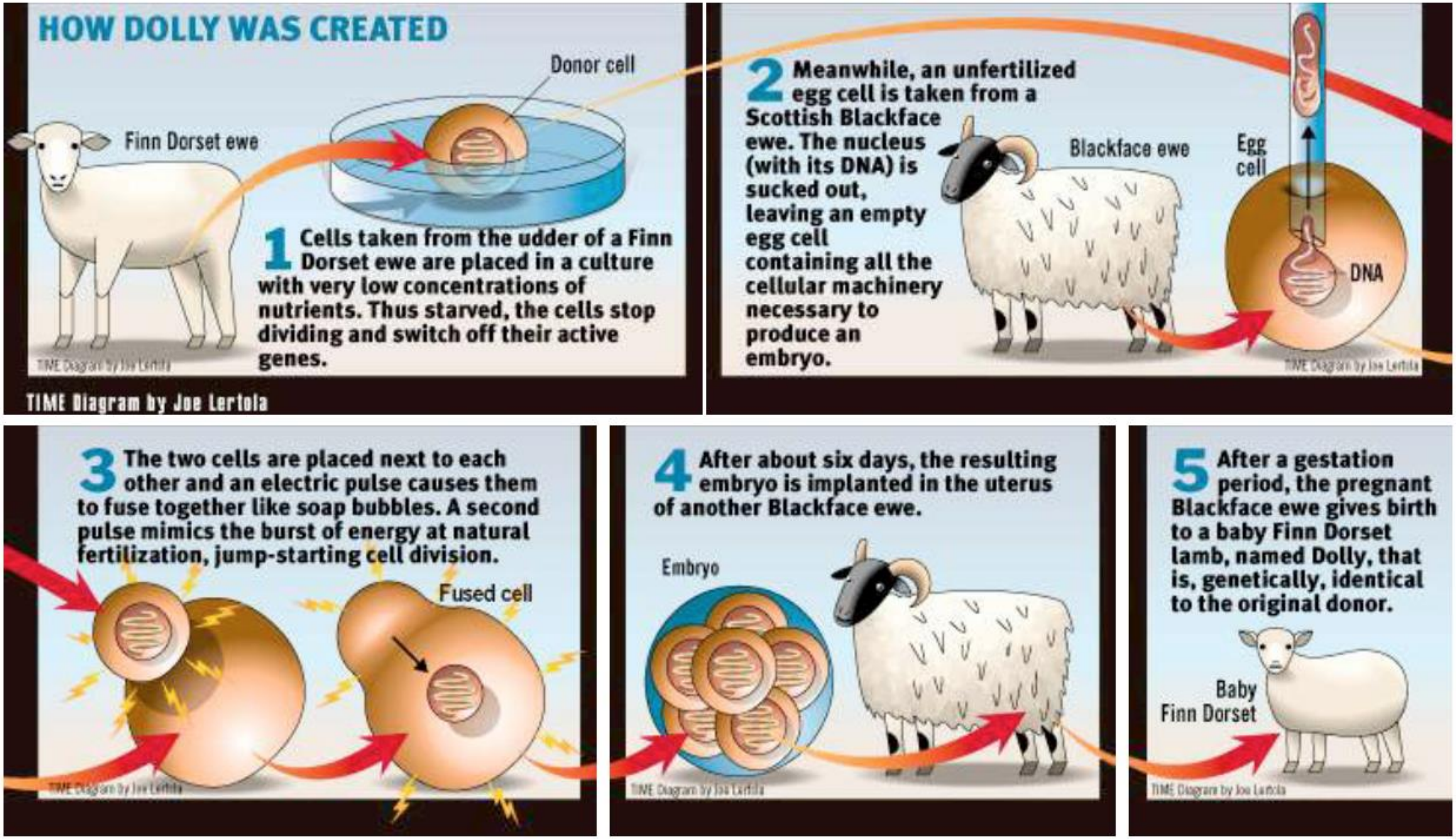
di **Paolo Virtuani**

Nel 1996 riuscì nell'impresa allora ritenuta impossibile ma che ha aperto nuove strade nella medicina rigenerativa. Il nome scelto in onore della cantante Dolly Parton

Ian Wilmut con la pecora Dolly (Un. di Edimburgo)

23 settembre 2023

Animal cloning by nuclear transfer



HOW DOLLY WAS CREATED

1 Cells taken from the udder of a Finn Dorset ewe are placed in a culture with very low concentrations of nutrients. Thus starved, the cells stop dividing and switch off their active genes.

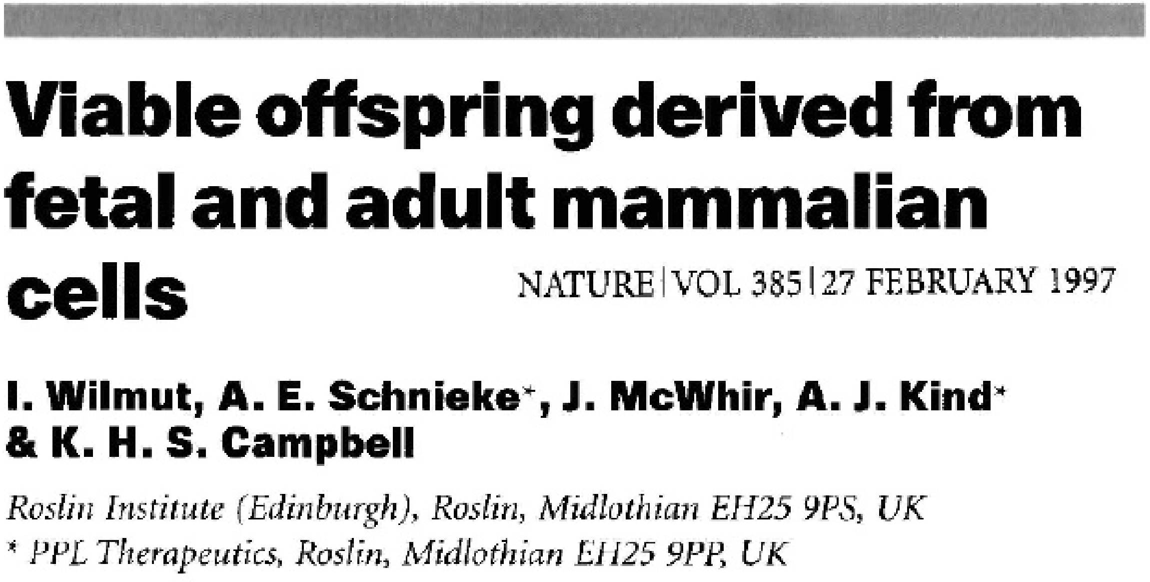
2 Meanwhile, an unfertilized egg cell is taken from a Scottish Blackface ewe. The nucleus (with its DNA) is sucked out, leaving an empty egg cell containing all the cellular machinery necessary to produce an embryo.

3 The two cells are placed next to each other and an electric pulse causes them to fuse together like soap bubbles. A second pulse mimics the burst of energy at natural fertilization, jump-starting cell division.

4 After about six days, the resulting embryo is implanted in the uterus of another Blackface ewe.

5 After a gestation period, the pregnant Blackface ewe gives birth to a baby Finn Dorset lamb, named Dolly, that is, genetically, identical to the original donor.

TIME Diagram by Joe Lertola



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.

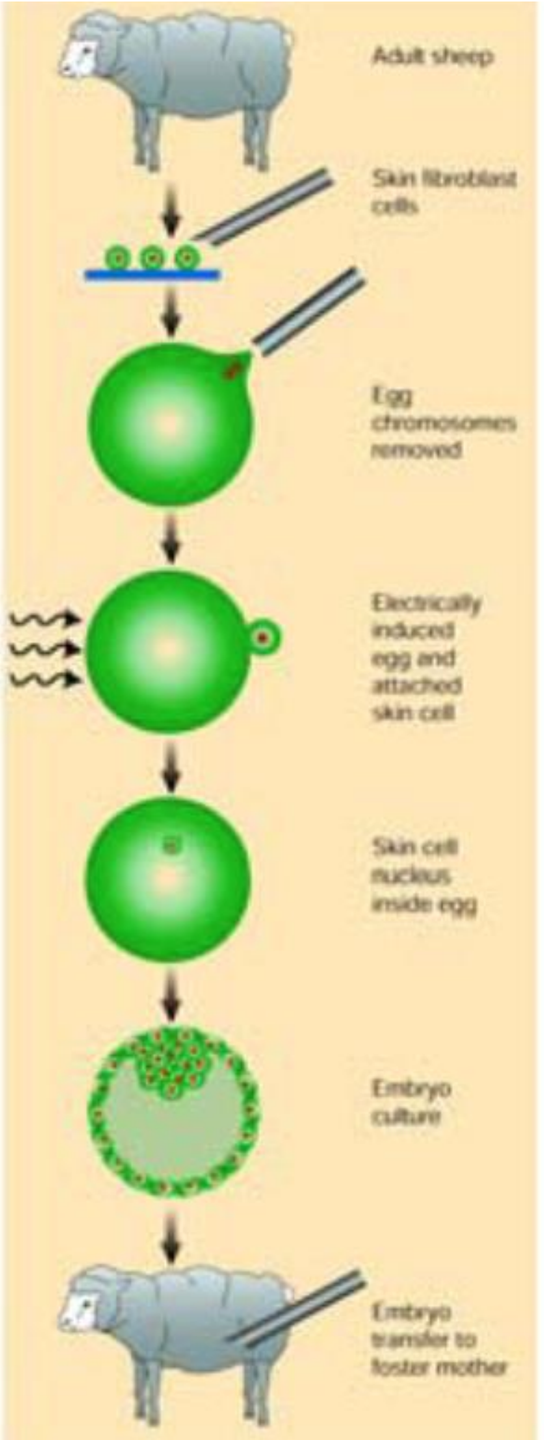
In tutti tre i casi, le cellule donatrici erano state indotte ad entrare uno stato di quiescenza replicativa (G_0) mediante riduzione della concentrazione di siero fetale bovino dal 10% a 5% per i 5 giorni precedenti il trasferimento nucleare. L'uscita dal ciclo era stata confermata mediante la ricerca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

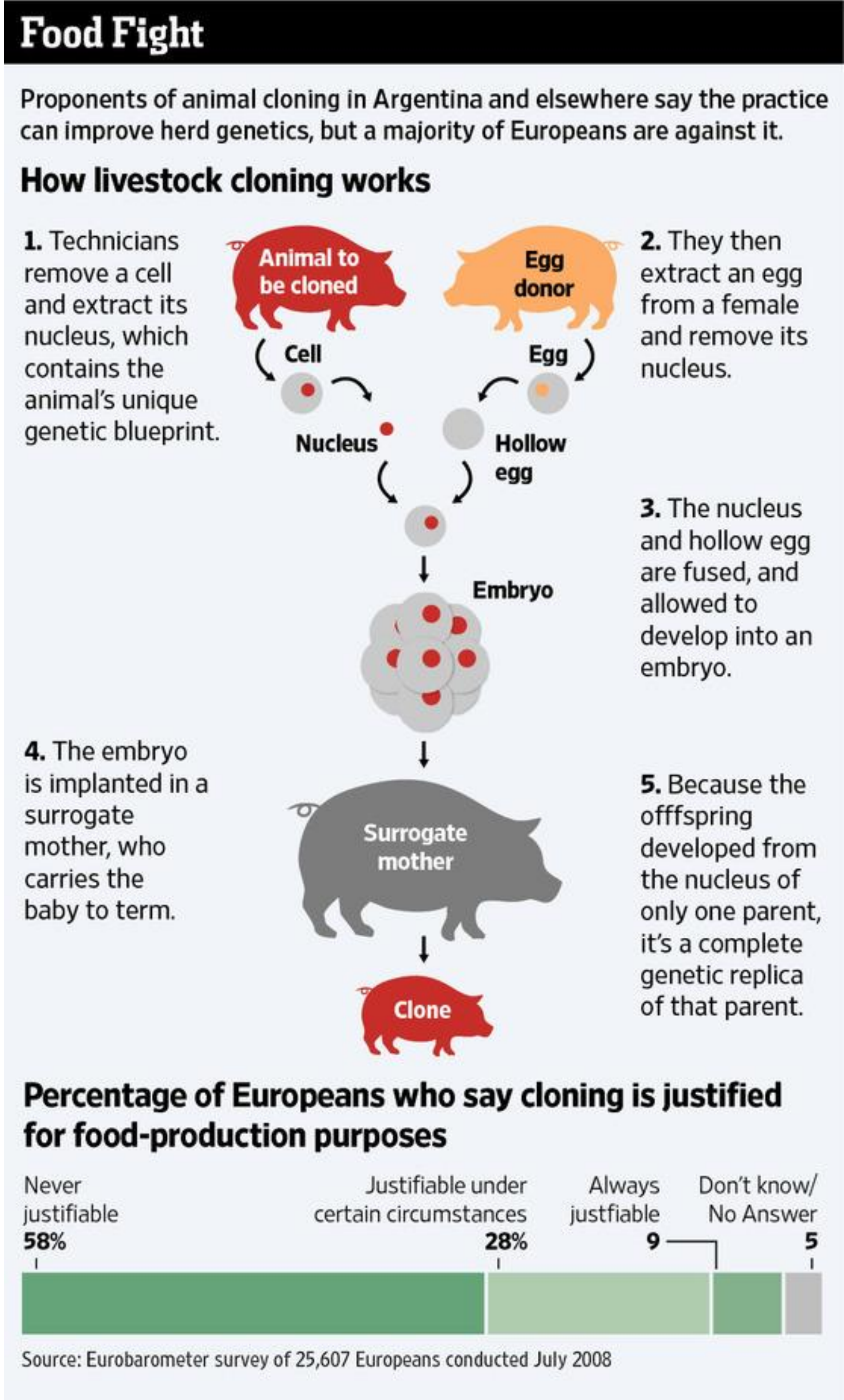
247 oociti ricostruiti coltivati all'interno delle ovidotti ligati di una pecora
29 (11.7%) progrediti allo stadio di morula/blastocisti dopo 6 giorni di coltura, e trasferiti in 13 pecore sincronizzate riceventi per lo sviluppo a termine.
1 embrione (0.4% del totale; 3.4% degli embrioni trasferiti)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



Perchè clonare le pecore?

La pecora Dolly fu prodotta al Roslin Institute come parte di una ricerca per la produzione di medicinali nel latte degli animali da allevamento. I ricercatori sono riusciti a trasferire i geni umani che producono utili proteine, nelle pecore e nelle mucche in modo che esse possano produrre, per esempio, il fattore IX agente coagulante del sangue per curare l'emofilia o la proteina alfa-1-antitripsina per curare la fibrosi cistica e altre patologie polmonari.



Food Fight

Proponents of animal cloning in Argentina and elsewhere say the practice can improve herd genetics, but a majority of Europeans are against it.

How livestock cloning works

1. Technicians remove a cell and extract its nucleus, which contains the animal's unique genetic blueprint.

2. They then extract an egg from a female and remove its nucleus.

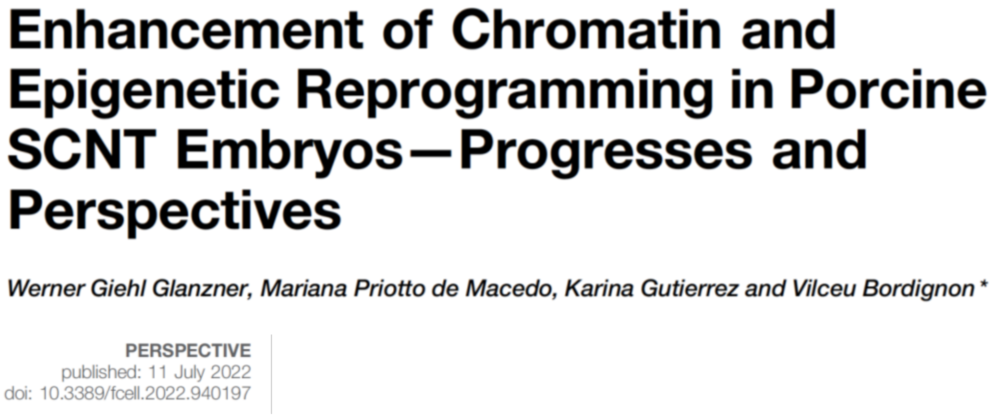
3. The nucleus and hollow egg are fused, and allowed to develop into an embryo.

4. The embryo is implanted in a surrogate mother, who carries the baby to term.

5. Because the offfspring developed from the nucleus of only one parent, it's a complete genetic replica of that parent.

Percentage of Europeans who say cloning is justified for food-production purposes

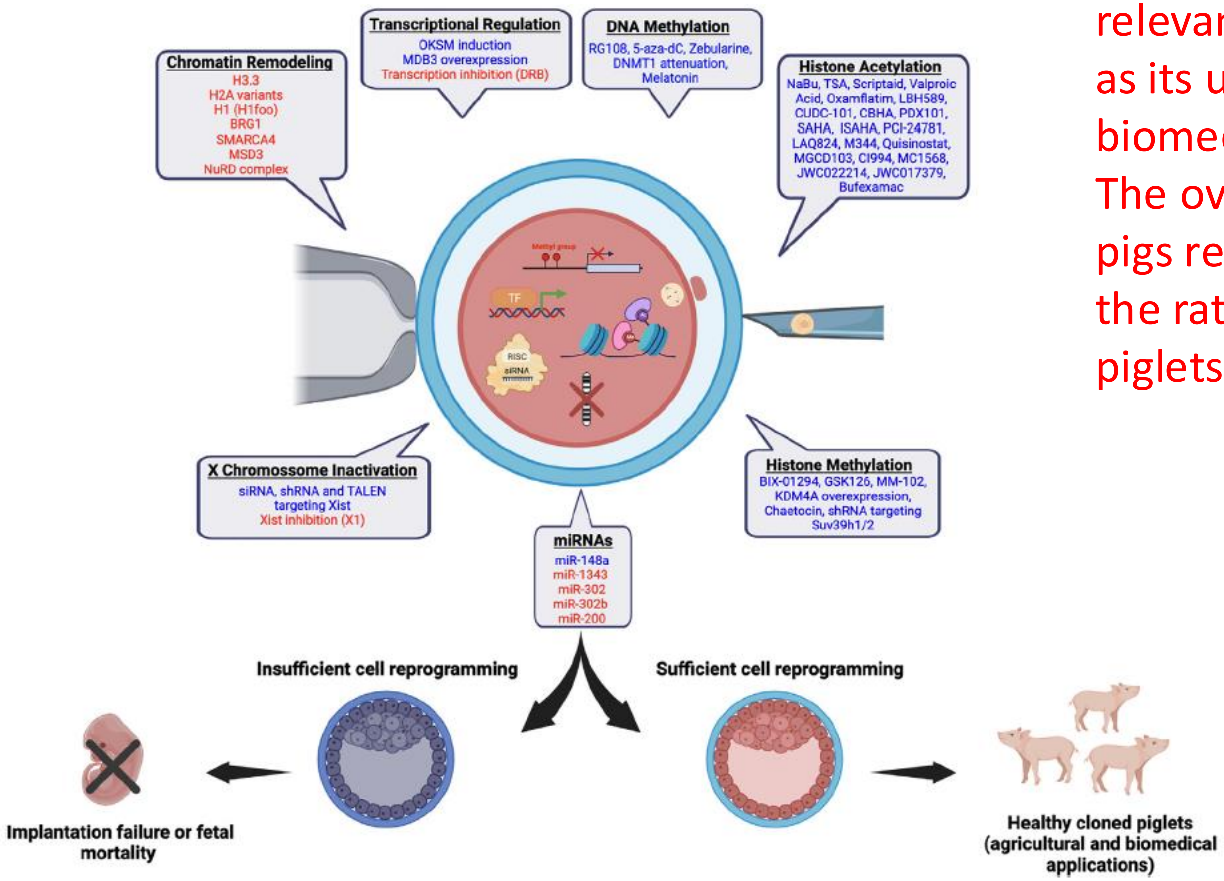
Source: Eurobarometer survey of 25,607 Europeans conducted July 2008



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



The greater interest in pig cloning has two main reasons, its relevance for food production and as its use as a suitable model in biomedical applications.

The overall efficiency of SCNT in pigs remains very low, based on the rate of healthy, live born piglets following embryo transfer.

CLONING TIMELINE

1952

Robert Briggs and Thomas King in Philadelphia, Pennsylvania, describe how they cloned frogs (*Rana pipiens*) by replacing the nuclei of eggs with cells from tadpoles and adult intestinal epithelium. A similar experiment was first proposed by Hans Spemann at the University of Freiberg, Germany, in 1938.

1984

Chinese researchers clone a fish — the crucian carp (*Carassius carassius*) — from cultured kidney cells.

1996

Researchers at the Roslin Institute in Scotland clone two lambs — Megan and Morag — from embryonic cells. This was a crucial step towards cloning an animal from an adult cell, and is seen by some scientists as a bigger breakthrough than Dolly herself.

1997

Roslin researchers announce the birth of Dolly the sheep, the first mammal to be cloned from an adult cell, igniting public debate about the prospects for cloning humans.

1998

Scientists at the University of Hawaii reveal the cloning of three generations of mice from the nuclei of adult cells, suggesting the technique could work on other mammals.

1998

Japanese researchers report cloning eight calves using adult cells from slaughterhouse entrails, raising the possibility that animals could be cloned for the quality of their meat.

1998

Scientists in New Zealand announce Elsie, a clone created from an adult cell from the last surviving Enderby Island cow (*Bos gaurus*). Attempts to clone endangered species have met with criticism that the technique will do little good without concurrent habitat preservation.

2000

PPL Therapeutics in Scotland unveils a litter of five cloned piglets. The firm says that genetically engineered cloned pigs could one day provide a source of organ transplants for humans.

2002

The first cloned cat (*Felis domesticus*), named cc for 'copycat', is announced by Texas A&M researchers. Cc's coat pattern is not the same as her genetic donor's, showing the impact on development of non-genetic effects.

2003

Italian scientists at the Laboratory of Reproductive Technology in Cremona announce Prometea, the first horse (*Equus caballus*) clone created from a skin cell, raising hopes that clones could one day perpetuate the genetic line of castrated geldings.

2003

French and Chinese scientists unveil Ralph the cloned laboratory rat (*Rattus norvegicus*). Rats had been tough to clone because rat eggs divide before the point at which the donor DNA is injected, so the technique relied on using drugs to inhibit division.

2004

Although Seoul National University researcher Woo Suk Hwang's claim to have derived stem-cell lines from cloned human embryos was later discredited, his group can still boast the most experience, and probably the highest number of cloned human embryos, but there is no hard evidence for this.

2005

Hwang's lab announces Snuppy the cloned dog. Although much of the stem-cell research from this lab has been discredited, Snuppy's clonal credentials have been confirmed.

Heidi Ledford

Clonazione riproduttiva: appplicazioni in:

Riproduzione animale

Animali transgenici

Moltiplicazione di specie minacciate di estinzione

..però….bassa efficienza della clonazione 1-5%

Clonate praticamente tutte le specie da reddito e di affezione

1996

1998

1998

1999

PPL Therapeutics Via AP

2000

2001

2003

2003

2003

Clonati anche animali “non convenzionali”

Medaka Fish

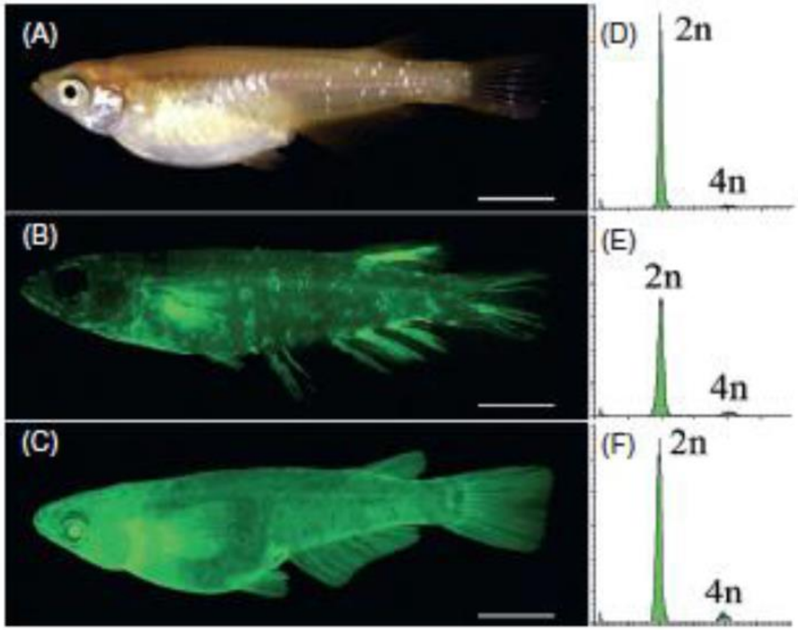


FIGURE 15.4 Three types of adult nuclear transplants generated in the second examination. (A) Donor clone, NT4. (B) Fluorescent image of chimeric fish, NT5. (C) Fluorescent image of NT fish originated from parthenogenesis of the recipient egg, NT1. (D–F) Ploidy analysis by flow cytometry showing the diploidy of adult fish: (D) NT4, (E) NT5, (F) NT1. Bars represent 5 mm. *From* Bubenshchikova et al., (2008).

zebrafish

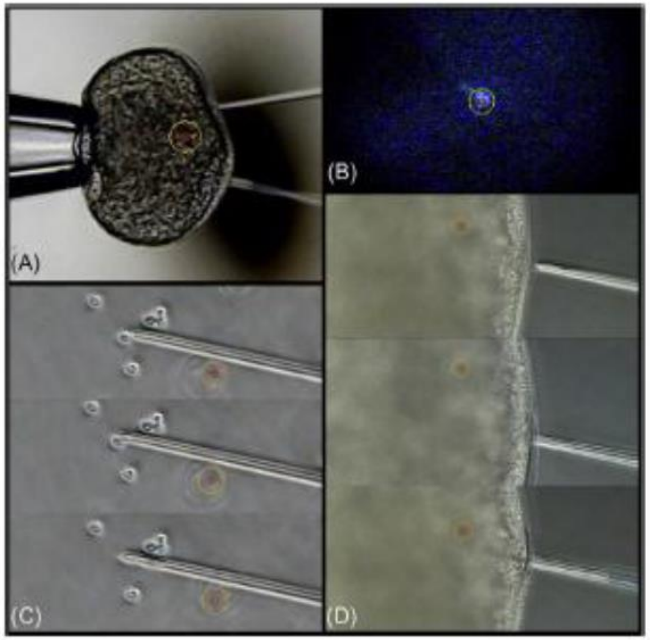


FIGURE 16.2 Method for somatic cell nuclear transfer in zebrafish. The egg micropyle is positioned facing the bottom of a Petri dish to visualize the metaphase plate (A). The Hoechst 33342-stained metaphase plate of the egg is ablated using the laser XY clone module (B). The areas under red and yellow circles, when the laser-pulse is introduced by a 40× laser-equipped objective lens, would be at 400°C and 100°C, respectively. An individual donor cell is picked up using a beveled-tip needle (C) and is then transferred through a micropyle to an animal pole of the egg (D).

Clone di bovini (Nuova Zelanda)

Clonazione riproduttiva: appplicazioni in:

Riproduzione animale

Animali transgenici

Moltiplicazione di specie minacciate di estinzione

..però….bassa efficienza della clonazione 1-5%


A
B
C
D
E
F
G
H
I

Clonare animali in via di estinzione

5485 animals specie animali in via di estinzione,
180 sono mammiferi

3500 razze locali minacciate di estinzione (FAOglobal survey 2005)

Current Biology 2002

News focus

Can cloning save endangered species?

SCIENCE'S COMPASS

POLICY FORUM: ECOLOGY

DNA Banks for Endangered Animal Species

Oliver A. Ryder, Anne McLaren, Sydney Brenner, Ya-Ping Zhang, Kurt Benirschke

Genetic rescue of an endangered mammal by cross-species
nuclear transfer using post-mortem somatic cells
Loi et al., Nature Biotechnology 2001

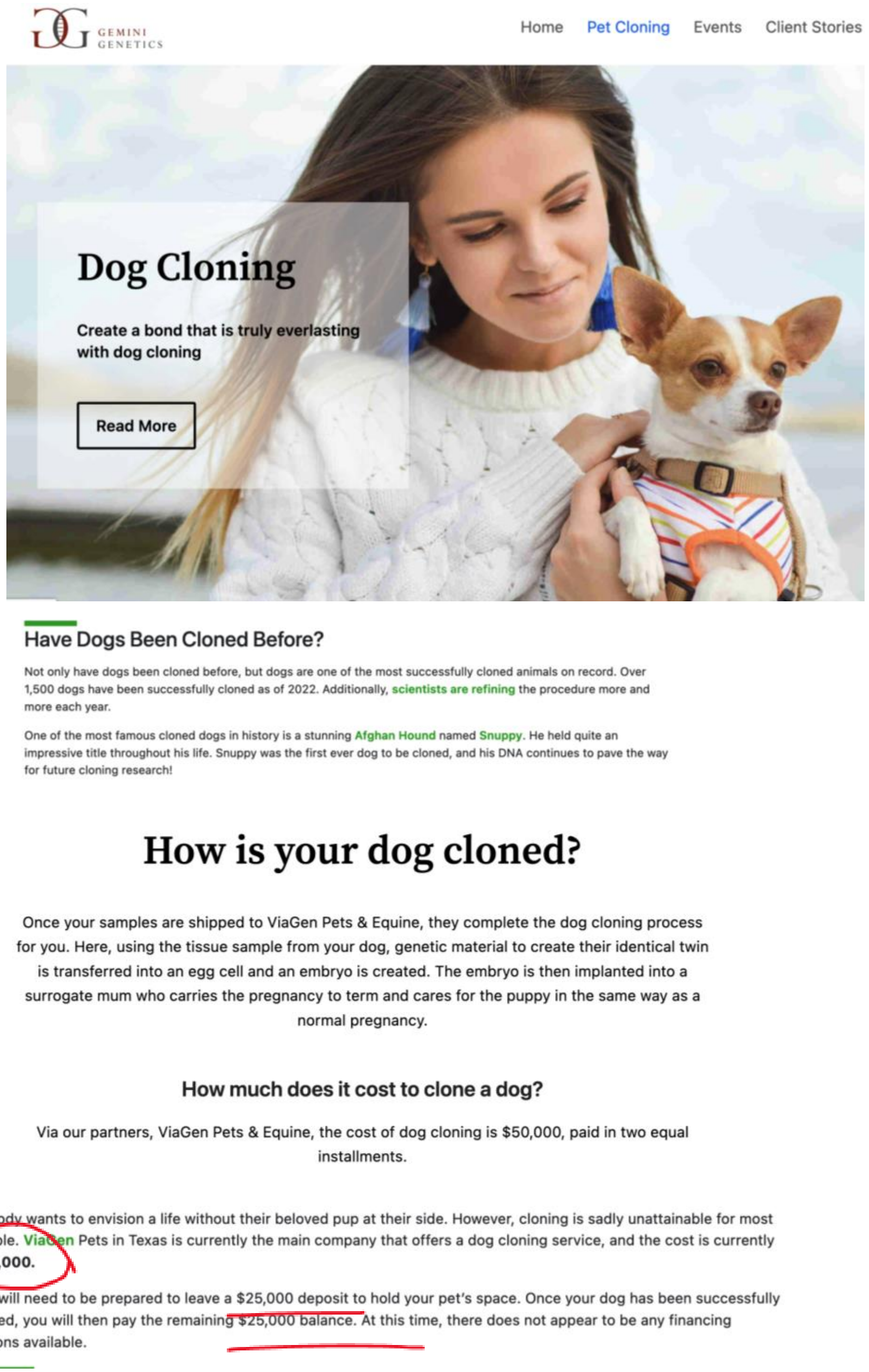
Elevata mortalità neonatale

Pet cloning is getting more popular despite the cost

4 April 2022

John Mendola had his former dog cloned to produce these two genetically identical replicas, pictured

Barbra Streisand pictured with her former dog Samantha in 2006



Dog Cloning

Create a bond that is truly everlasting with dog cloning

Have Dogs Been Cloned Before?

Not only have dogs been cloned before, but dogs are one of the most successfully cloned animals on record. Over 1,500 dogs have been successfully cloned as of 2022. Additionally, scientists are refining the procedure more and more each year.

One of the most famous cloned dogs in history is a stunning Afghan Hound named Snuppy. He held quite an impressive title throughout his life. Snuppy was the first ever dog to be cloned, and his DNA continues to pave the way for future cloning research!

How is your dog cloned?

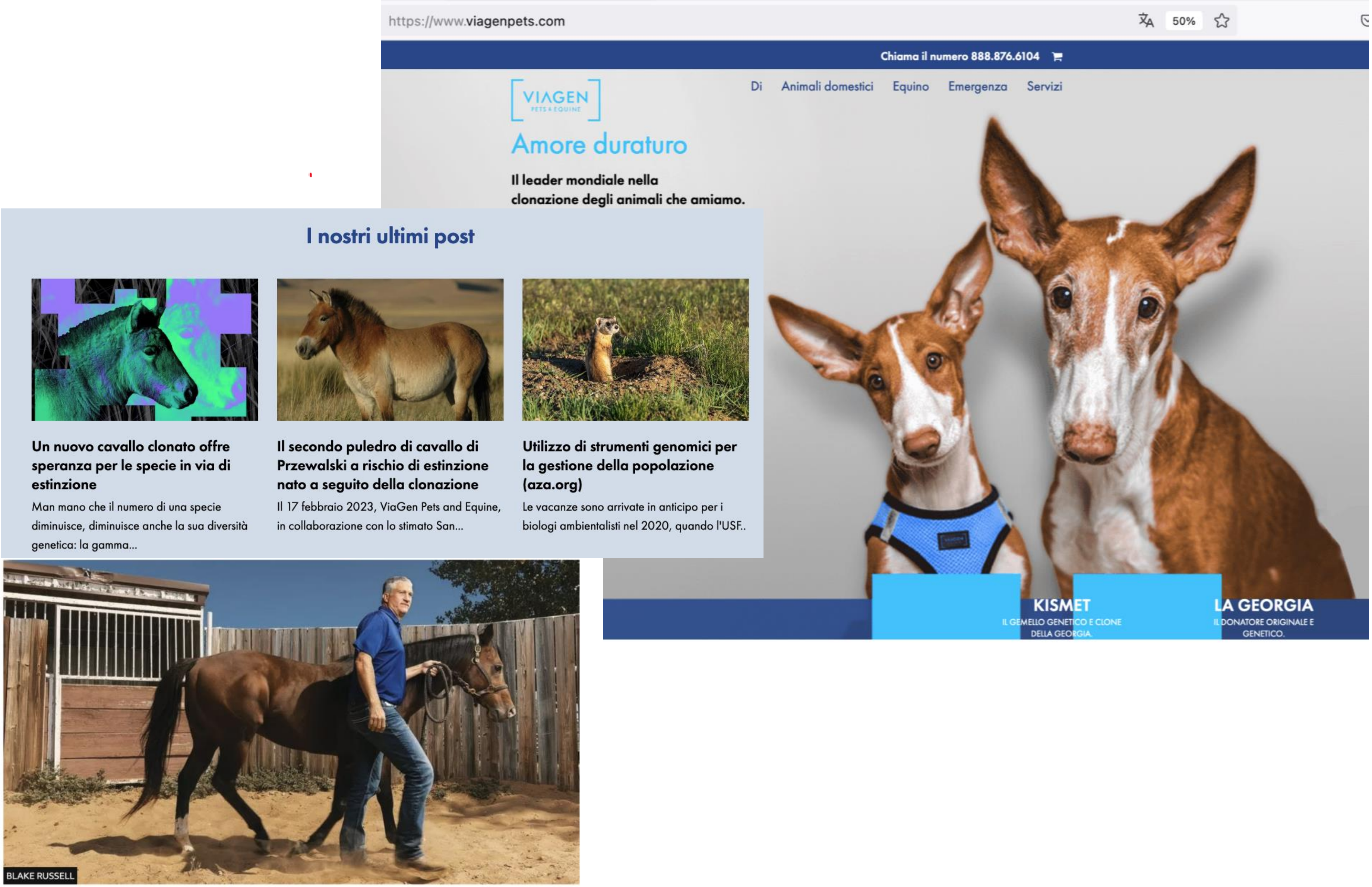
Once your samples are shipped to ViaGen Pets & Equine, they complete the dog cloning process for you. Here, using the tissue sample from your dog, genetic material to create their identical twin is transferred into an egg cell and an embryo is created. The embryo is then implanted into a surrogate mum who carries the pregnancy to term and cares for the puppy in the same way as a normal pregnancy.

How much does it cost to clone a dog?

Via our partners, ViaGen Pets & Equine, the cost of dog cloning is $50,000, paid in two equal installments.

Nobody wants to envision a life without their beloved pup at their side. However, cloning is sadly unattainable for most people. ViaGen Pets in Texas is currently the main company that offers a dog cloning service, and the cost is currently **$50,000.**

You will need to be prepared to leave a $25,000 deposit to hold your pet's space. Once your dog has been successfully cloned, you will then pay the remaining $25,000 balance. At this time, there does not appear to be any financing options available.



Chiama il numero 888.876.6104

Di Animali domestici Equino Emergenza Servizi

VIAGEN PETS & EQUINE

Amore duraturo

Il leader mondiale nella clonazione degli animali che amiamo.

I nostri ultimi post

Un nuovo cavallo clonato offre speranza per le specie in via di estinzione

Man mano che il numero di una specie diminuisce, diminuisce anche la sua diversità genetica: la gamma...

Il secondo puledro di cavallo di Przewalski a rischio di estinzione nato a seguito della clonazione

Il 17 febbraio 2023, ViaGen Pets and Equine, in collaborazione con lo stimato San...

Utilizzo di strumenti genomici per la gestione della popolazione (aza.org)

Le vacanze sono arrivate in anticipo per i biologi ambientalisti nel 2020, quando l'USF..

KISMET

IL GEMELLO GENETICO E CLONE DELLA GEORGIA.

LA GEORGIA

IL DONATORE ORIGINALE E GENETICO.

Blake Russell, pictured here with a horse clone, says genetic material can be safely stored for many years

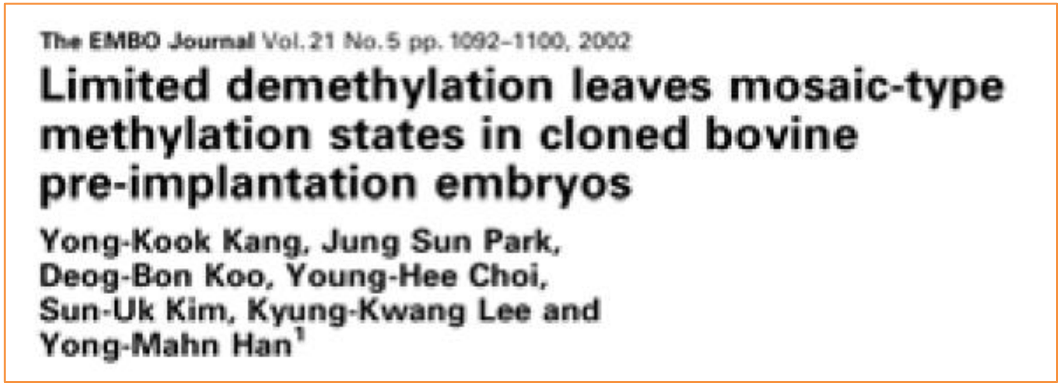
Yet animal welfare organisations have significant concerns about the sector. For example, a number of scientific studies have **suggested that cloned animals are more prone to disease.**

Other critics point to the industry's high failure rate - the large number of clones that are not born fit and healthy. One 2018 report by Columbia University in New York **put the average success rate at just 20%.** This means that you need numerous surrogate mums to allow for multiple attempts.

Che cosa è successo a Dolly?

Dolly, è stata viziata e coccolata al Roslin Institute. Si è accoppiata e ha partorito normalmente dimostrando che gli animali clonati si possono riprodurre. È nata il 5 luglio del 1996 e quando aveva sei anni e mezzo, il 14 febbraio del 2003, è morta con l'eutanasia. Le pecore possono vivere fino a 11 o 12 anni, ma Dolly soffriva di artrite all'articolazione dell'arto posteriore e di adenomatosi polmonare, un tumore del polmone provocato da virus al quale sono soggette le pecore allevate in ambiente chiuso.

I cromosomi di Dolly erano un po' più corti rispetto a quelli di altre pecore, ma sotto molti altri aspetti la pecora Dolly era uguale a qualunque altra pecora della sua eta cronologica.
Tuttavia, il suo precoce invecchiamento potrebbe essewre un'indicazione del fatto che essa è stata riprodotta dal nucleo di una pecora di 6 anni. Studi della sue cellule rivelarono anche che il DNA mitocondriale era stato ereditata dalla cellula uovo e non dal nucleo del donatore come il resto del DNA. Perciò essa non rappresenta una copia completamente identica



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]

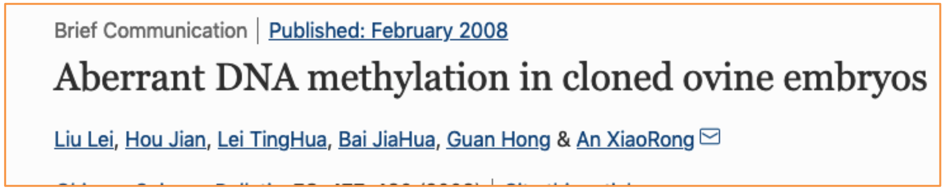
> Nat Genet. 2001 Jun;28(2):173-7. doi: 10.1038/88903.

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

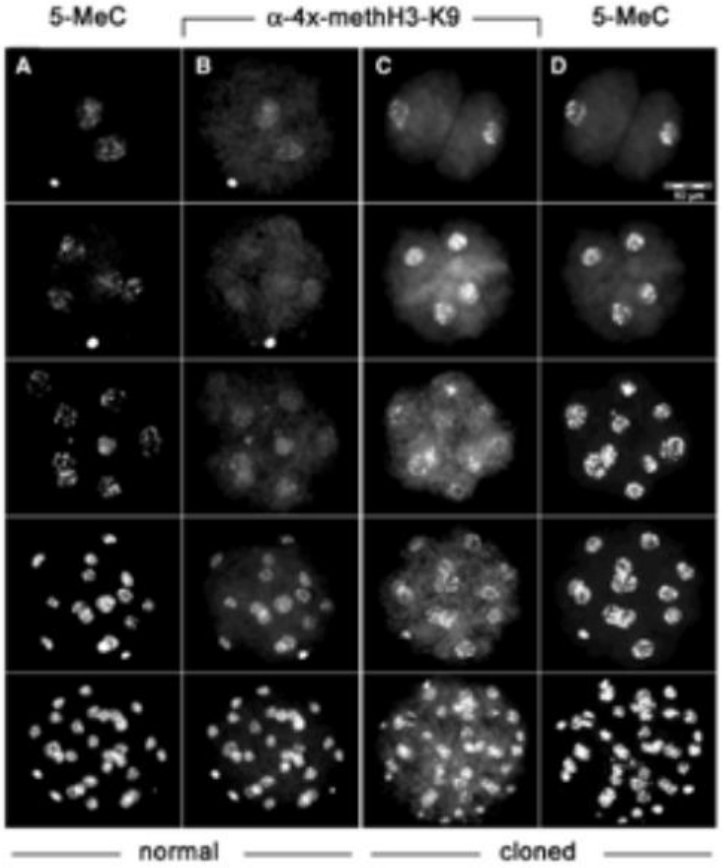
Liu Lei, Hou Jian, Lei TingHua, Bai JiaHua, Guan Hong & An XiaoRong ✉

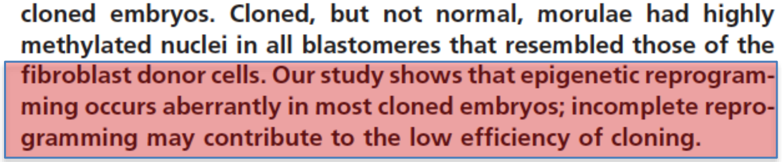
Conservation of methylation reprogramming in mammalian development: Aberrant reprogramming in cloned embryos

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

Dolly, la prima pecora clonata «rinasce» quattro volte

Gli animali, copie esatte, si trovano in un ovile nel Nottinghamshire e stanno bene

(dal web)

MILANO - La pecora più famosa del secolo, clonata circa quattordici anni fa in Scozia, è rinata: il ricercatore Keith Campbell dell'Università di Nottingham, uno dei papà della pecora Dolly, ha fatto nascere ben quattro cloni dell'animale morto nel 2003. Con lo stesso materiale genetico usato per creare Dolly. Sono stati ribattezzati con il nomignolo «The Dollies» e, oltre ad essere copie geneticamente esatte della loro precorritrice, non evidenziano problemi di salute.

NATI PIU' DI TRE ANNI FA - I quattro animali stanno bene e non mostrano nessun segno di artrosi precoce, l'infiammazione articolare per la quale era morta Dolly nel 2003, all'età di sei anni, soppressa dopo che esami veterinari avevano permesso di diagnosticare un'irreversibile malattia polmonare. Sono nati tre anni e mezzo fa, ma la notizia è stata annunciata solo ora. Campbell spera che i risultati raggiunti in questi anni nella tecnologia della clonazione possano avere un impatto diretto sulla salute dei «nuovi» animali. Con la sua venuta al mondo, Dolly - chiamata così in onore

ATTUALITÀ

Un furetto clonato ha dato alla luce dei cuccioli per la prima volta in assoluto

Un furetto dai piedi neri clonato dà alla luce cuccioli, segnando una svolta nella conservazione delle specie in via di estinzione e nel ripristino della diversità genetica.

Pubblicato il 5 Novembre 2024 - 11:12 · **Lucia Petrone**

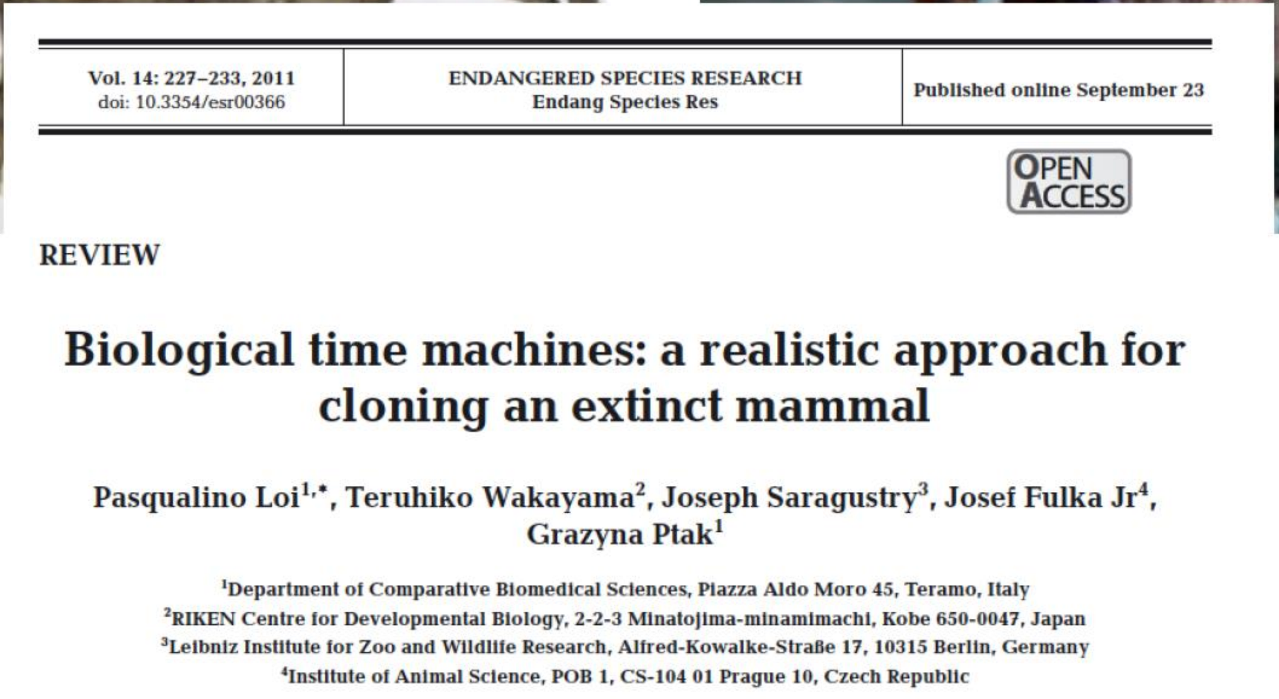
Per la prima volta in assoluto, un furetto clonato ha dato alla luce dei

Un furetto appartenente ad una specie in via di estinzione clonato, chiamato Antonia, ha dato alla luce dei cuccioli per la prima volta, segnando un successo per la salvaguardia di questa specie. Antonia è un clone di Willa, un furetto di cui sono stati conservati campioni genetici dal 1988. La sua nascita, contribuisce a incrementare la diversità genetica di questa specie, minacciata dalla perdita dell'habitat, malattie e dalla riduzione di fonti di cibo.

"Questa è la prima volta che una specie in via di estinzione clonata negli Stati Uniti ha prodotto prole, dimostrando un passo avanti fondamentale nell'uso della clonazione per migliorare la diversità genetica negli sforzi di conservazione. La riproduzione riuscita di una specie in via di estinzione clonata è una pietra miliare nella ricerca genetica sulla conservazione, dimostrando che la tecnologia di clonazione può non solo aiutare a ripristinare la diversità genetica, ma anche consentire la riproduzione futura, aprendo nuove possibilità per il recupero della specie. Ciò rappresenta un passo significativo nella salvaguardia del futuro dei furetti dai piedi neri e nel superamento delle sfide genetiche che hanno ostacolato gli sforzi di recupero".

http://www.nationalgeographic.com/deextinction

Recipe for a Resurrection
Scientists could try to clone a mammoth. Should they?



Vol. 14: 227–233, 2011
doi: 10.3354/esr00366

ENDANGERED SPECIES RESEARCH
Endang Species Res

REVIEW

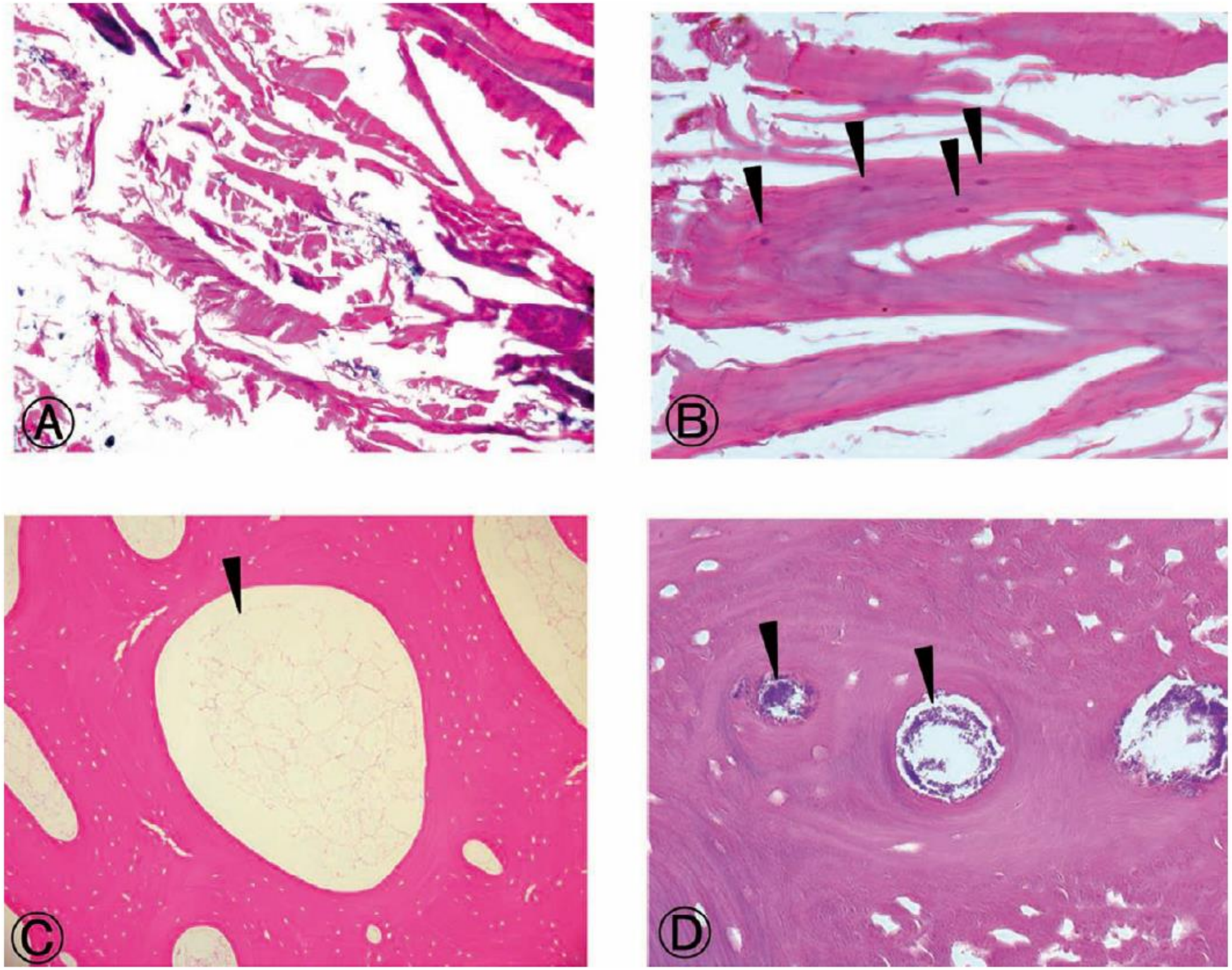
Biological time machines: a realistic approach for cloning an extinct mammal

Pasqualino Loi[1,*], Teruhiko Wakayama[2], Joseph Saragustry[3], Josef Fulka Jr[4], Grazyna Ptak[1]

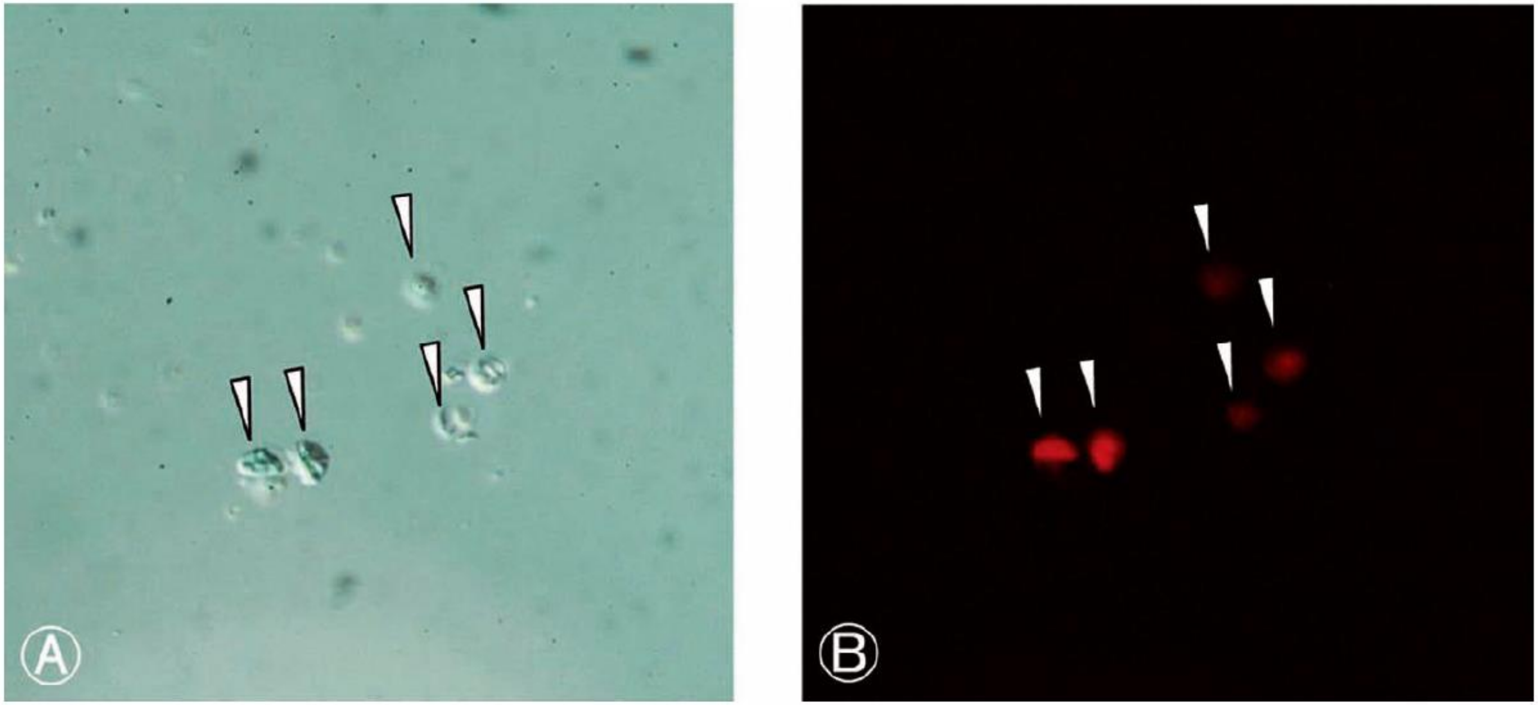
[1]Department of Comparative Biomedical Sciences, Piazza Aldo Moro 45, Teramo, Italy
[2]RIKEN Centre for Developmental Biology, 2-2-3 Minatojima-minamimachi, Kobe 650-0047, Japan
[3]Leibniz Institute for Zoo and Wildlife Research, Alfred-Kowalke-Straße 17, 10315 Berlin, Germany
[4]Institute of Animal Science, POB 1, CS-104 01 Prague 10, Czech Republic

Possiamo clonare il Mammut

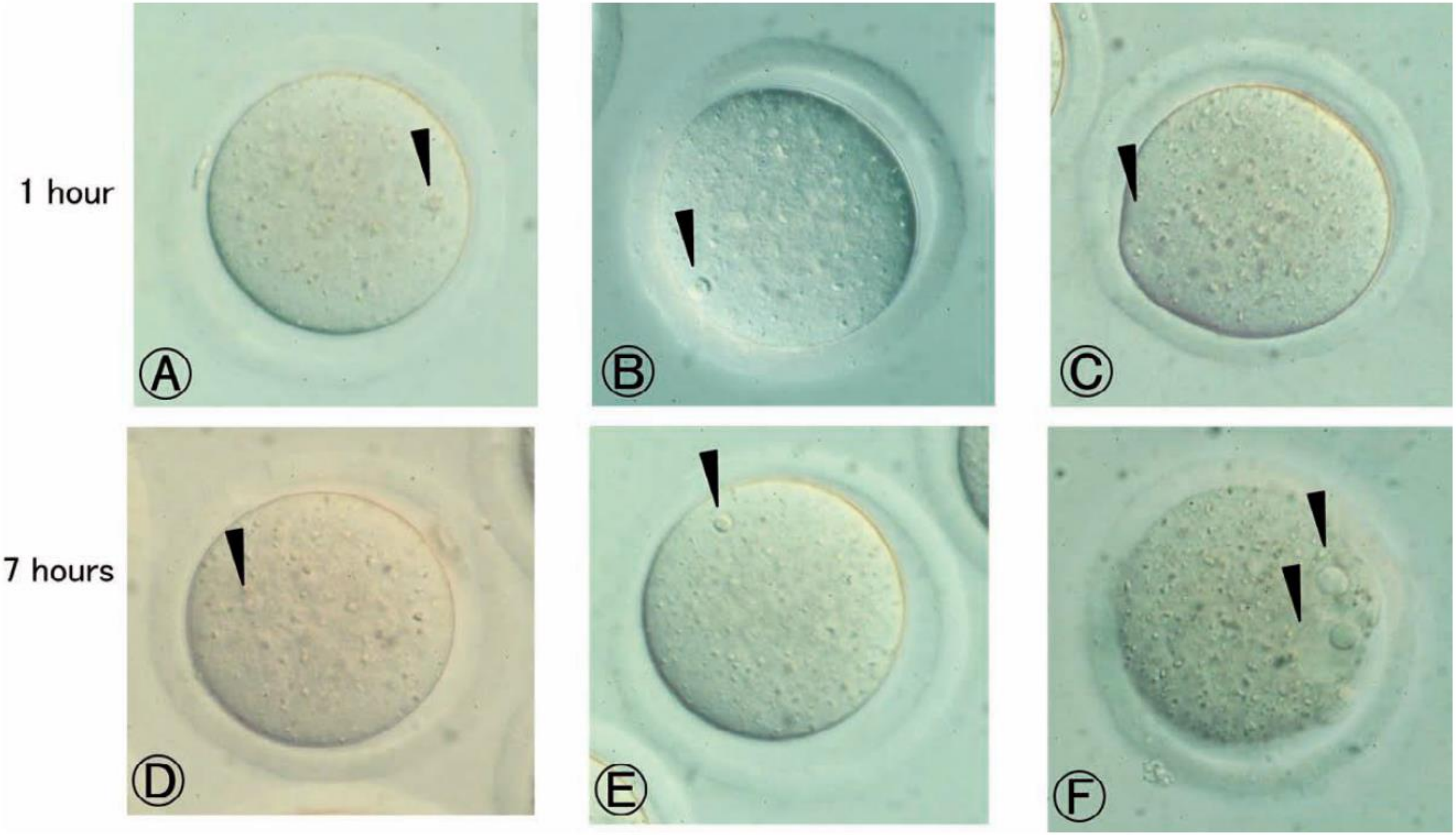
Sezioni istologiche tessuti (arto anteriore) di un Mammoth di 15000 anni trovato in Siberia



Nuclei isolati dai tessuti di mammut



Trapianto nucleare di nuclei di mammut in oociti enucleati di topo

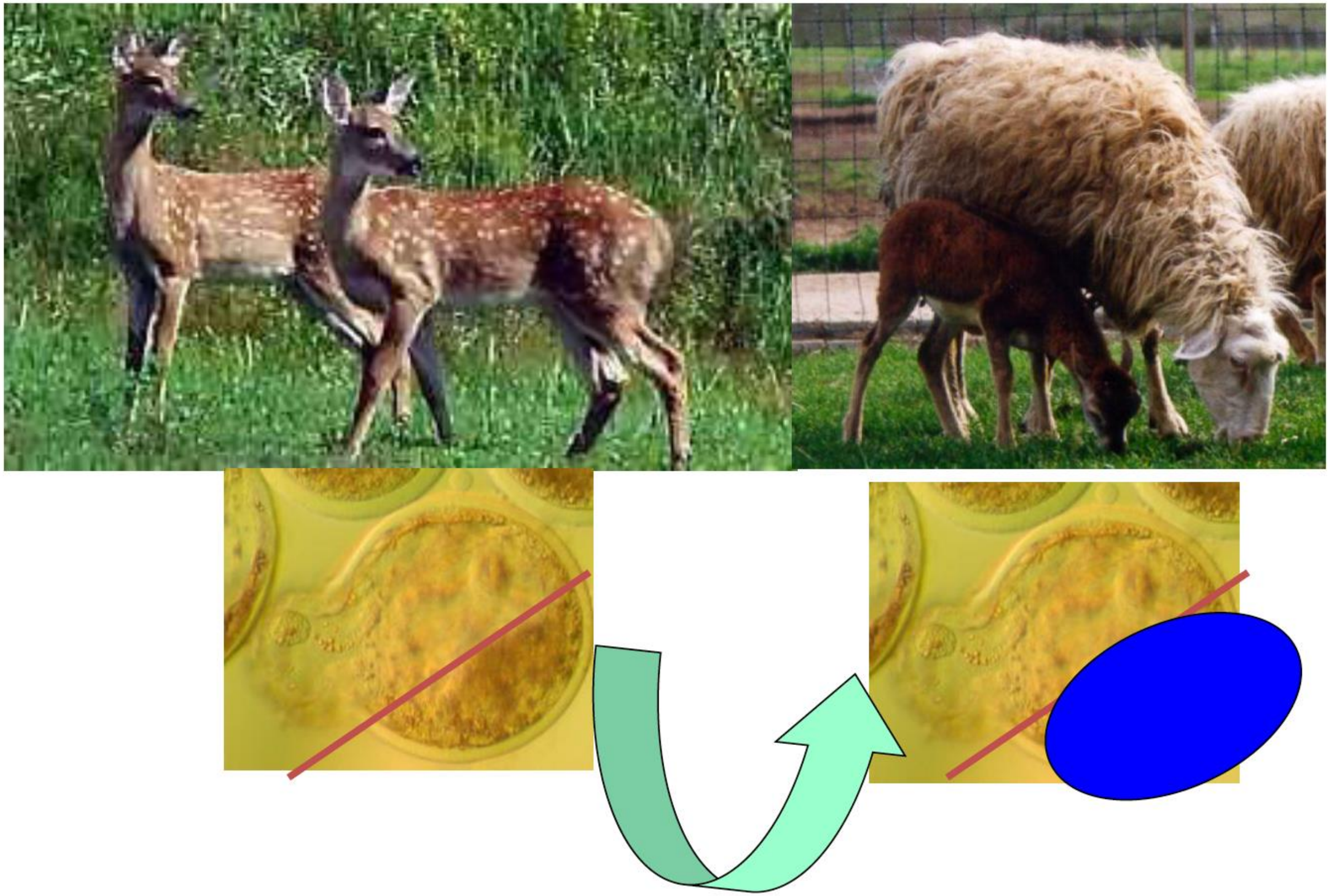


Altro problema: Disponibilità di femmine riceventi per l'embryo transfer

Semplice nel caso pecora-muflone……..

…….si può evitare il rigetto immunologico nel caso di trapianto embrionale intraspecifico?.......

Si può trasferire un embrione di daino Nell'utero di una pecora?



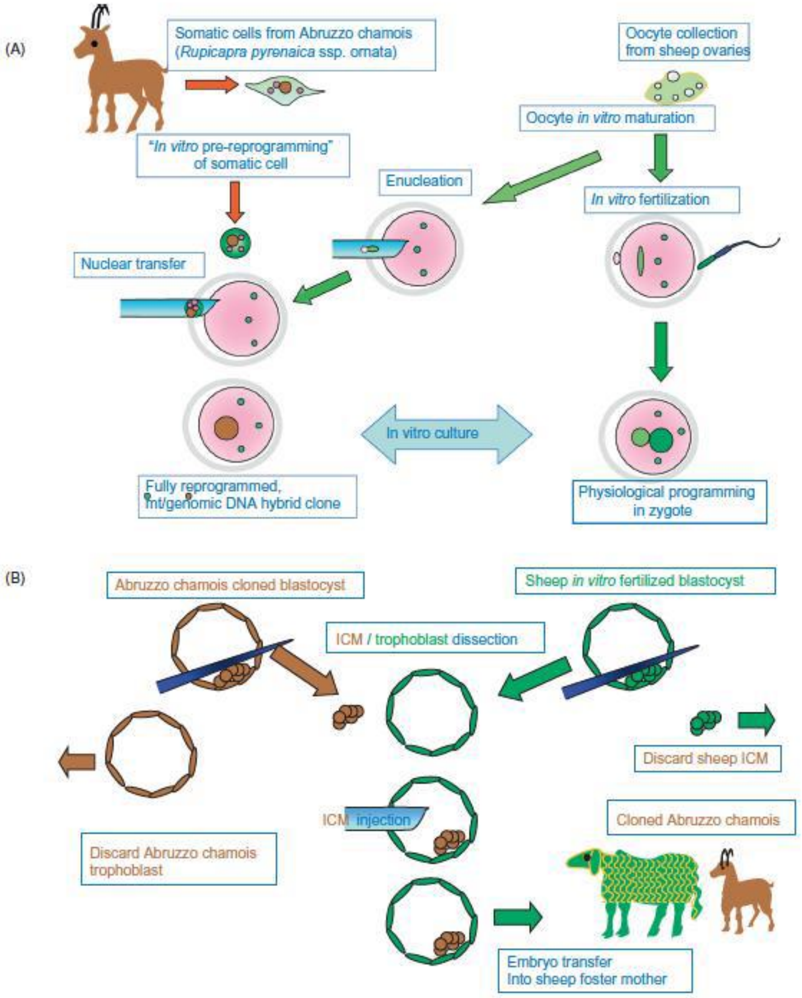


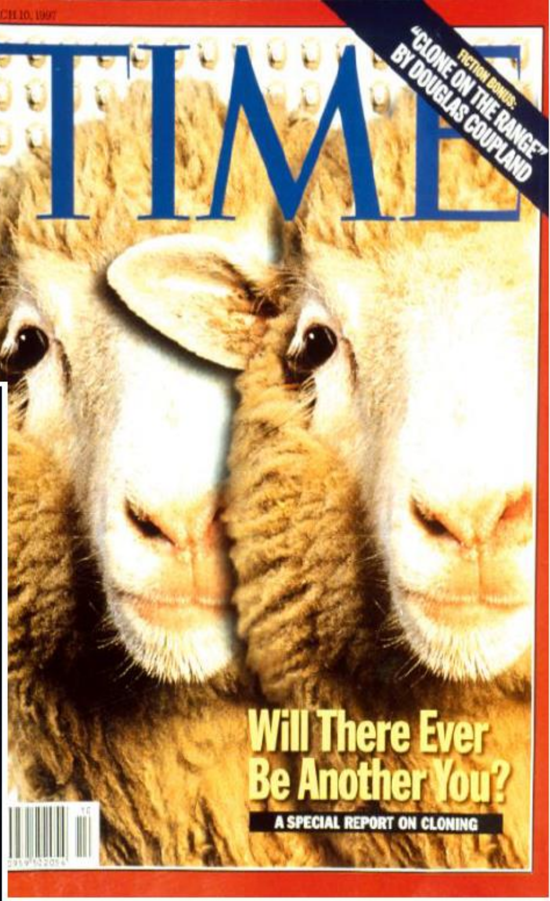
FIGURE 28.2 **"Idealized" procedure for cloning endangered animals** (A) Step 1: nuclear transfer. (B) Step 2: ICM–trophoblast exchange and interspecies embryo transfer. ICM, inner cell mass.

ACKNOWLEDGEMENTS

This work was supported by the program "Ideas" of the European Research Council, project "Angioplace," to G. Ptak; and the 7 FP EU project "NextGen," PRIN 2009 Miur, Tercas Foundation to P. Loi.

REFERENCES

Aizaki, H., Sawada, M., Sato, K., 2011. Consumers' attitudes toward consumption of cloned beef. The impact of exposure to technological information about animal cloning. Appetite 57, 459–466.




SIX APPEAL: Sandown Park meets Jurassic Park as half-a-dozen cloned Cigars fight out a finish in a race of the future.

Fancy another Cigar?

Shock cloning plans for barren stallion

By Richard Palmer

THE owners of Cigar, the record-breaking racehorse, sent shock waves through the sport of kings yesterday by revealing they are investigating the possibility of cloning him.

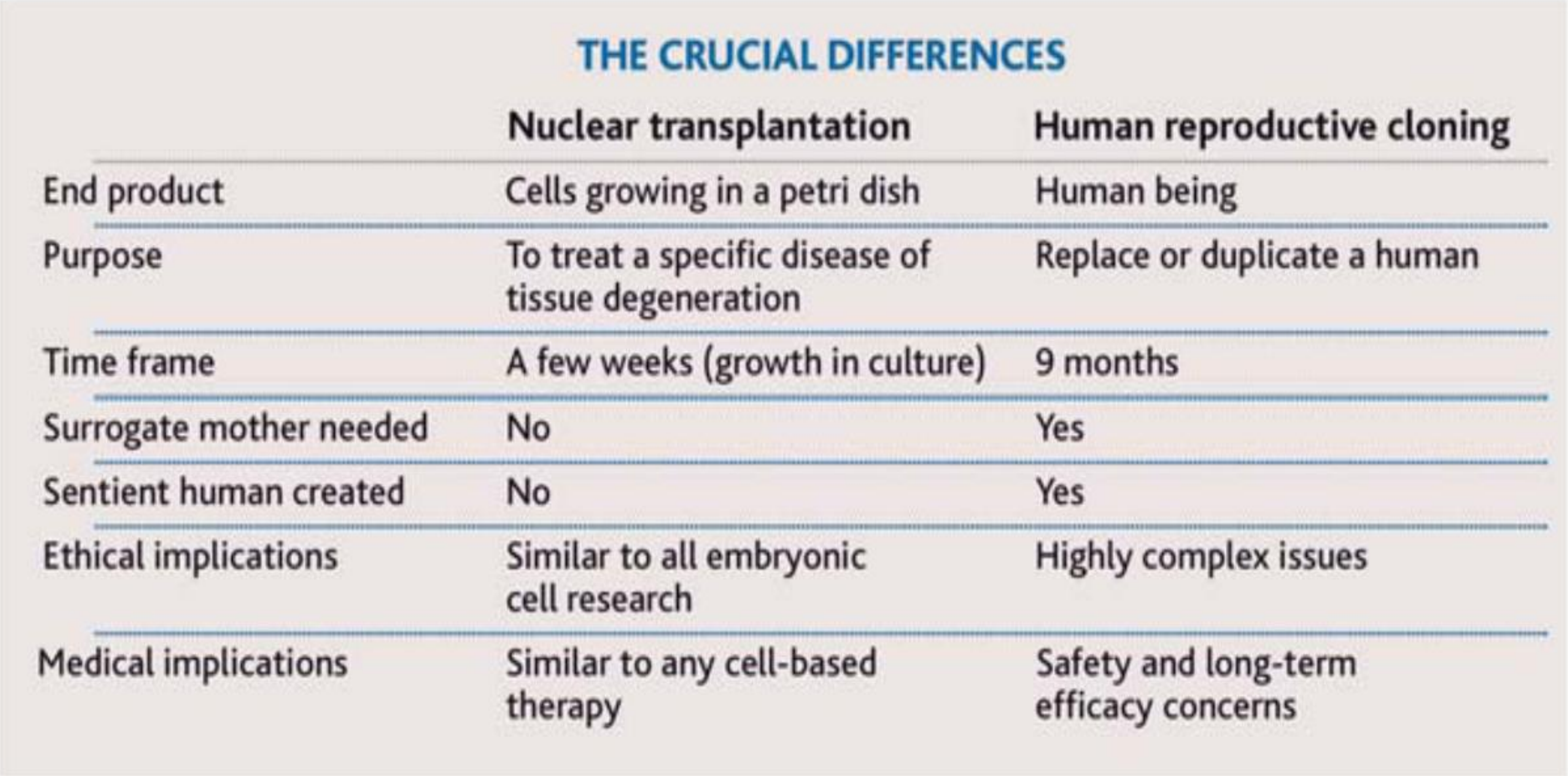
Cigar, who began a new career as a stallion in America this year

nowhere near as identical as many people think," said Griffin. "You would have genetically identical horses but whether one would develop into as good a racehorse as its originator is very unlikely.

"They would be stared several years apart. Then if you had different

Please Don't Call It Cloning!

Bert Vogelstein et al., Science 2002

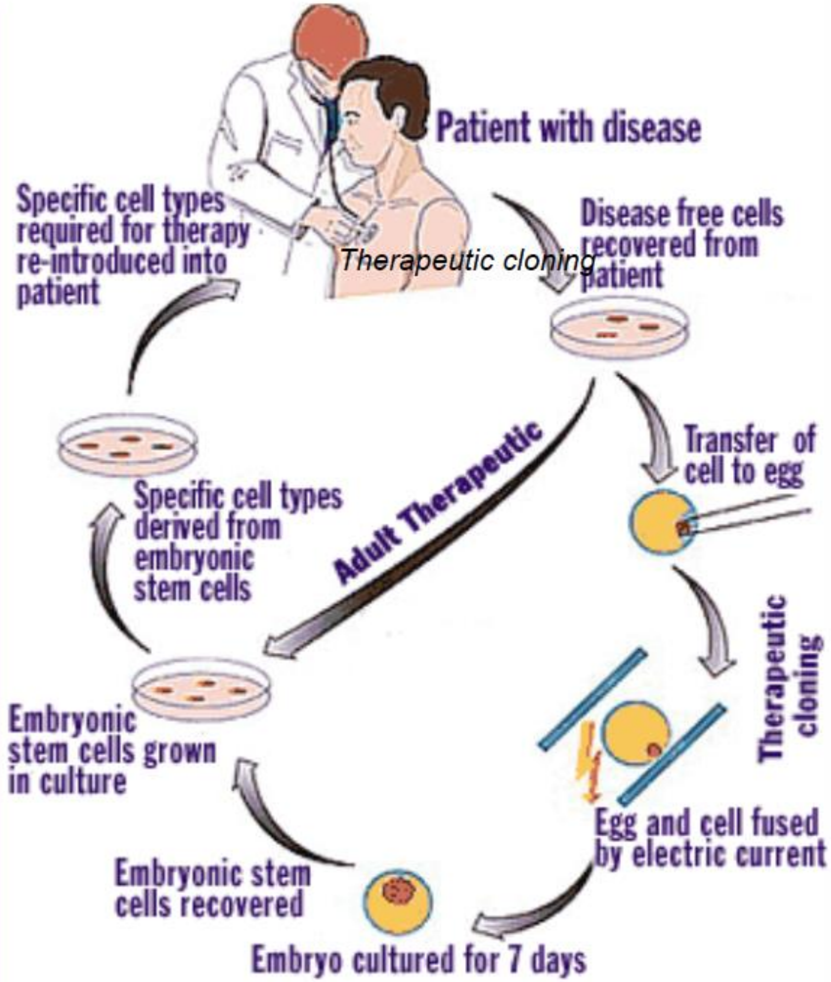


THE CRUCIAL DIFFERENCES

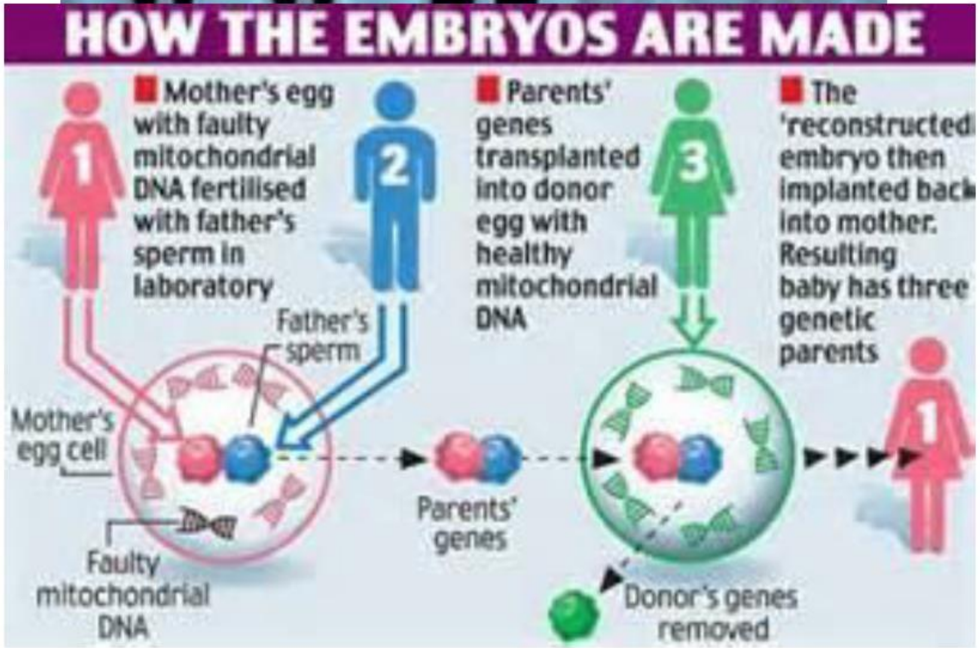
	Nuclear transplantation	Human reproductive cloning
End product	Cells growing in a petri dish	Human being
Purpose	To treat a specific disease of tissue degeneration	Replace or duplicate a human
Time frame	A few weeks (growth in culture)	9 months
Surrogate mother needed	No	Yes
Sentient human created	No	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

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



“Three parents” babies….?
Prevenzione patologie dovute a mutazioni del DNA mitocondriale



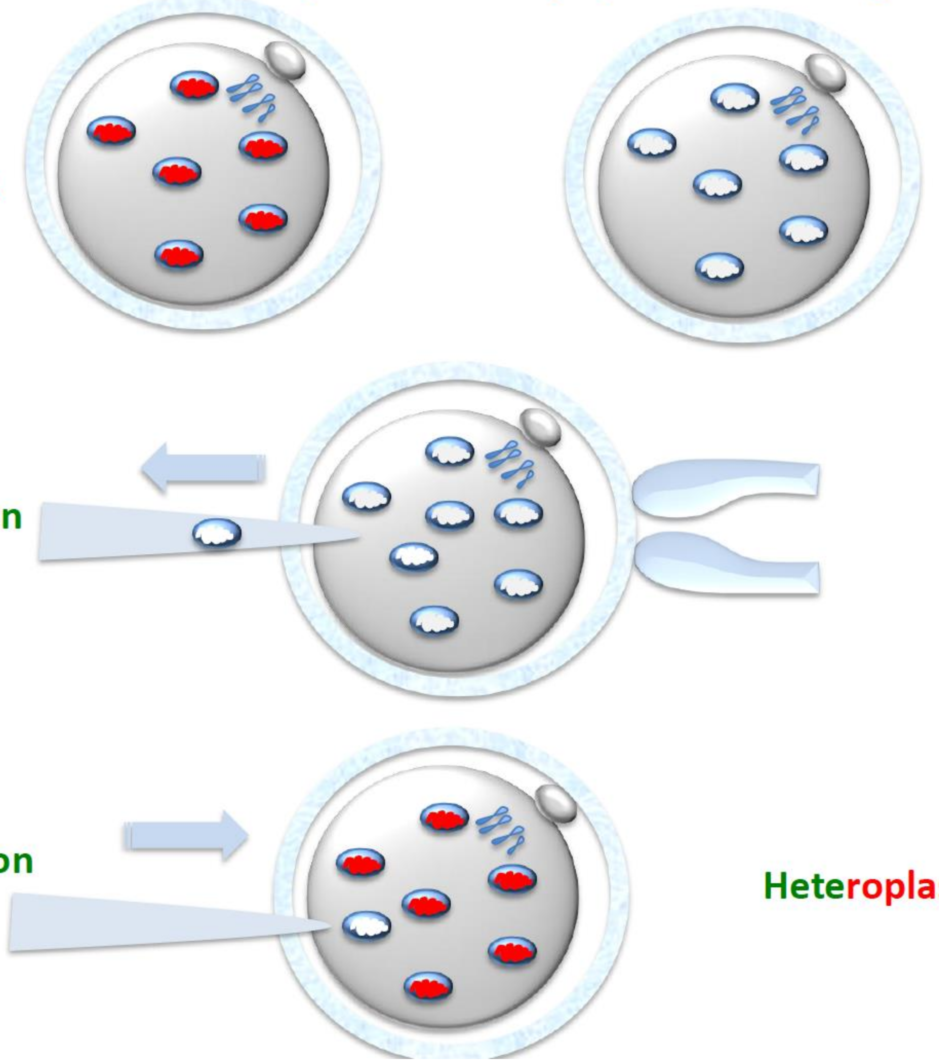
HOW THE EMBRYOS ARE MADE

Mother's egg with faulty mitochondrial DNA fertilised with father's sperm in laboratory

Parents' genes transplanted into donor egg with healthy mitochondrial DNA

The 'reconstructed' embryo then implanted back into mother. Resulting baby has three genetic parents

Micromanipulation for Cytoplasmic exchange



Oocyte with mutated mtDNA

"Healthy" oocyte

Cytoplasm aspiration

Cytoplasm injection

Heteroplasmic oocyte

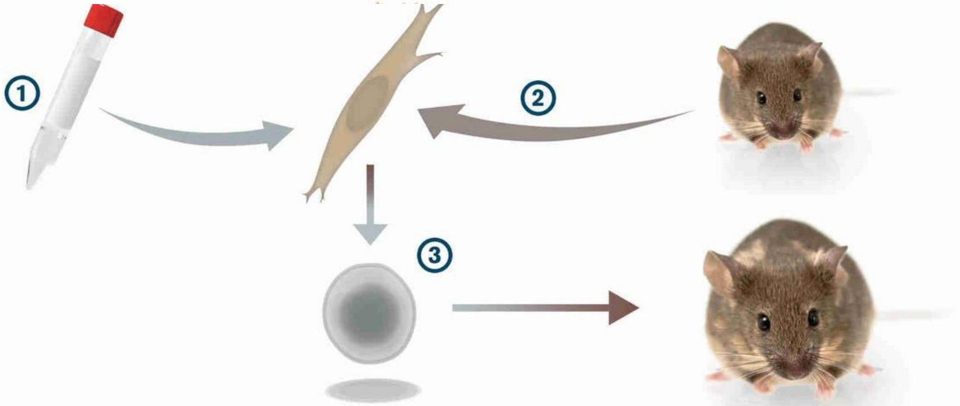
Premio Nobel 2012 per la medicina a John Gurdon e Yamanaka

IPS

Nuclear Transfer

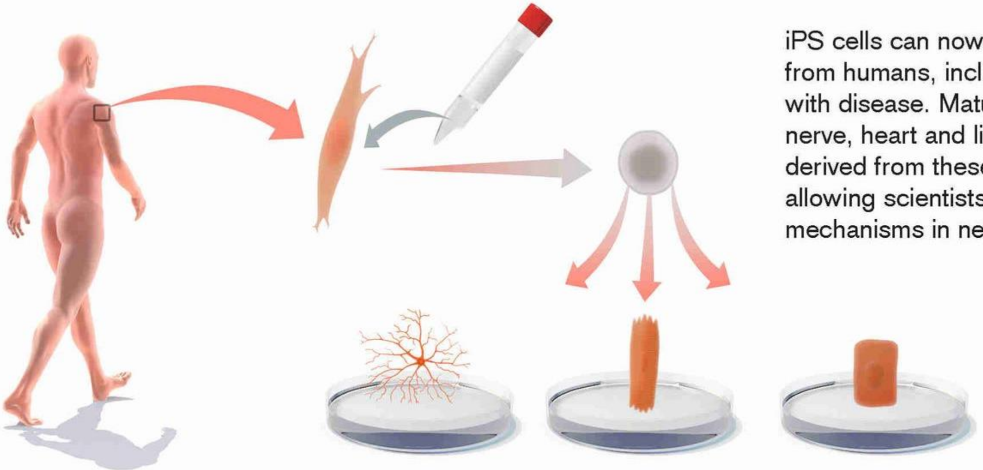
http://www.youtube.com/watch?v=cPvidAvzmx0

The Nobel Prize in Physiology or Medicine 2012



Shinya Yamanaka

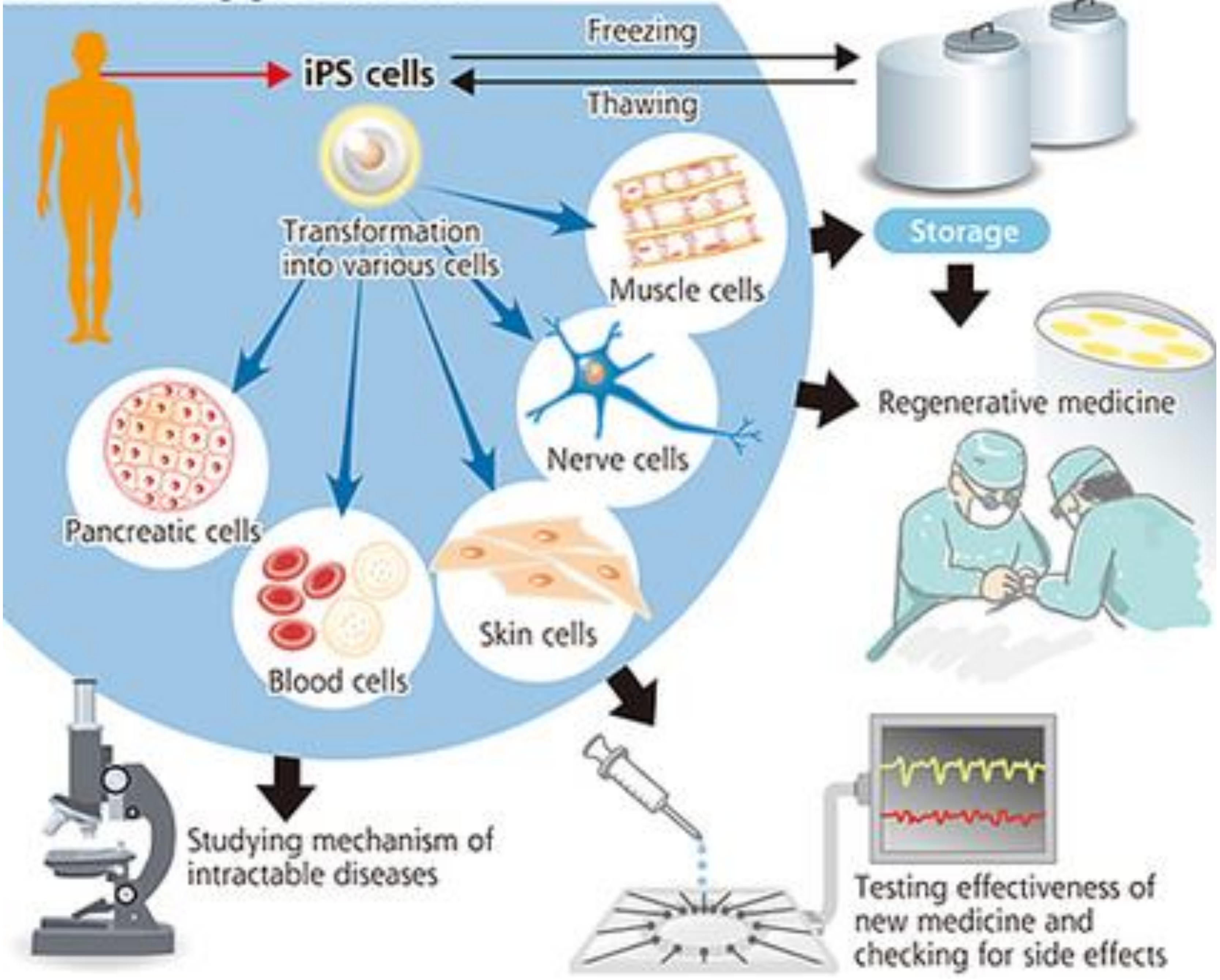
Shinya Yamanaka studied genes that are important for stem cell function. When he transferred four such genes (1) into cells taken from the skin (2), they were reprogrammed into pluripotent stem cells (3) that could develop into all cell types of an adult mouse. He named these cells induced pluripotent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

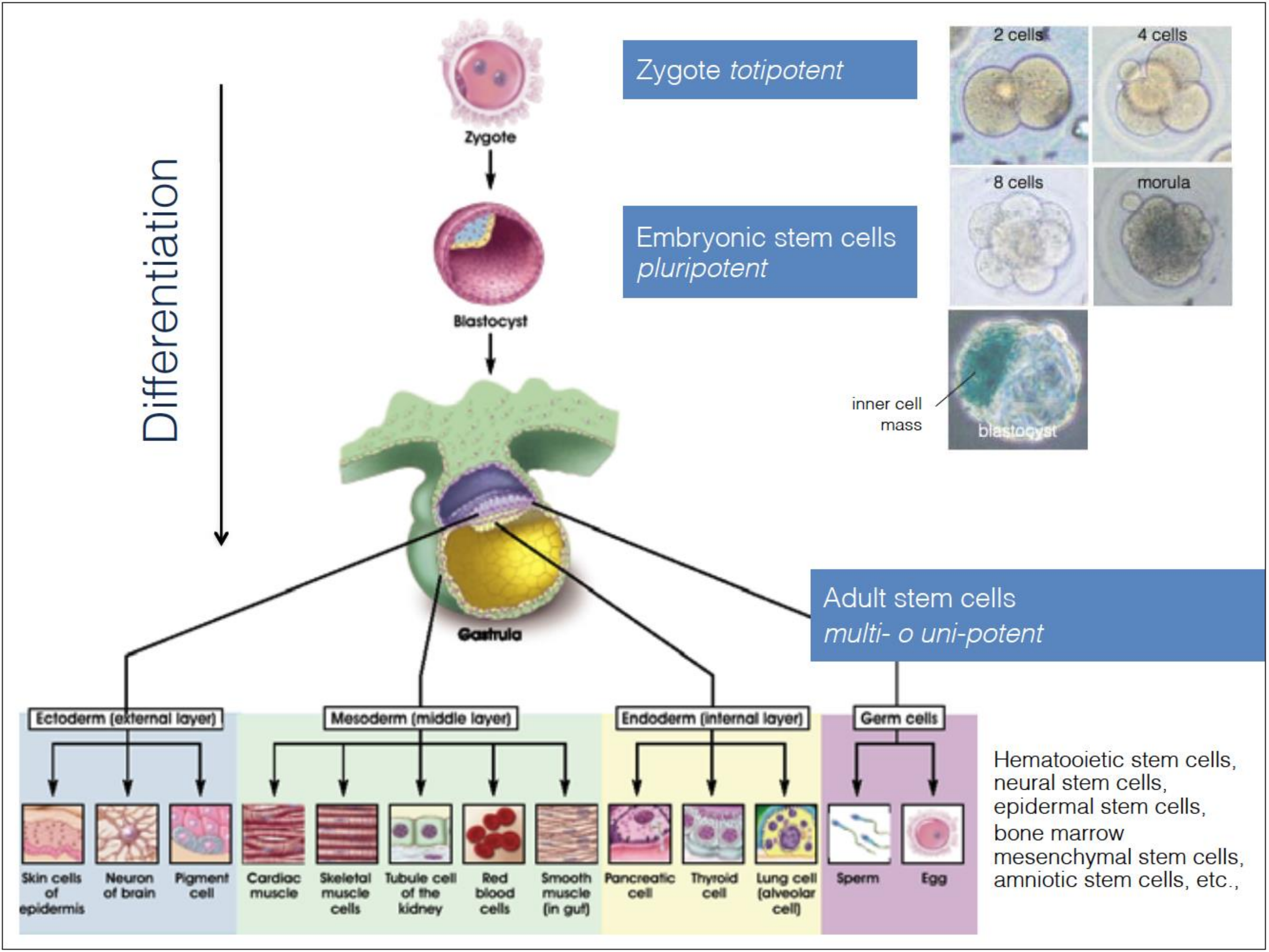


iPS cells can now be generated from humans, including patients with disease. Mature cells including nerve, heart and liver cells can be derived from these iPS cells, thereby allowing scientists to study disease mechanisms in new ways.

Illustration and layout: Mattias Karlén

iPS cell applications





Differentiation

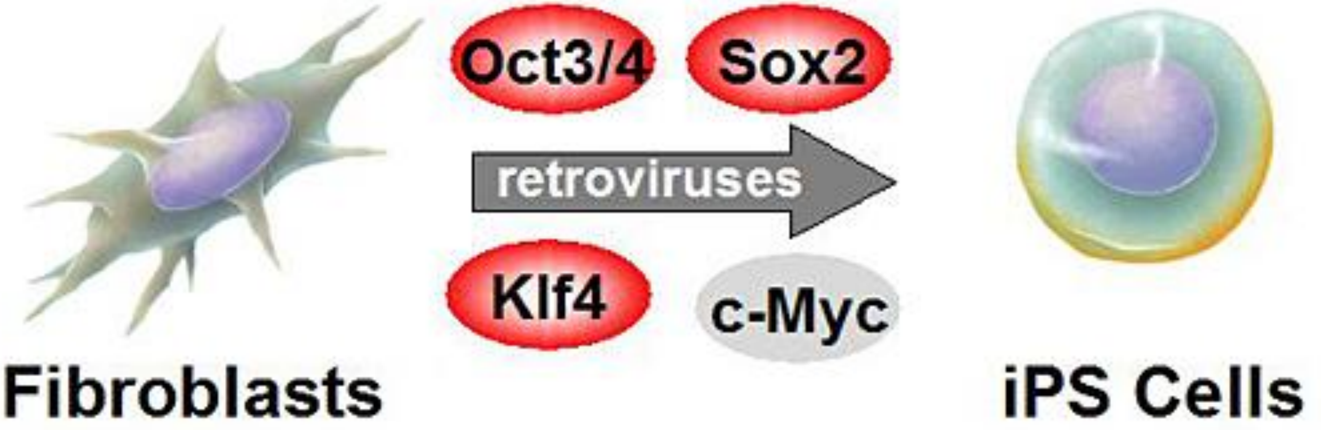
Zygote *totipotent*

Embryonic stem cells
pluripotent

Adult stem cells
multi- o uni-potent

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

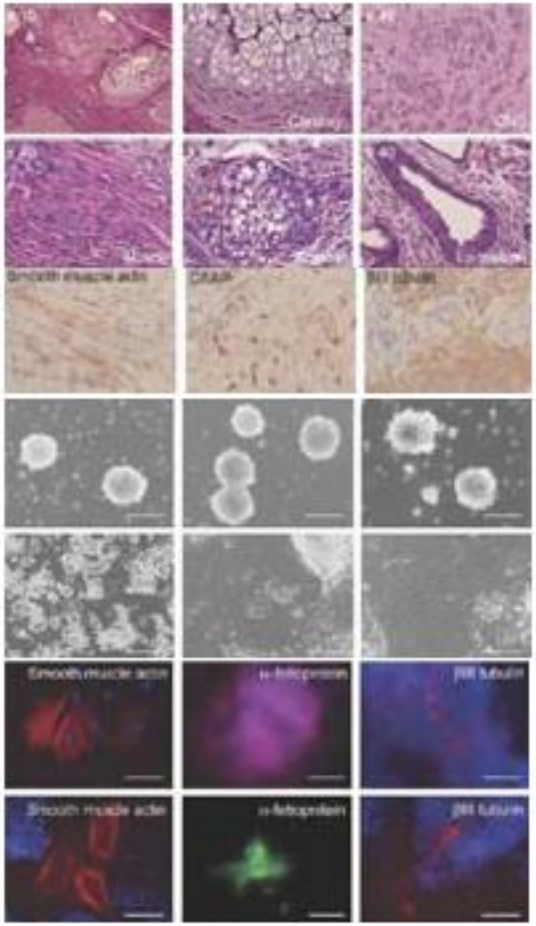
Human iPS cells reported in 2007

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
*Contact: yamanaka@frontier.kyoto-u.ac.jp

DOI 10.1016/j.cell.2006.07.024
Cell 126, 663-676, August 25, 2006 ©2006 Elsevier Inc.

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.

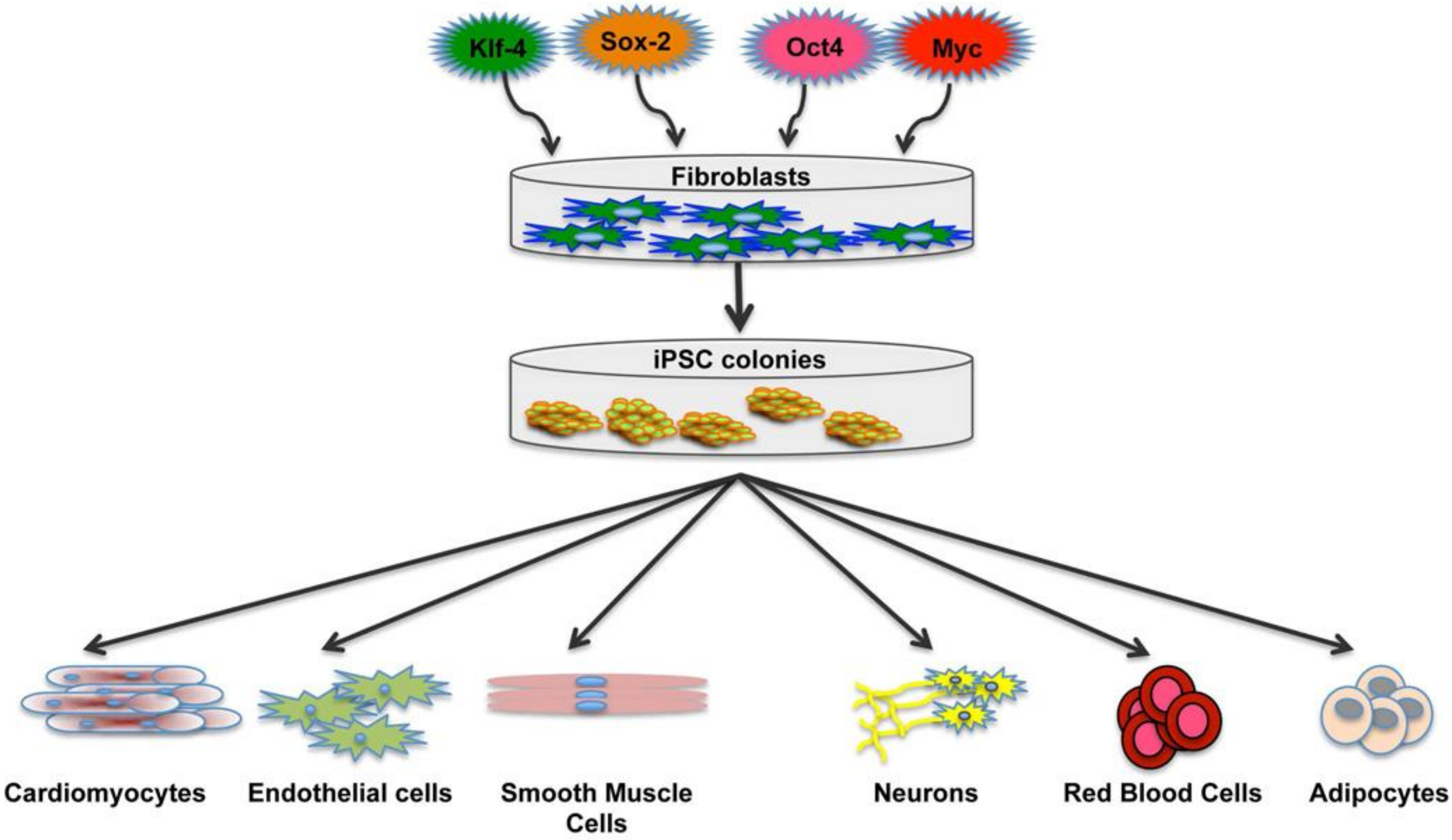
2- Following injection into blastocysts, iPS cells contributed to mouse embryonic development, but embryos failed to develop beyond mid-gestation stage.

Table 2

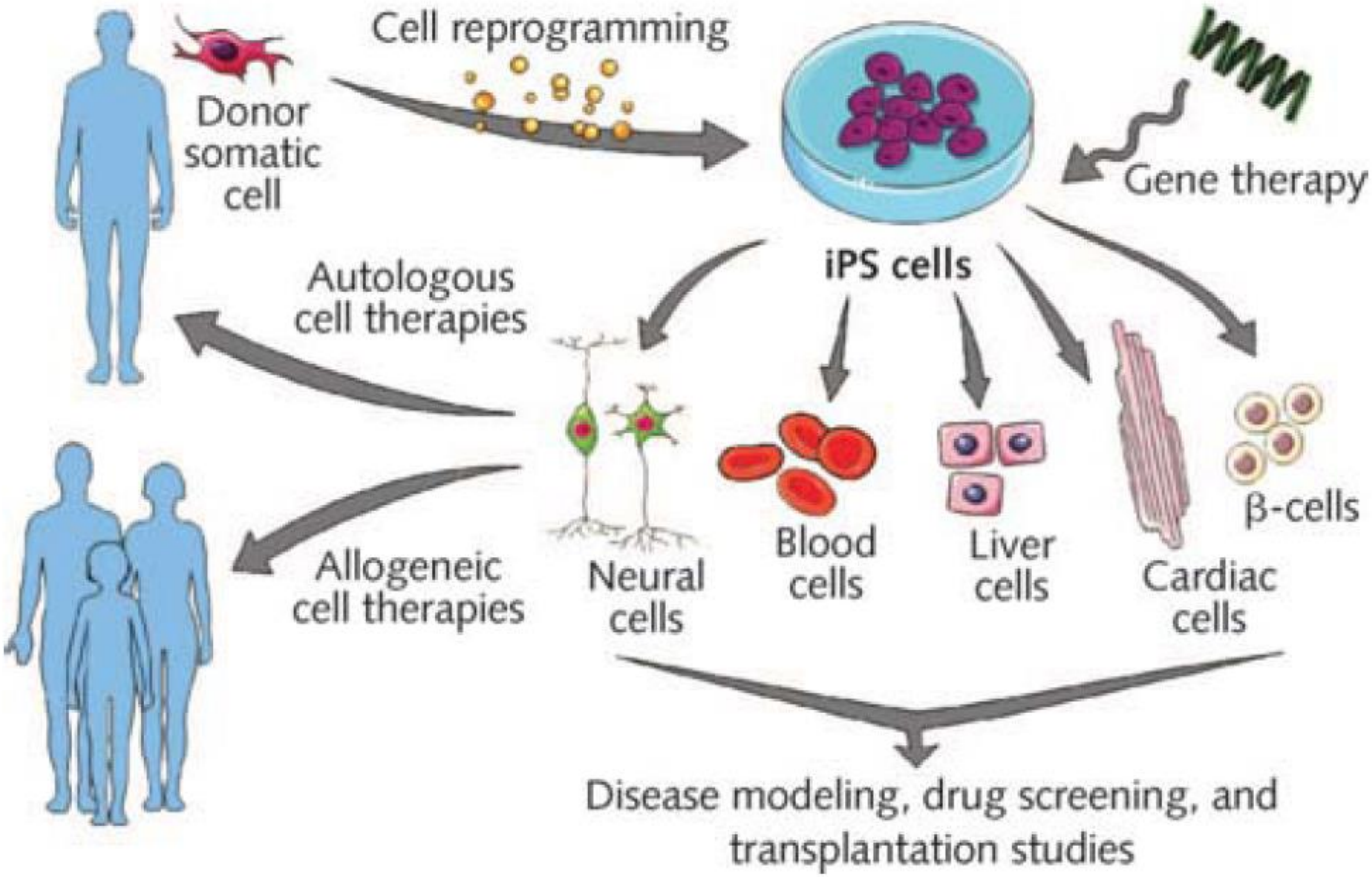
Minimum number of factors required for iPS cell generation

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.

iPSCs


Klf-4
Sox-2
Oct4
Myc
Fibroblasts
iPSC colonies
Cardiomyocytes
Endothelial cells
Smooth Muscle Cells
Neurons
Red Blood Cells
Adipocytes

Medical use of iPS cells: iPS as an ethical alternative to ES cells?

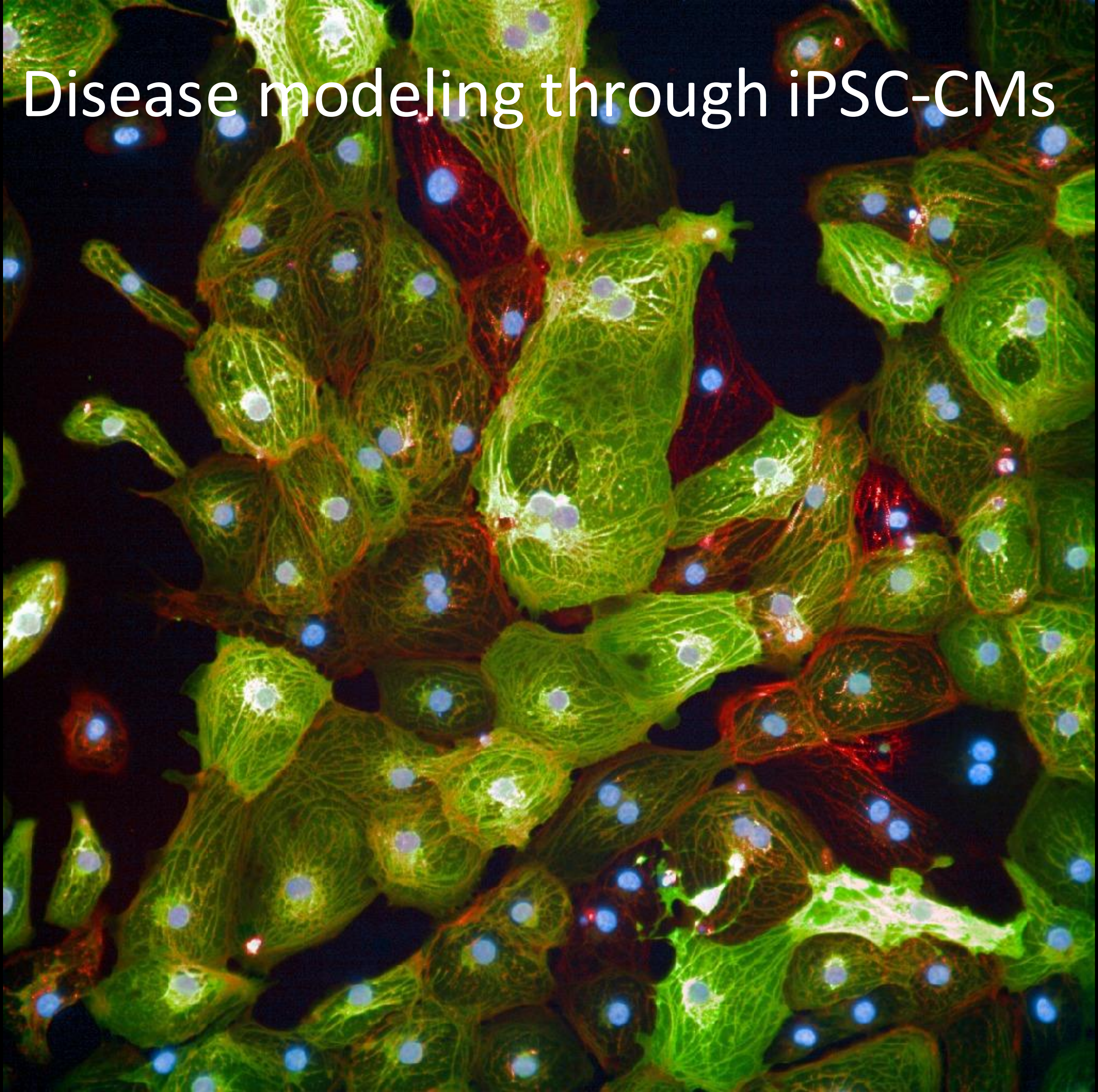


“Patient tailored therapy”

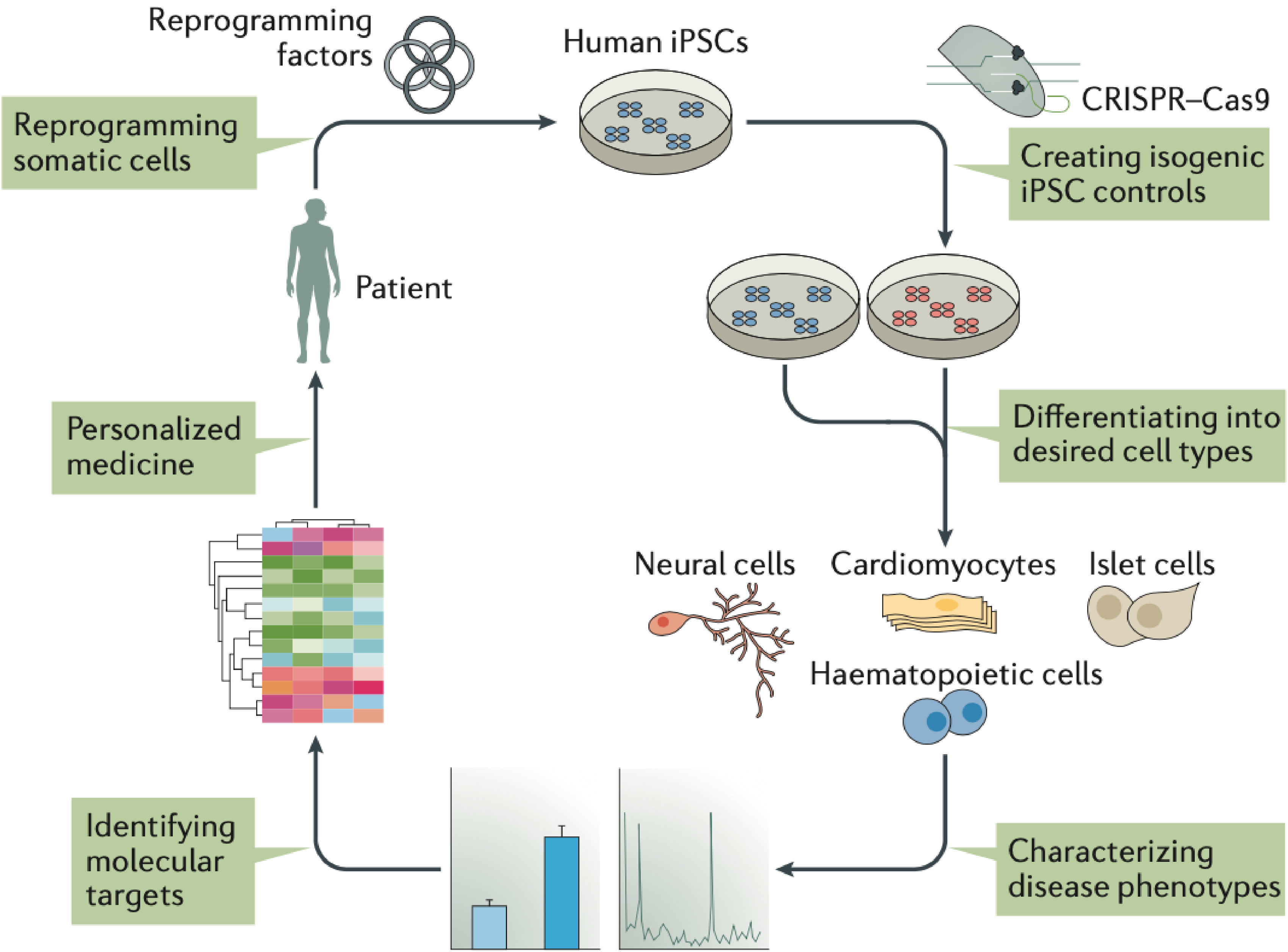
iPS vs ES cells

- Although they have similar phenotypes they are not strictly identical
- Differences in their gene expression profile
- iPS cells retain an epigenetic memory of their tissue of origin
- Several methods for reprogramming now exist
- iPS cells can cause teratoma - 2 of the 4 transgenes are known to be oncogenic; retroviruses and lentiviruses used as vectors can cause insertional mutagenesis
- The source and age of donor cells can affect reprogramming (the more differentiated the donor cell is the more difficult it is to wind back its developmental clock)

Disease modeling through iPSC-CMs



Disease modeling through iPSC-CMs



Reprogramming somatic cells

Creating isogenic iPSC controls

Personalized medicine

Differentiating into desired cell types

Identifying molecular targets

Characterizing disease phenotypes

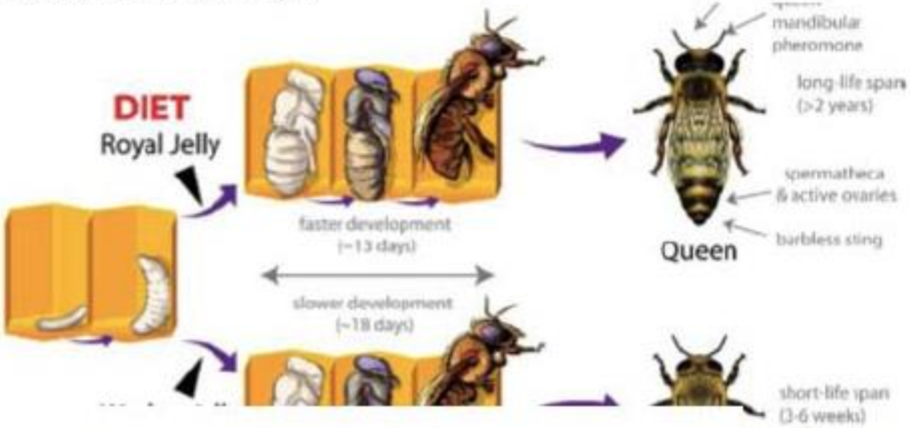
OPEN ACCESS Freely available online PLoS BIOLOGY

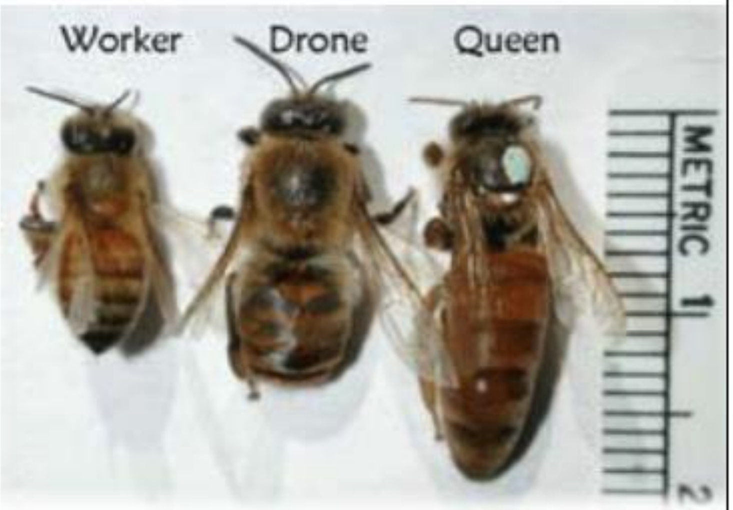
Primer

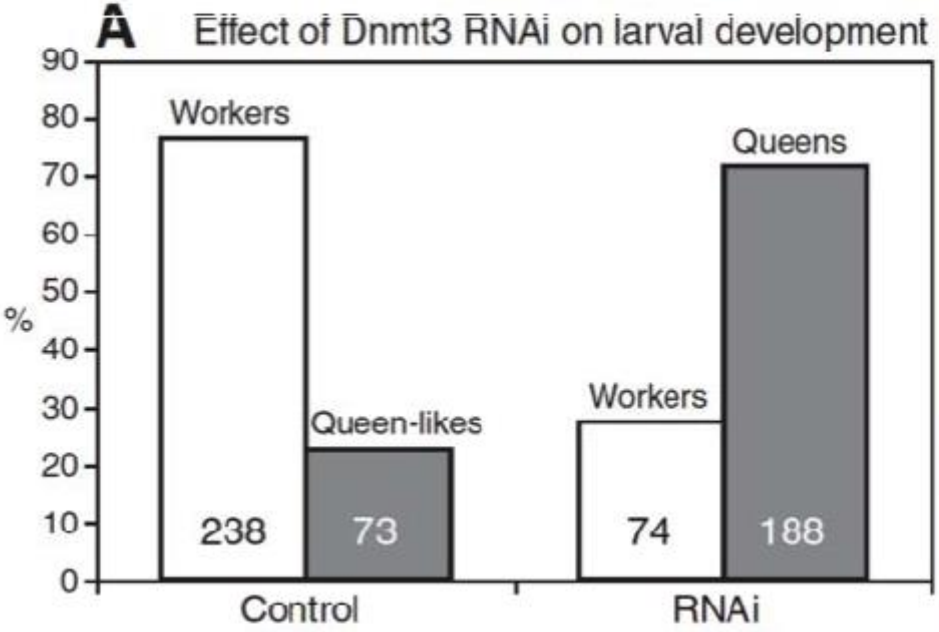
Epigenetics of Royalty

Alexandra Chittka[1*], Lars Chittka[2]

1 Wolfson Institute for Biomedical Research, University College London, London, United Kingdom, 2 Queen Mary University of London, Research Centre for Psychology, School of Biological and Chemical Sciences, London, United Kingdom







28 MARCH 2008 VOL 319 SCIENCE www.sciencemag.org

EPIGENETICS

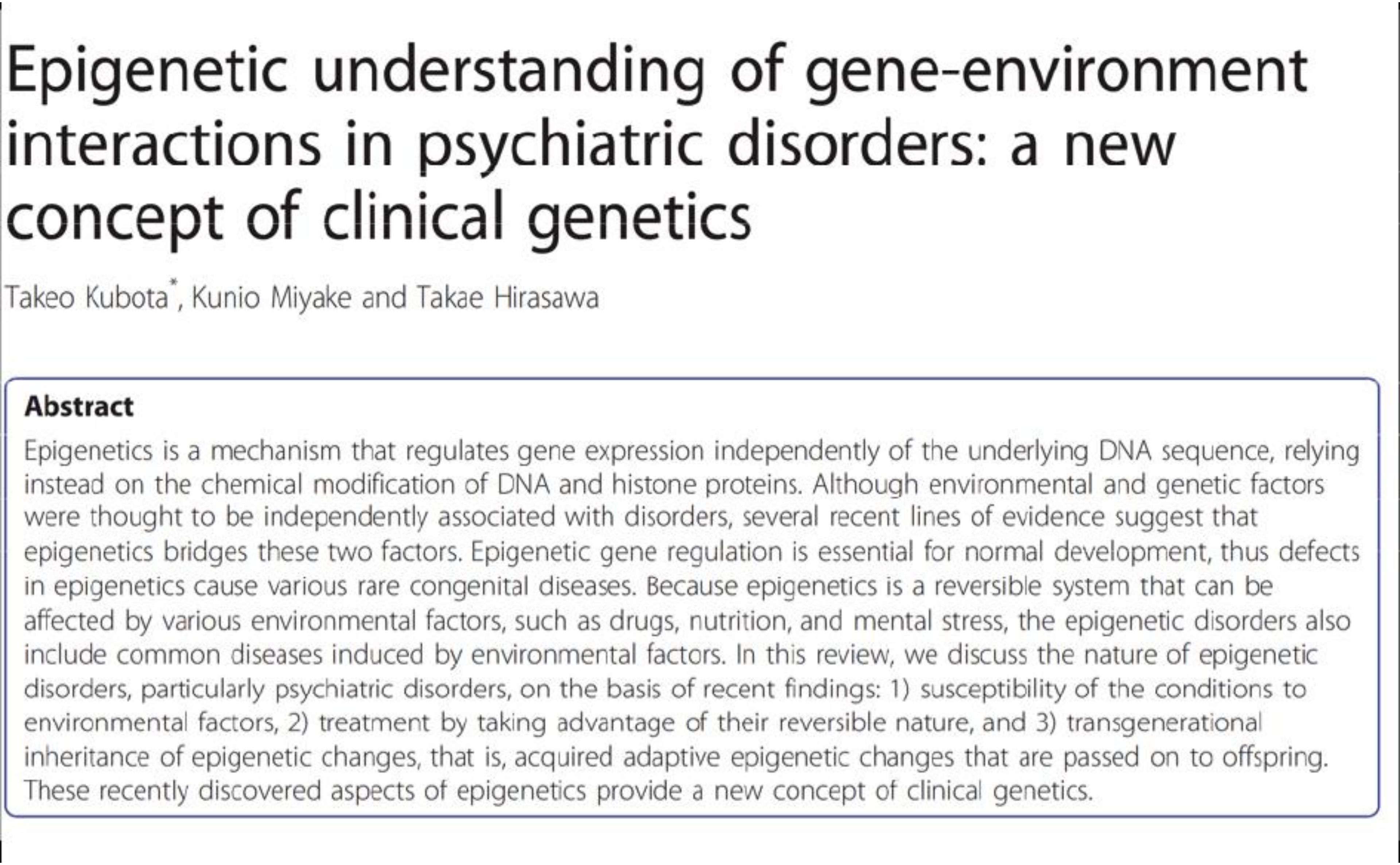
What makes a queen bee?

A queen honeybee and her female workers have identical DNA sequences but obvious differences in behaviour and reproductive ability. This can be explained, in part, by the attachment of methyl groups to the bees' DNA, which changes gene expression. Now researchers have found

University in Canberra and his team analysed genomes from the brain tissue of reproductive queens and sterile workers to reveal the genome-wide distribution of methyl groups. They found that methylation sites clustered in areas of genes where splicing — a form of

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

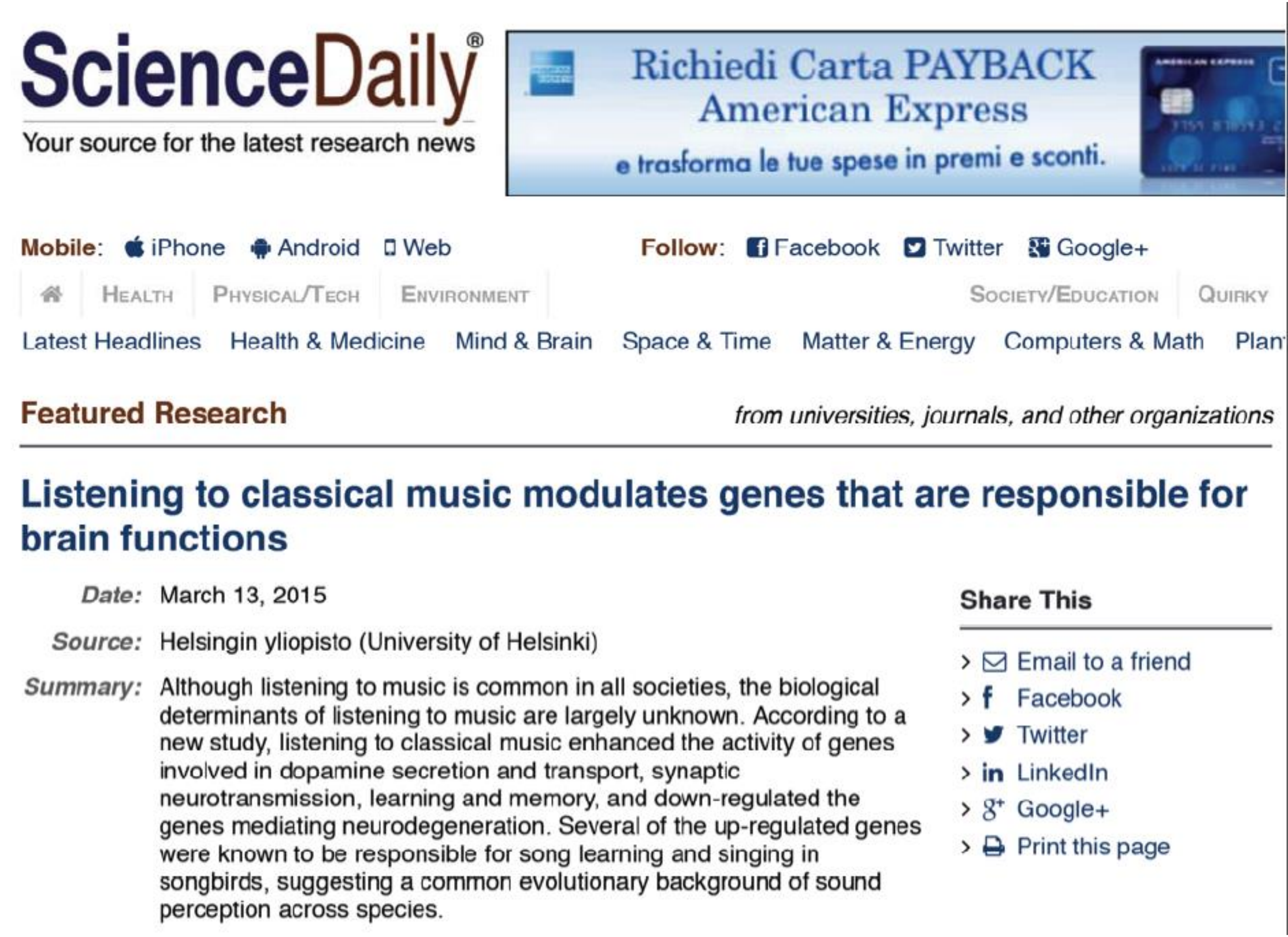


Epigenetic understanding of gene-environment interactions in psychiatric disorders: a new concept of clinical genetics

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.



ScienceDaily®
Your source for the latest research news

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.

Share This

- Email to a friend
- Facebook
- Twitter
- LinkedIn
- Google+
- Print this page

Study of transgenerational epigenetic inheritance on three generations of Holocaust survivor families, Cambodian genocide survivor families, and control populations across 25 years.

Specific, reproducible methylation changes are in stress-response genes

- present in trauma survivors,
- transmitted to their biological children
- detectable in grandchildren who never experienced trauma themselves.

The mechanism operates through the germline epigenome.

During the formation of sperm and eggs, the genome undergoes *near-complete* epigenetic reprogramming to remove parental marks. Certain loci, including stress-response gene promoters, resist this reprogramming when the parent's stress exposure has been sufficiently severe and prolonged, maintaining their trauma-induced methylation patterns and passing them to the offspring's genome.

The clinical implications are profound: Children and grandchildren of trauma survivors show elevated baseline cortisol levels and increased risk of PTSD, anxiety, and depression — not because of how they were raised, but because of how their grandparents suffered.

French scientists discovered emotional trauma physically alters DNA passed to future generations

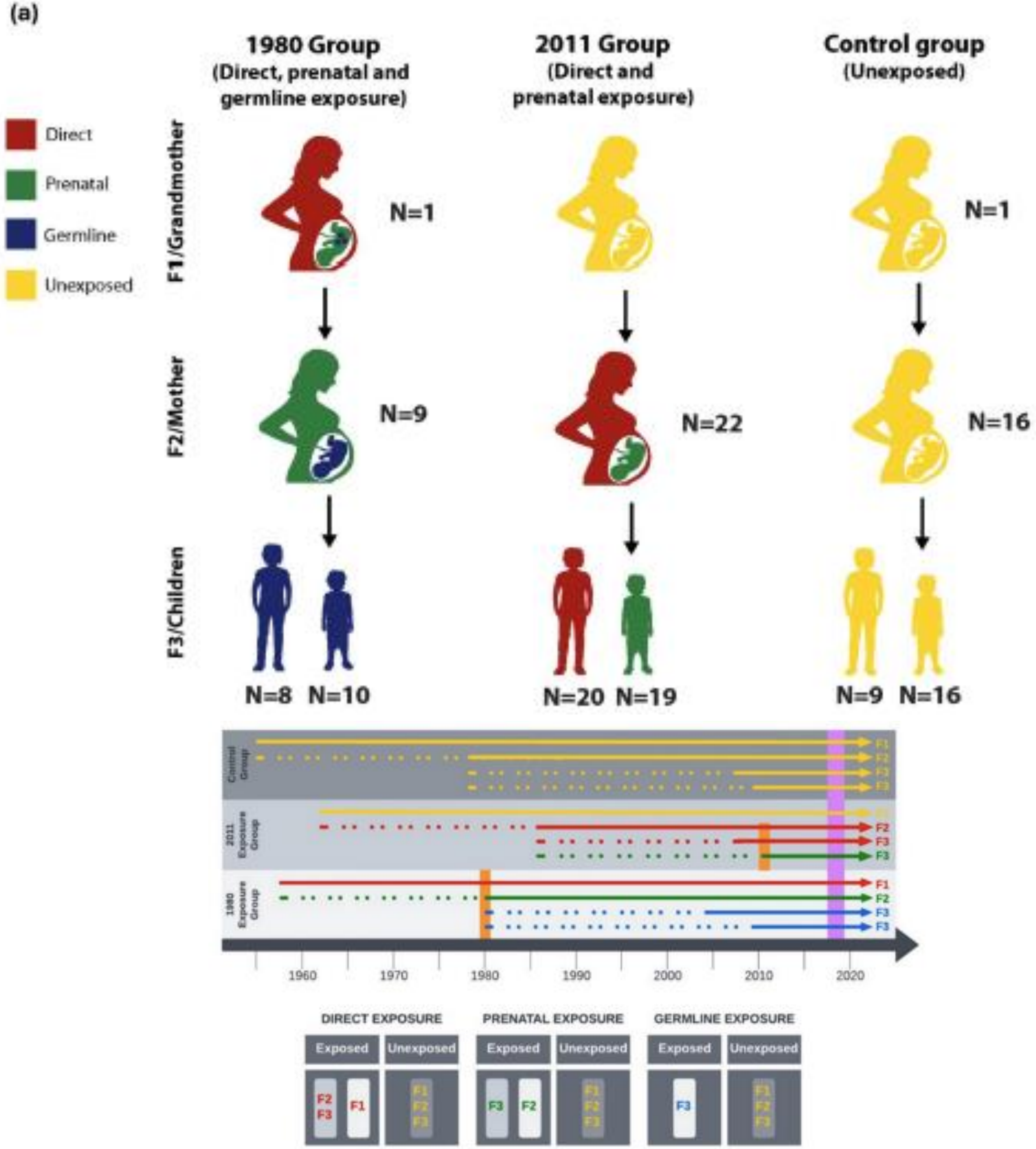
Scientific Reports | (2025) 15:5945 | https://doi.org/10.1038/s41598-025-89818-z

nature portfolio

Epigenetic signatures of intergenerational exposure to violence in three generations of Syrian refugees

Connie J. Mulligan[1,2], Edward B. Quinn[1,2,3], Dima Hamadmad[4], Christopher L. Dutton[1,2,5], Lisa Nevell[1,2], Alexandra M. Binder[6,7], Catherine Panter-Brick[8,9] & Rana Dajani[10]

Maternal trauma influences infant and adult health outcomes and may impact future generations through epigenetic modifications such as DNA methylation (DNAm). Research in humans on the intergenerational epigenetic transmission of trauma effects is limited. In this study, we assessed DNAm signatures of war-related violence by comparing germline, prenatal, and direct exposures to violence across three generations of Syrian refugees. We compared families in which a pregnant grandmother versus a pregnant mother was exposed to violence and included a control group with no exposure to war. We collected buccal swab samples and survey data from mothers and 1–2 children in each of 48 families ($n = 131$ participants). Based on an epigenome-wide association study (EWAS), we identified differentially methylated regions (DMPs): 14 were associated with germline and 21 with direct exposure to violence. Most DMPs showed the same directionality in DNAm change across germline, prenatal, and direct exposures, suggesting a common epigenetic response to violence. Additionally, we identified epigenetic age acceleration in association with prenatal exposure to violence in children, highlighting the critical period of in utero development. This is the first report of an intergenerational epigenetic signature of violence, which has important implications for understanding the inheritance of trauma.


(a)
1980 Group
(Direct, prenatal and germline exposure)
2011 Group
(Direct and prenatal exposure)
Control group
(Unexposed)
Direct
Prenatal
Germline
Unexposed
F1/Grandmother
F2/Mother
F3/Children
N=1
N=9
N=22
N=1
N=16
N=8
N=10
N=20
N=19
N=9
N=16
Control Group
2011 Exposure Group
1980 Exposure Group
F1
F2
F3
F3
F1
F2
F3
F3
F1
F2
F3
F3
1960
1970
1980
1990
2000
2010
2020
DIRECT EXPOSURE
PRENATAL EXPOSURE
GERMLINE EXPOSURE
Exposed
Unexposed
Exposed
Unexposed
Exposed
Unexposed
F2
F3
F1
F1
F2
F3
F3
F2
F1
F2
F3
F3
F1
F2
F3

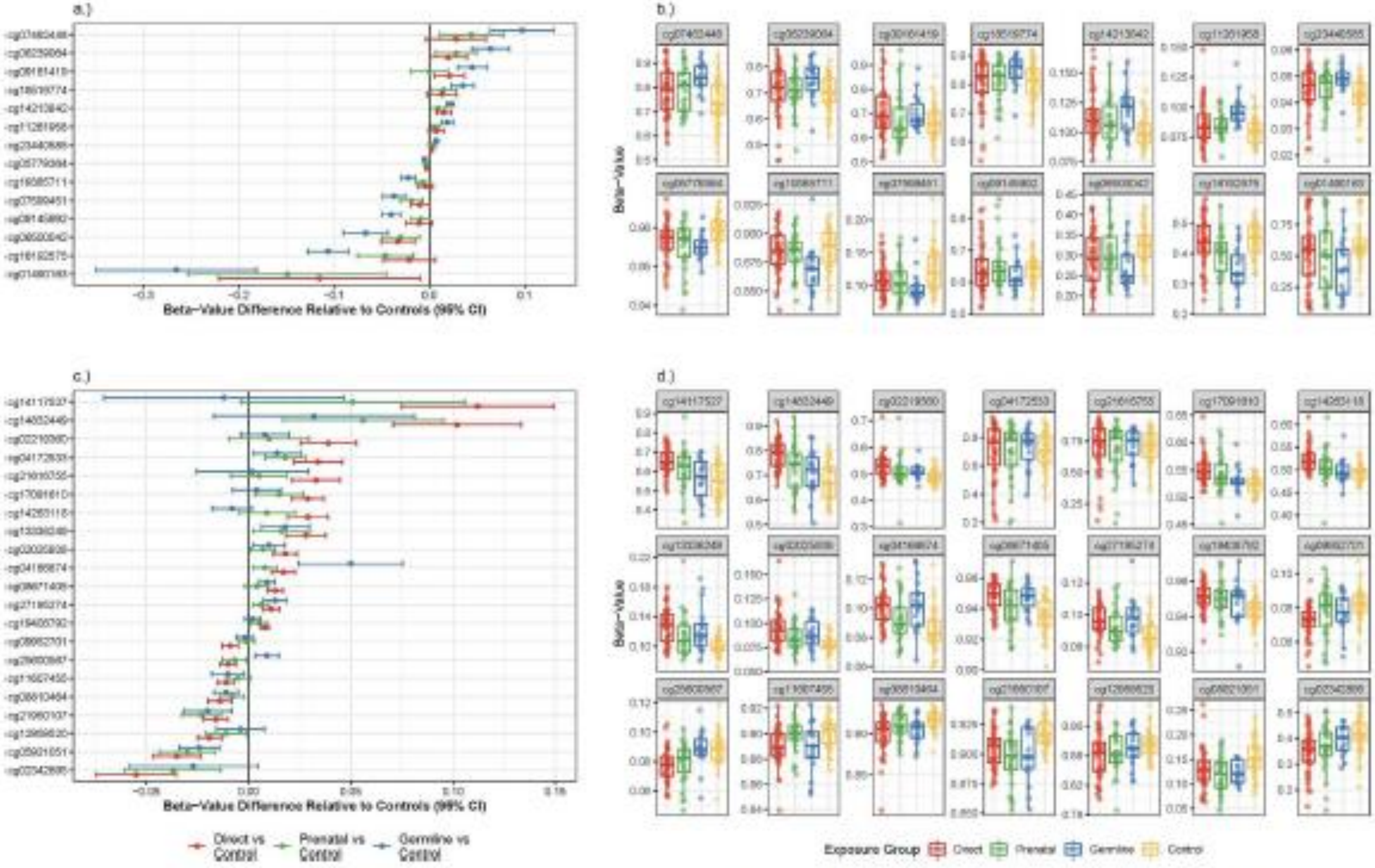
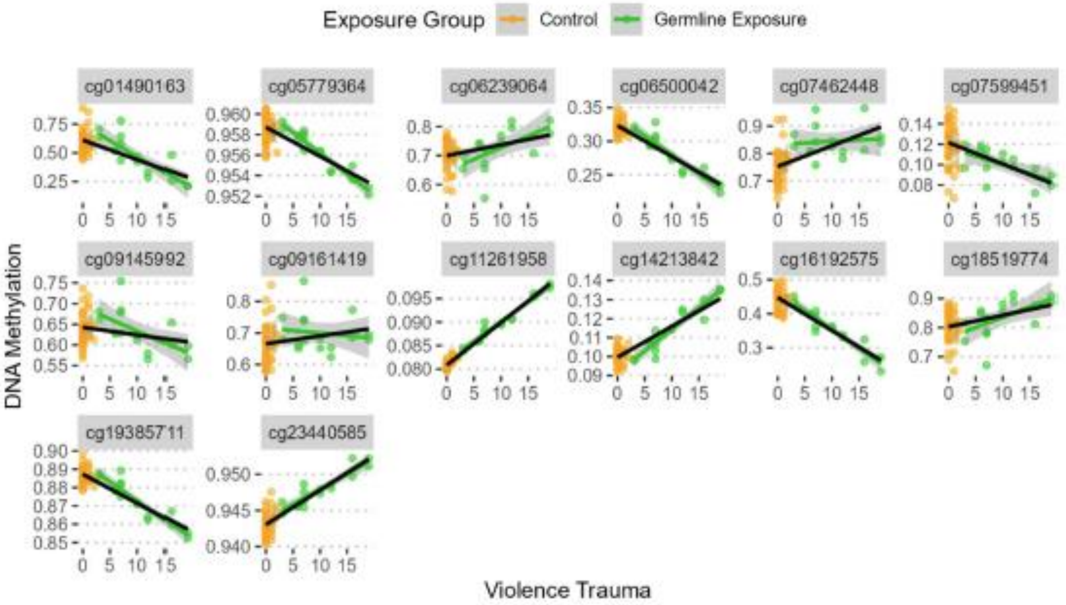


Fig. 2. Genome-wide significant differences in site-specific DNA methylation when comparing violence exposure groups and controls. Forest plots show DNAm levels for loci that reached genome-wide significance (p-value $< 6.5 \times 10^{-8}$) in both robust regression and GEE EWAS when comparing germline exposure to violence (**a,b**) and direct exposure to violence (**c,d**) to controls. Panels (**a**) and (**c**) are forest plots of the beta-value differences between all exposures relative to controls for all significant DMPs. The dots indicate the median difference and bars represent the 95% confidence intervals. Panels (**b**) and (**d**) are boxplots of the distribution of beta-values by violence exposure category; the middle line indicates the median, the box covers the interquartile range, and dots indicate observed beta-values. Exposure types are color coded: red = direct exposure, green = prenatal exposure, blue = germline exposure, and yellow = no exposure.

(a) Germline vs Control - 14 significant sites from EWAS



(b) Direct vs Control - 21 significant sites from EWAS

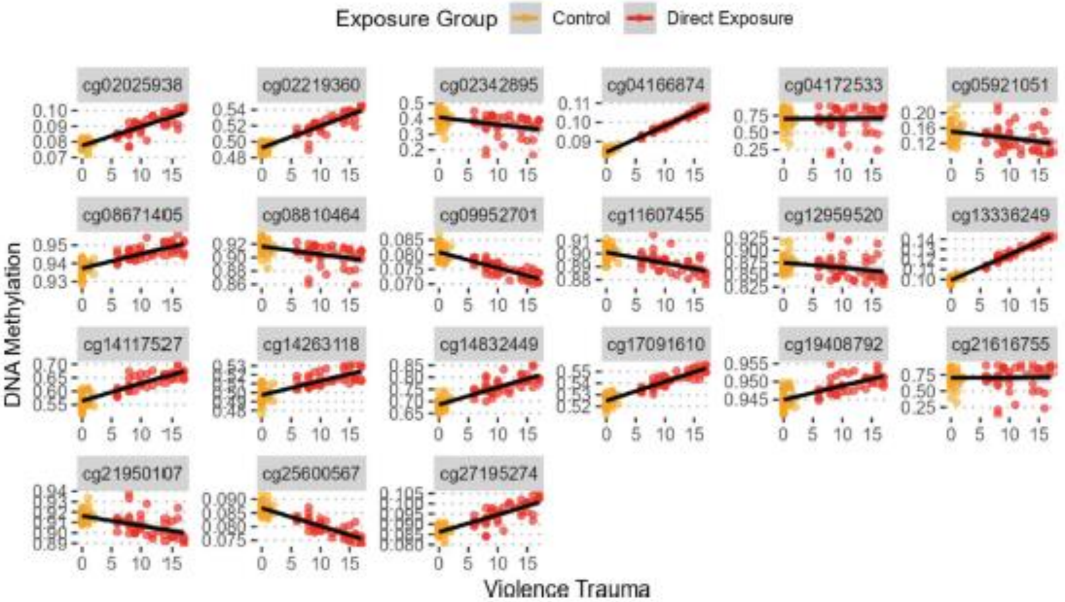


Fig. 3.. DNAm levels and violence trauma exposure scores. Plots show DNAm levels (Y axis) for individual Trauma Event scores (X axis) for (**a**) 14 germline exposure DMPs and (**b**) 21 direct exposure DMPs. Black lines are regression lines for all points and gray shading corresponds to 95% confidence intervals.

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

Lundi Ly[a,b], Donovan Chan[a,b], Jacquetta M. Trasler[a,b,*]

[a] Departments of Pediatrics, Human Genetics and Pharmacology & Therapeutics, McGill University, Canada
[b] Montreal Children's Hospital and Research Institute, McGill University Health Centre, Canada

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

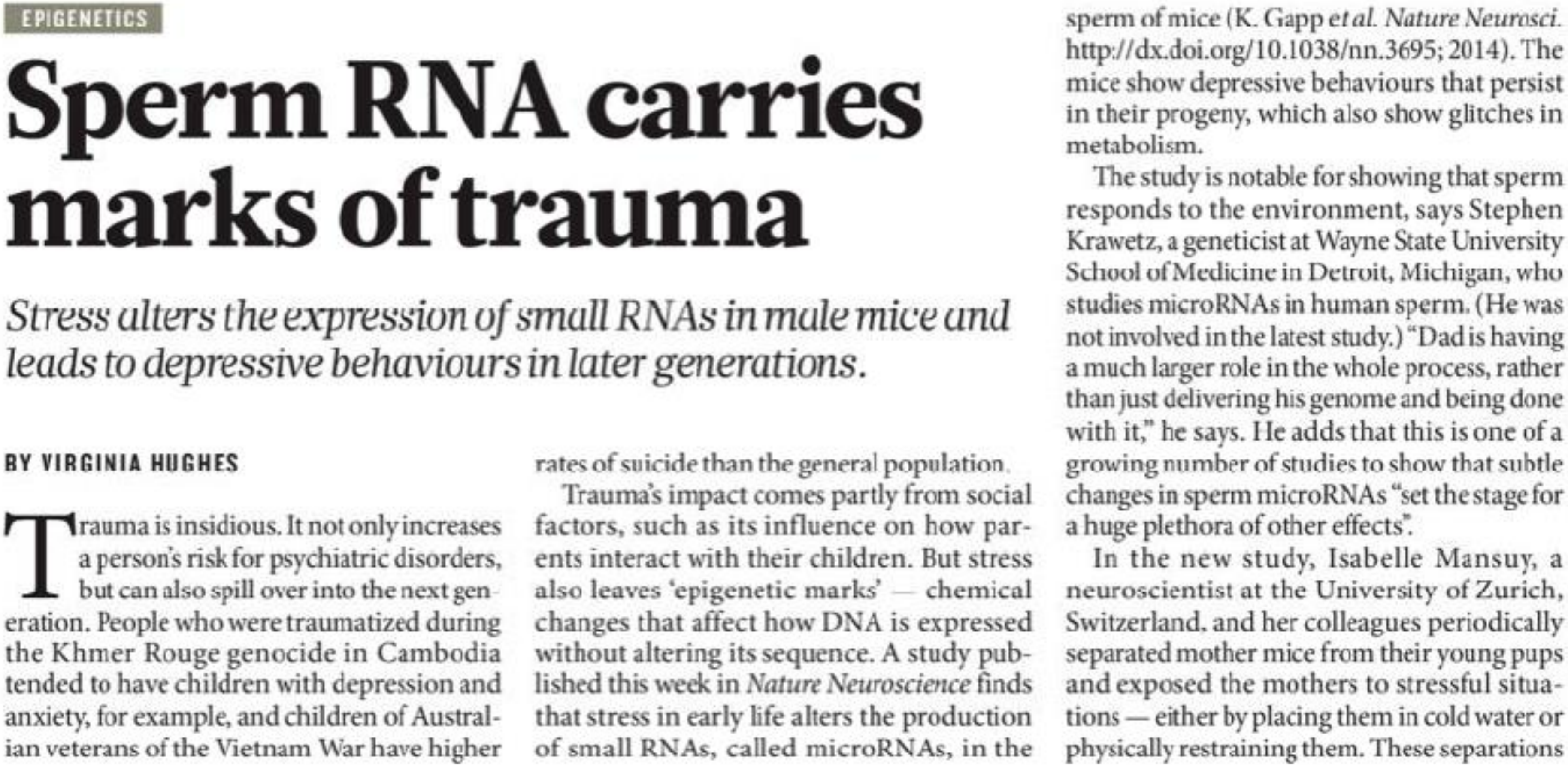
ABSTRACT

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."

CORRIERE DELLA SERA / NEUROSCIENZE

NEUROSCIENZE

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

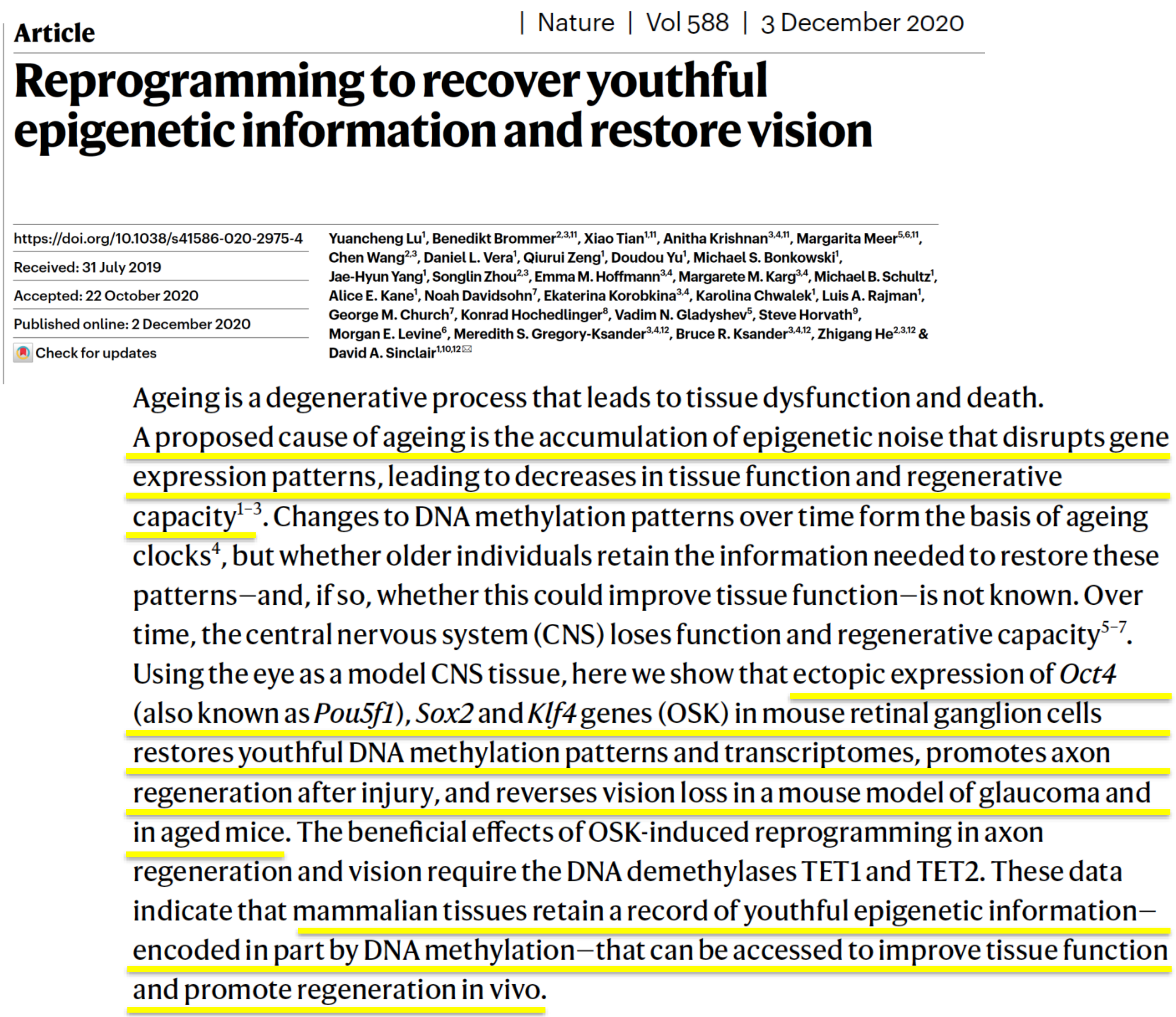
L'idea è quella di riprogrammare le cellule 'vecchie' in modo che recuperino le sane caratteristiche giovanili. Così i ricercatori hanno ridato la vista ad animali di laboratorio

di **Adriana Bazzi**

Gettyimages

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



Article | Nature | Vol 588 | 3 December 2020

Reprogramming to recover youthful epigenetic information and restore vision

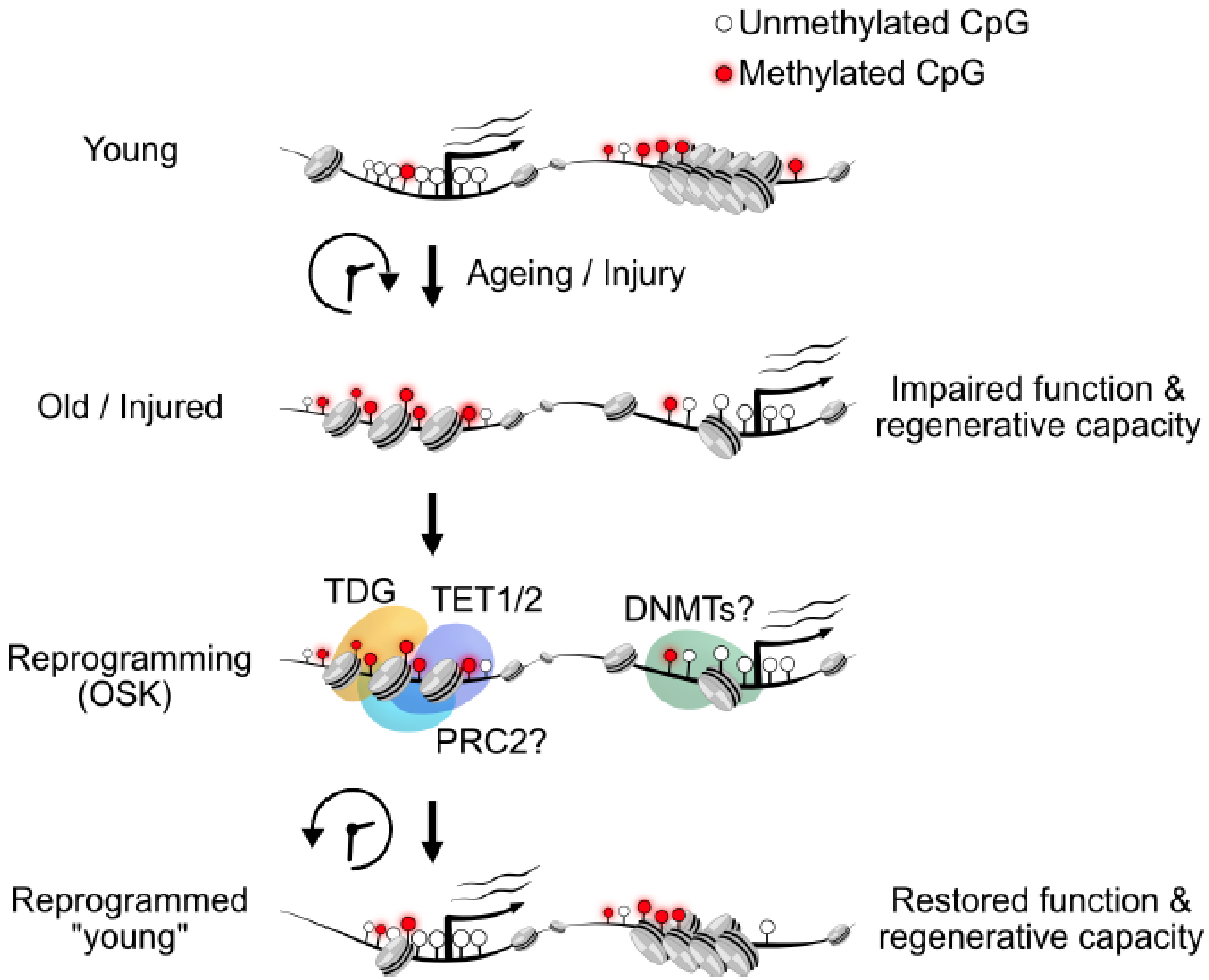
https://doi.org/10.1038/s41586-020-2975-4

Received: 31 July 2019

Accepted: 22 October 2020

Yuancheng Lu[1], Benedikt Brommer[2,3,11], Xiao Tian[1,11], Anitha Krishnan[3,4,11], Margarita Meer[5,6,11], Chen Wang[2,3], Daniel L. Vera[1], Qiurui Zeng[1], Doudou Yu[1], Michael S. Bonkowski[1], Jae-Hyun Yang[1], Songlin Zhou[2,3], Emma M. Hoffmann[3,4], Margarete M. Karg[3,4], Michael B. Schultz[1], Alice E. Kane[1], Noah Davidsohn[7], Ekaterina Korobkina[3,4], Karolina Chwalek[1], Luis A. Rajman[1], George M. Church[7], Konrad Hochedlinger[8], Vadim N. Gladyshev[5], Steve Horvath[9], Morgan E. Levine[6], Meredith S. Gregory-Ksander[3,4,12], Bruce R. Ksander[3,4,12], Zhigang He[2,3,12] & David A. Sinclair[1,10,12] ✉

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.

ALL THESE FACTORS INFLUENCE EPIGENETICS:

Social interactions
Alternative medicine
Therapeutic drugs
Microbiome
Exercise
Financial status
Diurnal/seasonal correlations
Disease exposures
Toxic chemicals
Drugs of abuse
Diet