
EPIGENETICA

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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.**

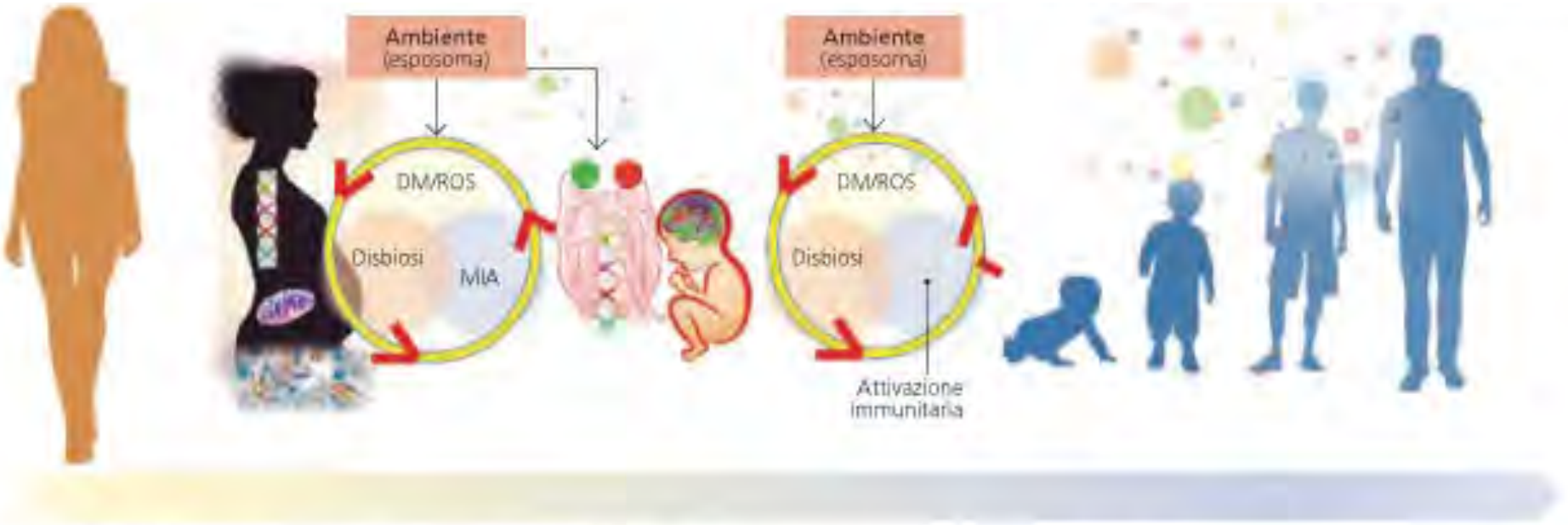
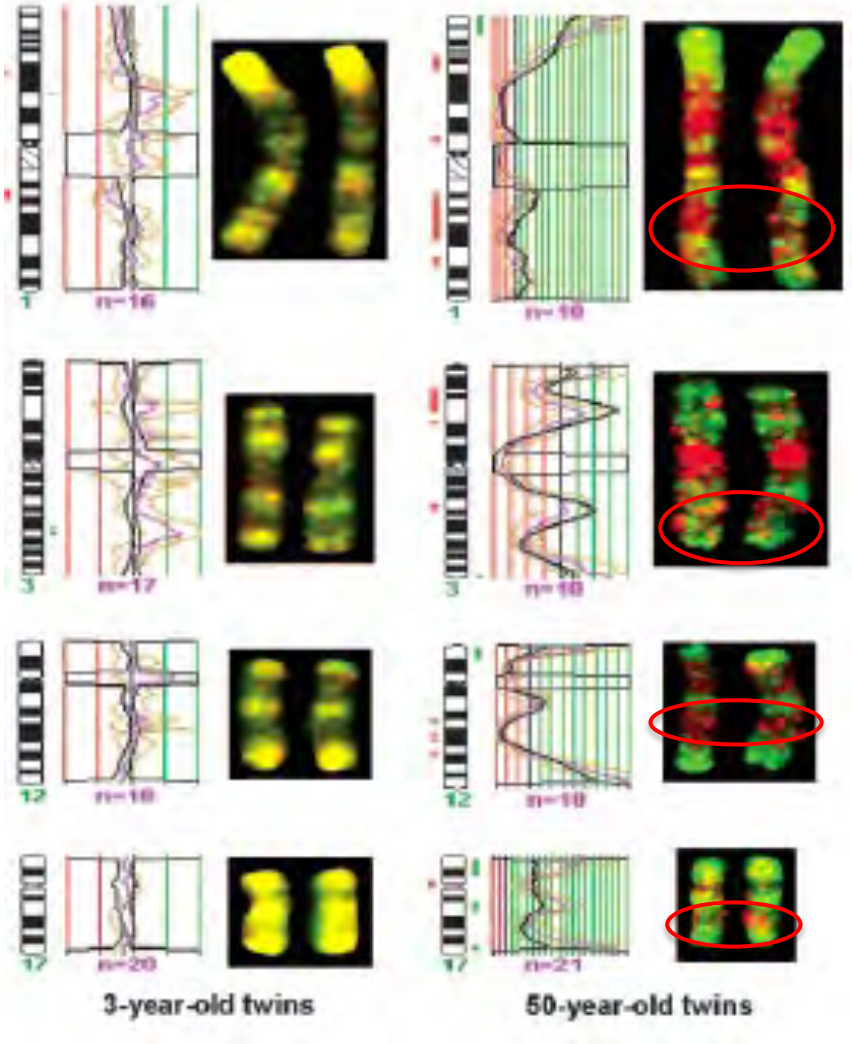


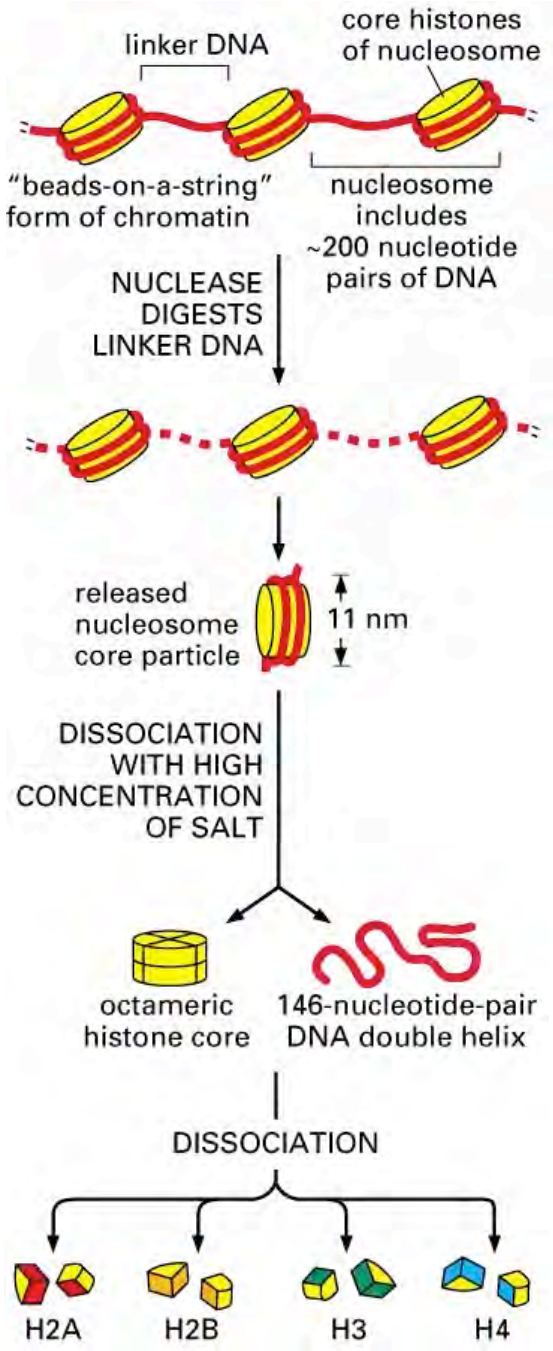
Figura 2. **Durante l'ontogenesi, i fattori ambientali (esposoma) influiscono sulla programmazione epigenetica embriofetale, direttamente o attraverso i meccanismi del "trio cattivo" costituito da disbiosi/attivazione immunitaria materna (MIA)/disfunzione mitocondriale (DM)-stress ossidativo (ROS). Dopo 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.*[4]).

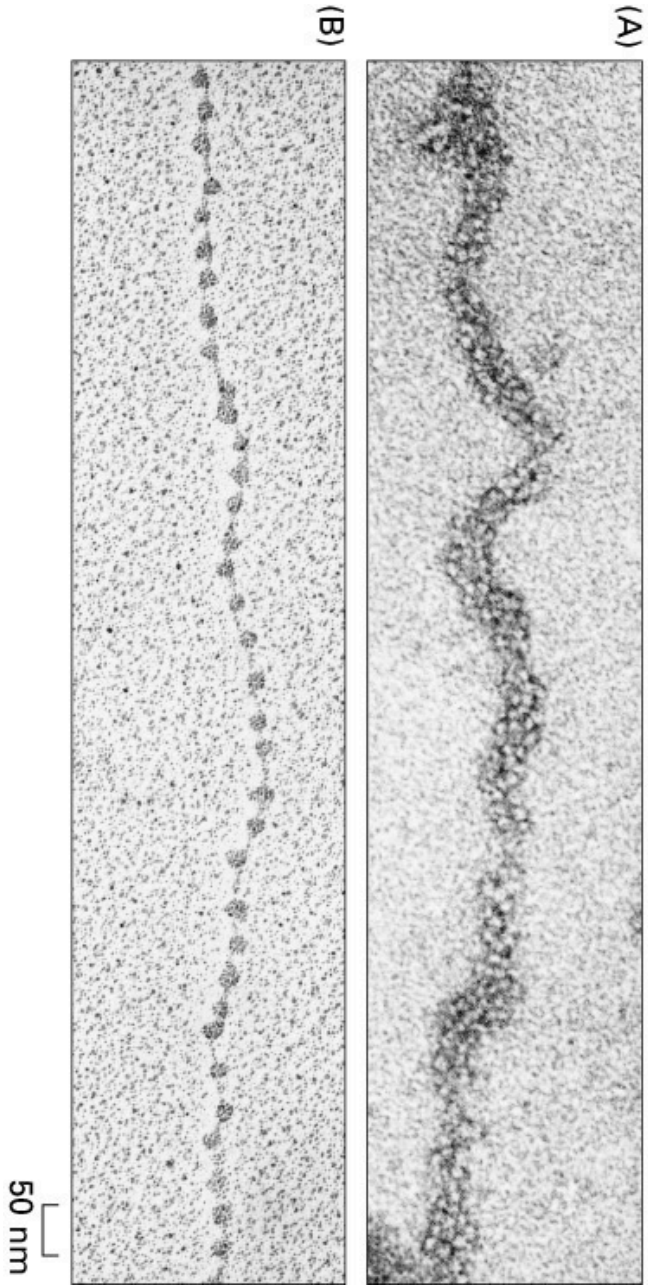
Epigenetic differences arise during the lifetime of monozygotic twins

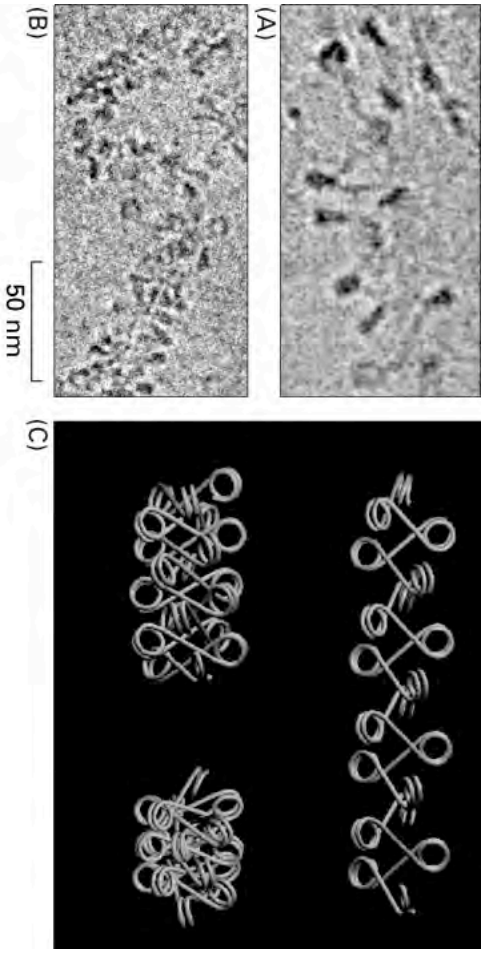
Mario F. Fraga*, Esteban Ballestar*, Maria F. Paz*, Santiago Ropero*, Fernando Setien*, Maria L. Ballestar†, Damia Heine-Suñer‡, Juan C. Cigudosa§, Miguel Urioste¶, Javier Benitez¶, Manuel Boix-Chornet†, Abel Sanchez-Aguilera†, Charlotte Ling||, Emma Carlsson||, Pernille Poulsen**, Allan Vaag**, Zarko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

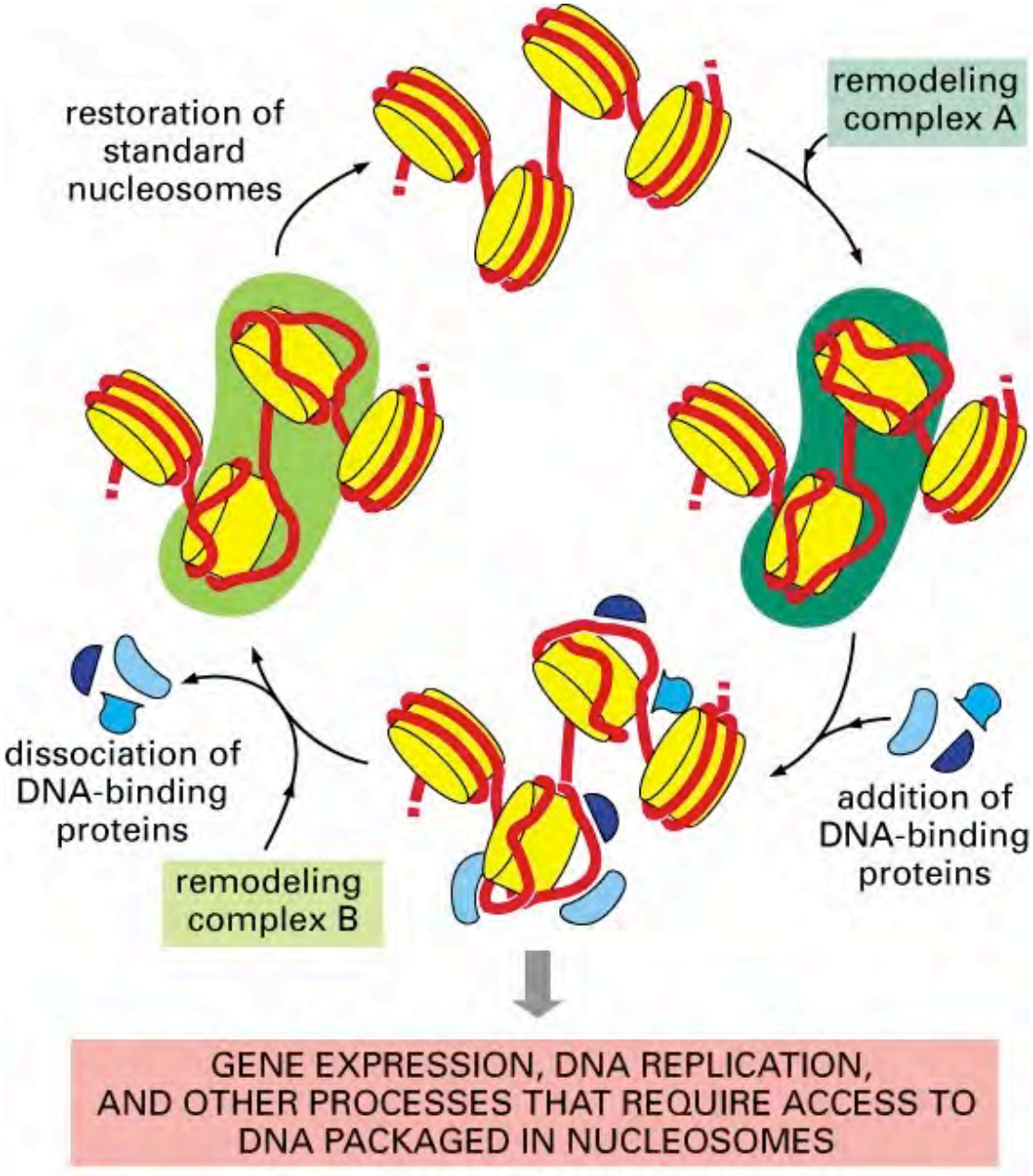
"Epigenomics is where genomics was 30 years ago, when everyone was working on part of the puzzle."
— Peter Jones




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


(B)
(A)
50 nm


(B)
(A)
50 nm
(C)

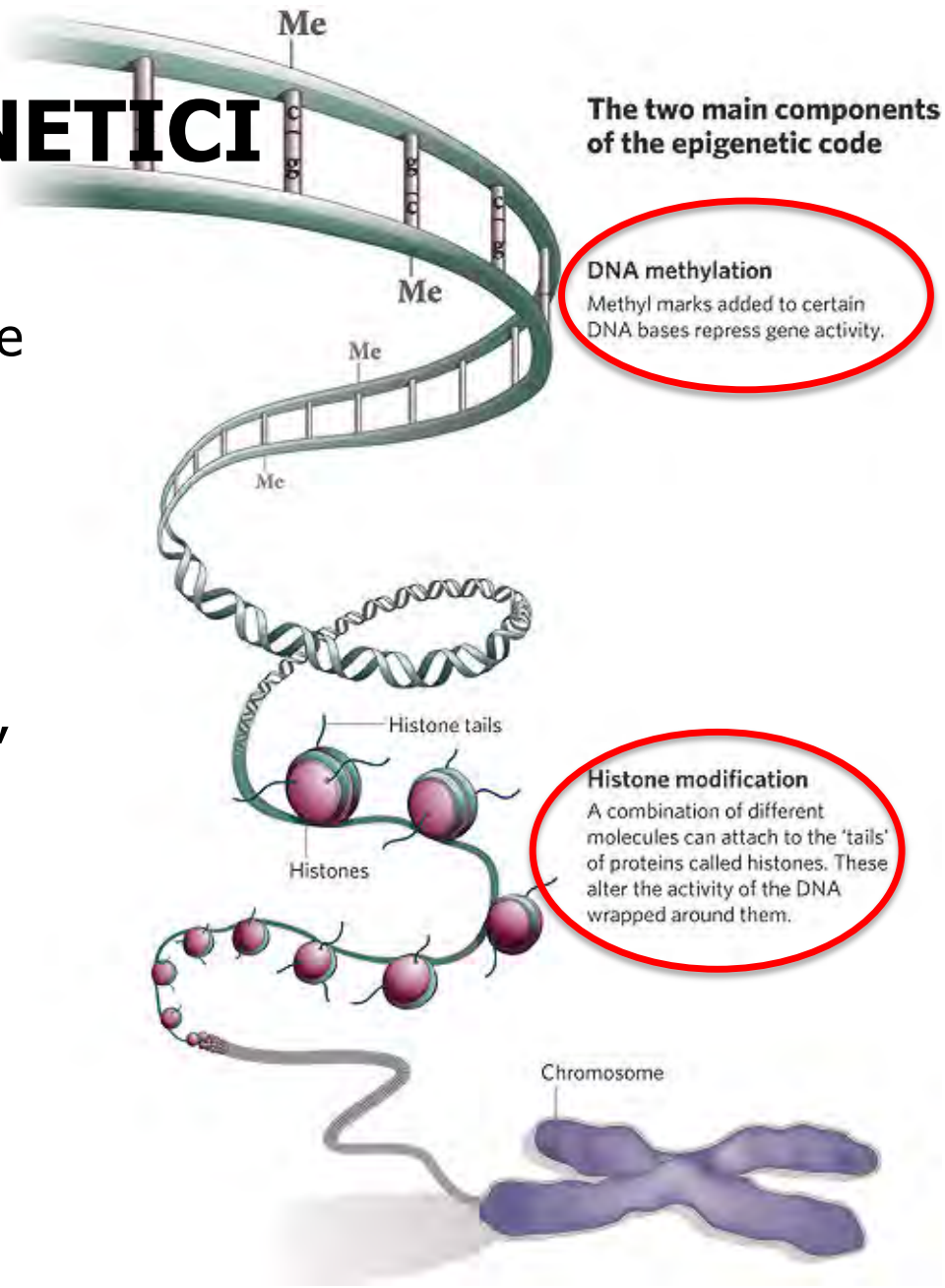

restoration of standard nucleosomes
remodeling complex A
dissociation of DNA-binding proteins
addition of DNA-binding proteins
remodeling complex B
GENE EXPRESSION, DNA REPLICATION, AND OTHER PROCESSES THAT REQUIRE ACCESS TO DNA PACKAGED IN NUCLEOSOMES

MECCANISMI EPIGENETICI

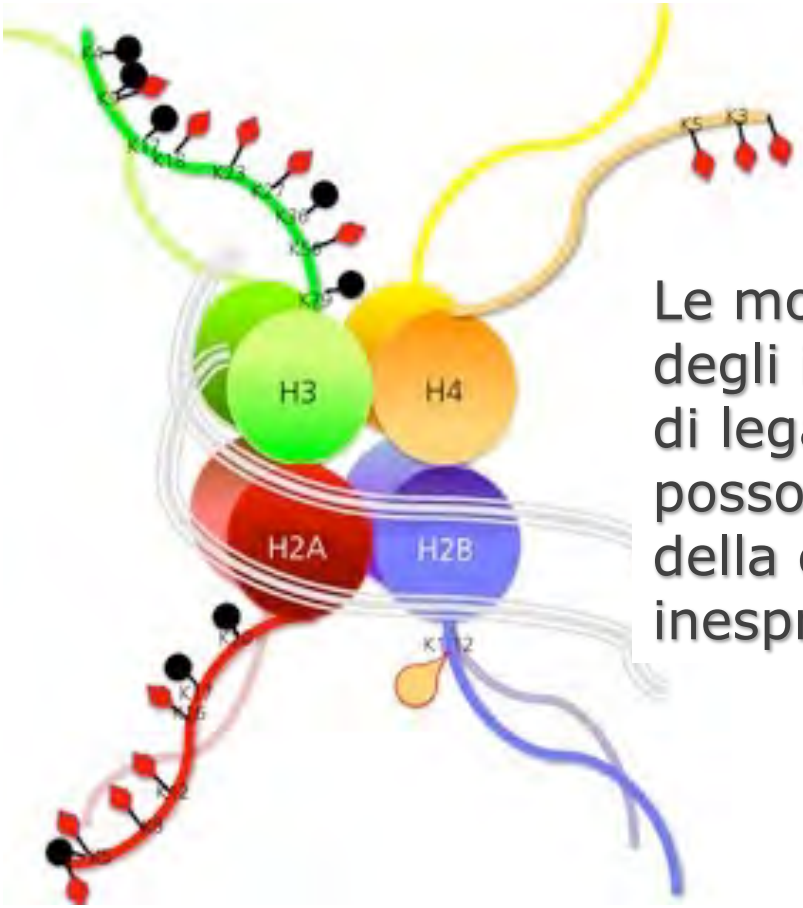
Fattori che influenzano l'espressione genica, trasmessi alla progenie, ma che non sono direttamente attribuibili a sequenze di DNA.

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

<u>METILAZIONE DEL DNA</u>



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

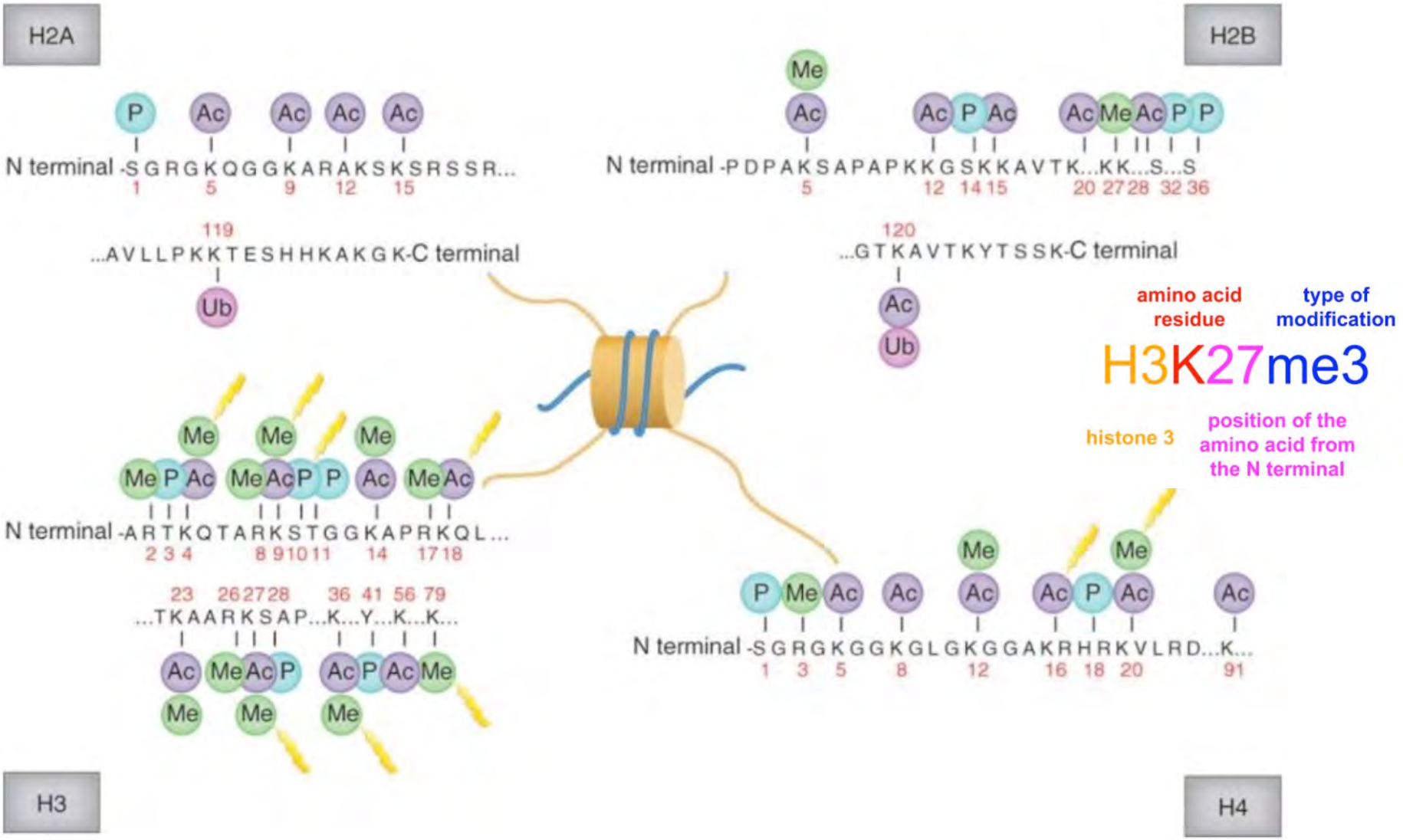


Le modificazioni chimiche degli istoni forniscono siti di legame per proteine che possono cambiare lo stato della cromatina in attivo o inespresso

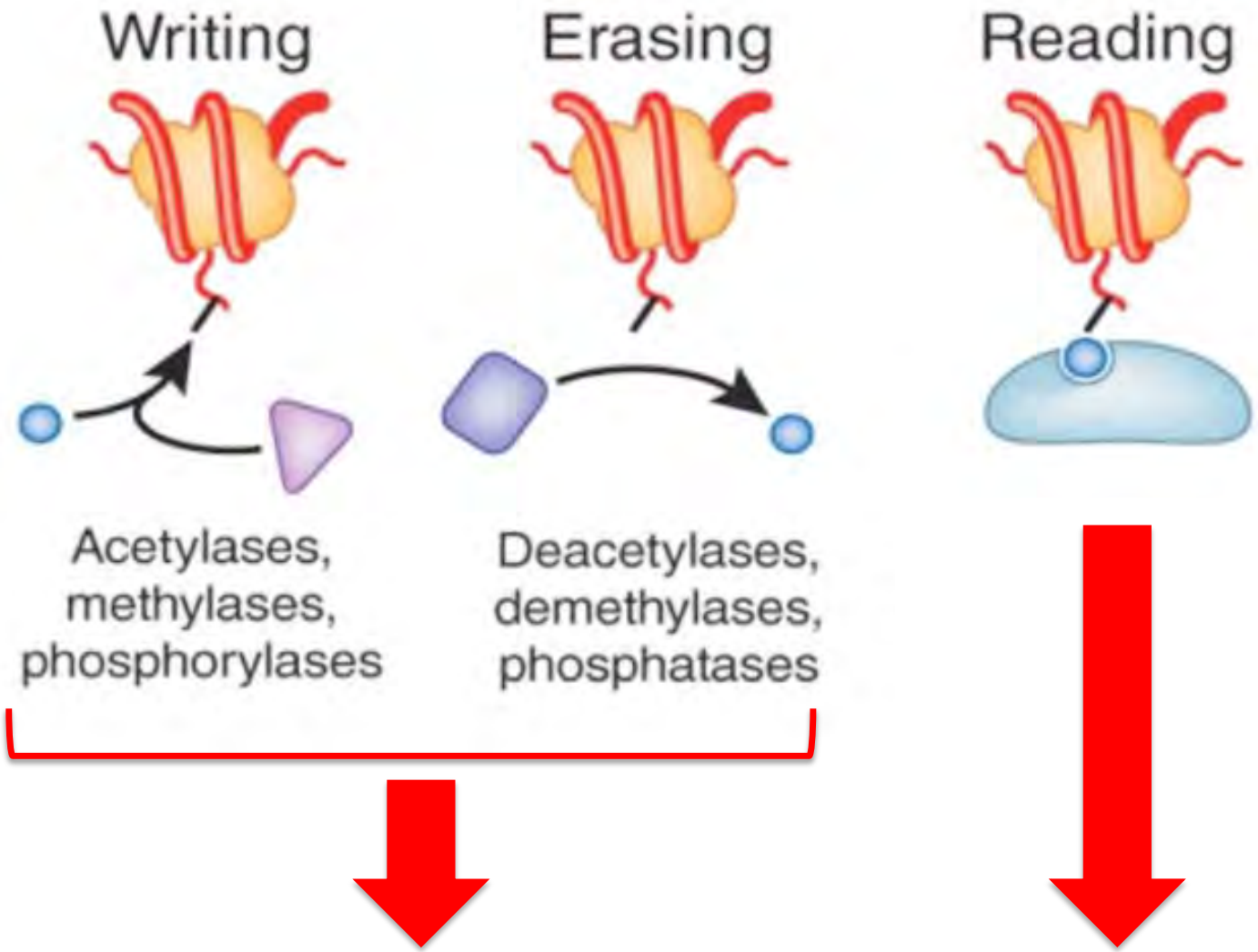
Una particolare combinazione di tali modificazioni ha un significato biologico (CODICE ISTONICO)

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

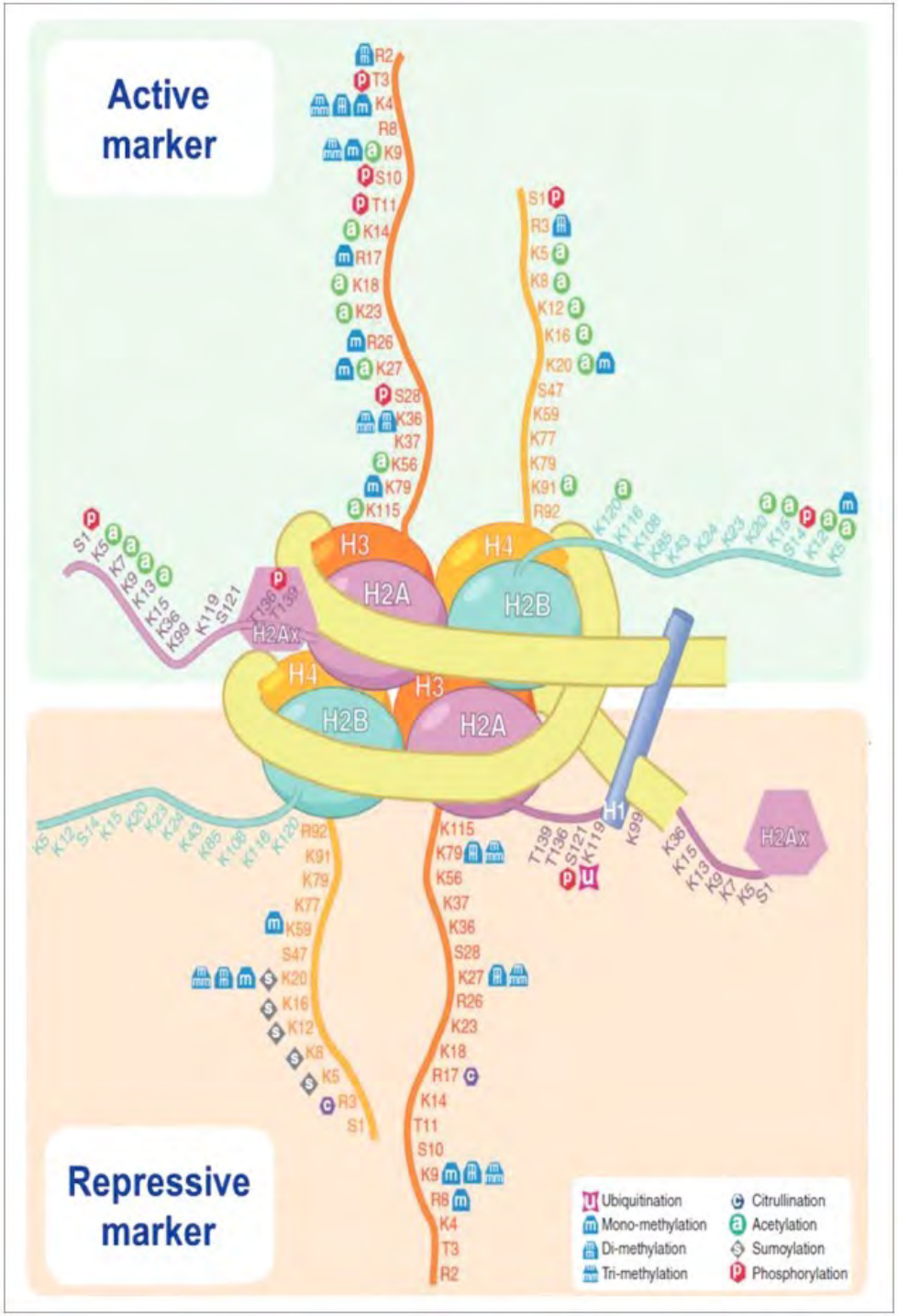


L'ipotesi del **codice istonico** propone che modificazioni covalenti post-traduzionali delle code degli istoni vengano "lette" dalla cellula portando ad un risultato trascrizionale combinatorio complesso



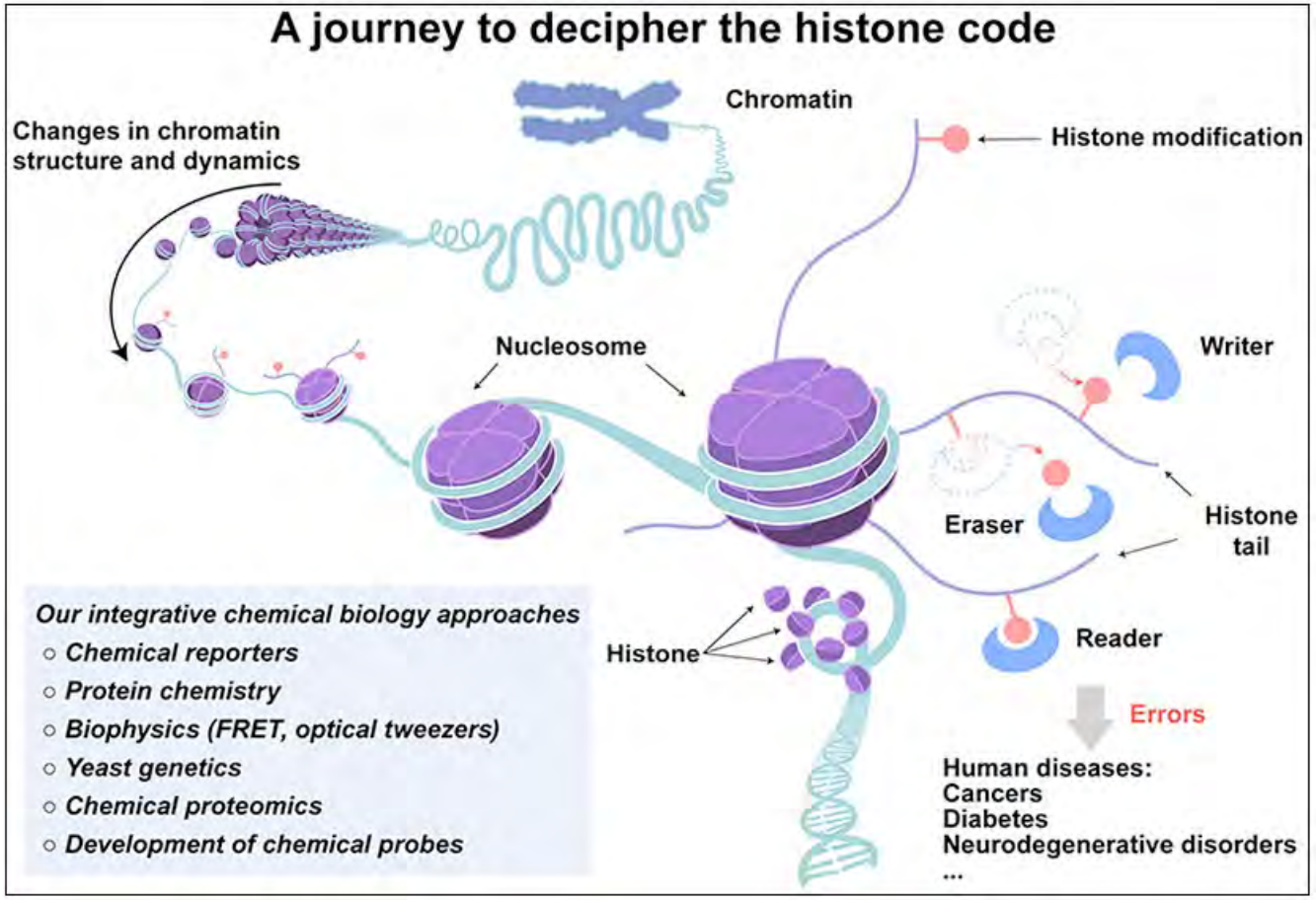
Modificazioni prot-traduzionali
DINAMICHE

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?



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

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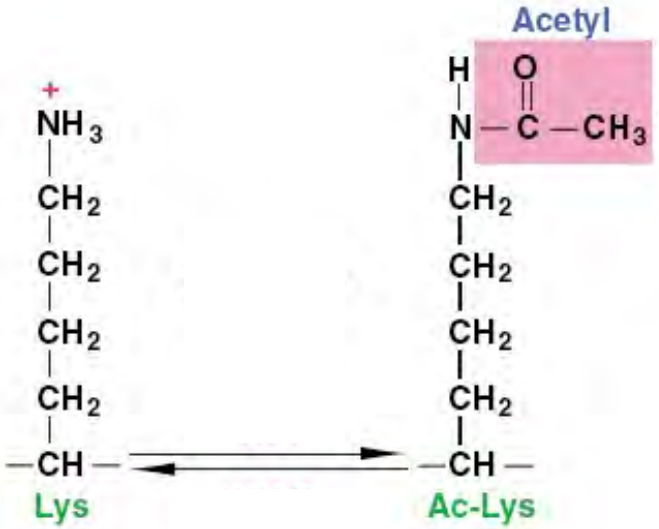
CHI AGISCE?

COMPLESSI DI MODIFICAZIONE DELLA CROMATINA:

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

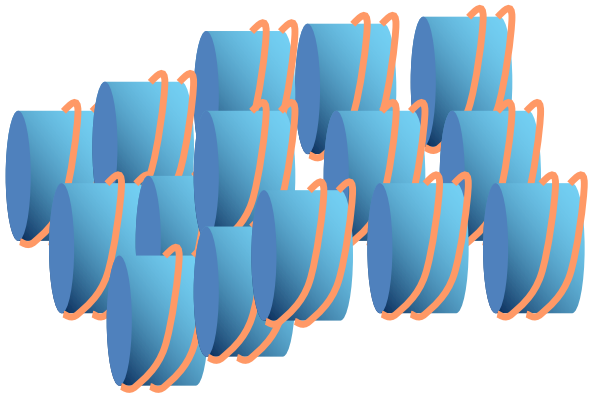
Acetylation is very dynamic and rapidly changing



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

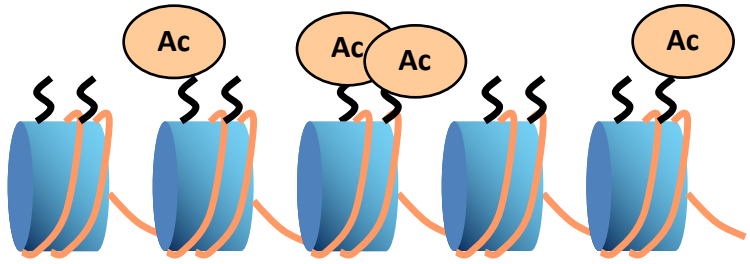
HDAC promotes the removal of the acetyl group from the acetyl-lysine regenerating the ε– amino group and releasing the acetate molecule

heterochromatin
(transcriptionally inactive/condensed)



HAT

euchromatin
(transcriptionally active/accessible)



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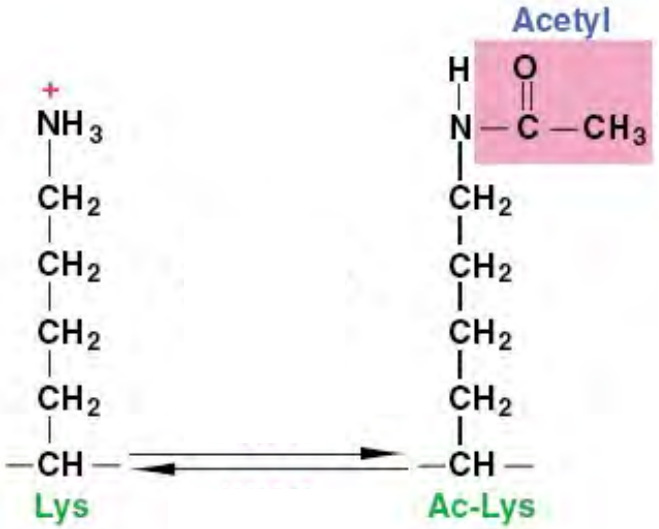

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Writing, erasing and reading histone lysine methylations

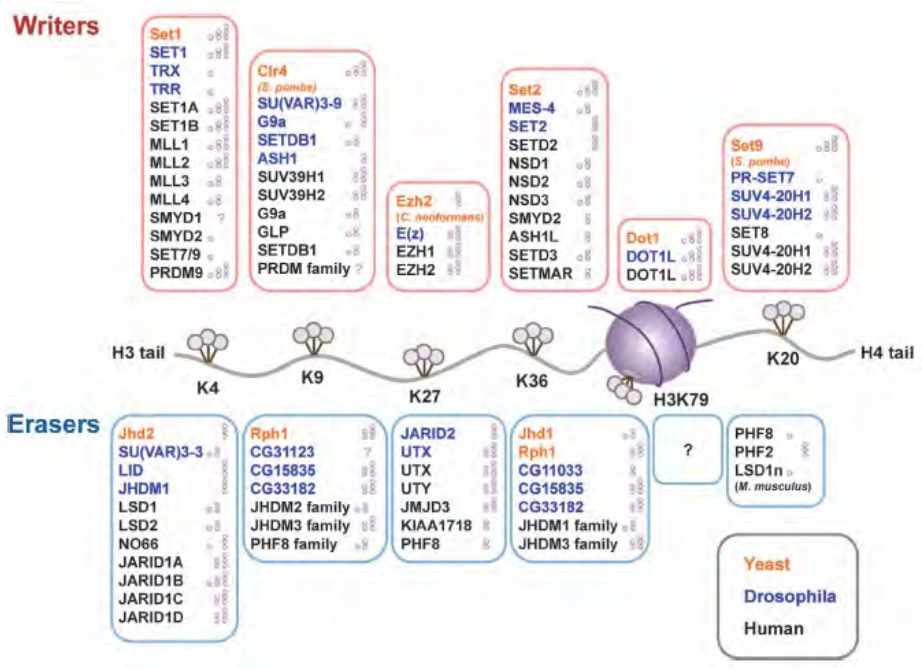
Experimental & Molecular Medicine (2017) 49, e324; doi:10.1038/emm.2017.11

www.nature.com/emm

Histone lysine methylations confer active or repressive transcription depending **on their positions** and methylation states.

H3K4, H3K36 and H3K79 methylations = active transcription

H3K9, H3K27 and H4K20 methylations = silenced chromatin states.



Histone lysine methylation functions are exerted by effector molecules that specifically recognize the methylated site.

These '**reader**' proteins contain methyl-lysine-binding motifs the ability to distinguish target methyl-lysines and surrounding amino-acid sequence.

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Mechanisms for the Inheritance of Chromatin States

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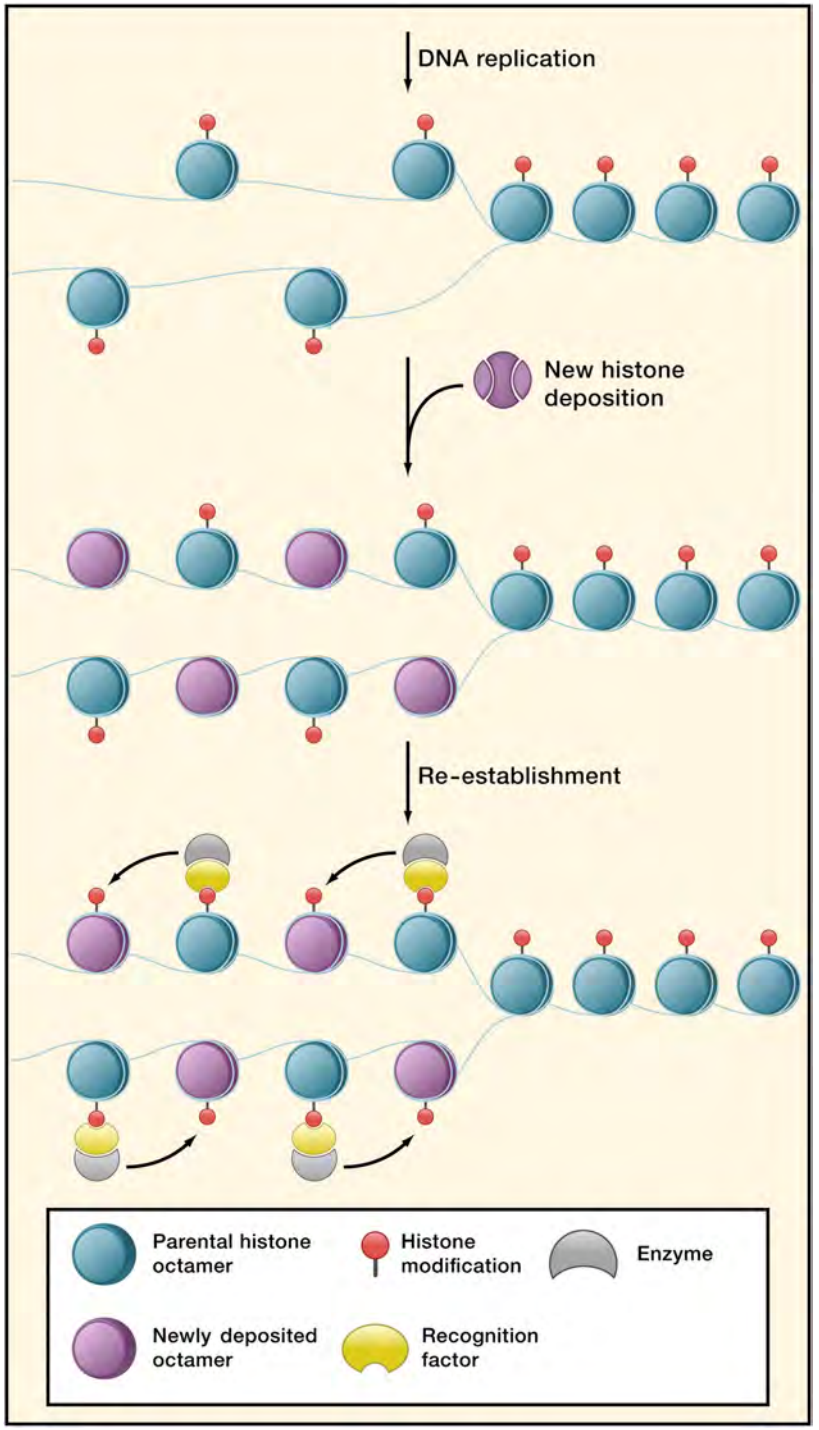
DOI 10.1016/j.cell.2011.07.013

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**.



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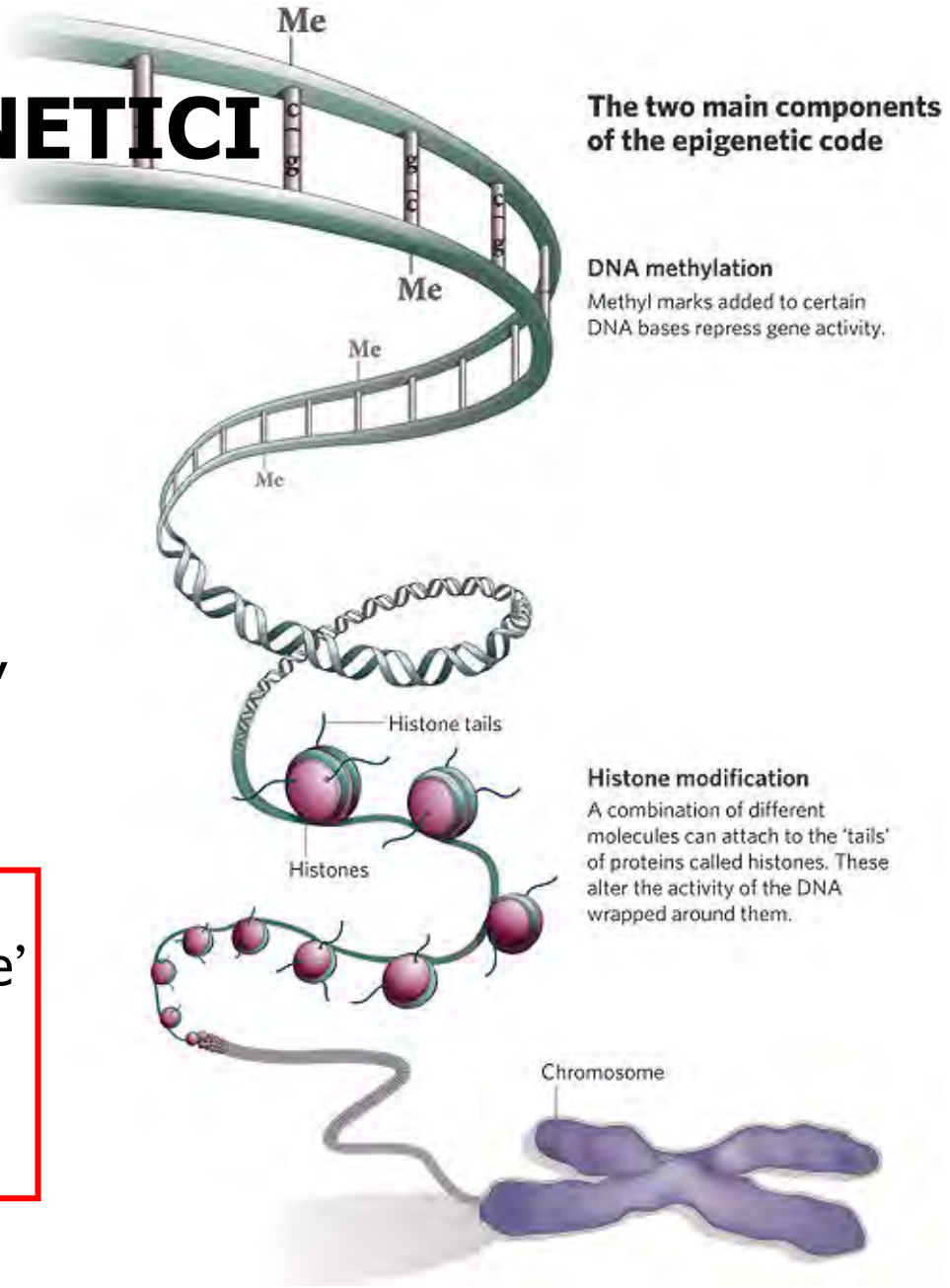
<u>METILAZIONE DEL DNA</u>
Nelle cells eucariotiche la metilazione e' a carico della G. Solo il 3% delle C e' metilato; in genere e' bersaglio della metilazione la C delle doppiette CpG.



DNA methylation

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

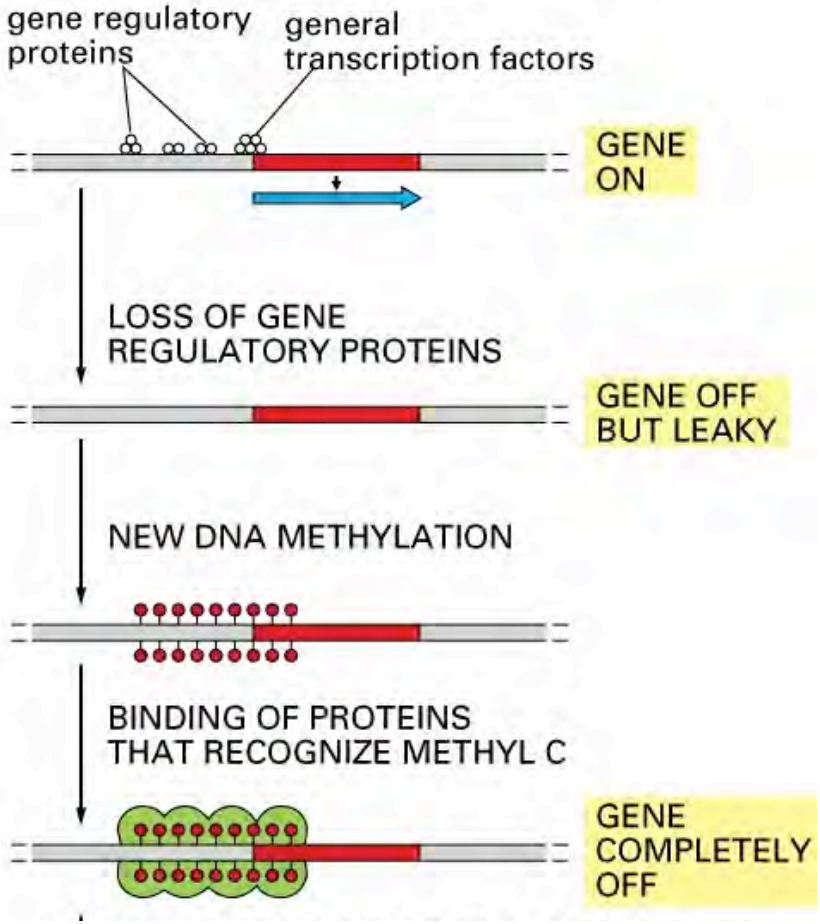
Given the robust stability of DNA methylation, tight regulation is of crucial importance, for proper cellular function (Greenberg, 2021).

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).

Vertebrates Use DNA Methylation to Lock Genes in a **Silent** State

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

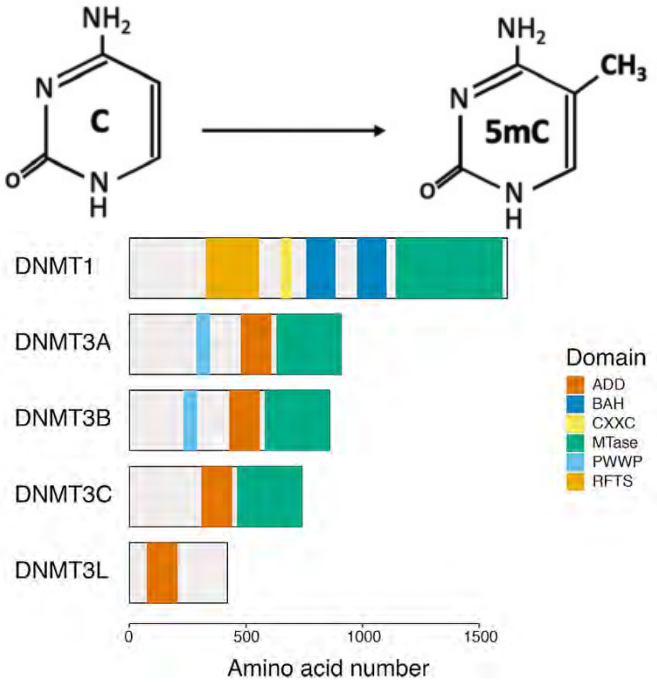
These DNA-binding proteins, in turn, interact with **chromatin remodeling complexes** and **histone deacetylases** that condense chromatin so it becomes transcriptionally inactive.



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

DNA Methyl-transferases

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



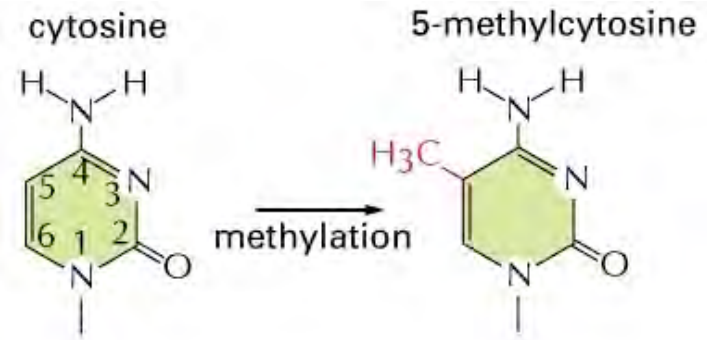
In mammals, there are 2 families of **DNA Methyl-transferases**:

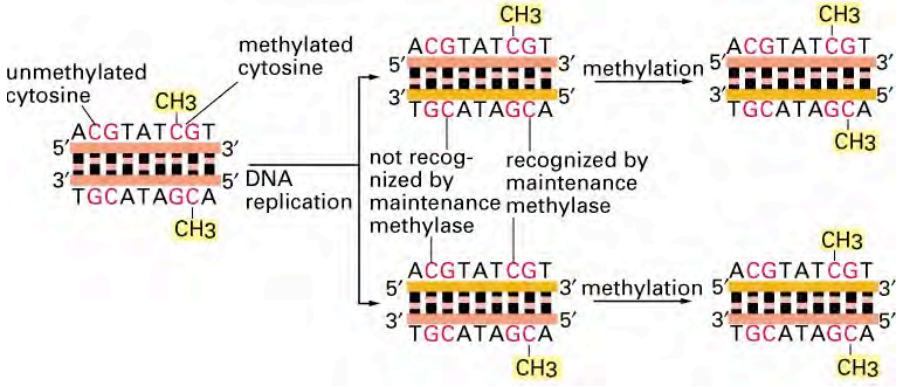
a) **DNMT1**, the maintenance methyltransferase that is responsible for the methylation of hemi-methylated CpG sites during DNA replication.

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

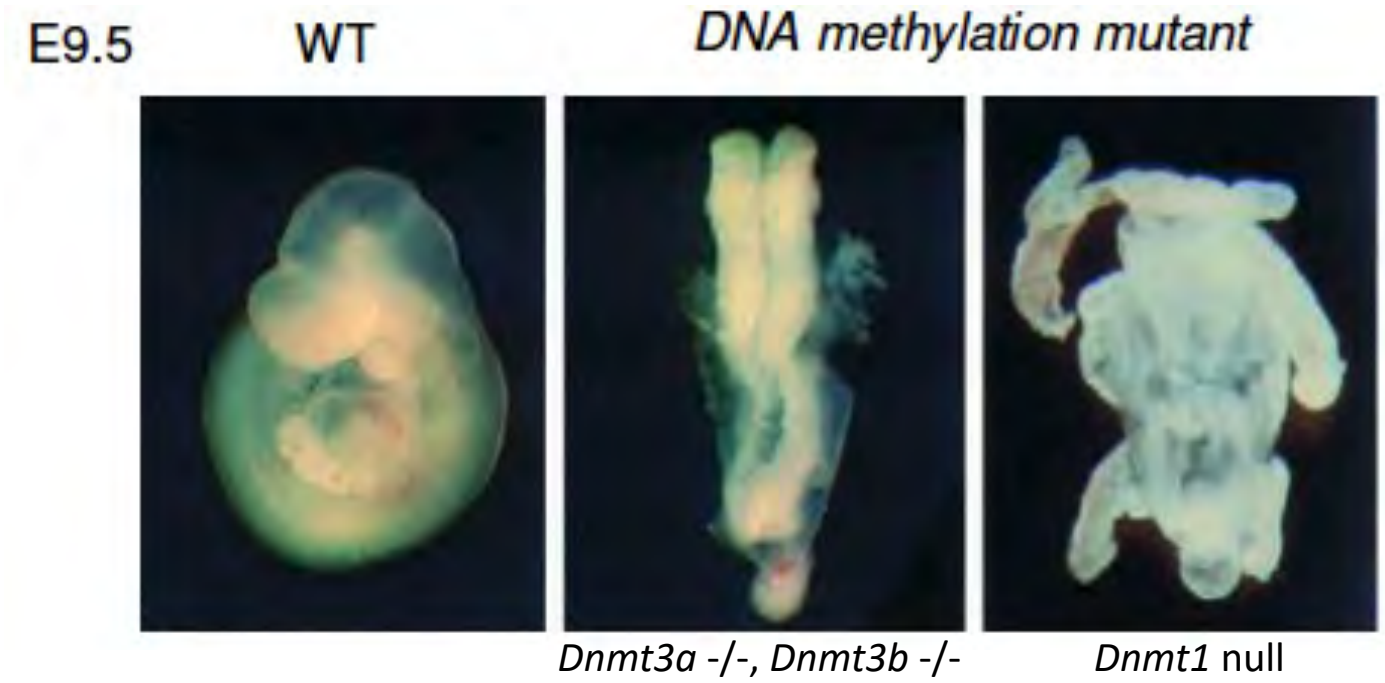
Maintenance of DNA methylation

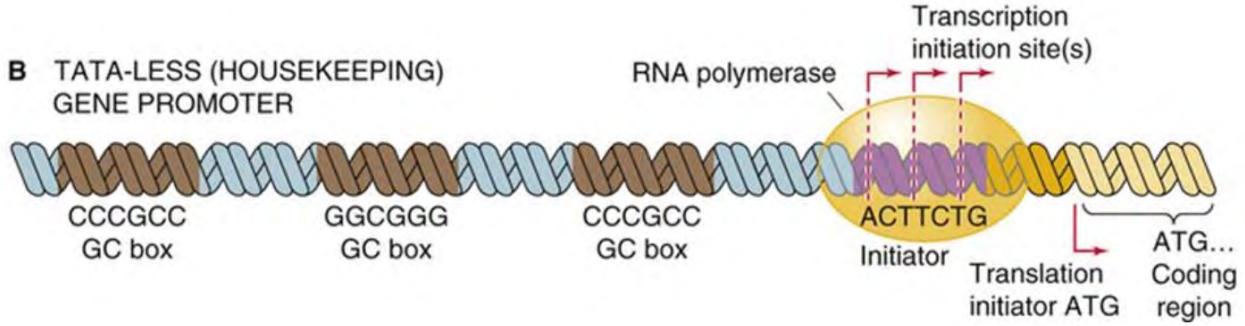
1. **Dnmt1** maintains the methyl-CpG content of both daughter DNA duplexes following replication (higher affinity for hemimethylated mCpG DNA)
2. Dnmt1 Methyltransferase is localized to the chromosomal replication complex
3. Methylation of newly synthesized DNA takes place less than one minute following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



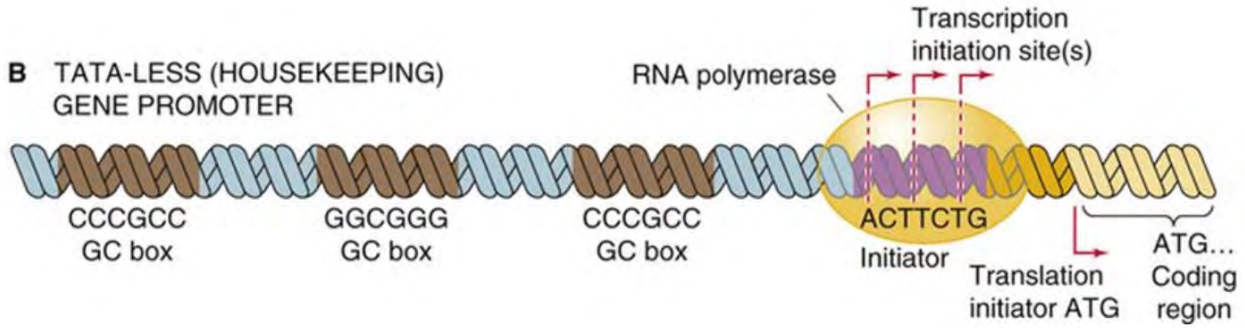


The essential role of DNA methylation for a proper differentiation is supported by the severe developmental defects and embryonic lethality exhibited in DNMT-deficient mice.





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



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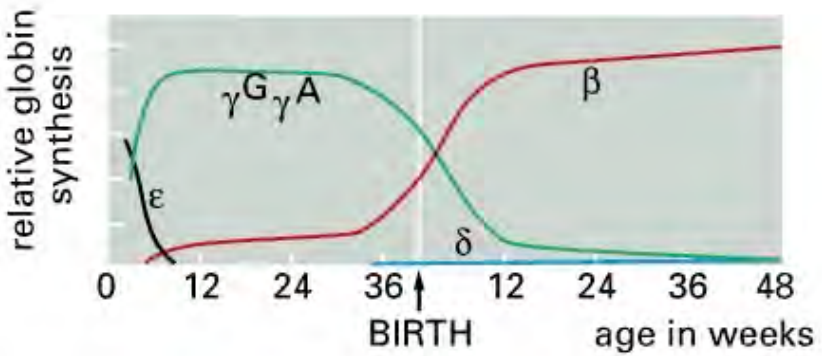
CpG island (CGI) promoters are not methylated

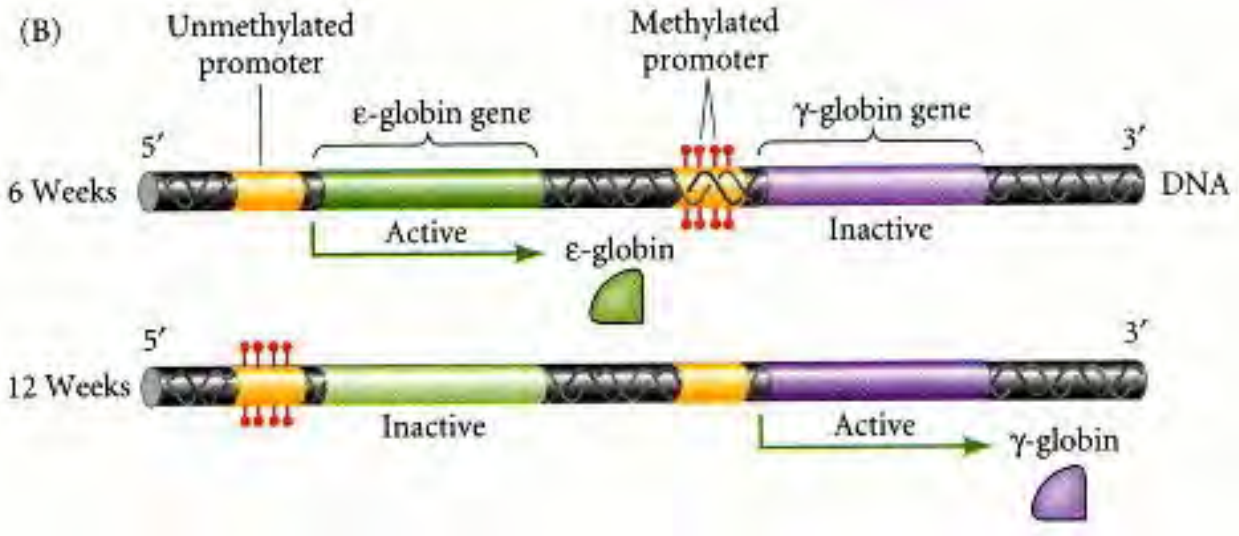
- Roughly two-thirds of promoters are CpG-Islands, and comprise most housekeeping and developmental genes.
- ALL CpG islands are associated with transcribed genes
- Keeping promoters free of methylation is absolutely crucial for proper cellular function.
- X-linked CpG islands become methylated upon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

The amazing demethylase

Howard Cedar and Gregory L. Verdine

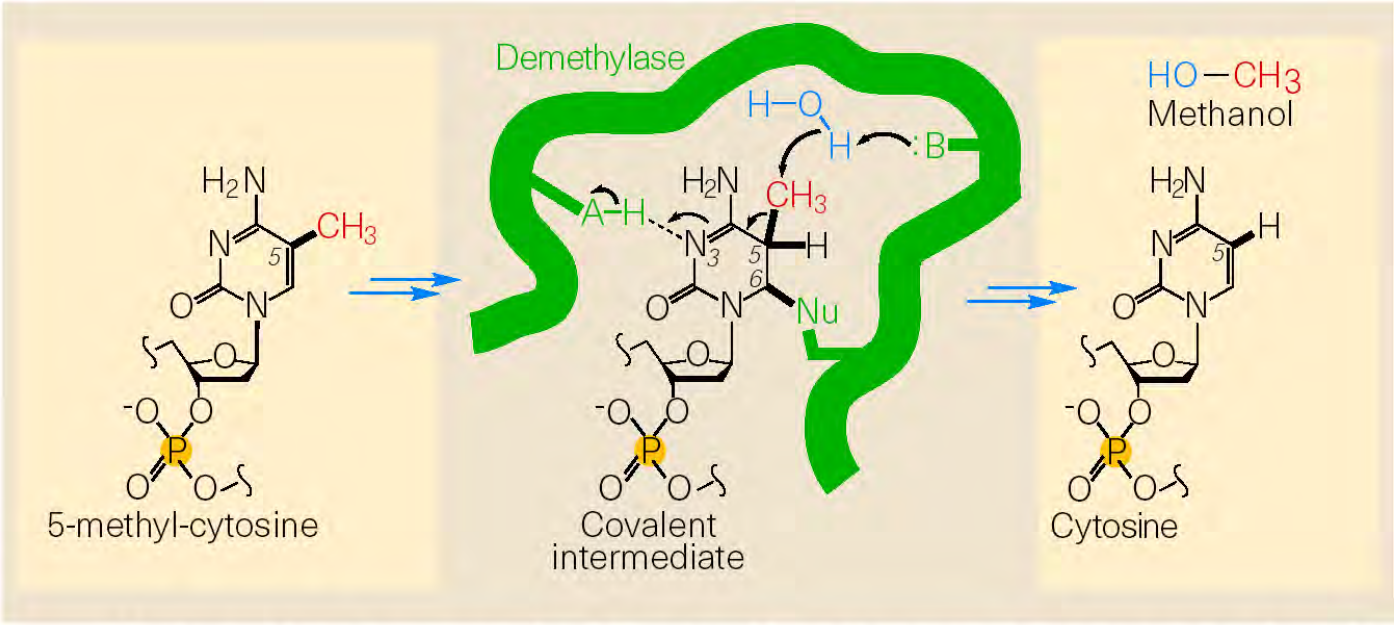


Figure 1 **Mechanism for the enzymatic demethylation of 5-methyl-cytosine. The demethylase (green) is envisaged to form a covalent intermediate by addition of an enzymatic nucleophile (Nu–H) across the 5,6 double bond, assisted by proton shuffling at N3. This intermediate is poised to attack the hydroxide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

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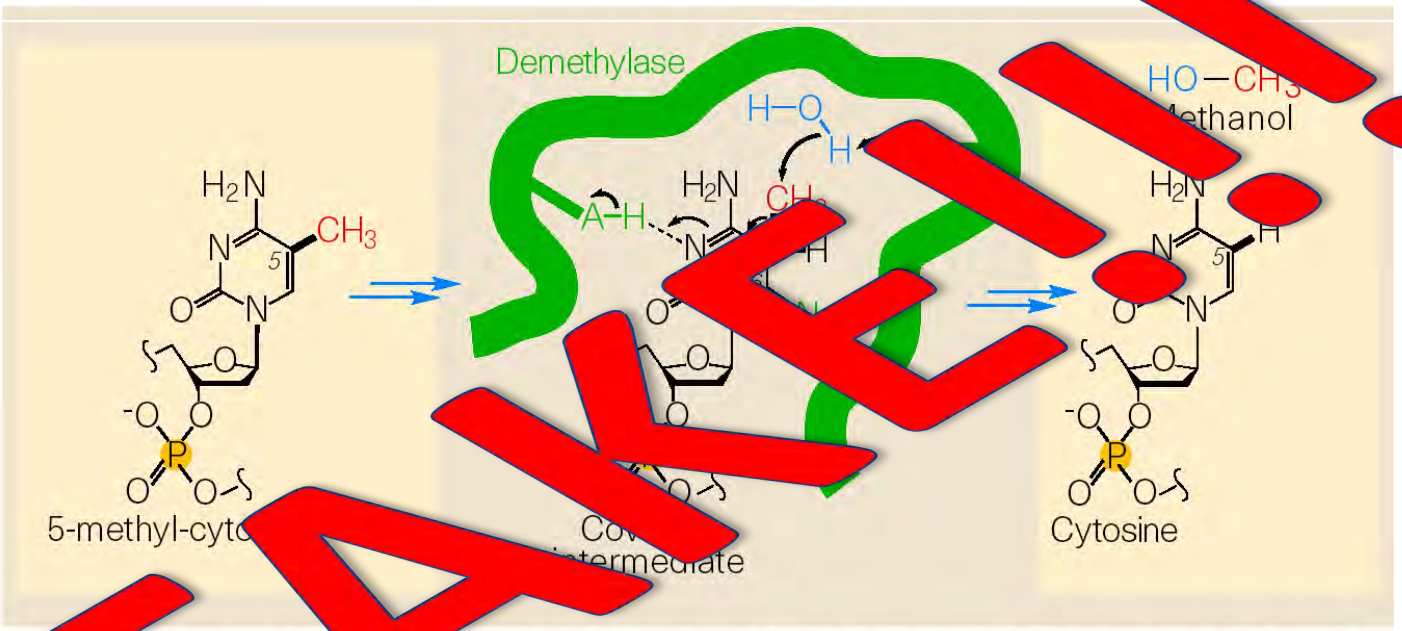
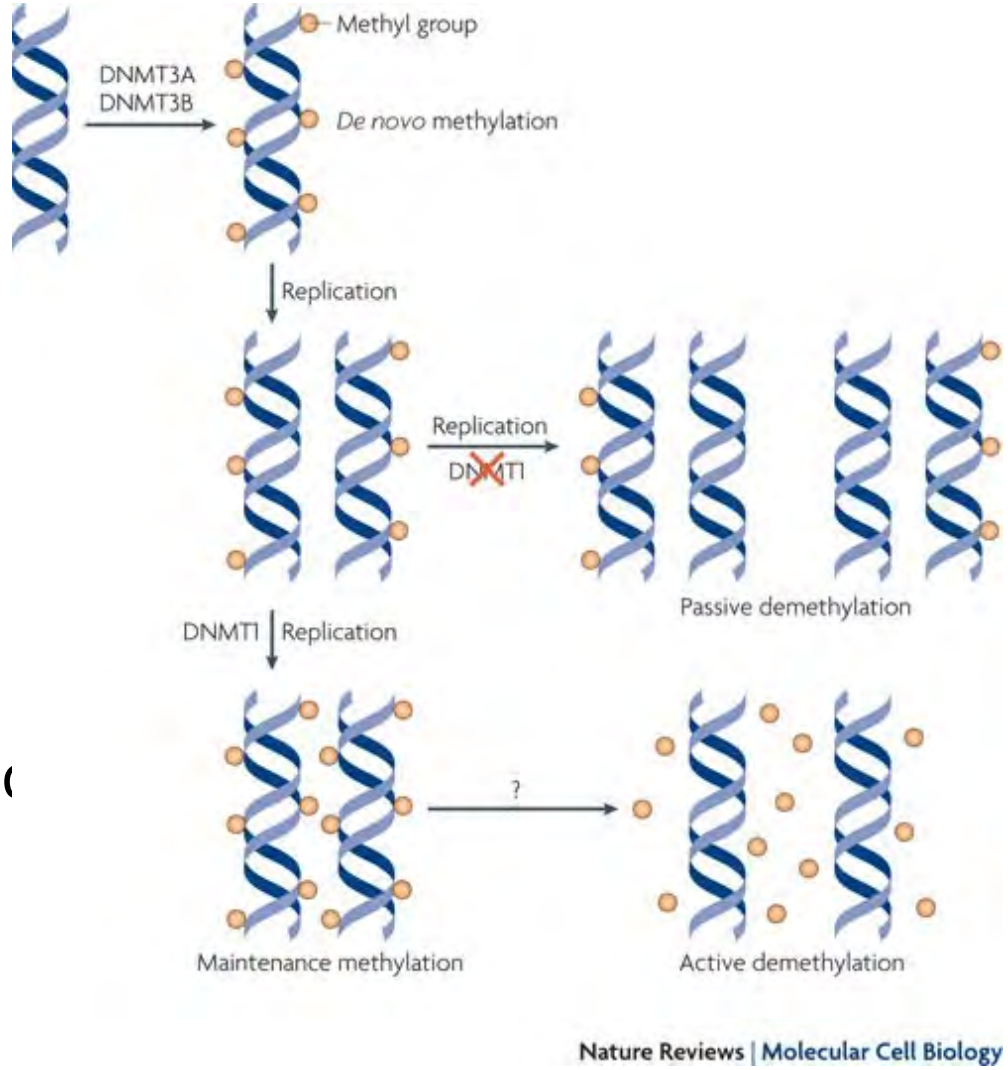


FAKE!!!

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

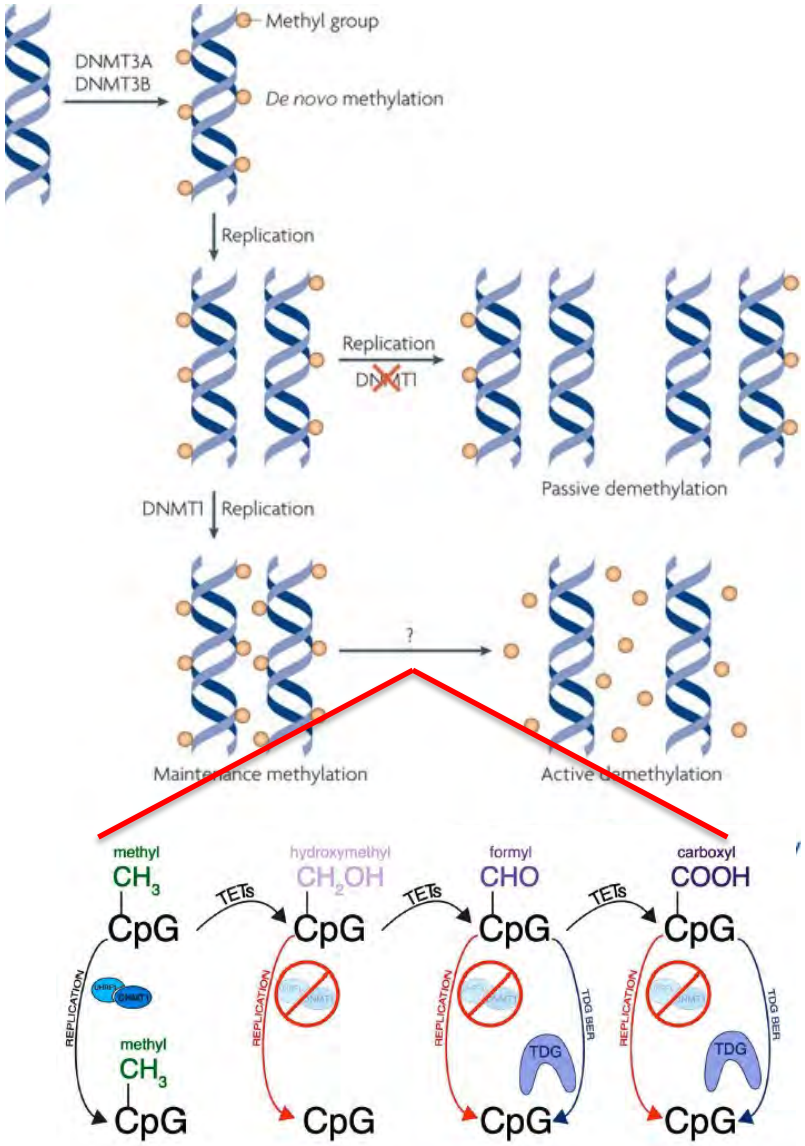
There are different mechanisms of DNA demethylation: both **passive** and **active** processes can occur.

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



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

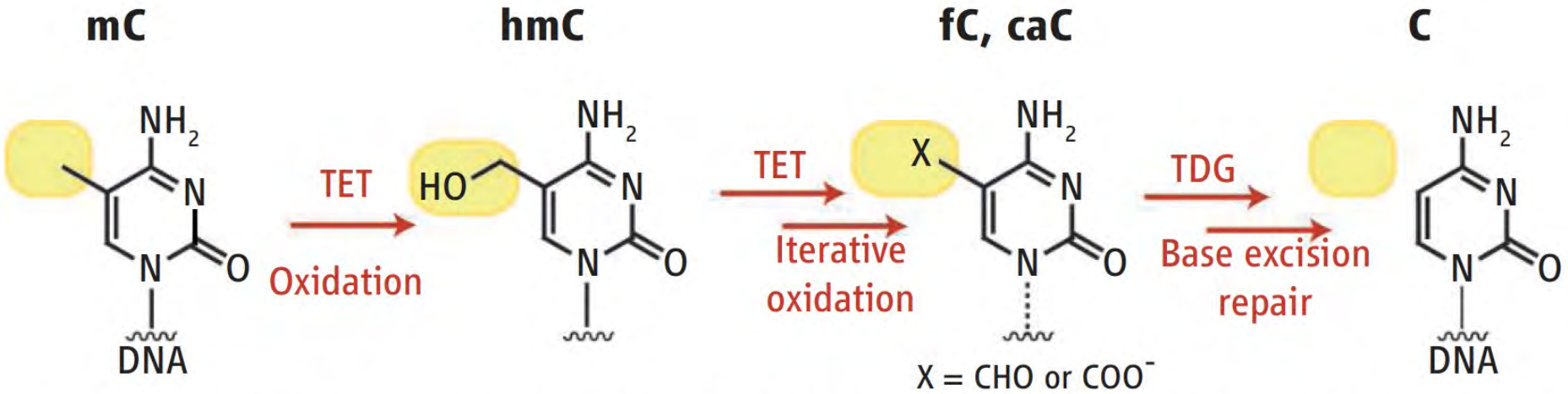
- Active DNA demethylation in mammals involves the action of Ten-eleven translocase (TET) family of dioxygenases.



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.

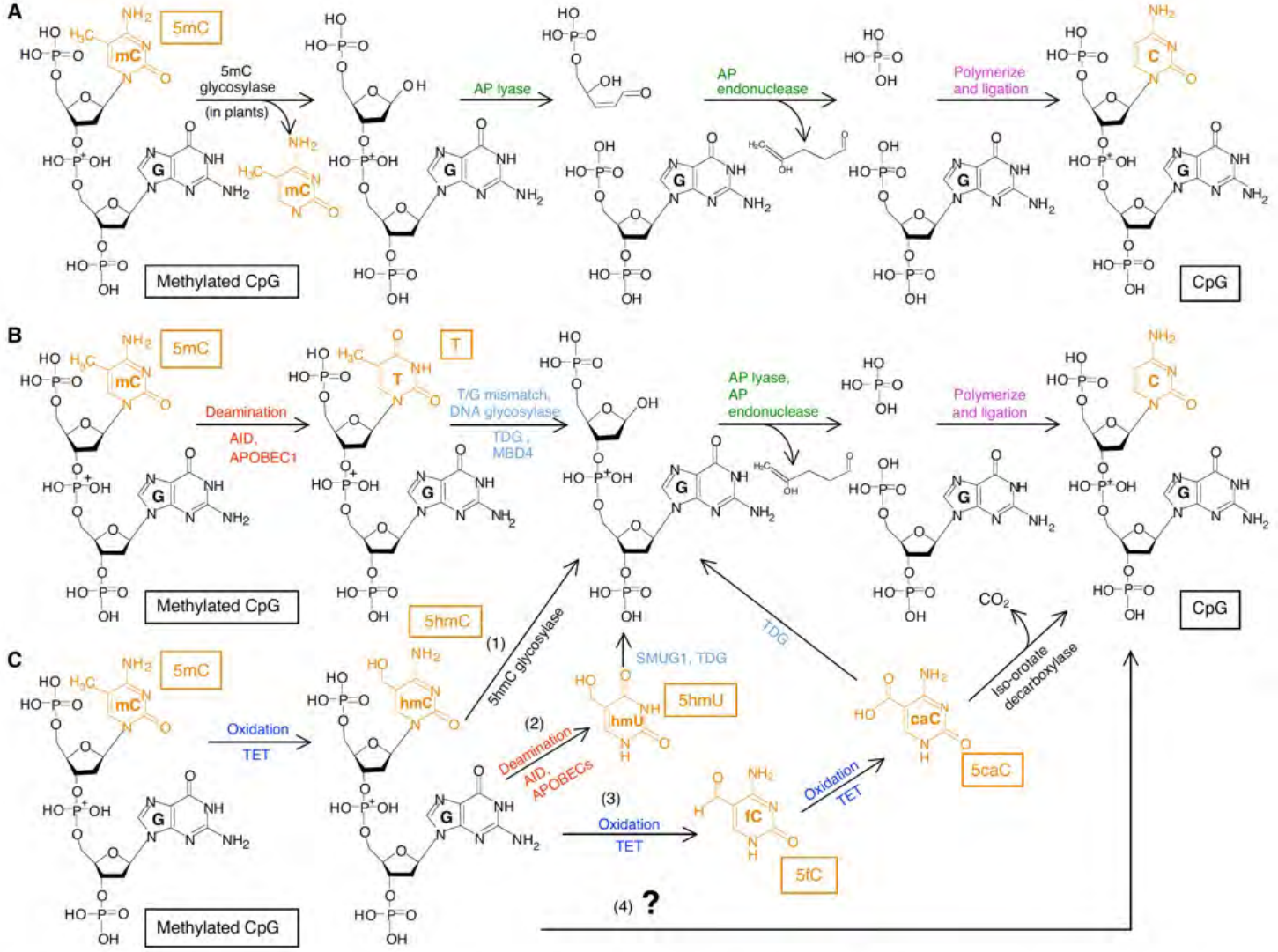
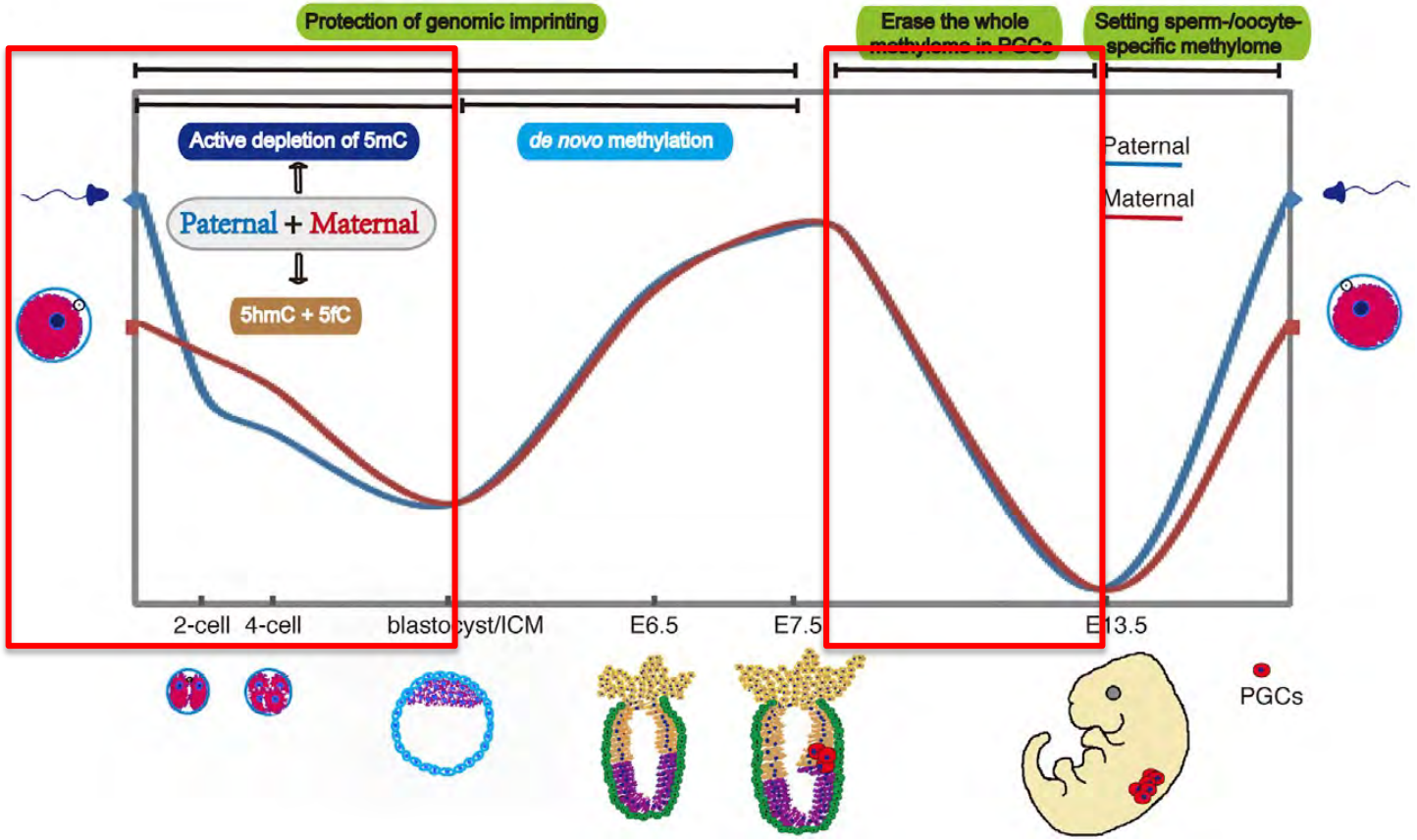


Fig. 4. Potential chemical pathways for active DNA demethylation. (**A**) Direct excision of 5mC (orange) by a 5mC glycosylase followed by repair via the base excision repair (BER) pathway (green and pink), as occurs in plants. (**B**) Cytosine deamination by AID/APOBEC1 (red), followed by base excision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

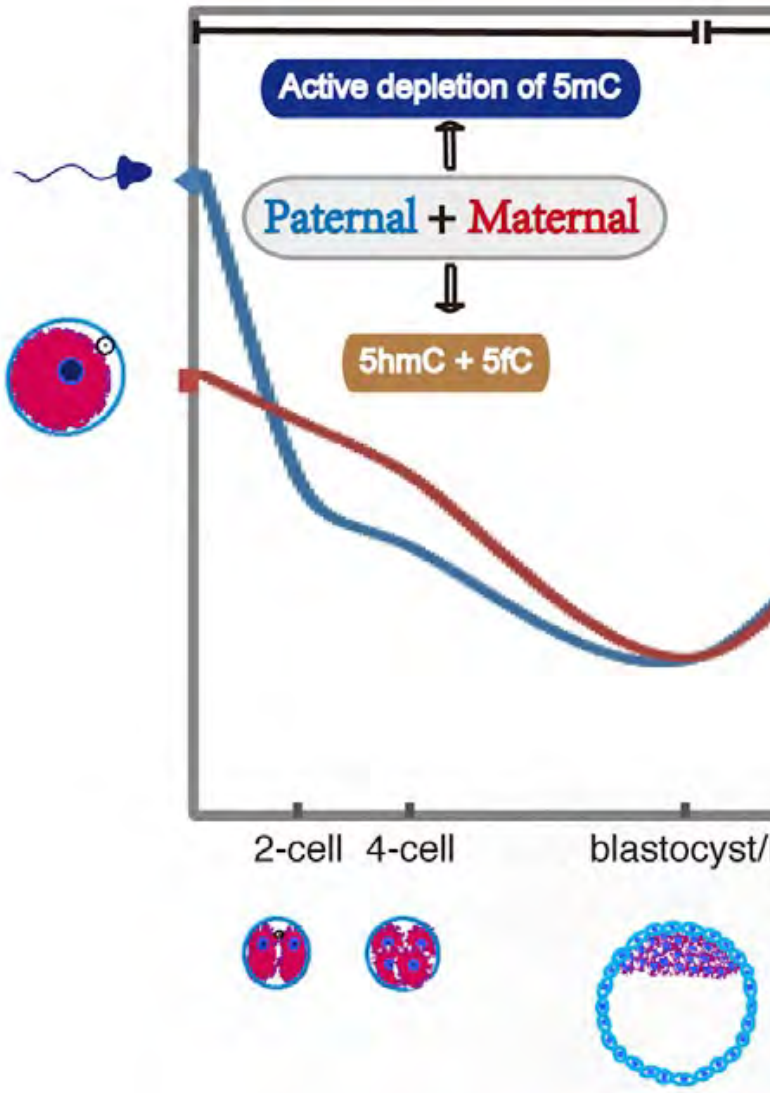
Mammals exhibit two rounds of dramatic DNA methylation reprogramming during embryonic development:

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



Such reprogramming is found exclusively in mammals.

Methylation patterning in development



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

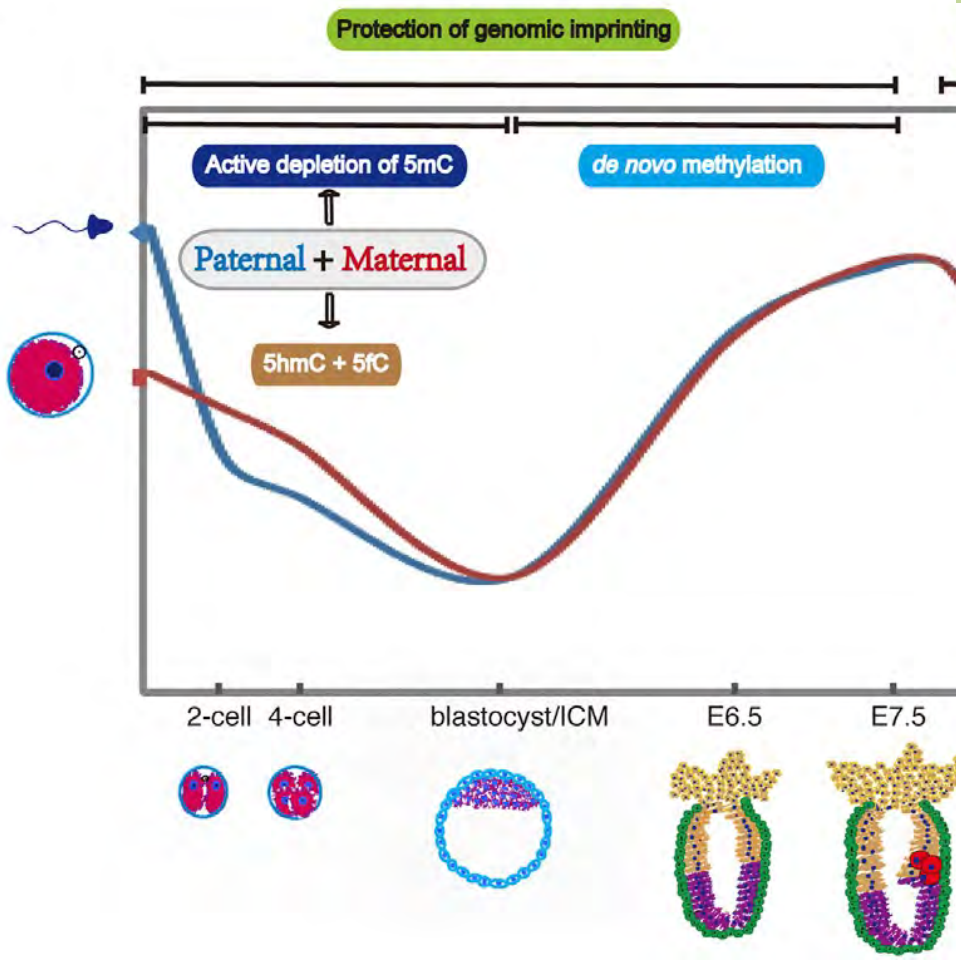
During post- fertilization reprogramming, the embryo loses gamete- specific DNA methylation patterns inherited from the oocyte and the sperm.

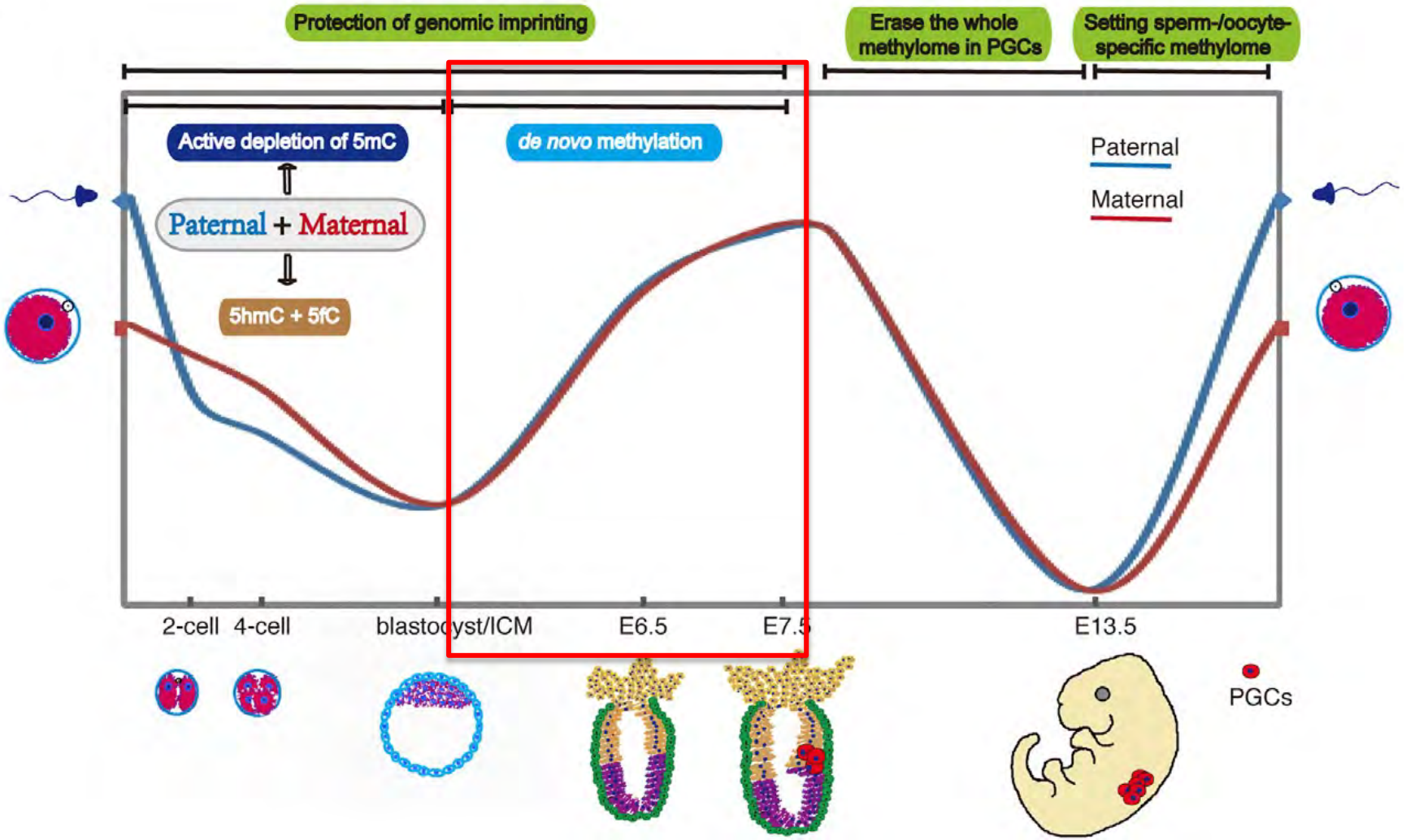
The paternal genome is actively demethylated within a few hours after fertilization.

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

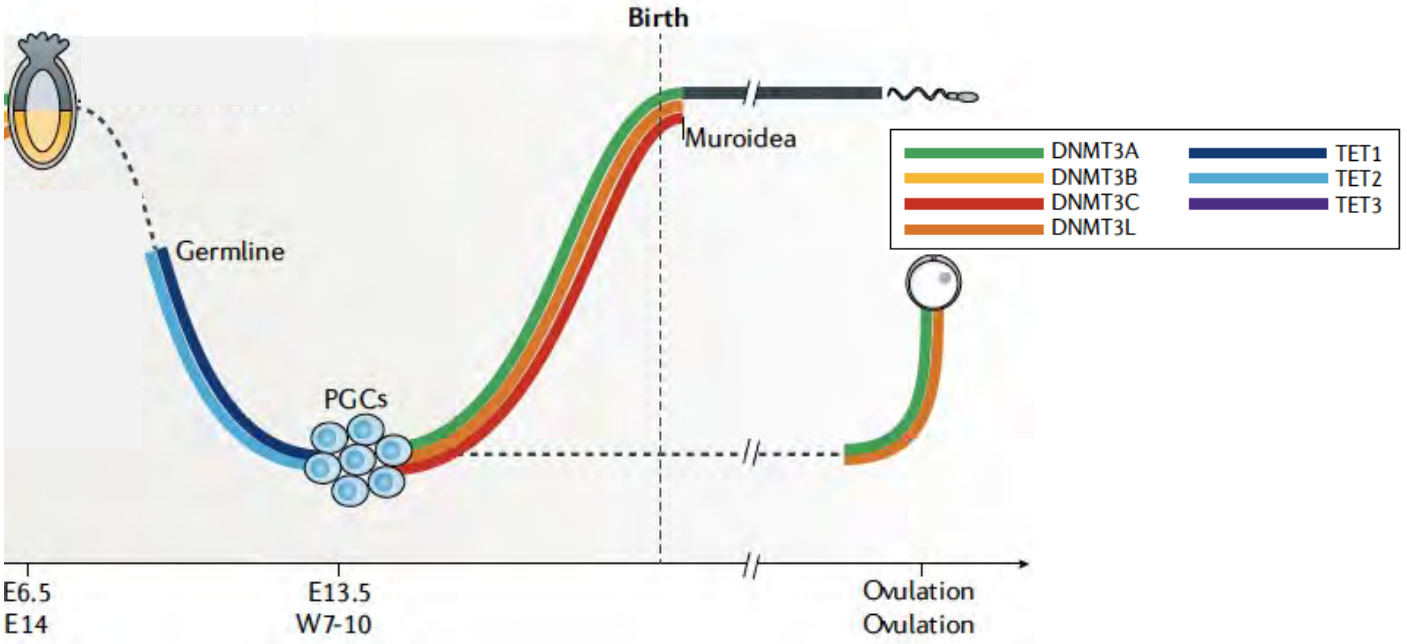




Following this dramatic global demethylation wave, the *de novo* methyltransferases, **DNMT3A** and **DNMT3B**, rapidly re-establish high levels of methylation.
By E6.5, the primed stem cells in the epiblast will then further differentiate into the somatic lineages, which will globally maintain the pattern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

What about germ cells?

Methylation patterning in PGCs development



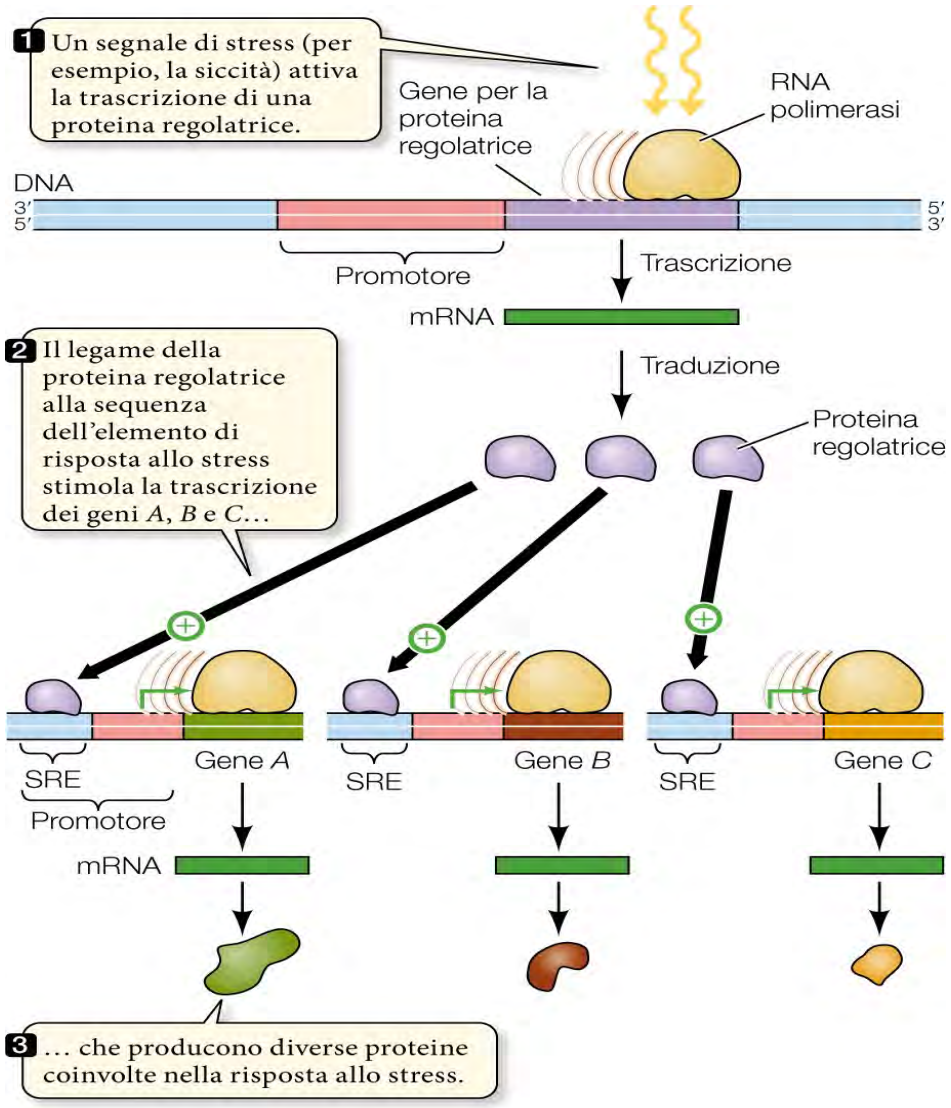
- Demethylation occurs in developing PGCs (primordial germ cells), as a prerequisite for subsequent acquisition of sex- specific DNA methylation patterns during male and female germline differentiation.
- Post implantation, in the epiblast, a subset of stem cells is specified for the germline, where 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.

- 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.

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

2012 Nobel Prize in Physiology or Medicine

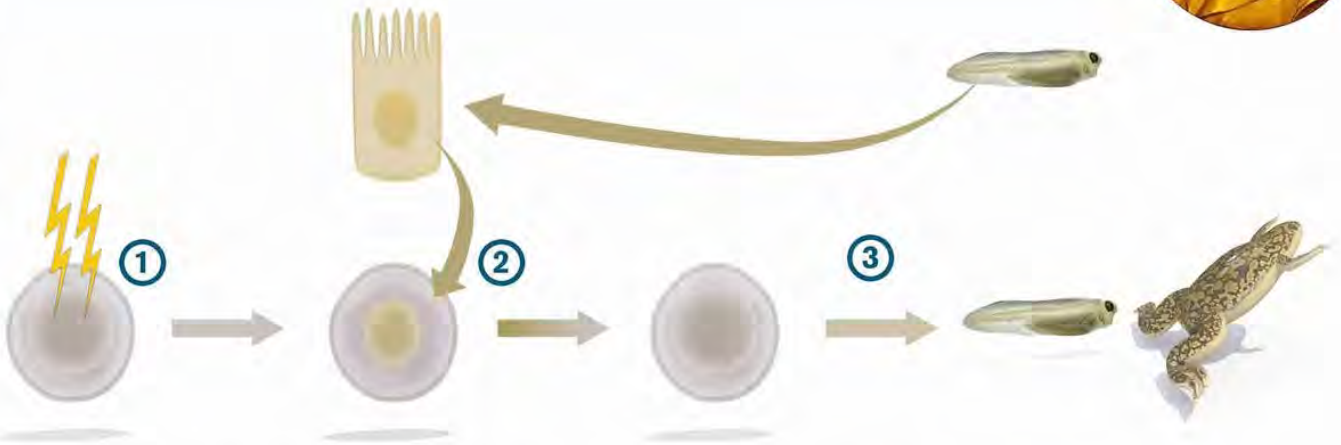
Shinya Yamanaka
University of Kyoto, Japan

Photo Credit:
Center for iPS cell Research and Application, Kyoto University

John B. Gurdon
Gurdon Institute in Cambridge, UK

The Nobel Prize in Physiology or Medicine 2012

John B. Gurdon



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

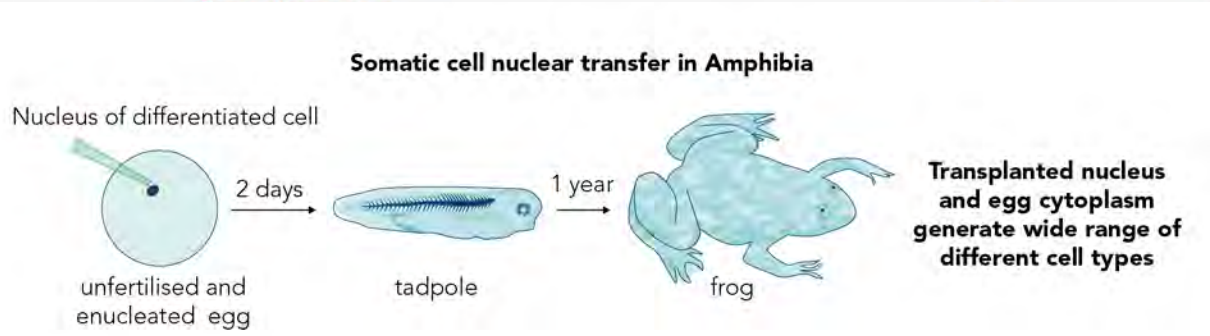
John Gurdon

Distinguished group leader

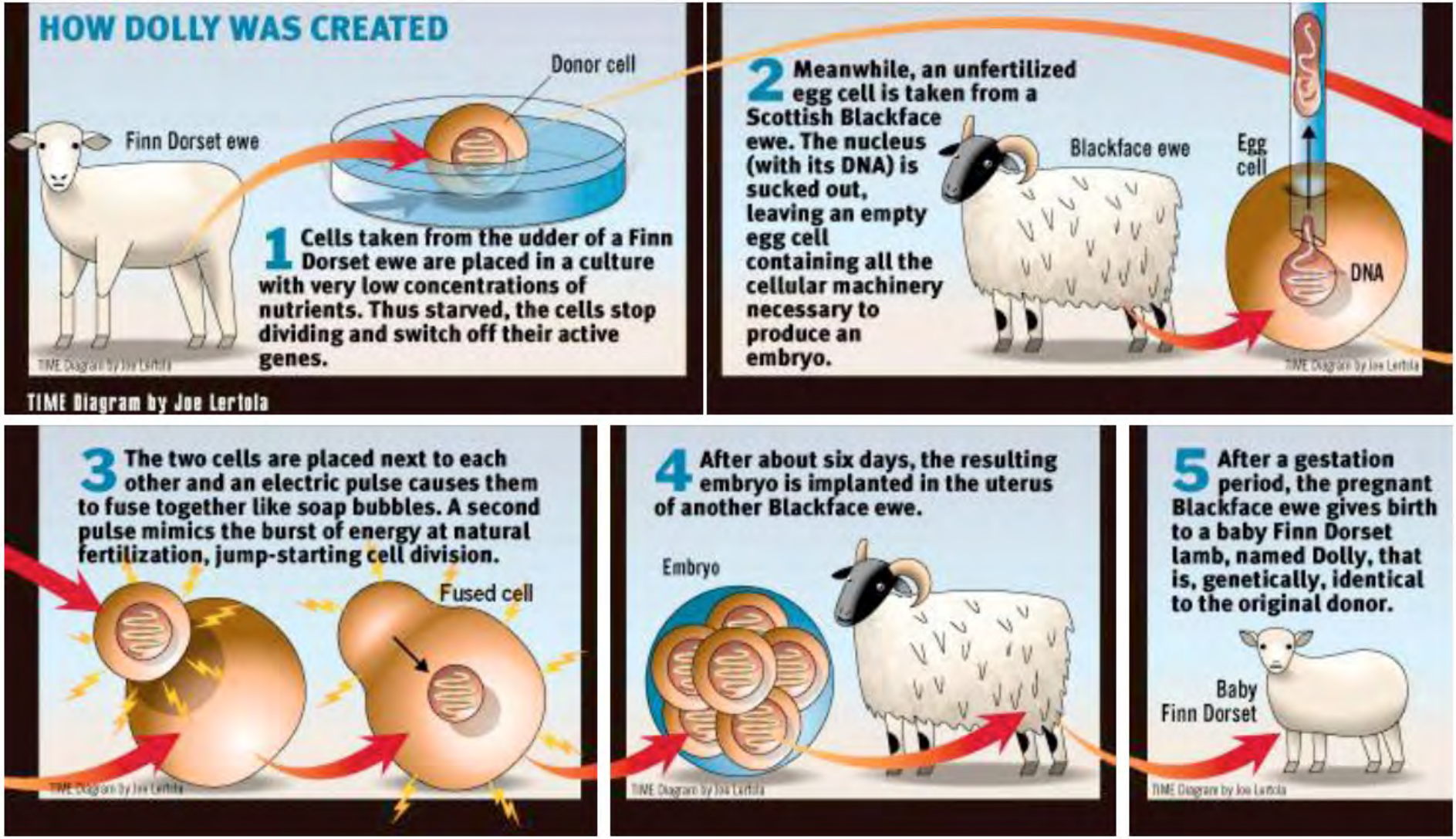
Research summary

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.



Animal cloning by nuclear transfer



Viable offspring derived from fetal and adult mammalian cells

NATURE | VOL 385 | 27 FEBRUARY 1997

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

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

Nuclei donatori:

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

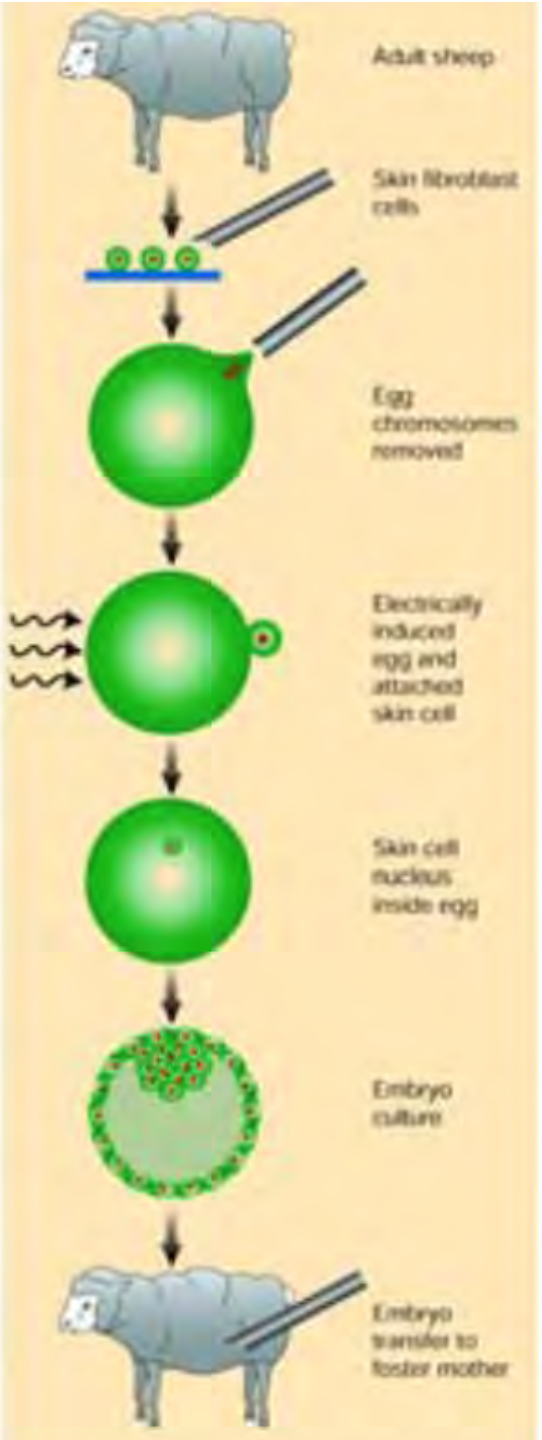
In tutti tre i casi, le cellule donatrici erano state indotte ad entrare uno stato di quiescenza replicativa (G_0) mediante riduzione della concentrazione di siero fetale bovino dal 10% a 5% per i 5 giorni precedenti il trasferimento nucleare. L'uscita dal ciclo era stata confermata mediante la ricerca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

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

Risultati (nuclei da cellule ghiandola mammaria):

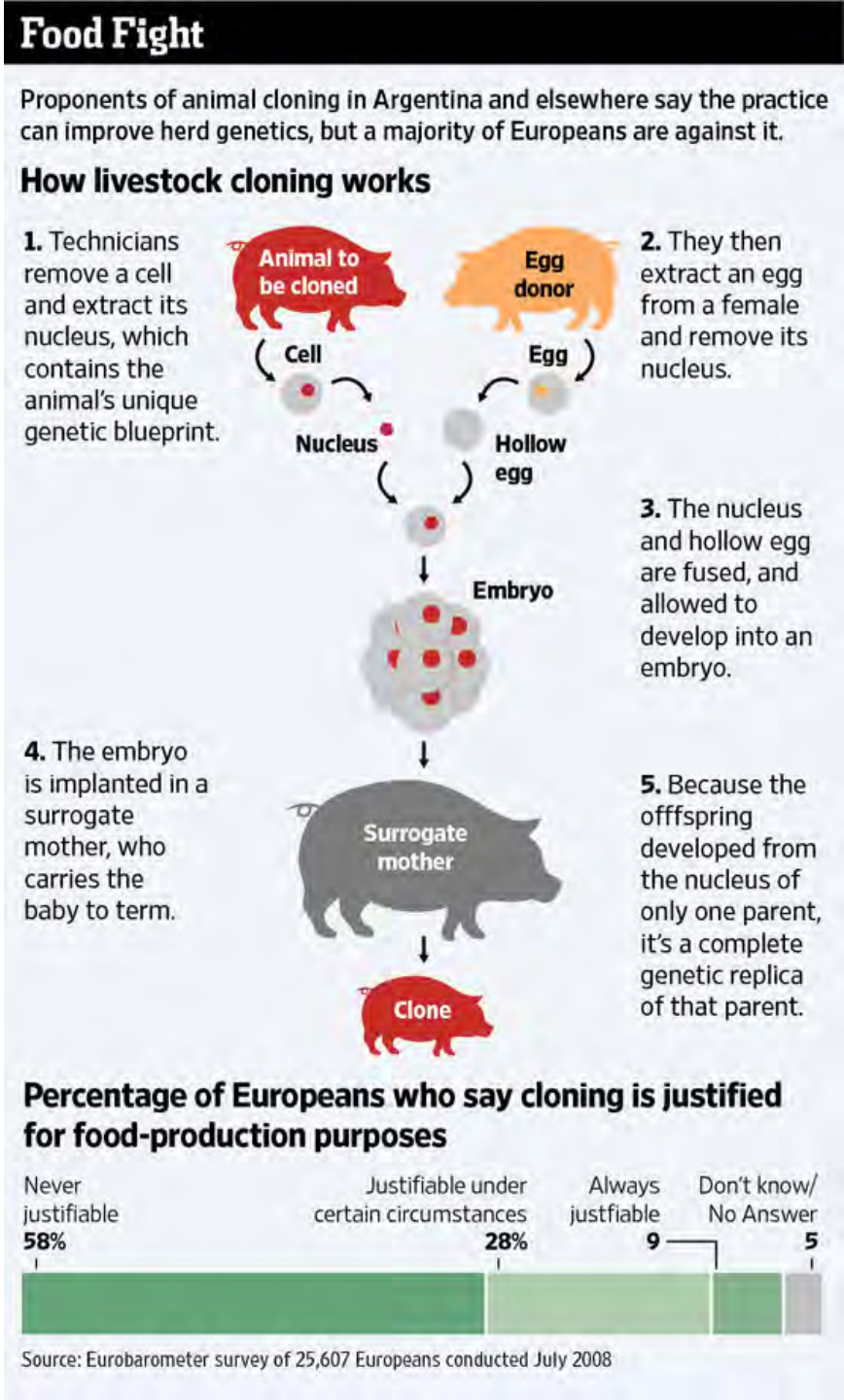
247 oociti ricostruiti coltivati all'interno delle ovidotti ligati di una pecora
29 (11.7%) progrediti allo stadio di morula/blastocisti dopo 6 giorni di coltura, e trasferiti in 13 pecore sincronizzate riceventi per lo sviluppo a termine.
1 embrione (0.4% del totale; 3.4% degli embrioni trasferiti)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

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



Perchè clonare le pecore?

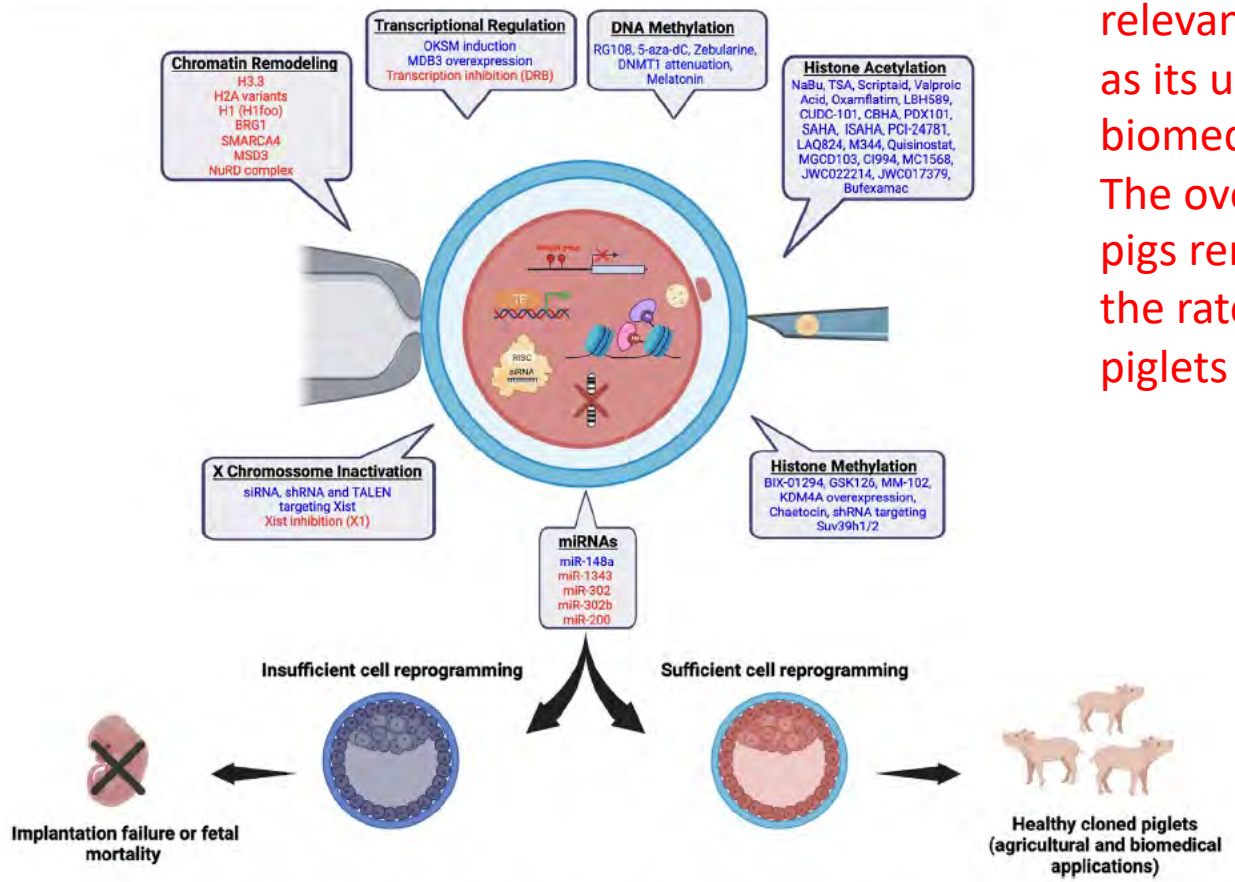
La pecora Dolly fu prodotta al Roslin Institute come parte di una ricerca per la produzione di medicinali nel latte degli animali da allevamento. I ricercatori sono riusciti a trasferire i geni umani che producono utili proteine, nelle pecore e nelle mucche in modo che esse possano produrre, per esempio, 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.



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

Pet cloning is getting more popular despite the cost

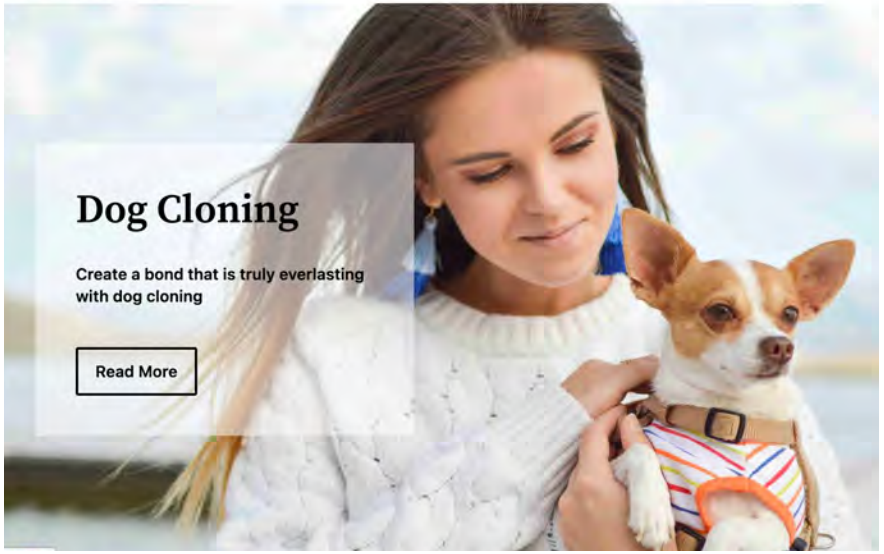
4 April 2022

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

GEMINI GENETICS

Home Pet Cloning Events Client Stories



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.

https://www.viagenpets.com 50%

Chiama il numero 888.876.6104

VIAGEN PETS & EQUINE

Di Animali domestici Equino Emergenza Servizi

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..

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

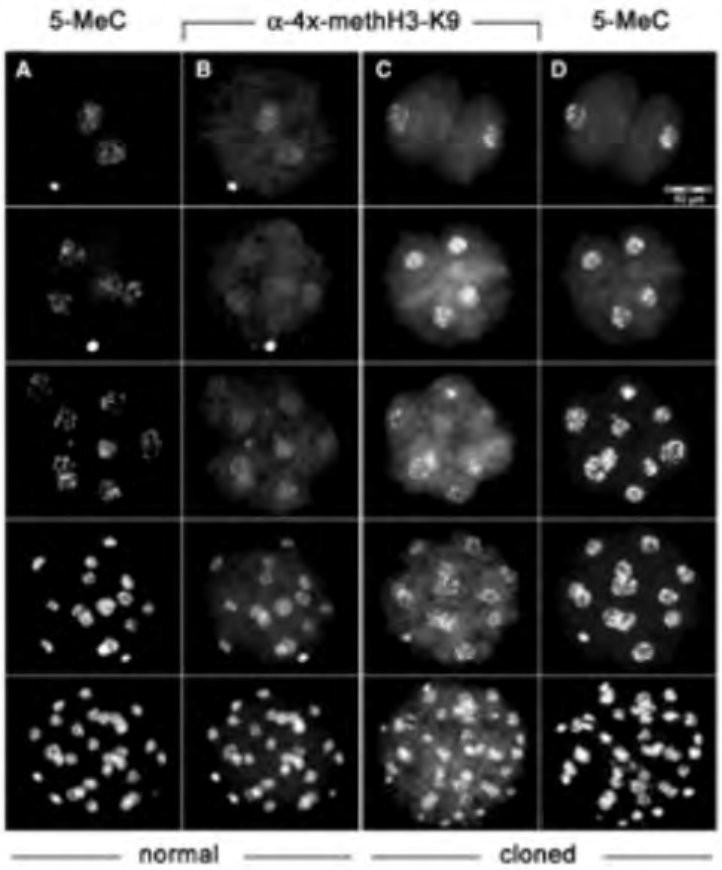
Affiliations + expand

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".

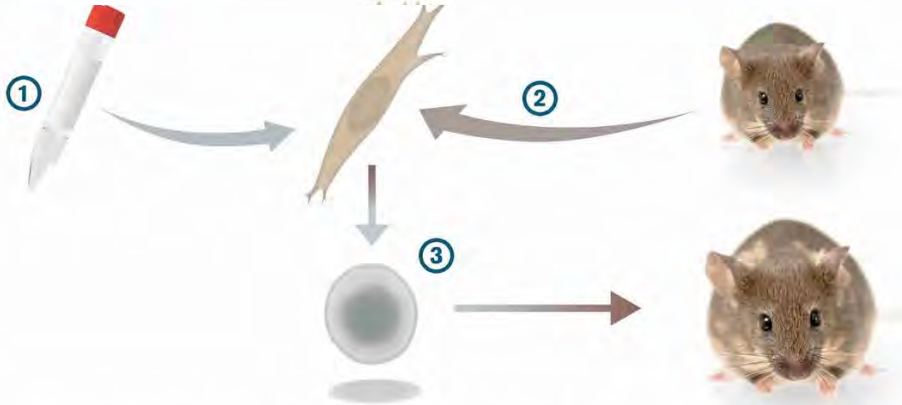
Please Don't Call It Cloning!

Bert Vogelstein et al., Science 2002

THE CRUCIAL DIFFERENCES

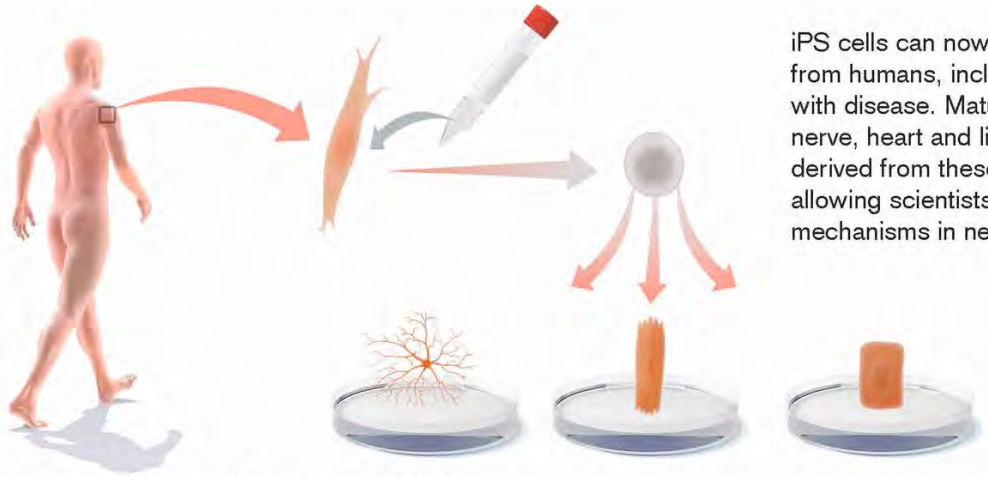
	Nuclear transplantation	Human reproductive cloning
End product	Cells growing in a petri dish	Human being
Purpose	To treat a specific disease of tissue degeneration	Replace or duplicate a human
Time frame	A few weeks (growth in culture)	9 months
Surrogate mother needed	No	Yes
Sentient human created	No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

The Nobel Prize in Physiology or Medicine 2012



Shinya Yamanaka

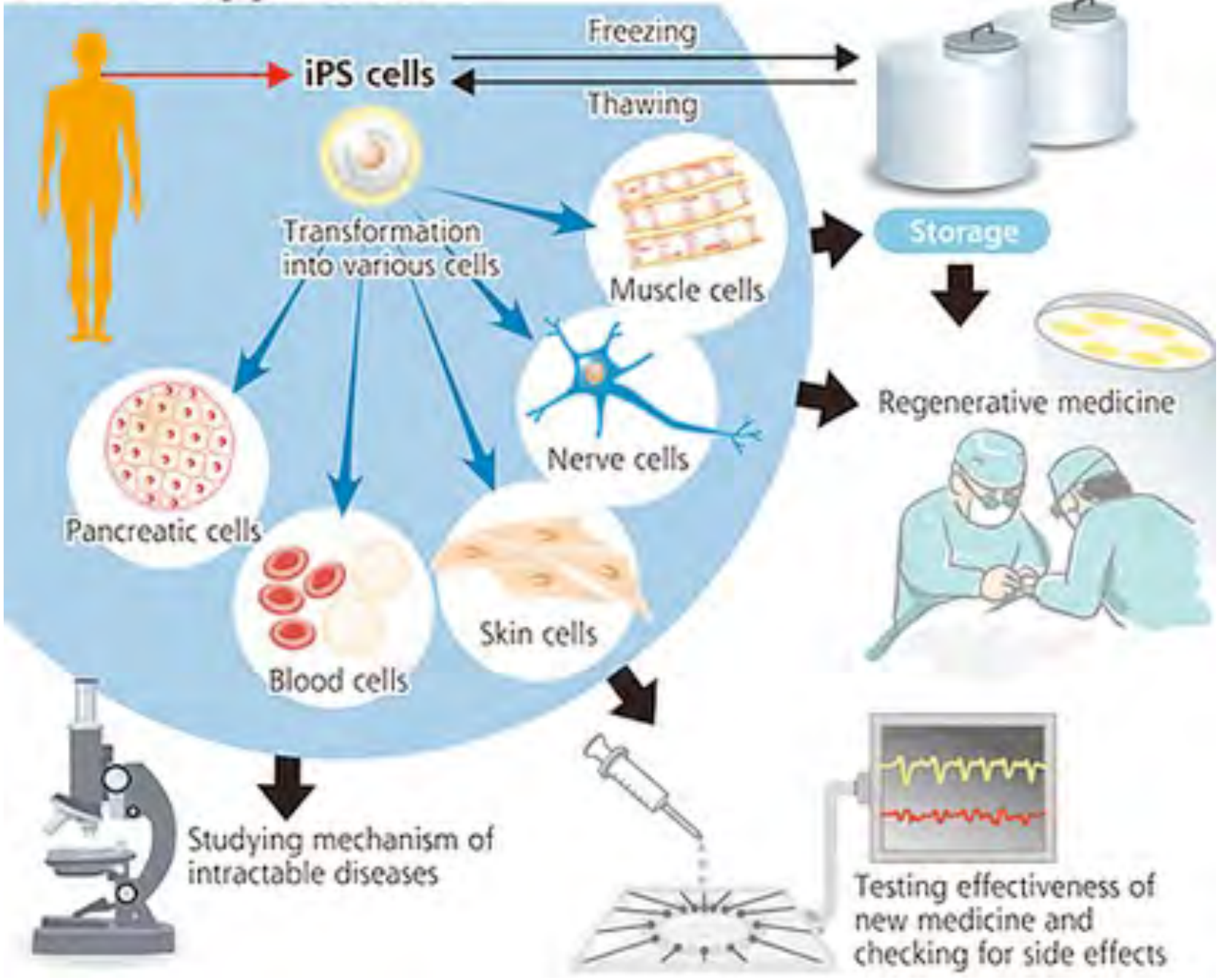
Shinya Yamanaka studied genes that are important for stem cell function. When he transferred four such genes (1) into cells taken from the skin (2), they were reprogrammed into pluripotent stem cells (3) that could develop into all cell types of an adult mouse. He named these cells induced pluripotent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

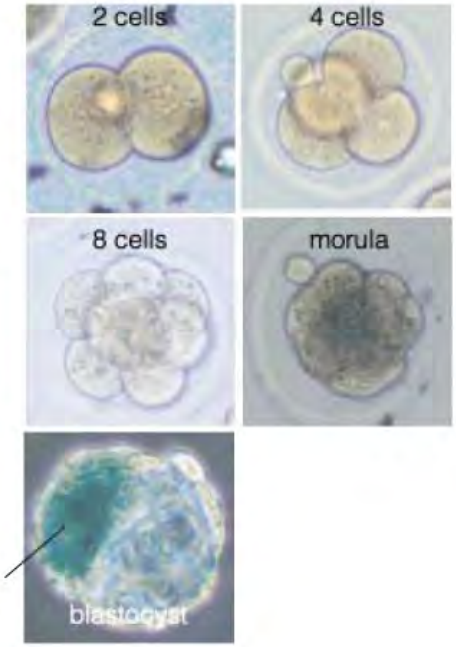
Zygote

Blastocyst

Gastrula

Zygote *totipotent*

Embryonic stem cells
pluripotent



Adult stem cells
multi- o uni-potent

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

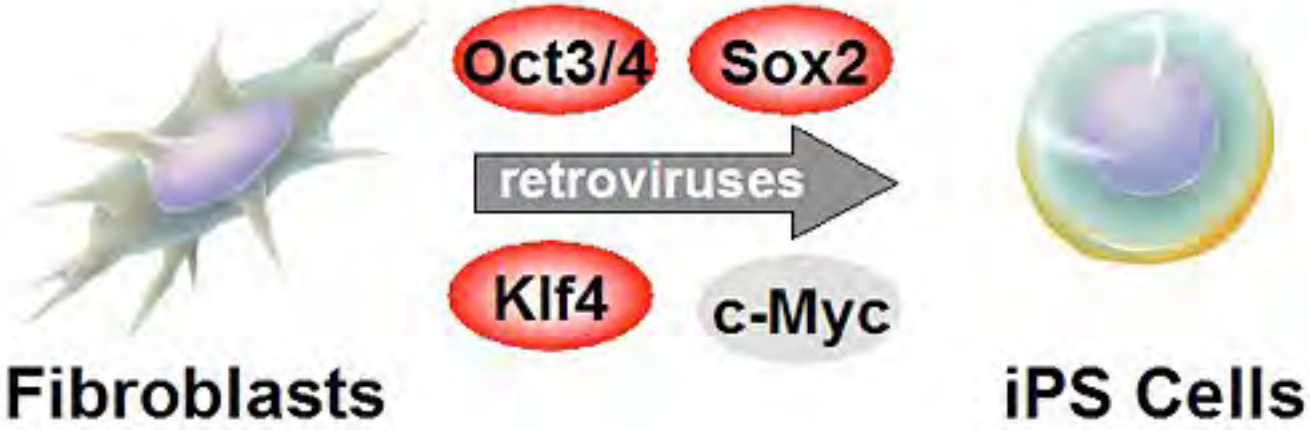
Mesoderm (middle layer)
Cardiac muscle
Skeletal muscle cells
Tubule cell of the kidney
Red blood cells
Smooth muscle (in gut)

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

Germ cells
Sperm
Egg

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

Induced Pluripotent Stem (iPS) Cells



Mouse iPS cells reported in 2006

Human iPS cells reported in 2007

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

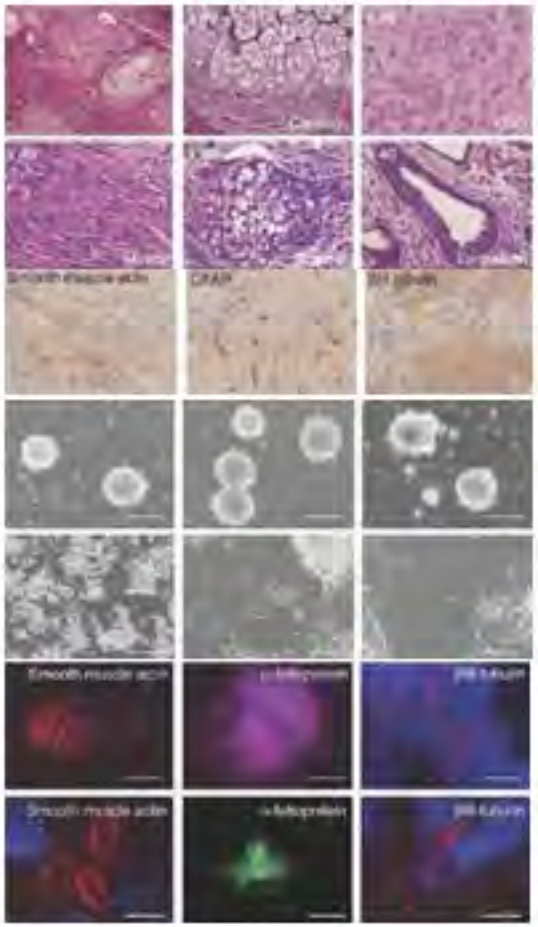
Induction of pluripotent stem cells from mouse embryonic or adult fibroblasts by introducing four factors, **Oct3/4, Sox2, c-Myc**, and **Klf4** in the FBX15 locus, under ES cell culture conditions.

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.

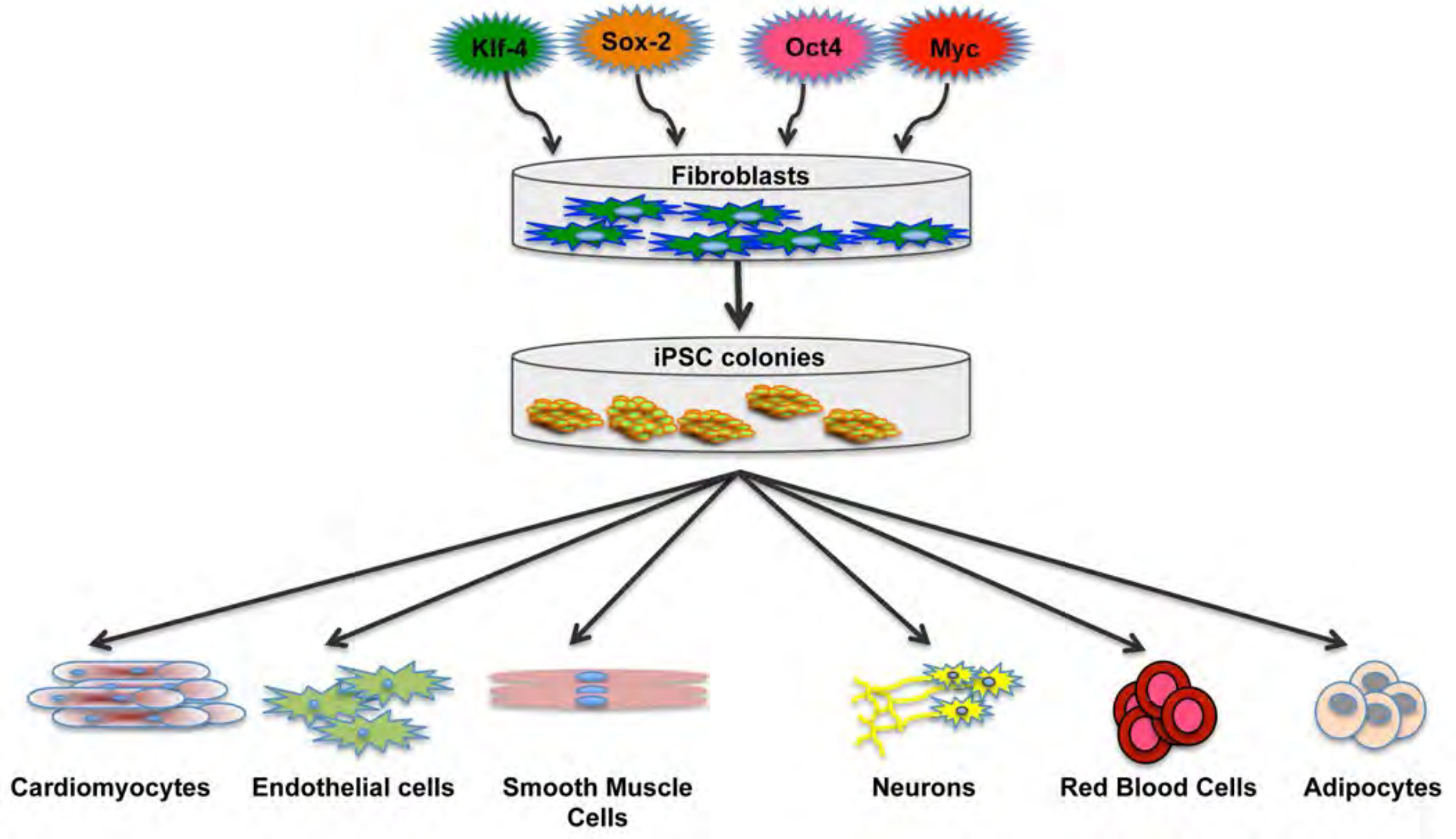
2- Following injection into blastocysts, iPS cells contributed to mouse embryonic development, but embryos failed to develop beyond mid-gestation stage.

Table 2

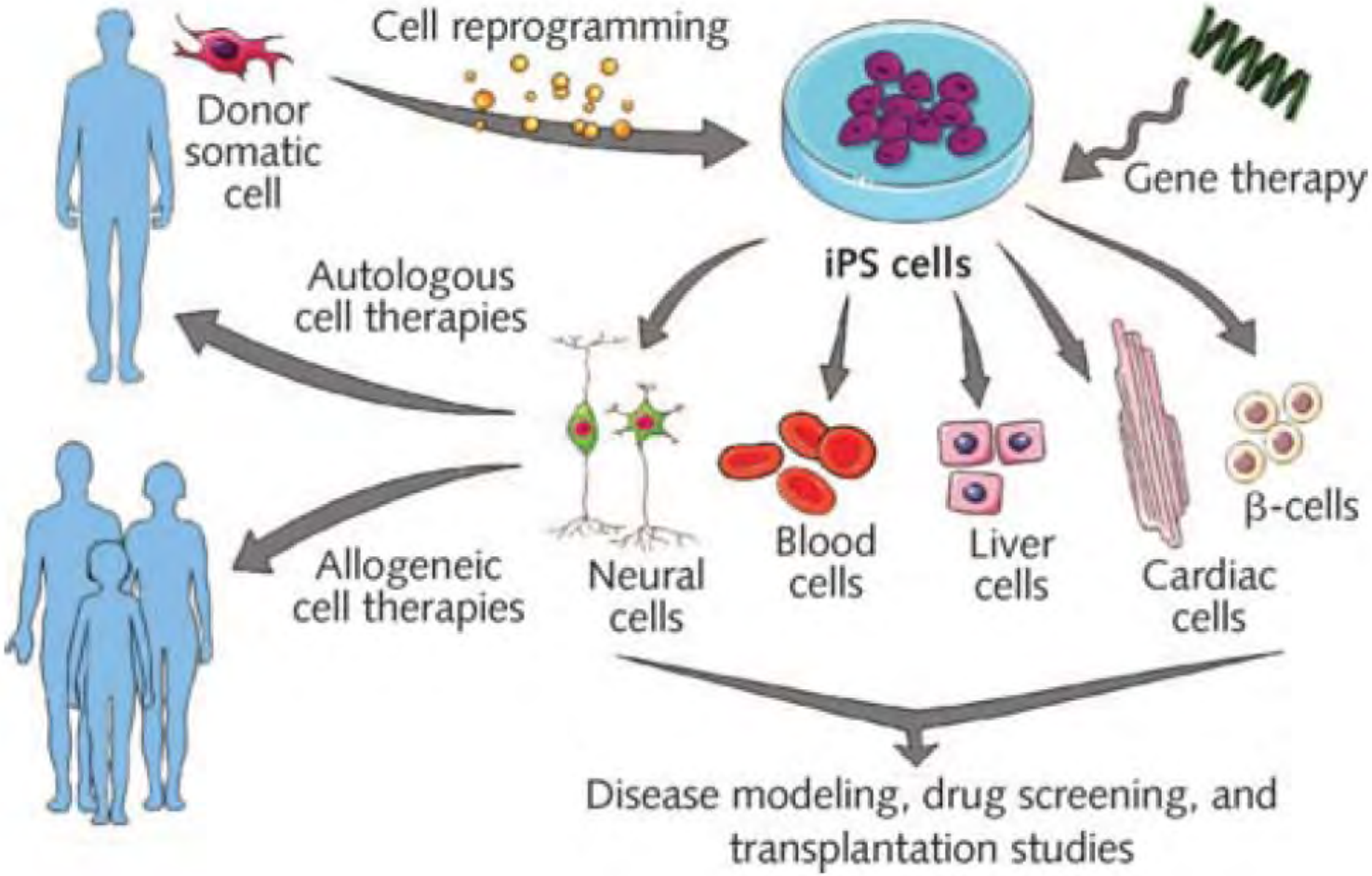
Minimum number of factors required for iPS cell generation

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

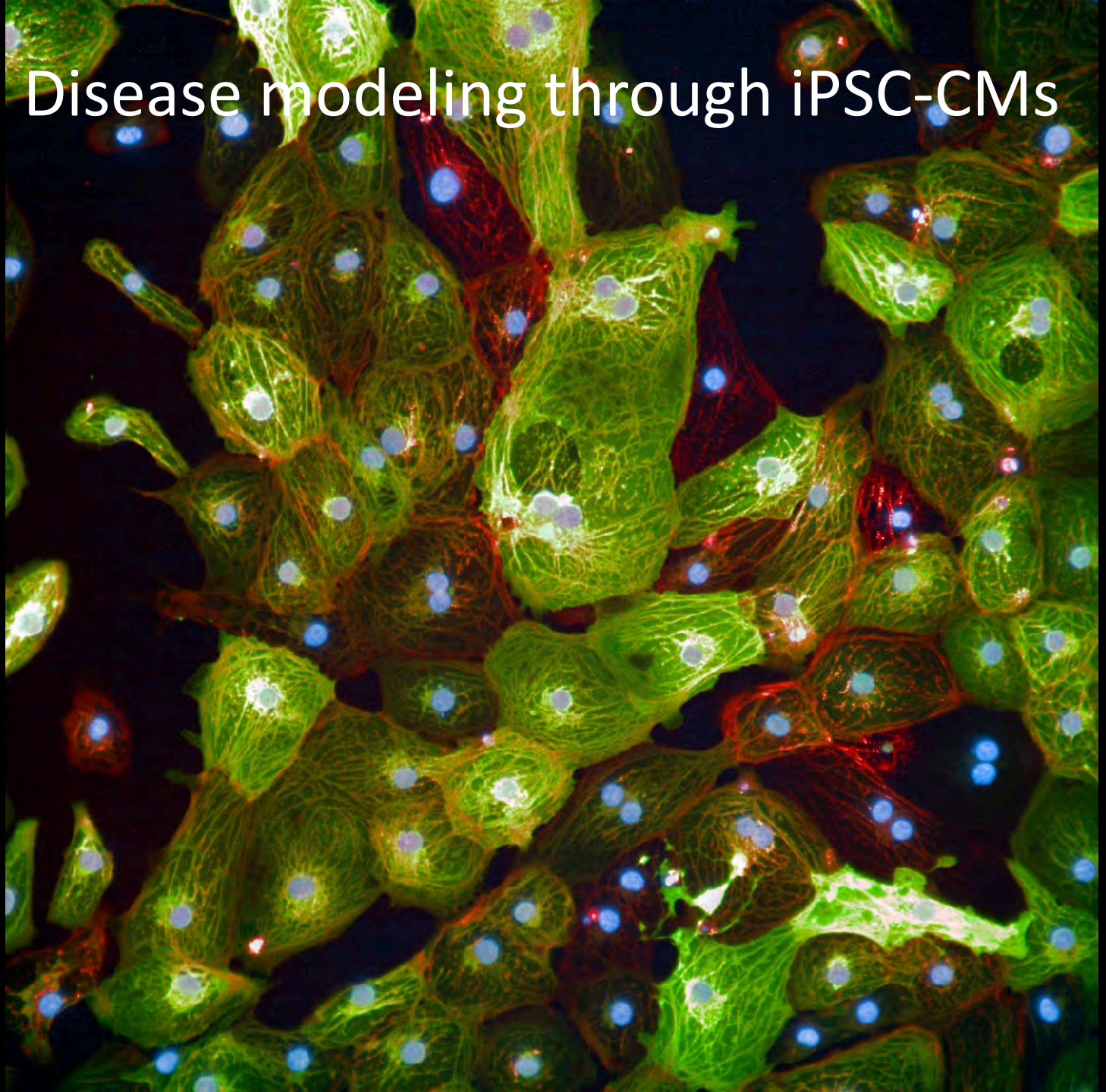

Klf-4
Sox-2
Oct4
Myc
Fibroblasts
iPSC colonies
Cardiomyocytes
Endothelial cells
Smooth Muscle Cells
Neurons
Red Blood Cells
Adipocytes

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

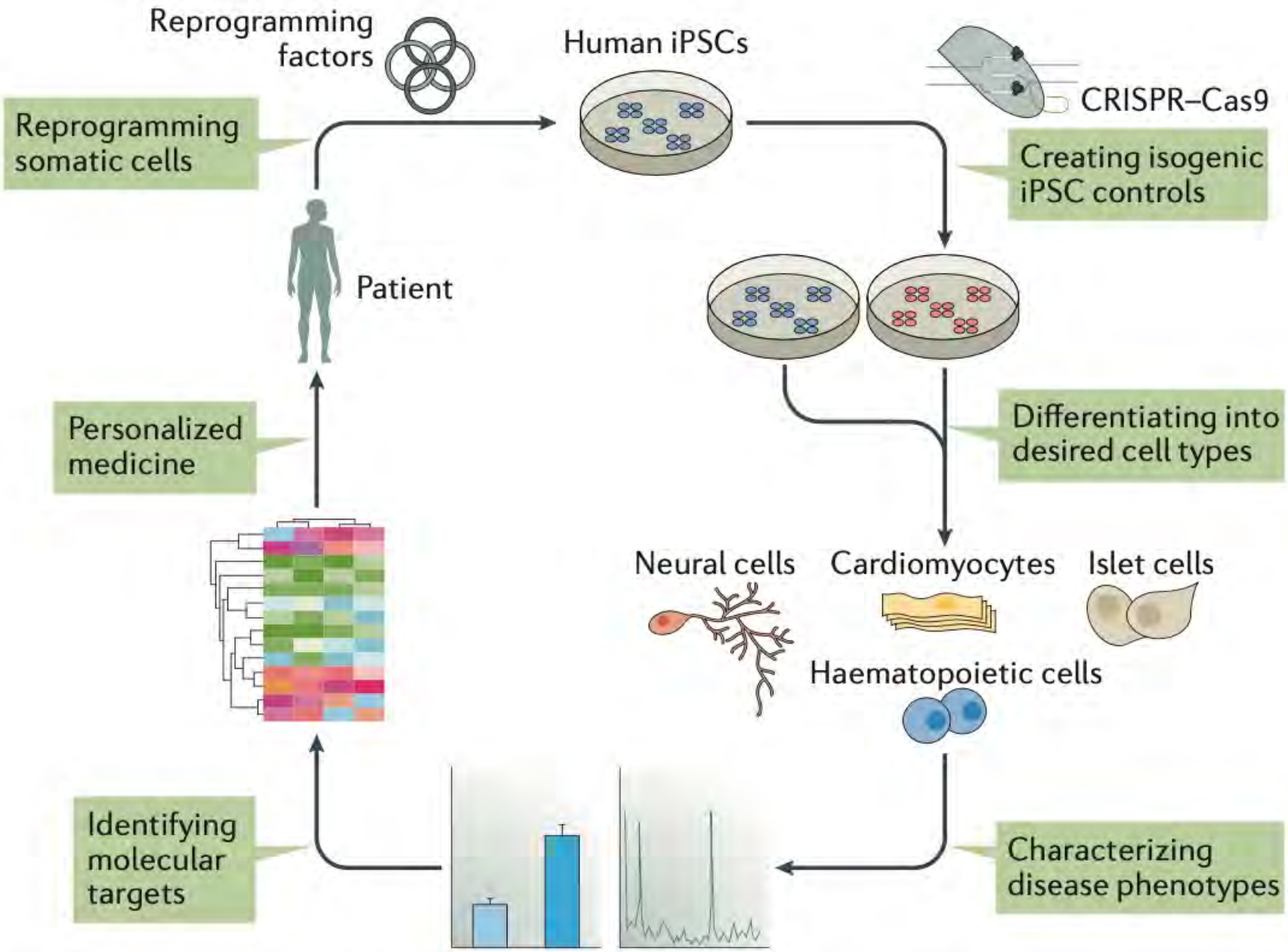


“Patient tailored therapy”

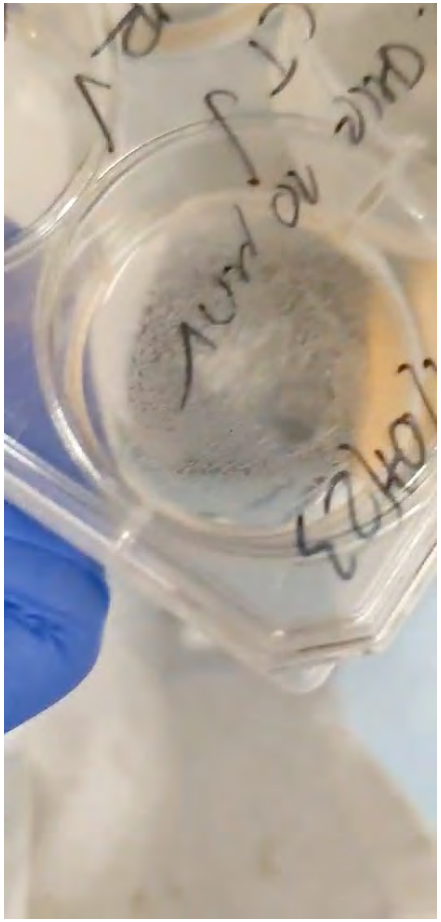
Disease modeling through iPSC-CMs

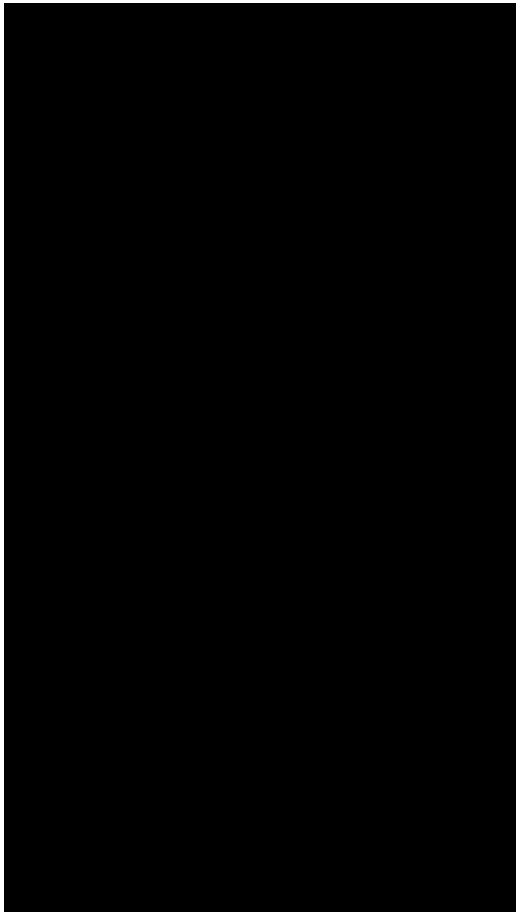


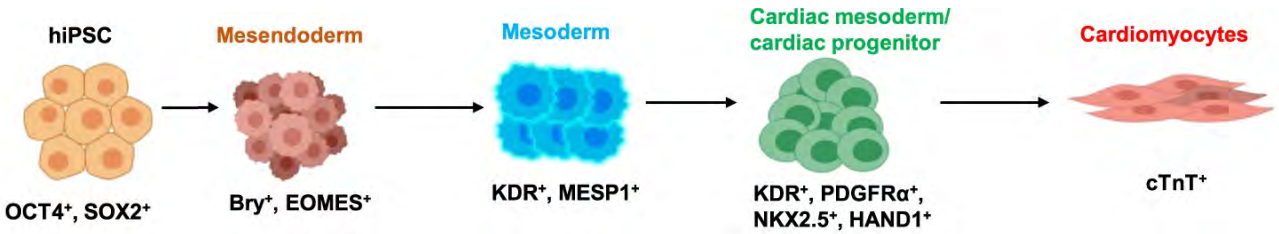
Disease modeling through iPSC-CMs

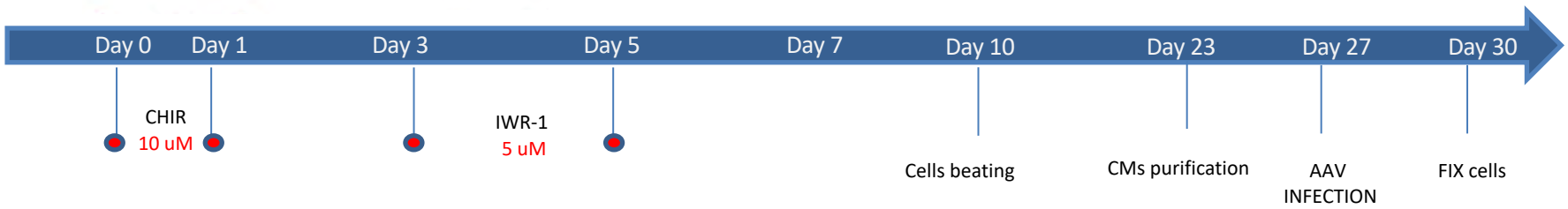


Cardiac differentiation









ENGINEERED HEART TISSUE