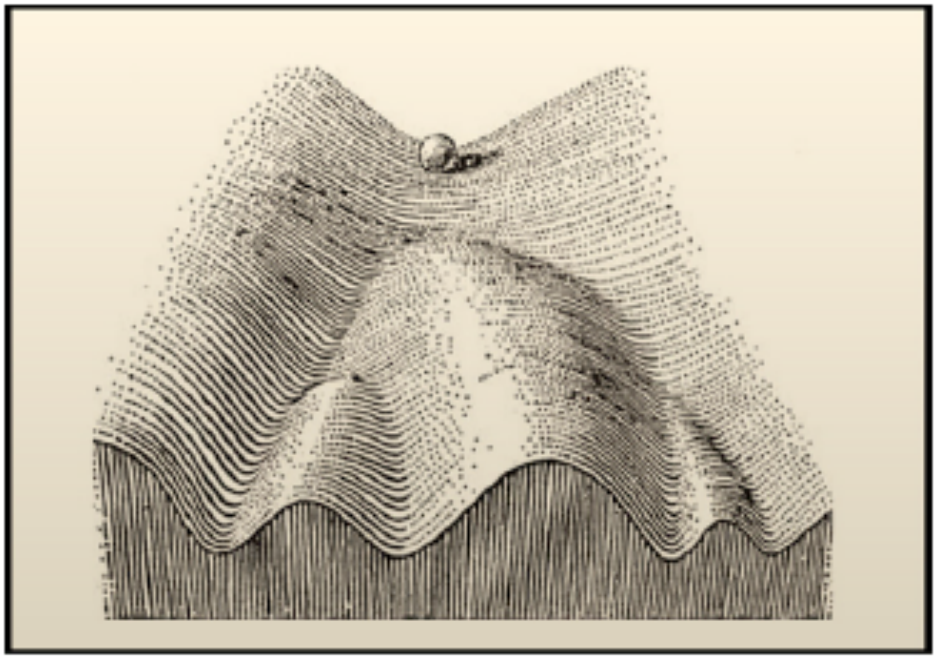
Epigenetics- Interaction of the genes with their environment

Heritable changes in phenotype or gene expression caused by mechanisms other than changes in the underlying DNA sequence. These changes may remain through cell divisions for the remainder of the cell's life and may also last for multiple generations.



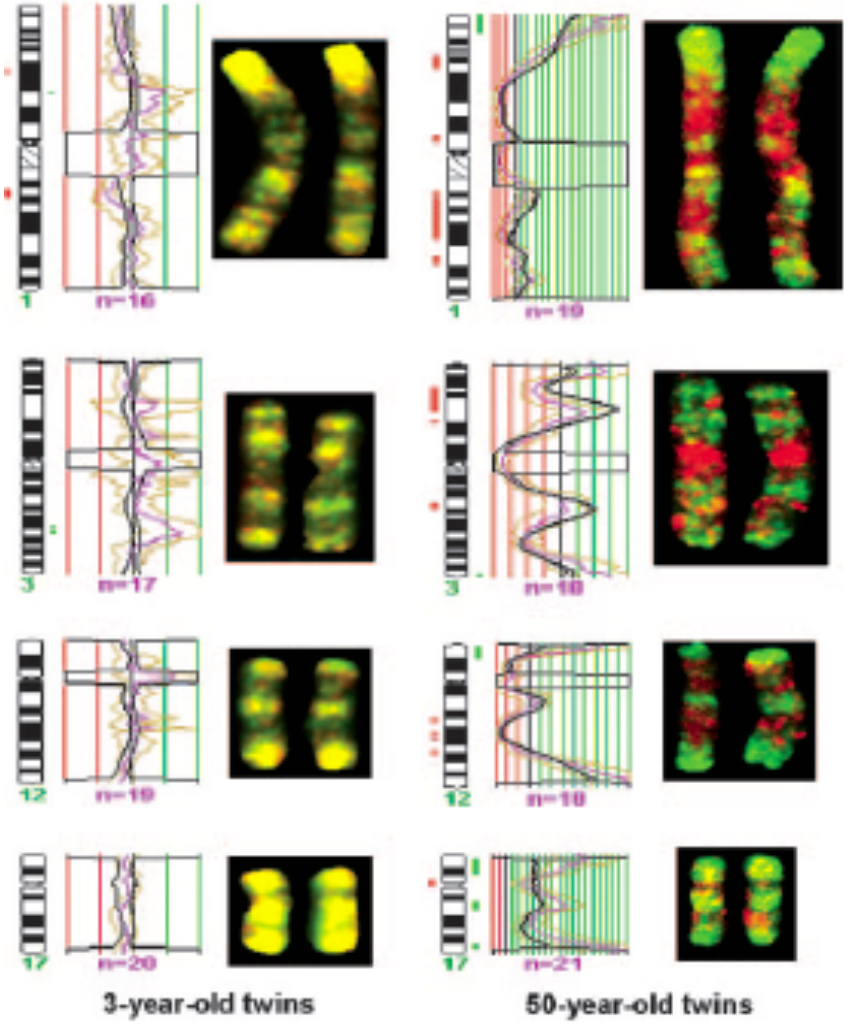
Waddington's classical epigenetic landscape

The word "epigenetics" (as in "epigenetic landscape") was coined by C. H. Waddington in 1942 as a fusion of the words "genetics" and "epigenesis". Epigenesis is an older word used to describe the differentiation of cells from a totipotent state in embryonic development. At the time Waddington first used the term "epigenetics," the physical nature of genes and their role in heredity was not known. **Epigenetics was Waddington's model of how genes within a multicellular organism interact with their surroundings to produce a phenotype.** Because all cells within an organism inherit the same DNA sequences, cellular differentiation processes crucial for epigenesis rely strongly on epigenetic rather than genetic inheritance.

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*§§

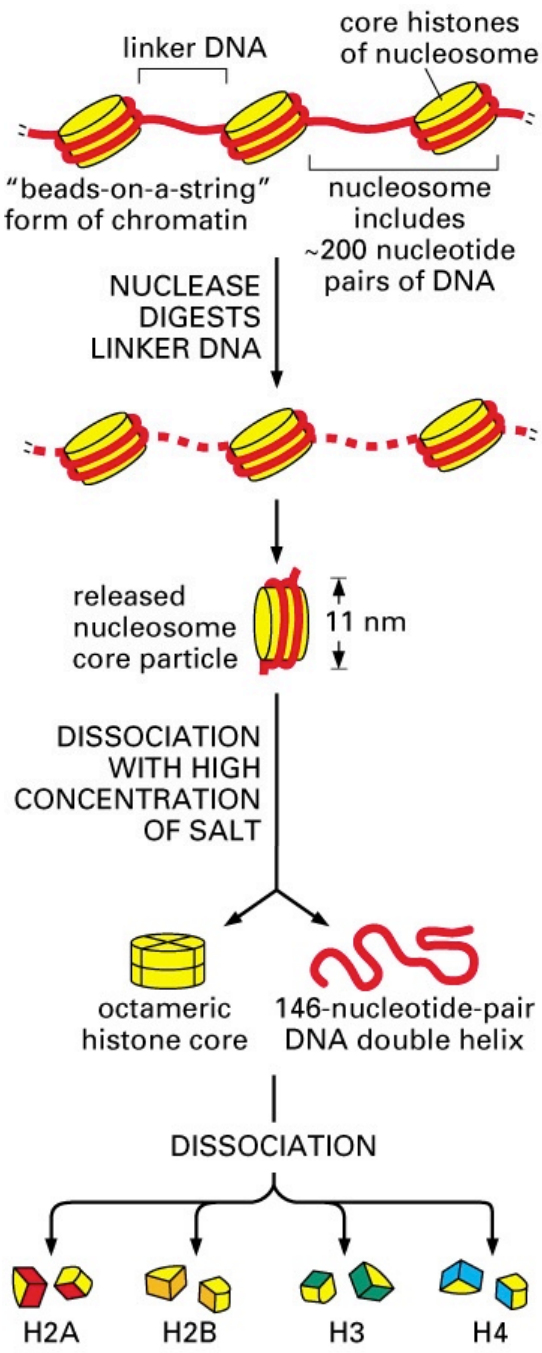
"Epigenomics is where genomics was 30 years ago, when everyone was working on part of the puzzle."
— Peter Jones

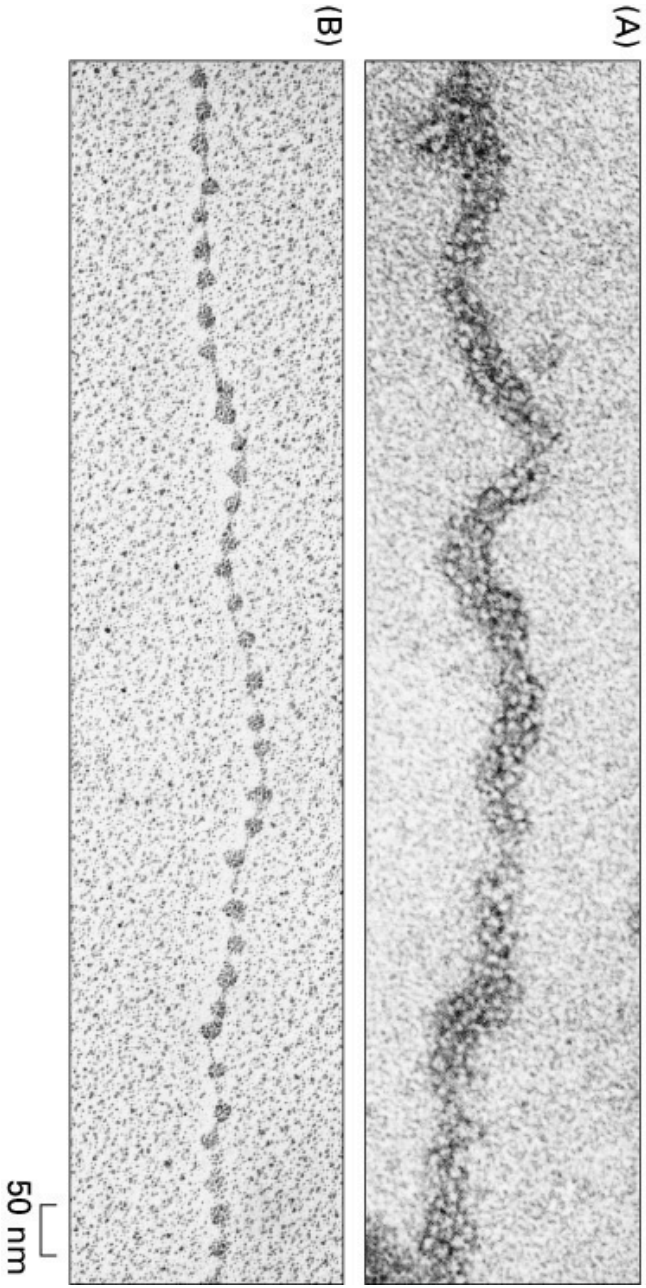


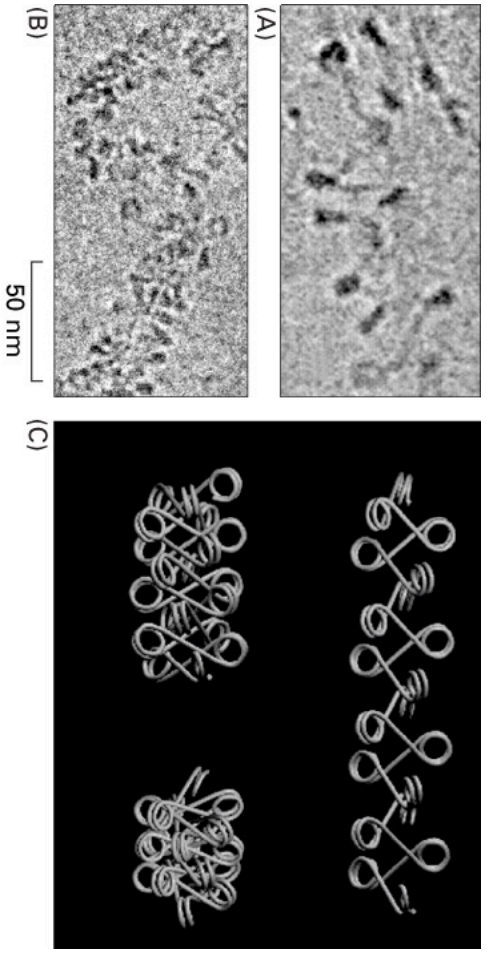
The proteins that bind to the DNA to form eucaryotic chromosomes are traditionally divided into two general classes: the **histones** and the **nonhistone chromosomal proteins**. The complex of both classes of protein with the nuclear DNA of eucaryotic cells is known as **chromatin**.

Histones are present in such enormous quantities in the cell (about 60 million molecules of each type per human cell) that their total mass in chromatin is about equal to that of the DNA.

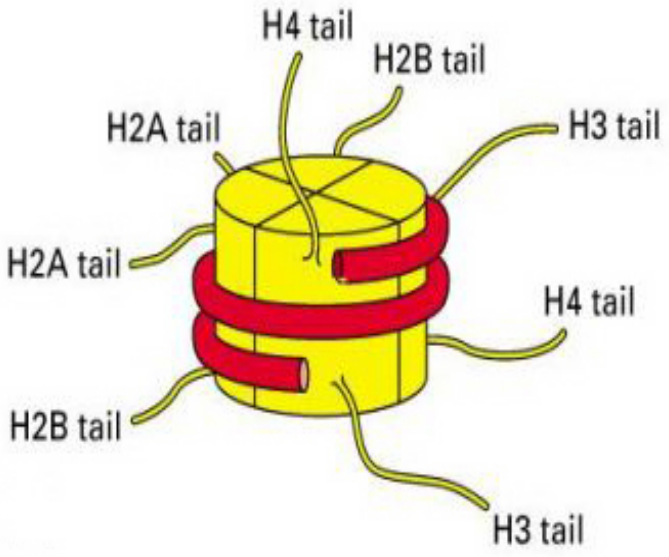
Histones are responsible for the first and most basic level of chromosome organization, the nucleosome


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

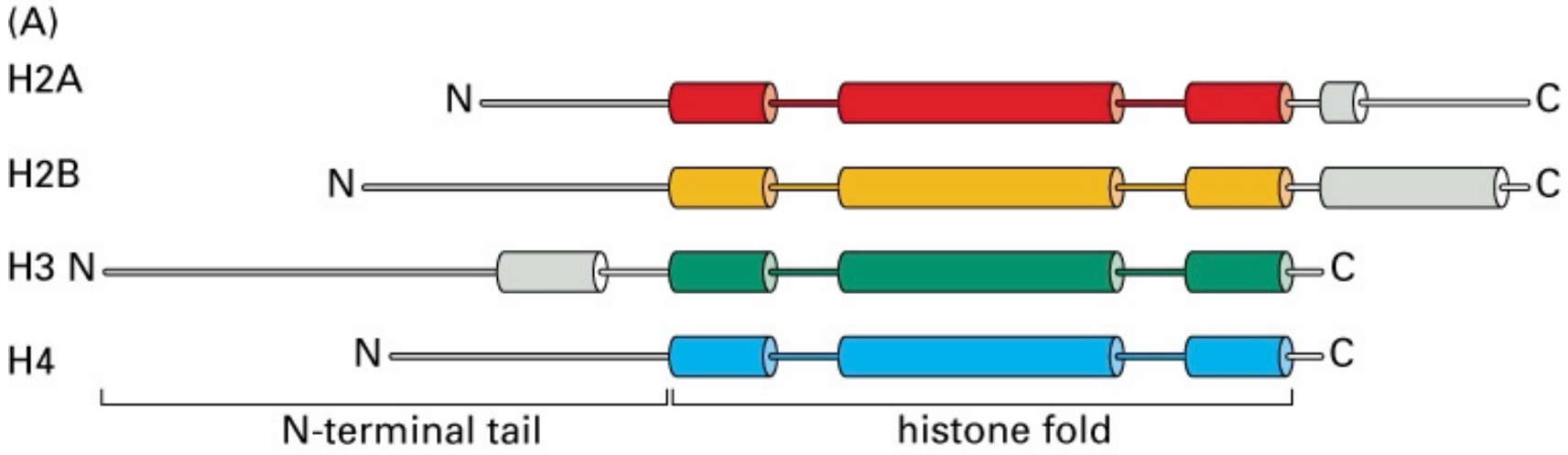

(B)
(A)
50 nm
(C)

NUCLEOSOME - THE FUNDAMENTAL UNIT OF CHROMATIN

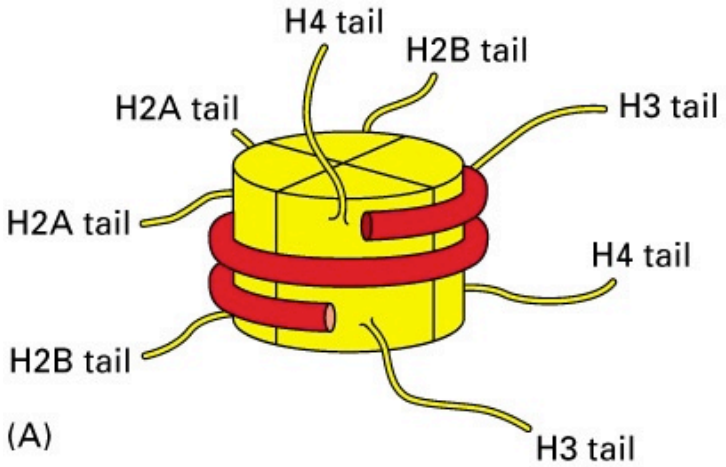


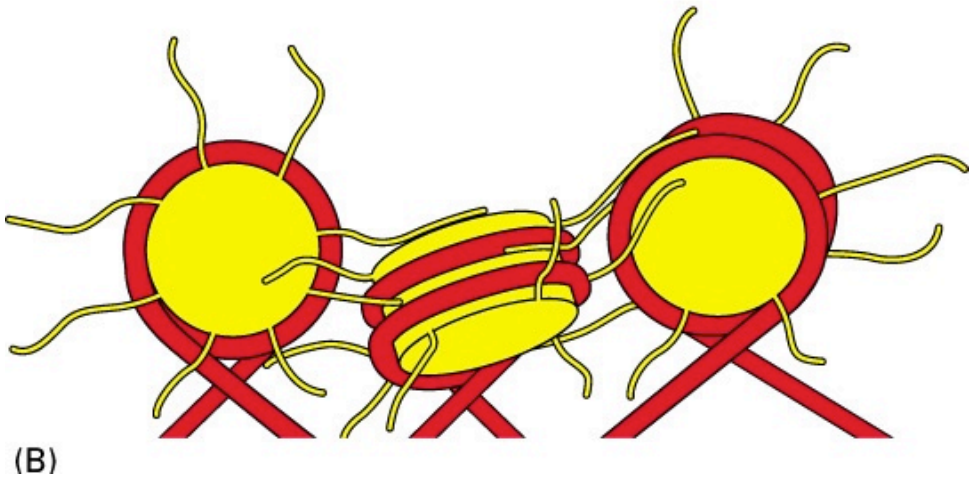
Histone modifications occur at the N-terminal tails of histones and are highly dynamic processes

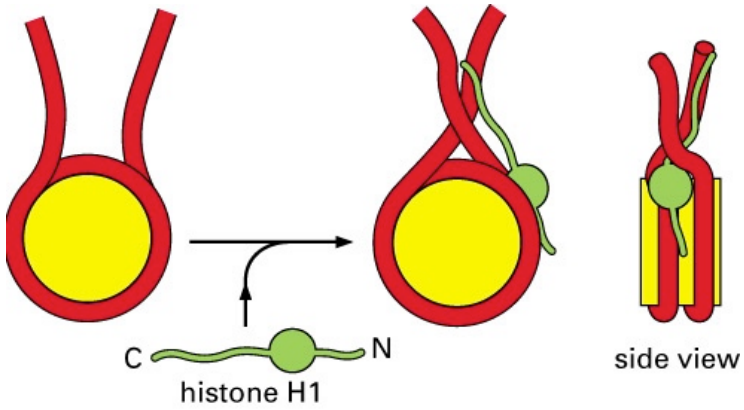
- Octamer of four core histones (H3 H4 H2A and H2B) with 147 base pairs of DNA wrapped around
- Core histones are predominantly globular except for their N-terminal tails which are unstructured



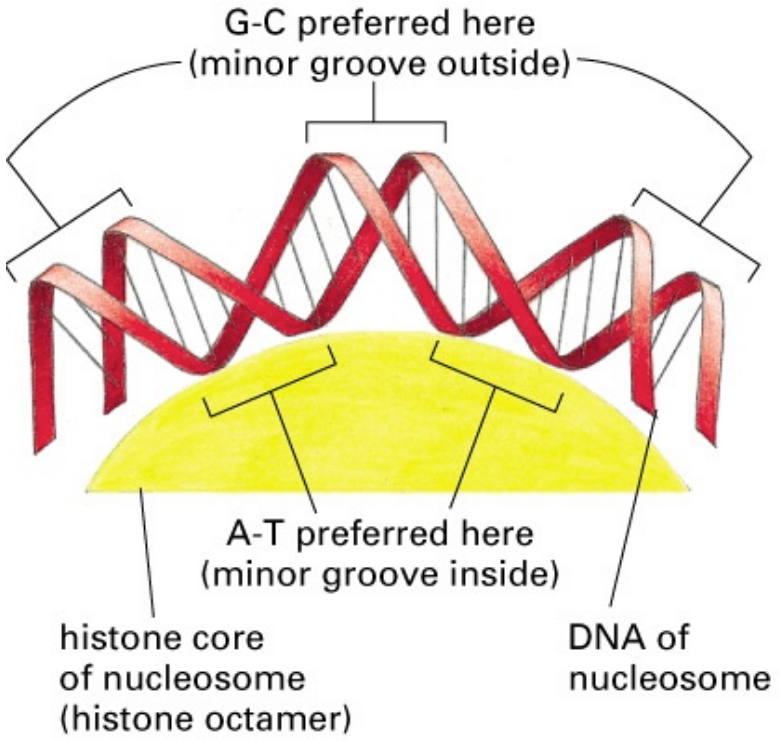
H1 hystone



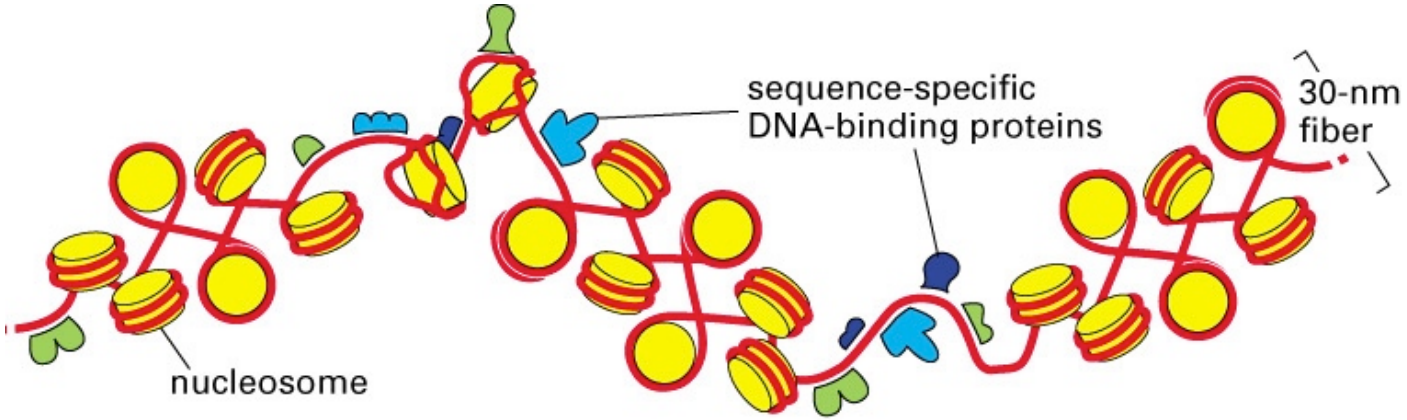


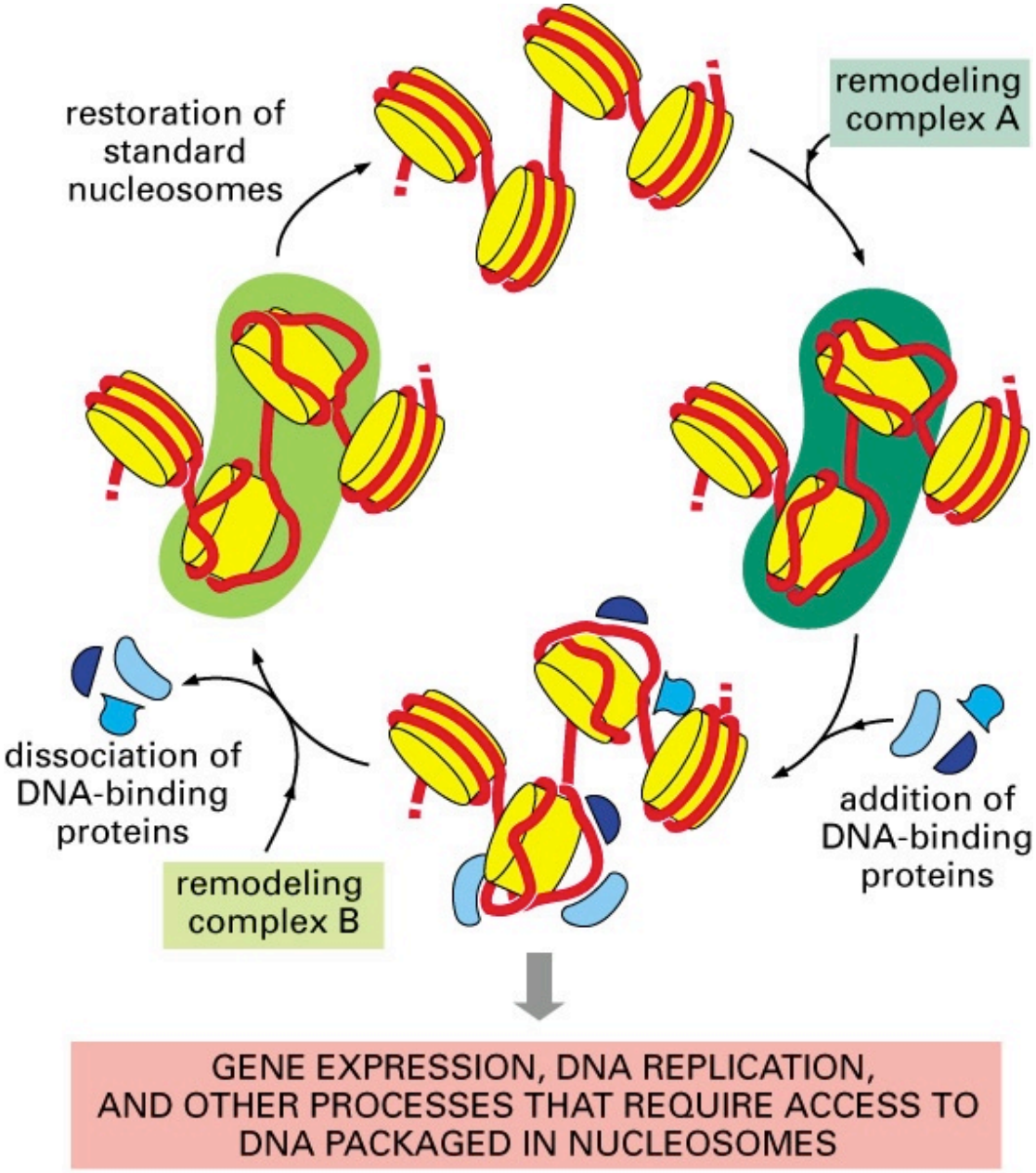


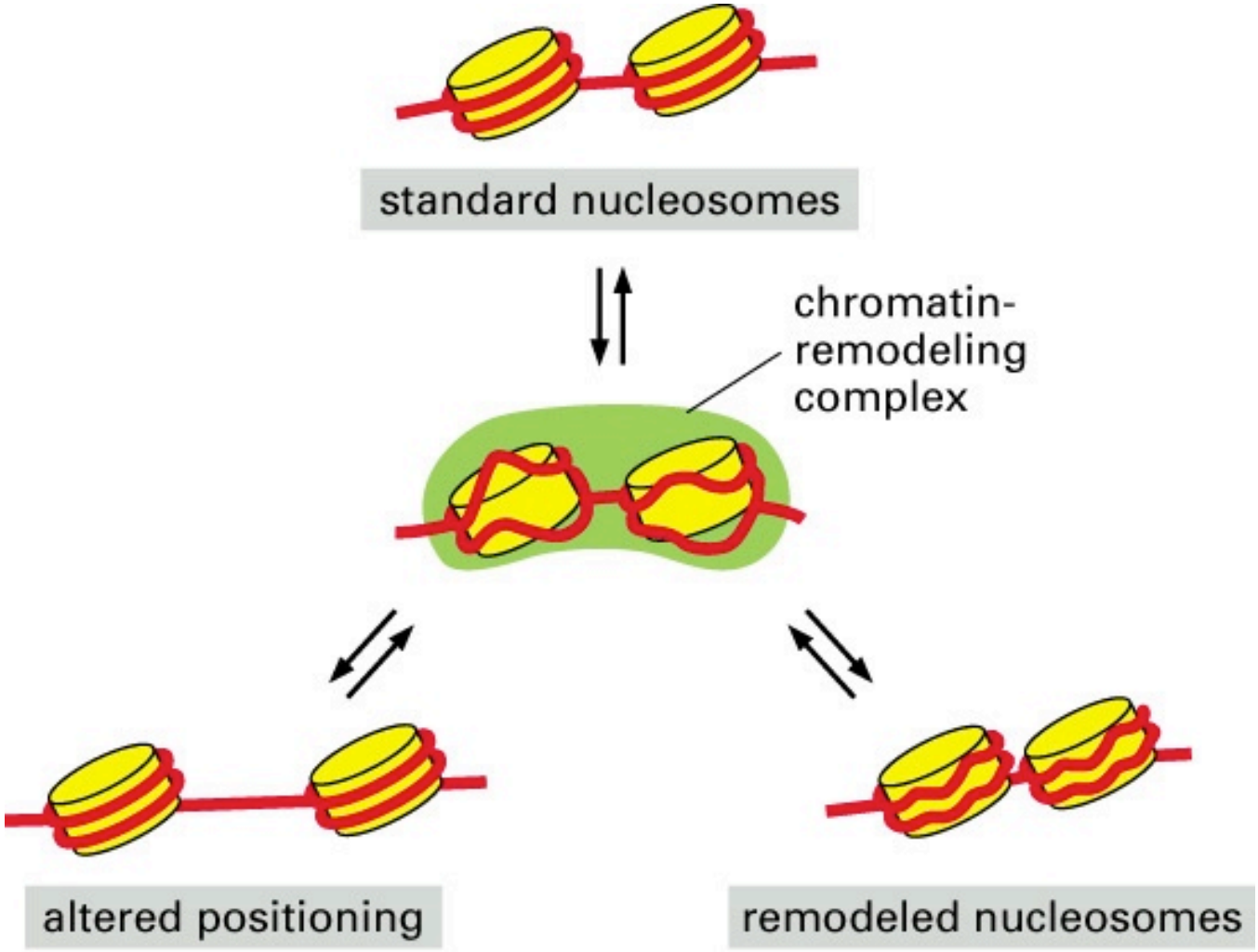
Histone H1 consists of a globular core and two extended tails.
Part of the effect of H1 on the **compaction** of nucleosome organization may result from *charge neutralization*: like the core histones, H1 is positively charged (especially its C-terminal tail), and this helps to compact the negatively charged DNA.
Unlike the core histones, H1 **does not seem to be essential** for cell viability; in one ciliated protozoan the nucleus expands nearly twofold in the absence of H1, but the cells otherwise appear normal



Each nucleosome core particle is separated from the next by a region of linker DNA, which can vary in length : on average, therefore, nucleosomes repeat at intervals of about 200 nucleotide pairs.

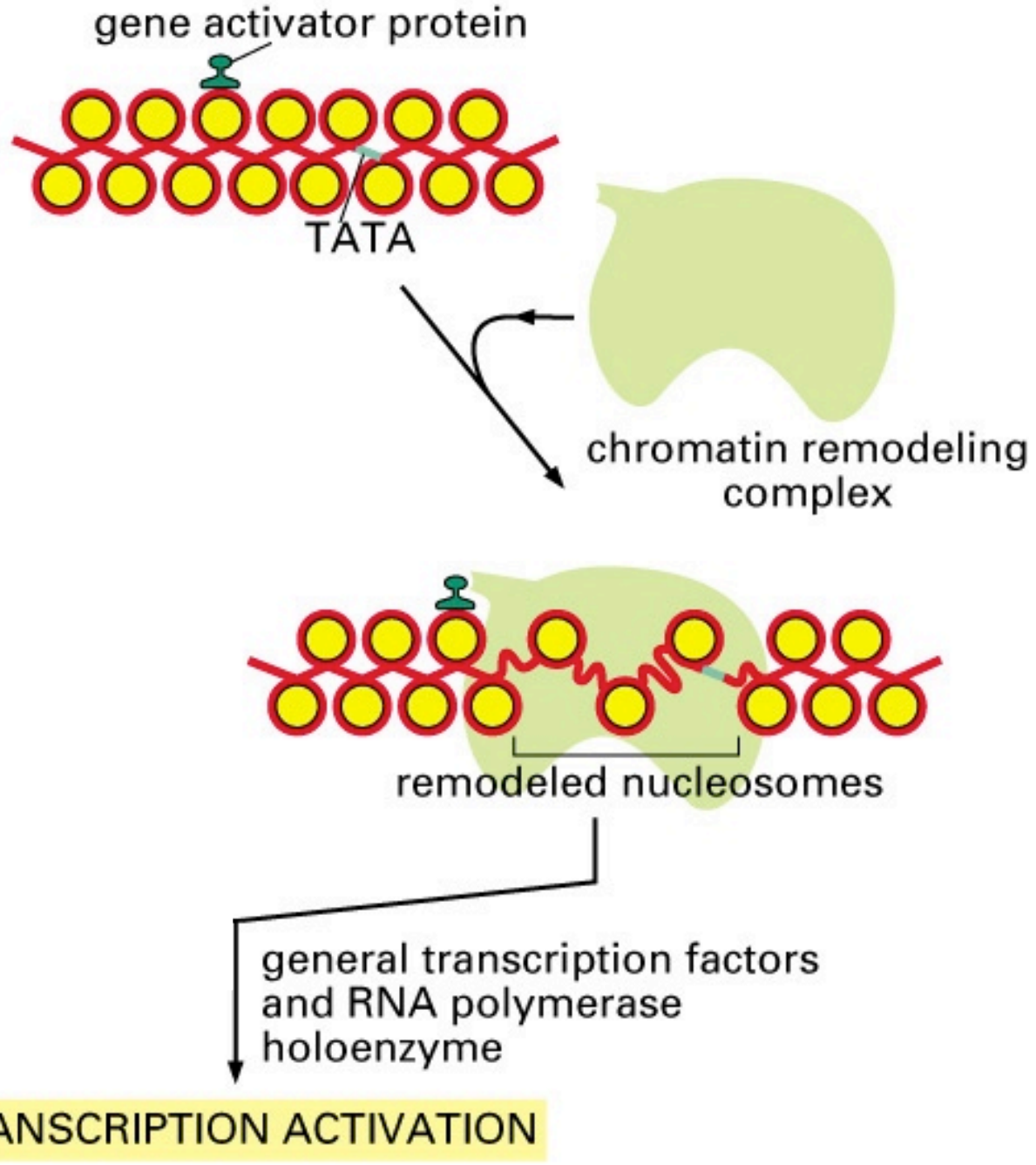


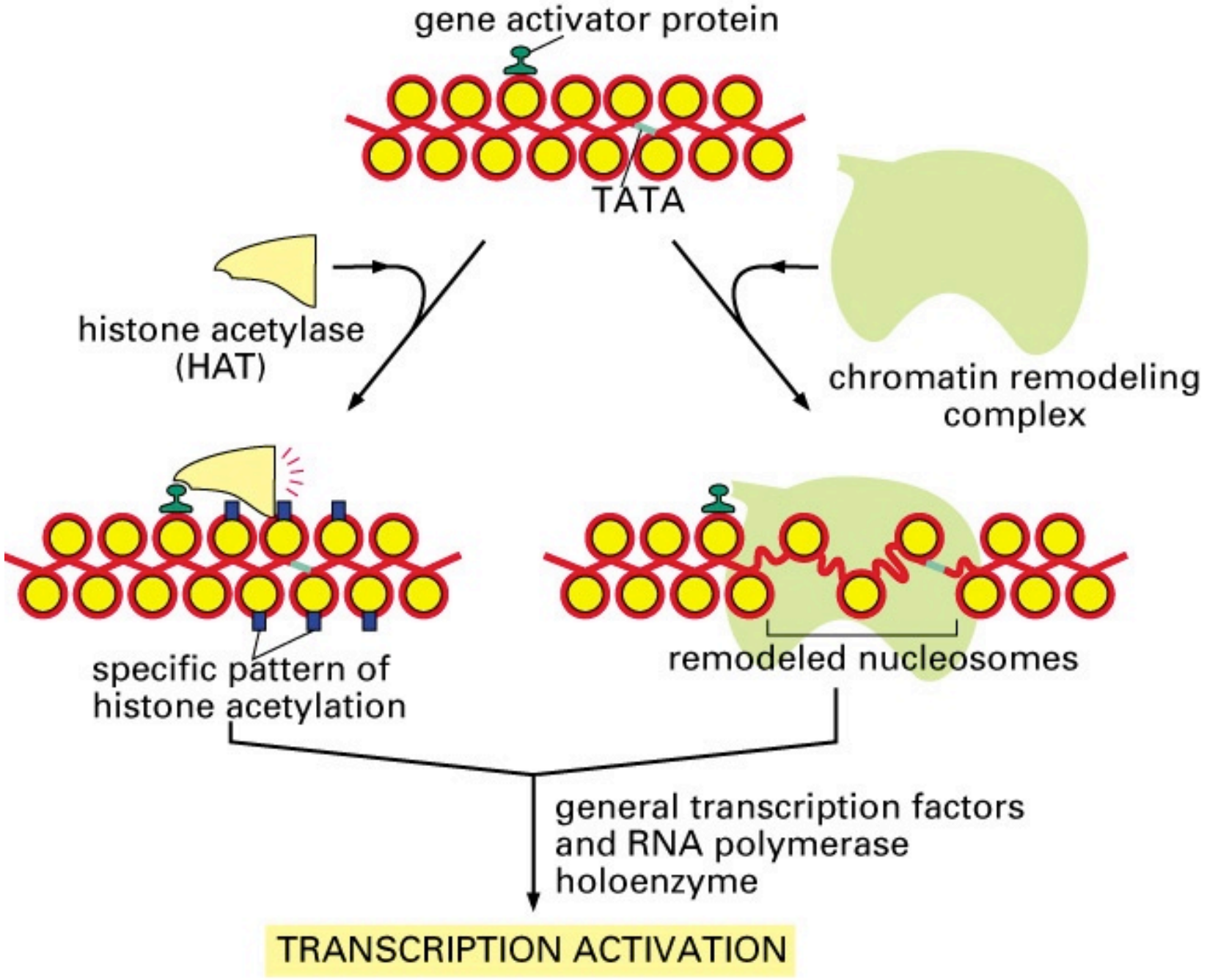

restoration of standard nucleosomes
remodeling complex A
dissociation of DNA-binding proteins
remodeling complex B
addition of DNA-binding proteins
GENE EXPRESSION, DNA REPLICATION, AND OTHER PROCESSES THAT REQUIRE ACCESS TO DNA PACKAGED IN NUCLEOSOMES



Chromatin remodelling complexes:

protein machines that use the energy of ATP hydrolysis to change the structure of nucleosomes temporarily so that DNA becomes less tightly bound to the histone core


gene activator protein
TATA
chromatin remodeling complex
remodeled nucleosomes
general transcription factors and RNA polymerase holoenzyme
TRANSCRIPTION ACTIVATION

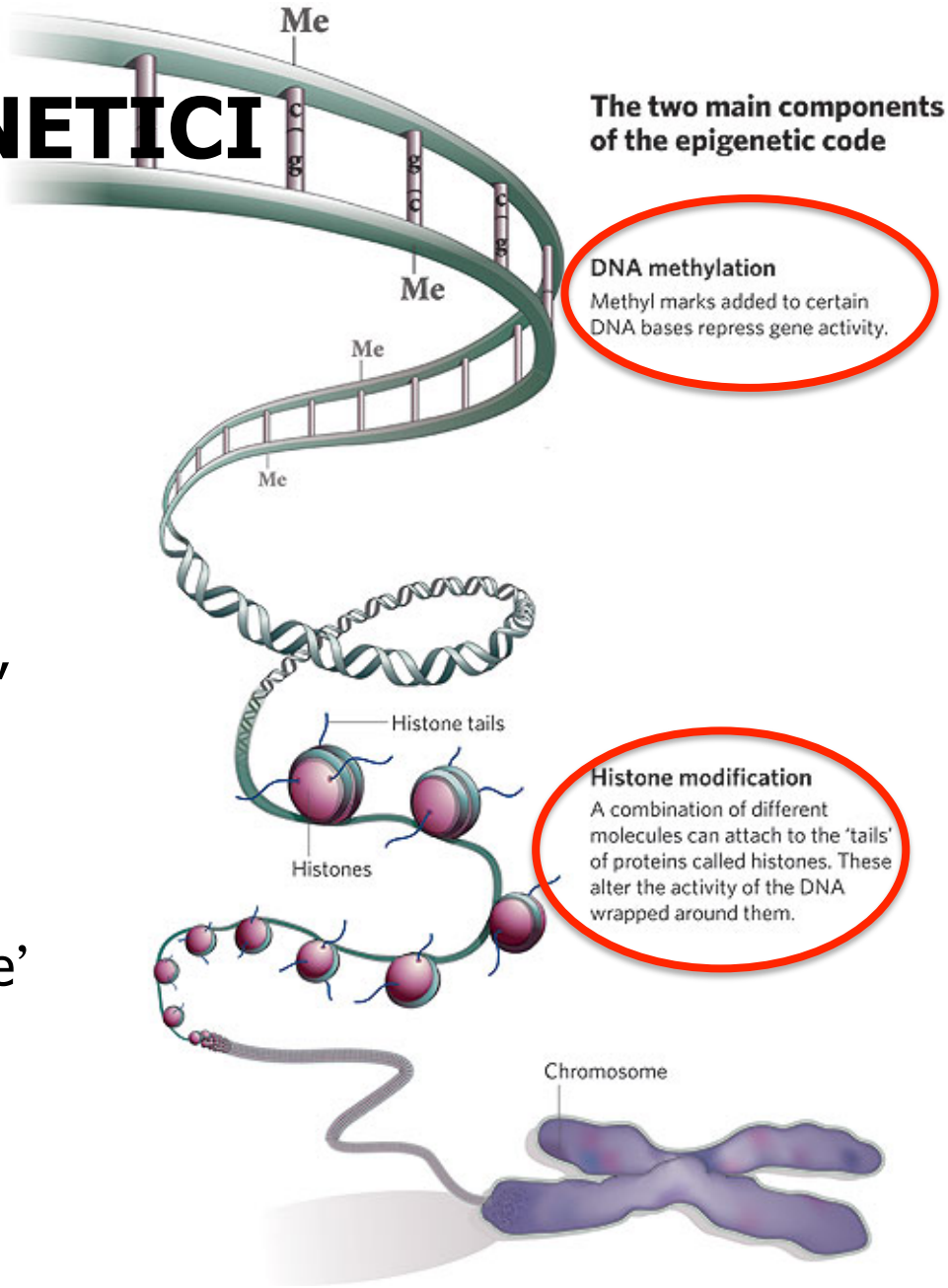

gene activator protein
TATA
histone acetylase
(HAT)
chromatin remodeling
complex
specific pattern of
histone acetylation
remodeled nucleosomes
general transcription factors
and RNA polymerase
holoenzyme
TRANSCRIPTION ACTIVATION

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.



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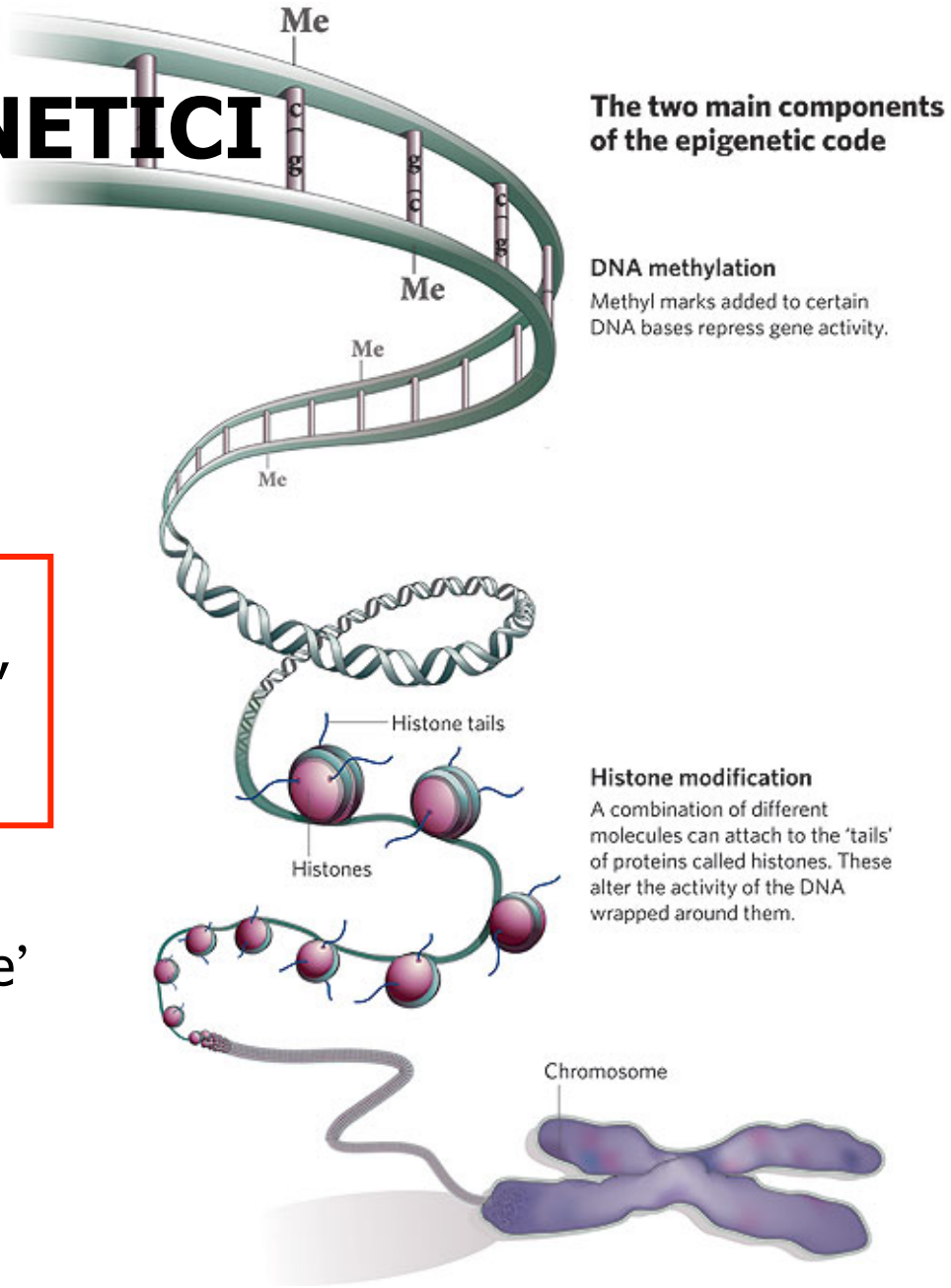
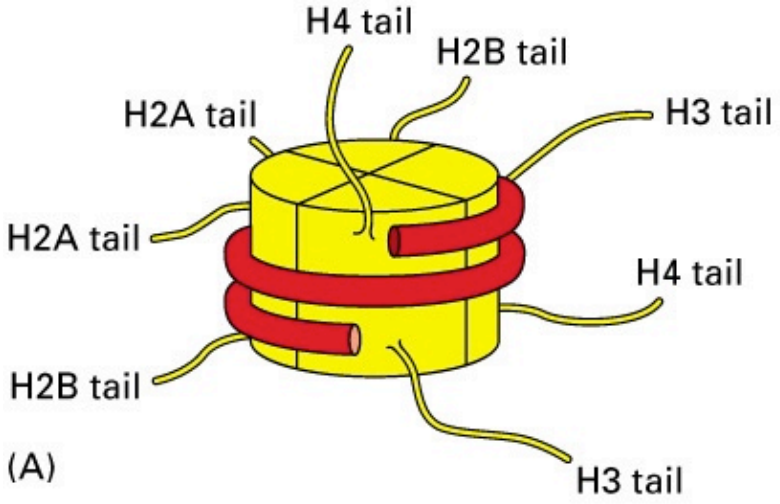
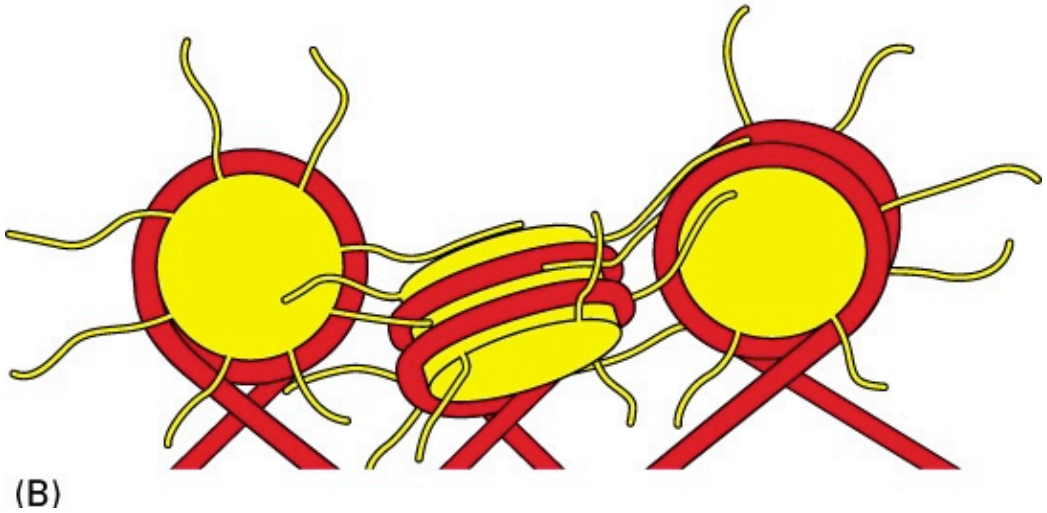


Le **code N-terminali** degli istoni sporgono dal nucleo dell'ottamero

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

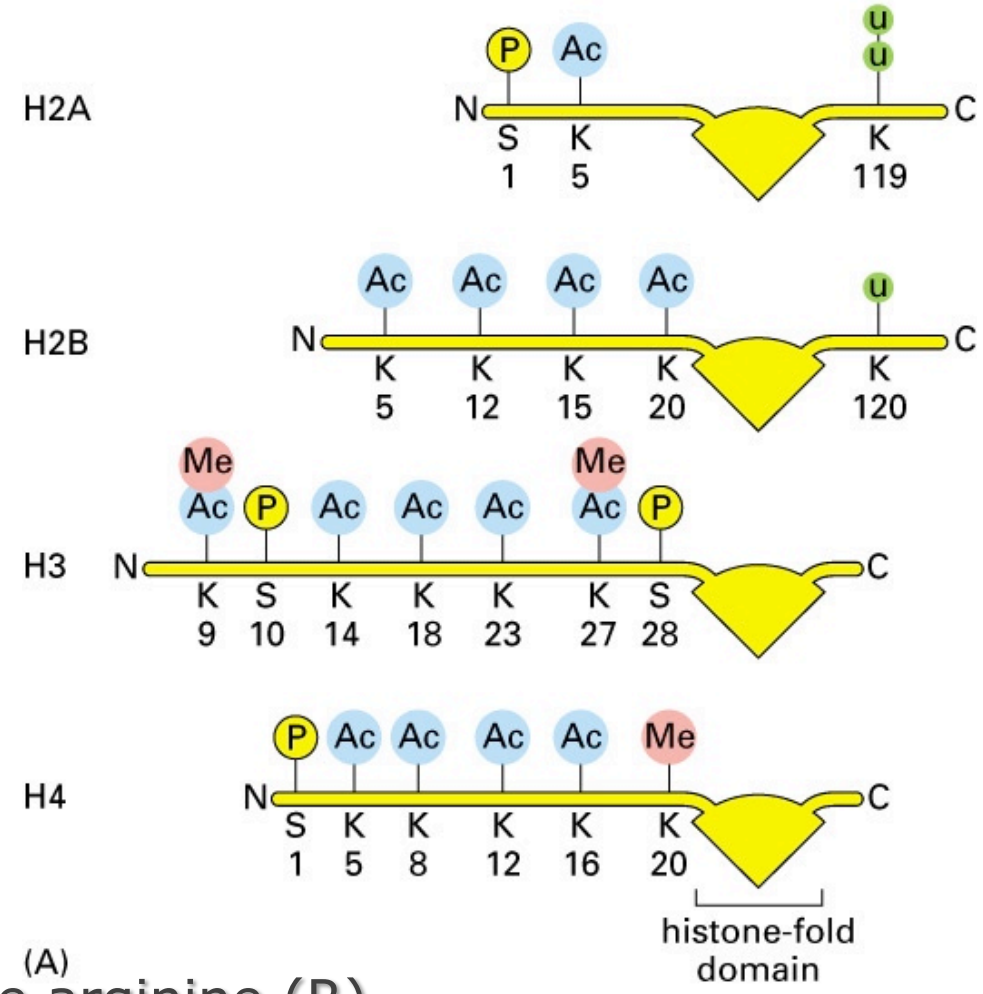


Una particolare combinazione di tali modificazioni ha un significato biologico (CODICE ISTONICO)



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 possibili:

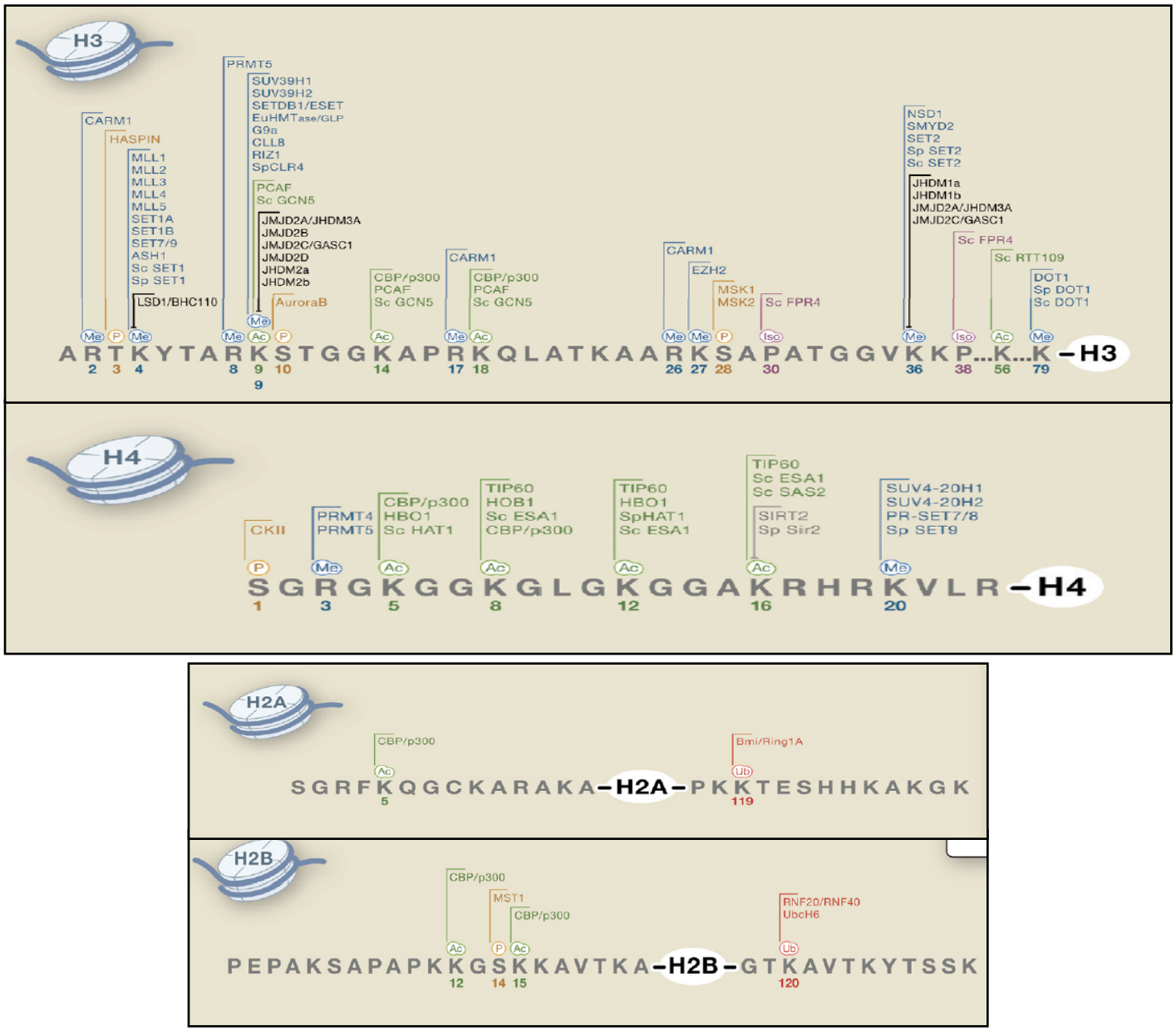
A = Acetilazione di lisine (K)

M = Metilazione di lisine (K) e arginine (R)

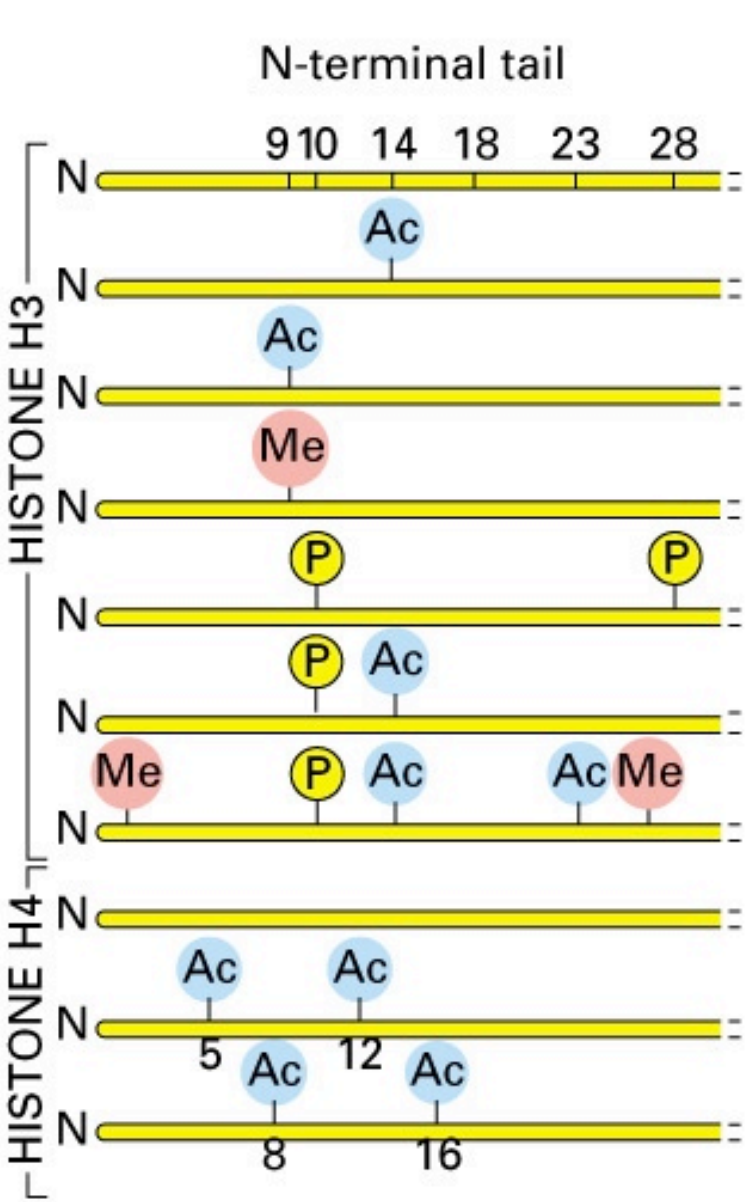
P = Fosforilazione di serine e treonine (S/T)

U = Ubiquitinazione di lisine (K)

60 different residues on histones can be modified



Cell snapshot on histone modifications 2007



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

MODELS OF THE FUNCTIONS OF HISTONE MODIFICATIONS

3 POPULAR MODELS that attempt to explain the function of post-translational histone modifications in gene regulation:

1) **Charge neutralization** - specific modifications of histone acetylation and histone phosphorylation change the overall charge of the chromatin structure. The acetylation neutralizes positive charge on DNA and phosphorylation adds a negative charge. According to this model, these modifications can lead to a general decondensation of the chromatin fiber

2) **Histone code** was originally introduced to explain how multiple histone modifications occurring in the same region could control gene regulation and it hypothesizes that multiple modifications can function combinatorially or sequentially to regulate downstream functions.

3) **Signalling pathway** model postulates that histone modifications serve as signalling platforms to facilitate binding of enzymes for their function on chromatin. It is more general than the histone code model, and suggests that multiple histone modifications provide stability, robustness and specificity through feedback loops, redundancy and combination.

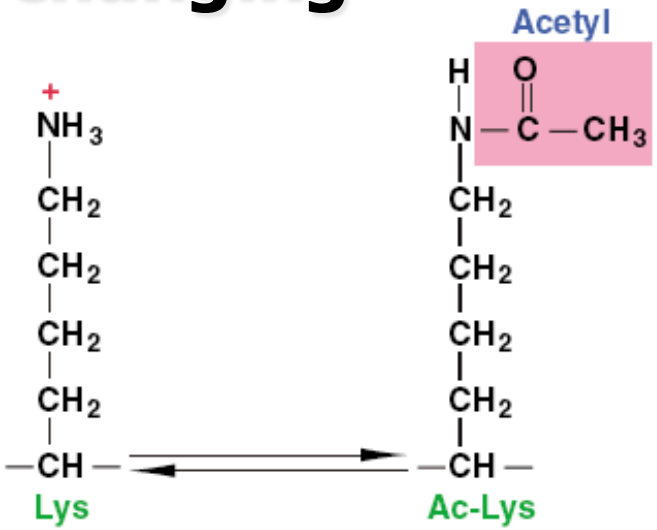
Modificazioni possibili:
A = Acetilazione di lisine (K)
M = Metilazione di lisine (K) e arginine (R)
P = Fosforilazione di serine e treonine (S/T)
U = Ubiquitinazione di lisine (U)

CHI AGISCE?

COMPLESSI DI MODIFICAZIONE DELLA CROMATINA:

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

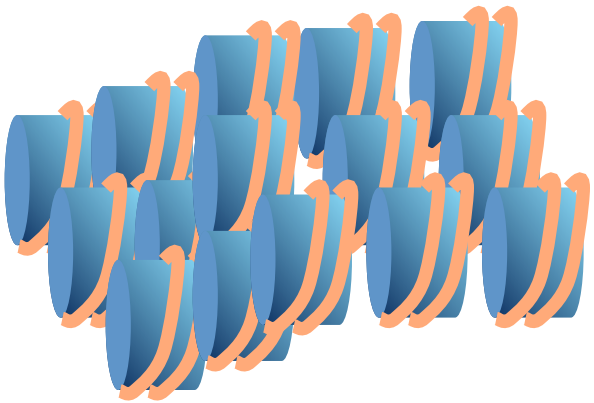
Acetylation is very dynamic and rapidly changing



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

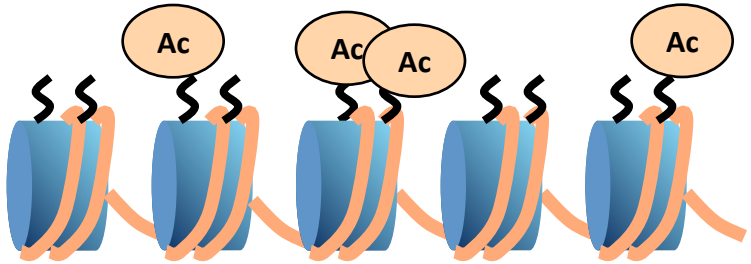
HDAC promotes the removal of the acetyl group from the acetyl-lysine regenerating the ε– amino group and releasing the acetate molecule

heterochromatin
(transcriptionally inactive/condensed)



HAT

euchromatin
(transcriptionally active/accessible)



Writing, erasing and reading histone lysine methylations

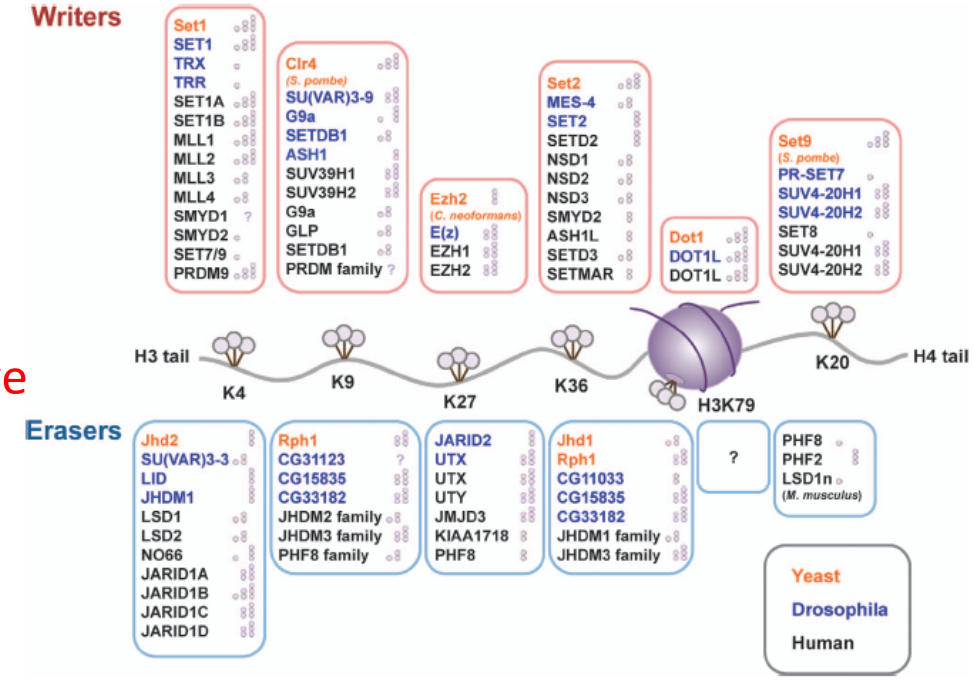
Experimental & Molecular Medicine (2017) 49, e324; doi:10.1038/emm.2017.11

www.nature.com/emm

Kwangbeom Hyun, Jongcheol Jeon, Kihyun Park and Jaehoon Kim

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

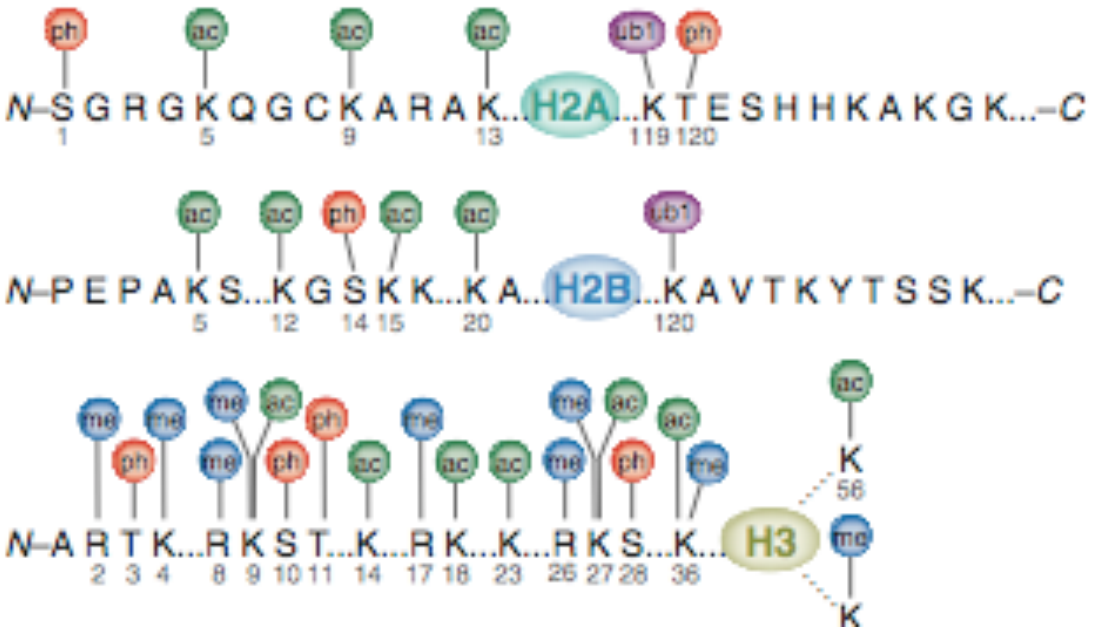
Generally, H3K4, H3K36 and H3K79 methylations are considered to mark active transcription, whereas H3K9, H3K27 and H4K20 methylations are associated with silenced chromatin states.



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.

Histone ubiquitination

Histone ubiquitination occurs on histones H2A, H2B and H3



Histone ubiquitination is formed as isopeptide bond between the carboxy-terminal glycine of ubiquitin and a lysine residue on histones; this bond is formed with the catalytic actions of E1, E2 and E3 ligases.

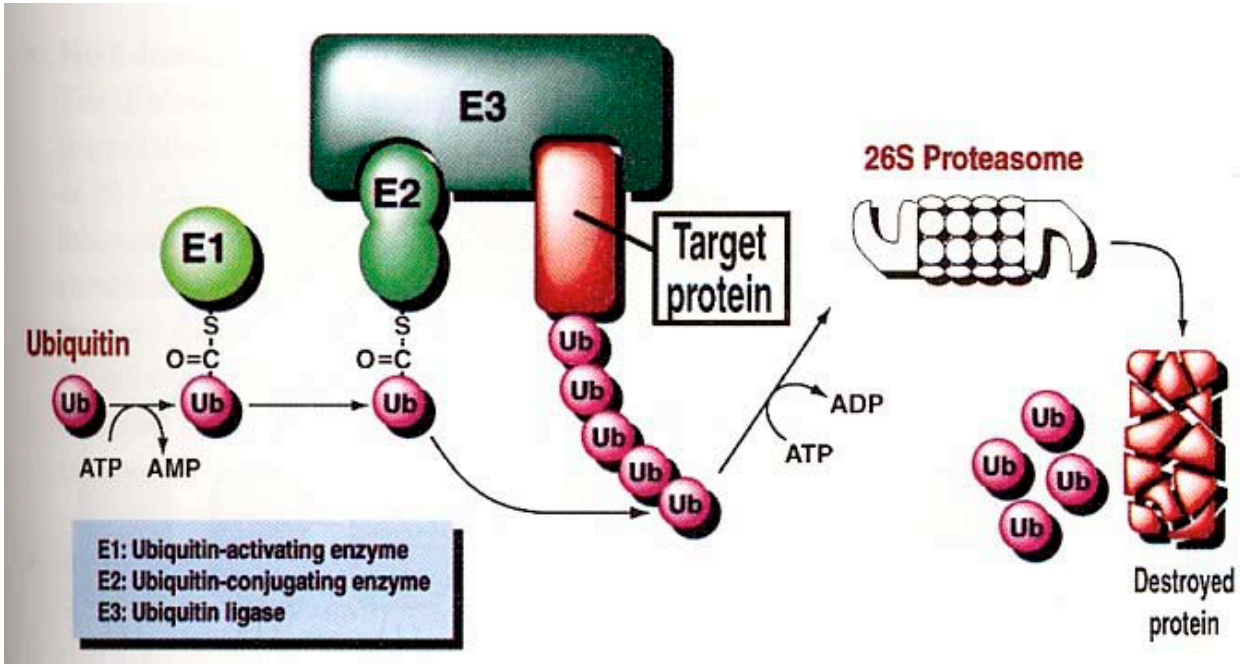
Histone ubiquitination can be reversed by deubiquitinases

Ubiquitin is a small, highly-conserved regulatory protein that is *ubiquitously* expressed in eukaryotes.

Ubiquitination (or ubiquitylation) refers to the post-translational modification of a protein by the covalent attachment (via an isopeptide bond) of one or more ubiquitin monomers.

The most prominent function of ubiquitin is **labeling proteins for proteasomal degradation**.

Besides this function, MONO-ubiquitination also controls the stability, function, and intracellular localization of a wide variety of proteins.



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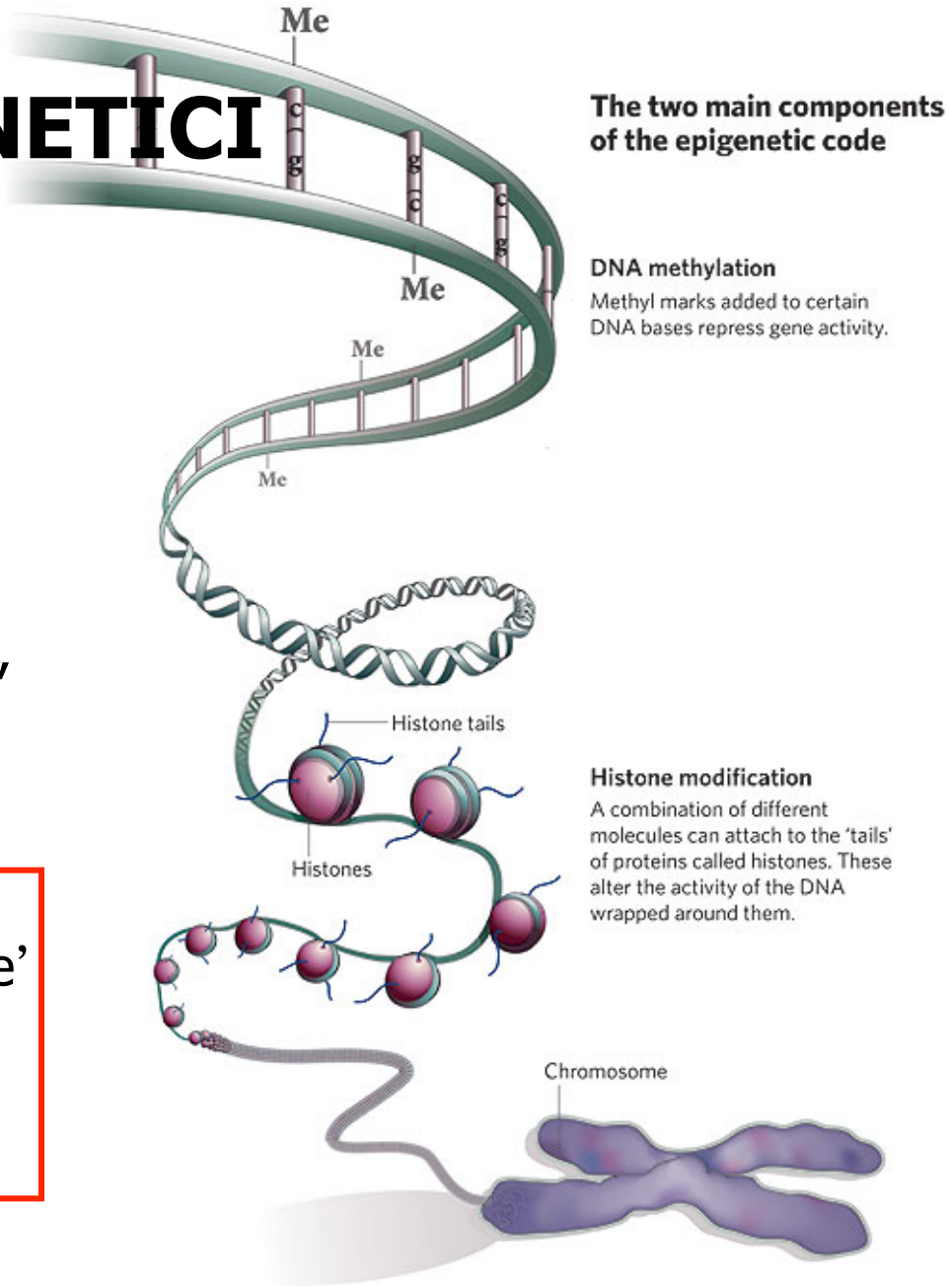
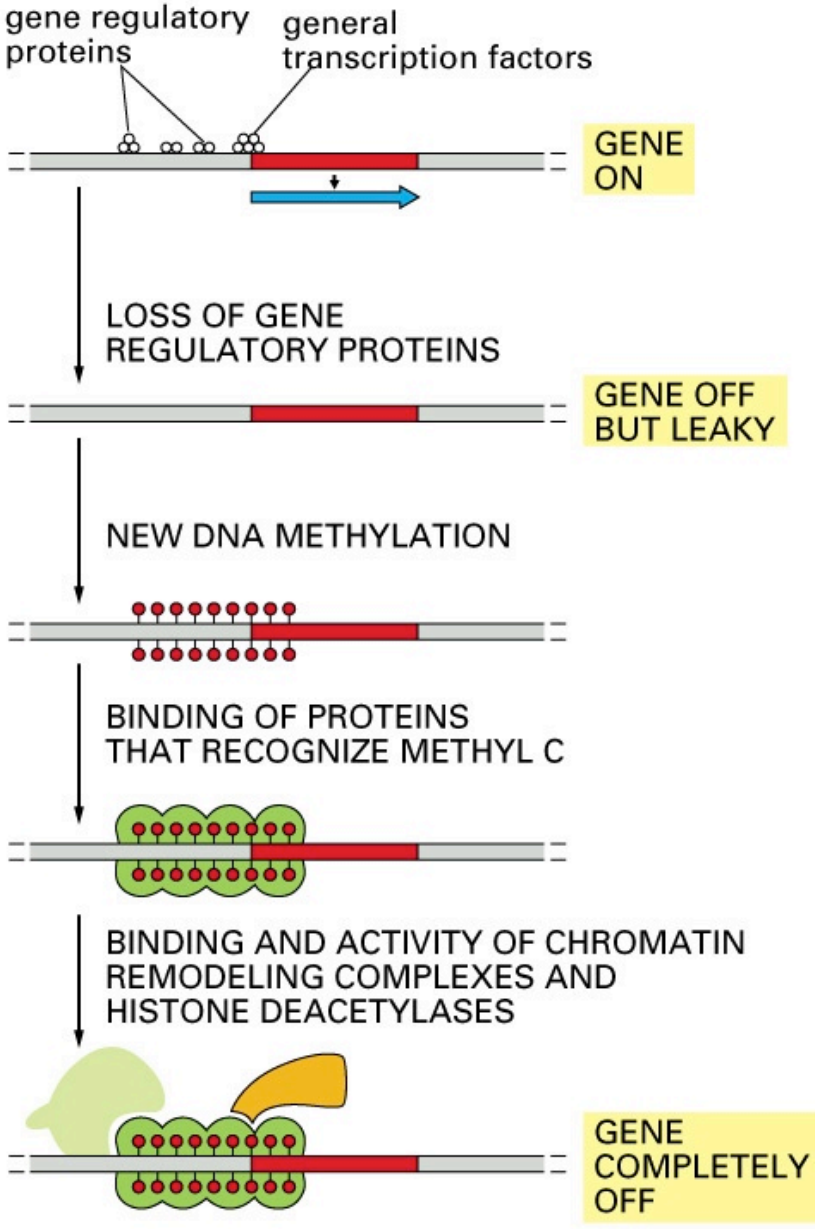


Vertebrates Use DNA Methylation to Lock Genes in a Silent State

In vertebrates **DNA methylation** (by **Dnmt1**) is found primarily on transcriptionally silent regions of the genome, such as the inactive X chromosome or genes that are inactivated in certain tissues, suggesting that it plays a role in gene silencing.

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.



Transcriptional repression by the methyl-CpG-binding protein MeCP2 involves a histone deacetylase complex

Xinsheng Nan*†, Huck-Hui Ng*†, Colin A. Johnson‡, Carol D. Laherty§, Bryan M. Turner‡, Robert N. Eisenman§ & Adrian Bird*

NATURE | VOL 393 | 28 MAY 1998

Cytosine residues in the sequence 5'CpG (cytosine–guanine) are often postsynthetically methylated in animal genomes. CpG methylation is involved in long-term silencing of certain genes during mammalian development[1,2] and in repression of viral genomes[3,4]. The methyl-CpG-binding proteins MeCP1 (ref. 5) and MeCP2 (ref. 6) interact specifically with methylated DNA and mediate transcriptional repression[7–9]. Here we study the mechanism of repression by MeCP2, an abundant nuclear protein that is essential for mouse embryogenesis[10]. MeCP2 binds tightly to chromosomes in a methylation-dependent manner[11,12]. It contains a transcriptional-repression domain (TRD) that can function at a distance *in vitro* and *in vivo*[9]. We show that a region of MeCP2 that localizes with the TRD associates with a corepressor complex containing the transcriptional repressor mSin3A and histone deacetylases[13–19]. Transcriptional repression *in vivo* is relieved by the deacetylase inhibitor trichostatin A[20], indicating that deacetylation of histones (and/or of other proteins) is an essential component of this repression mechanism. The data suggest that two global mechanisms of gene regulation, DNA methylation and histone deacetylation, can be linked by MeCP2.

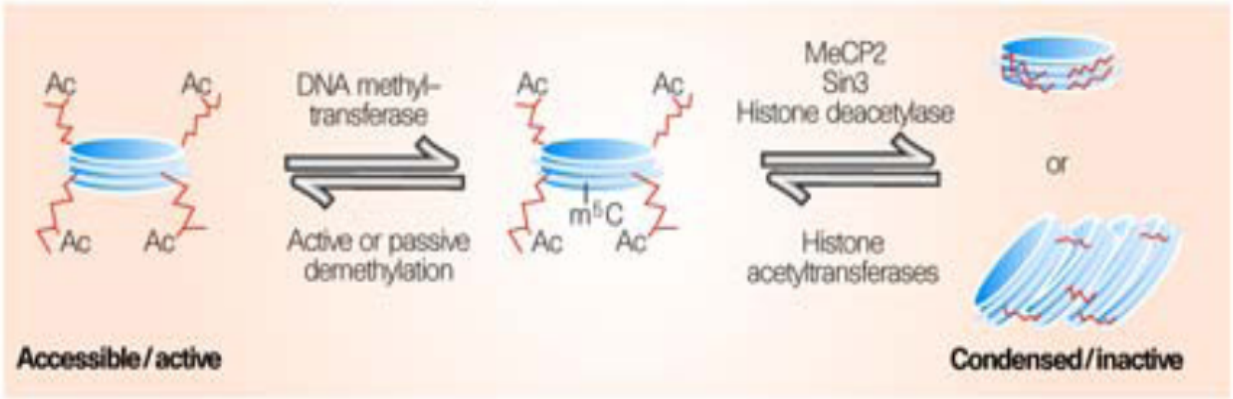


Figure 1 **The effects of cytosine methylation and histone deacetylation on transcription.** Transcriptional silencing in vertebrates is usually associated with the presence of 5-methylcytosine (m^5C) in the DNA. Nan *et al.*[1] and Jones *et al.*[2] have now discovered a link between methylation and histone deacetylation — MeCP2 (a protein that binds methylated DNA) exists in a complex with histone deacetylase.

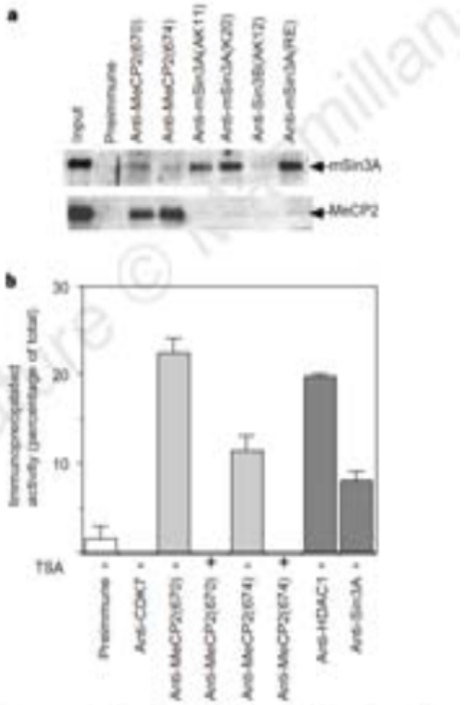
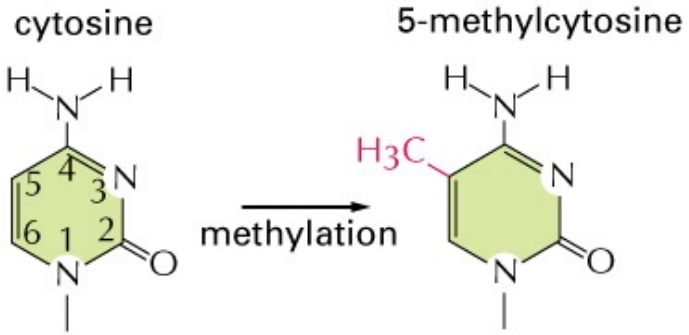
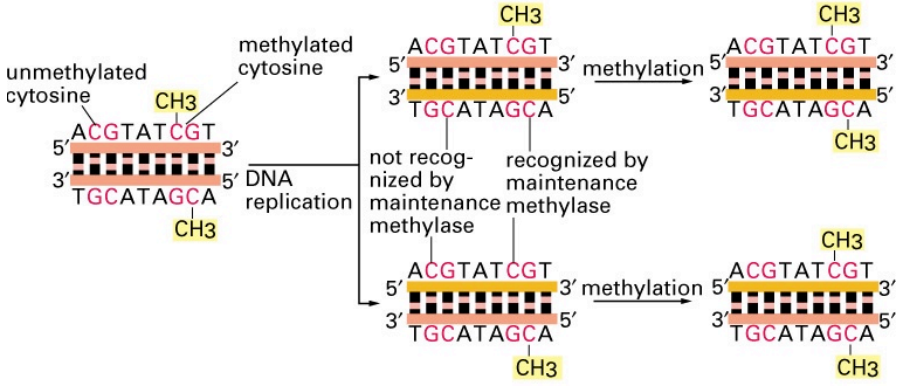


Figure 2 Co-immunoprecipitation of mSin3, MeCP2 and histone deacetylase activity from rat brain nuclear extracts. **a**, Western blot of immunoprecipitates prepared using antibodies against MeCP2 (antibodies 670 and 674) or mSin3 (antibodies AK11, AK12, K-20 and RE) or using preimmune serum. Probes were anti-mSin3A (RE) and anti-MeCP2 (670 and 674) antibodies. **b**, Immunoprecipitation of histone deacetylase activity from rat brain nuclear extracts with antibodies against MeCP2 (670 and 674), HDAC1, mSin3A or cyclin-dependent kinase 7 (CDK7) or with preimmune serum, in the presence or absence of 5 ng ml^{-1} TSA. Precipitated activities from duplicate parallel experiments are shown as percentage total activity added to each tube (19,200 d.p.m. per 2 h in the experiment shown).

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. Methyltransferase 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)





DNA cytosine methyltransferases/1

Dnmt1

- 1620 aa
- 5- to 30-fold preference for hemimethylated substrates (maintenance of methylation)
- De novo methylation in embryo lysates - lack of specificity beyond CpG dinucleotide
- Homologues of Dnmt1 in all eukaryotes show DNA bears m^5C
- C-term domain related to bacterial restriction methyltransferases
- N-term domain contain information for nuclear import, localization in replication foci and suppression of de novo methylation
- Found in nucleoplasm in G1 and redistributed in replication foci in S
- Dnmt1 knock-out recessive lethal (ES cells grow normally with demethylated genome but undergo apoposis when induced to differentiate; embryos show biallelic expression of several imprinted genes and ectopic expression of all copies of Xist and transient inactivation of all X chromosomes)
- Dnmt1 has sex-specific promoters and 5' exons; oocyte specific form

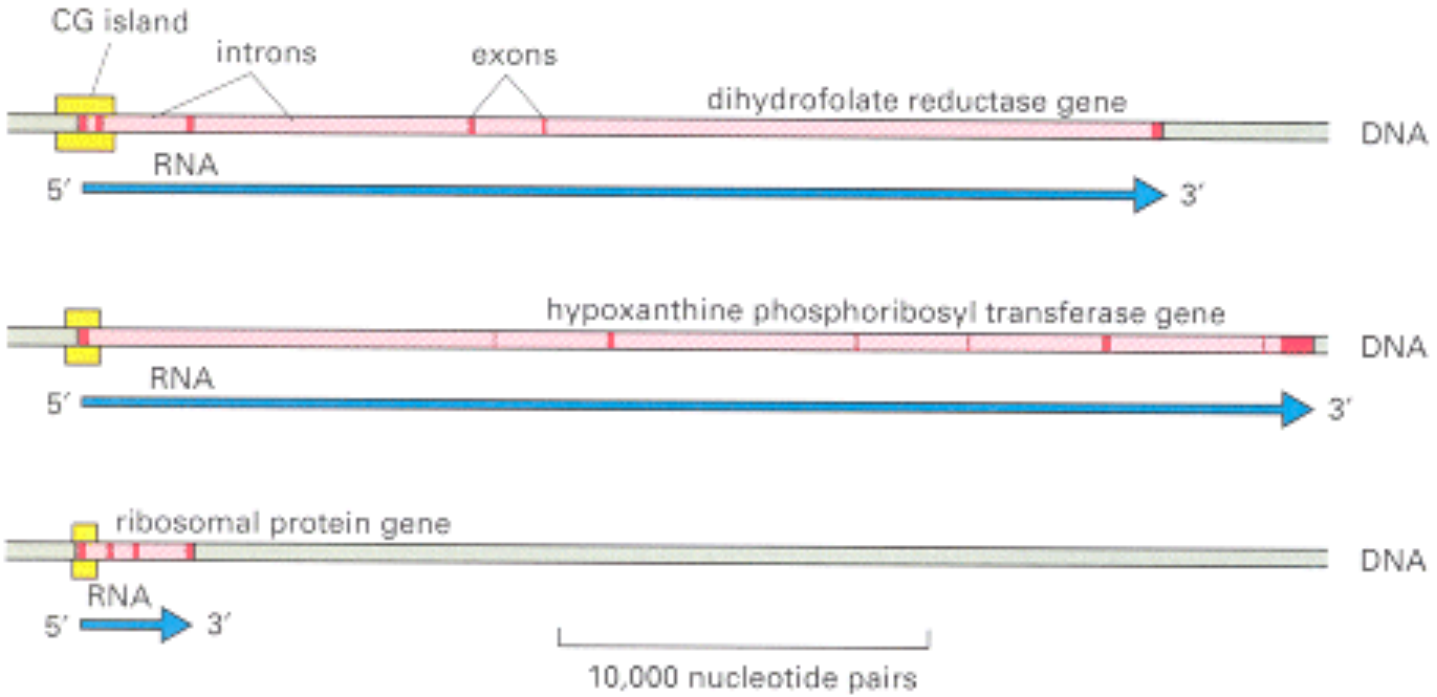
Why is DNA methylation required?

- To program tissue-specific and stage-specific gene expression?
- To prevent transcription of selfish DNA elements? (the vast majority of methyl-CpG is in transposons, which are abundant genomic elements - 106 elements, >40% of the genome) - most cellular genes contain transposons within introns. Methylation provides a mean for transcriptional repression and permanent inactivation
- To limit unscheduled transcription throughout the genome?

- Mice lacking the maintenance methyltransferase gene (**Dnmt1**) die early during development

DNA methylation and CpG islands

- 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

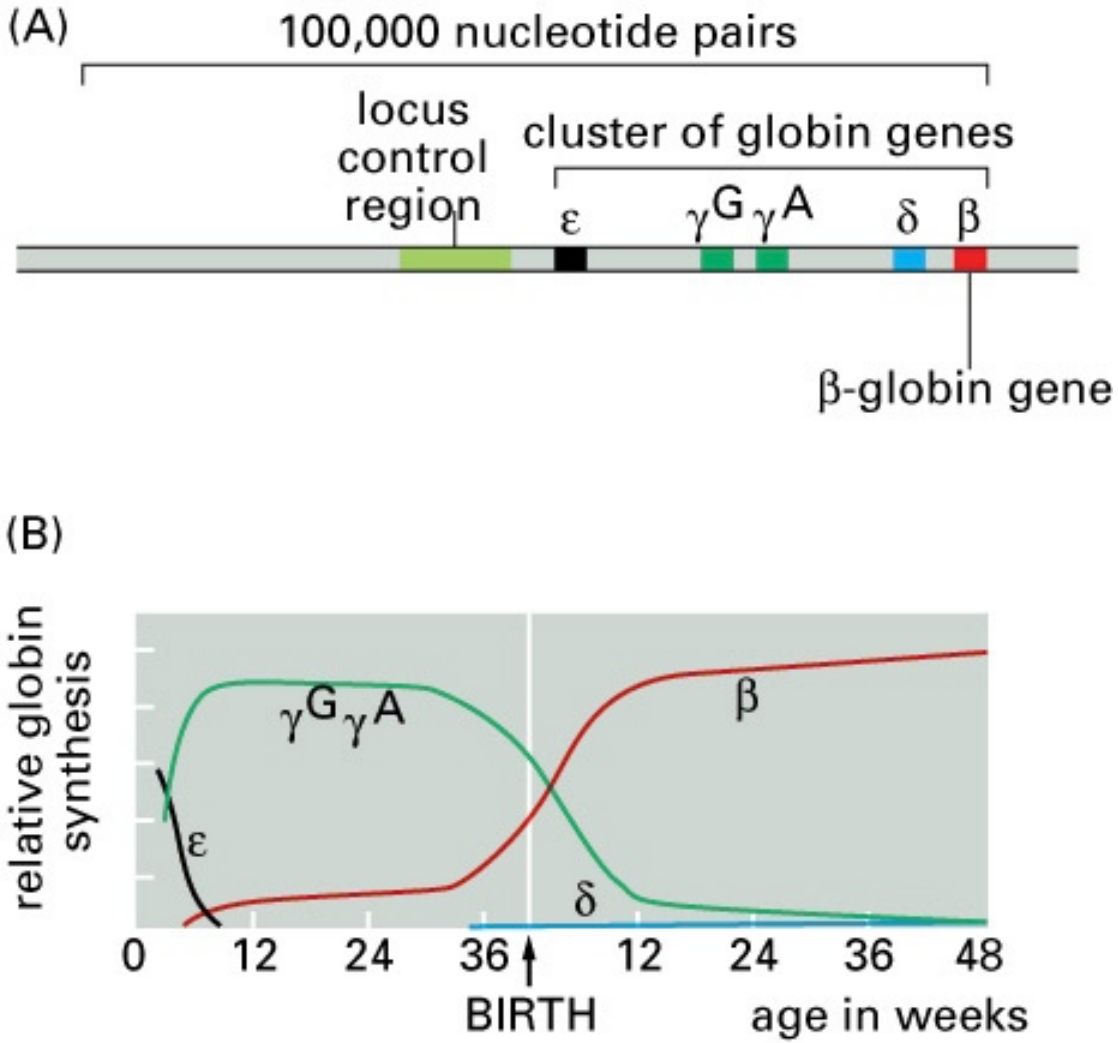


A transcription factor called SP1 recognizes these CG rich regions: TATA-less promoters

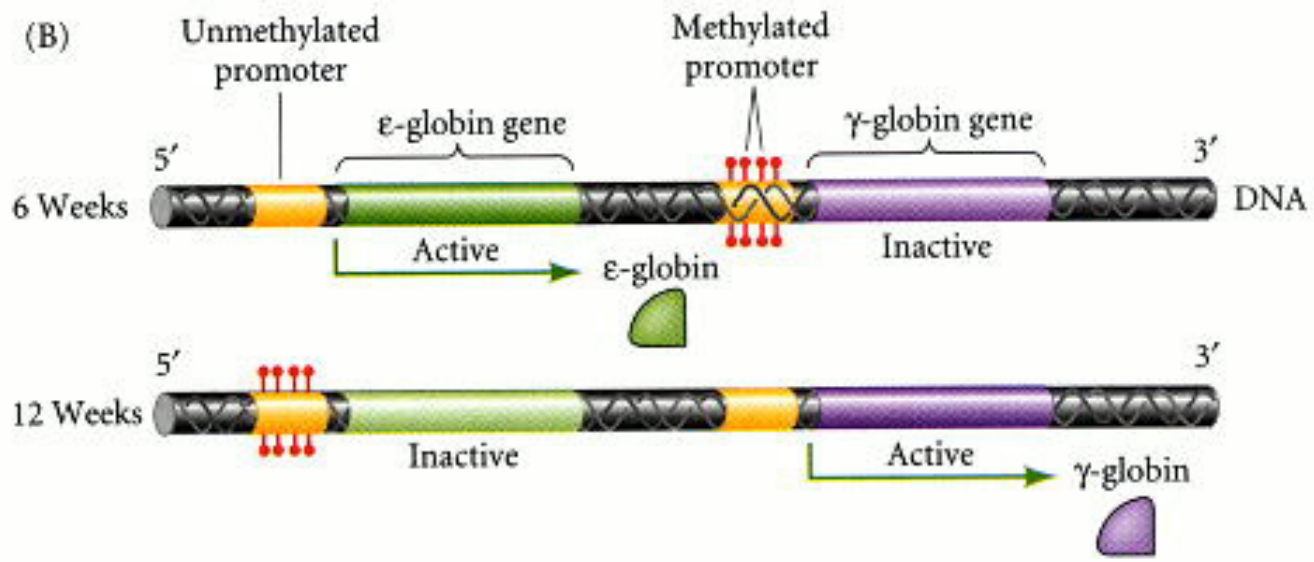
CpG islands and transcription

- All house-keeping genes have CpG islands associated with their promoters
- All CpG islands are associated with transcribed genes
- Lack of methylation of CpG islands is essential for expression of the associated gene
- CpG islands at tissue-specific genes are non-methylated regardless of the activity of the gene, with the exception of the X-chromosome
- X-linked CpG islands become methylated upon X inactivation; DNA methylation at CpG islands is essential for maintenance of inactivated state

The β-globin gene cluster



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



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

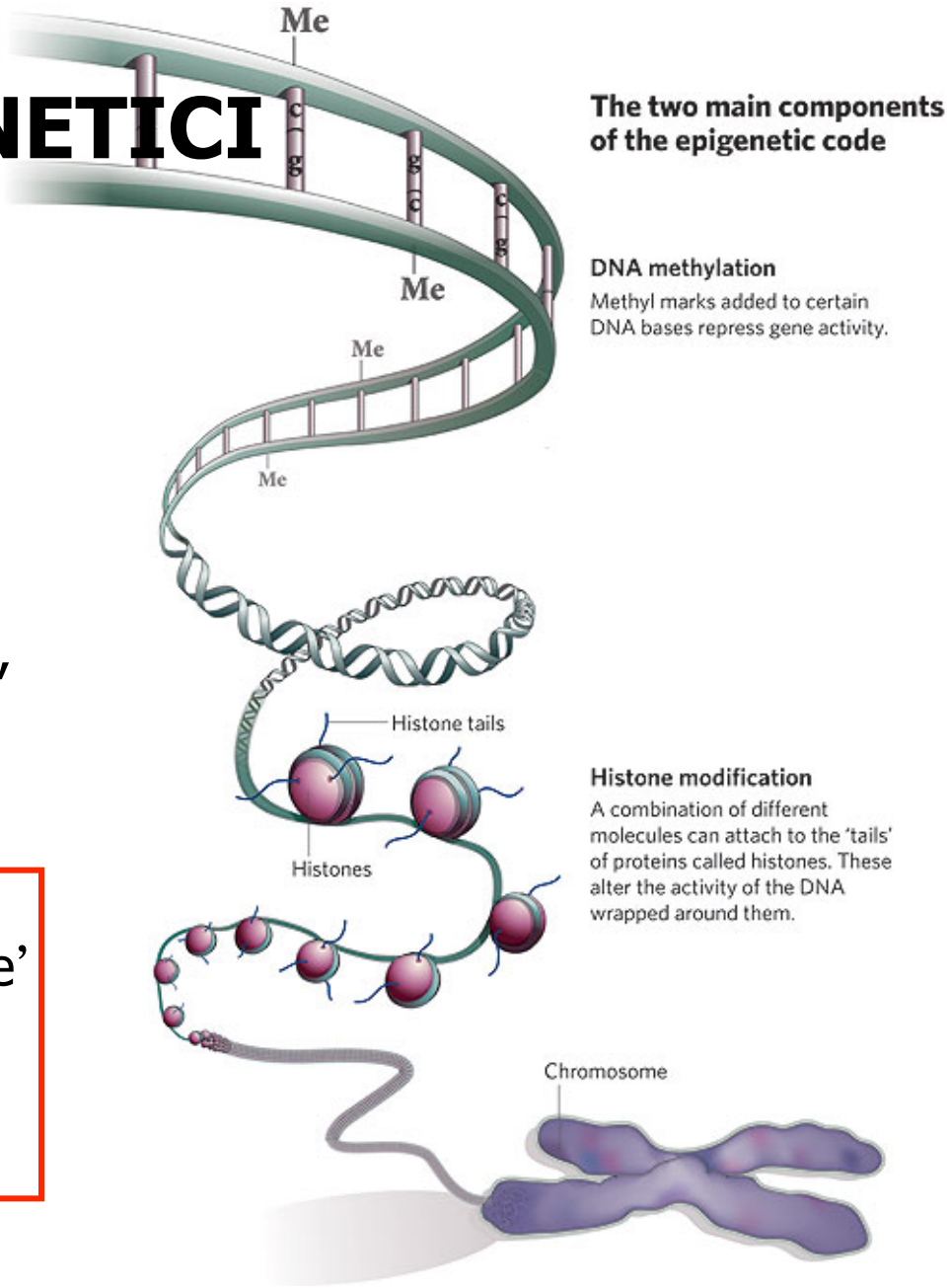
Moreover, the methylation pattern changes during development. The cells that produce hemoglobin in the human embryo have unmethylated promoters for the genes encoding the ε-globins of embryonic hemoglobin. These promoters become methylated in the fetal tissue. Similarly, when the fetal globin gives way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

MECCANISMI EPIGENETICI

Fattori che vengono 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
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.



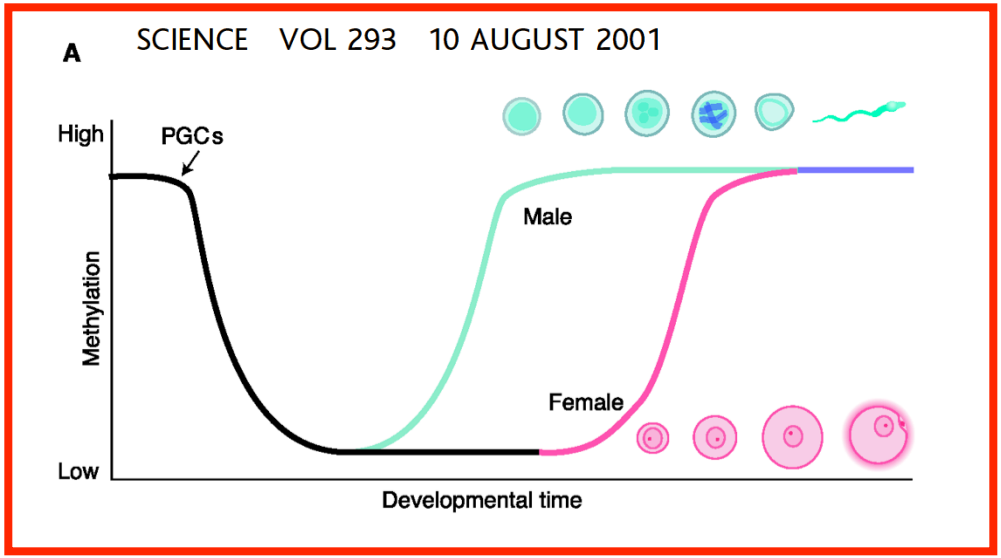
Global demethylation of the genome occurs *immediately after fertilization* and de novo remethylation follows implantation

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

DNA demethylation in germ cell differentiation



- The genomes of mature egg and sperm cells in mammals are highly methylated
- Genomewide demethylation occurs early in the development of primordial germ cells (completed by E13-E14)
- Remethylation takes place several days later
- Remethylation in the **male** at E15-E16 and onwards at the prospermatogonia state preceding mitosis and meiosis
- Remethylation in the **female** after birth during the growth of the oocyte
- **Dnmt3A** and **Dnmt3B** are the enzymes responsible for de novo methylation at specific stages of gametogenesis and early development to establish methylation patterns

Methylation reprogramming in preimplantation embryos

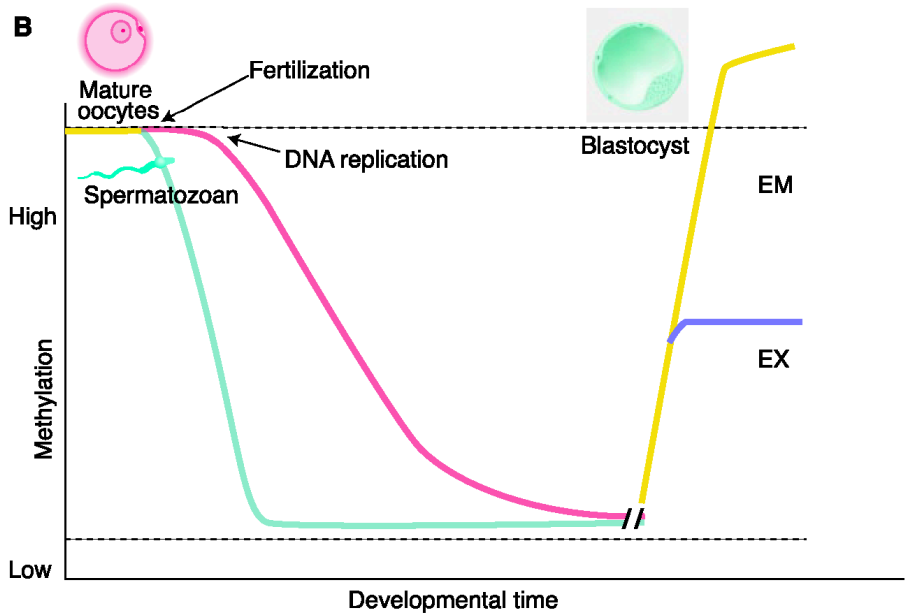
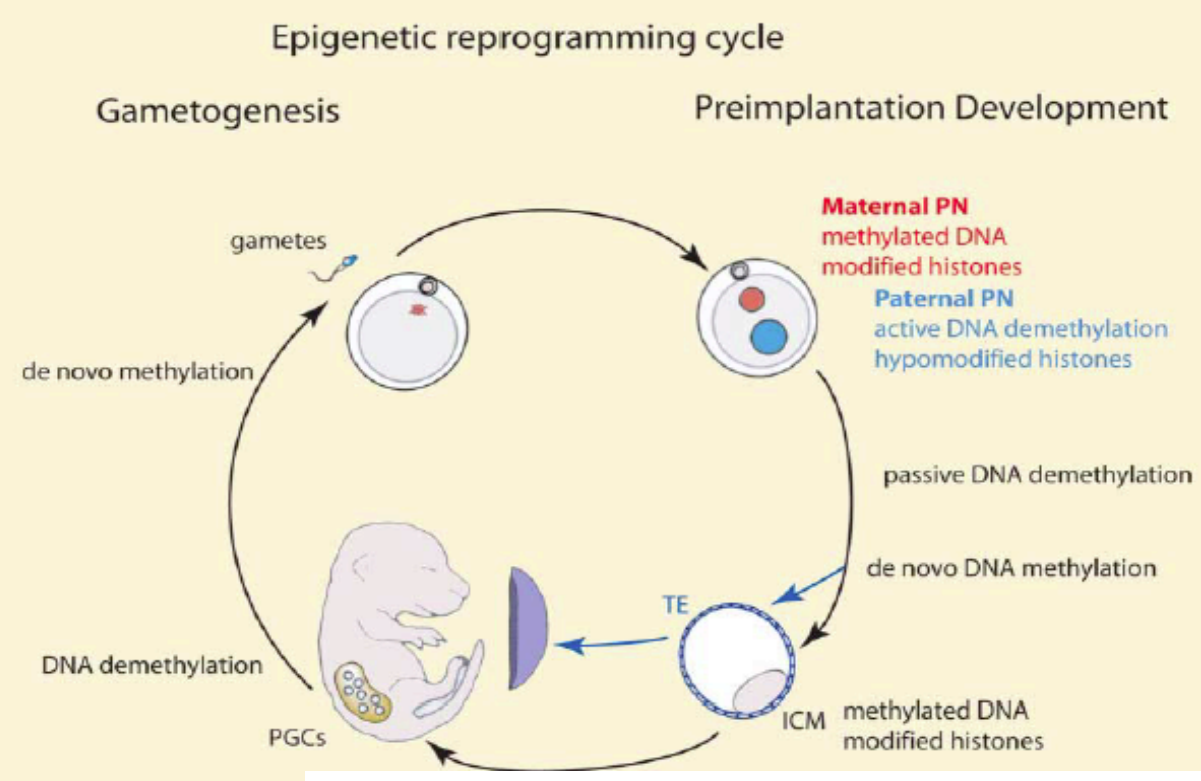


Fig. 1. (**A**) Methylation reprogramming in the germ line. Primordial germ cells (PGCs) in the mouse become demethylated early in development. Remethylation begins in prospermatogonia on E16 in male germ cells, and after birth in growing oocytes. Some stages of germ cell development are shown [modified from (*29*)]. (**B**) Methylation reprogramming in preimplantation embryos. The paternal genome (blue) is demethylated by an active mechanism immediately after fertilization. The maternal genome (red) is demethylated by a passive mechanism that depends on DNA replication. Both are remethylated around the time of implantation to different extents in embryonic (EM) and extraembryonic (EX) lineages. Methylated imprinted genes and some repeat sequences (dashed line) do not become demethylated. Unmethylated imprinted genes (dashed line) do not become methylated.

- The paternal genome undergoes a remarkable tranformation in the egg cytoplasm, with remodeling of sperm chromatin through removal of protamines and replacement by acetylated histones. These events are followed by genome wide demethylation, which is complete before DNA replication commences.
- Re-methylation carried out by Dnmt3a and Dnmt3b with protection of the unmethylated allele of imprinted genes.
- Timing of re-methylation differs. In mouse embryos, de novo methylation occurs in the inner cell mass (ICM) cells of the expanded blastocyst; in bovine embryos in cells embryos at the 8-16 cell stage.



Human Molecular Genetics, 2005, Vol. 14, Review Issue 1

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

These genome-wide demethylation waves may have a role in reprogramming of the genetically sperm and egg chromatin for somatic development

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

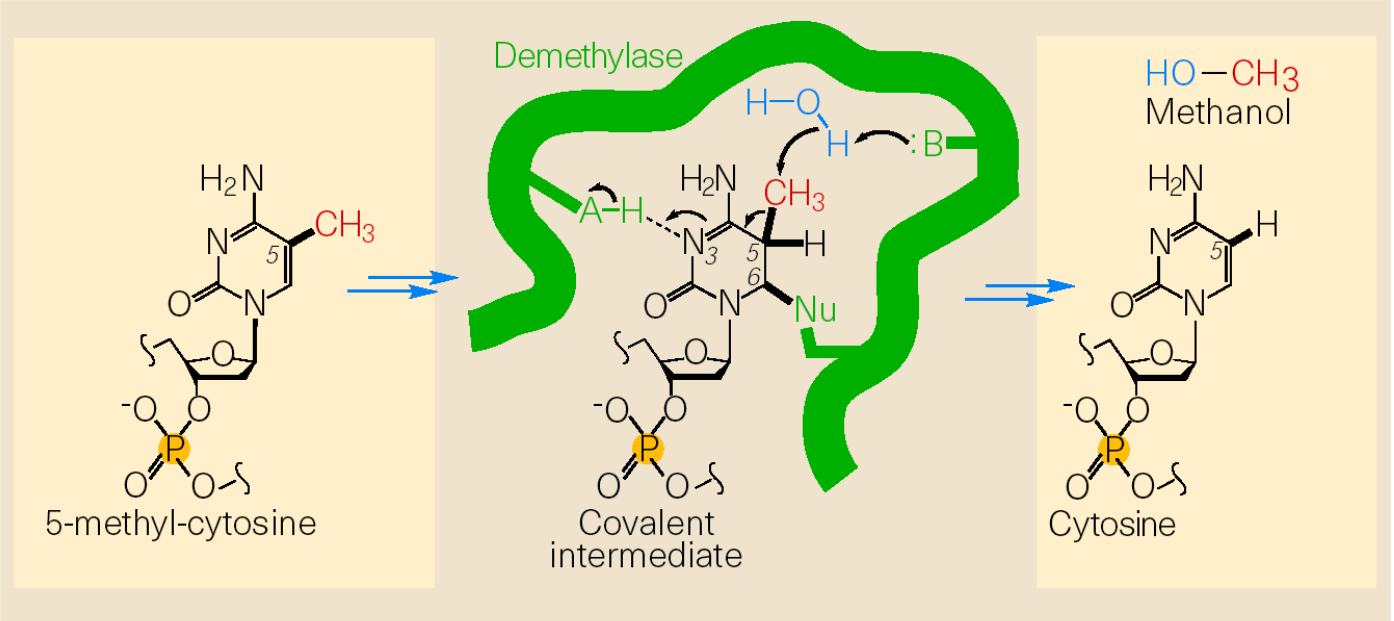


Figure 1 **Mechanism for the enzymatic demethylation of 5-methyl-cytosine. The demethylase (green) is envisaged to form a covalent intermediate by addition of an enzymatic nucleophile (Nu–H) across the 5,6 double bond, assisted by proton shuffling at N3. This intermediate is poised to attack the hydroxide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

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.

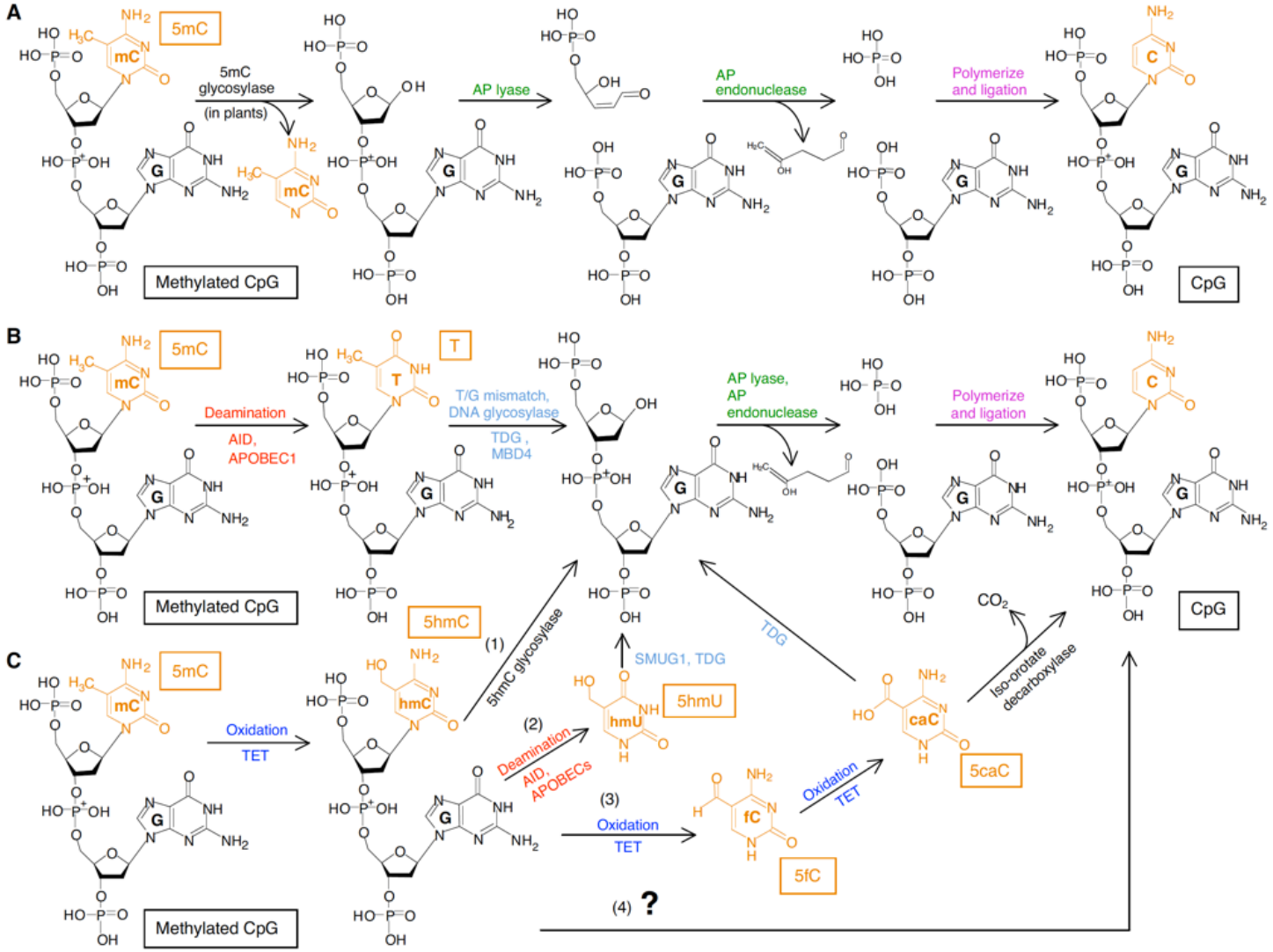
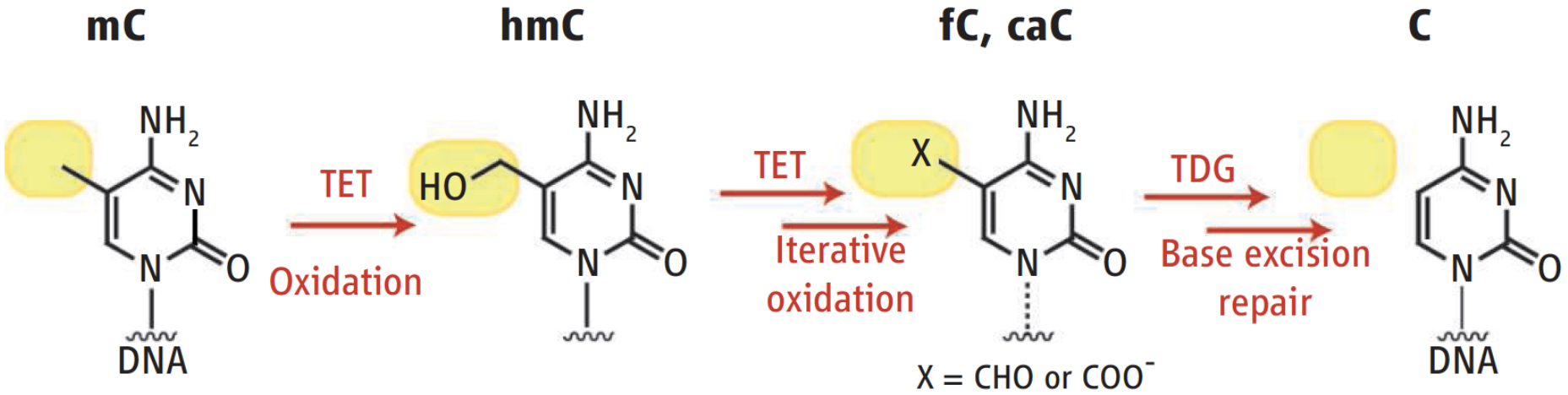


Fig. 4. Potential chemical pathways for active DNA demethylation. (**A**) Direct excision of 5mC (orange) by a 5mC glycosylase followed by repair via the base excision repair (BER) pathway (green and pink), as occurs in plants. (**B**) Cytosine deamination by AID/APOBEC1 (red), followed by base excision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

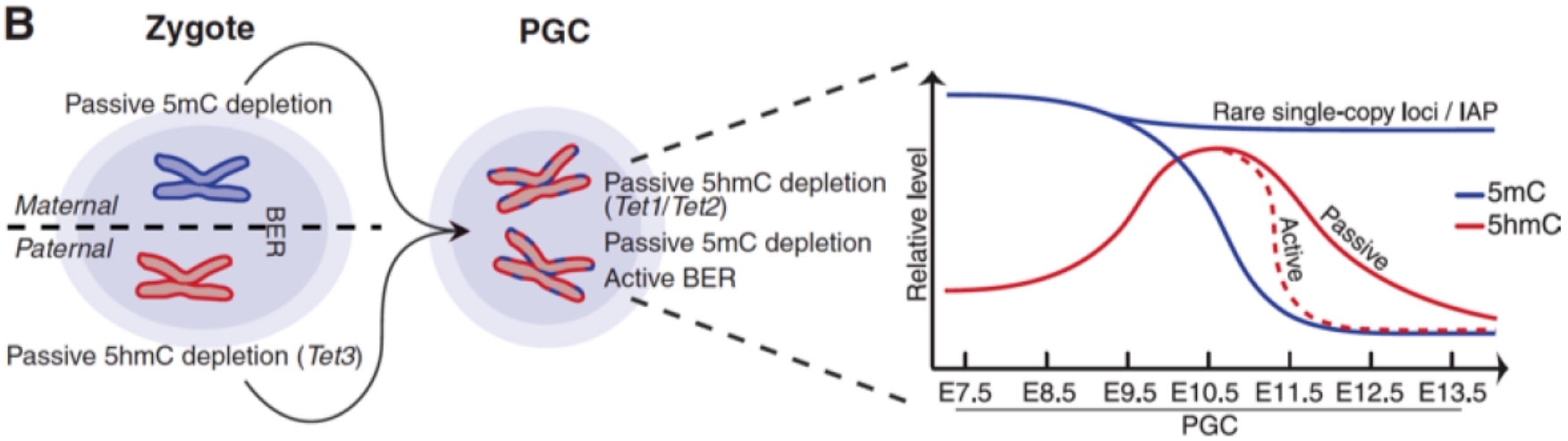


DNA demethylation. TET enzymes are proposed to oxidize 5-methylcytosine (mC) to 5-hydroxymethylcytosine (hmC) and subsequently to generate the higher oxidation substituents 5-formylcytosine (fC) and 5-carboxylcytosine (caC) (shown as the structure with the 5-X substituent). Unmodified cytosine (C) is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

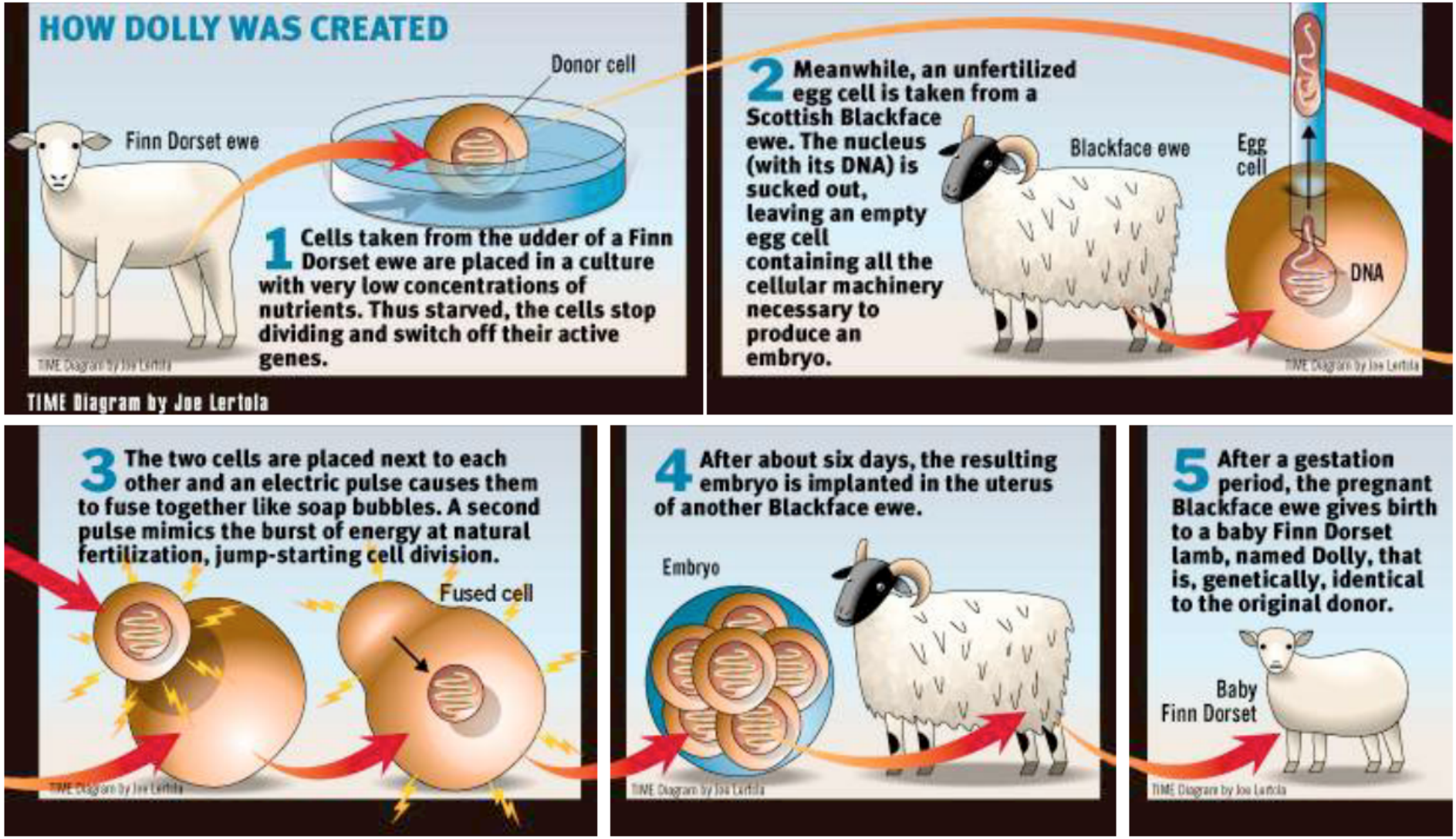
Germline DNA Demethylation Dynamics and Imprint Erasure Through 5-Hydroxymethylcytosine

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

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



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.

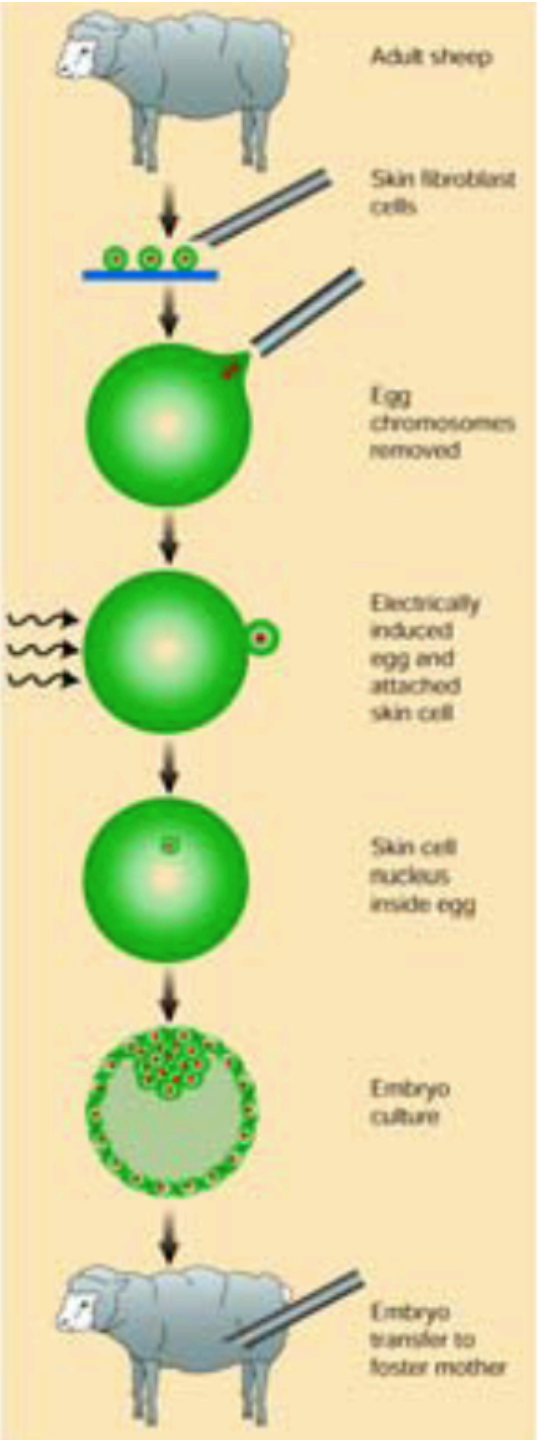
In tutti tre i casi, le cellule donatrici erano state indotte ad entrare uno stato di quiescenza replicativa (G_0) mediante riduzione della concentrazione di siero fetale bovino dal 10% a 5% per i 5 giorni precedenti il trasferimento nucleare. L'uscita dal ciclo era stata confermata mediante la ricerca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



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

Aberrant H3-K9 methylation and DNA methylation in cloned embrios

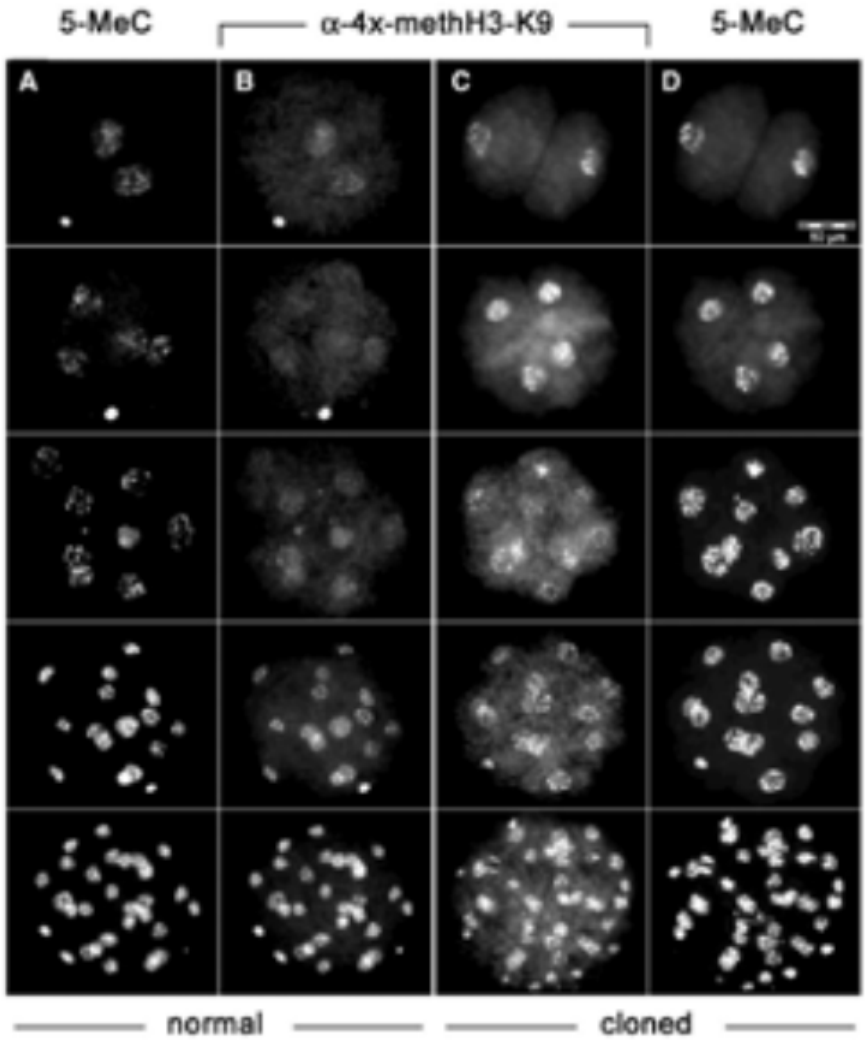


Figure 1. Epigenetic Marking Systems in Normal and Bovine Preimplantation Embryos Produced by Nuclear Transfer (NT)

Normal and NT preimplantation staged embryos were double labeled for DNA and histone H3-K9 methylation. (A) Anti-5-methyl-cytosine (5-MeC) and (B) anti-ω-4x-methH3-K9 immunofluorescence of normal bovine embryos. Staged embryos are, from top to bottom: 2 cell embryos (n >10); 4 cell embryos (n = 15); 8 cell embryos (n = 15); approximately 16 cell embryos (n >10); and morulae (n > 10). (C) Anti-ω-4x-methH3-K9 and (D) anti-5-methyl-cytosine immunofluorescence of cloned bovine embryos from fetal-fibroblast nuclear donors. Staged reconstructed embryos are from top to bottom: 2 cell embryos (n = 6/16); 4 cell embryos (n = 8/16); 8 cell embryos (n = 10/15); approximately 16 cell embryos (n = 2/6); and morulae (n = 8/14). The scale bar represents 50 μm.

Current Biology, Vol. 13, 1116–1121, July 1, 2003,

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

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

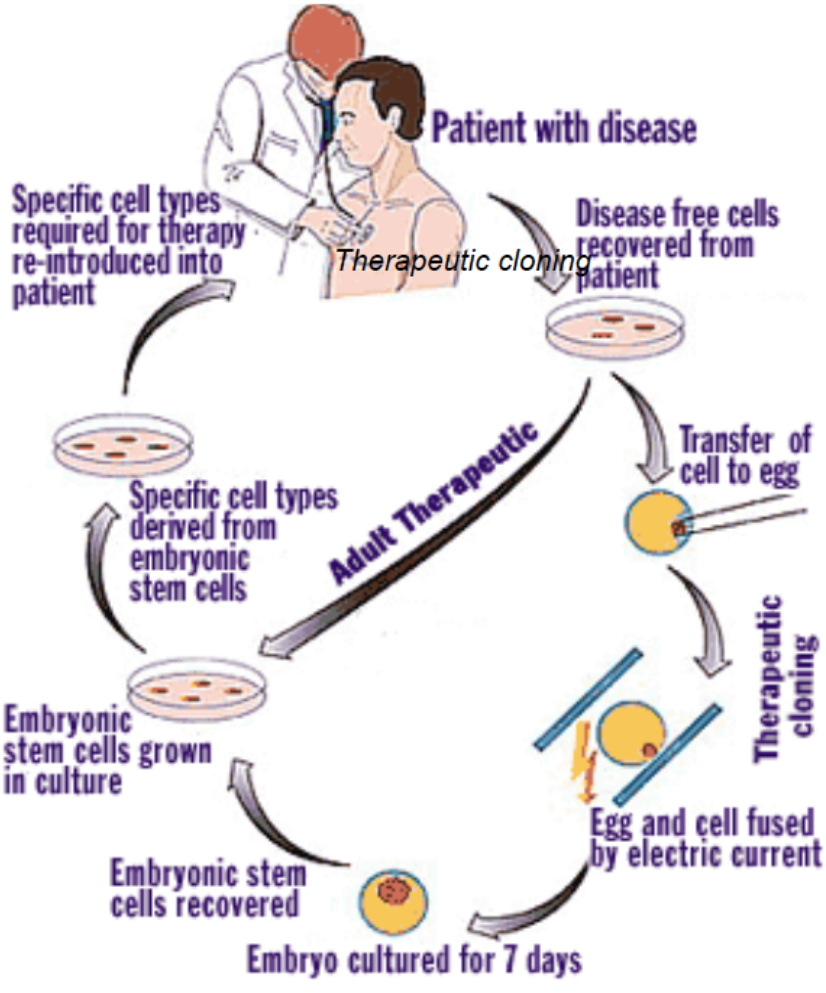
Yong-Kook Kang, Jung Sun Park, Deog-Bon Koo, Young-Hee Choi, Sun-Uk Kim, Kyung-Kwang Lee and Yong-Mahn Han[1]

Cloning by nuclear transfer (NT) has been riddled with difficulties: most clones die before birth and survivors frequently display growth abnormalities. The cross-species similarity in abnormalities observed in cloned fetuses/animals leads us to suspect the fidelity of epigenetic reprogramming of the donor genome. Here, we found that single-copy sequences, unlike satellite sequences, are demethylated in pre-implantation NT embryos. The differential demethylation pattern between genomic sequences was confirmed by analyzing single blastocysts. It suggests selective demethylation of other developmentally important genes in NT embryos. We also observed a reverse relationship between methylation levels and inner cell mass versus trophectoderm (ICM/TE) ratios, which was found to be a result of another type of differential demethylation occurring in NT blastocysts where unequal methylation was maintained between ICM and TE regions. TE-localized methylation aberrancy suggests a widespread gene dysregulation in an extra-embryonic region, thereby resulting in placental dysfunction familiar to cloned fetuses/animals. These differential demethylations among genomic sequences and between differently allocated cells produce varied overall, but specified, methylation patterns, demonstrating that epigenetic reprogramming occurs in a limited fashion in NT embryos.

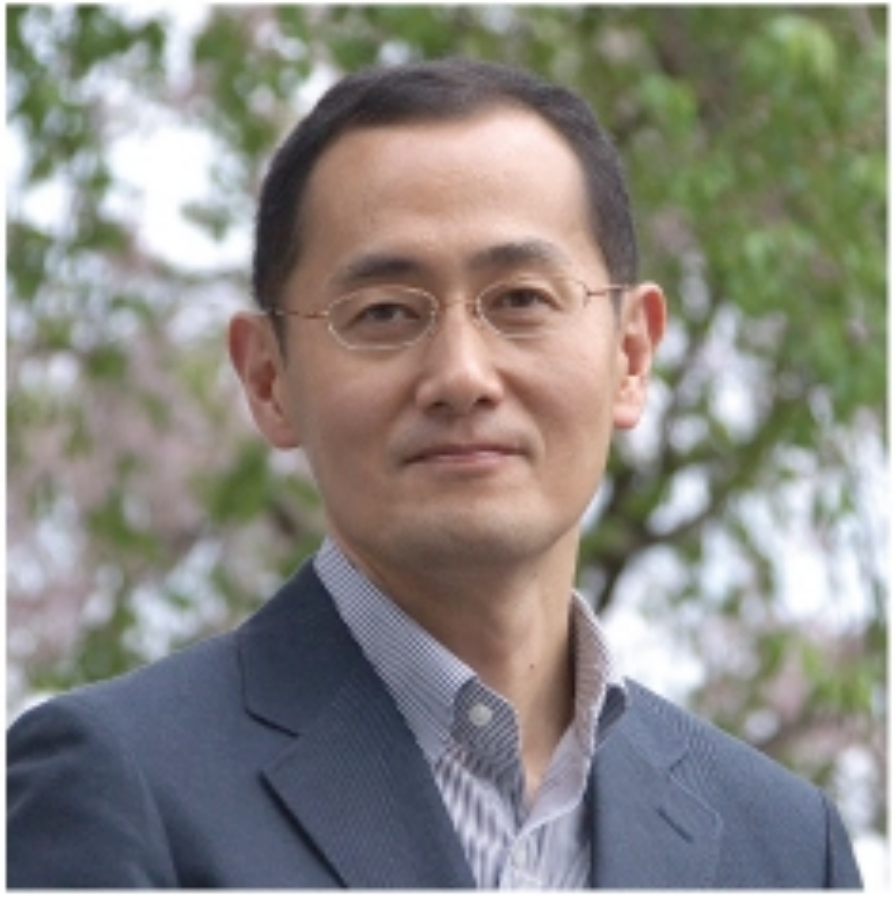
“Patient tailored therapy”

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



2012 Nobel Prize in Physiology or Medicine

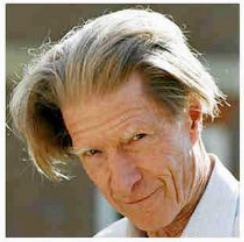


Shinya Yamanaka
University of Kyoto, Japan

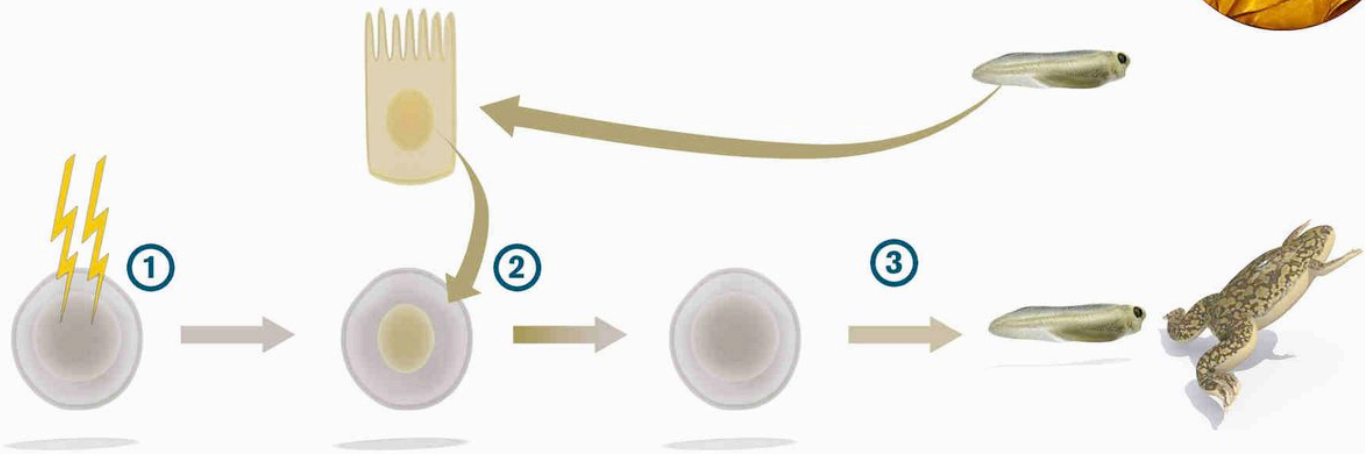
Photo Credit:
Center for iPS cell Research and Application, Kyoto University

John B. Gurdon
Gurdon Institute in Cambridge, UK

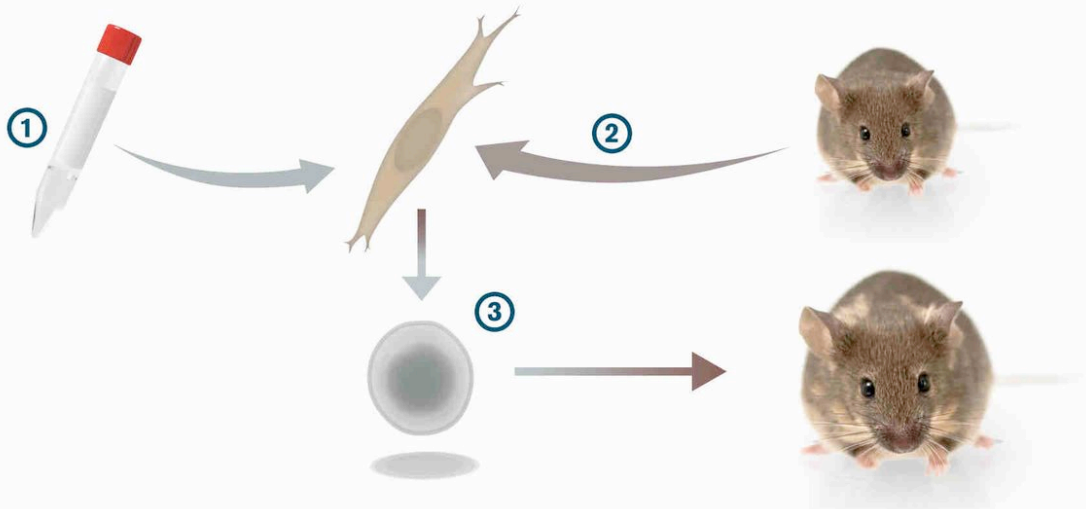
The Nobel Prize in Physiology or Medicine 2012



John B. Gurdon

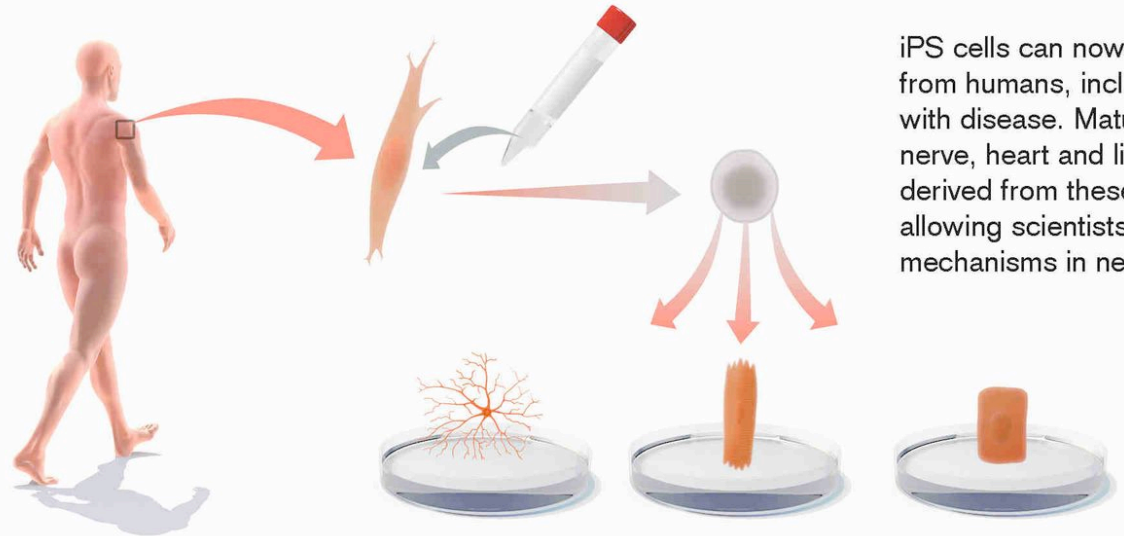


John B. Gurdon eliminated the nucleus of a frog egg cell (1) and replaced it with the nucleus from a specialised cell taken from a tadpole (2). The modified egg developed into a normal tadpole (3). Subsequent nuclear transfer experiments have generated cloned mammals (4).



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

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.

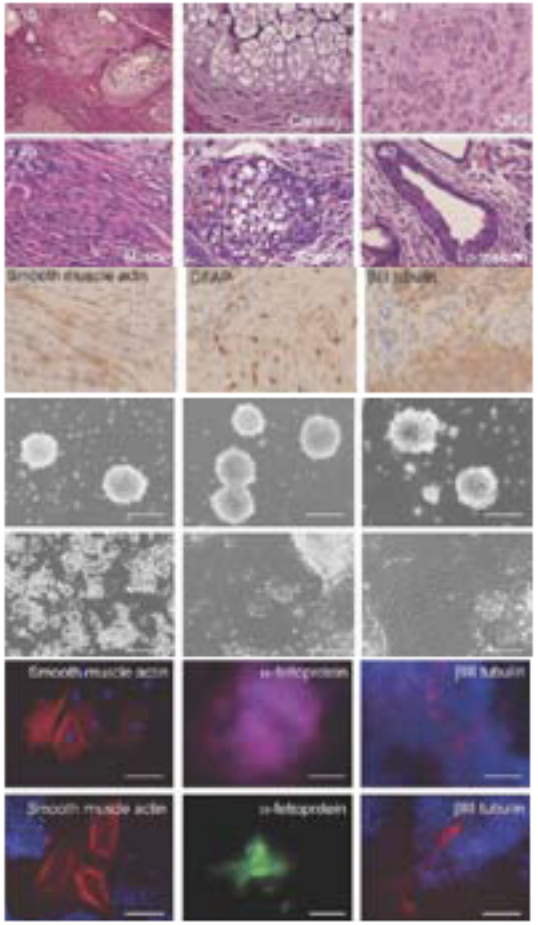
Induction of pluripotent stem cells from mouse embryonic or adult fibroblasts by introducing four factors, **Oct3/4, Sox2, c-Myc**, and **Klf4** in the FBX15 locus, under ES cell culture conditions.

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.

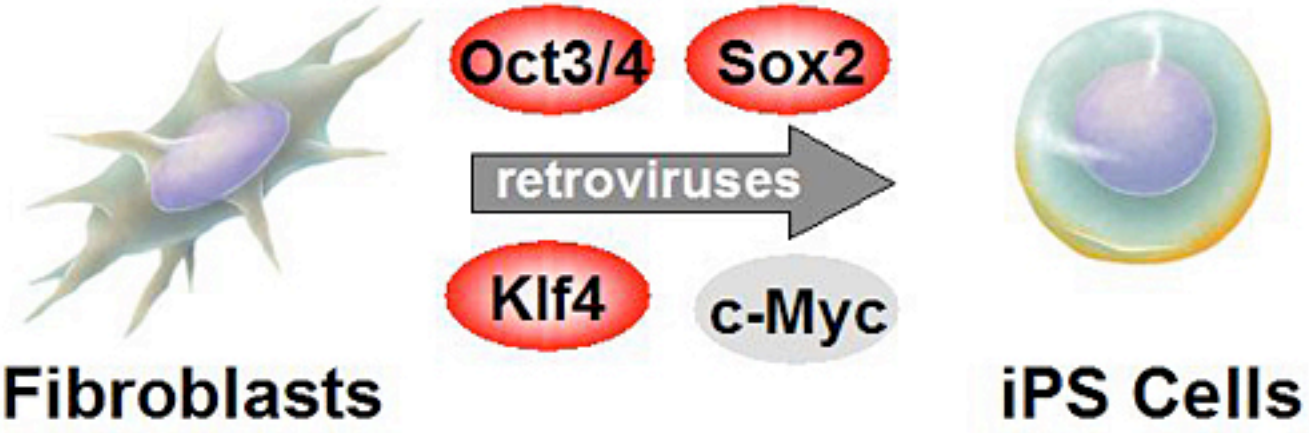


These cells, which were designated iPS (induced pluripotent stem) cells, exhibit the morphology and growth properties of ES cells and express ES cell marker genes.

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.

Induced Pluripotent Stem (iPS) Cells



Mouse iPS cells reported in 2006

Human iPS cells reported in 2007

Table 2

Minimum number of factors required for iPS cell generation

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

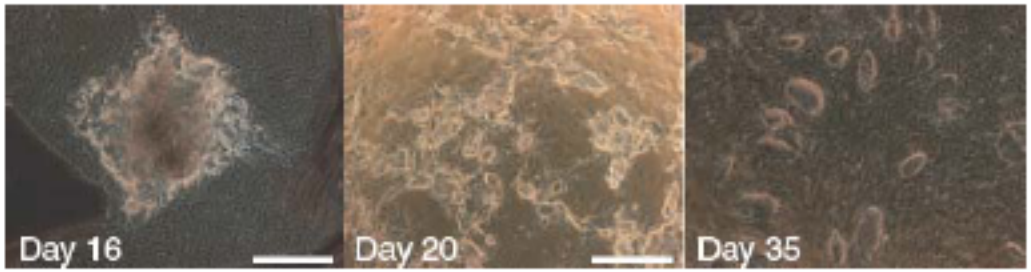
NATURE BIOTECHNOLOGY VOLUME 25 NUMBER 10 OCTOBER 2007

Direct reprogramming of genetically unmodified fibroblasts into pluripotent stem cells

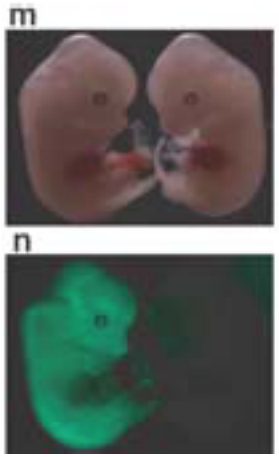
Alexander Meissner[1,3], Marius Wernig[1,3] & Rudolf Jaenisch[1,2]

In vitro reprogramming of somatic cells into a pluripotent embryonic stem cell–like state has been achieved through retroviral transduction of murine fibroblasts with Oct4, Sox2, c-myc and Klf4. In these experiments, the rare 'induced pluripotent stem' (iPS) cells were isolated by stringent selection for activation of a neomycin-resistance gene inserted into the endogenous Oct4 (also known as Pou5f1) or Nanog loci. Direct isolation of pluripotent cells from cultured somatic cells is of potential therapeutic interest, but translation to human systems would be hindered by the requirement for transgenic donors in the present iPS isolation protocol.

Here they demonstrate that reprogrammed pluripotent cells can be isolated from genetically unmodified somatic donor cells solely based upon morphological criteria.

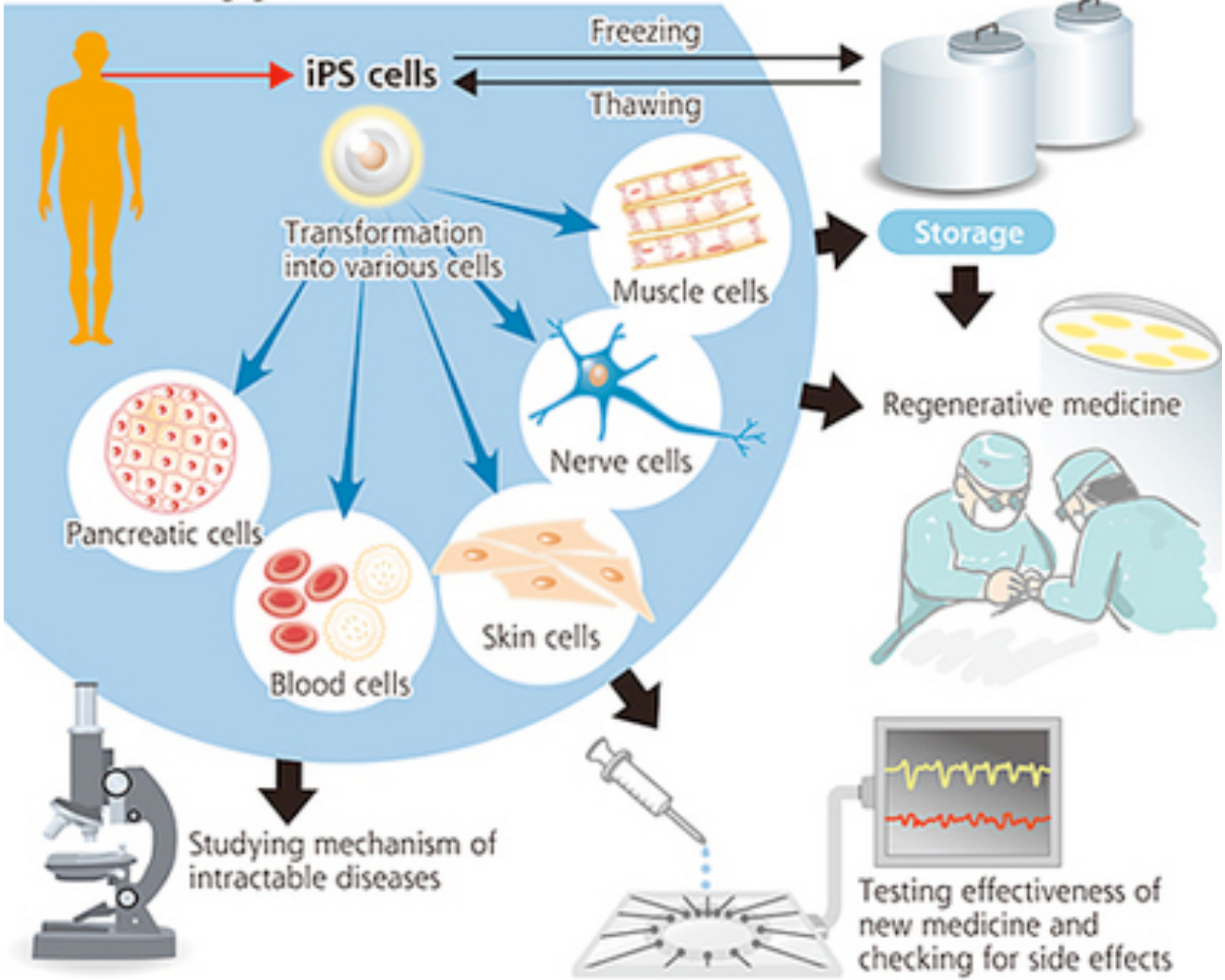


iPS cells from genetically unmodified embryonic and adult fibroblasts. (a) Primary colony 16 d after infection of wild-type F1 MEFs and wild-type adult tail-tip fibroblasts.

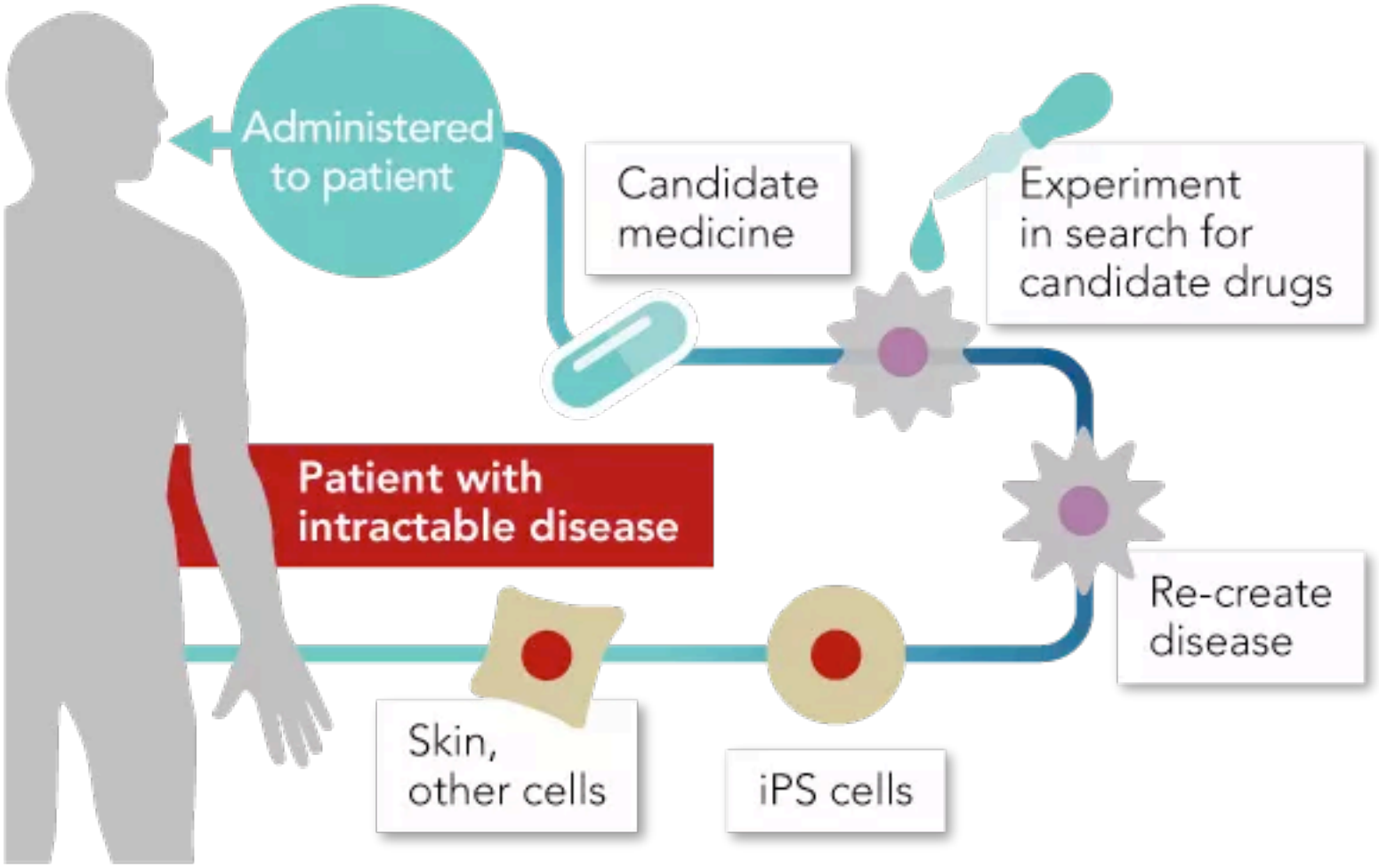


E12.5 chimera generated from the F1 MEF iPS line shown in (C) The F1 MEF iPS line was labeled with a EGFP expressing lentivirus. Shown are the same chimeras as in m. Left iPS and right control littermate. (o) Adult chimera derived from BALB/c iPS cells (white hair indicates donor contribution).

iPS cell applications

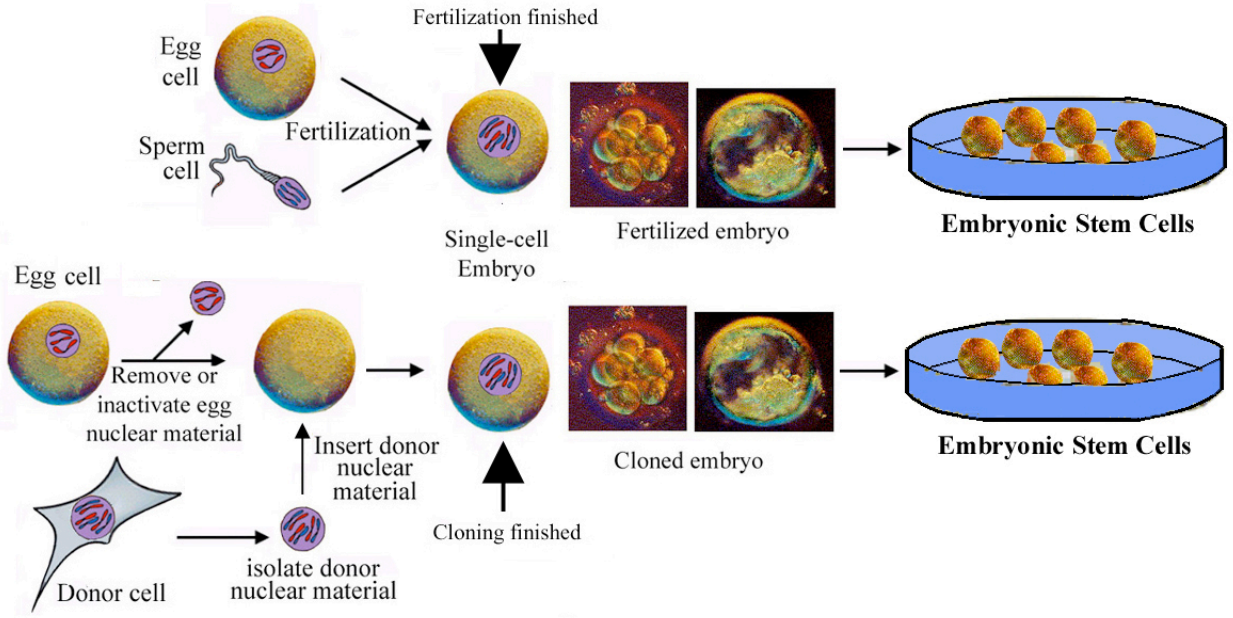


How iPS cells can help develop new drugs



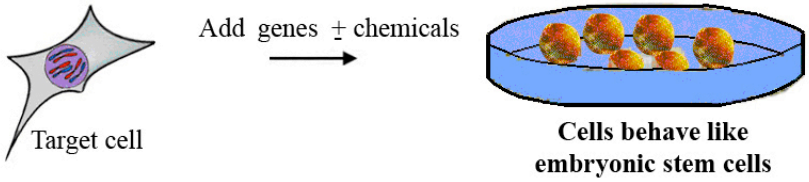
Embryonic Stem Cells

from Embryos created by Fertilization or by Cloning (Somatic Cell Nuclear Transfer)



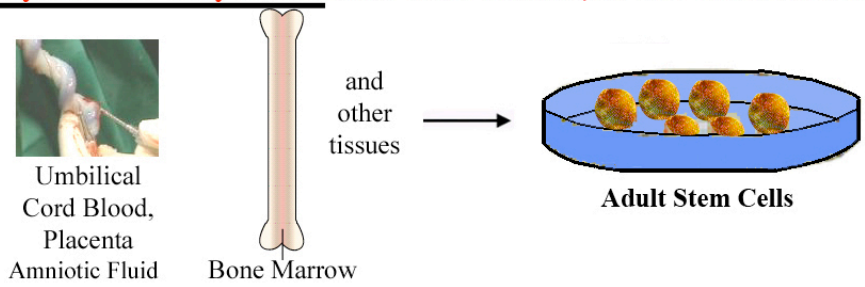
Induced Pluripotent Stem Cells (iPS cells)

from Normal Cells that are Reprogrammed to behave like Embryonic Stem Cells



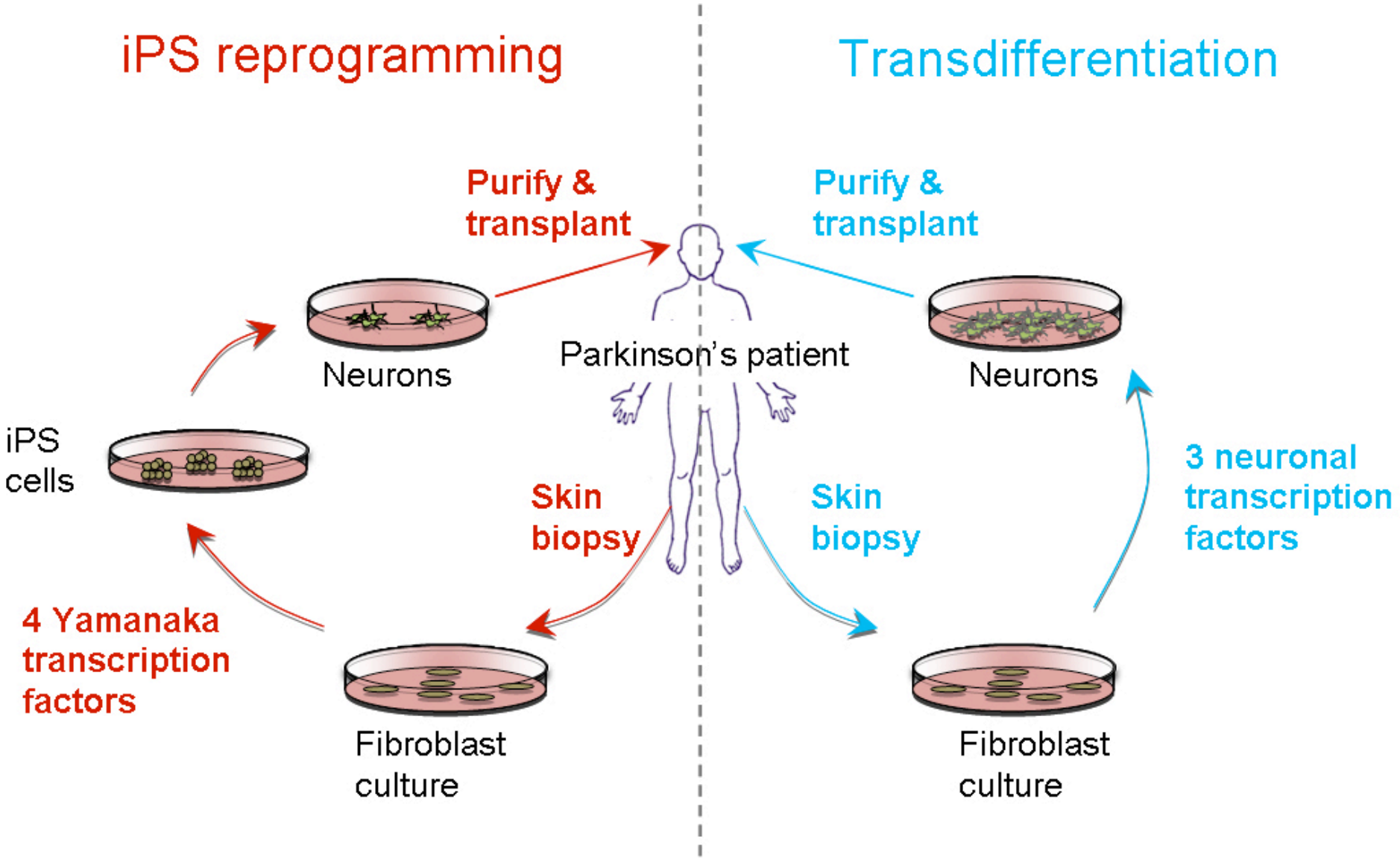
Adult Stem Cells

Stem Cells normally found in body tissues from birth onward, as well as umbilical cord, etc.



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)


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

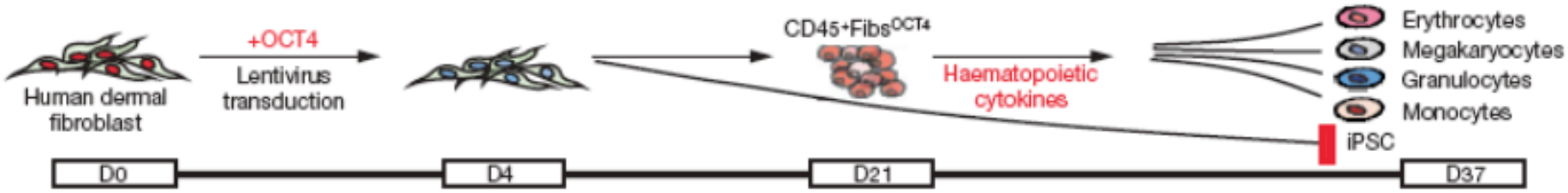
Transdifferentiation: the direct reprogramming of one somatic cell type into another without a stem cell intermediate

ARTICLE

doi:10.1038/nature09591

Direct conversion of human fibroblasts to multilineage blood progenitors

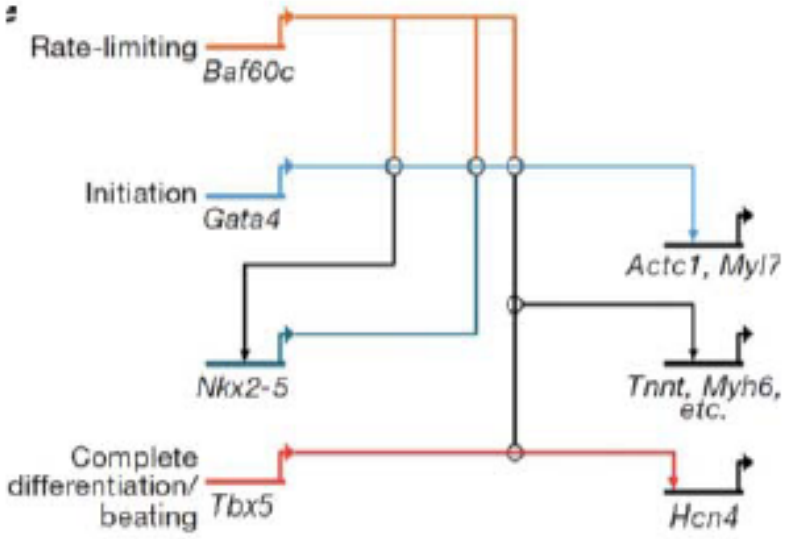
Eva Szabo[1], Shravanti Rampalli[1], Ruth M. Risueño[1], Angelique Schnerch[1,2], Ryan Mitchell[1,2], Aline Fiebig-Comyn[1], Marilyne Levadoux-Martin[1] & Mickie Bhatia[1,2]



Directed transdifferentiation of mouse mesoderm to heart tissue by defined factors

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

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



NATURE | Vol 459 | 4 June 2009

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Direct Reprogramming of Fibroblasts into Functional Cardiomyocytes by Defined Factors

Masaki Ieda,[1,2,3,6,*] Ji-Dong Fu,[1,2,3] Paul Delgado-Olguin,[1,2,4] Vasanth Vedantham,[1,5] Yohei Hayashi,[1] Benoit G. Bruneau,[1,2,4] and Deepak Srivastava[1,2,3,*]
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DOI 10.1016/j.cell.2010.07.039

ARTICLE

31 MAY 2012 | VOL 485 | NATURE | 599

doi:10.1038/nature11139

Heart repair by reprogramming non-myocytes with cardiac transcription factors

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

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

ARTICLE

doi:10.1038/nature11044

In vivo reprogramming of murine cardiac fibroblasts into induced cardiomyocytes

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

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

Circulation Research

JOURNAL OF THE AMERICAN HEART ASSOCIATION

American Heart Association. Learn and Live.

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

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

Rationale: Repopulation of the injured heart with new, functional cardiomyocytes remains a daunting challenge for cardiac regenerative medicine. An ideal therapeutic approach would involve an effective method of achieving direct conversion of injured areas to functional tissue in situ.

Objective: The aim of this study was to develop a strategy that identified and evaluated the potential of specific microRNAs (miRNAs) capable of inducing reprogramming of cardiac fibroblasts directly to cardiomyocytes in vitro and in vivo.

Methods and Results: Using a combinatorial strategy, we identified a combination of miRNAs 1, 133, 208, and 499 capable of inducing direct cellular reprogramming of fibroblasts to cardiomyocyte-like cells in vitro. Detailed studies of the reprogrammed cells demonstrated that a single transient transfection of the miRNAs can direct a switch in cell fate as documented by expression of mature cardiomyocyte markers, sarcomeric organization, and exhibition of spontaneous calcium flux characteristic of a cardiomyocyte-like phenotype. Interestingly, we also found that miRNA-mediated reprogramming was enhanced in the presence of JAK inhibitor I treatment. Importantly, administration of miRNAs into ischemic mouse myocardium resulted in evidence of direct conversion of cardiac fibroblasts to cardiomyocytes in situ. Genetic tracing analysis using Fsp1Cre-traced fibroblasts from both cardiac and noncardiac cell sources strongly suggests that induced cells are most likely of fibroblastic origin.

Conclusions: The findings from this study provide proof of concept that miRNAs have the capability of directly converting fibroblasts to a cardiomyocyte-like phenotype in vitro. Also of significance is that this is the first report of direct cardiac reprogramming in vivo. Our approach may have broad and important implications for therapeutic tissue regeneration in general. (*Circ Res.* 2012;110:1465-1473.)

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

Direct conversion of human fibroblasts to dopaminergic neurons

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

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

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

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

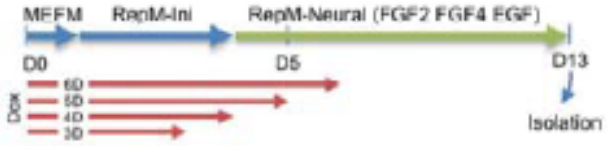
Direct reprogramming of mouse fibroblasts to neural progenitors

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

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

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

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



Direct conversion of fibroblasts to functional neurons by defined factors

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

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

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

Forcing cells to change lineages

Thomas Graf[1] & Tariq Enver[2]

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

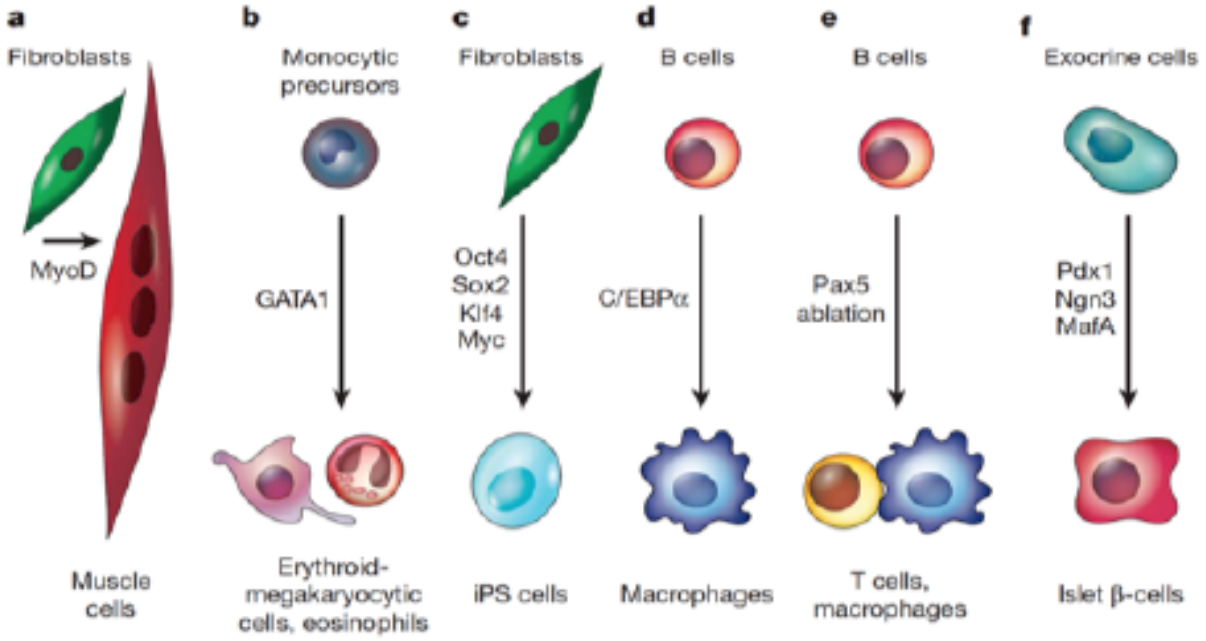


Figure 1 | Examples of transcription factor overexpression or ablation experiments that result in cell fate changes. For explanation of panels **a–f** see text.

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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

| Nature | Vol 588 | 3 December 2020

Reprogramming to recover youthful epigenetic information and restore vision

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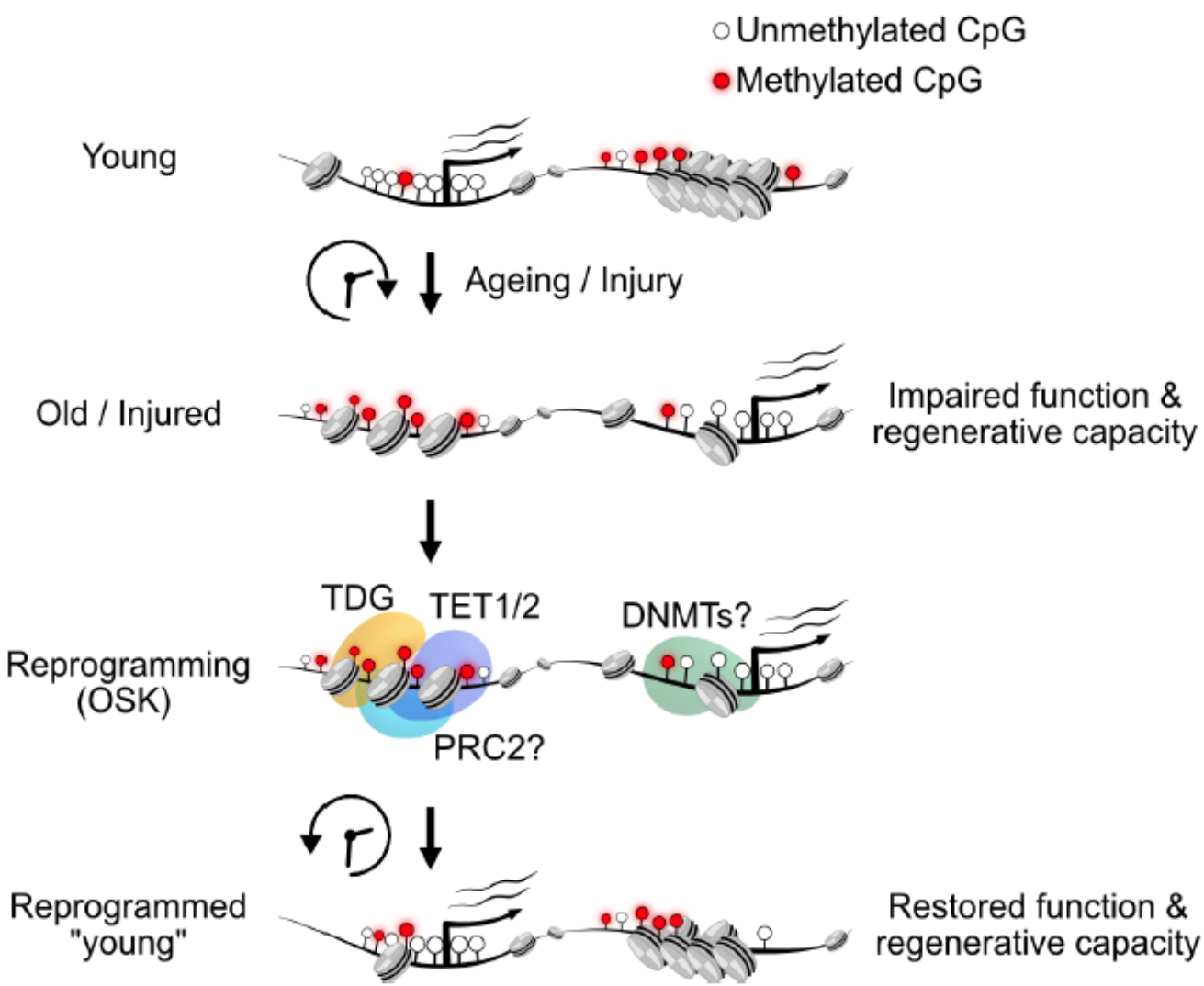
Check for updates

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.