Epigenetics
~from mechanism to therapy~

Literature seminar

January 31, 2012

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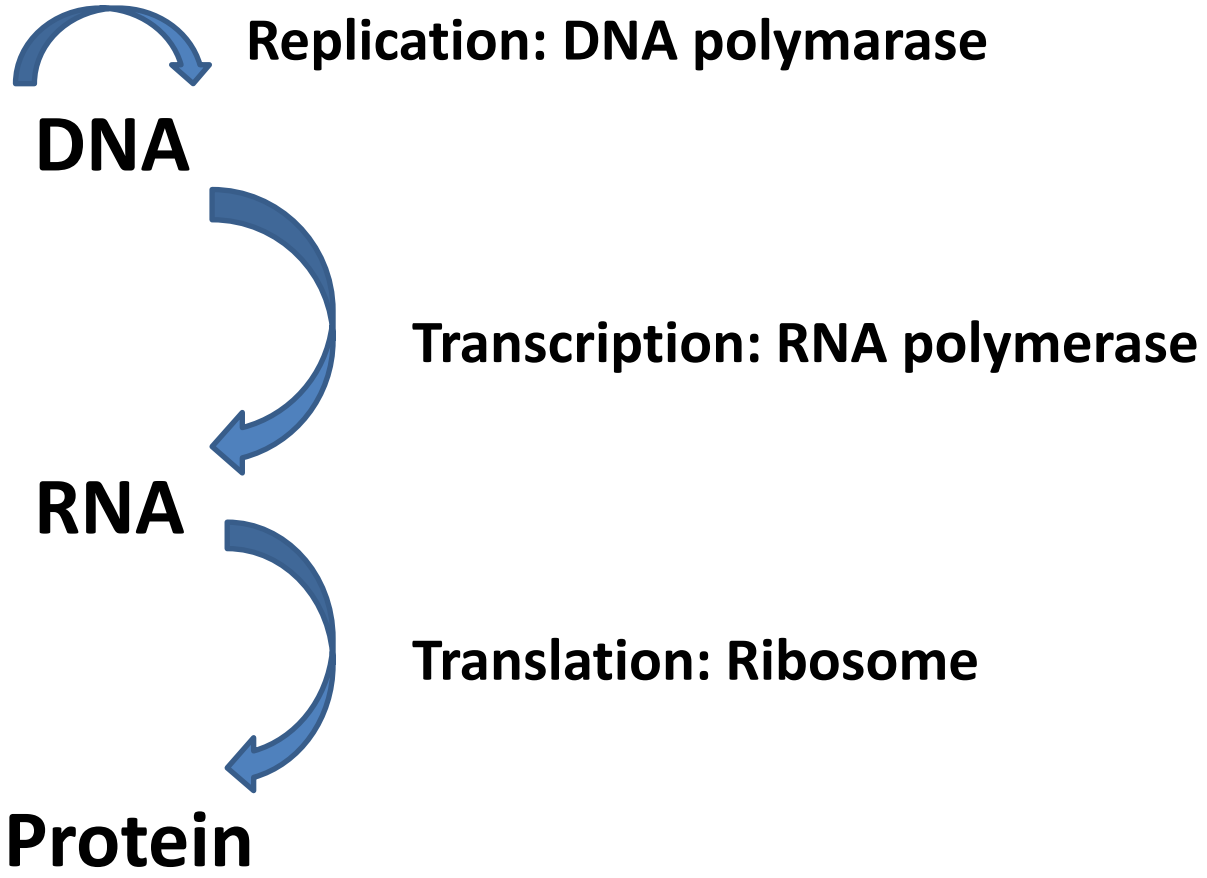
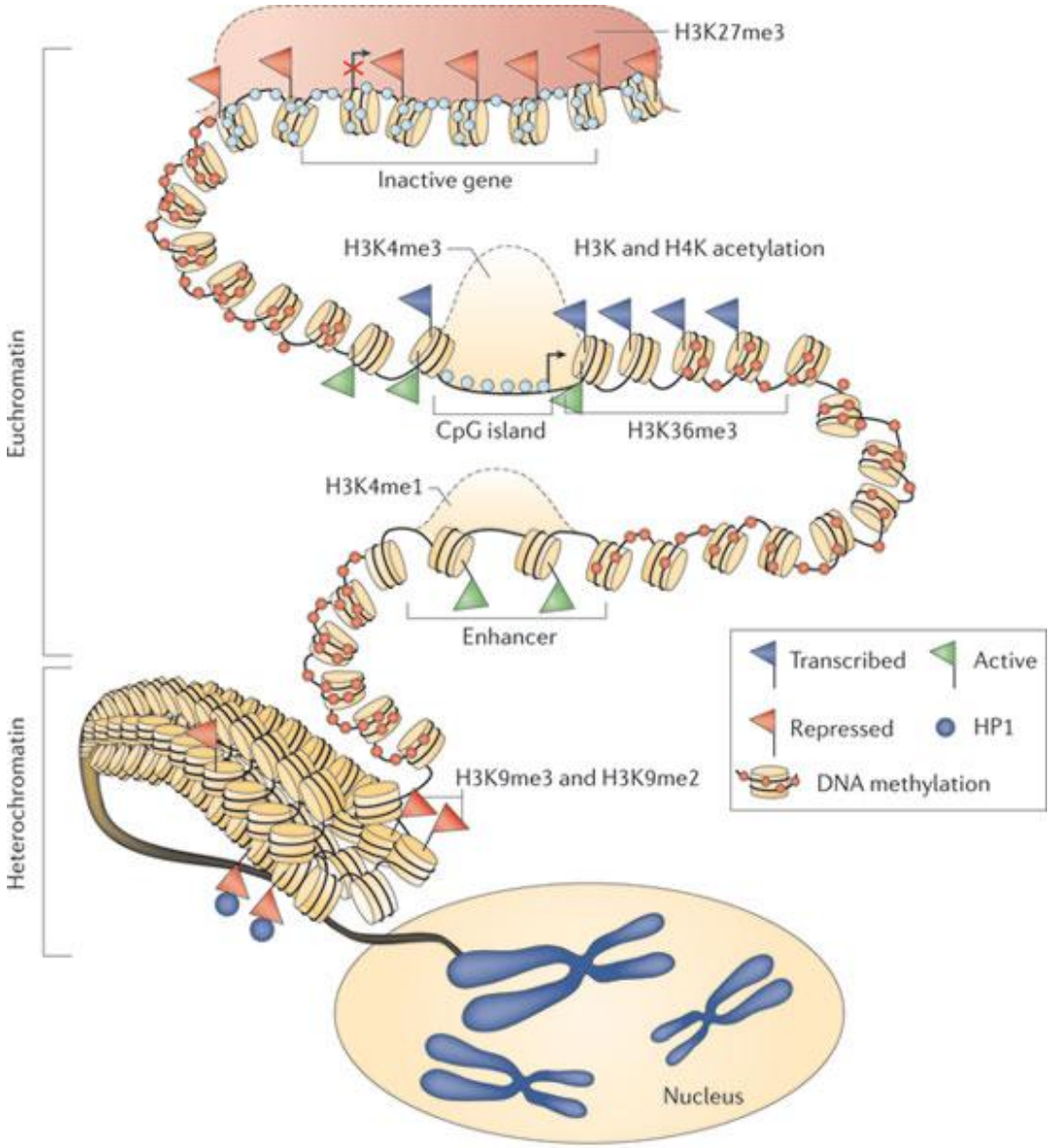
2. Histone modification
3. Epigenetic modifications and disease
4. Epigenetic therapy of cancer
5. Perspective

Introduction

Central Dogma



Chromatin structure



Stephen B. Baylin *et al.*
Nature Reviews Cancer , 2011,*11*, 726-734

What is the epigenetics?

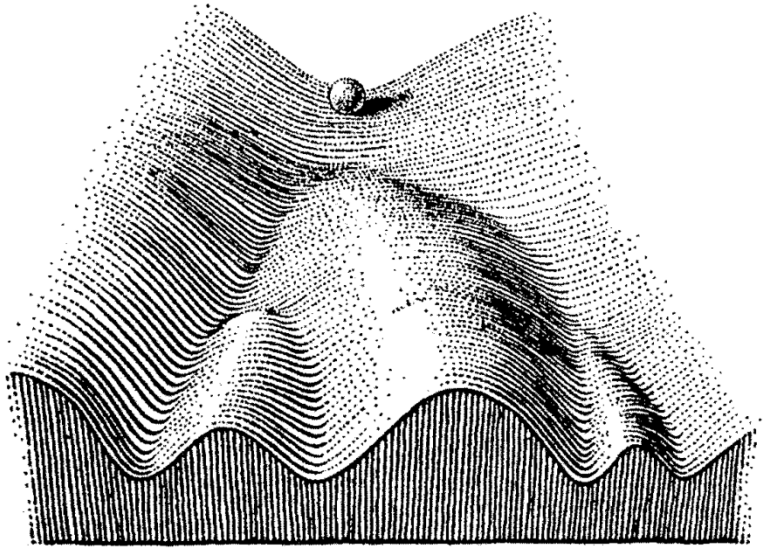


FIGURE 1. Waddington's epigenetic landscape. (Reproduced from Waddington,[5] p. 29, with permission from Taylor & Francis, London.)

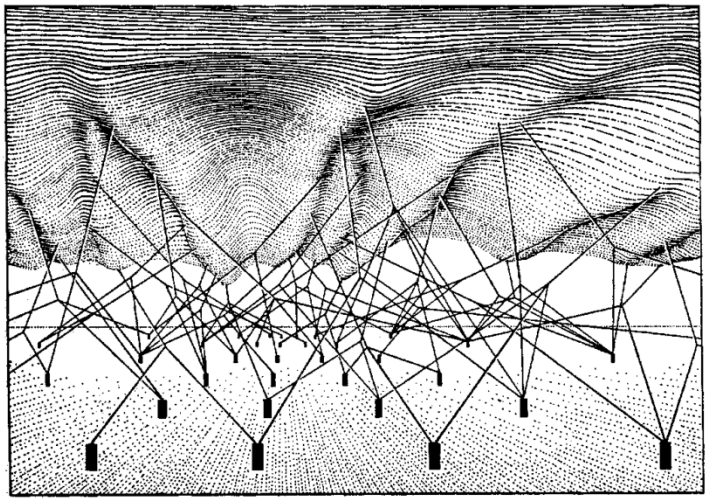


FIGURE 2. The interactions underlying the epigenetic landscape. (Reproduced from Waddington,[5] p. 36, with permission from Taylor & Francis, London.)

Waddingtonian epigenetics(1940s～) was located at the junction of genetics, developmental Biology, and ecology, all of which were rooted in evolutionary biology.

Holliday's definition

"The study of the changes in gene expression, which occur in organisms with differentiated cells, and the mitotic inheritance of given patterns of gene expression."

"Nuclear inheritance which is not based on differences in DNA sequence."

Epigenetic modification

- DNA modifications
- Histone modifications
- Nucleosome positioning

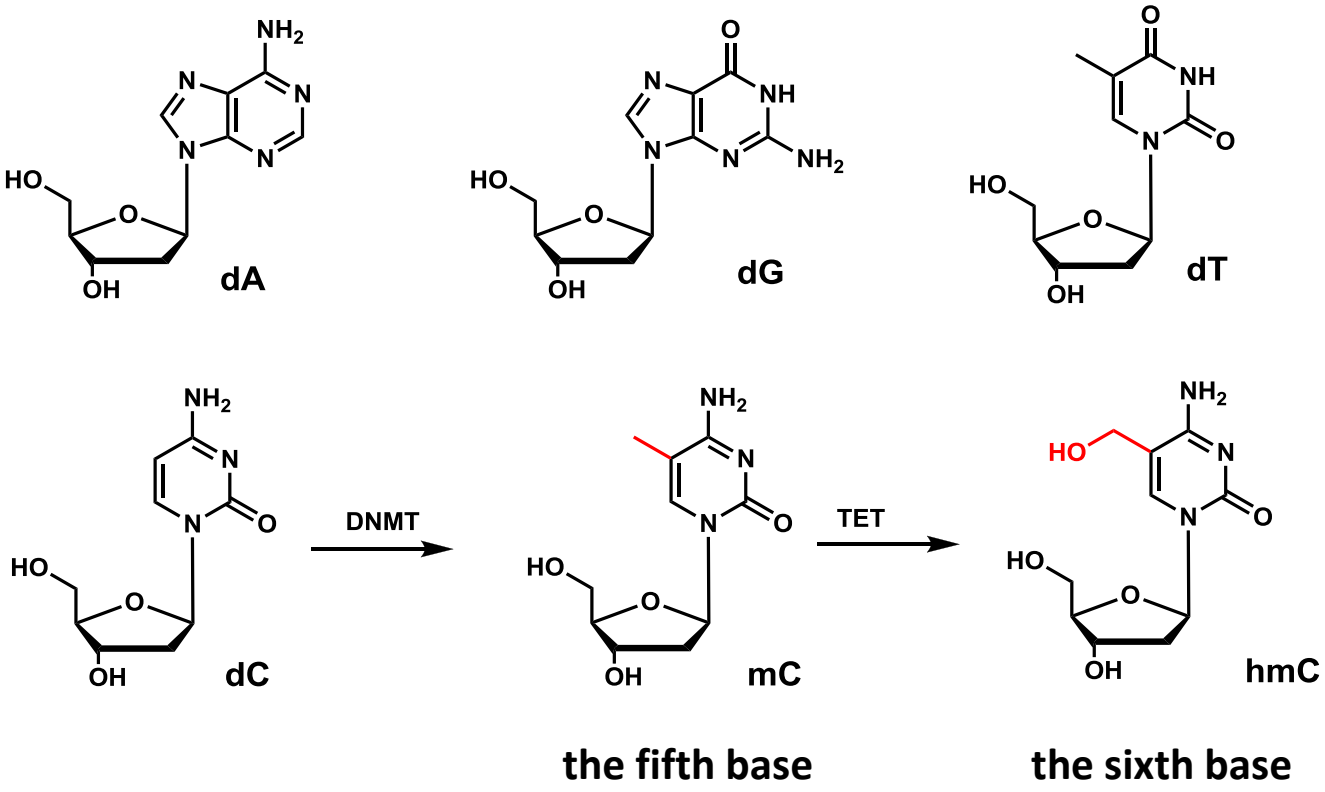
regulate

- Gene and microRNA expression
- DNA-protein interactions
- suppression of transposable element mobility
- cellular differentiation
- embryogenesis
- X-chromosome inactivation
- genomic imprinting etc.

Topics

1. **DNA methylation and demethylation**
2. Histone modification
3. Epigenetic modifications and disease
4. Epigenetic therapy of cancer
5. Perspective

DNA methylation: What is methylated?



The canonical DNA nucleosides : dA, dG, dT, dC
Cytosine can be modified to mC and hmC in mammalian tissues.
DNMT : DNA methyltransferase
TET enzyme: ten eleven translocation enzyme

Where is 5-methylcytosine?

- DNA methylation mainly occurs in the context of CpG dinucleotides.
- 60～90% of CpG is methylated in mammals (corresponding to 3～8% of all cytosine residues).

➢ The remaining unmethylated CpG dinucleotides are mostly found near gene promoters in dense clusters known as "CpG islands".

➢ The definition of CpG island is "a sequence of more than 200 bases with a C+G content of at least 50% and a ratio of observed to statistically expected CpG frequencies of at least 0.6. (CpG dinucleotides are usually quite rare in mammalian genomes (～1%))

➢ About 60% of human gene promoters are associated with CpG islands and are usually unmethylated in normal cells, although some of them (～6%) become methylated in a tissue-specific manner during early development or in differentiated tissues.

✓ **Unmethylated CpG islands at promoters of genes allow transcription.**

The function of DNA methylation

- Methylated cytosine can block binding of transcriptional factors.
- Methylated cytosine can promote the recruitment of methyl-CpG-binding domain (MBD) proteins.
- MBD family members recruit histone-modifying and chromatin-remodeling complexes to methylated sites.

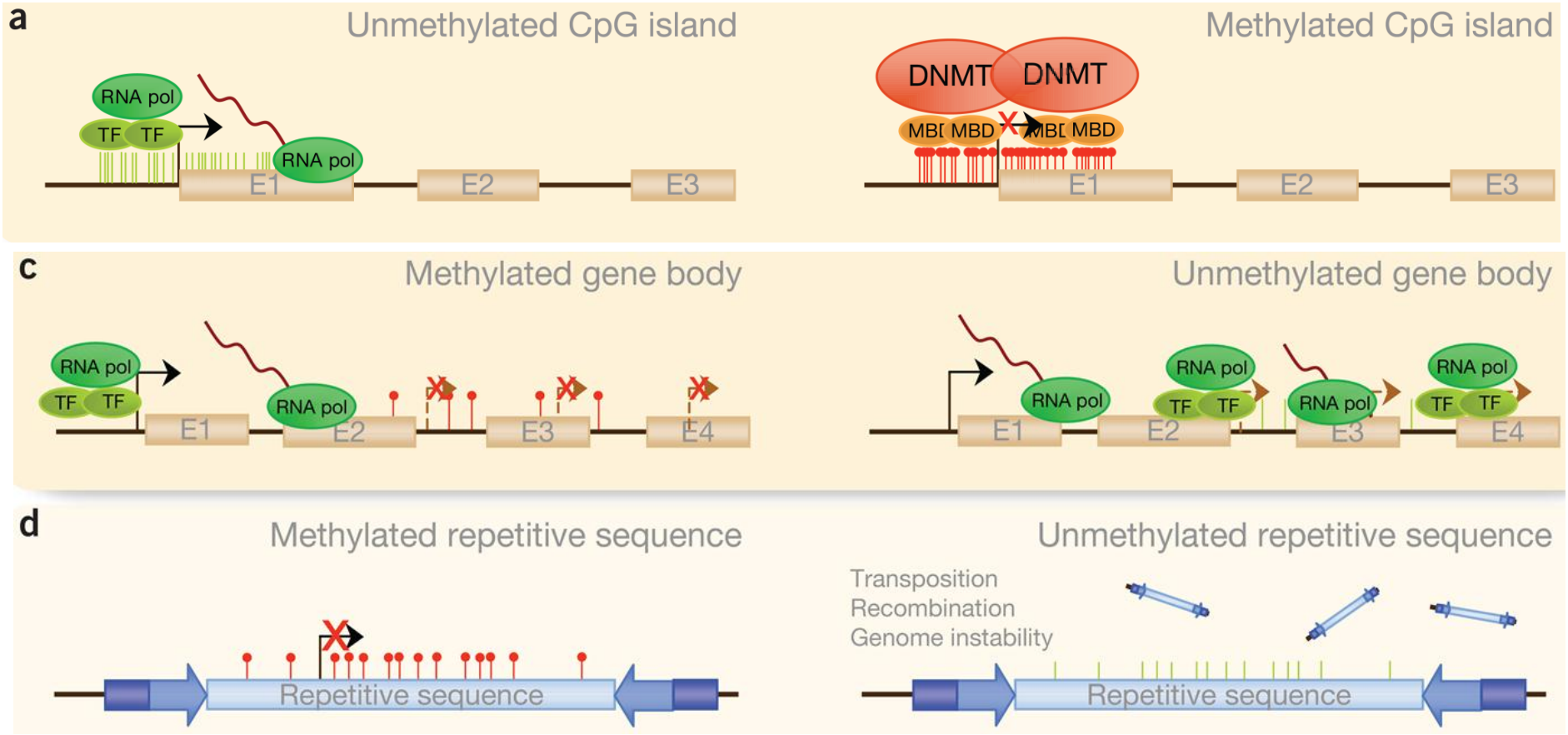
- In general, methylation of CpG islands is associated with gene silencing.

*Genomic imprinting

Hypermethylation at one of the two parallel alleles leads to monoallelic expression.

A similar gene-dosage reduction is observed in X-chromosome inactivation in females.

DNA methylation patterns



Anna Portela *et al.Nature Biotechnology,*2010, *28*, 1057–1068.

The types of methylation

- ***De novo* methylation**

The DNA methylation pattern is largely established during early embryogenesis, at around the time of implantation.

De novo DNMTs: DNMT3A, DNMT3B, DNMT3L(non-catalytic paralogue)

*DNMT3A and DNMT3B may participate in maintenance methylaion.

- **Maintenance methylation**

Global DNA metylation patterns must be stably maintained during replication to ensure that transposons remain in a silenced state and to preserve cell type identity.

Maintenance DNMT: DNMT1

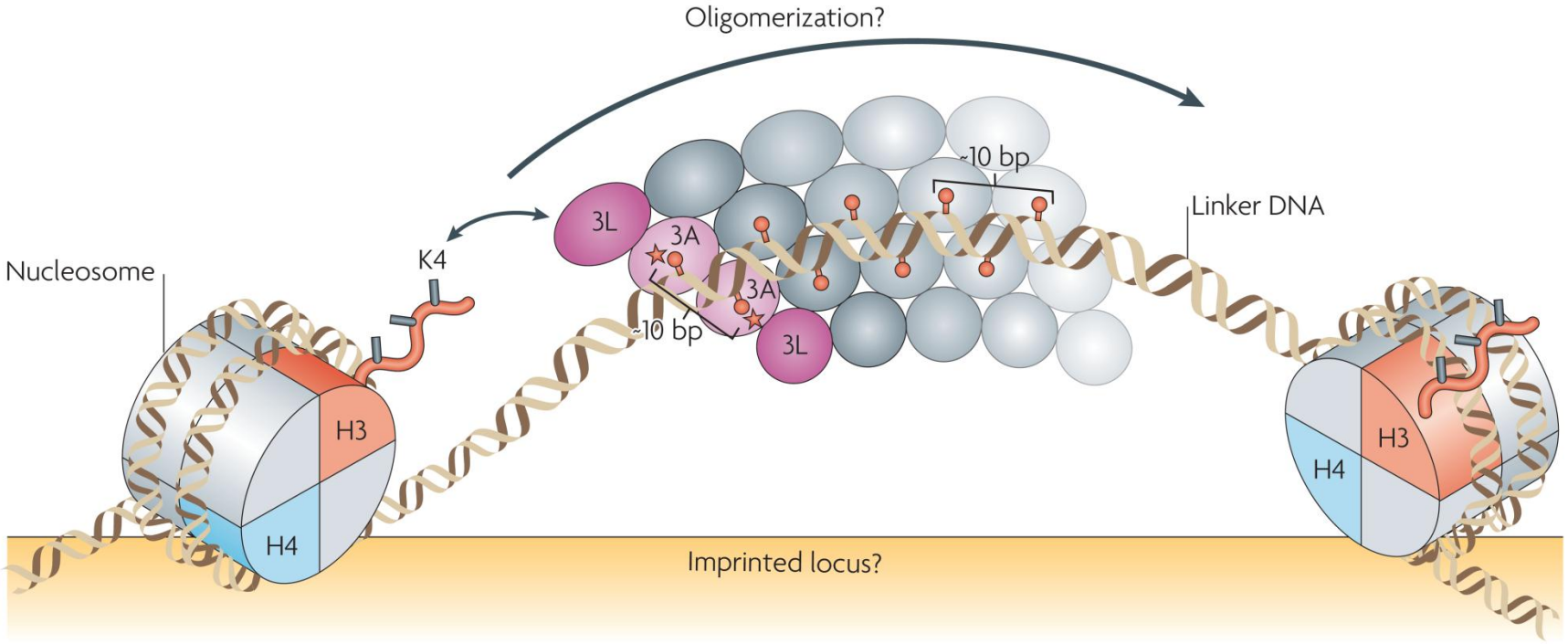
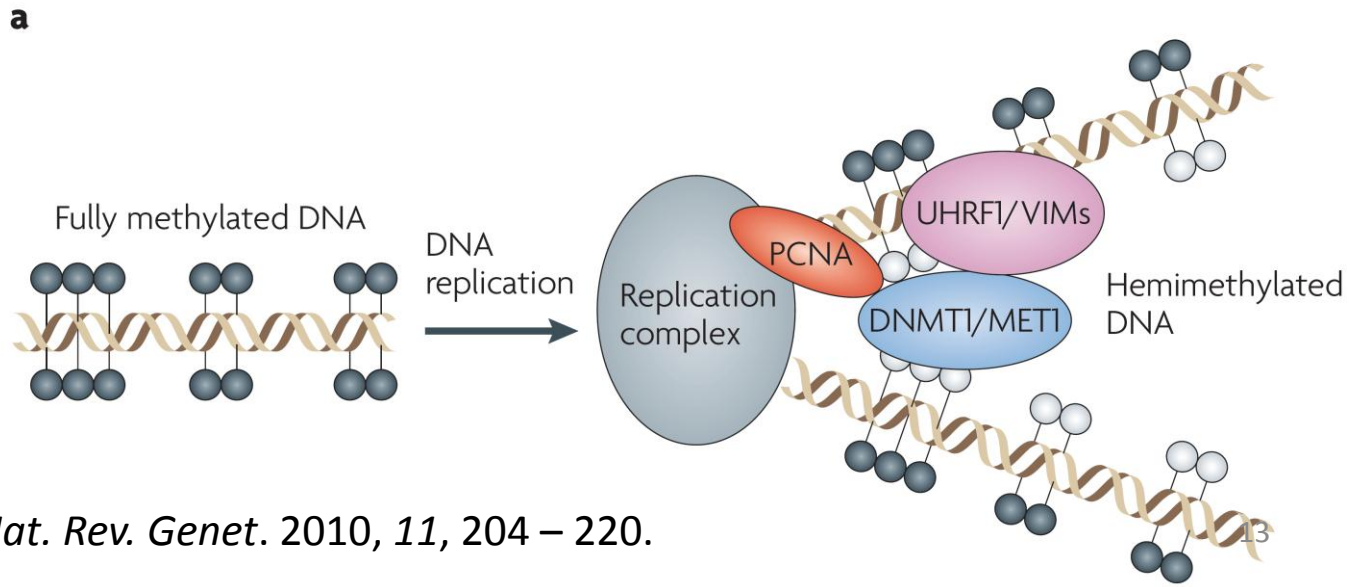


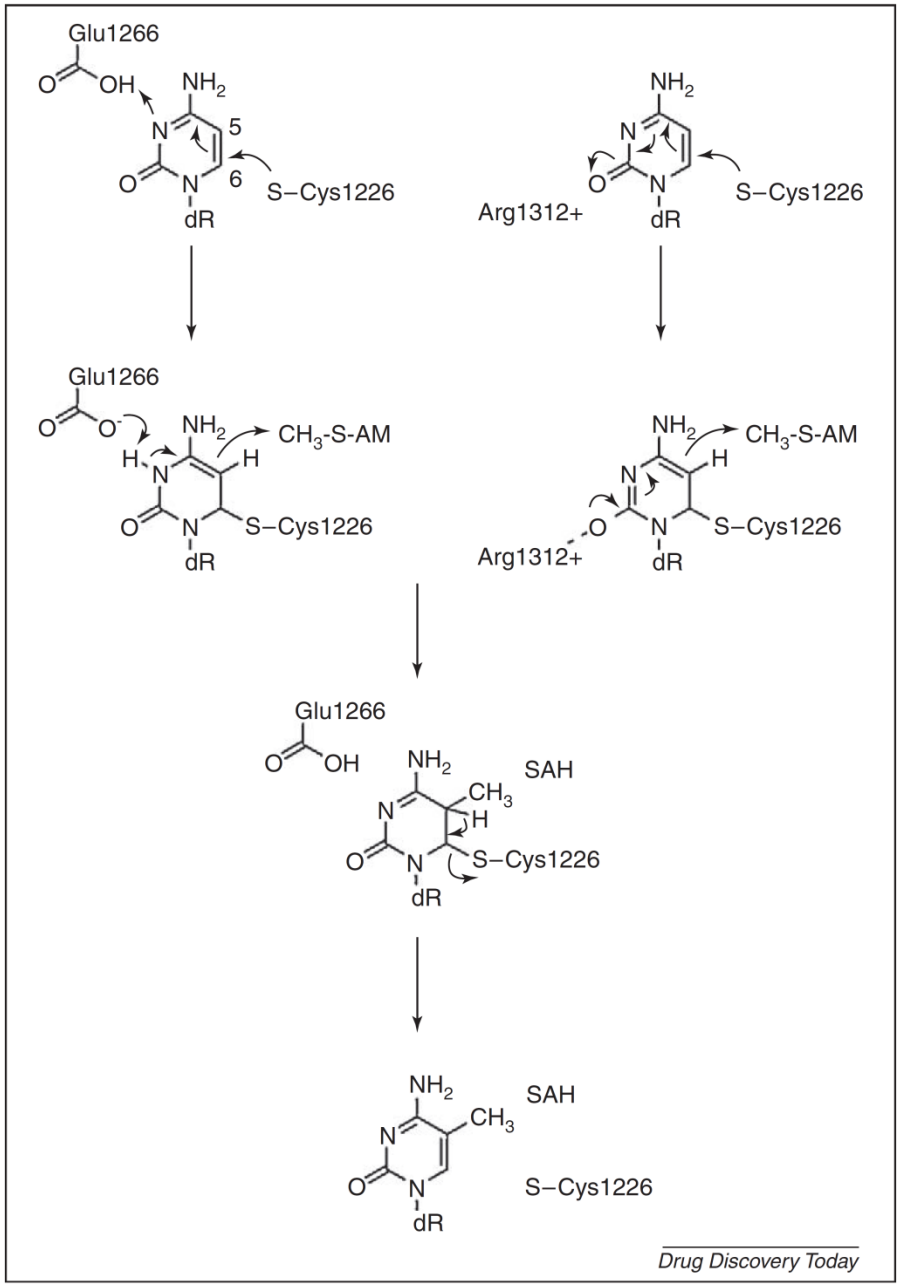
Figure 3 | **Model of recruitment of the *de novo* methylation machinery by unmethylated histone 3 lysine 4 tails.**

Maintenance of DNA methylation



J. A. Law, S. E. Jacobsen, *Nat. Rev. Genet*. 2010, *11*, 204 – 220.

the mechanism of methylation



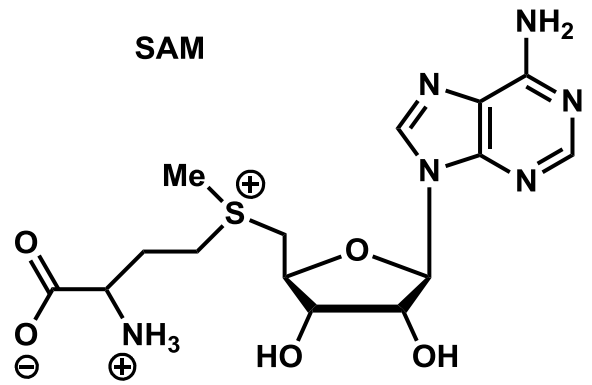


FIGURE 2

Mechanism of DNA methylation. After DNMT forms a complex with DNA and the cytosine that will be methylated flips out of the DNA, the thiol of the catalytic cysteine acts as a nucleophile that attacks the 6-position of the target cytosine to generate a covalent intermediate. The 5-position of the cytosine is activated and performs a nucleophilic attack on the methyl group of SAM to form the 5-methyl covalent adduct and SAH. The attack on the 6-position is assisted by transient protonation of the cytosine ring at the endocyclic nitrogen atom N3, which is stabilized by a glutamate residue. The carbanion might also be stabilized by resonance, where an arginine residue might participate in the stabilization. The covalent complex between the methylated base and the DNA is resolved by deprotonation at the 5-position to generate the methylated cytosine and the free enzyme. The 5-methylated cytosine base then flips back into its original position within the DNA.

José L. Medina-Franco and Thomas Caulfield

Drug Discovery Today, 2011, *16*, 418-425.

Summary of DNA methylation

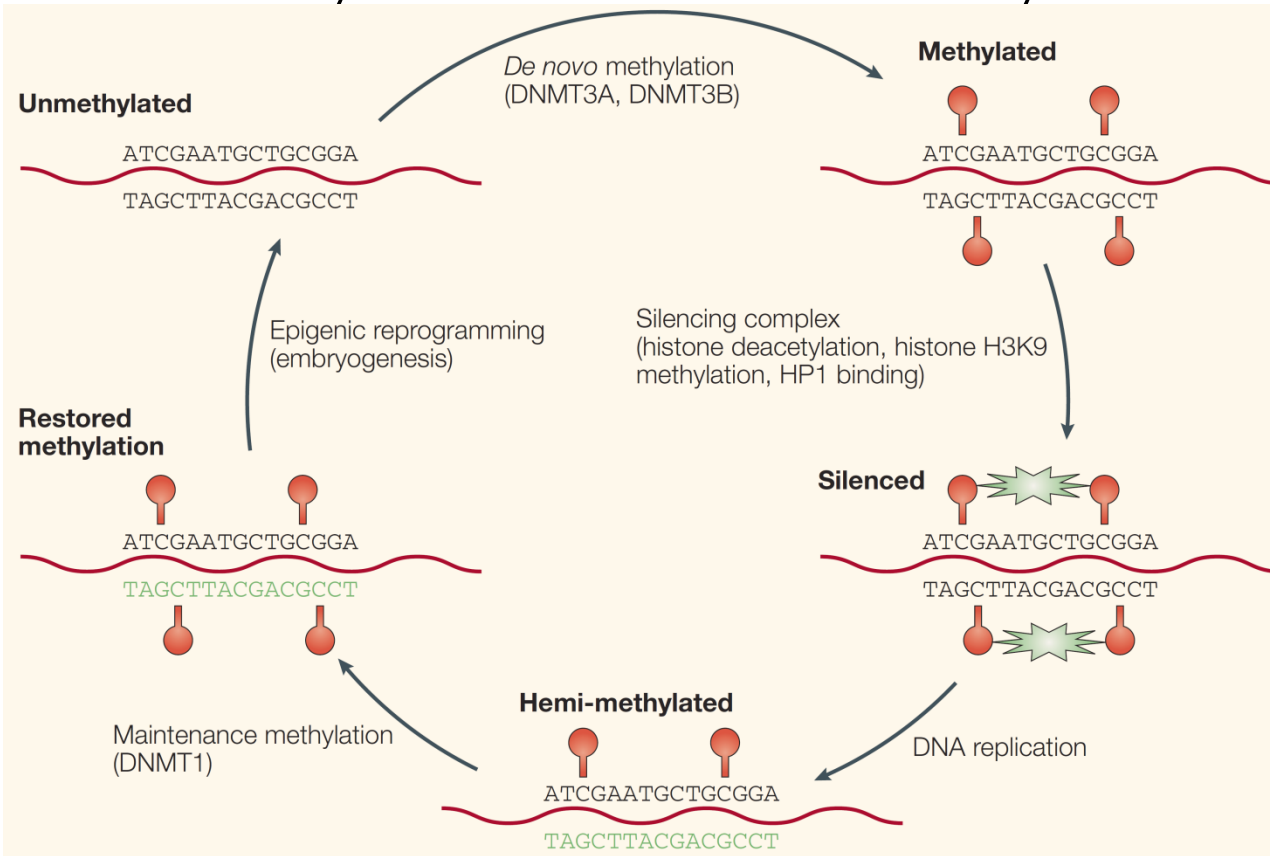
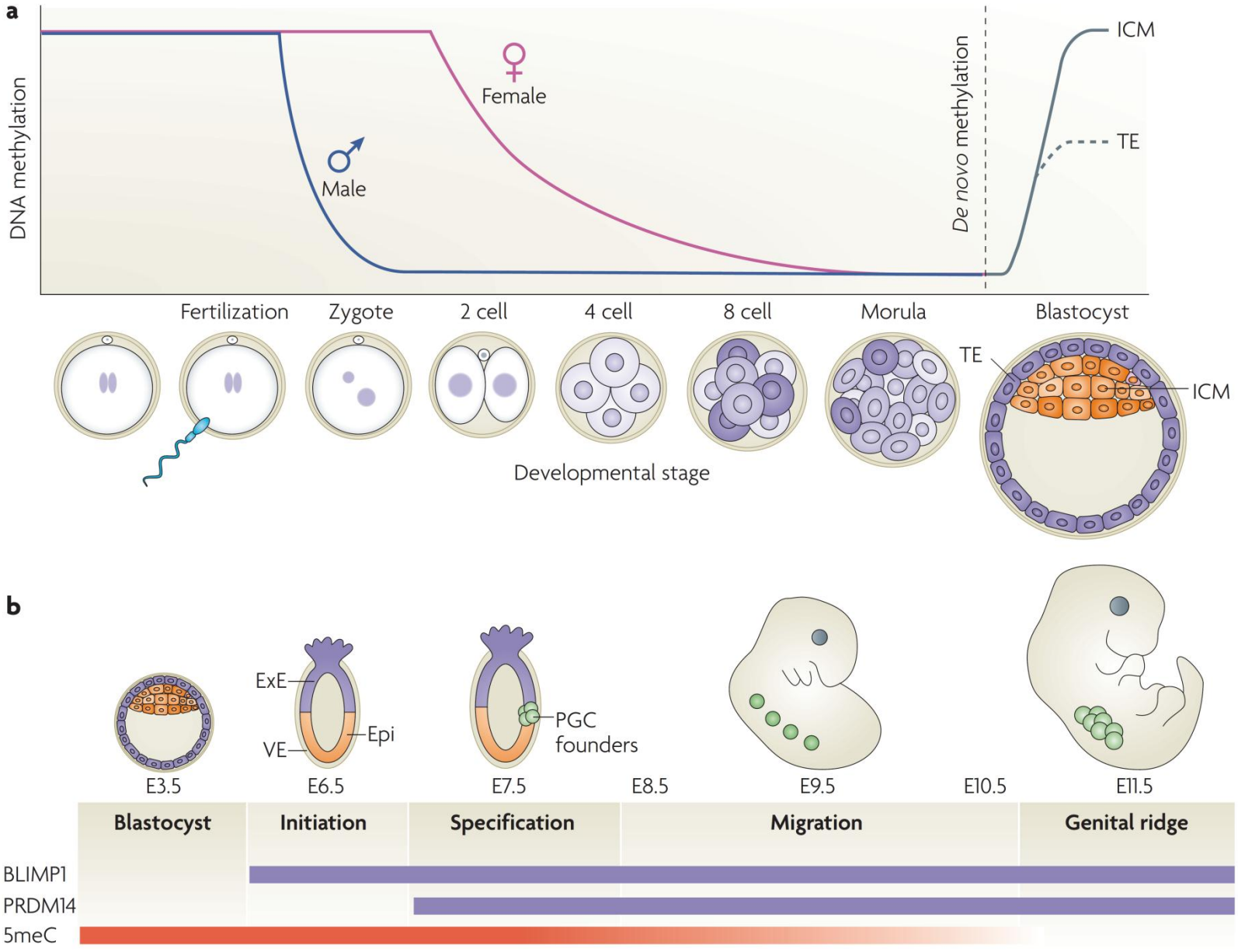


Figure 1 | **DNA methylation and gene silencing.** In early embryogenesis, DNA is largely devoid of methylation (top left). Post implantation, *de novo* methylation begins (red circles), mediated primarily by DNA (cytosine-5-)-methyltransferase-3α (DNMT3A) and -3β (DNMT3B) (top). When methylation affects CpG islands, methyl-binding proteins trigger a silencing cascade (activity illustrated by green stars) whereby histone H3K9 is sequentially deaceylated and then methylated, allowing heterochromatin protein 1 (HP1) to bind; eventually resulting in closed chromatin (bottom right). After DNA replication, newly synthesized DNA (in green) is unmethylated. However, DNMT1 rapidly scans DNA and deposits methyl groups on newly synthesized DNA, opposite methyl groups present on the old DNA strand. This results in faithful replication of methylation patterns (bottom left) and the maintenance of silencing. Adult patterns of methylation are erased by epigenetic reprogramming in early embryogenesis (top left).

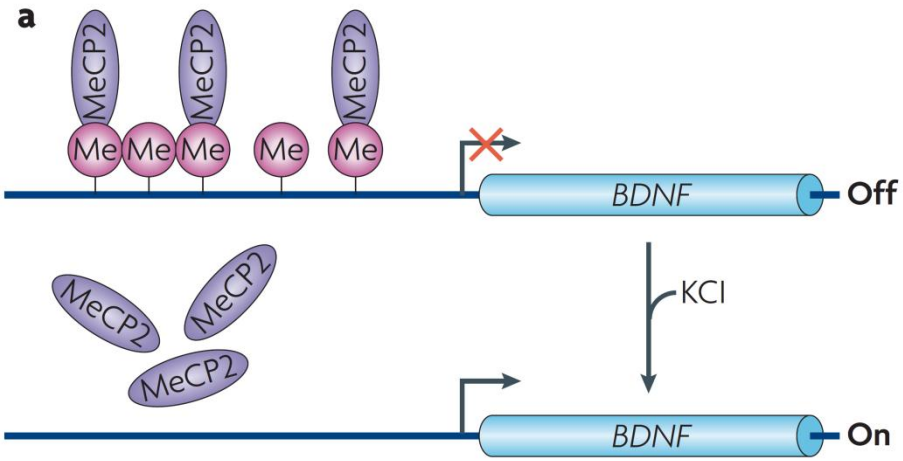
Randy L. Jirtle , Michael K. Skinner, *Nat. Rev. Genet*. 2007, *8*, 253 – 262.

DNA demethylation during development

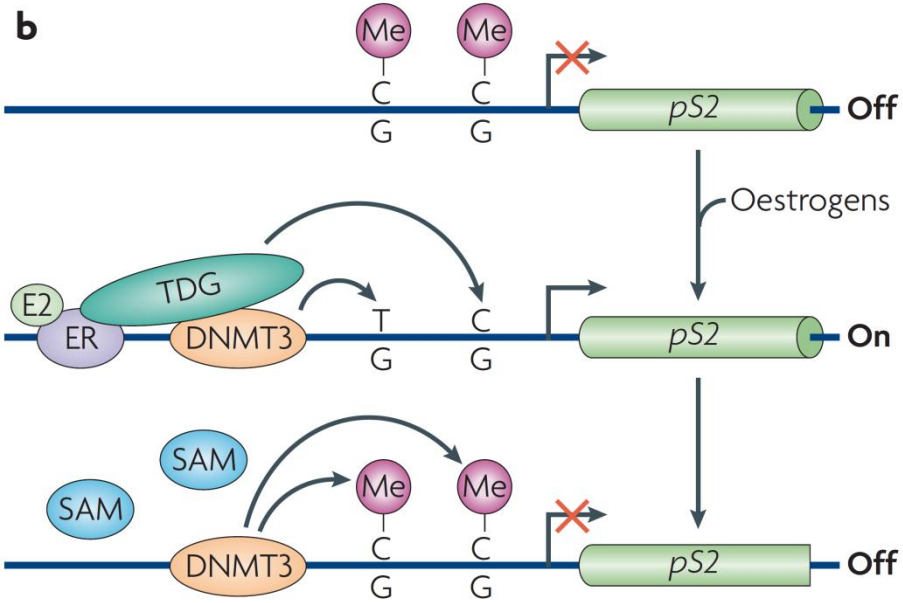


S. C. Wu, Y. Zhang, *Nat. Rev. Mol. Cell Biol*. 2010, *11*, 607 –620.

Locus-specific active demethylation in somatic cells



Active demethylation at the brain-derived neurotrophic factor (*BDNF*) promoter.



Active demethylation at nuclear receptor Target promoters.

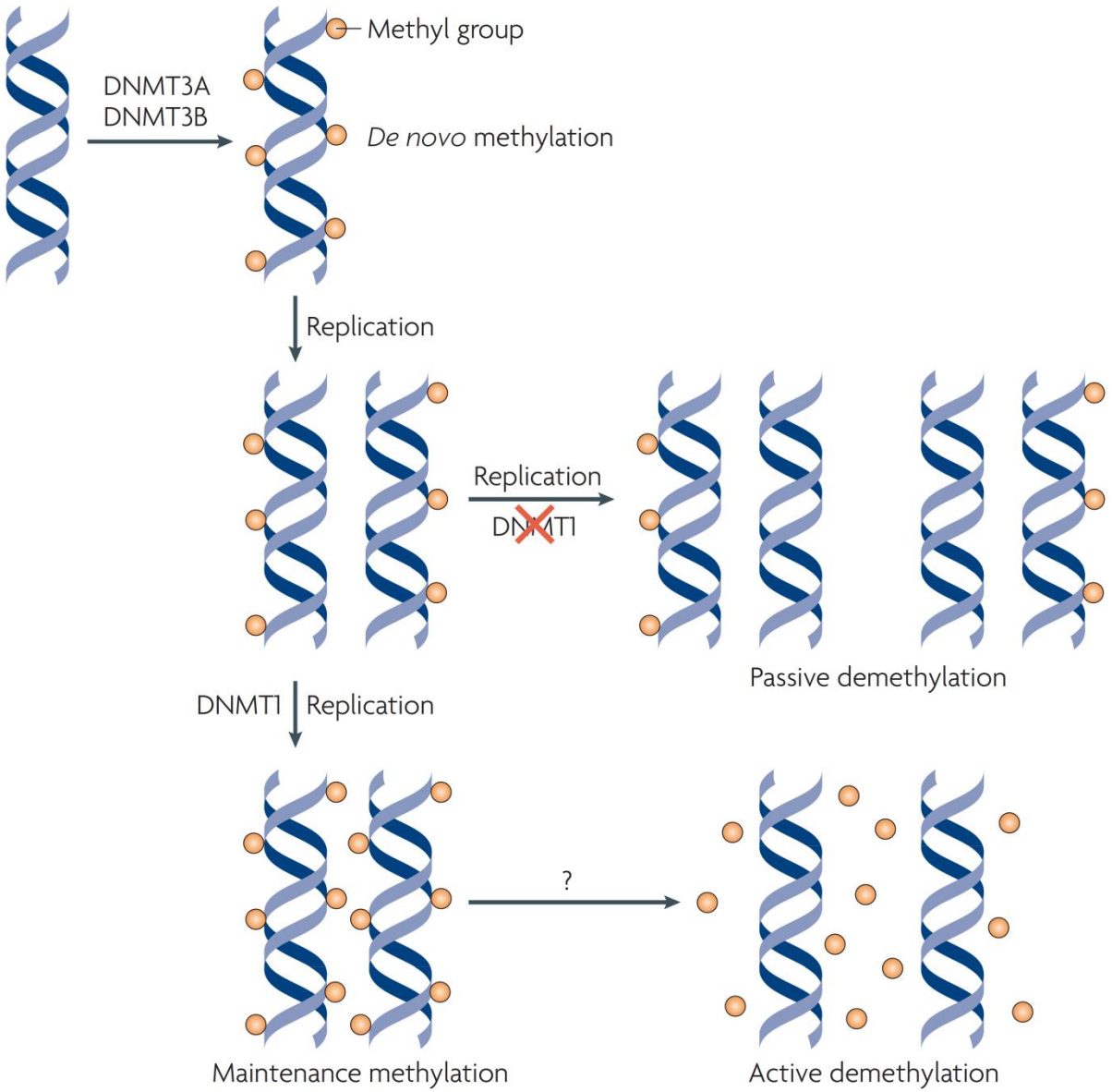
DNA demethylation

- **Passive demethylation**
- Passive demethylation occurs during replication in the absence of maintenance methyltransferase DNMT1.
- A rather slow process
- **Active demethylation**
- Active demethylation occurs through the enzymatic replacement of 5meC with C.

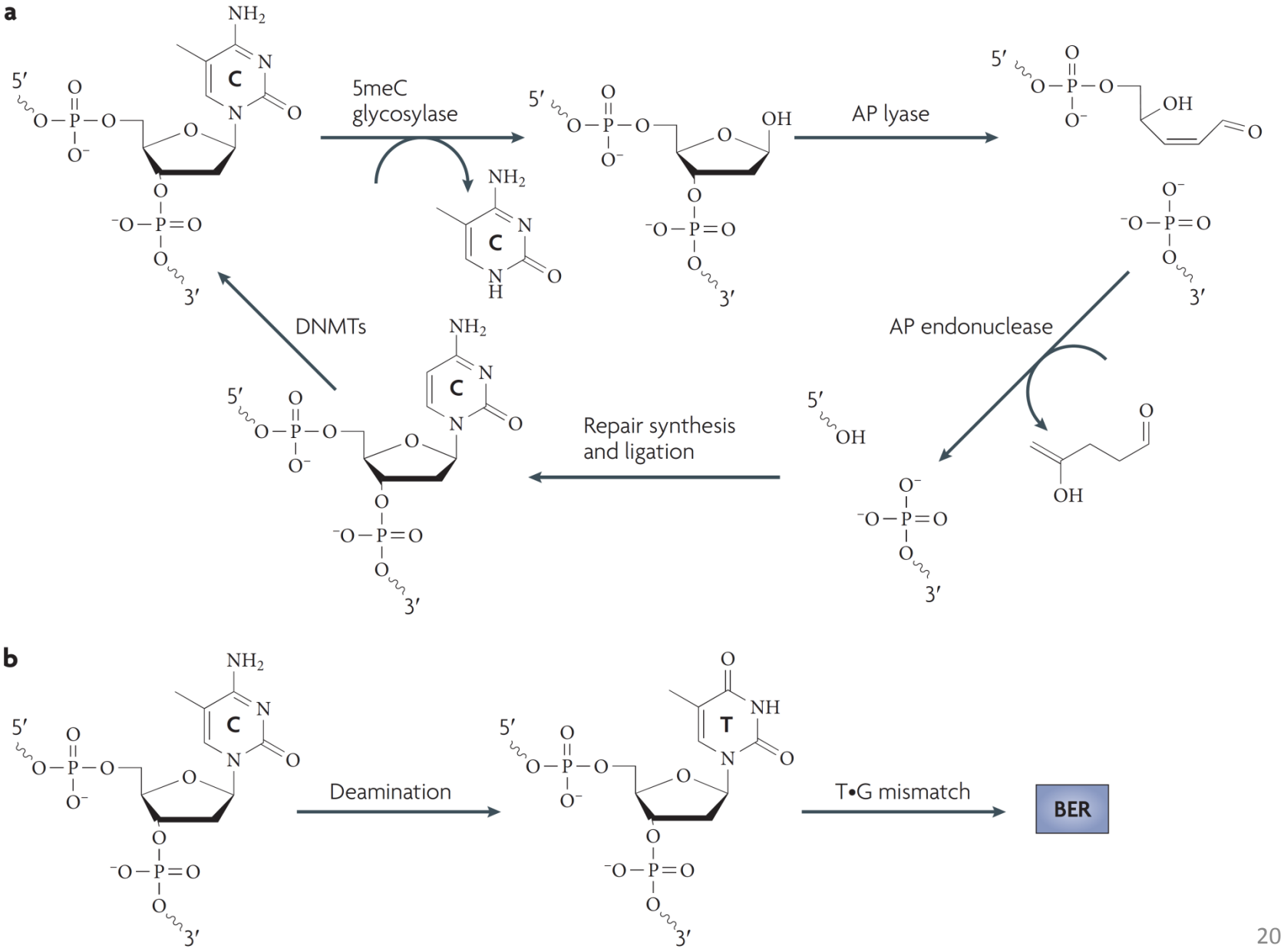
Proposed mechanisms

- ➢ deamination of 5meC to T followed by BER(base excision repair)
- ➢ oxidative demethylation (adult mouse brain in vivo, mouse ES cell?)
- ➢ Radical SAM mechanism (zygotic paternal genome?) etc.
- ✓ A consensus has yet to be achieved.

Passive demethylation



BER mechanisms for DNA demethylation



Oxidative demethylation

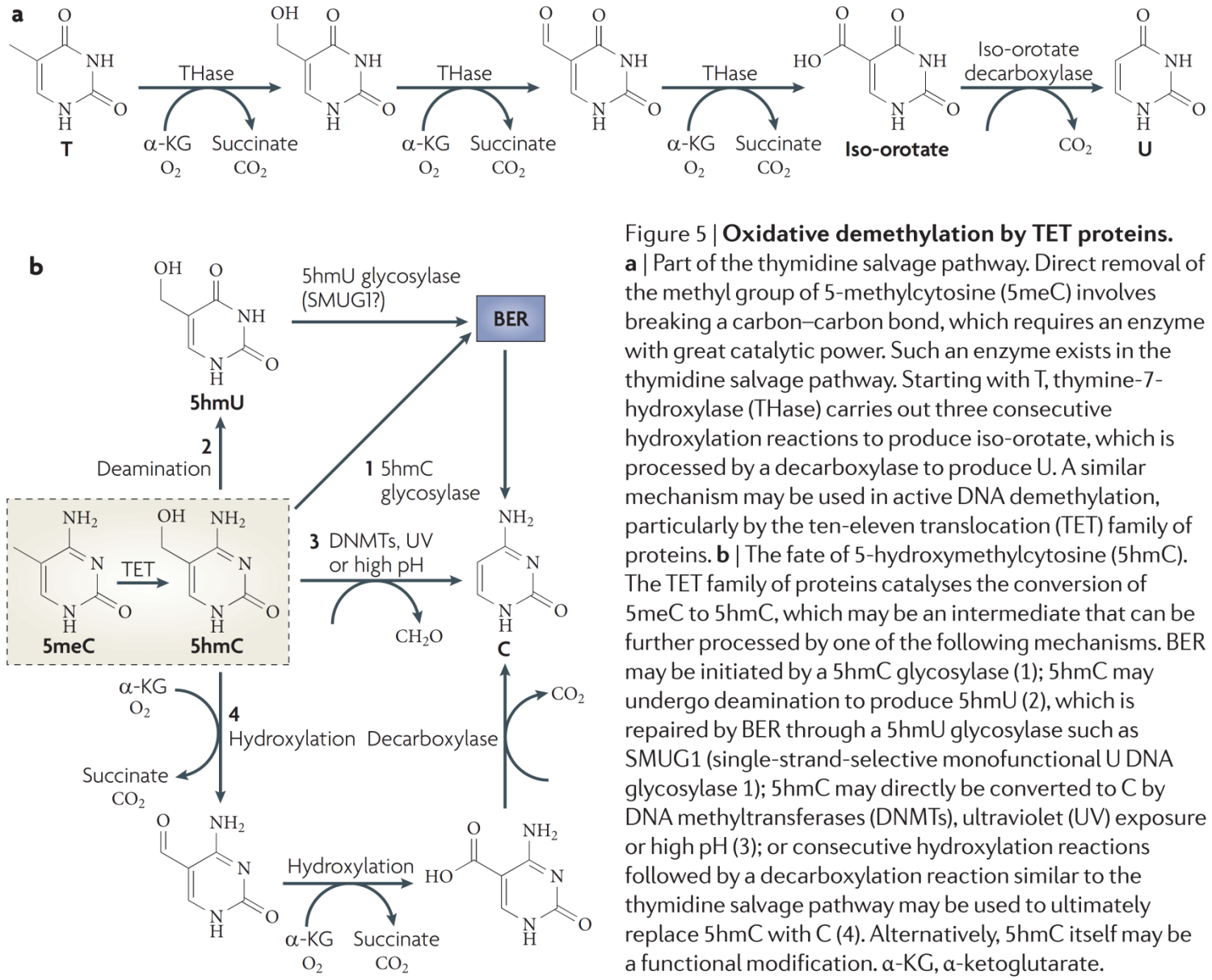


Figure 5 | **Oxidative demethylation by TET proteins.** **a** | Part of the thymidine salvage pathway. Direct removal of the methyl group of 5-methylcytosine (5meC) involves breaking a carbon–carbon bond, which requires an enzyme with great catalytic power. Such an enzyme exists in the thymidine salvage pathway. Starting with T, thymine-7-hydroxylase (THase) carries out three consecutive hydroxylation reactions to produce iso-orotate, which is processed by a decarboxylase to produce U. A similar mechanism may be used in active DNA demethylation, particularly by the ten-eleven translocation (TET) family of proteins. **b** | The fate of 5-hydroxymethylcytosine (5hmC). The TET family of proteins catalyses the conversion of 5meC to 5hmC, which may be an intermediate that can be further processed by one of the following mechanisms. BER may be initiated by a 5hmC glycosylase (1); 5hmC may undergo deamination to produce 5hmU (2), which is repaired by BER through a 5hmU glycosylase such as SMUG1 (single-strand-selective monofunctional U DNA glycosylase 1); 5hmC may directly be converted to C by DNA methyltransferases (DNMTs), ultraviolet (UV) exposure or high pH (3); or consecutive hydroxylation reactions followed by a decarboxylation reaction similar to the thymidine salvage pathway may be used to ultimately replace 5hmC with C (4). Alternatively, 5hmC itself may be a functional modification. α-KG, α-ketoglutarate.

Radical SAM mechanism

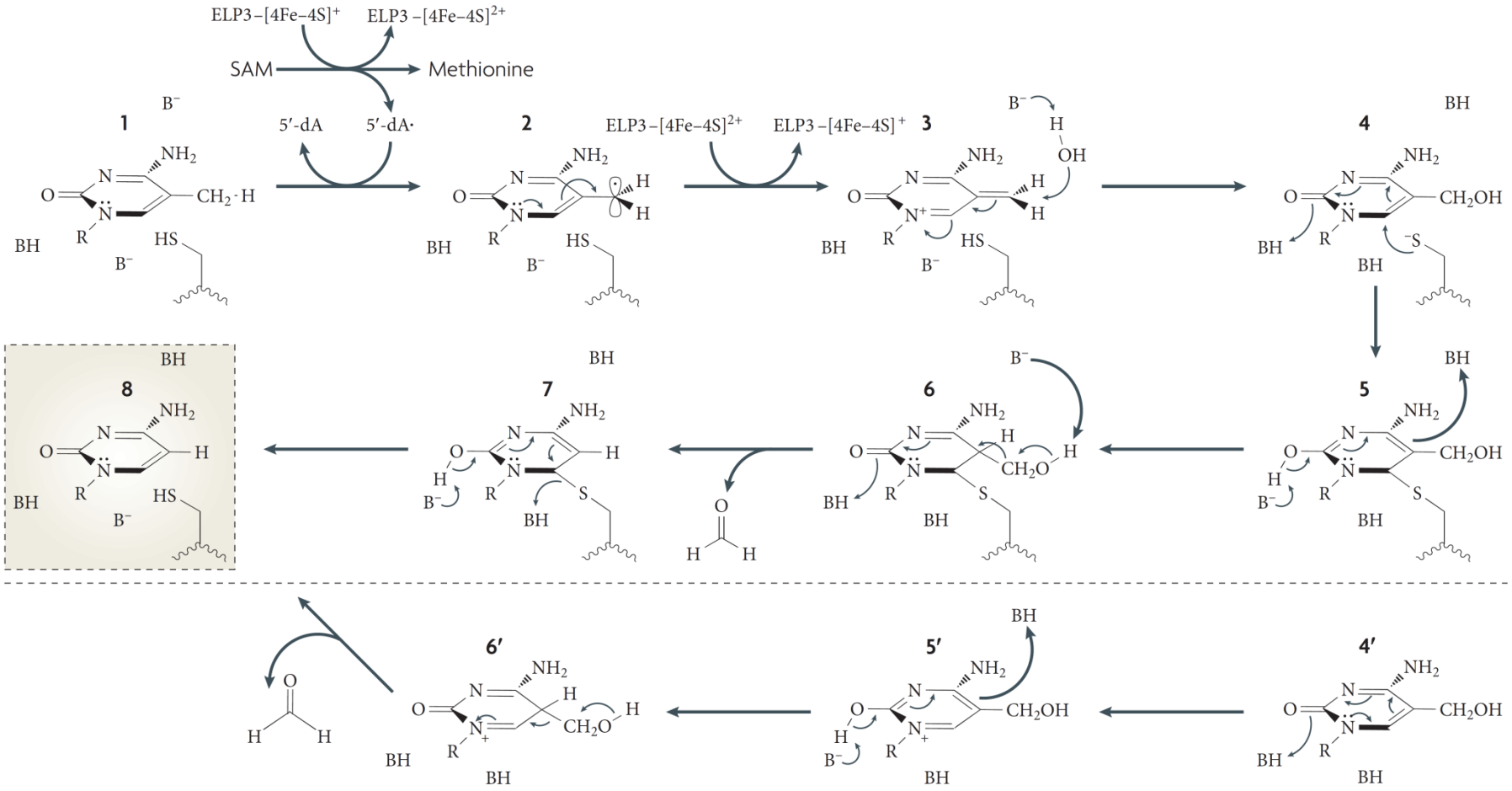
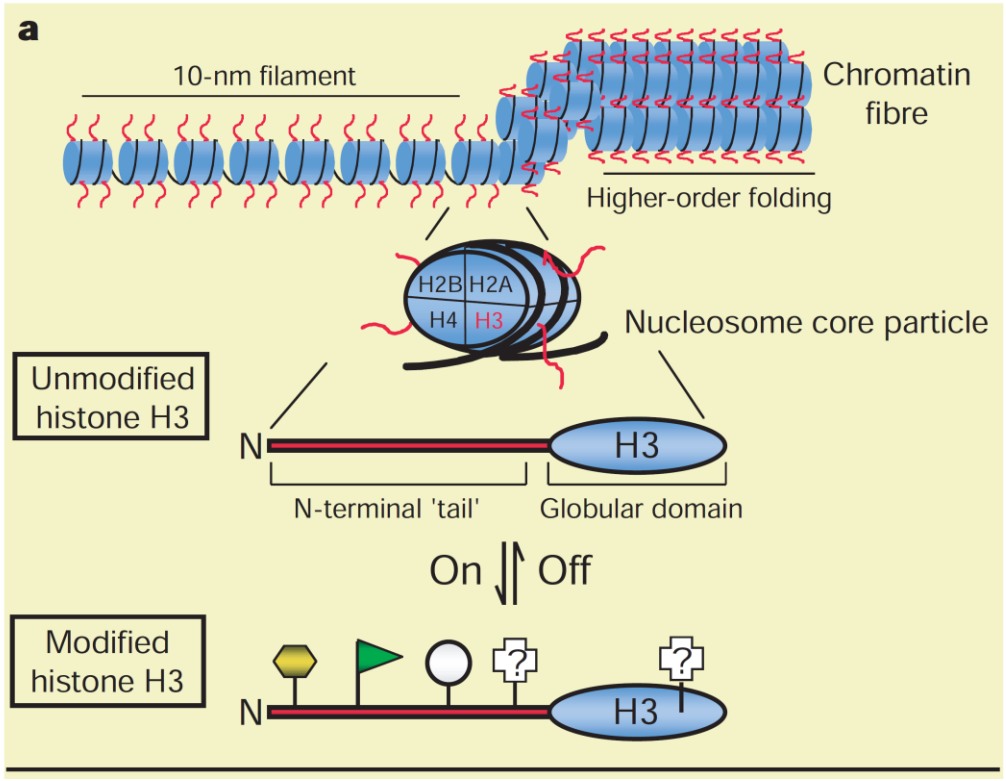


Figure 6 | **Proposed mechanism for ELP3-mediated DNA demethylation.** Mammalian elongator complex protein 3 (ELP3) contains an Fe–S radical S-adenosylmethionine (SAM) domain that is important for active DNA demethylation of the zygotic paternal genome. If ELP3 is indeed a functional radical SAM protein, it may directly carry out DNA demethylation through the following mechanism. First, ELP3 uses SAM to generate a 5′-deoxyadenosyl radical, which could extract a hydrogen atom from the 5-methyl group of 5-methylcytosine (5meC; 1) to form a 5meC radical (2). After an electron is donated back to the Fe–S to create the third intermediate (3), a water molecule would promote the formation of 5-hydroxymethylcytosine (5hmC) (4). A nucleophilic attack at carbon 6 can result in the carbon–carbon bond breaking to release formaldehyde (5–7). In the absence of an external nucleophile, an alternative pathway (4′–6′) that leads to the release of formaldehyde can also take place. Finally, an elimination step would produce an end product of C (8).

Topics

1. DNA methylation and demethylation
2. **Histone modification**
3. Epigenetic modifications and disease
4. Epigenetic therapy of cancer
5. Perspective

Histones in chromatin



Brian D. Strahl *et al*. *NATURE,* 2000, *403*,41-45.

- Core histones

An octamer consisting of H3/H4 tetramer and two H2A/H2B dimers.

147 bp of DNA wrap in 1.65 turns around it.

- Linker histone

Histone H1.

A Linker histone binds to the linker DNA(~50 bp of free DNA separating nucleosomes.)

Histone modification(1)

- The histone tails protrude out of nucleosomes and are subject to a number of post-translational modifications.

✓ Acetylation (K:lys)
✓ Methylation (K:lys, R:arg)
✓ Phosphorylation (S:ser, T:thr)
✓ Ubiquitylation (K:lys)
✓ Sumoylation (K:lys) etc.

Acetyl groups neutralize the positive charges on the basic histone tails.
As a result, electrostatic interactions between the histones and the negatively charged phosphate backbone of DNA.

Play important roles

◆ Transcriptional regulation
◆ Chromosome condensation
◆ DNA repair
◆ DNA replication
◆ Alternative splicing

Histone modification(2)

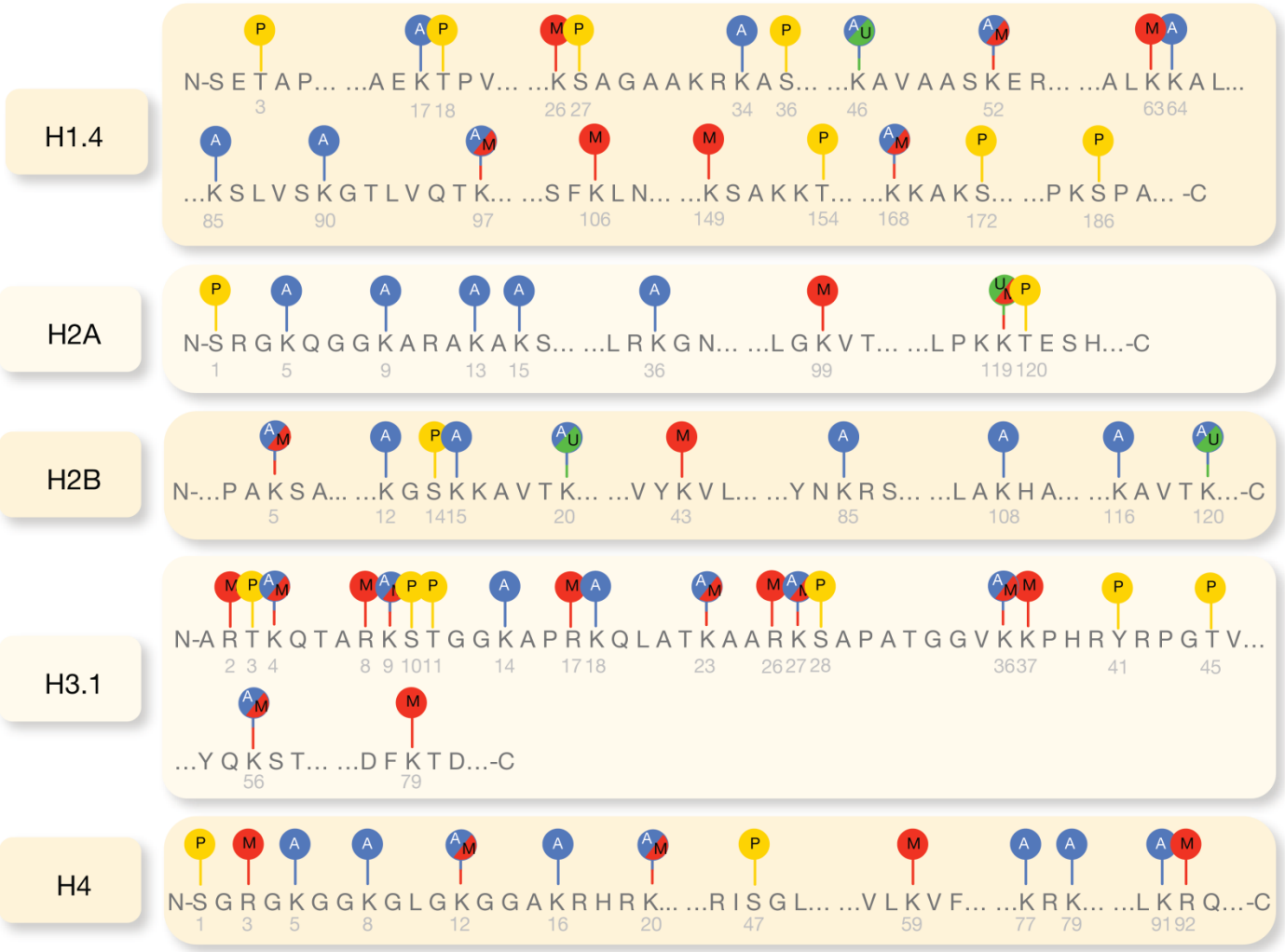


Figure 3 Histone modifications. All histones are subject to post-transcriptional modifications, which mainly occur in histone tails. The main post-transcriptional modifications are depicted in this figure: acetylation (blue), methylation (red), phosphorylation (yellow) and ubiquitination (green). The number in gray under each amino acid represents its position in the sequence.

Histone modification(3)

- Euchromatin (actively transcribed)
  - High levels of acetylation (K in H2A, H2B, H3, H4)
  - High levels of trimethylated H3K4, H3K36 and H3K79
- Heterochromatin (transcriptionally inactive)
  - Low levels of acetylation
  - High levels of H3K9, H3K27 and H4K20 methylation

◆Actively transcribed genes

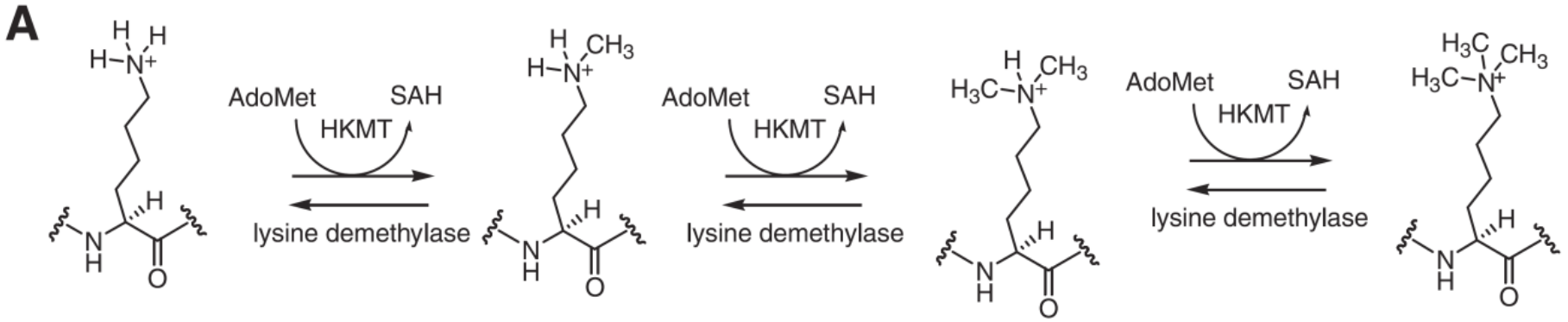
In the promoter

- High levels of H3K4me3, H3K27ac, H2BK5ac and H4K20me1

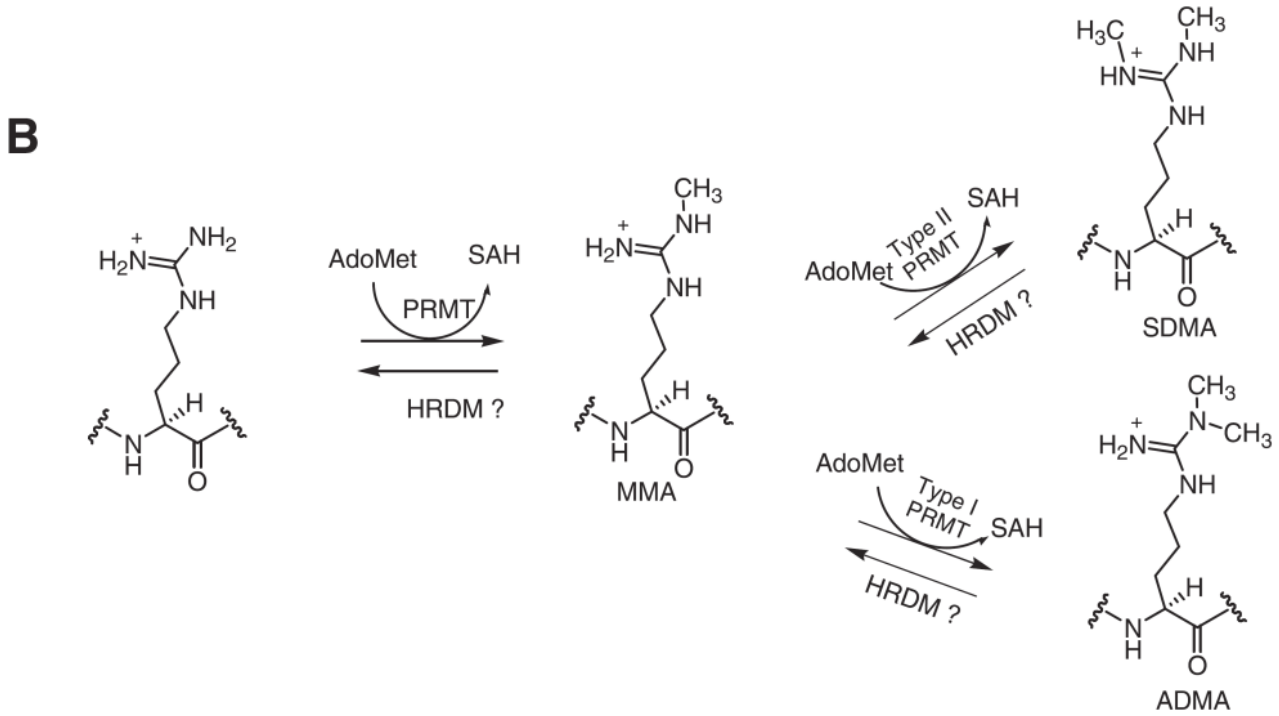
Along the gene body

- High levels of H3K79me1, H4K20me1

Histone methylation

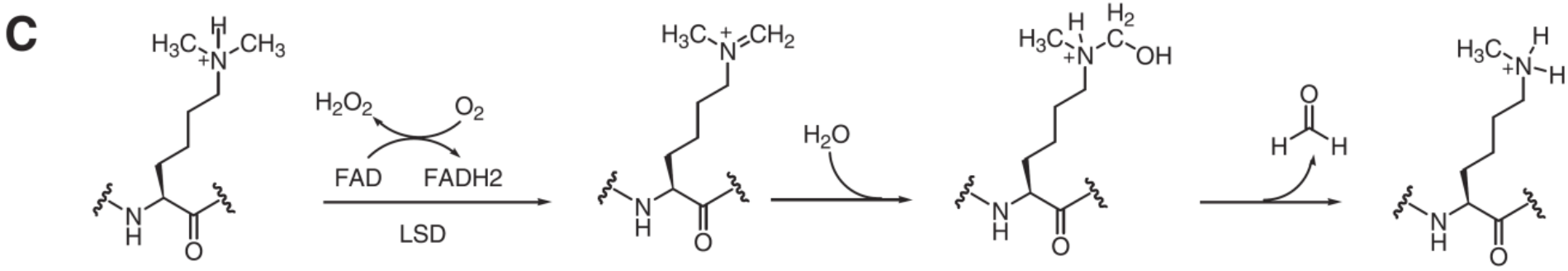


(A) Lysine is methylated by HKMTs

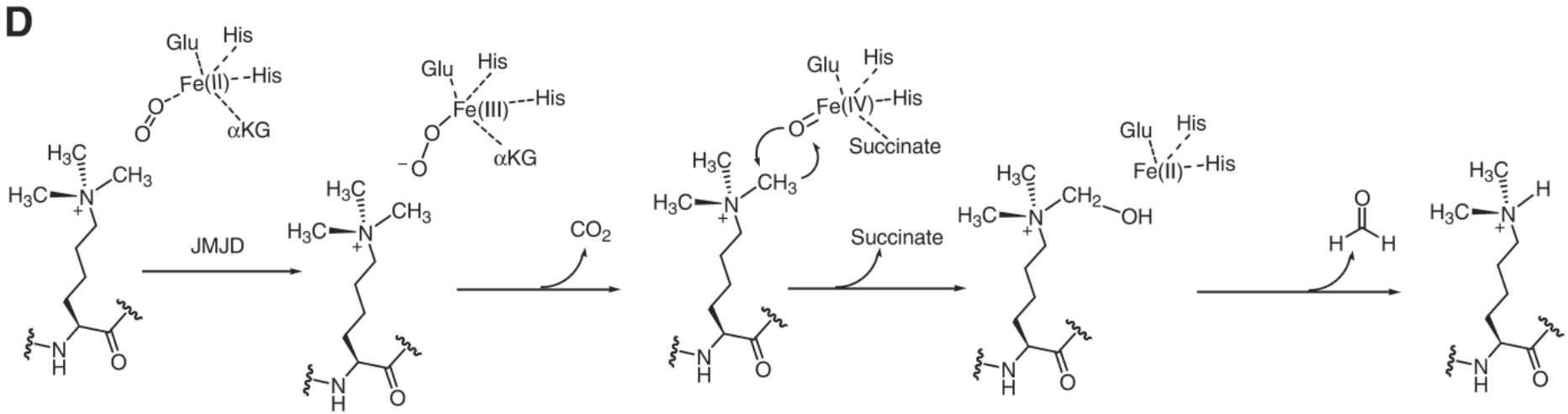


(B) Type I and II PRMTs methylate arginines and generate MMA, ADMA, and SDMA.

Histone demethylation



(C) LSD demethylates lysine via an amine oxidation reaction using FAD as a cofactor.



(D) JMJD enzymes use aKGand Fe(II) as cofactors to demethylate the methylated lysines.

Keqin Kathy Li *et al. Medicinal Research Reviews* , 2010, DOI: 10.1002/med.20228

Histone acetylation (by HATs)

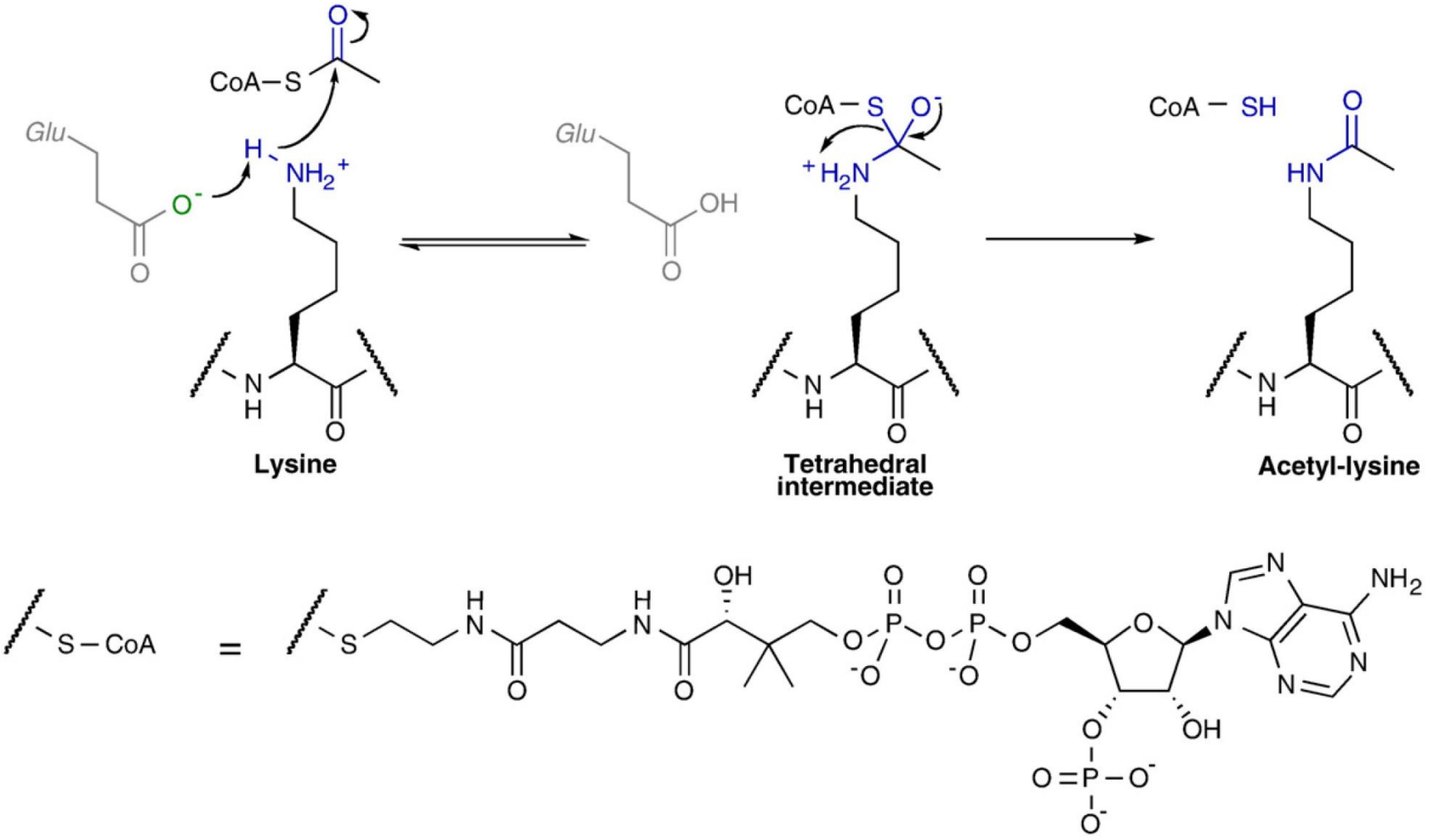
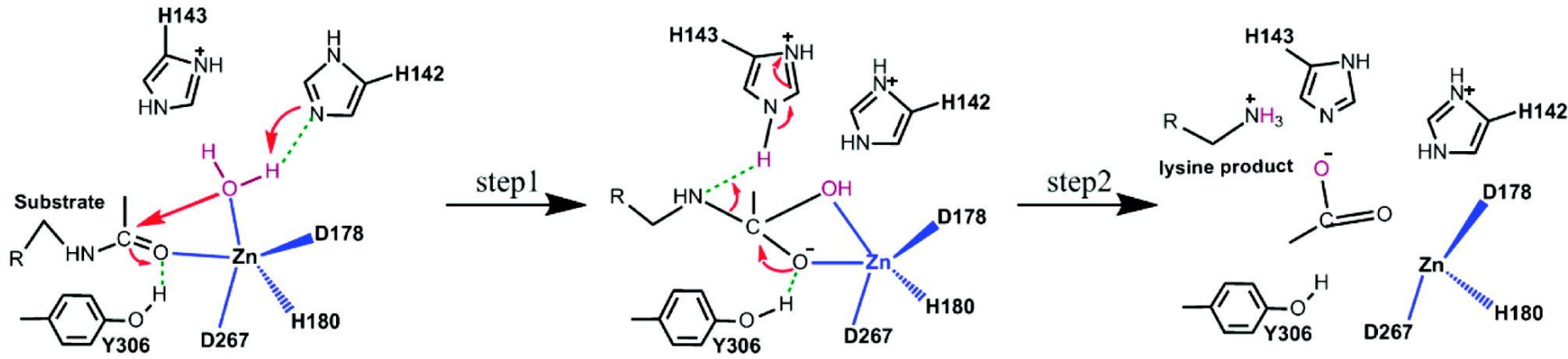


Fig. 4. Proposed chemical mechanism of histone acetyltransferases.

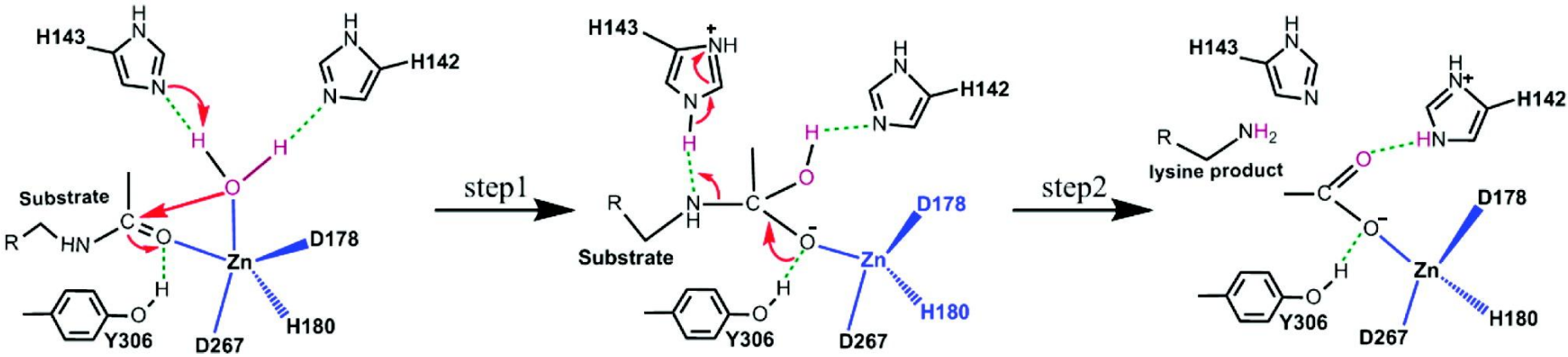
Brian C. Smith *et al*. *B. B. A*, 2009, *1789*, 45-47.

Histone deacetylation (by class I/II/IV HDACs)

Scheme 1:

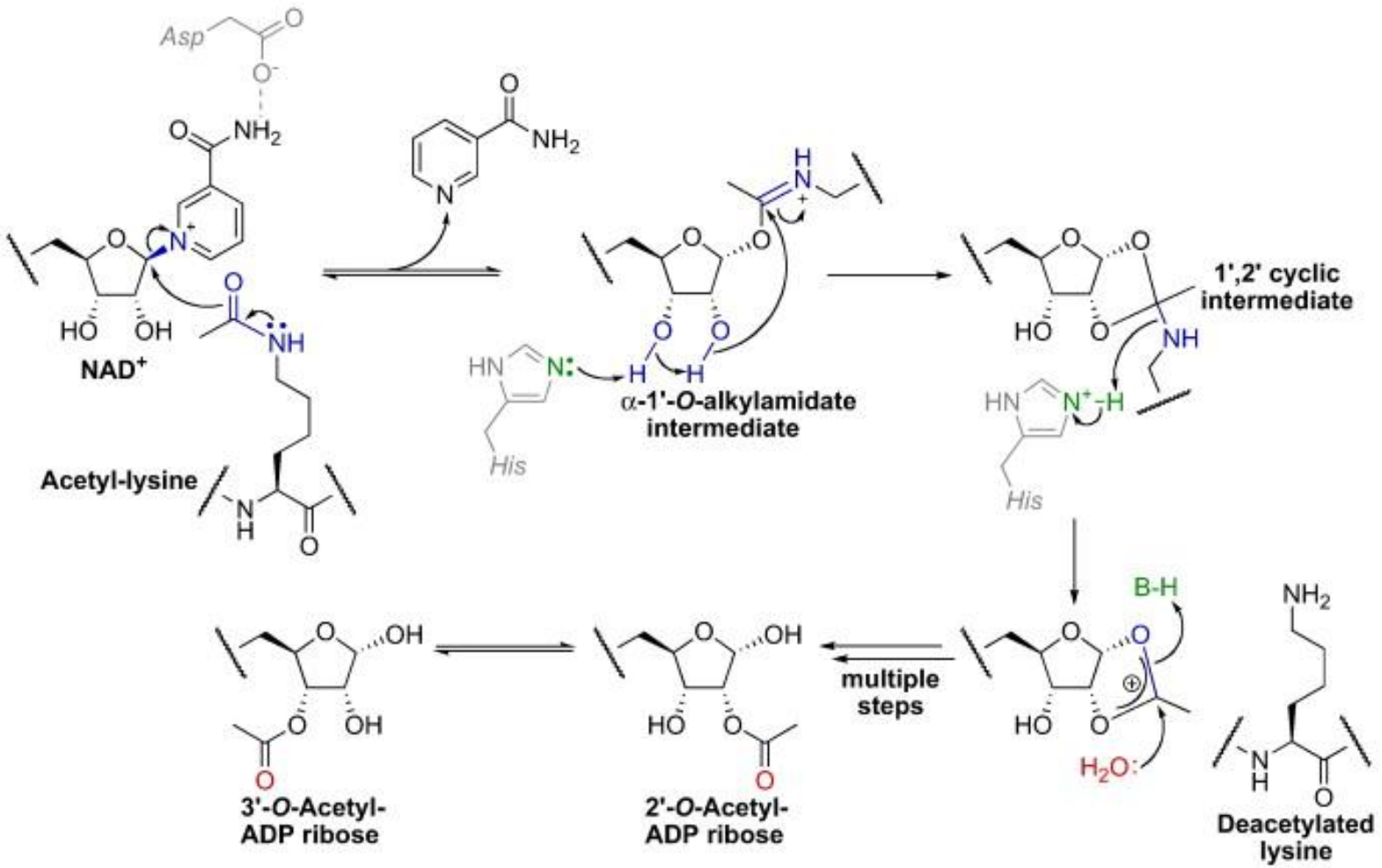


Scheme 2:



Ruibo Wu *et al. J. Am. Chem. Soc.*, **2010**, *132* (27), pp 9471–9479.

Histone deacetylation (by class Ⅲ HDACs)



Brian C. Smith *et al. B.B.A*. 2009, *1789,* 45-47

‘histone code’ hypothesis

‘Distinct histone modifications, on one or more tails, act sequentially or in combination to form a ‘histone code’ that is read by other proteins to bring about distinct downstream events’

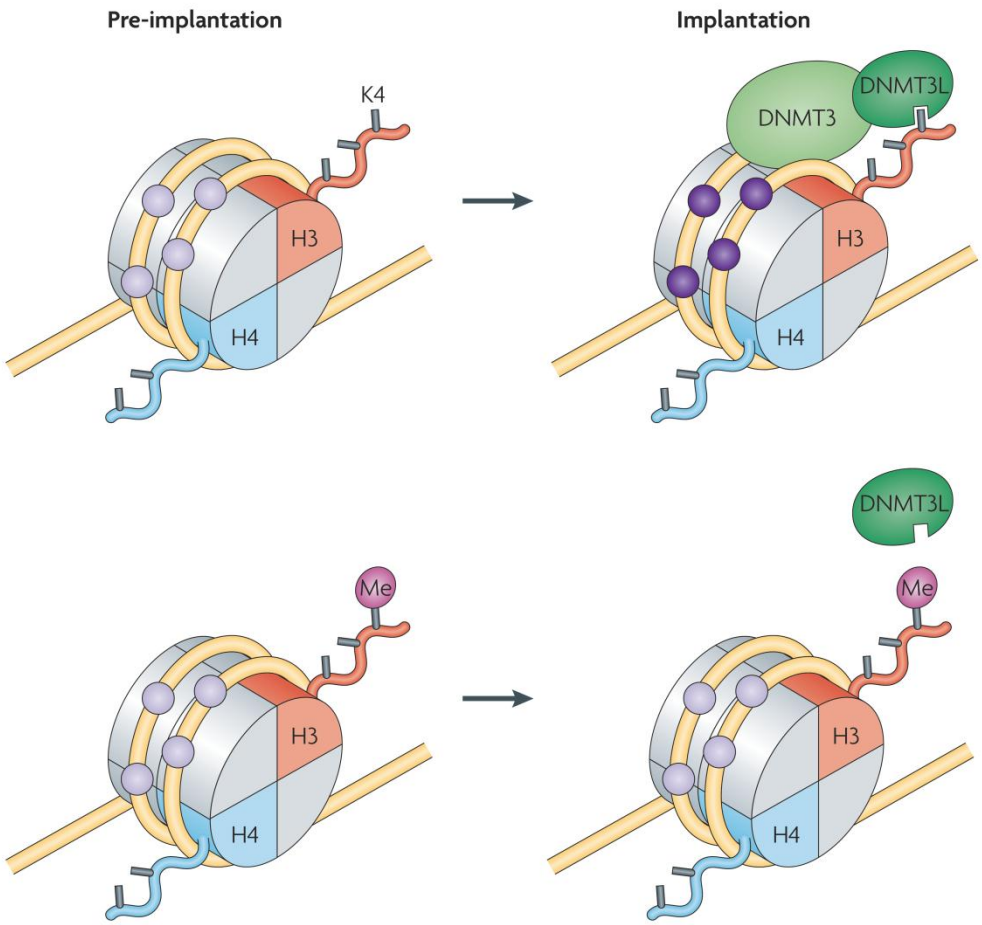
A single histone mark does not determine outcome alone.

It is the combination of all marks in a nucleosome or region that specifies outcome.

Example

The H3K4me3 active mark is found together with the H3K27me3 repressive mark at promoters of developmentally important genes within the ‘bivalent domains’ in ES cells. Bivalent domains enable ES cells to tightly regulate and rapidly activate gene expression during different developmental processes, but lost with cell commitment.

Establishment of bimodal methylation



DNMT3L binds to chromatin by recognizing H3K4, and recruit DNMT3.

If H3K4 is methylated, DNMT3L cannot bind and the underlying DNA region is protected from *de novo* methylation.

This may be one of the mechanisms used to generate a bimodal methylation pattern characterized by methylation over most of the genome, but not at CpG islands.

Howard Cedar *et al*. *Nat. Rev. Gen*. 2009, *10*, 295-304.

Turning off pluripotency genes

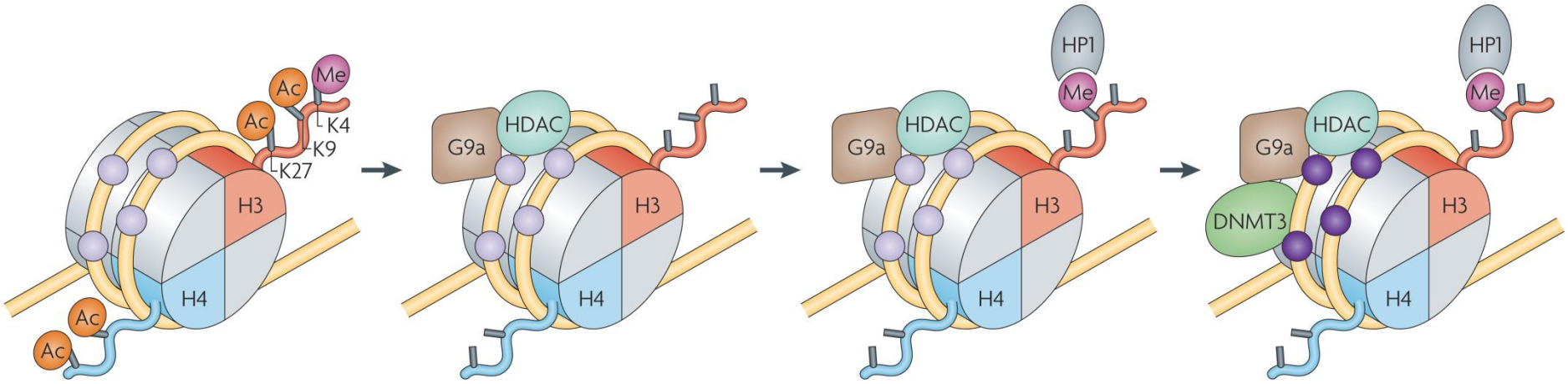


Figure 2 | **Turning off pluripotency genes.** In embryonic stem cells, pluripotency genes such as *Oct3/4* and *Nanog* have unmethylated CpG islands (light purple circles) and are packaged with acetylated (Ac) histone H3 and H4 and methylated (Me) lysine 4 of histone H3 (H3K4). With the onset of differentiation the SET domain-containing histone methyltransferase G9a is recruited, together with a histone deacetylase (HDAC), and this causes deacetylation of local histones. In addition, H3K4 is demethylated, but the enzymatic machinery responsible for this has not yet been identified. In the next step, G9a catalyses the methylation of H3K9, and this modification serves as a binding site for the chromodomain protein heterochromatin protein 1 (HP1), thus generating a form of local heterochromatin. Finally, G9a recruits the methylases DNMT3A and DNMT3B, which mediate *de novo* methylation (dark purple circles) of the underlying DNA[21,22].

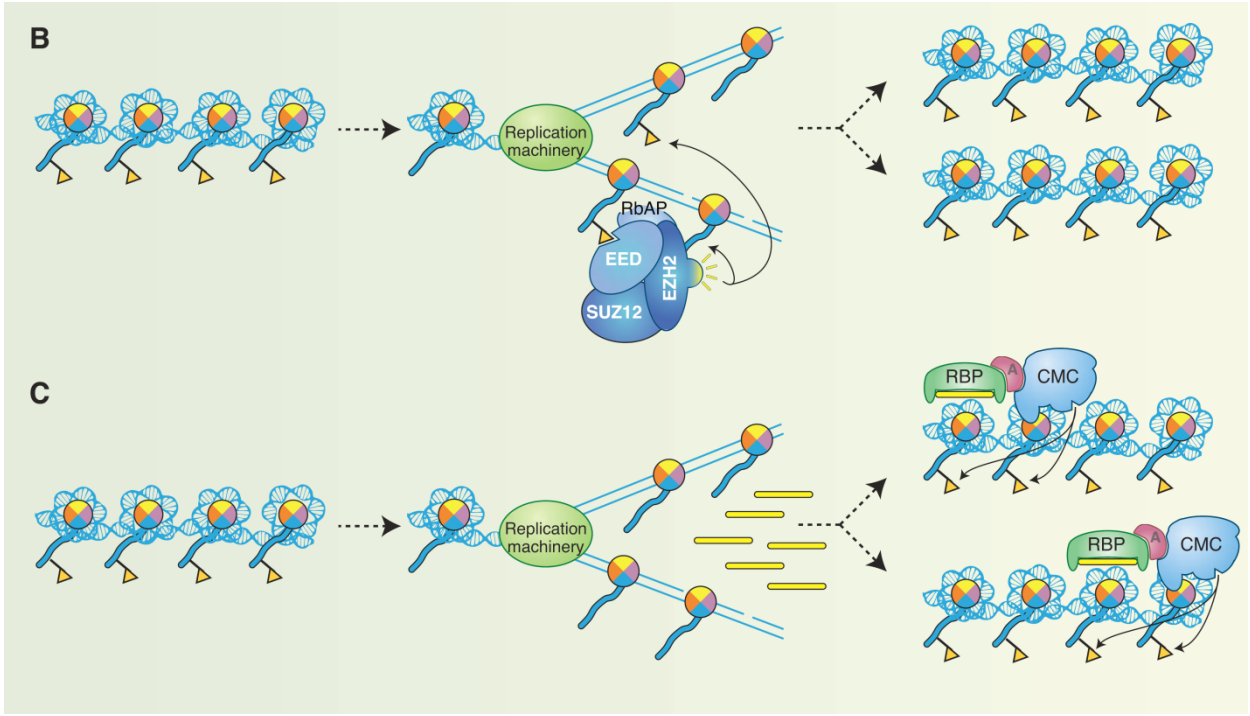
G9a: histone methyltransferase

HDAC: histone deacetylase

HP1: heterochromatin protein 1

Questions of Epigenetic inheritance

- ✓ Can histone modifications serve as templates for autonomously reproducing these same structures on newly incorporated nucleosomes following replication?
- It is known that the presence of methyl group in DNA affects chromatin packaging.
- ✓ How is the DNA methylation pattern actually translated to produce the correct histone modification profile?



Hypothetical model

H3K9me in
S. pombe heterochromatin

Nucleosome positioning

- Nucleosome positioning plays an important role in transcriptional regulation.

➢ Nucleosomes block access of activators and transcription factors to their sites on DNA.

➢ Nucleosomes inhibit elongation of the transcripts by engaged polymerases.

➢ Transcriptionally active gene promoters possess a nucleosome-free region at the 5’ and 3’ untranslated region, providing space for assembly and disassembly of the transcription machinery.

✓ The nucleosome remodeling machinery is influenced by DNA methylation and has been linked with specific histone modifications.

Topics

1. DNA methylation and demethylation
2. Histone modification
3. **Epigenetic modifications and disease**
4. Epigenetic therapy of cancer
5. Perspective

Aberrant epigenetic mark and disease

- Cancer

Global changes in DNA methylation, histone modification patterns and chromatin-modifying enzyme-expression profiles

- Neurodevelopmental disorders

➢ Rett syndrome: point mutations in the MBD protein MeCP2

➢ Rubinstein-Taybi syndrome: dysfunction of a HAT

➢ Coffin-Lowry syndrome: loss-of-function mutations in RSK2

*RSK2 promotes gene transcription through chromatin opening

- Neurodegenerative and neurological diseases

Hyper- and hypomethylation of DNA, histone hypoacetylation

- Autoimmune diseases

Hyper- and hypomethylation of DNA

Epigenetic modifications in cancer

Aberrant epigenetic mark	Alteration	Consequences	resulting disease
Cancer			
DNA methylation	CpG island hypermethylation	Transcription repression	*MLH1* (colon, endometrium, stomach[11]), *BRCA1* (breast, ovary[11]), *MGMT* (several tumor types[11]), *p16^{INK4a}* (colon[11])
	CpG island hypomethylation	Transcription activation	*MASPIN* (pancreas[92]), *S100P* (pancreas[92]), *SNCG* (breast and ovary[92]), *MAGE* (melanomas[92])
	CpG island shore hypermethylation	Transcription repression	*HOXA2* (colon[20]),*GATA2* (colon[20])
	Repetitive sequences hypomethylation	Transposition, recombination genomic instability	*L1* (ref. 11), *IAP*[11], *Sat2* (ref. 107)
Histone modification	Loss of H3 and H4 acetylation	Transcription repression	*p21^{WAF1}* (also known as *CDKN1A*)[11]
	Loss of H3K4me3	Transcription repression	*HOX* genes
	Loss of H4K20me3	Loss of heterochromatic structure	*Sat2*, *D4Z4* (ref. 107)
	Gain of H3K9me and H3K27me3	Transcription repression	*CDKN2A, RASSF1* (refs. 115–116)
Nucleosome positioning	Silencing and/or mutation of remodeler subunits	Diverse, leading to oncogenic transformation	*BRG1, CHD5* (refs. 127–131)
	Aberrant recruitment of remodelers	Transcription repression	*PLM-RARa*[103] recruits NuRD
	Histone variants replacement	Diverse (promotion cell cycle/destabilization of chromosomal boundaries)	H2A.Z overexpression/loss

Anna Portela *et al*. *Nature Biotechnology,* 2010*, 28*, 1057–1068.

Epigenetic modifications in Neurological disorders and Autoimmune diseases

Neurological disorders			
DNA methylation	CpG island hypermethylation	Transcription repression	Alzheimer's disease (*NEP*)[135]
	CpG island hypomethylation	Transcription activation	Multiple sclerosis (*PADI2*)[135]
	Repetitive sequences aberrant methylation	Transposition, recombination genomic instability	ATRX syndrome (subtelomeric repeats)[13]
Histone modification	Aberrant acetylation	Diverse	Parkinson's and Huntington's diseases[13]
	Aberrant methylation	Diverse	Huntington's disease and Friedreich's ataxia[135]
	Aberrant phosphorylation	Diverse	Alzheimer's disease[135]
Nucleosome positioning	Misposition in trinucleotide repeats	Creation of a 'closed' chromatin domain	Congenital myotonic dystrophy[151]
Autoimmune diseases			
DNA methylation	CpG island hypermethylation	Transcription repression	Rheumatoid arthritis (*DR3*)[154,155]
	CpG island hypomethylation	Transcription activation	SLE (*PRF1*, *CD70*, *CD154*, *AIM2*)[6]
	Repetitive sequences aberrant methylation	Transposition, recombination genomic instability	ICF (*Sat2*, *Sat3*), rheumatoid arthritis (*L1*)[152,155]
Histone modification	Aberrant acetylation	Diverse	SLE (*CD154*, *IL10*, IFN-γ)[6]
	Aberrant methylation	Diverse	Diabetes type 1 (*CLTA4*, *IL6*)[159]
	Aberrant phosphorylation	Diverse	SLE (NF-κB targets)
Nucleosome positioning	SNPs in the 17q12-q21 region	Allele-specific differences in nucleosome distribution	Diabetes type 1 (*CLTA4*, *IL6*)
	Histone variants replacement	Interferes with proper remodeling	Rheumatoid arthritis (histone variant macroH2A at NF-κB targets)[157]

Topics

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Cancer epigenetics: *DNA methylation*

- Hypermethylation at the CpG islands of certain promoters

→transcriptional inactivation of genes involved in the main cellular pathway

◆DNA repair, vitamin response, Ras signaling, cell cycle control,p53 network, apoptosis among others

✓DNMT1 and DNMT3B are overexpressed in many tumor types

- Global hypomethylation (20-60% less overall 5meC)

➢ At repetitive sequences

→ reactivation of endoparasitic sequeneces

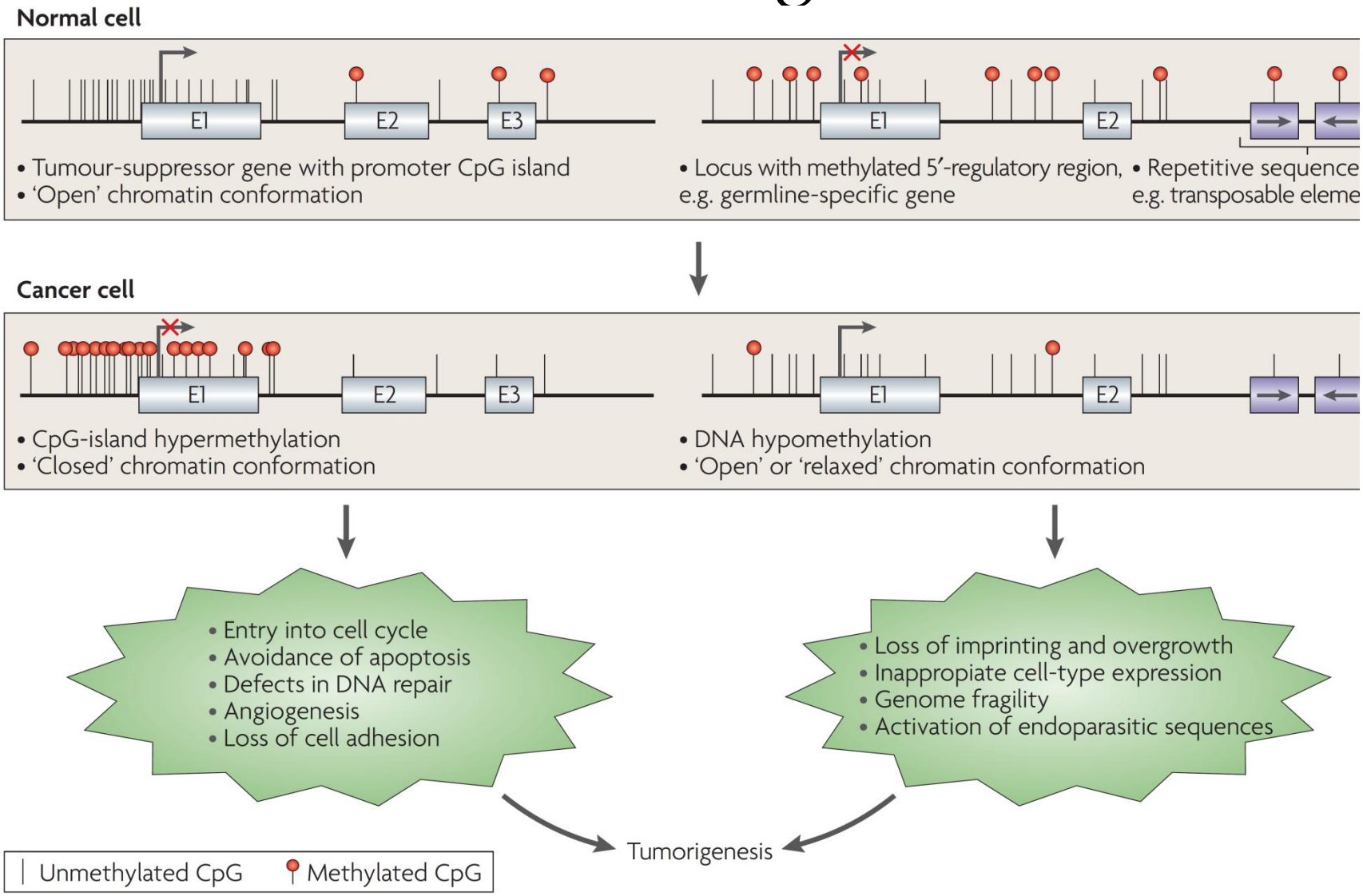
→chromosomal instability, translocations and gene disruption

➢ At specific promoters

→aberrant expression of oncogenes and loss of imprinting (LOI)

*LOI of *IGF2* is the most common LOI event across the widest range of tumor types.

Altered DNA-methylation patterns in tumorigenesis



Manel Esteller *Nature Reviews Genetics,*2007, *8,* 286-298.

Epigenetic therapy of cancer (1)

1. DNMT inhibitor

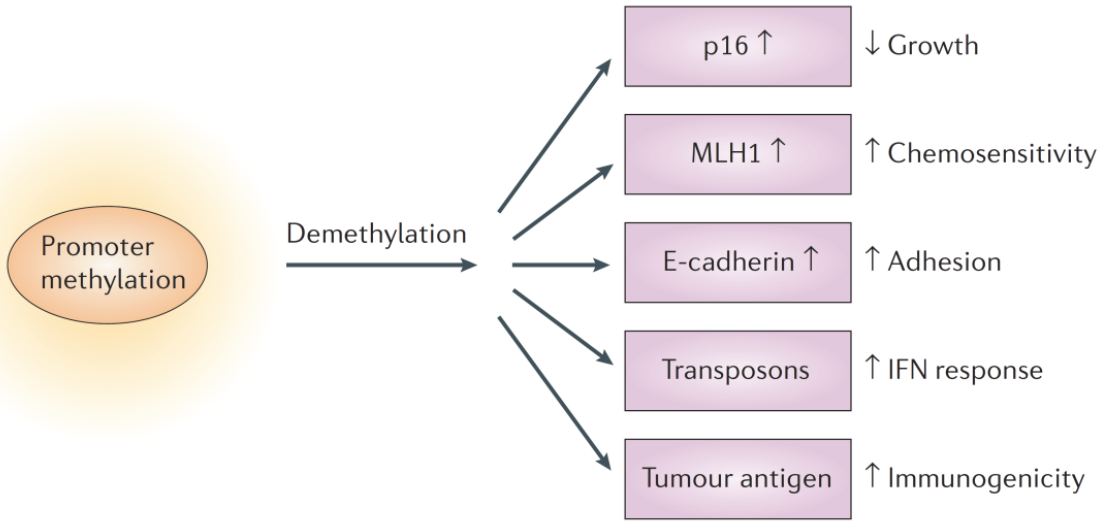


Figure 2 | **Reactivation of aberrantly silenced genes by DNA methylation inhibitors.**

Christine B. Yoo *et al*.
Nat. Rev. Drug Discovery, 2006, *5*, 37-50.

I. Inhibition of DNMTs
II. Inhibition of methylation of DNA
III. Passive demethylation
IV. Reactivation of genes including apoptotic genes and cell-cycle regulators
V. Cell death and cell-cycle arrest

DNMT inhibitors (1)

◆Nucleoside analogues

- They have a modified cytosine ring that is attached to either ribose or deoxyribose.

➢ They are metabolized by kinases and converted into nucleotides for incorporation into DNA and/or RNA.

➢ Ribonucleotide diphosphates can be reduced by ribonucleotide reductase into a deoxy-diphosphate, and can then be incorporated into DNA.

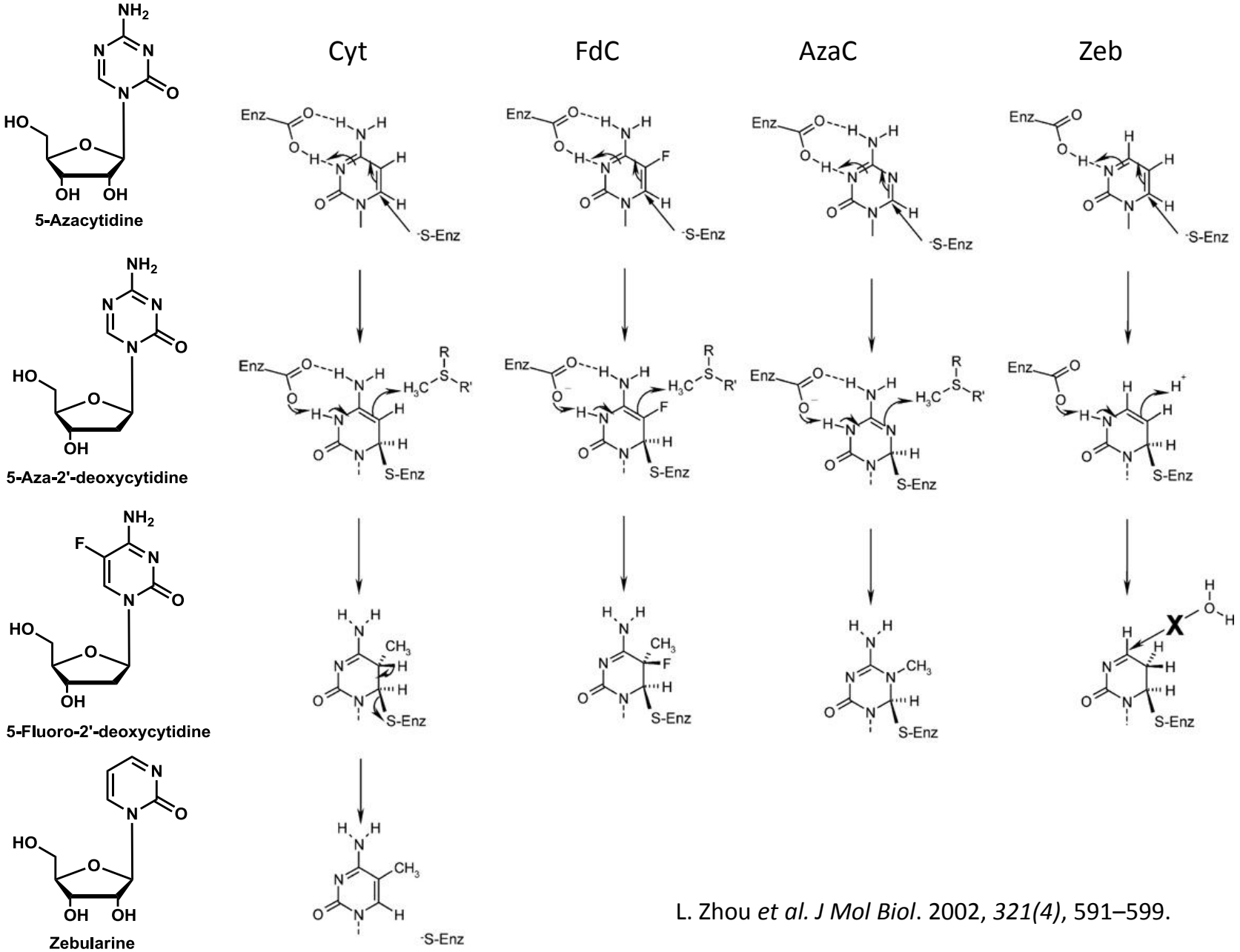
- Aniti cancer activity is mediated by

I. Cytotoxicity resulting from incorporation into the RNA and/or DNA

II. Restoring normal growth and differentiation by demethylation of tumor suppressor genes

➢ Cytosine analogues form a covalent complex with DNMTs.

DNMT inhibitors (2)



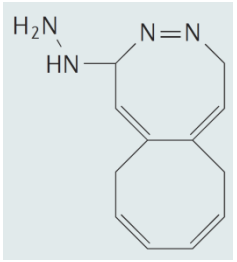
L. Zhou *et al. J Mol Biol*. 2002, *321(4)*, 591–599.

DNMT inhibitors (3)

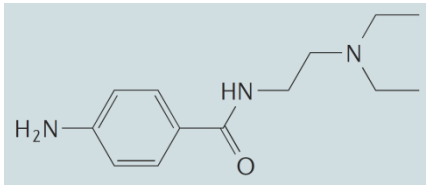
- Nucleoside analogues carry a considerable concern on cytotoxicity, which is probably associated with the drugs' incorporation into DNA.

→Non-nucleoside DNMT inhibitors

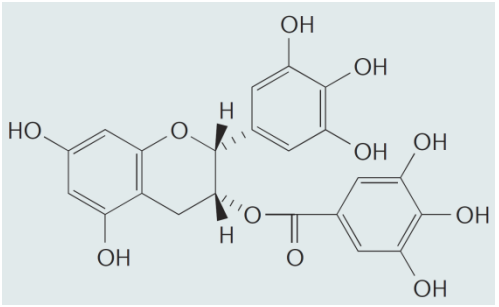
- These small-molecule inhibitors inhibit DNA methylation by binding directly to the catalytic region of the DNMTs, without incorporation into DNA.



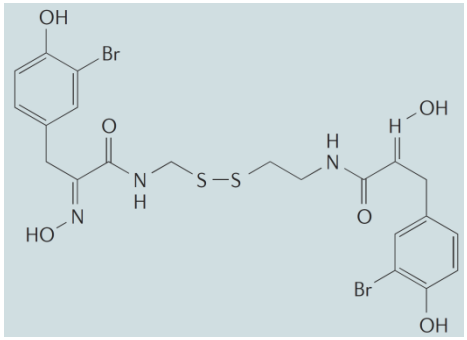
Hydralazine



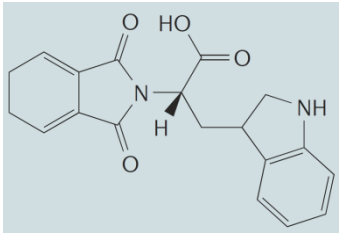
Procainamide



EGCG



Psammaplin A



RG108

MG98: 20-bp anti-sense oligonucleotide of human DNMT1, which prevents the translation of *DNMT1* mRNA

→ Despite promising results in preclinical studies, the clinical use of MG98 has not been validated

Cancer epigenetics: *histone modification*

- A global loss of H4K16ac

➢ mediated by overexpressed or mutated HDACs in different tumor types

➢ Several cancer types (e.g., colon, uterus, lung and leukemia) also bear translocations leading to the formation of aberrant fusion proteins, mutations, or deletions in HATs and HAT-related genes.

→global imbalance of histone acetylation

➢ Loss of acetylation does not only result in gene silencing, but can also lead to decreased DNA repair

→accelerating molecular events leading to the development of cancer

- A global loss of H4K20me3

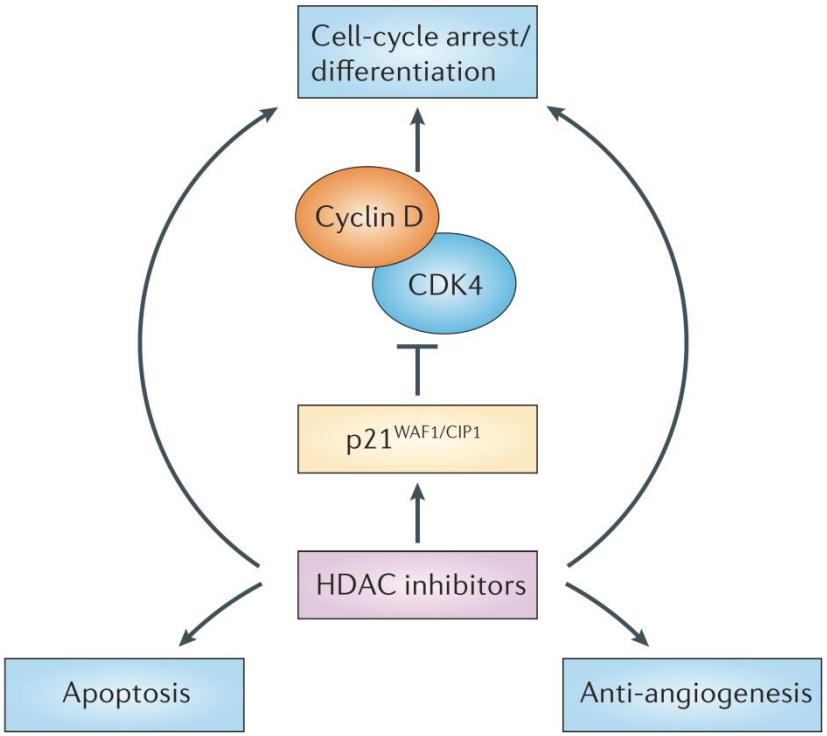
→loss of the constitutive heterochromatin structure

- A global loss of H3K4me3 and a gain in H3K9me and H3K27me3

→transcription repression

Epigenetic therapy of cancer (2)

2. Histone deacetylase inhibitor



1. Inhibition of histone deacetylase enzyme
2. Accumulation of acetylation in histones
3. Chromatin remodelling
4. Transcriptional activity
5. Restoration of malignant cells to a more normal state

3. Histone methylase or demethylase inhibitor→ not ideal

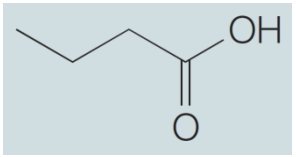
- The methyl marks exist as both active and inactive markers.
- Histone demethylase such as LSD1 demethylates both H3K4 (active) and H3K9 (inactive).
- The loss of histone acetylation seems to be the primary event leading to gene silencing, whereas the accumulation H3K9 methylation plays a secondary role.

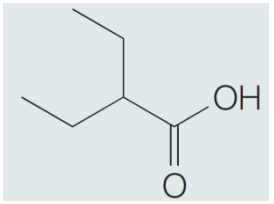
Histone deacetylase(class Ⅰ / Ⅱ /Ⅳ) inhibitors (1)

- Short-chain fatty acids

➢ The first known HDAC inhibitors

➢ Generally, they are not very potent in inhibiting HDACs.

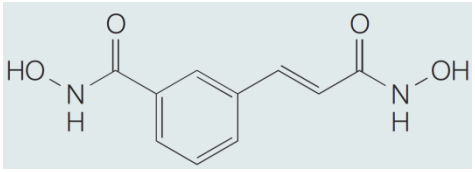
Butyrate 

Valproic acid 

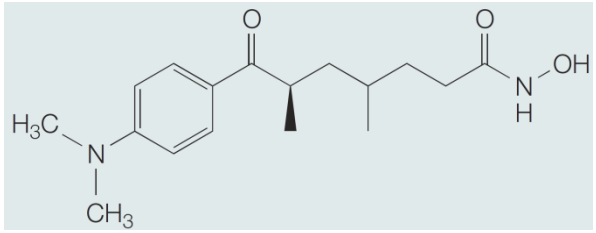
- Hydroxamic acids

➢ They are potent inhibitors of HDACs.

➢ It is postulated that hydroxamates inhibit HDAC by binding to a zinc ion in the catalytic domain of the enzyme, thereby inhibiting the deacetylation of histones.



m-Carboxy cinnamic Acid bishydroxamic Acid (CBHA)



Trichostatin A (TSA)

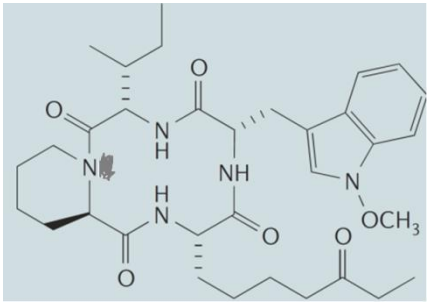
Suberoylanilide hydroxamic acid (SAHA)

Histone deacetylase(class Ⅰ / Ⅱ /Ⅳ) inhibitors (2)

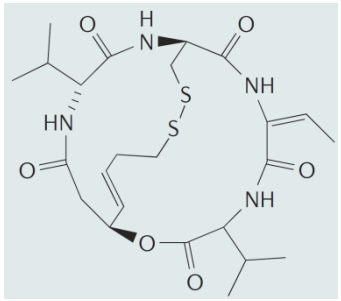
- Cyclic tetrapeptides

➢ They are potent inhibitors of HDACs.

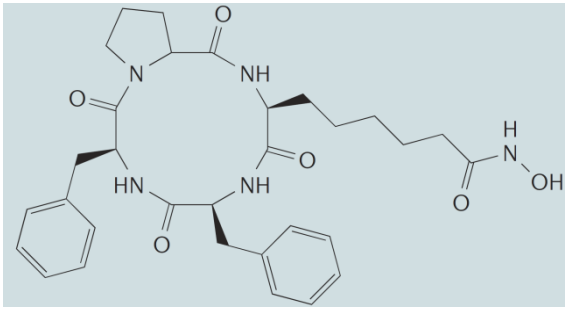
➢ Their target is the zinc ion in the HDAC catalytic domain.



Apicidin



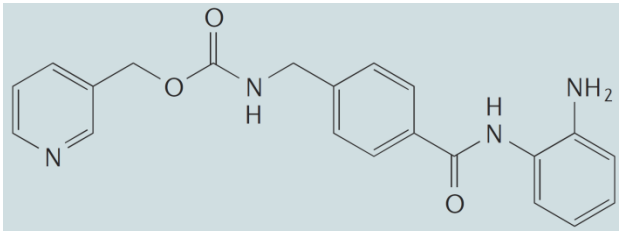
Depsipeptide



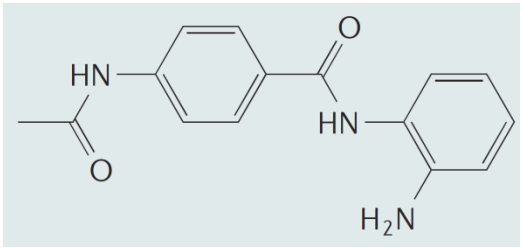
TPX-HA analogue

- Benzamides

➢ The benzamide group of MS275 inhibits HDACs by binding to the catalytic zinc ion.



MS-275



CI-994

Combination therapy

- Reactivation of tumor-suppressor genes and/or genes that are crucial for normal functioning cells is the target of epigenetic drugs.

➢ DNA methylation and histone modification represent the two most important pathways involved in gene silencing.

→combination of DNMT inhibitor and HDAC inhibitor

- Combination therapies are expected to achieve the following goals

1. To enhance or extend the molecular effects of the inhibitors
2. To reduce the side effects through applying lower dosages of one or both drugs.

Topics

1. DNA methylation and demethylation
2. Histone modification
3. Epigenetic modifications and disease
4. Epigenetic therapy
5. **Perspective**

Perspective (1)

- *Combination of epigenetic therapy and other therapeutic modalities, such as chemotherapy, immunotherapy or radiotherapy*

➢ The reactivation of tumor-suppressor genes and restoration of DNA-repair pathways by epigenetic drugs results in more chemosensitive cells.

- *Biomarker for cancer*

➢ Epimutations , or aberrant DNA methylation and histone modification patterns, are observed in individuals with no history of malignancy, and can be used as an indicator of the likelihood of developing cancer.

- *Drugs designed to target a specific region of the genome*

➢ The lack of specificity is a disadvantage of current epigenetic drugs.

DNMTi results in demethylation of many regions of the genome.

HDACi have a very broad specificity.

- *The detailed map of specific epigenetic patterns in each tissue type in their normal and in cancerous states*

Perspective (2)

- ‘Artificial *de novo* DNA-methylating agents’ and ‘artificial histone-acetylating agents’ as drugs?

➢ Current epigenetic drugs inhibit enzymes that are responsible for DNA-methylation or histone-deacethylation, resulting in ‘passive’ DNA-demethylation or histone-acetylation.

➢ However, ‘active’ DNA-demethylation and histone-acetylation of silenced genes seem to be very difficult because the chromatins of the silenced regions are highly compacted.

✓ Sequence-specific artificial *de novo* DNA-methylation and/or histone-deacetylation of aberrantly expressed oncogenes and imprinted genes would result in silencing of the unwanted genes, and this might be an effective cancer treatment.

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