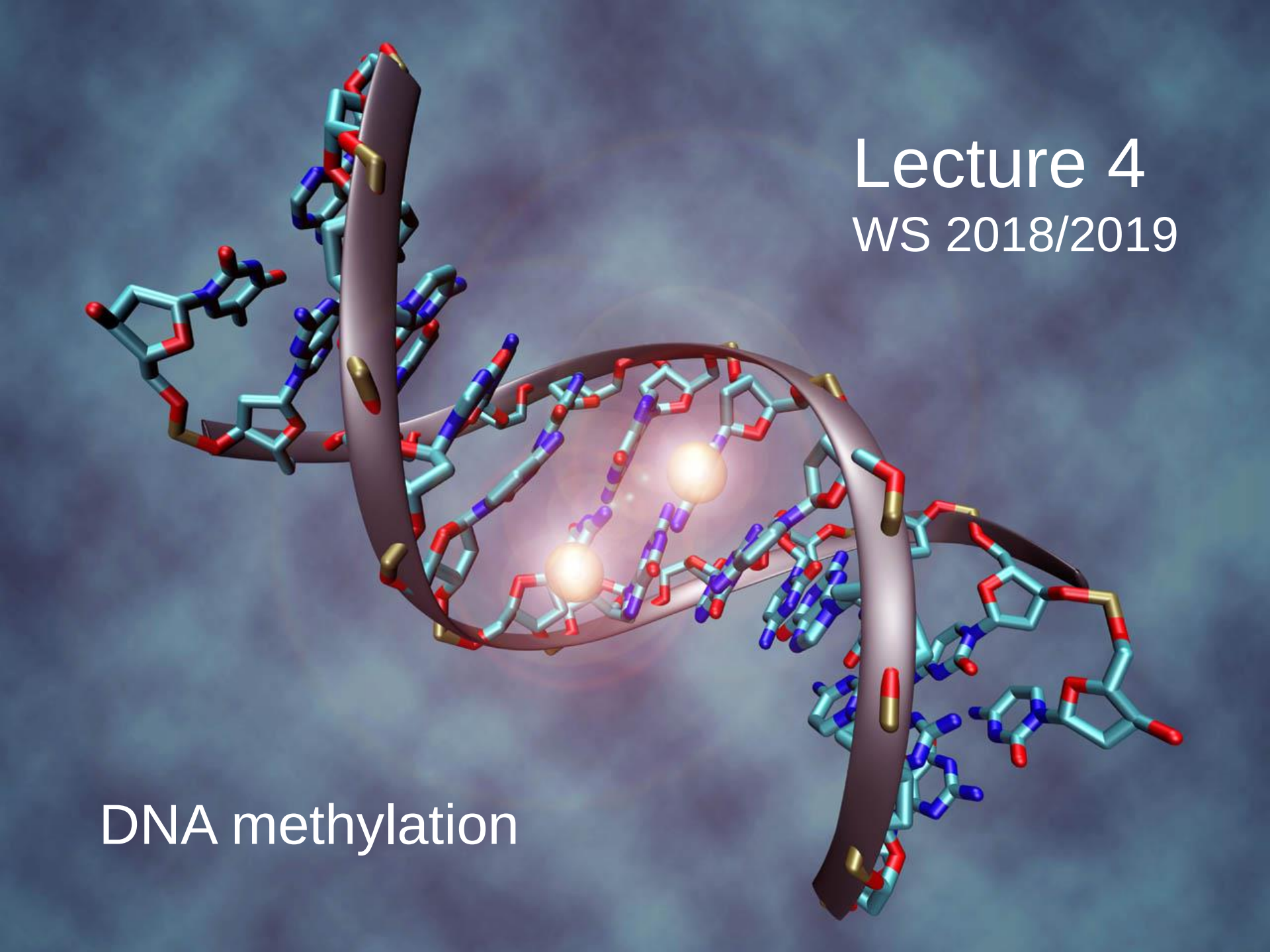


Lecture 4

WS 2018/2019

DNA methylation

DNA-methylation

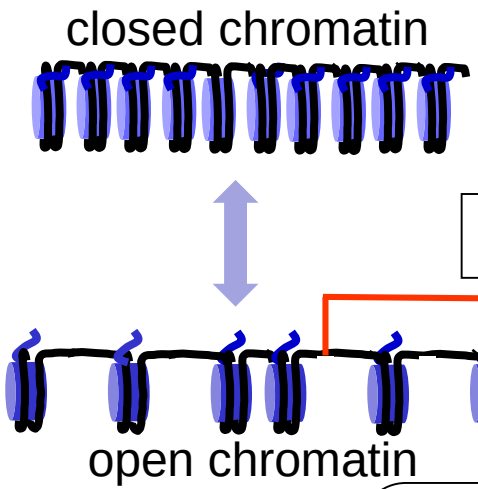
- Evolution of DNA-methylation: from viral defense to gene regulation
- Introduction into DNA-methylation reactions: the general principles and diversities of DNA-base modifications.
- DNA-methylation in mammals: Concept of establishment, maintenance (inheritance) and erasure of DNA-methylation.
- Function of DNA-methylation in mammals: genome wide distribution, changes during development and disease impact on gene regulation and on the transcriptional control of transposable elements.
- Role of oxidised forms of 5mC.

Molecular interaction levels in epigenetics

4 DNA-methylation
(5^{me}cytosine, 5^{hme}cytosine, $5^{carboxyl}$cytosine)

> 10 classes of RNAs
(mi-, pi-, si- and long non-coding RNAs)

>140 Histone Mod‘s
(Methylation, Acetylation, Phosphorylation,...)



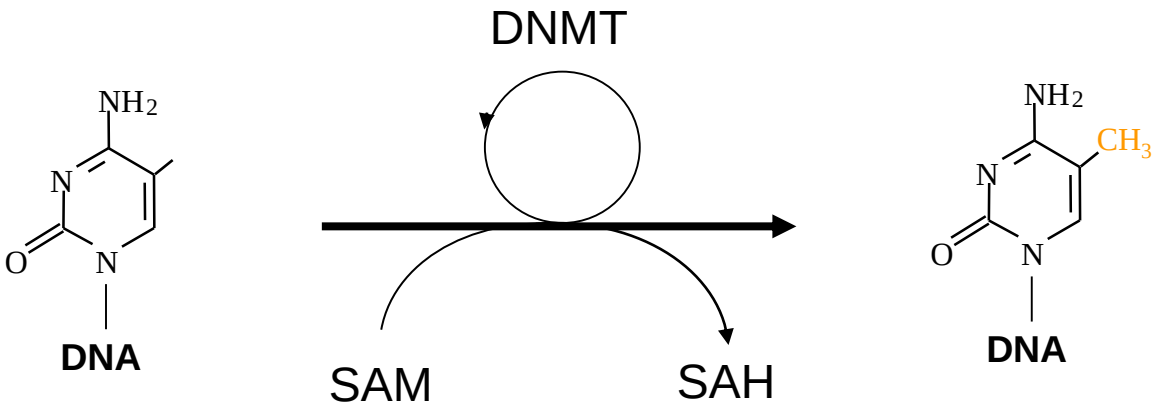
DNA-sequence

DNA-structure

>1400 other proteins
Transcriptional control proteins
Chromatin-Remodelling proteins,
Structural proteins

>8 Histone variants
(Protamines, H1.1, H2AX, H2AZ, H3.3, CenpA..)

DNA-methylation: the enzymatic reaction



A 5‘CpG3‘ dinucleotide in the DNA of mammals is recognized by the DNA-methyltransfrease (DNMT) and a methyl group is transferred from the methylgroup donor S-adenosyl-methionine (SAM) to the carbon 5 of the cytosine ring. SAM is converted in S-adenosyl-homocysteine (SAH).

Note: S-adenosyl-methionine is also abbreviated as „AdoMet“

DNA-methylation in found in nearly the entire flora and fauna

- Almost all bacteria (gram negative und gram positive)
- Many Archaebacteria (Thermophiles, Halophiles)
- Most fungi (Filamentous fungi, Some ascomycetes)
- All Plants (Mono- and dicotyledones)
- Most Insects (Diptera (Bees), Coccides (Beatles)
- All Vertebrates, (Birds, Fishes, Amphibia)
- All Mammals (mouse, rat, primates, human)

- *DNA-methylation is not found some exceptional organisms:*
  Some Yeasts :S.cerevisiae and S.pombe,
  Most Nematodes: C.elegans (but in Annilides and higher worms)
- *Some Insects: Drosophila melanogaster*

DNA-methylation in found in nearly the entire flora and fauna

Birds do it, bees do it, worms and ciliates do it too:

DNA methylation from unexpected corners of the tree of life

Soojin V Yi

Genome Biology 2012, **13**:174 doi:10.1186/gb-2012-13-10-174

http://genomebiology.com/2012/13/10/174

DNA-methylation specificity

- DNA of most organisms contains modified cytosine bases = C5-Methyl-Cytosin (5mC)
- Bacteria also have enzyme modifying N4-Methyl-Cytosin (N4-mC) or N6-Methyl-Adenin (N6-mA = e.g. "*Dam" enzyme in E.coli*).
- All DNA-methylation occurs after replication (post-replicative) and is enzymatically catalyzed by DNA-methyltransferases (DNMTs).

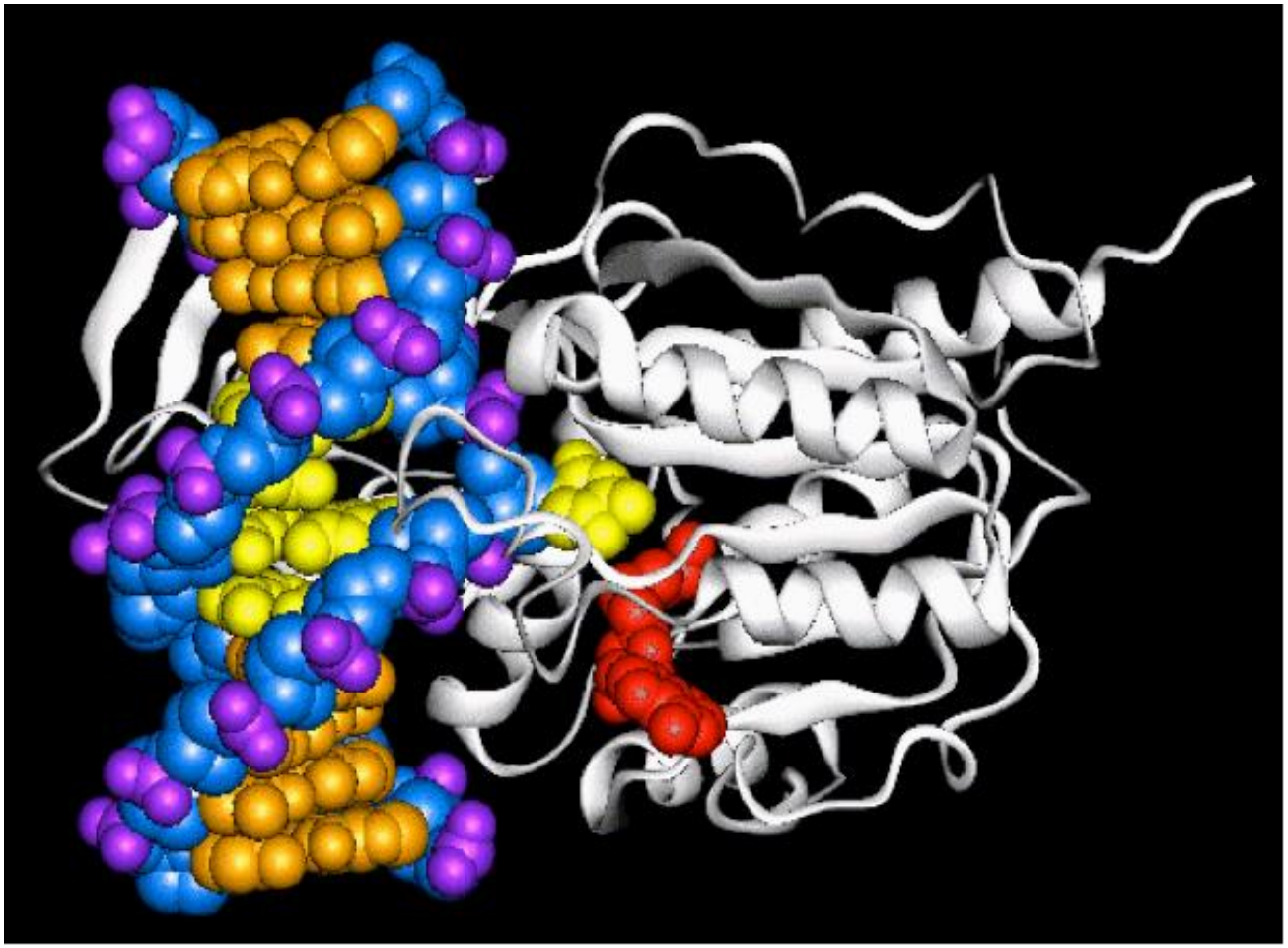
DNA-methylation: base specificity

- DNA-methylation occurs in a sequence context and usually is symmetrical on both strands:

- In bacteria many different enzymes are found which all have different sequence specificities:e.g. GATC, CCCGGG, GGCC or CCGG (methylated base and position in red).

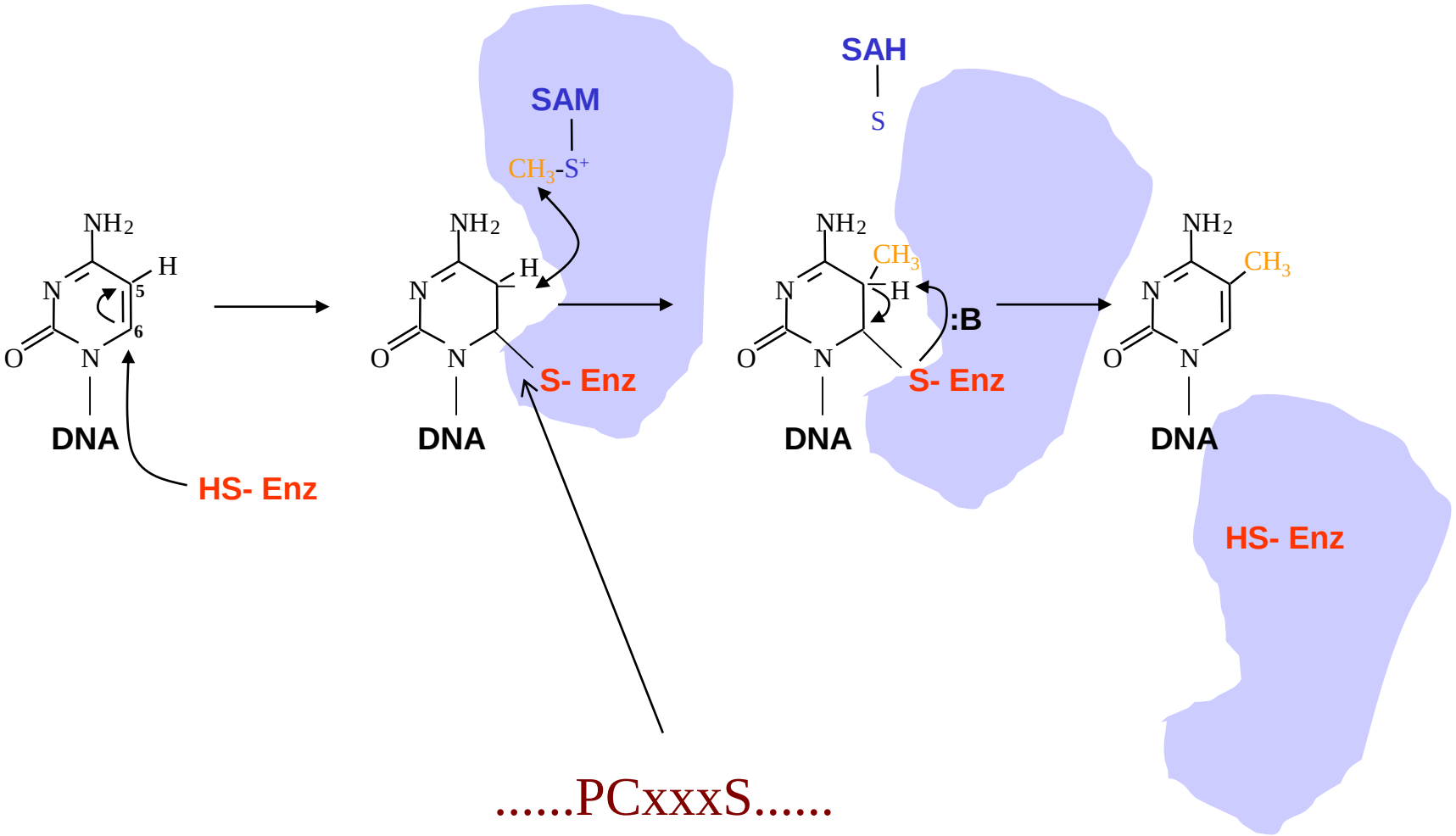
- DNA-methylation in mammals is almost exclusively confined to the CpG sequence context.

DNA-methyltransferase binding to DNA



Cytosine base in yellow,
Co-factor S-AdenosylMethionine (SAM) in red

DNA-methylation: enzymatic reaction

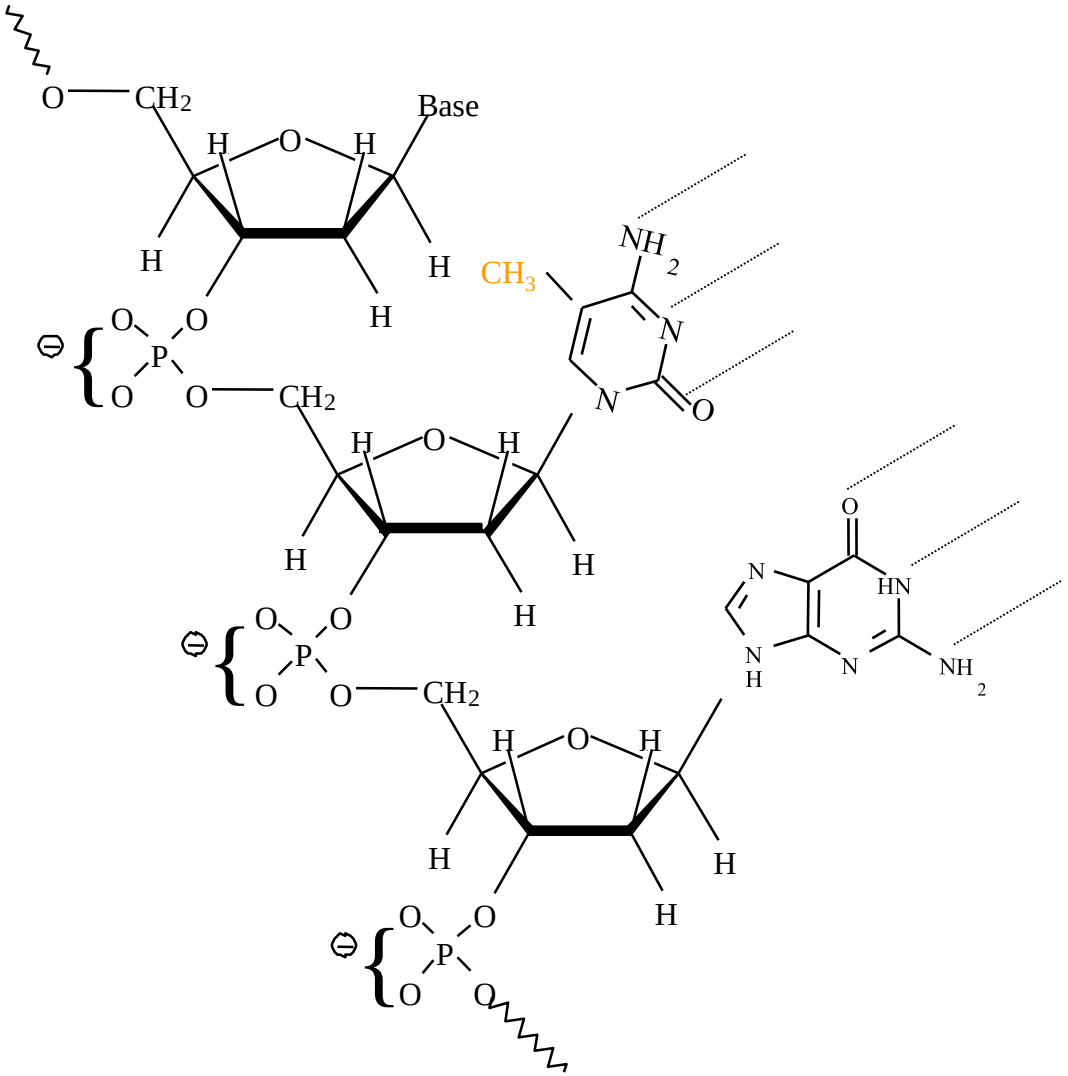


.....PCxxxS.....

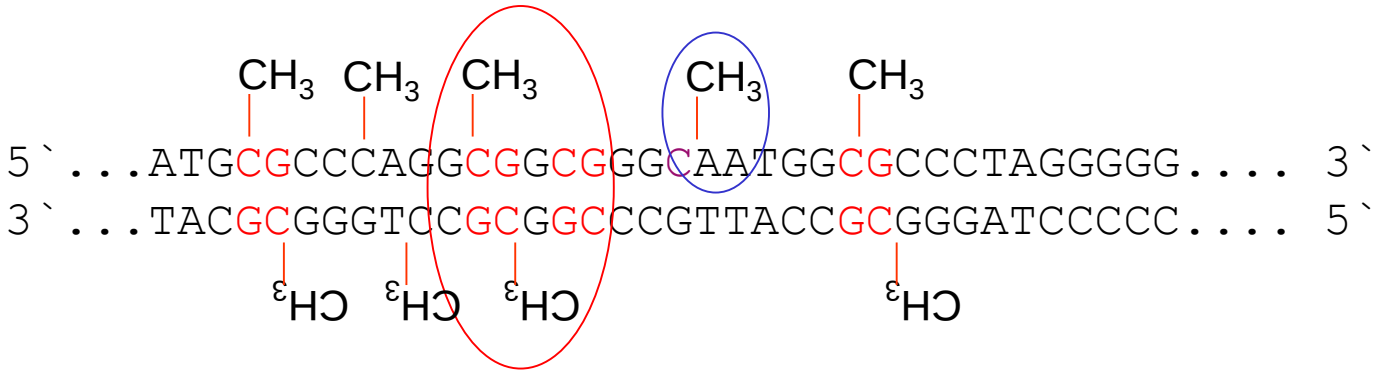
Amino acid in the catalytic center in DNA-methyltransferases

The enzyme binds to the C6 position covalently through the cysteine

DNA-methylation does not affect base pairing



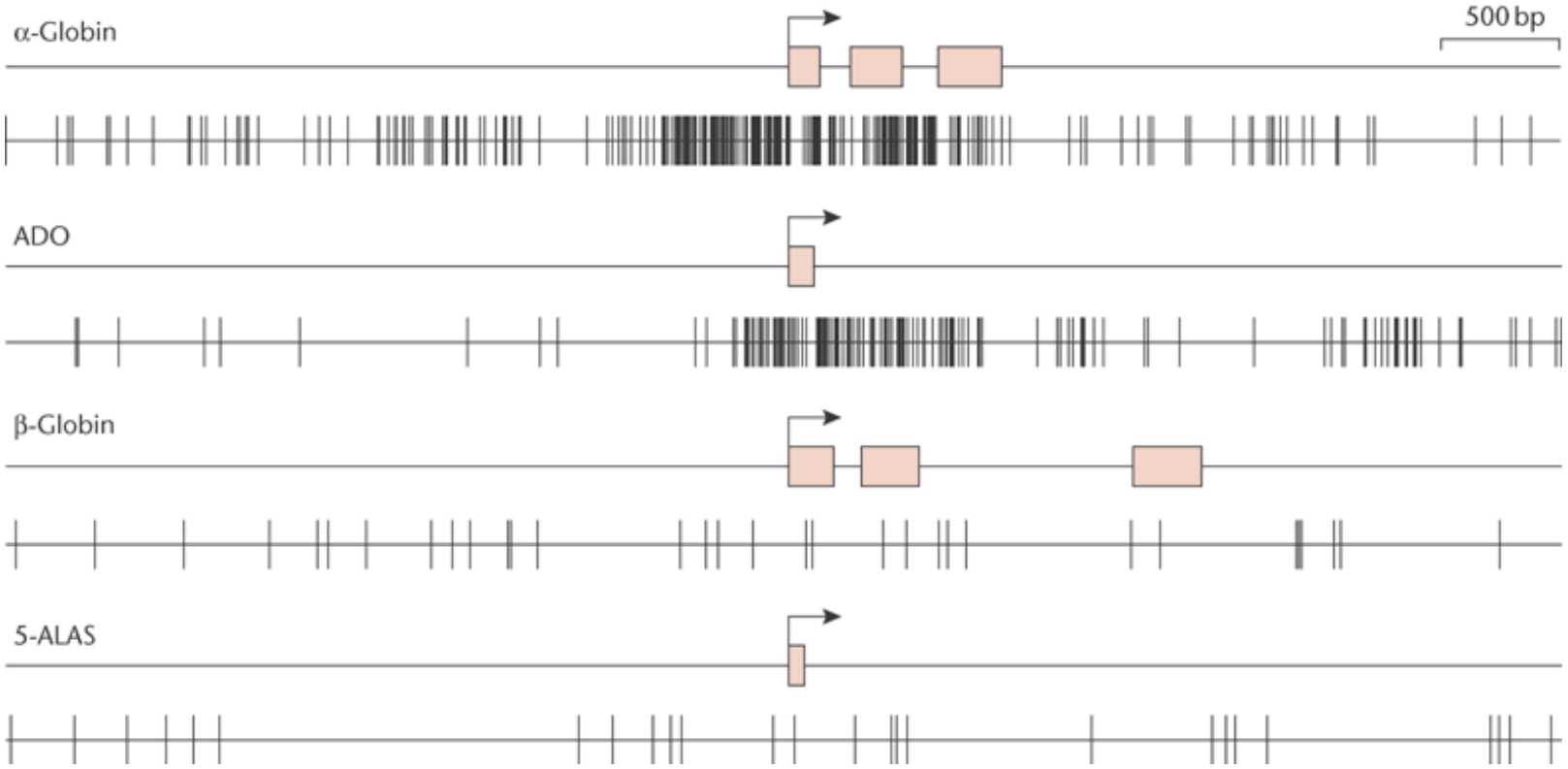
cytosine methylation in mammals/human



DNA methylation in mammals occurs almost exclusively at CpG dinucleotides.

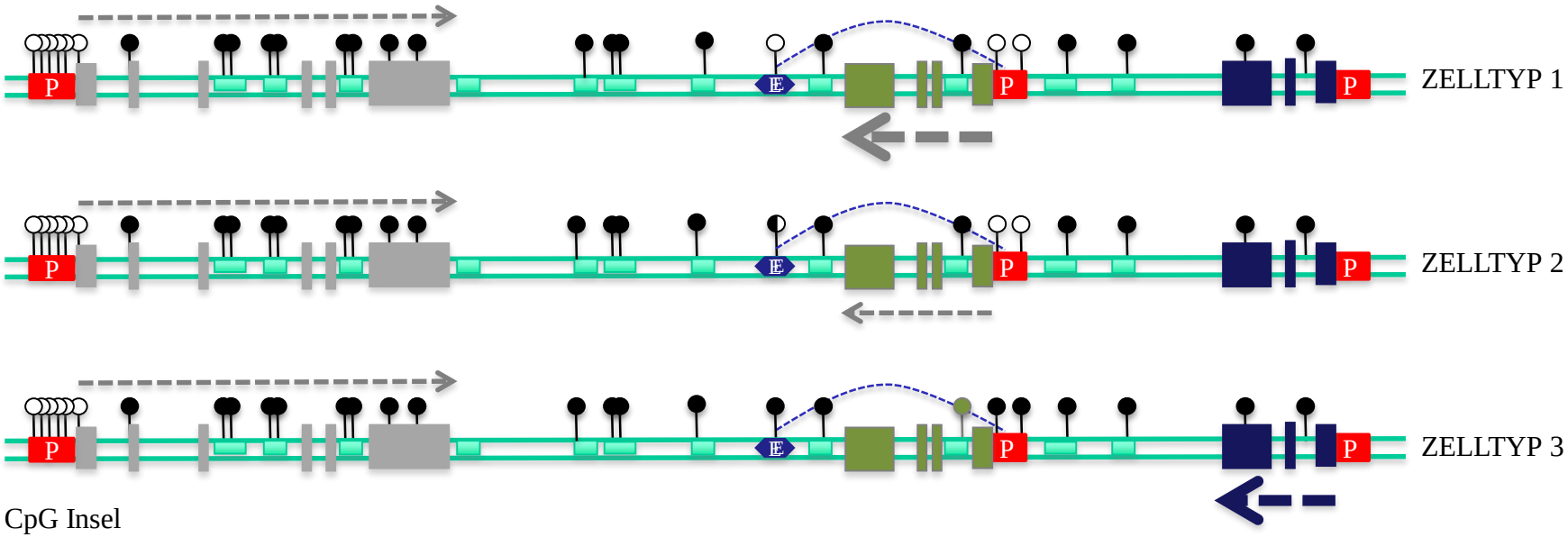
In stem cells cytosines can be methylated at non-CpG positions such as CNG sequence context or any CA(N)

The CpG target site for DNA methylation are not equally distribution in mammalian genomes



The picture outlines the unequal distribution of CpGs in the genome of mammals aroung three genes – the dense regions are CpG islands

DNA methylation distribution in mammalian genes



Promoter P
mit CpG Insel

Repetetives
Element

enhancer E
CpG-arm

Promoter P
CpG-arm

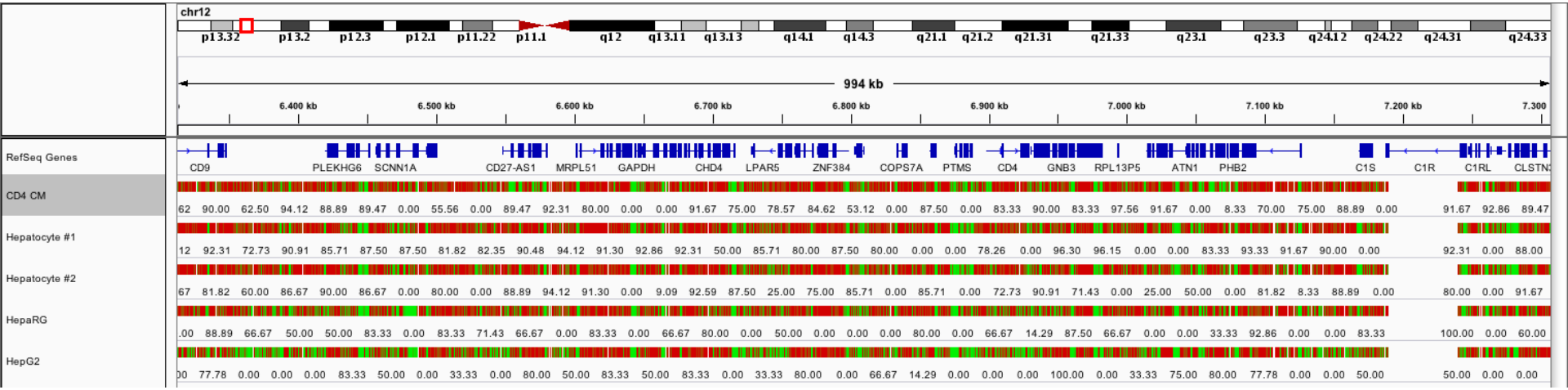
Promoter P
ohne CpG

methyliertes CpG

unmethyliertes CpG

Transkript

Exon



Green: low DNA-methylation

Red: high DNA- methylation

DNA-methylation in human/mammals

- The CpG dinucleotide is relatively rare in the DNA.
- Less than 2% of all nucleotides are CpG's and their genomic distribution is non-random.
- Most CpGs are found in clusters, so called CpG islands which are mostly not methylated.
- CpG islands are found in promoters/ 5' end of about 50% of all genes.
- An exception are CpG islands on the Xi and some imprinted genes which are methylated in one of the alleles.

DNA-methyltransferases in mammals/human

DNA-Methylation is catalysed by different DNA-methyl-transferases (= DNMTs)

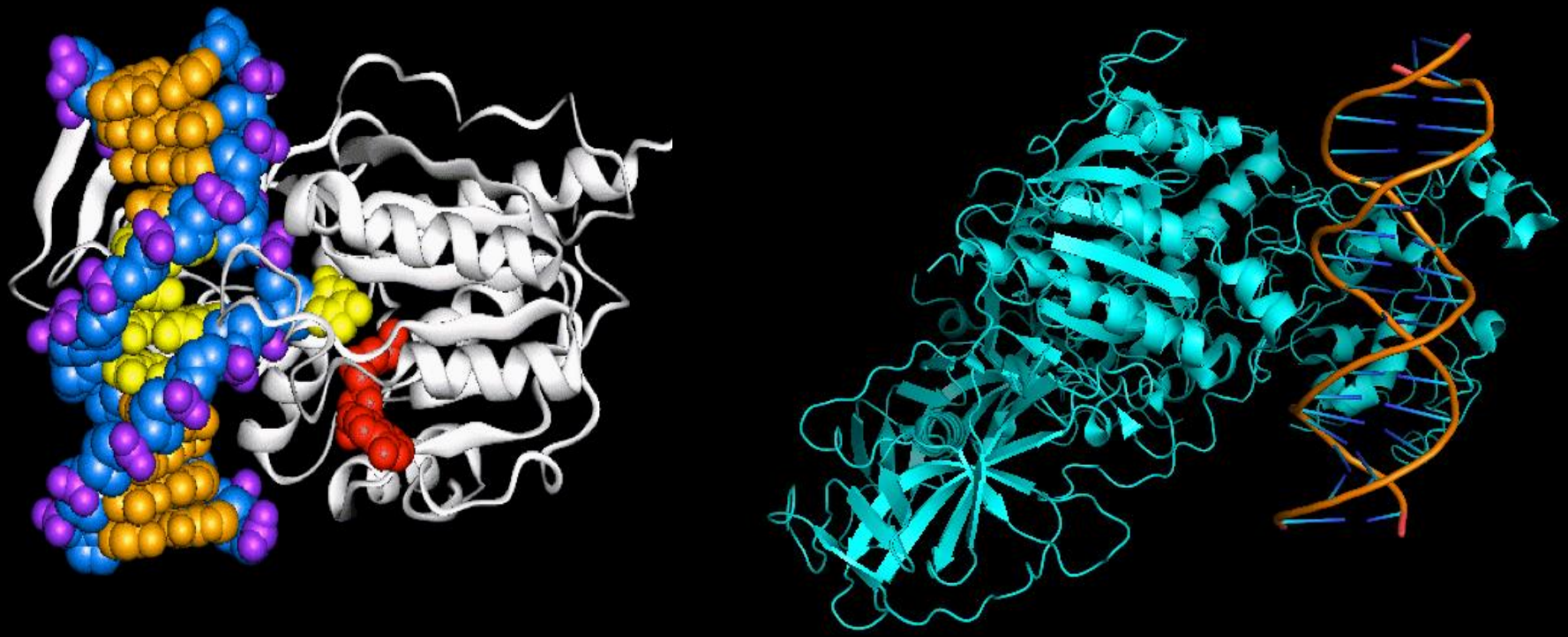
All five know DNMTs methylate cytosine in CpG‘s

DNMTs interact with other proteins which direct them to the „place“ where they methylate

DNMTs in mammals have different functions

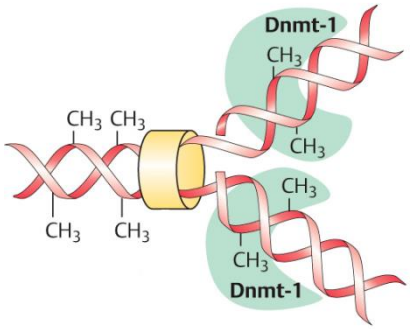
Bacterial M.HhaI

Human Dnmt1

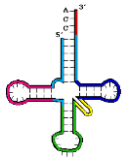


Mammalian DNA-Methyltransferases (DNMT ‘s)

‚Maintenance‘ Methyltransferase



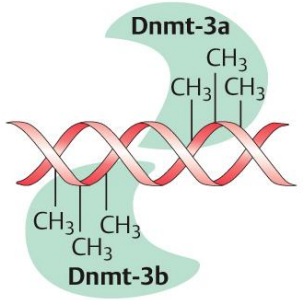
t-RNA methylatransferase‘



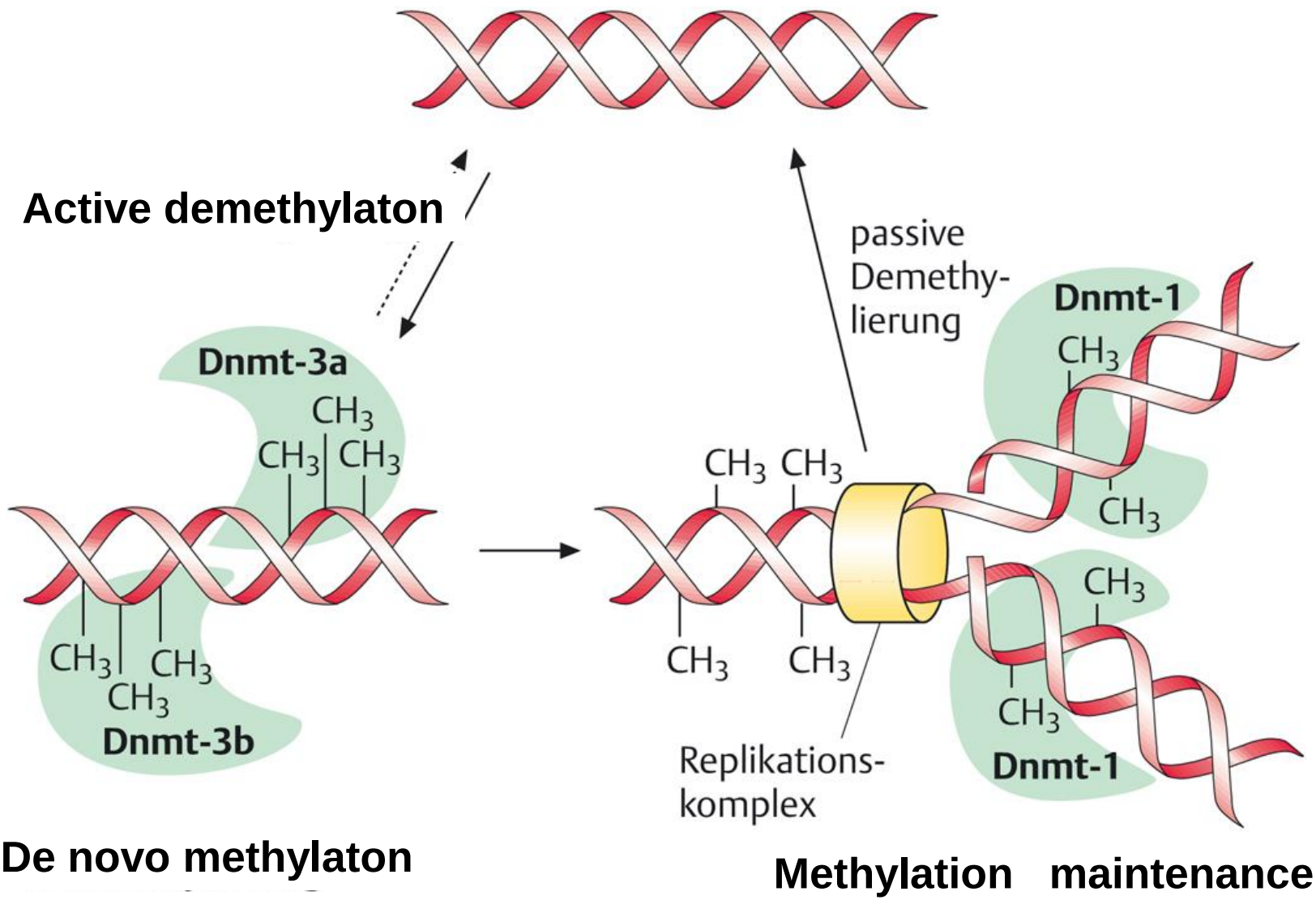
tRNA

‚De novo methyltransferases‘

reg.



DNA-methylation: general steps



Georg Thieme Verlag, Stuttgart
Knippers: Molekulare Genetik, 9. Auflage · 2006

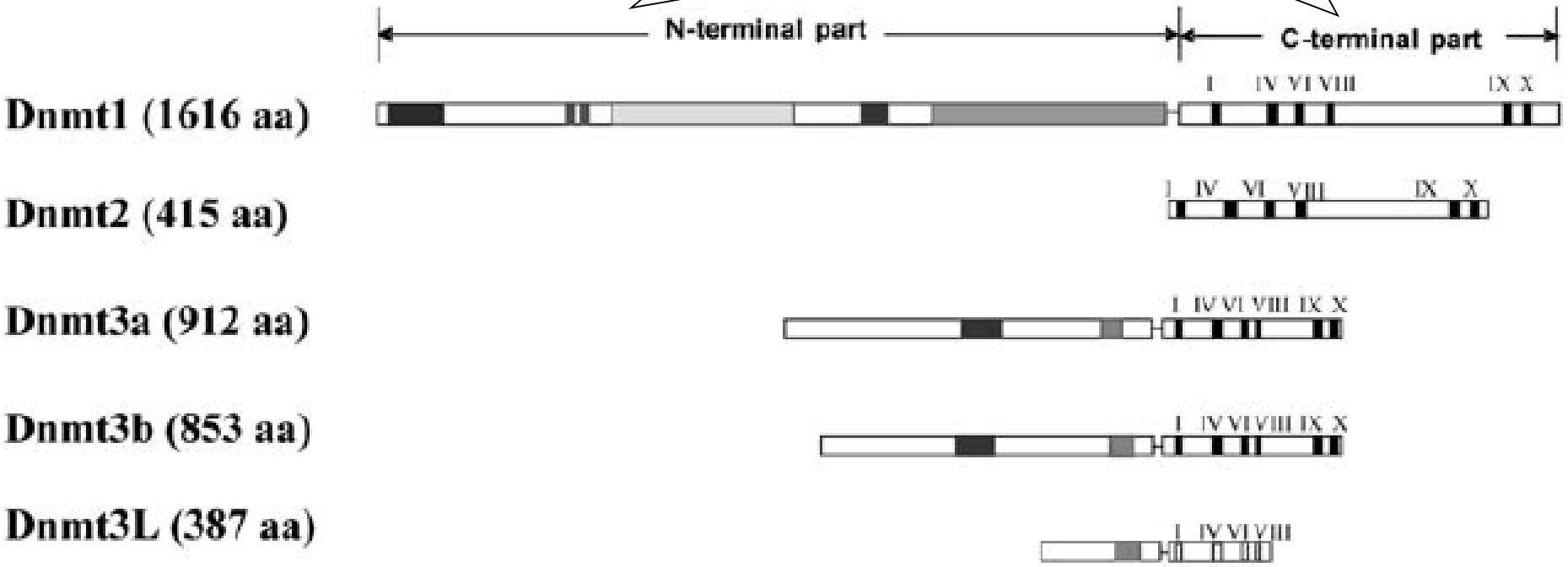
Mammalian DNA-methyltransferases

Regulatory domain:

- Association with other proteins and targeting to sequences
- Nuclear import/export
- Recognition of methylation status

Catalytic domain:

- DNA-binding
- Co-factor binding
- Catalytic center



DNA-Methyltransferase 1 = DNMT 1

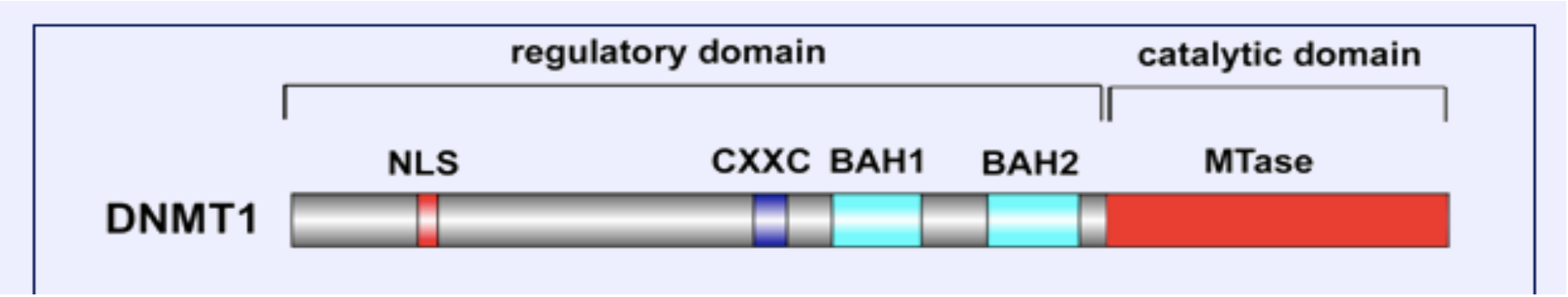
→ DNMT1 is a maintenance methyltransferase responsible for the inheritance (copying) of methylation patterns upon cell division

→ DNMT1 prefers hemimethylated DNA as a substrate

→ DNMT1 has a major function during **S-Phase** (DNA-Replication)

- The DNMT1 maintenance function is important to:
- regulate coordinated gene expression during development
- silence promoters of „junk DNA“
- stabilize genomic mprinting

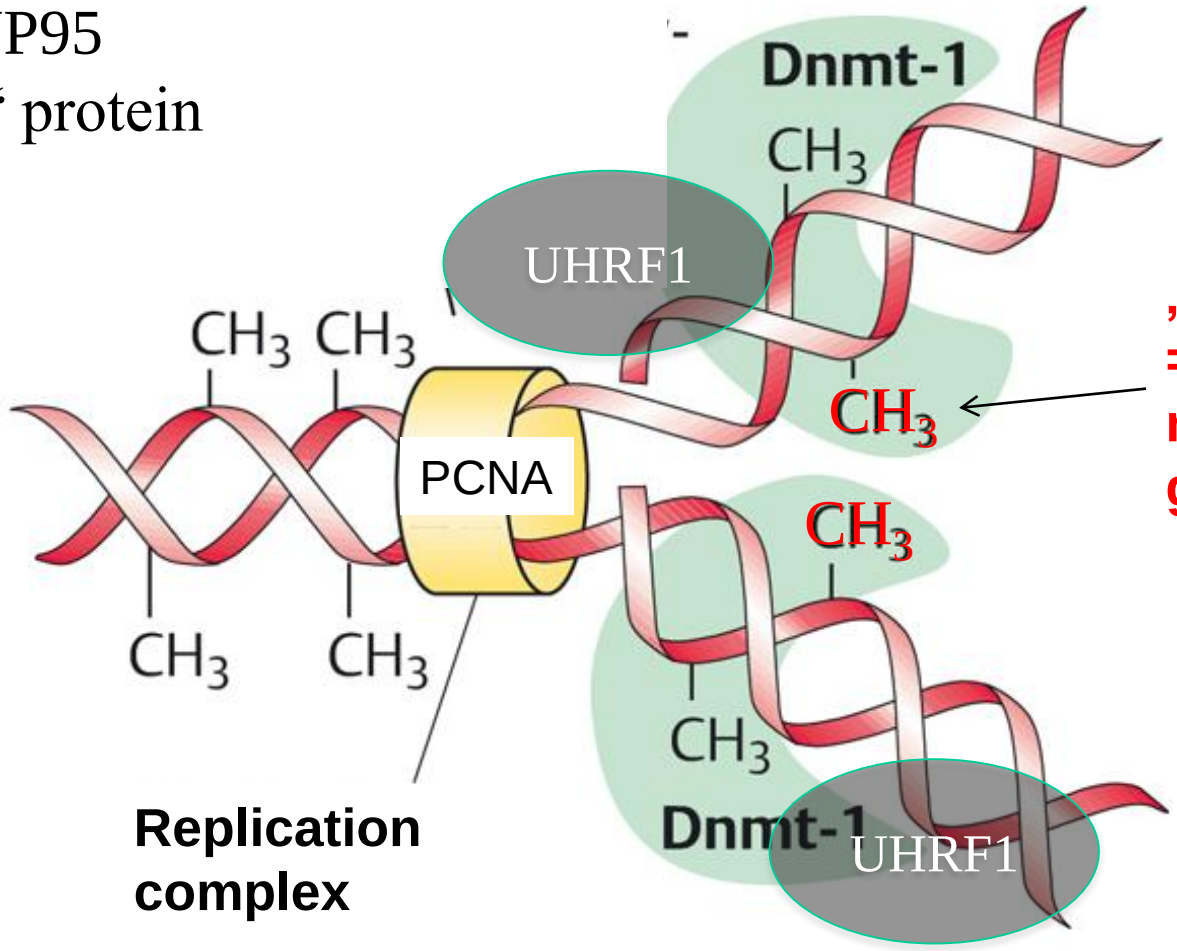
DNMT 1 domain structure



Dnmt1 protein domains:
NLS, nuclear localization signal;
CXXC, a cysteine rich region;
BAH1 and 2, bromo-adjacent homology domains;
MTase, a methyltransferase domain.

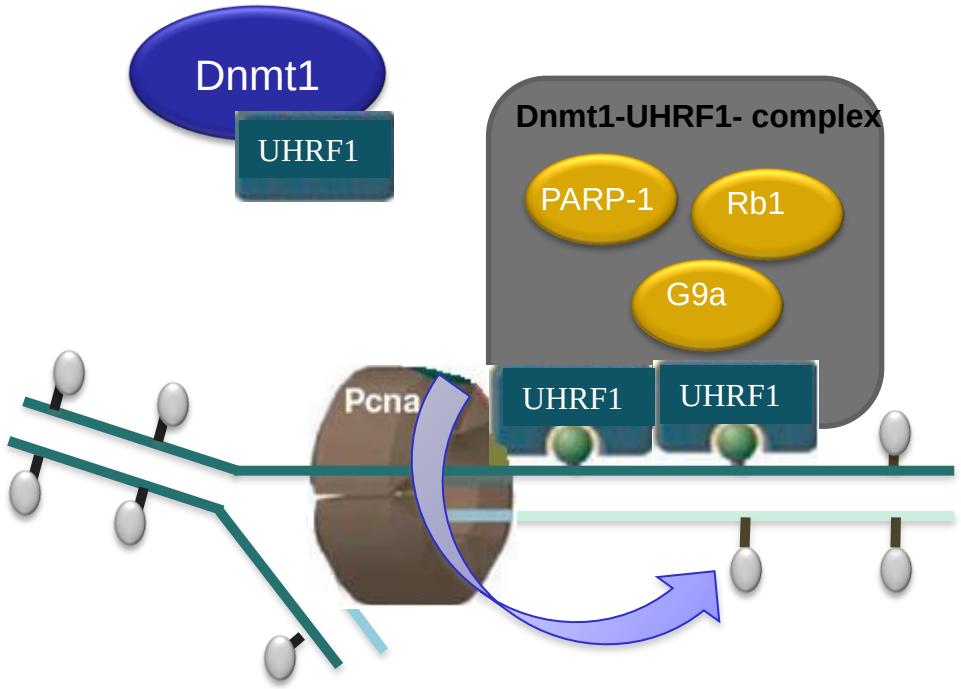
Dnmt1 and DNA-methylation maintenance

UHRF1 = NP95
is a „helper“ protein
for Dnmt1



Methylation maintenance

Regulation of DNMT1 maintenance activity by UHRF1



PARP1 =
PolyADP ribosylation protein

G9A =
HMT modifying H3K9me3

Rb1 =
Retinoblastoma associated Protein
Cell cycle dependent
transcription factor

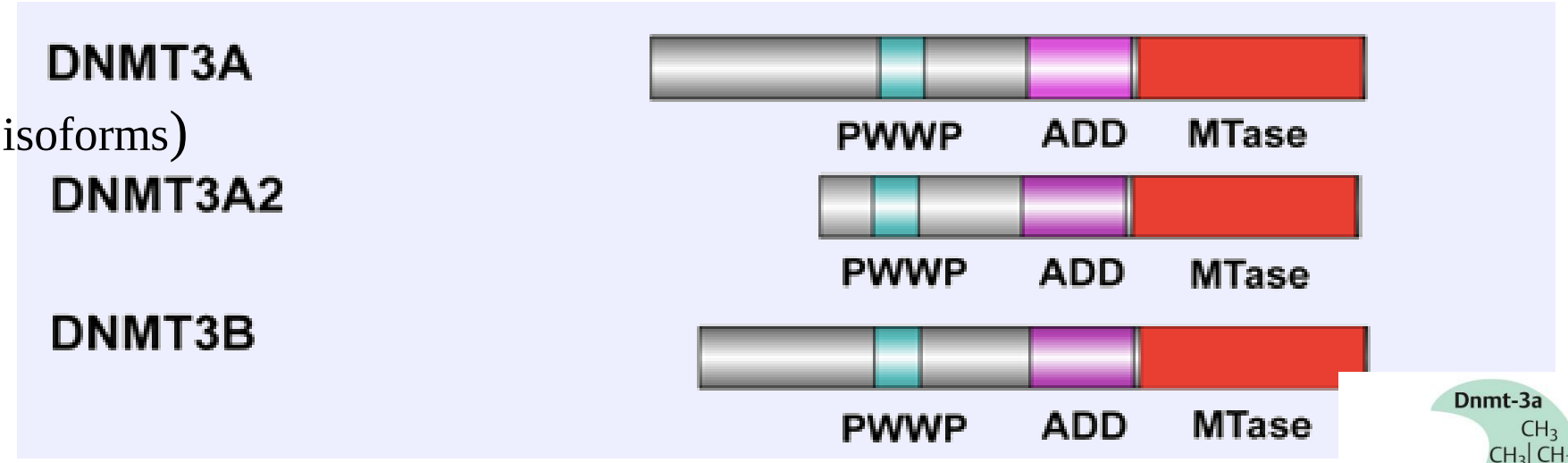
PCNA =
Proliferation ccell nuclear antigen

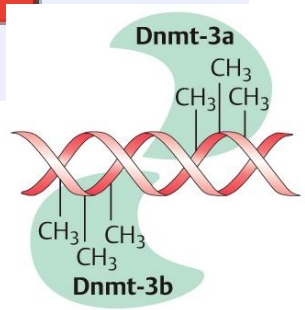
UHRF1) coordinates the setting and spreading of heterochromatic information through interaction with chromatin modifiers such as G9A (a H3K9me2/3 specific histone methyltransferase)

DNA-methyltransferase DNMT1

- The maintenance methyltransferase DNMT1 comprises about 1620 aa – it has three isoforms (different regulatory N-terminus).
- DNMT1 has a long amino-terminal domain for interactions with regulatory/modifying proteins/complexes such as URHF1/PCNA (replication) HDACs (changes of histone-modification) and co-repressors (targeting of the methylation reaction) and others.
- The C-terminal catalytic domain of DNMT1 is very similar to bacterial enzymes.
- DNMT1 prefers „in vivo“ hemimethylated DNA as a substrate - but has the capacity to methylate unmethylated DNA de novo „in vitro“.
- Mutations (KO) of DNMT1 cause a genome wide demethylation and results in an embryonic lethality

DNMT3 A/B DNA-Methyltransferases





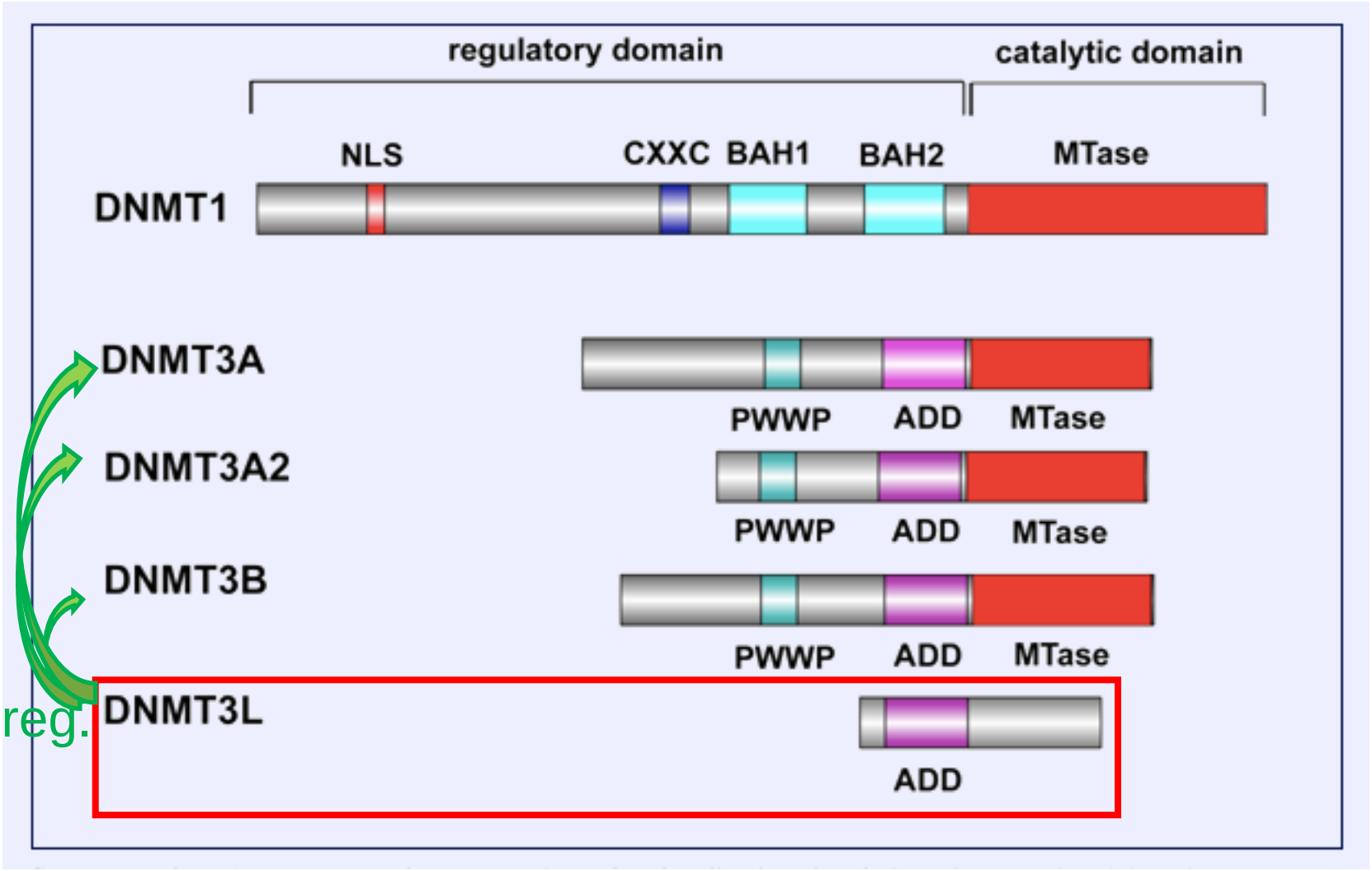
DNMT3A/B protein domains

NLS, nuclear localization signal;
PWWP, a proline-tryptophan-tryptophan-proline domain;
ADD, an ATRX-DNMT3-DNMT3L-type zinc finger domain;
MTase, a methyltransferase domain.

DNMTs 3a and 3b (and Dnmt3c)

- Both DNMT3A and DNMT3B can modify unmethylated DNA „de novo“, but can also methylate hemimethylated DNA substrates.
- Their NH2 terminal regulatory region is shorter that that of DNMT1.
- DNMT3A and B have distinct and overlapping functions during development.
- Both enzymes are very important to recognize and methylate repetetive elements in the genome.
- DNMT 3a and b are essential for development but their functions are partially redundant
- Single knockouts (mutations) are partially viable but double KO‘s (mutants) cause an early embryonic lethality with defects in organ development.

DNMT3L

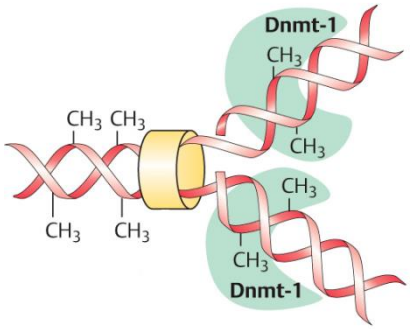

regulatory domain
catalytic domain
NLS
CXXC
BAH1
BAH2
MTase
DNMT1
DNMT3A
PWWP
ADD
MTase
DNMT3A2
PWWP
ADD
MTase
DNMT3B
PWWP
ADD
MTase
reg.
DNMT3L
ADD

DNMT3L

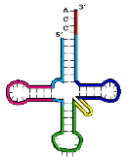
- DNMT-like protein of 387AS has no similartity in the N-terminal domain to other DNMTs.
- Has some sequence similarity in the catalysitc domain but lacks important motifs such as the catalytic center and the SAM binding domain.
- DNMT3L has no own intrisinc methyltransferase activity.
- Dnmt3L guides the *de-novo*-DNMTs 3a and 3b to targets.
- Is important for the establishment of maternal imprints during oogenesis and paternal imprints during spermatogenesis.
- Binds to unmodified Histone H3K4.

DNA-Methyltransferasen (DNMT ‘s)

‚Maintenance‘ Methyltransferase



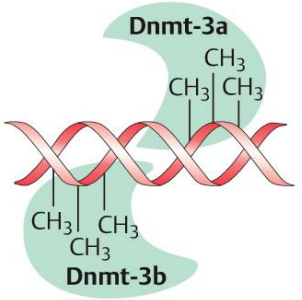
t-RNA methyltransferase‘



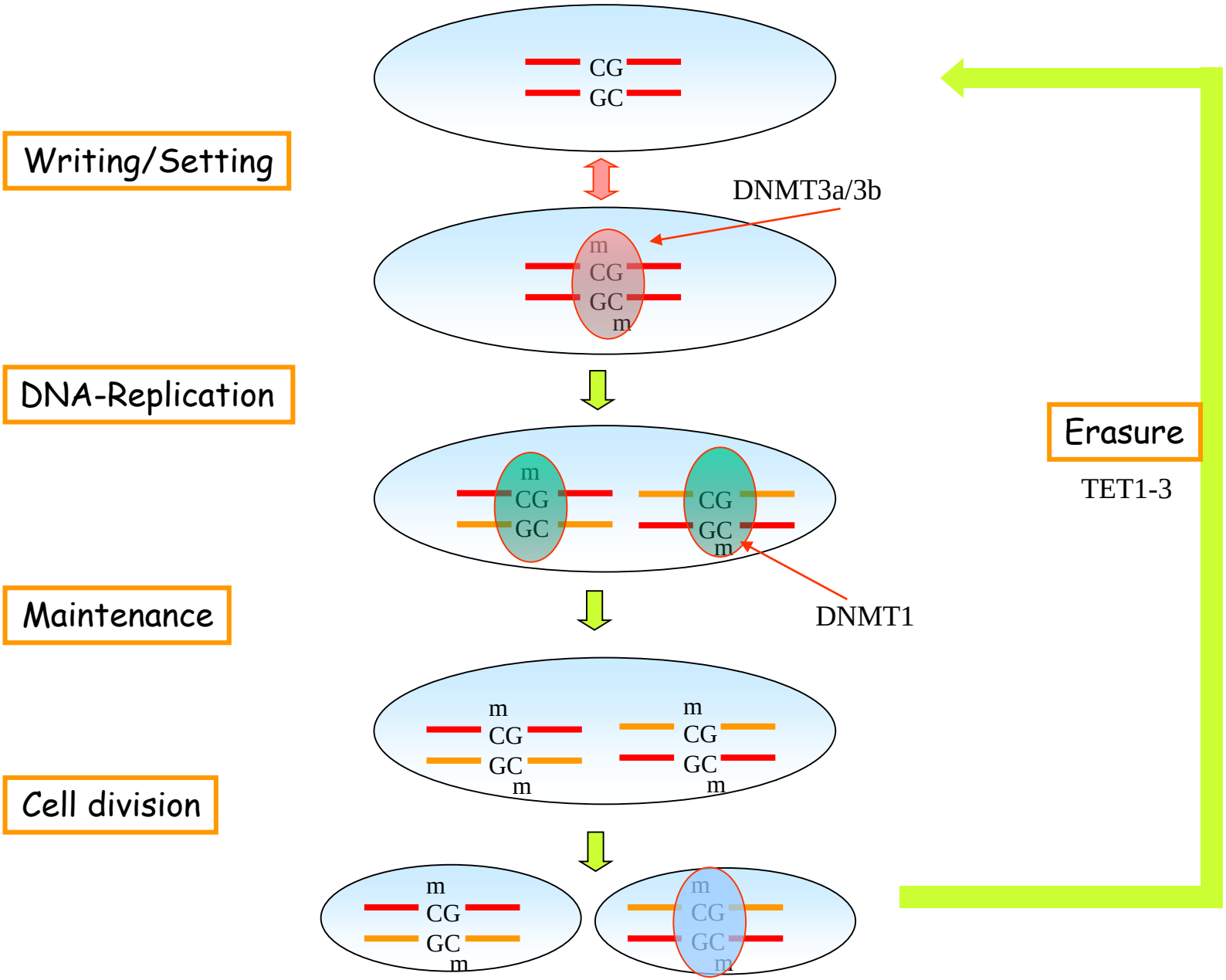
tRNA

‚De novo methyltransferases‘

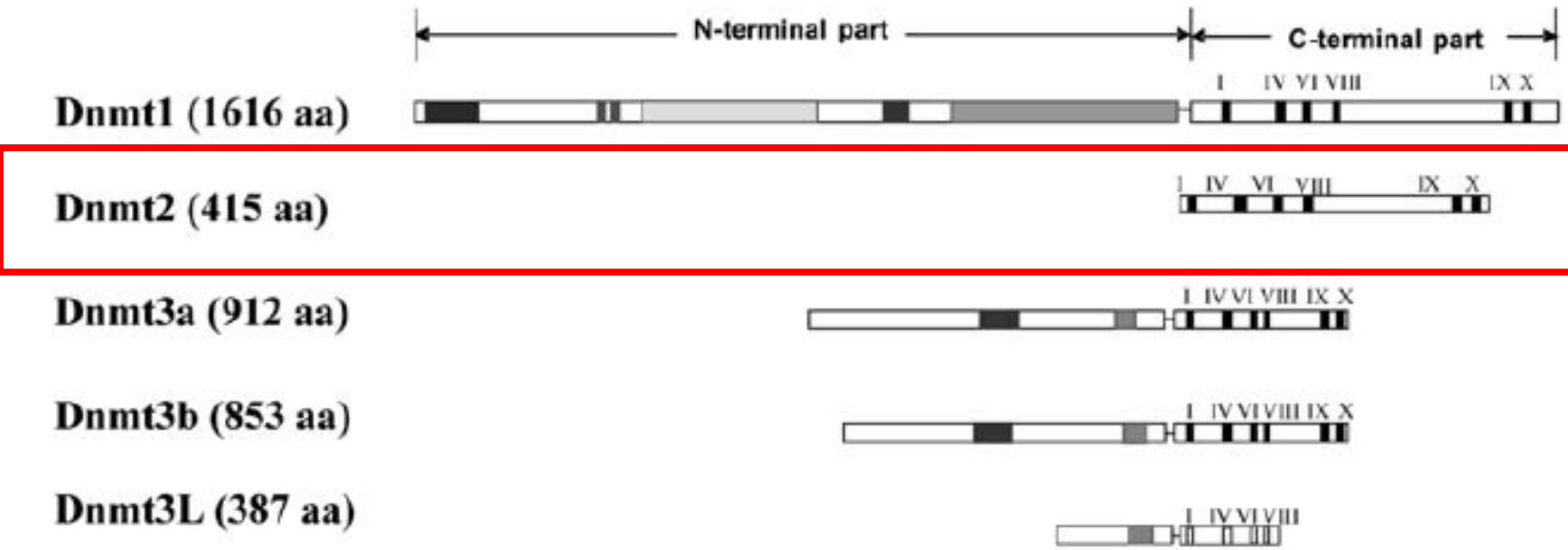
reg.



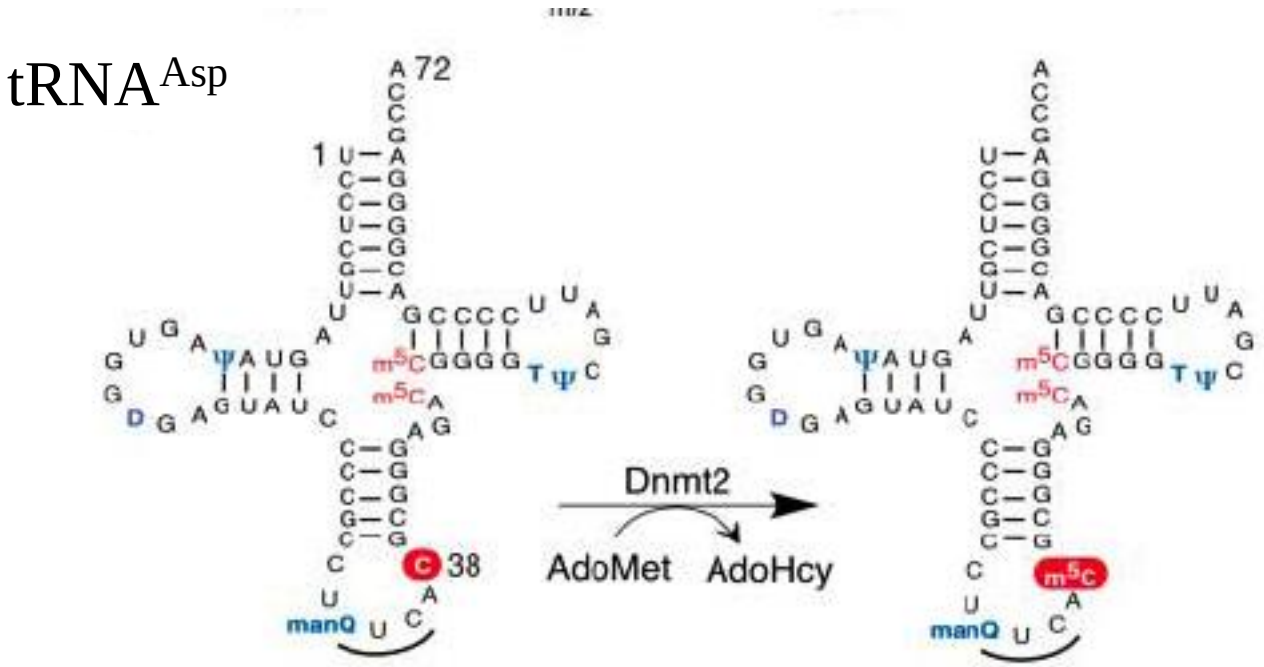
The „on“ and „off“ of DNA-Methylation



DNMT2


N-terminal part
C-terminal part
Dnmt1 (1616 aa)
I IV VI VIII IX X
Dnmt2 (415 aa)
I IV VI VIII IX X
Dnmt3a (912 aa)
I IV VI VIII IX X
Dnmt3b (853 aa)
I IV VI VIII IX X
Dnmt3L (387 aa)
I IV VI VIII

DNMT2 looks like a DNA-methyltranferase but acts as a tRNA methyltransferase



DNMT2 has a typical DNA-Methyltransferase structure. But it specifically methylates a cytosine at position 38 near the anticodon loop in tRNA^{Asp} . The methylation protects the tRNA from degradation.

Reading and interpretation of DNA-methylation:

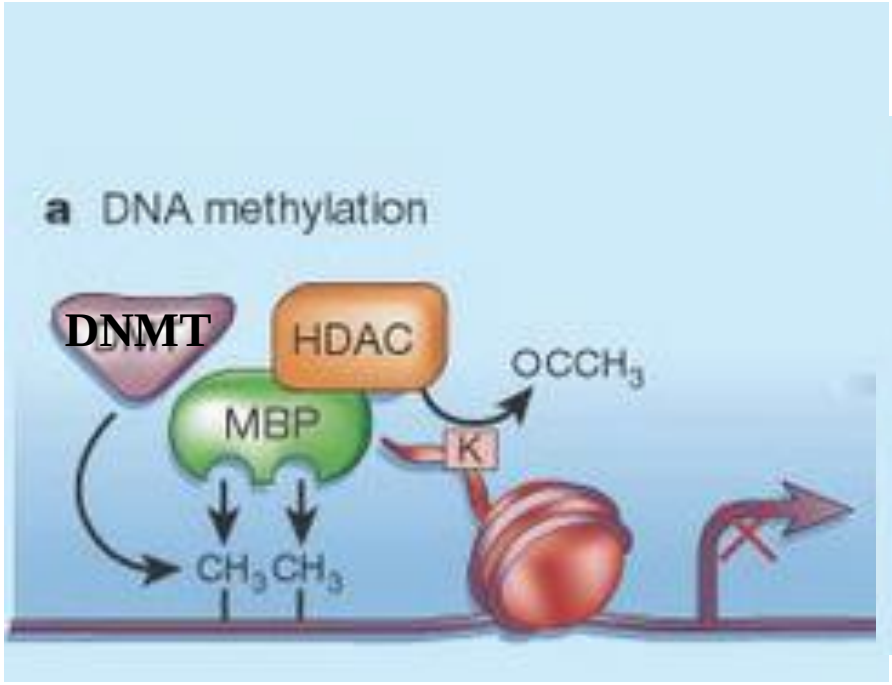
DNA-methylation is recognized by specific binding proteins: **M**ethylated DNA **B**inding **D**omain
MBD1, MDB2, MBD3, MBD4, MeCP2

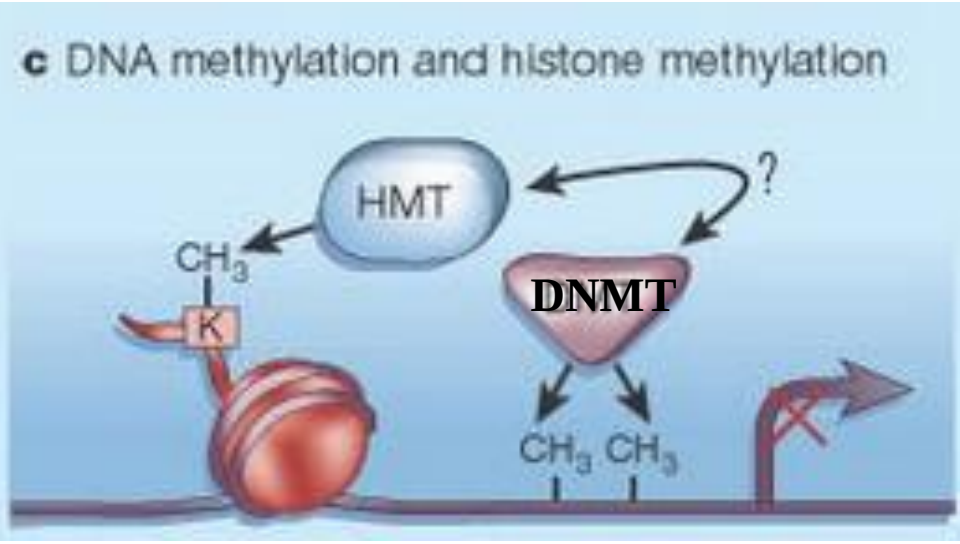
and some methylation specific transcription factors:
Kaiso, ZBTB33,....

MBD proteins are often present in chromatin modifying (repressor) complexes (such as NuRD).

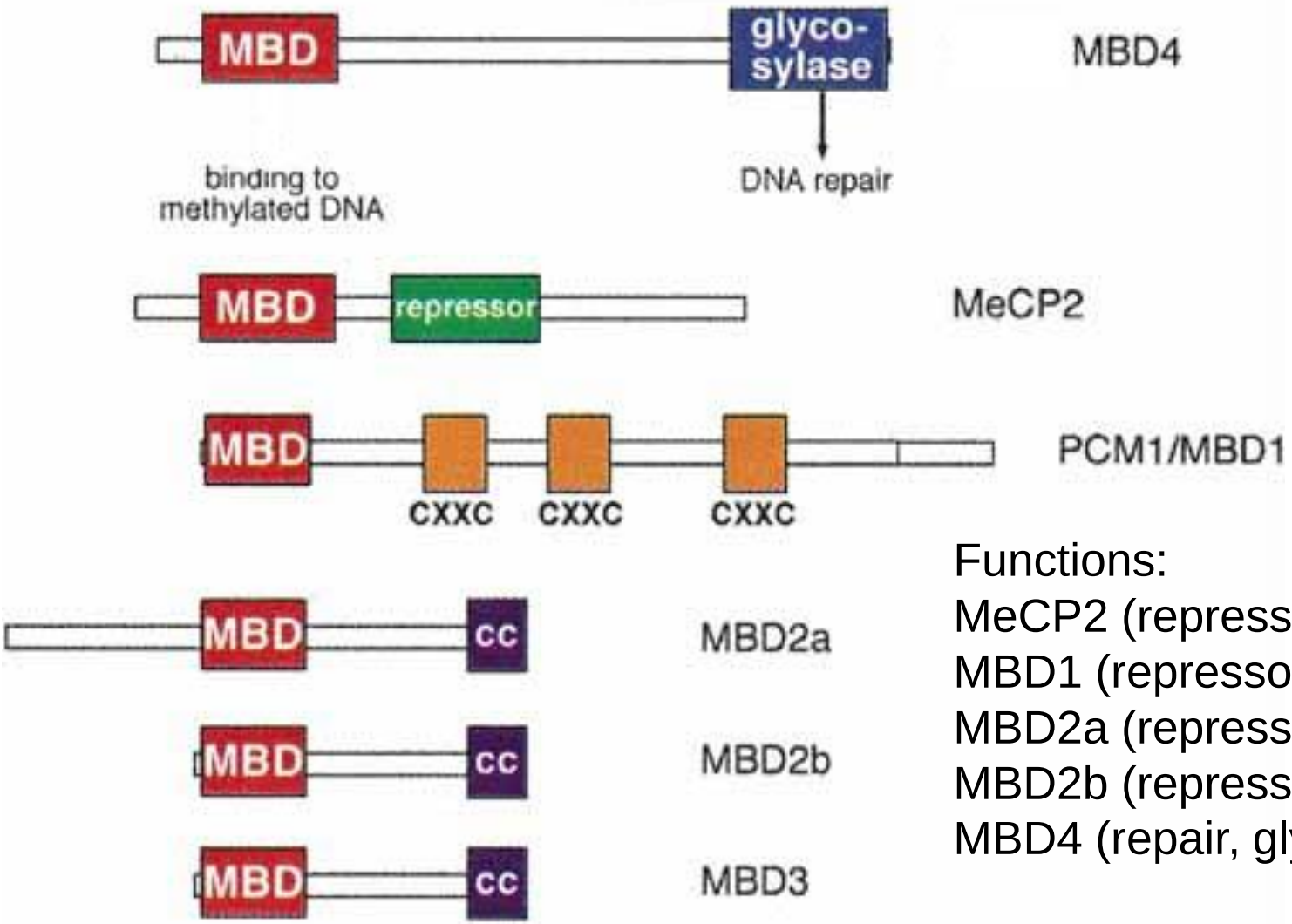
They mediate a „crosstalk“ between heterochromatic histone- and DNA-modifications.

DNA-methylation readers: Methyl binding proteins (MBDs)





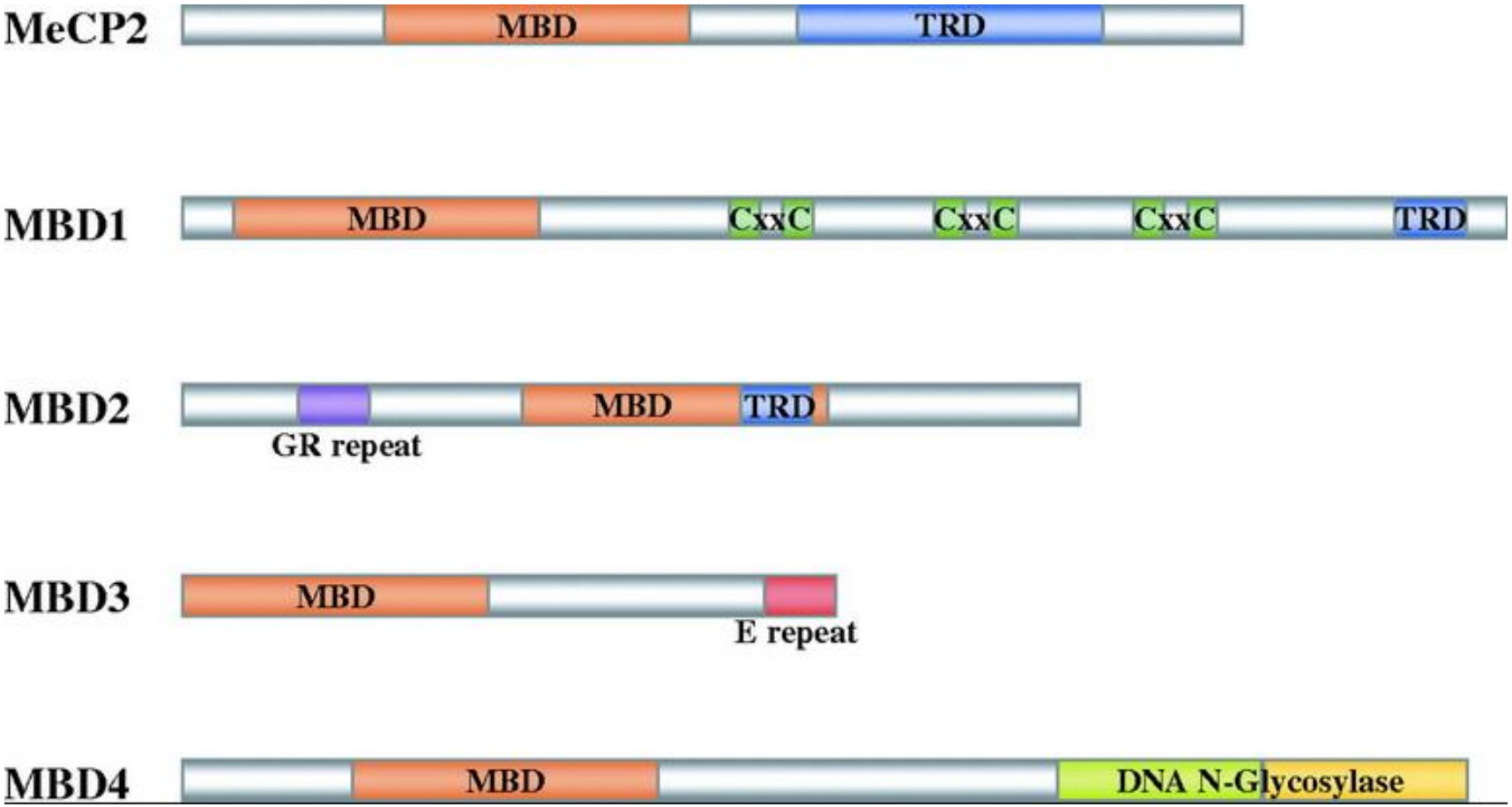
DNA-methylation binding (MBD domain) proteins

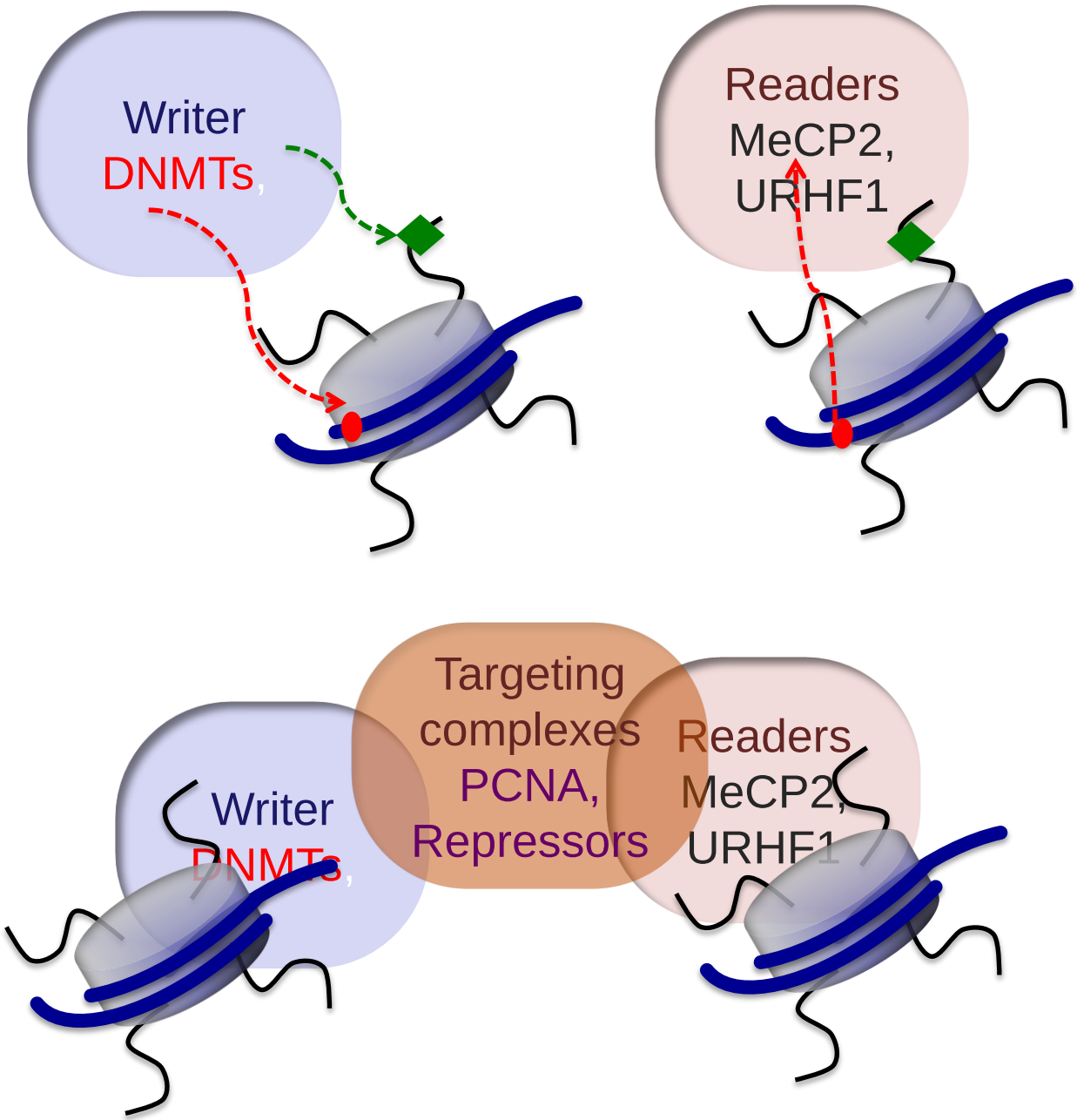


Functions:
MeCP2 (repressor)
MBD1 (repressor)
MBD2a (repressor)
MBD2b (repressor)
MBD4 (repair, glycosylase)

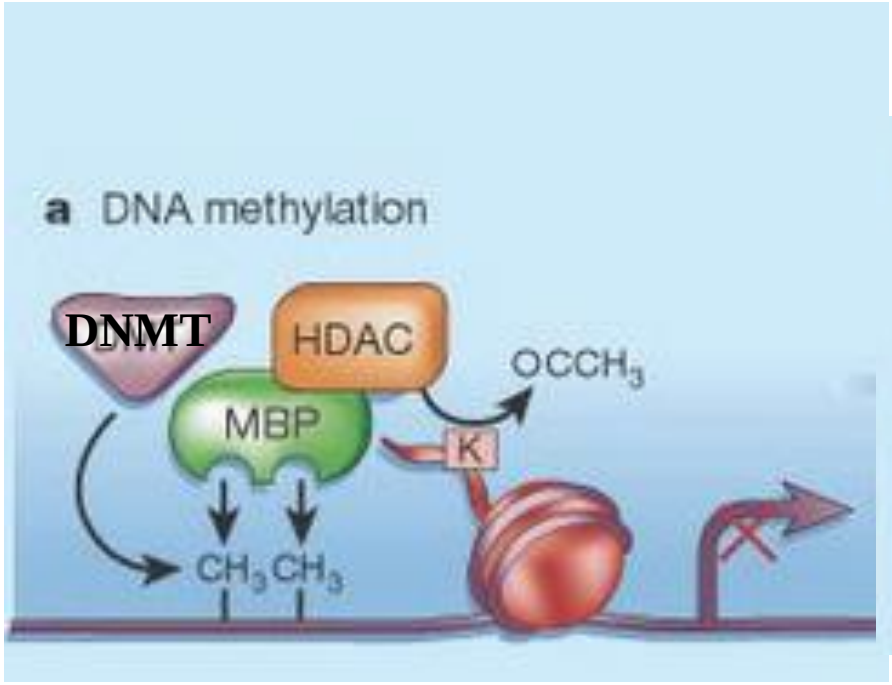
Interpretation and reading of DNA methylation is mediated by methyl-CpG binding proteins containing MBD domains for 5meCytosine recognition

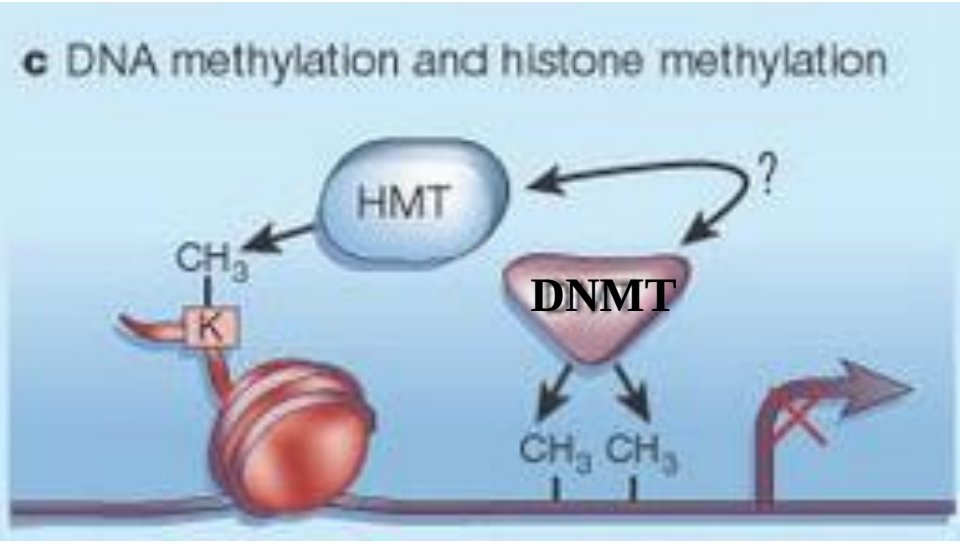
DNA-methylation binding (MBD domain) proteins



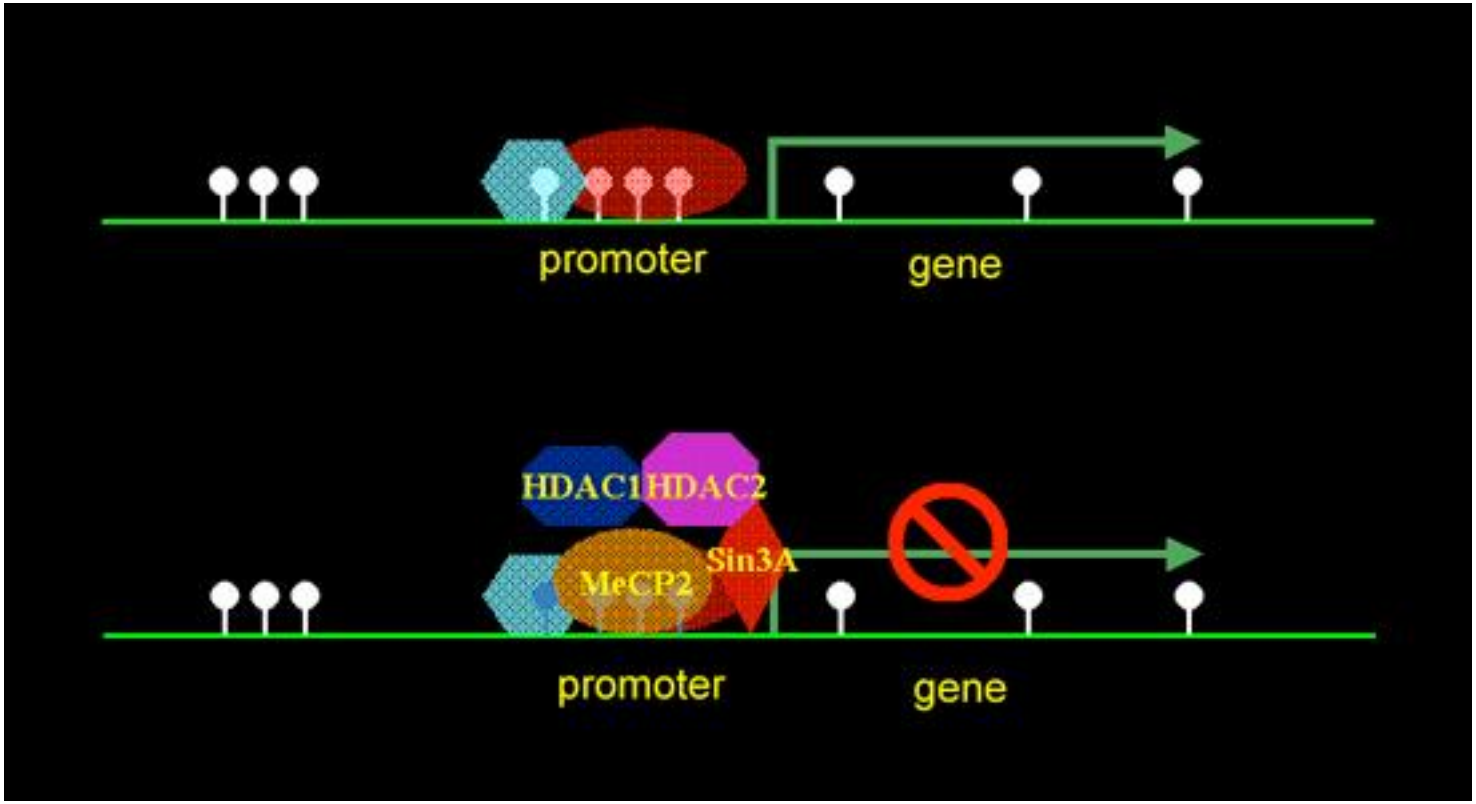

Writer
DNMTs,
Readers
MeCP2,
URHF1
Targeting
complexes
PCNA,
Repressors
Writer
DNMTs,
Readers
MeCP2,
URHF1

DNA-methylation readers: Methyl binding proteins (MBDs)



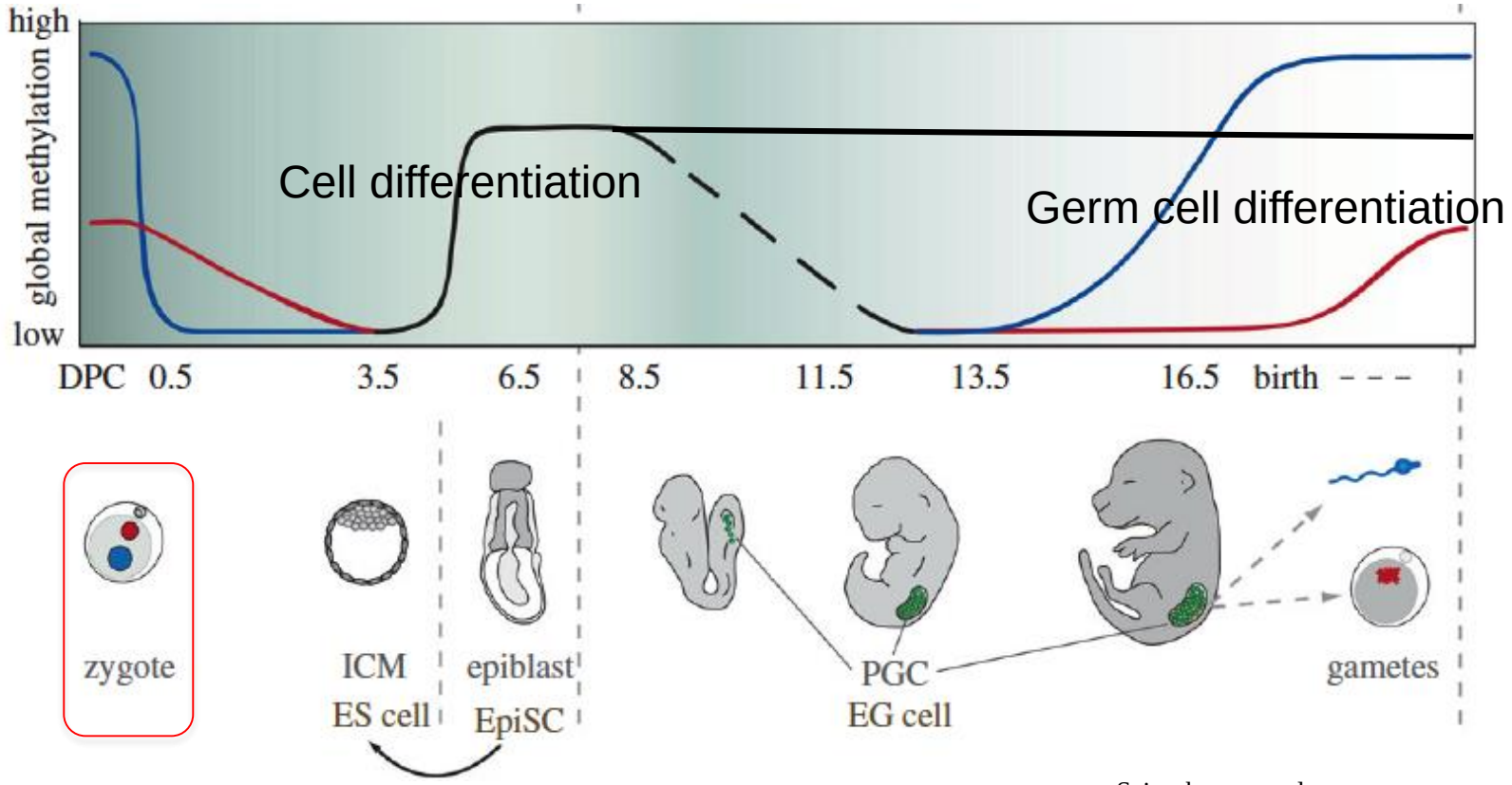


DNA-methylation: sequential model for its regulatory role at promoters

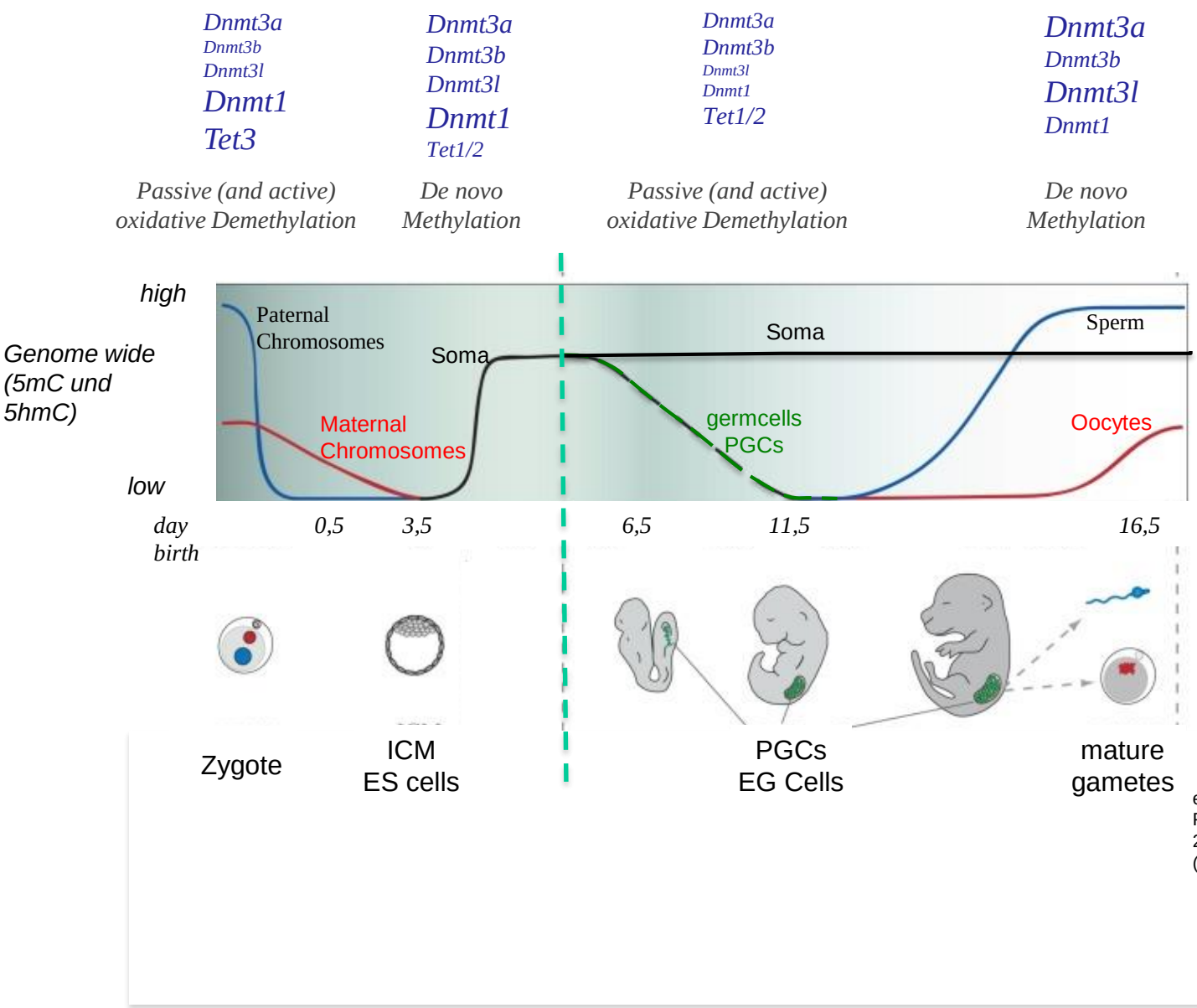


How is DNA methylation regulated:
Which mechanisms regulate the removal of DNA-methylation?

Epigenetic reprogramming during development: active loss and gain of DNA demethylation



Seisenberger et al
Philos Trans R Soc Lond B Biol Sci.
2013 January 5; 368(1609).



eisenberger et al
Philos Trans R Soc Lond B Biol Sci. 2013 January 5; 368(1609).
(Modifies)

DNA-methylation of cytosines can be altered by oxidation

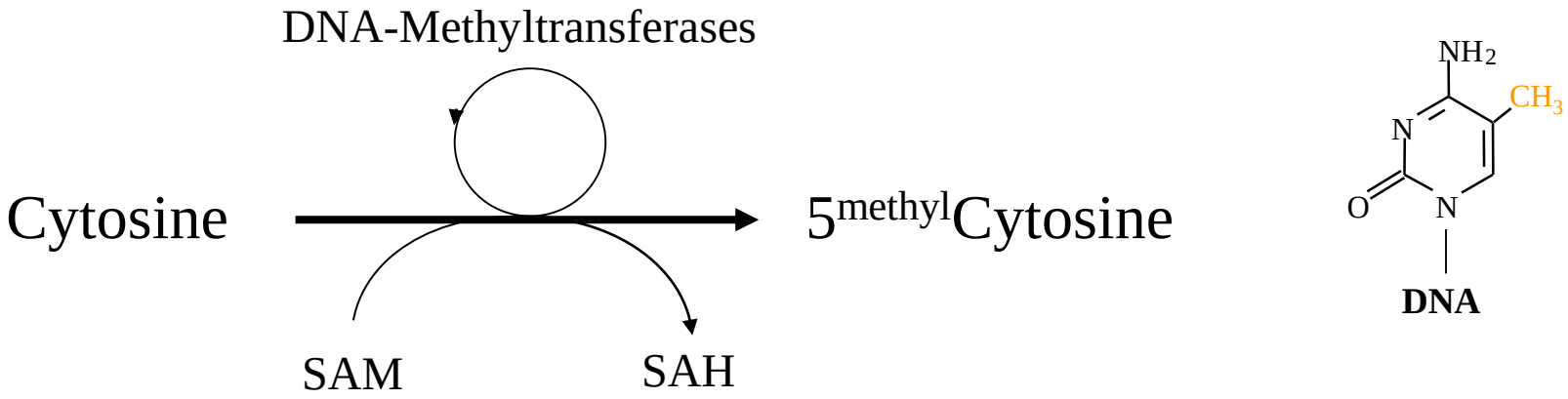
1. The oxidative forms of 5mC (5 methyl-cytosine)

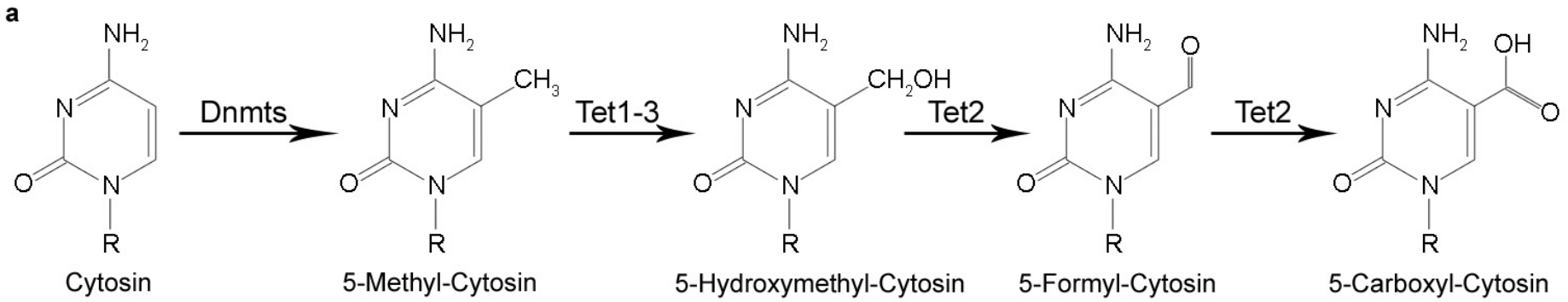
2. The enzymes catalysing this modification.

3. The proteins recogning (reading) this oxidation.

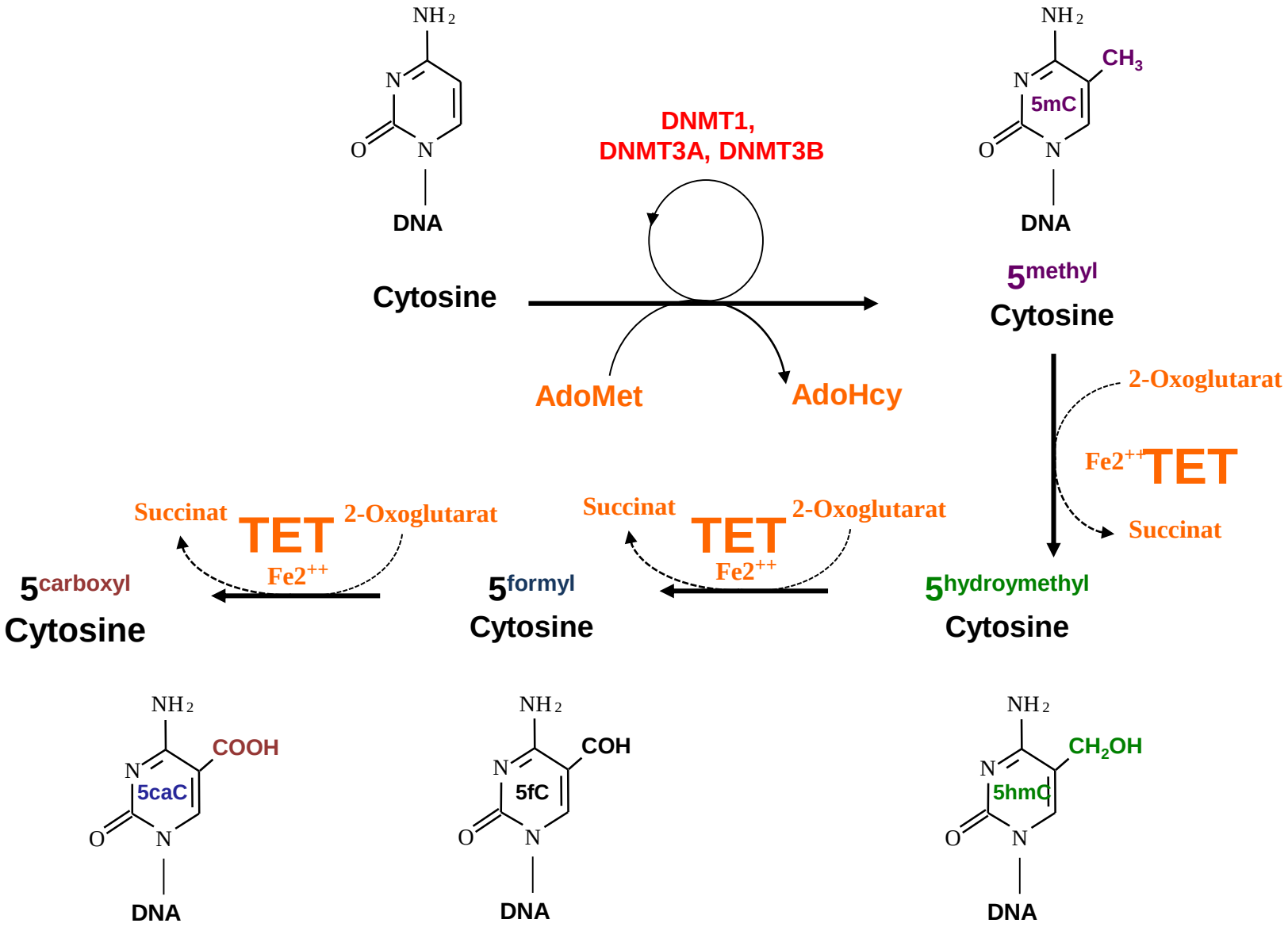
1. The function of oxidized forms in epigenetic control

DNA-methylation comes in different flavours





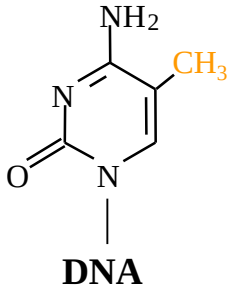
Oxidised forms of DNA-methylation



5-methylcytosine:

closed chromatin transcriptional repression

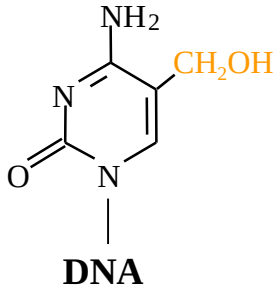
can be copied (maintained after replication)



5-hydroxymethylcytosine:

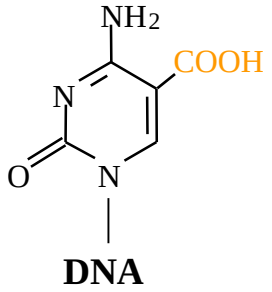
transcriptional effect unclear most likely non-repressive signal

unclear if 5hmC is recognized during replication to copy 5mC methylation on the new DNA strand



5-carboxyl-cytosine:

intermediate for DNA repair mediated demethylation



Presence of oxidized forms of DNA methylation

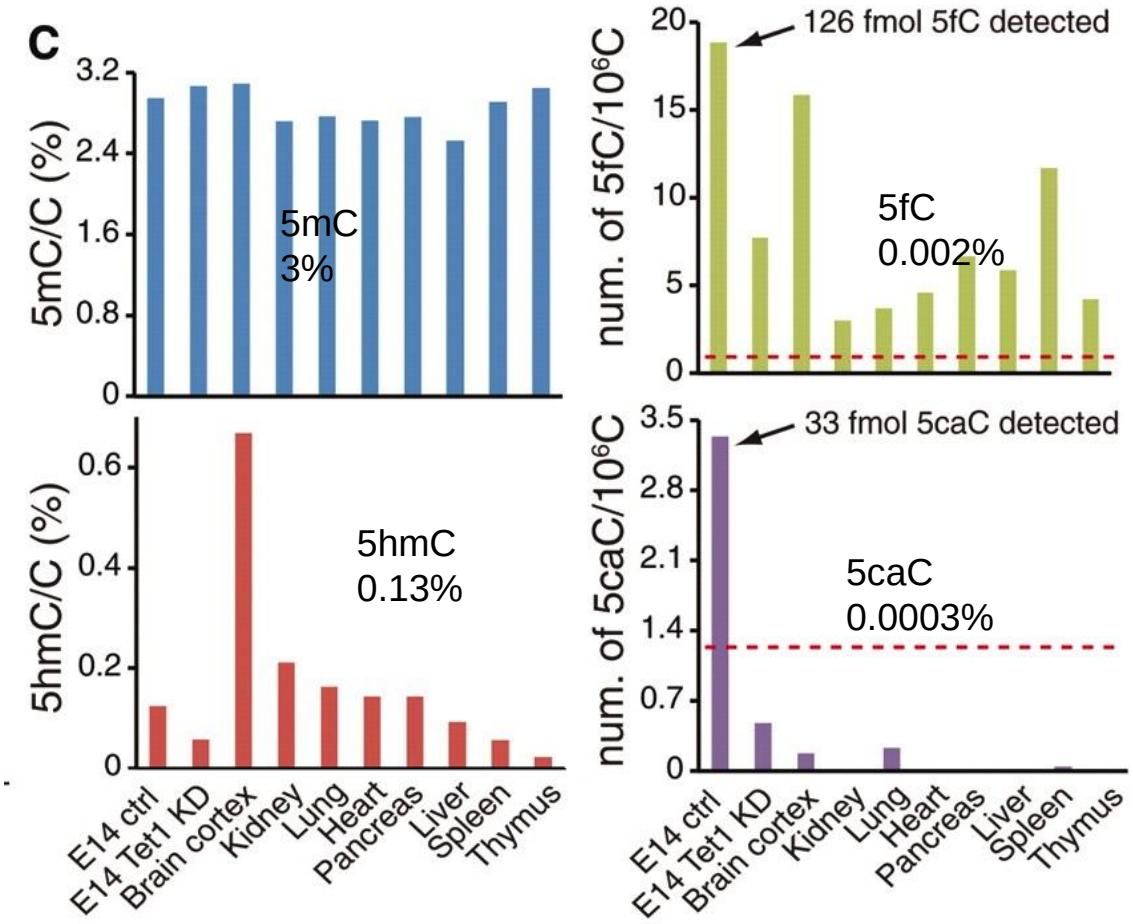
Oxidized forms are exclusively formed on 5mC (i.e. on methylated cytosine)

5mC > 5hmC > 5fC >5caC – the higher oxidised forms are barely detectable

Unlike 5mC the presence of 5hmC, 5fC and 5caC vary greatly from cell type to cell type

Highest amounts of 5hmC (and 5fC) are found in embryonic stem cells and in adult brain (neurons)

Presence of oxidized forms of DNA methylation in various cell types



Ito *et al.* 2011

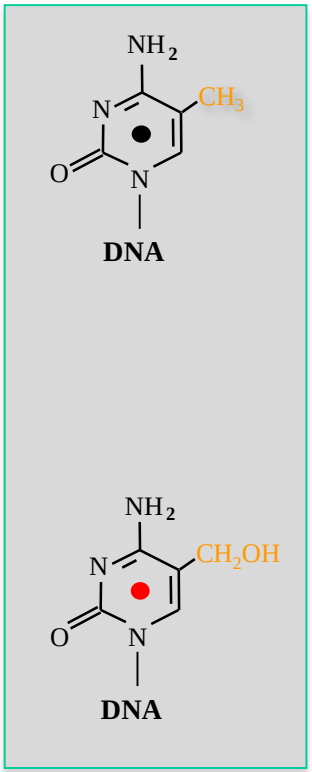
Functions of oxidised forms of 5mC: 5hmC, 5fC and 5caC

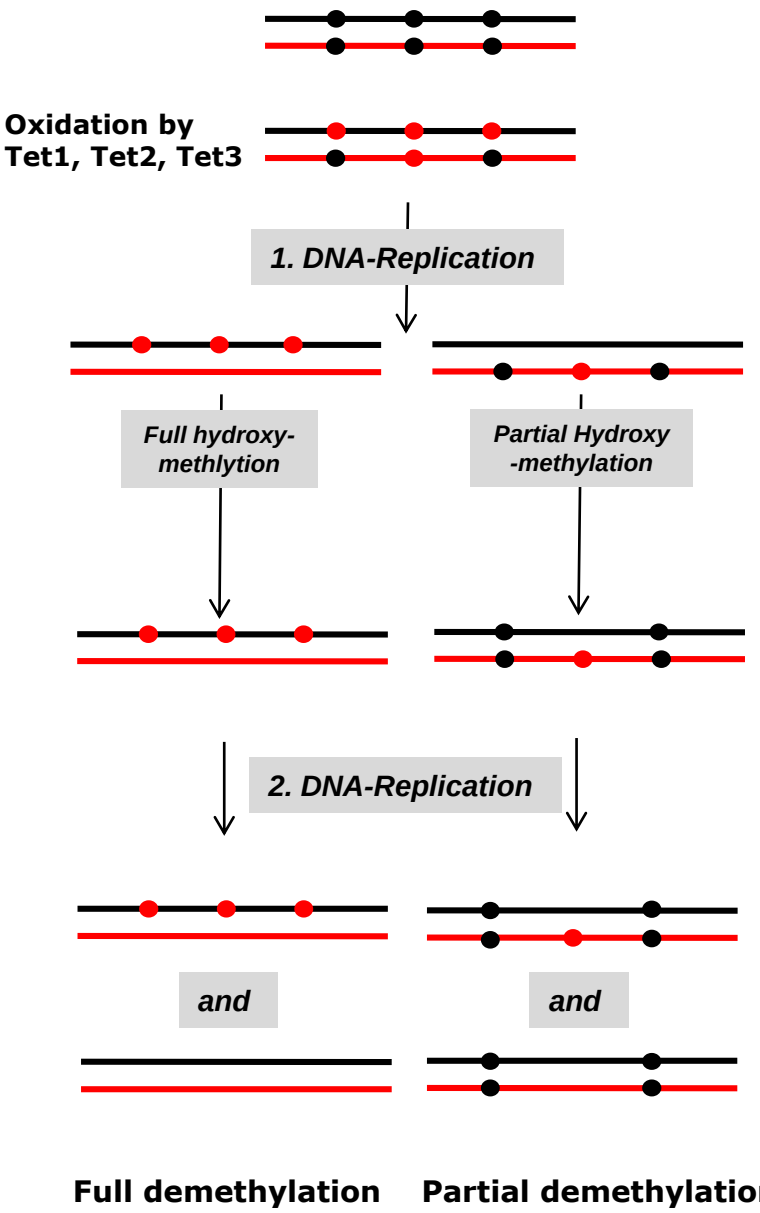
1. Control of gene expression

1. Control of Dnmt1 dependent epigenetic inheritance (maintenance)

2. Induction of (active) demethylation

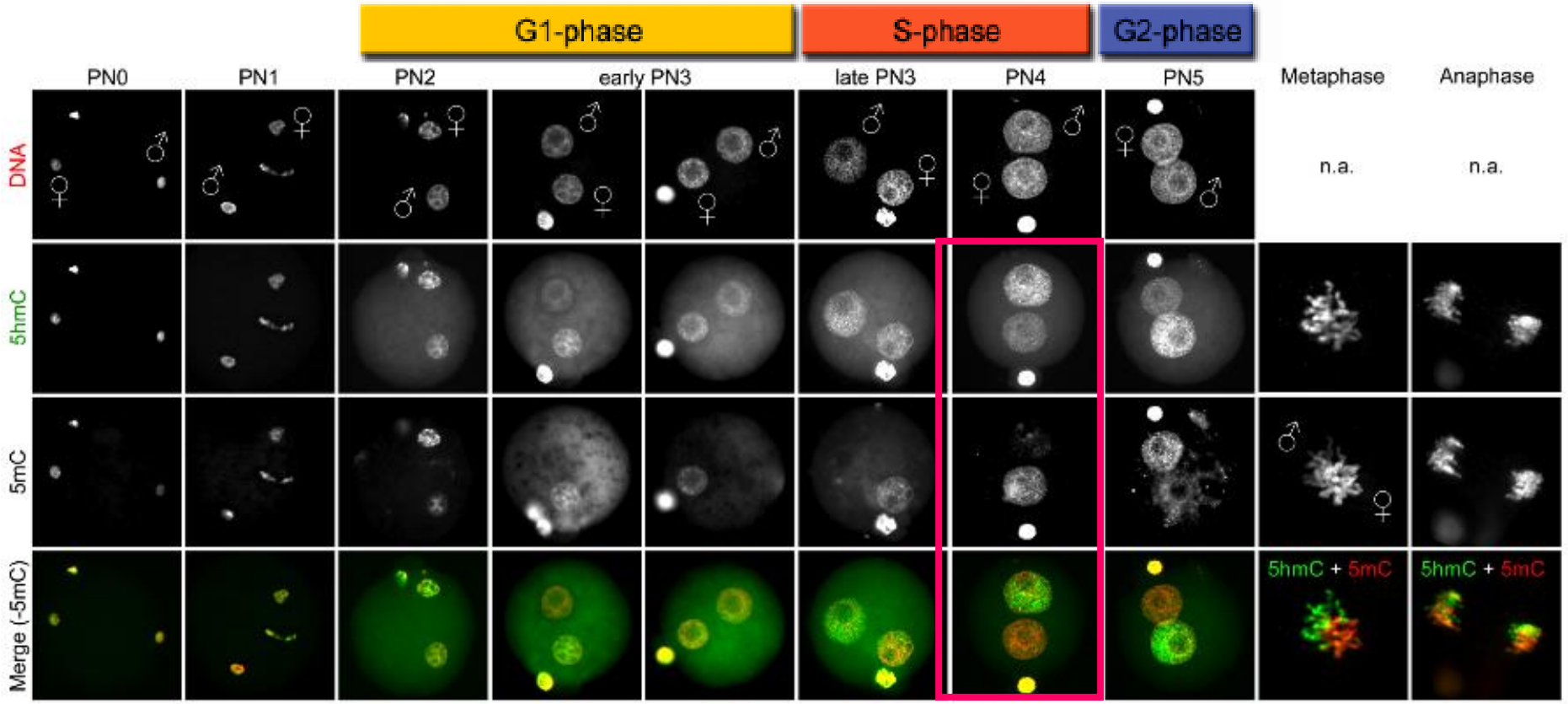
Model for the influence of 5-hydroxy-methylation on the maintenance of DNA-methylation

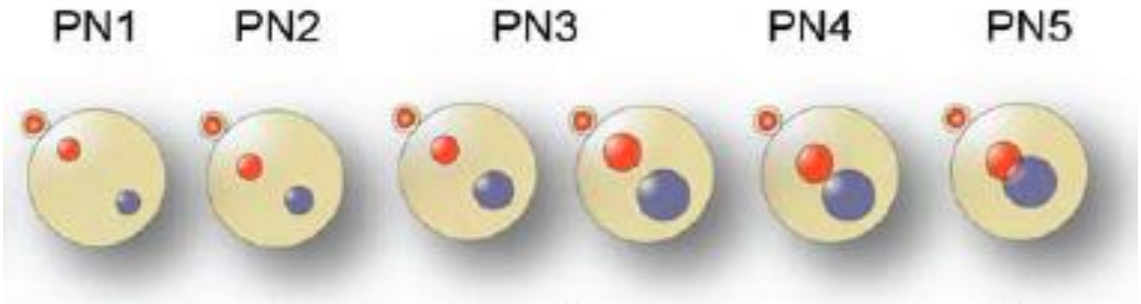




Impaired maintenance by Dnmt1

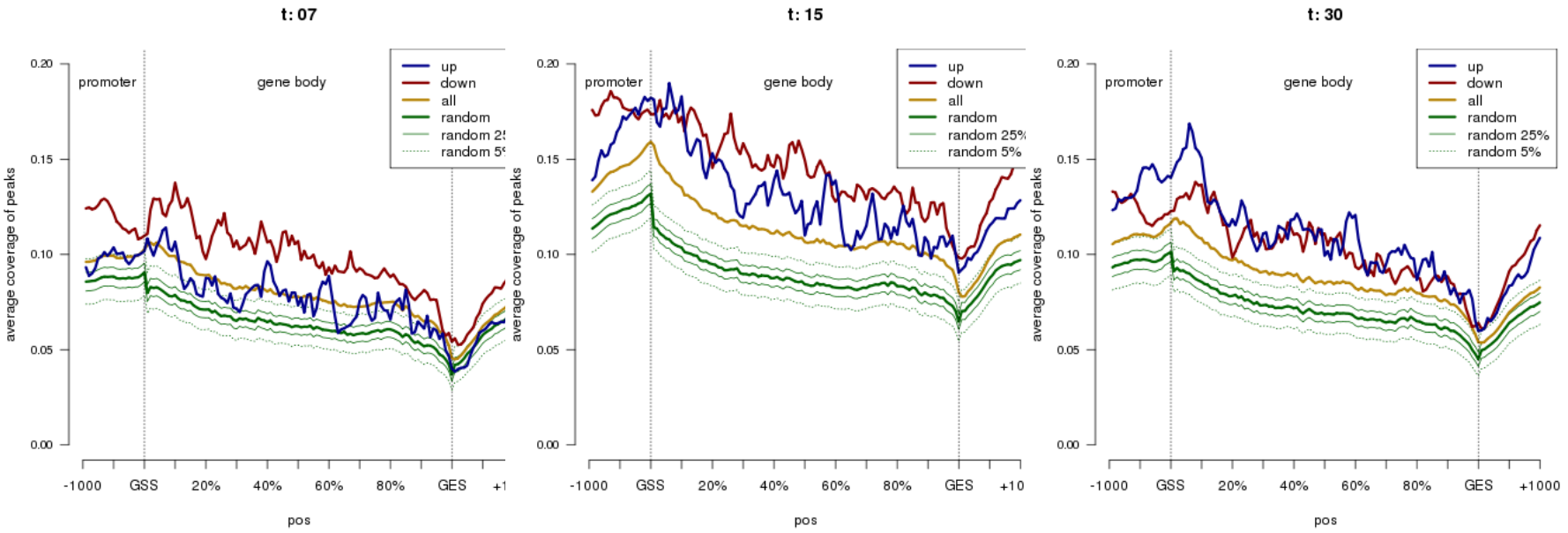
5mC is oxidized to 5hmC in the mammlian zygotes





Wossidlo et al.
Nature Comm.
2011

5hmC in genes and gene expression



Functions of oxidised forms of 5mC: 5hmC, 5fC and 5caC

1. Control of gene expression

1. Control of Dnmt1 dependent epigenetic inheritance (maintenance)

2. Induction of (active) demethylation

Mechanisms of active DNA-demethylation