Nucleosome, Chromosome, and Epigenetic regulations

09/15/2020

Outline

- Introduction to the Structures of Chromatin and Chromosome
- Introduction to Epigenetics
- Chromatin Remodeling Factors:
  - Histone modification enzymes, mode of actions, and small molecule inhibitors/activators
  - DNA Methylation

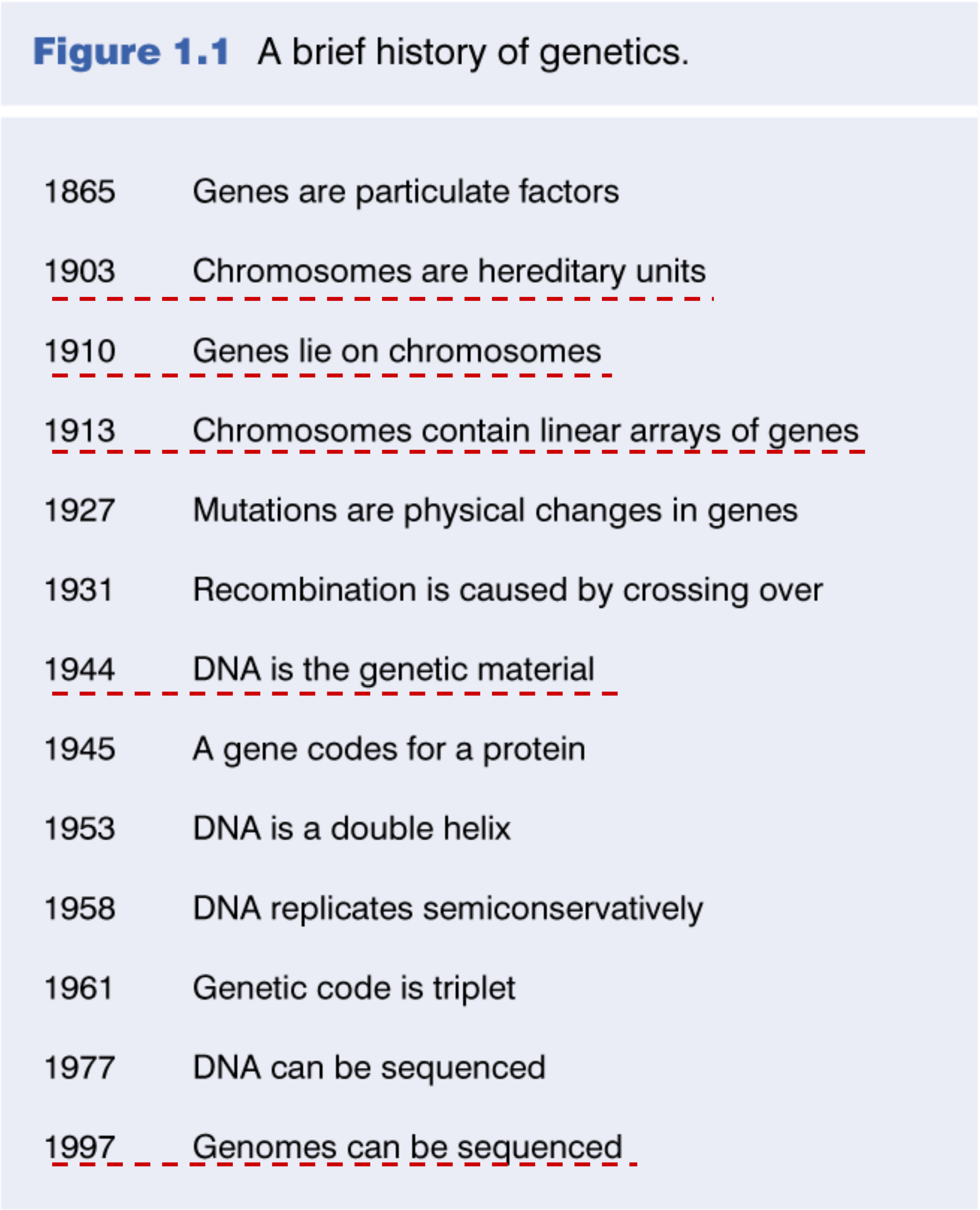
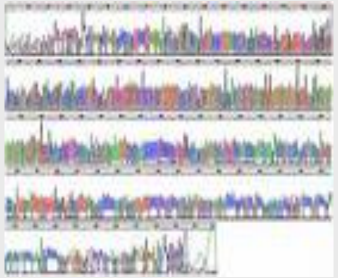


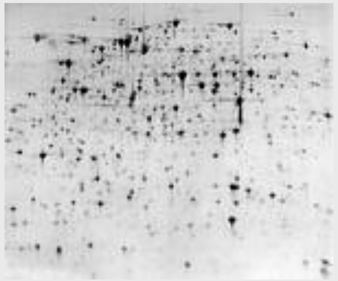
Figure 1.1 A brief history of genetics.

1865	Genes are particulate factors
1903	Chromosomes are hereditary units
1910	Genes lie on chromosomes
1913	Chromosomes contain linear arrays of genes
1927	Mutations are physical changes in genes
1931	Recombination is caused by crossing over
1944	DNA is the genetic material
1945	A gene codes for a protein
1953	DNA is a double helix
1958	DNA replicates semiconservatively
1961	Genetic code is triplet
1977	DNA can be sequenced
1997	Genomes can be sequenced

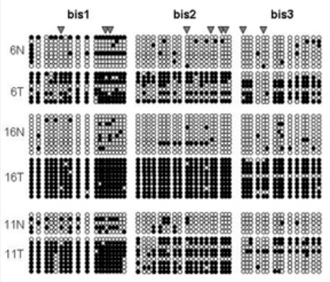
Current Biology



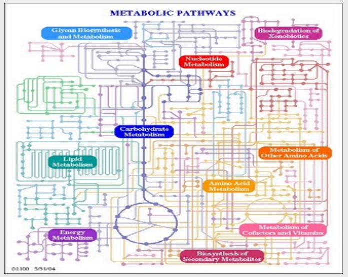
Genome



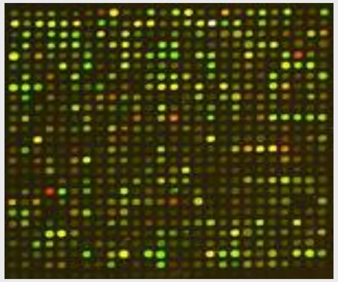
Proteome



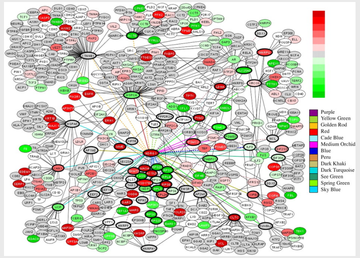
Epigenome



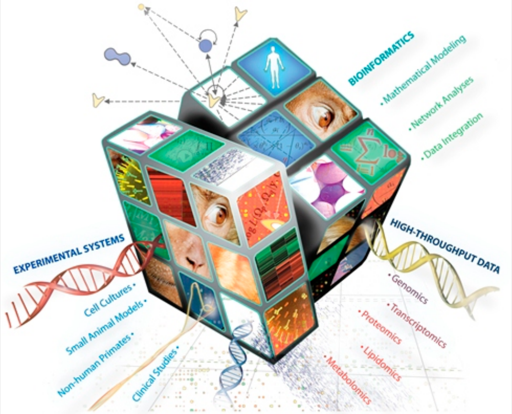
Metabolome



Transcriptome



Interactome

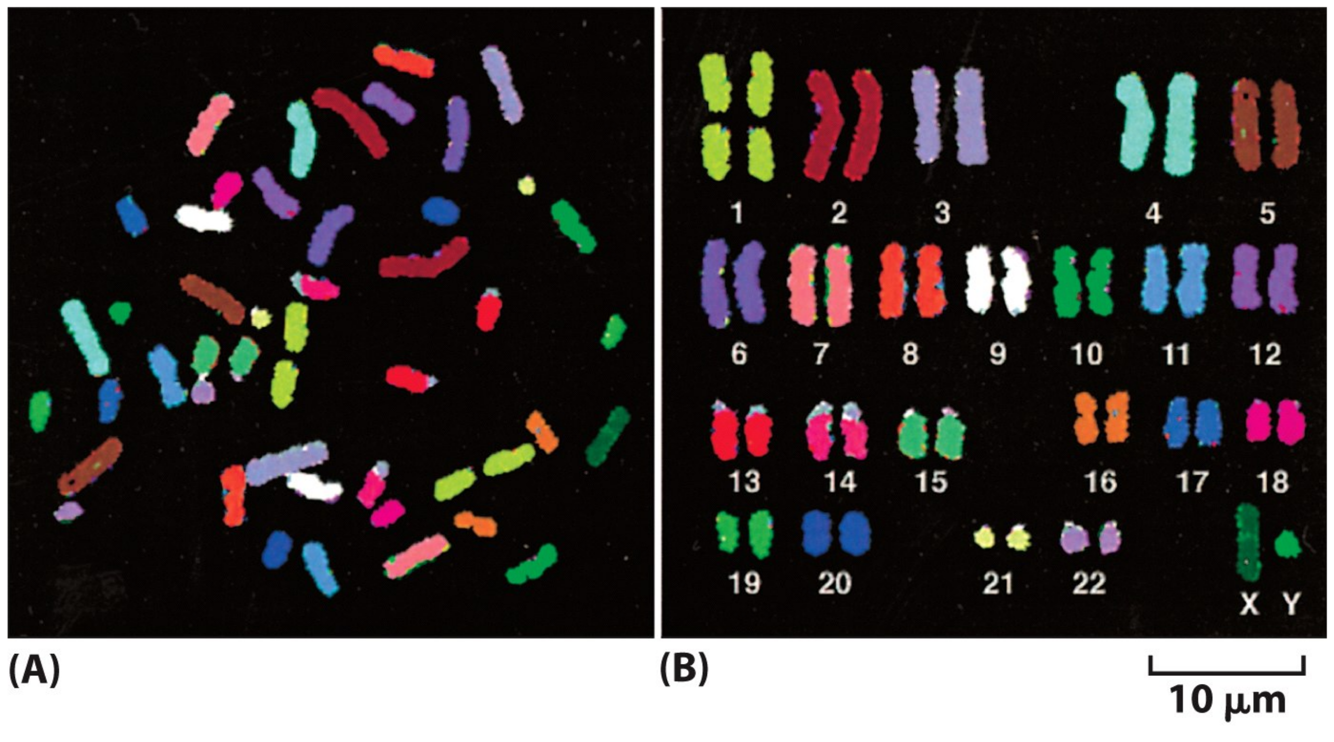


Systems Biology

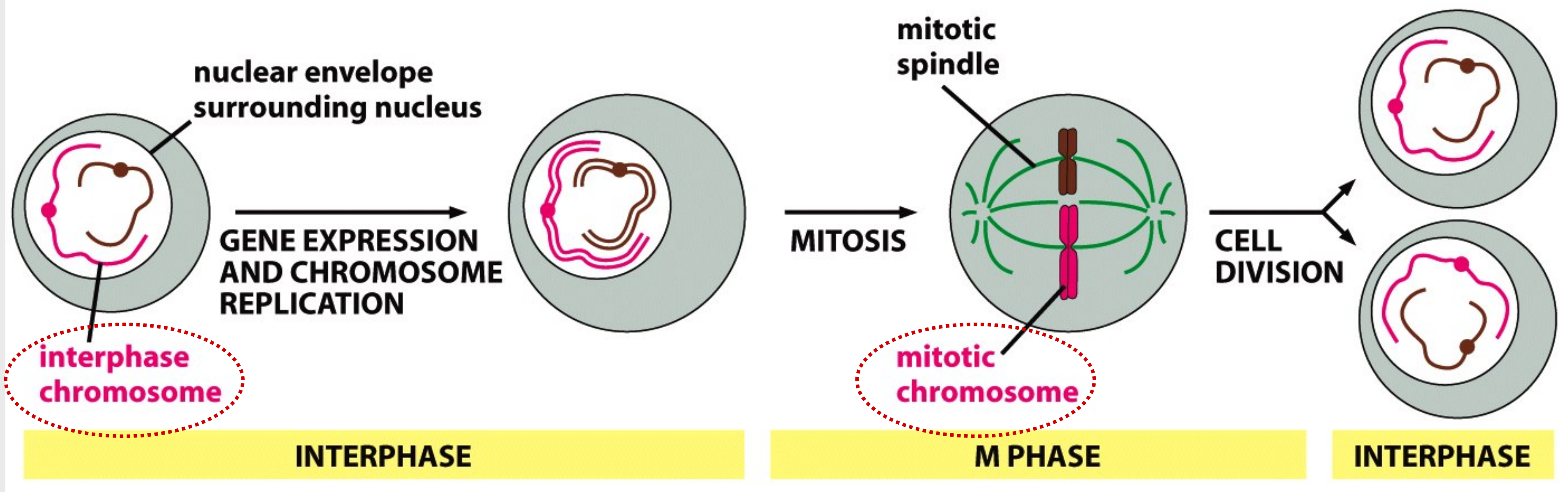
Eucaryotic DNA is packaged into a set of chromosomes

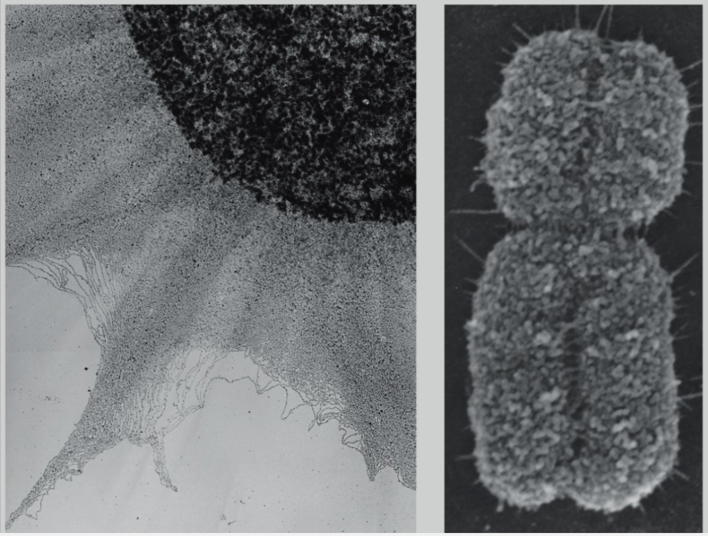
-**Chromatin: A filamentous complex of DNA, histones, and other proteins, constituting the eukaryotic chromosome.**

- **Karyotype**: the full chromosome set
- **Chromosome painting**: DNA hybridization with fluorescent labeled probes



Chromosomes exist in different states



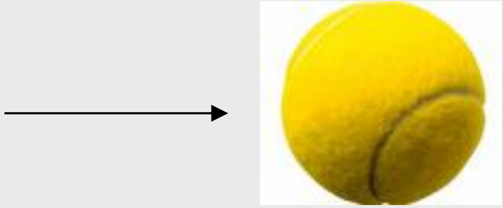


Packing of DNA into Nucleus

46 human chromosomes linked together—2 meters packed into nucleus—6 um

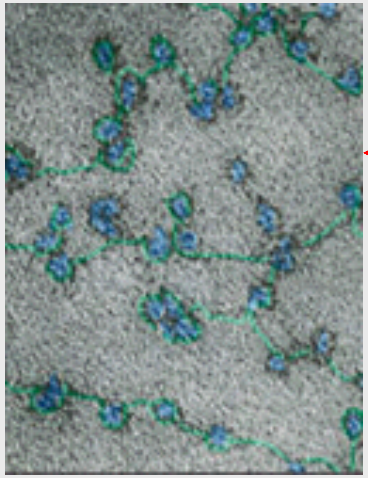
40 km of extreme fine thread

6.7 cm in diameter



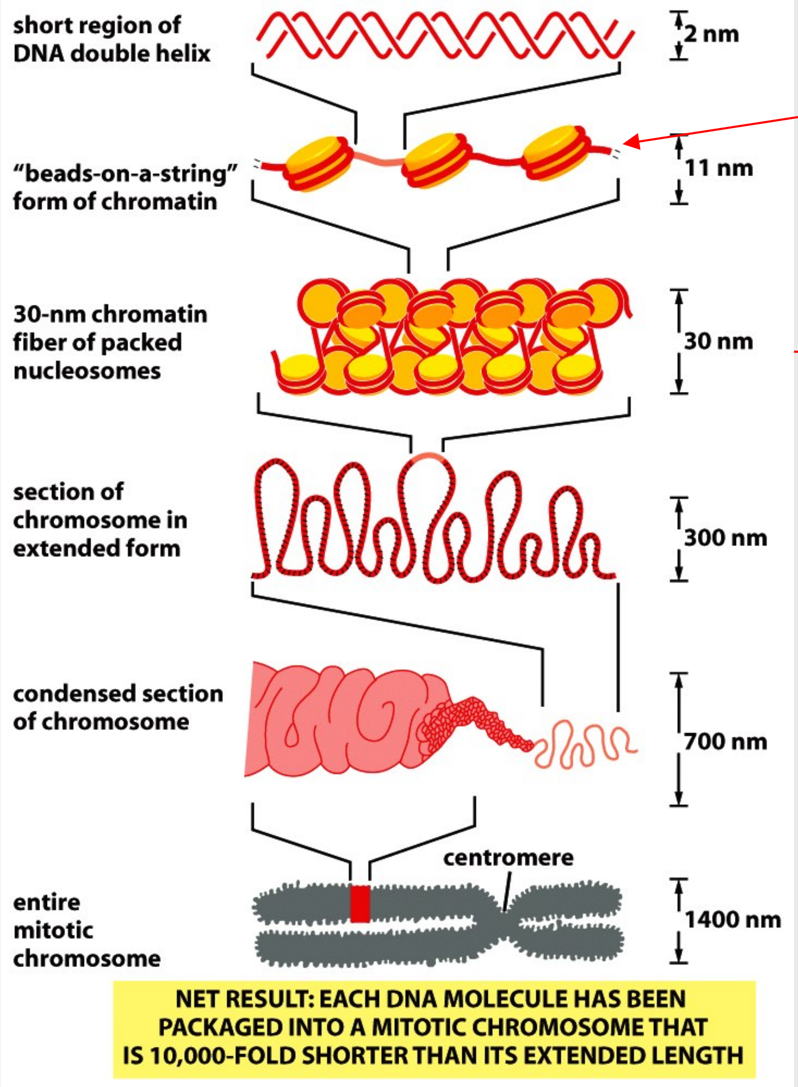
Packing of Mitotic Chromosome

- In eukaryotic cells, DNA double helix can be packed by **histone proteins** into a structure, called **Nucleosome**.



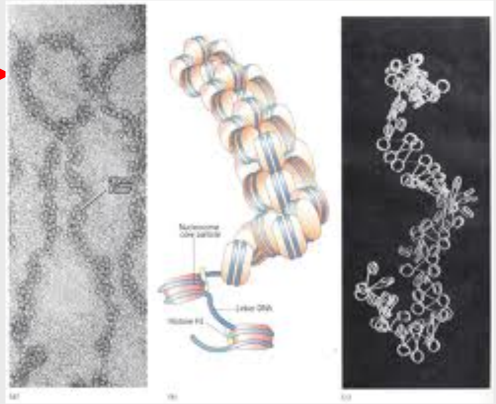
11 nm fiber

(**Beads on a string**)



NET RESULT: EACH DNA MOLECULE HAS BEEN PACKAGED INTO A MITOTIC CHROMOSOME THAT IS 10,000-FOLD SHORTER THAN ITS EXTENDED LENGTH

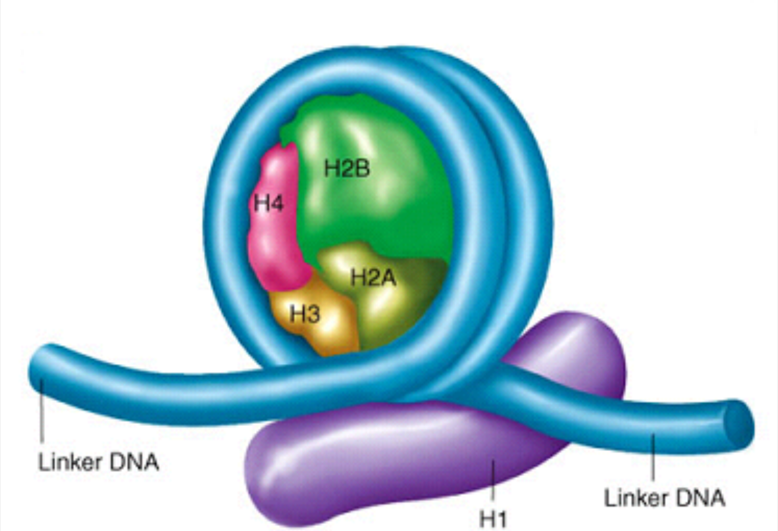
Nucleosome



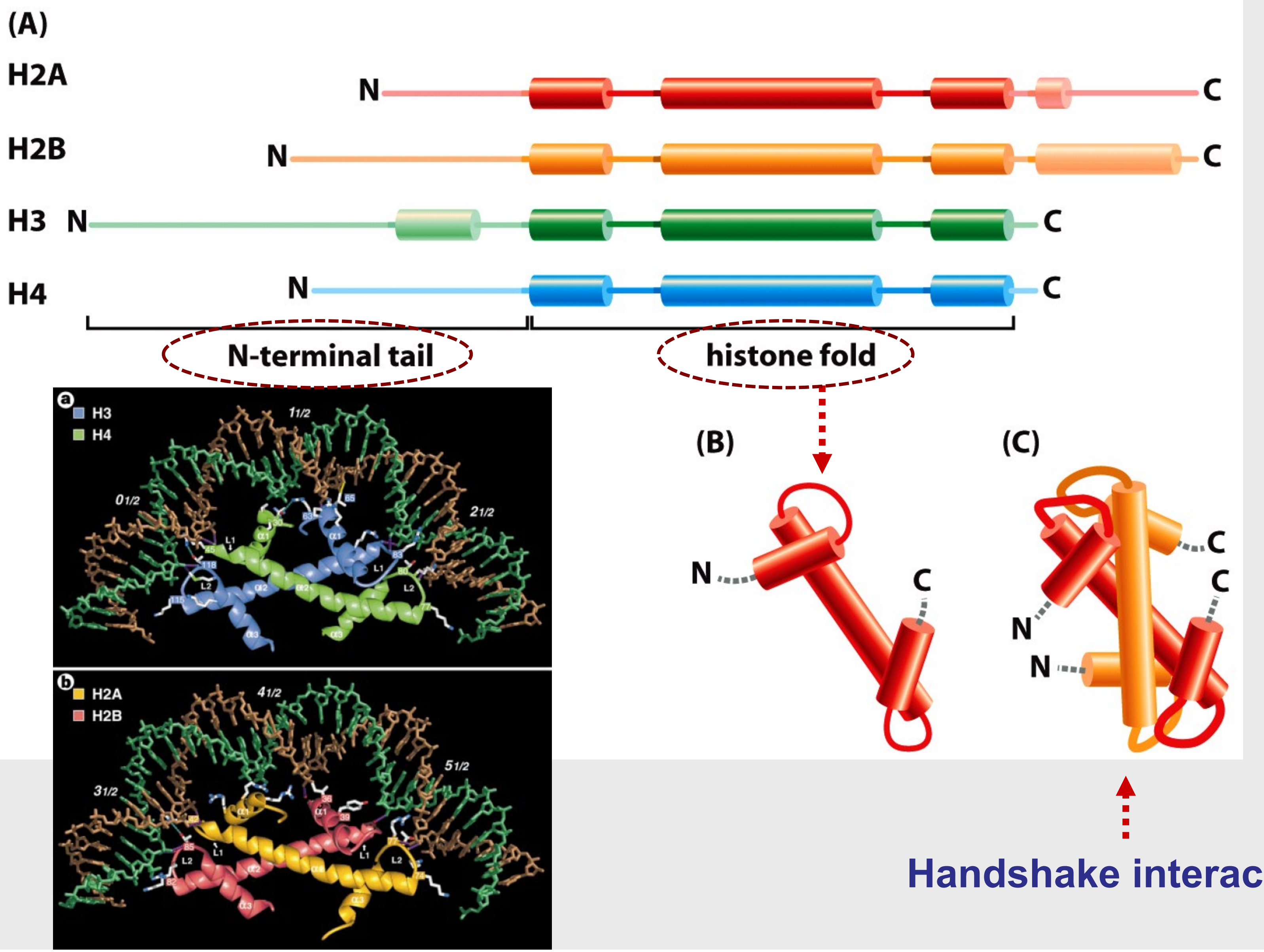
The Structure of Nucleosome

- The DNA makes **1.7 turns** around the **Histone Octamer (H2A, H2B, H3, and H4)** to form an overall particle with a disk-like structure, called **Nucleosome**. **H1** and ~8-114 bp. of linker DNA between nucleosomes.

- Interactions between DNA and the histone core:
  1. 142 **hydrogen bonds** are formed
  2. **Hydrophobic interactions**
  3. **Salt linkages**: positive charge amino acids (**K** or **R**) and the negatively charged DNA backbone



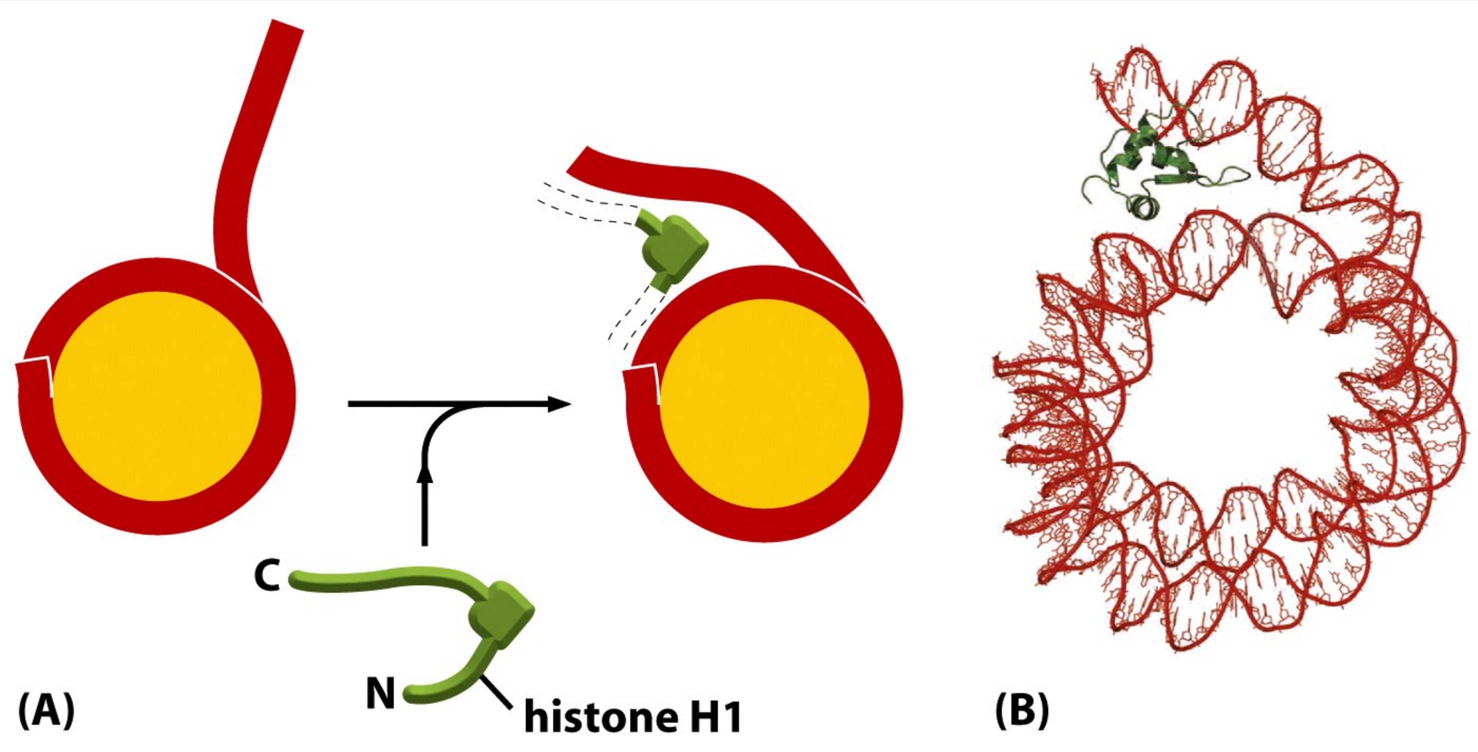
Domain organization of the Core Histones



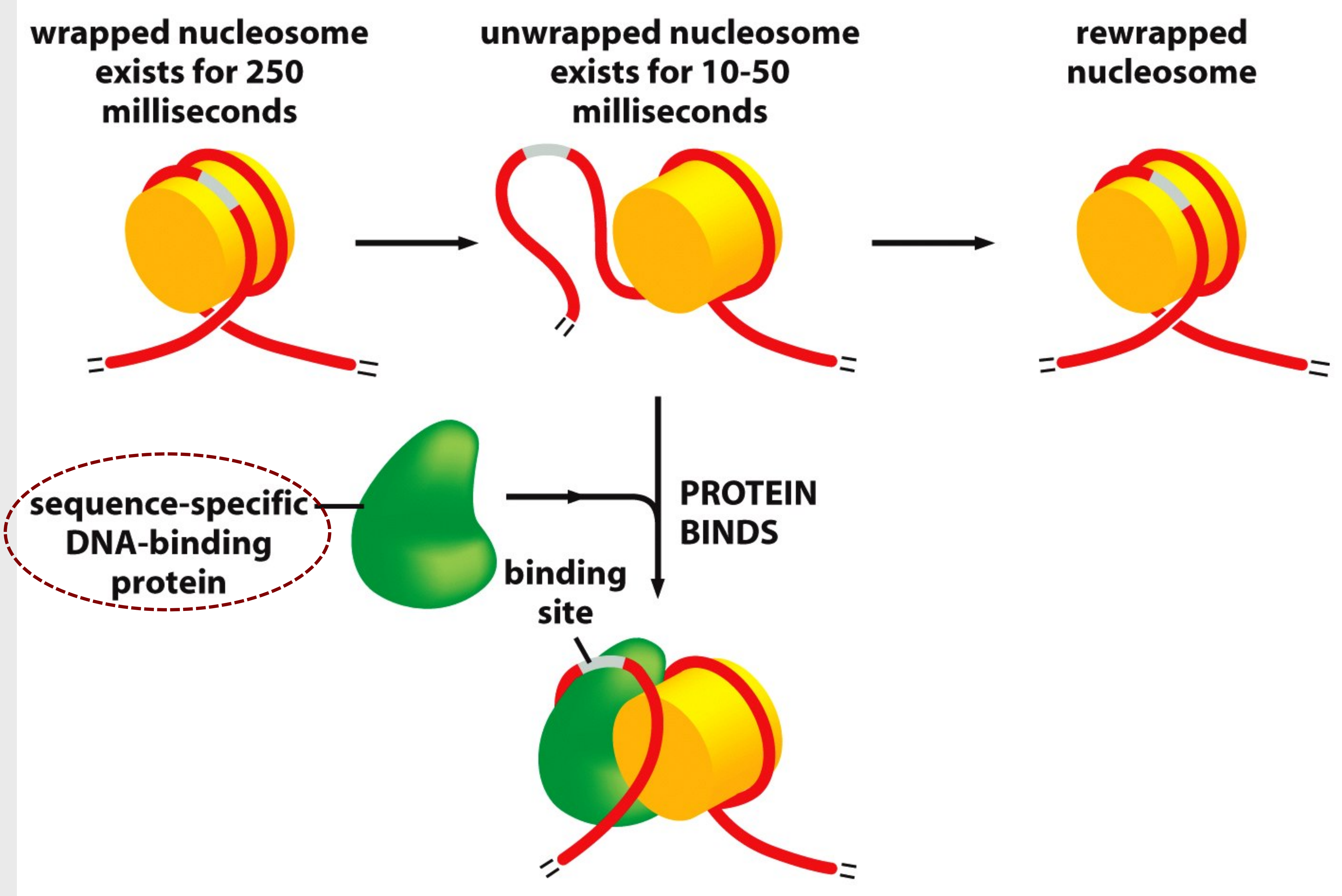
Handshake interaction

Linker Histone, Histone H1

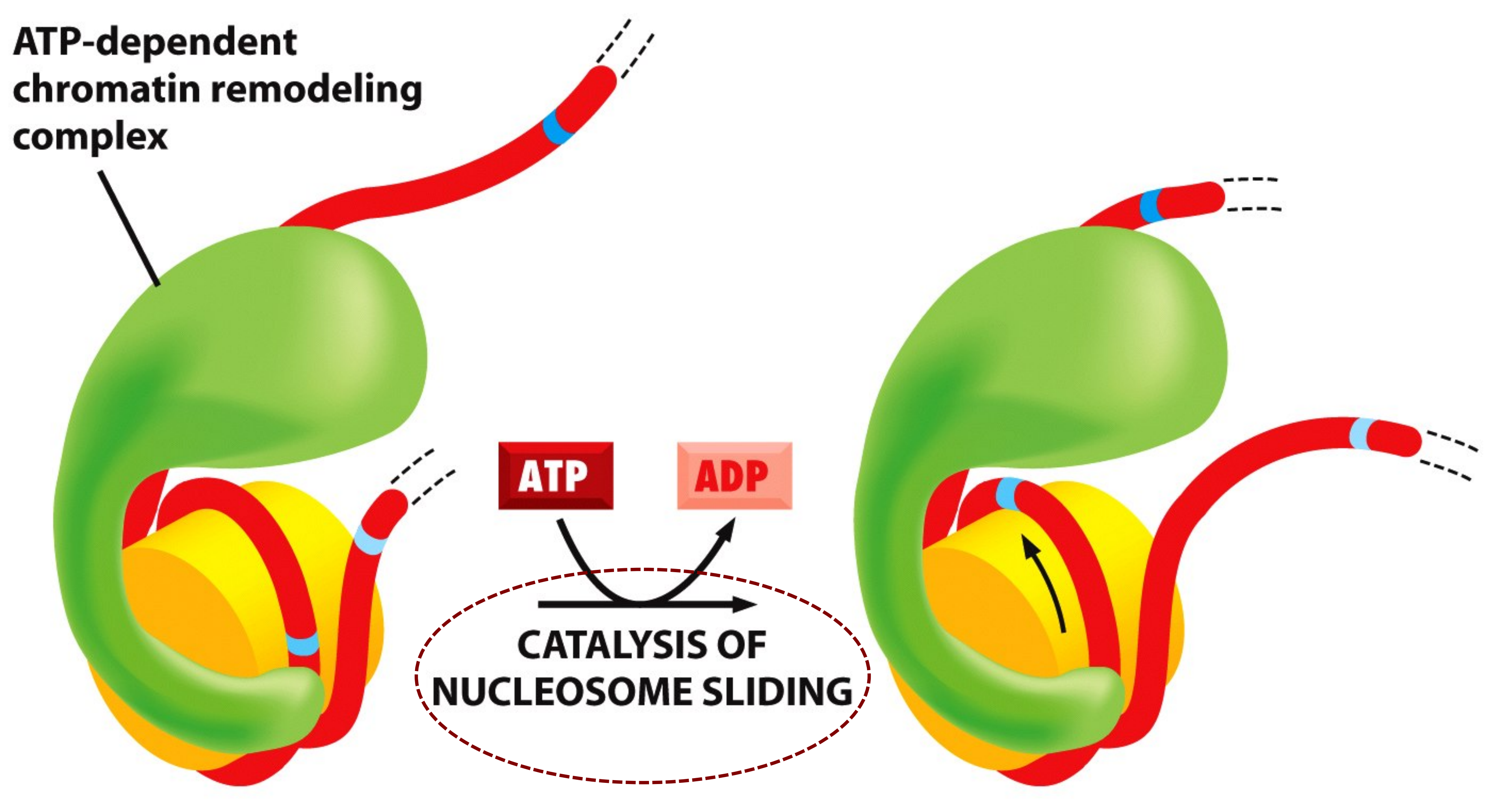
- **1-to-1 ration with nucleosome cores**
- **Less well conserved during evolution**
- **H1 is important for forming 30-nm chromatin**



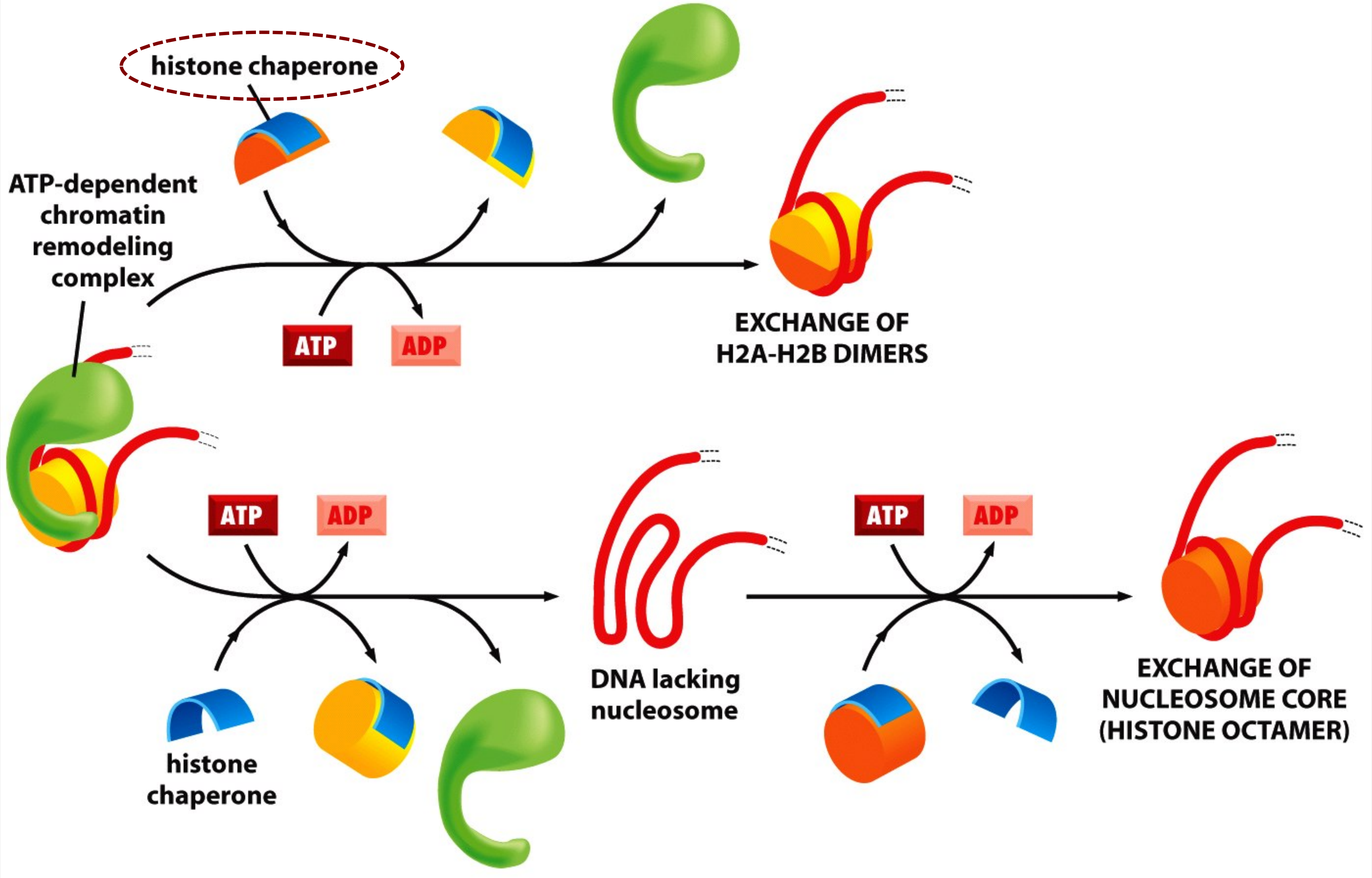
Dynamic Nucleosomes



ATP-dependent chromatin remodeling complex

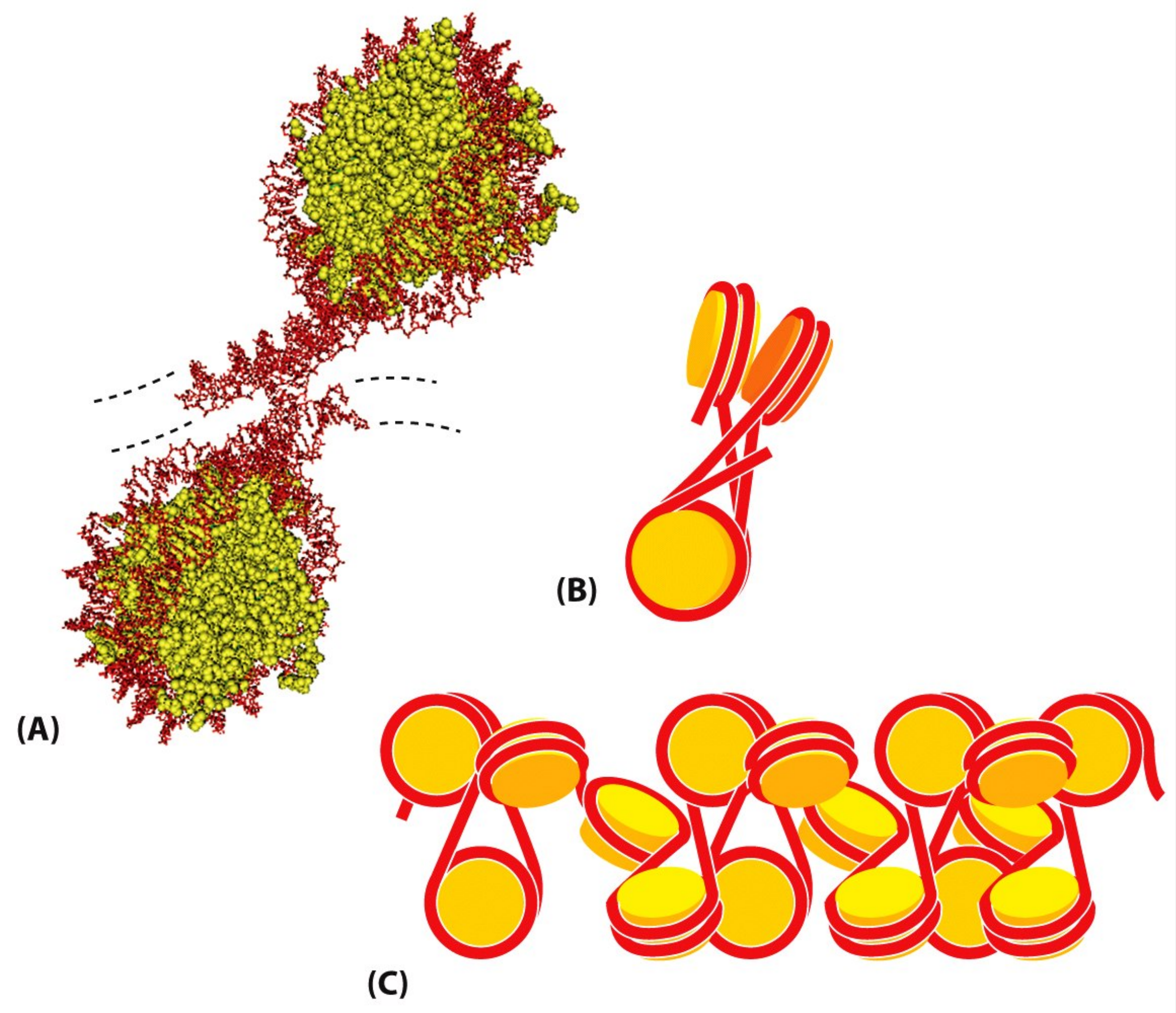


Nucleosome removal and Histone exchange



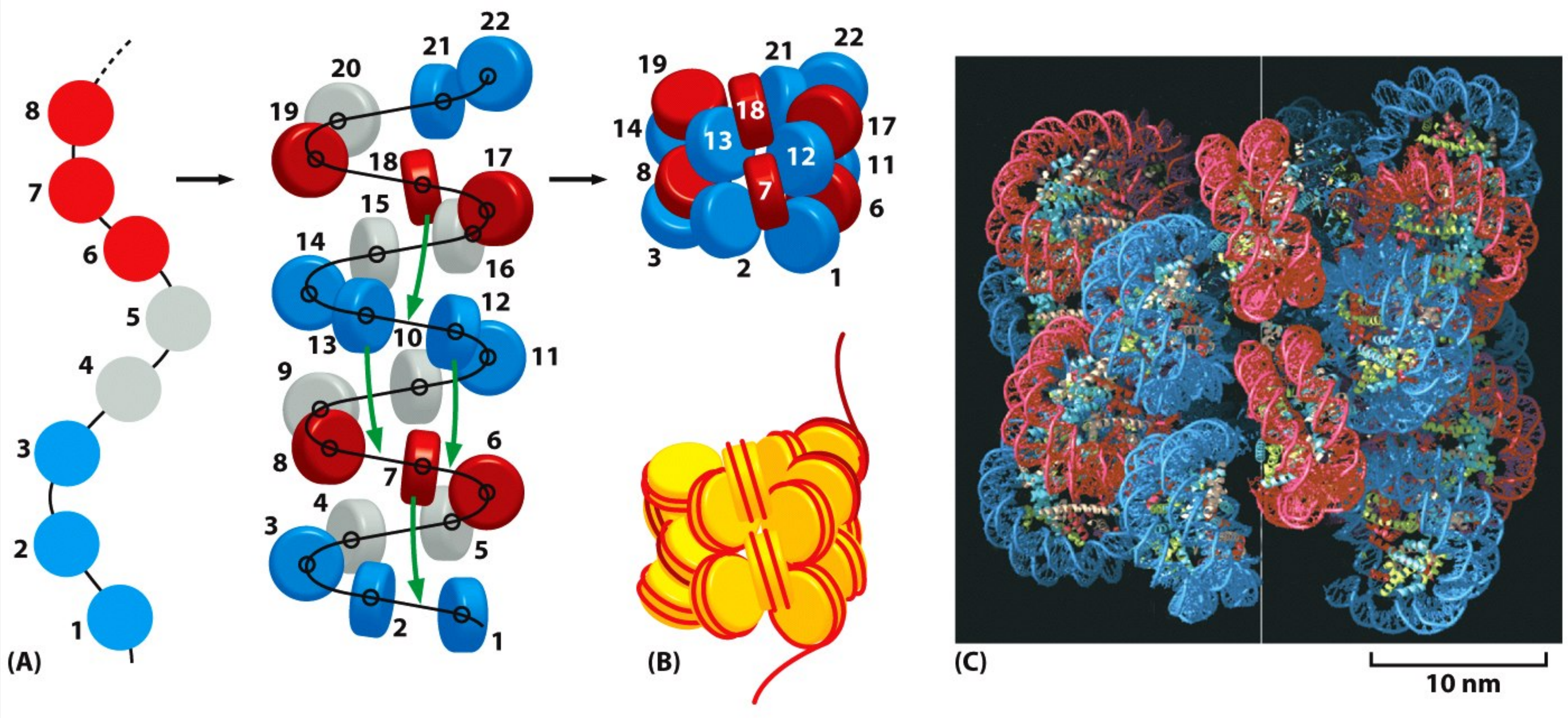
Zigzag model for the 30-nm chromatin fiber

- Supported by χ-ray crystallography

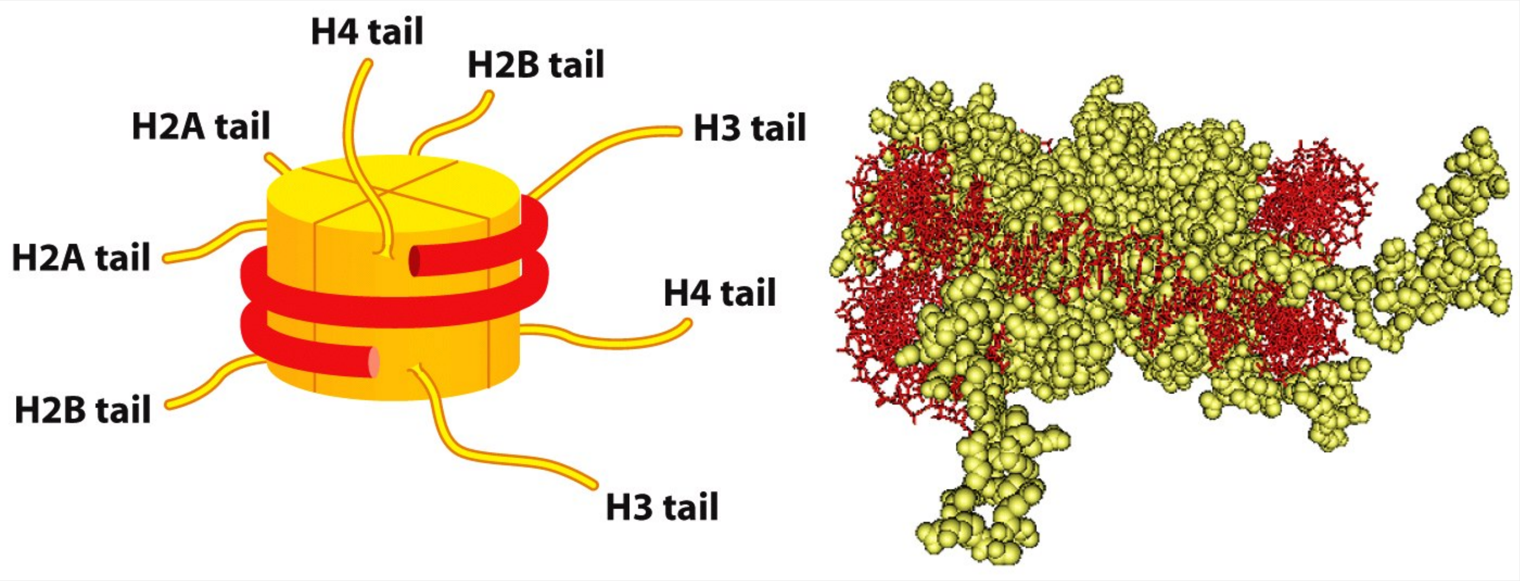


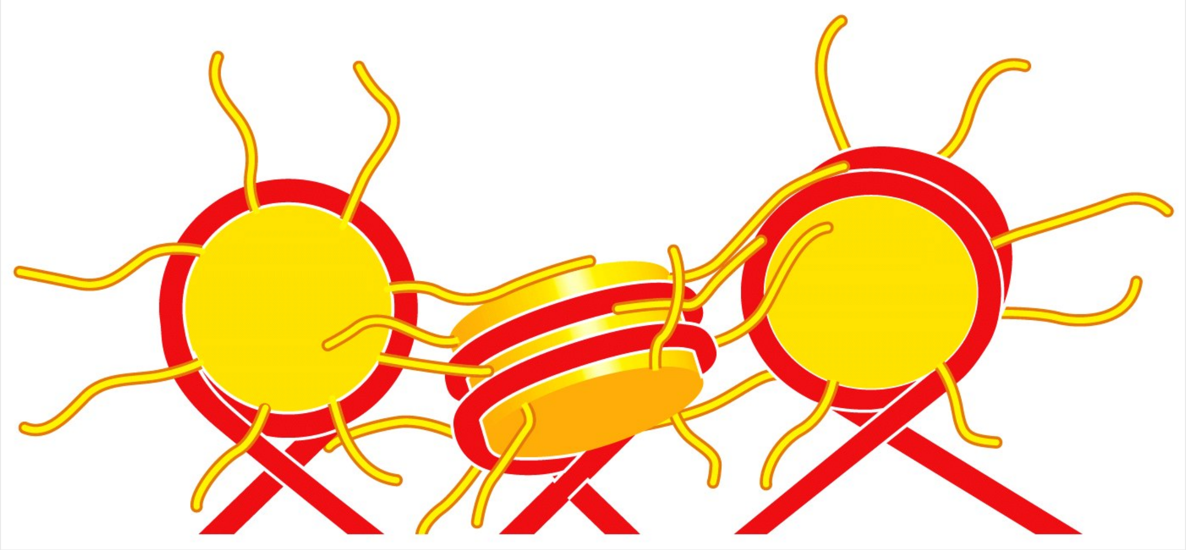
Solenoid model for the 30-nm chromatin fiber

- Supported by Cryo-electron microscopy

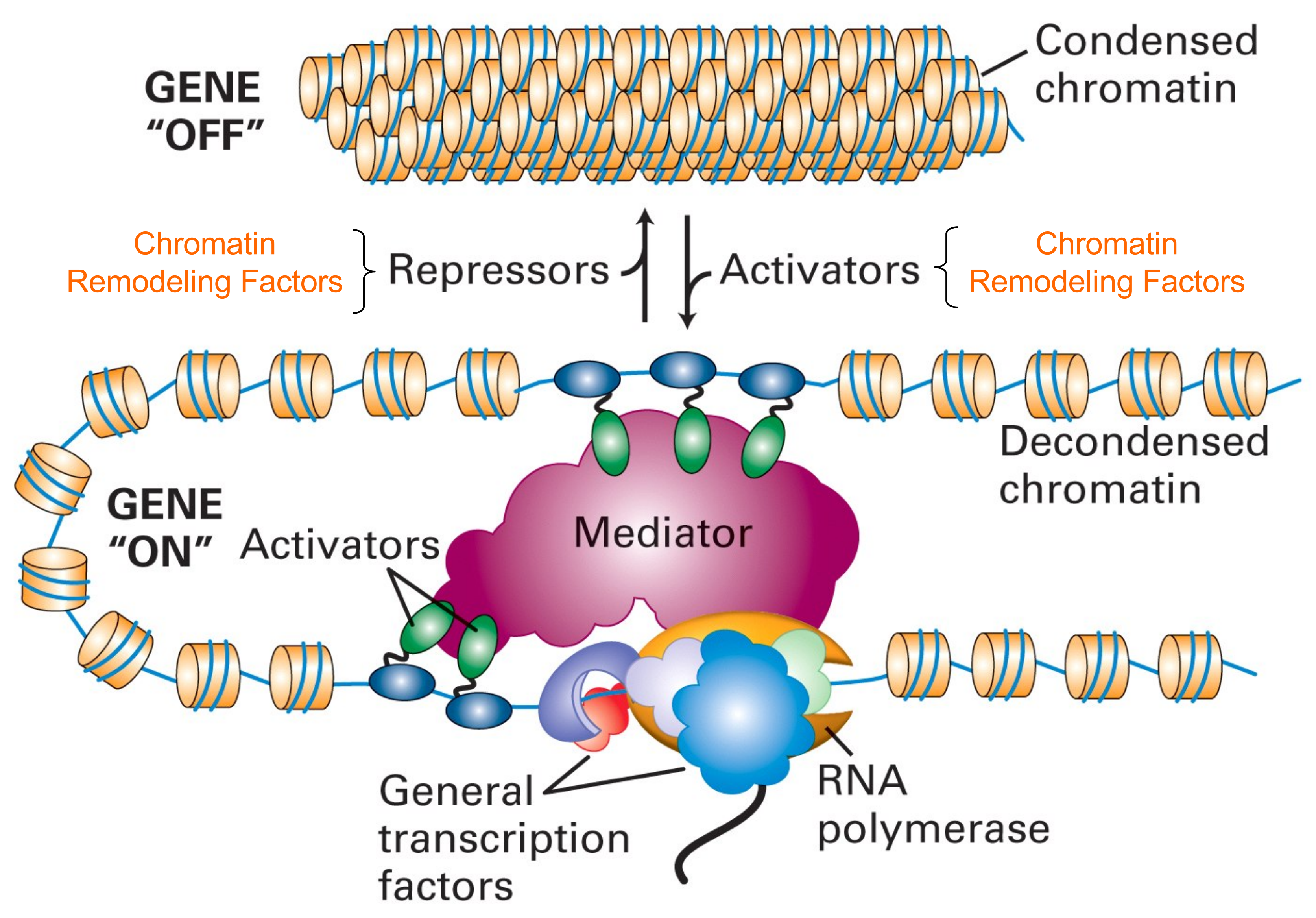


Histone tails in the formation of the 30-nm fibers

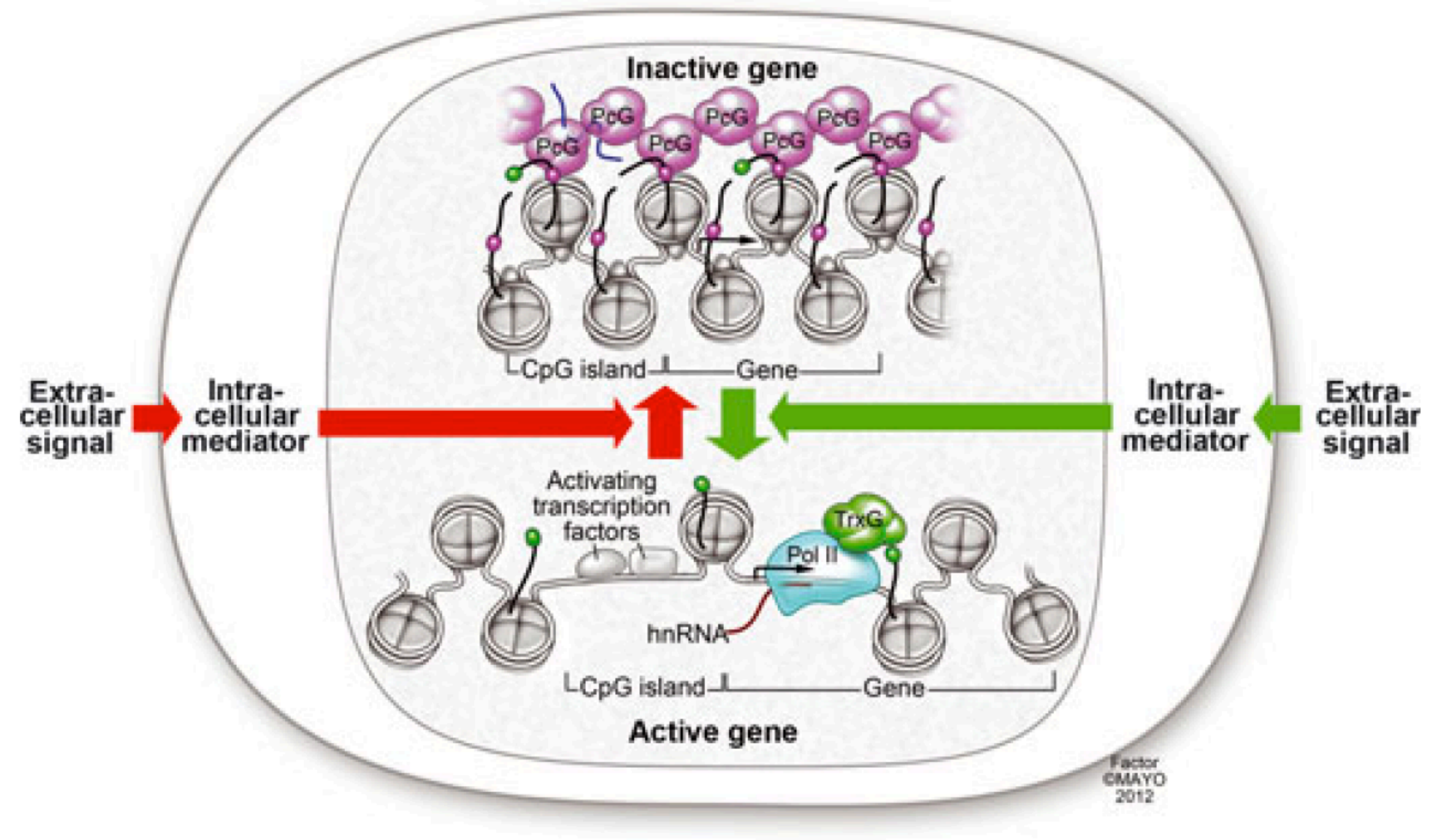




Chromatin Remodeling and Transcriptional initiation



Chromatin Remodeling and Transcriptional initiation



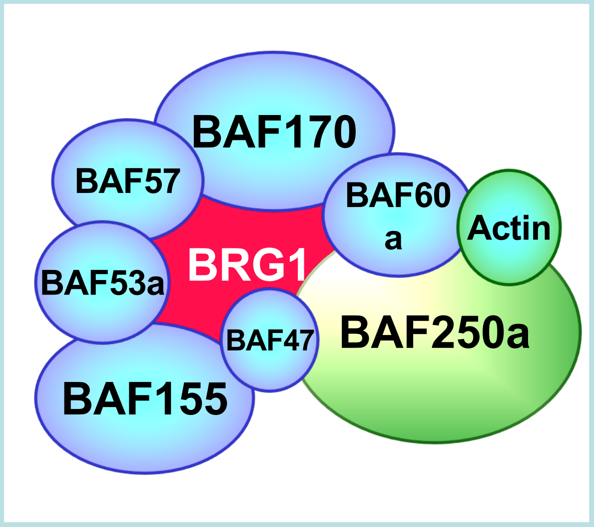
Ordog T. *et al.*, Neurogastroenterol Motil 2012

The SWI/SNF Complex

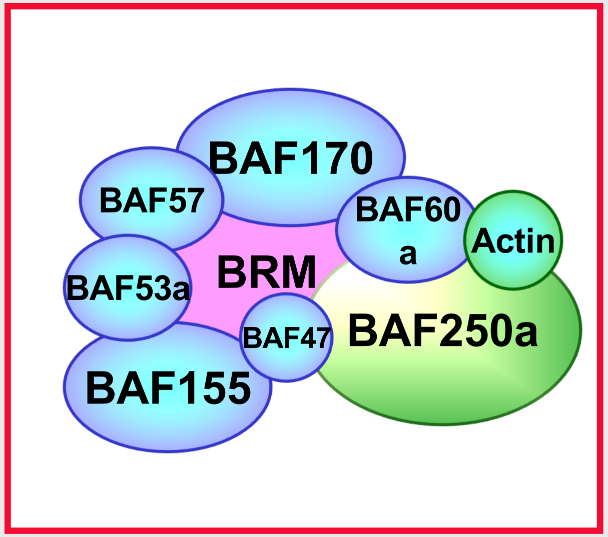
- ATP-dependent chromatin remodeling complex
- Yeast mating type switching (SWI) and sucrose non-fermenting (SNF)
- Expression of 6% of yeast genes require SWI/SNF function
- 9-12 subunits - 2MDa
- Each subunit is required for function of the entire complex
- Evolutionarily conserved: *C. elegans*, yeast, fly, rat, mouse, human

Variations in Human SWI/SNF Complexes

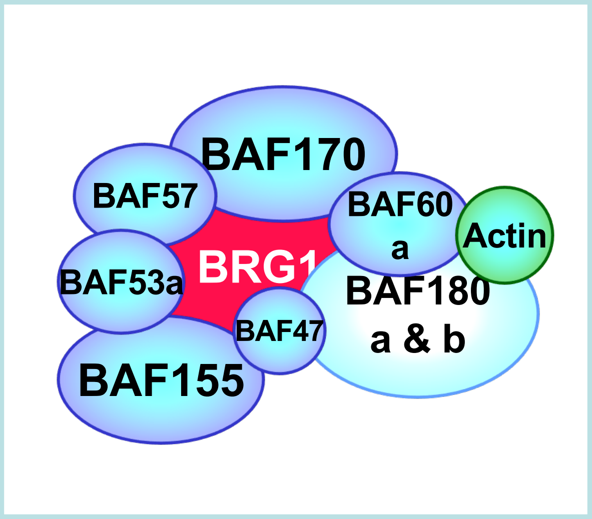
BRG1/BAF



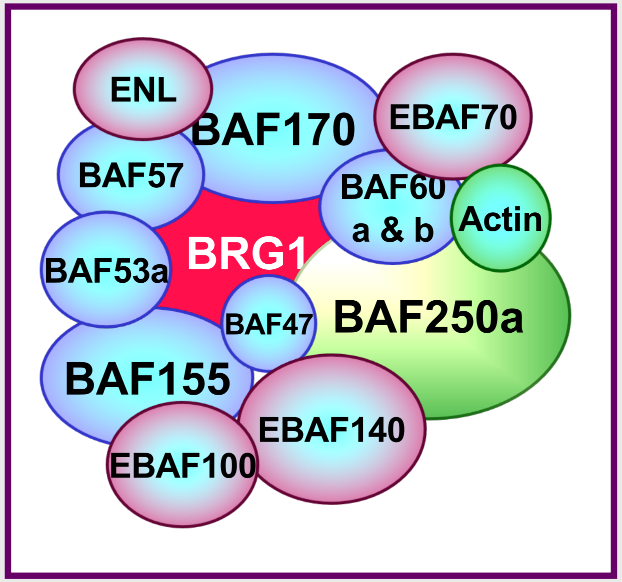
BRM/BAF



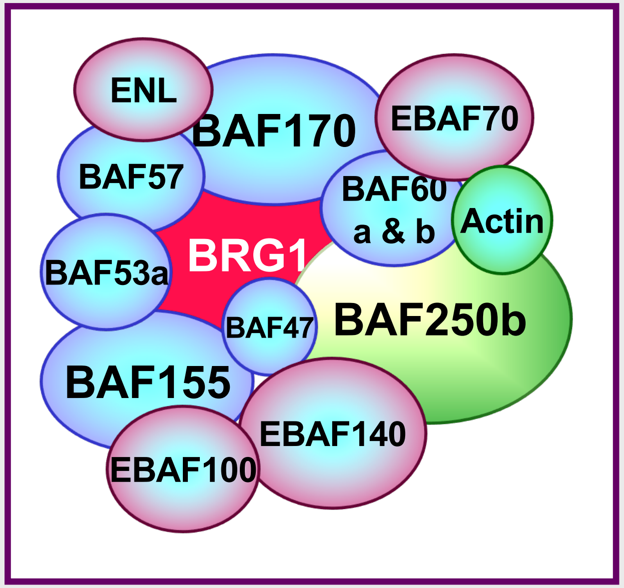
PBAF



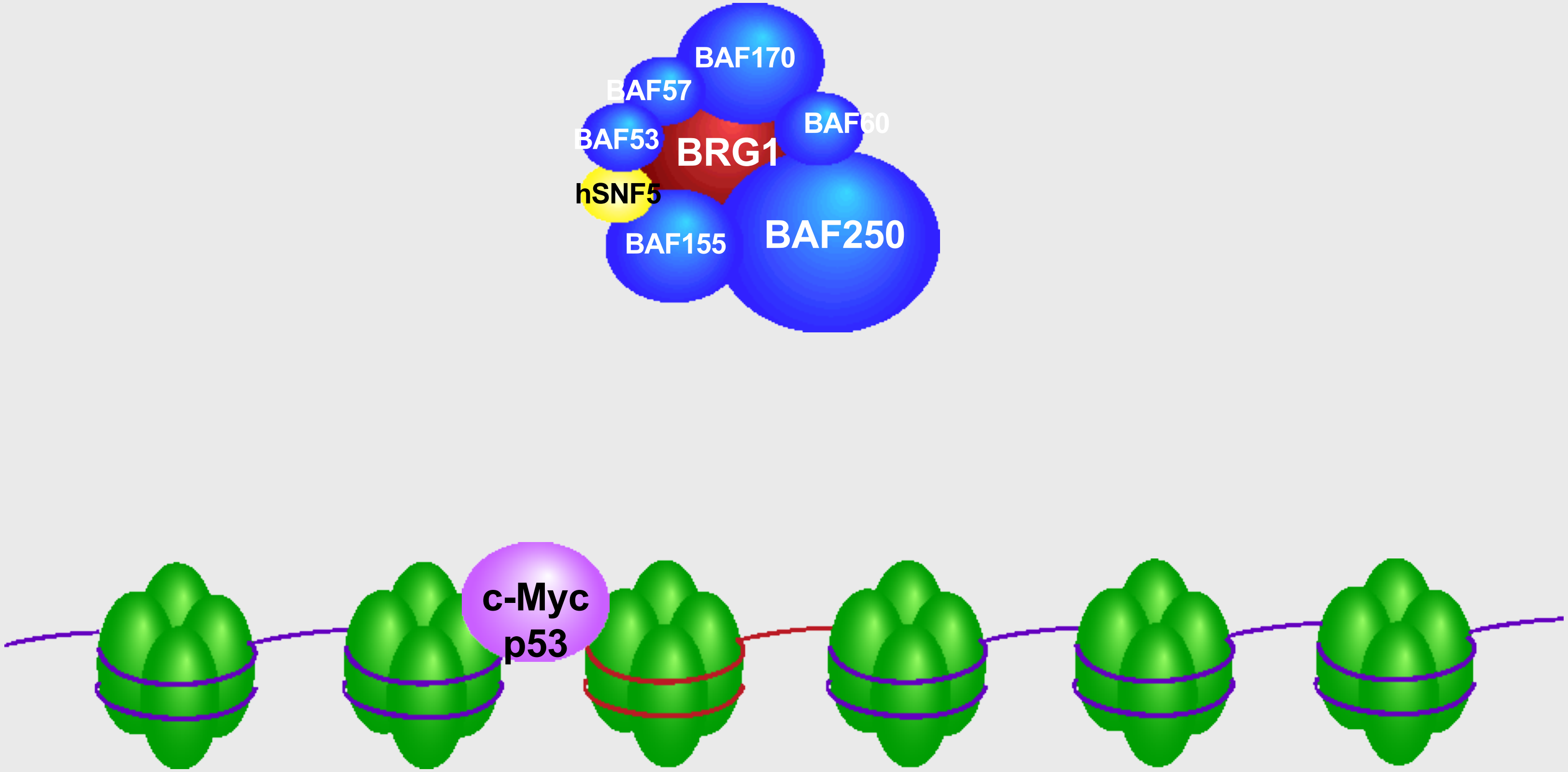
EBAFa



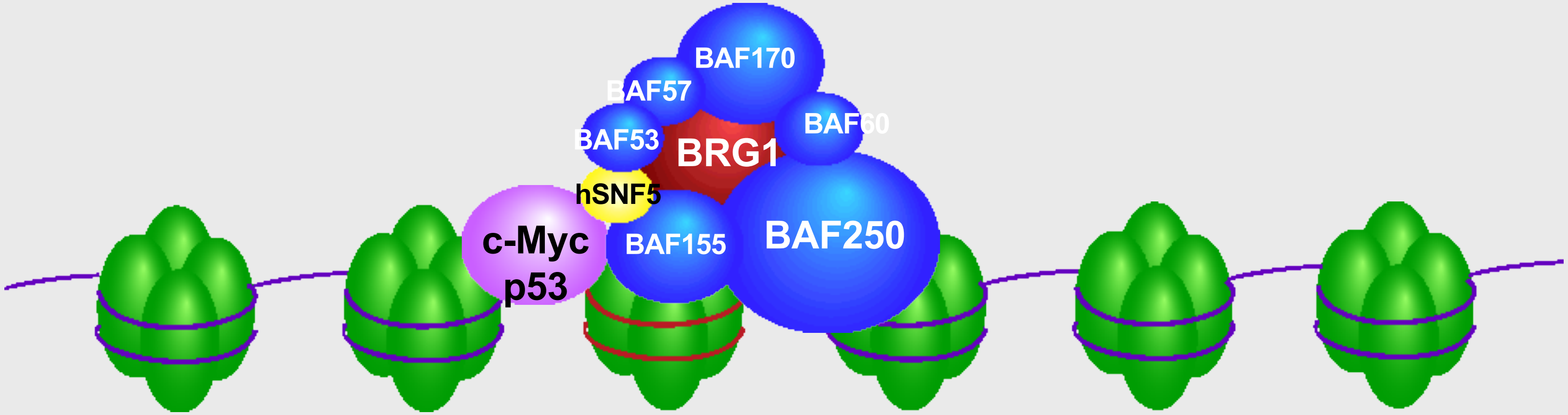
EBAFb



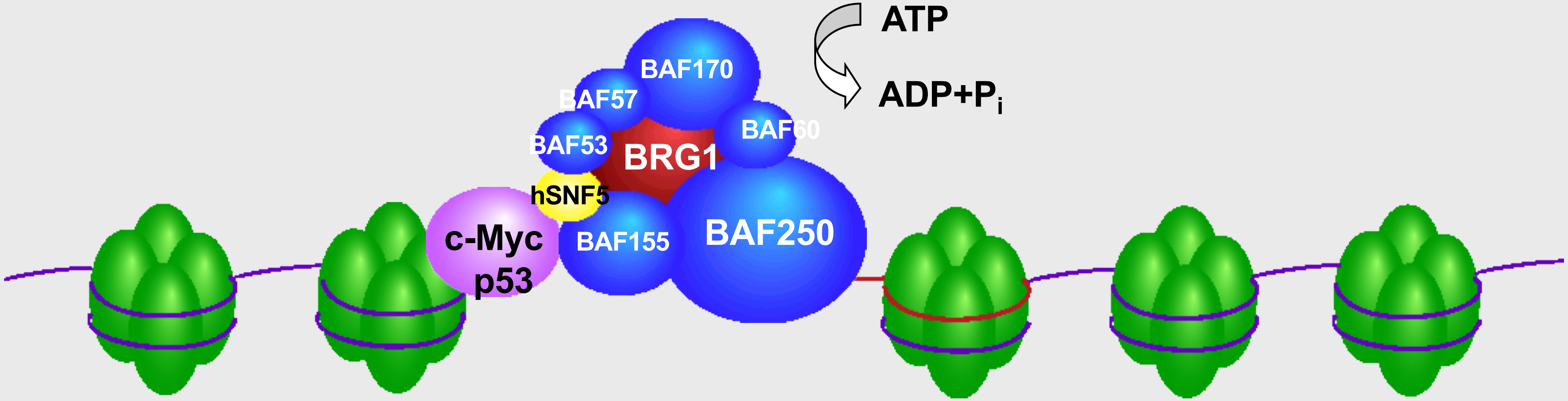
SWI/SNF-Mediated Chromatin Remodeling



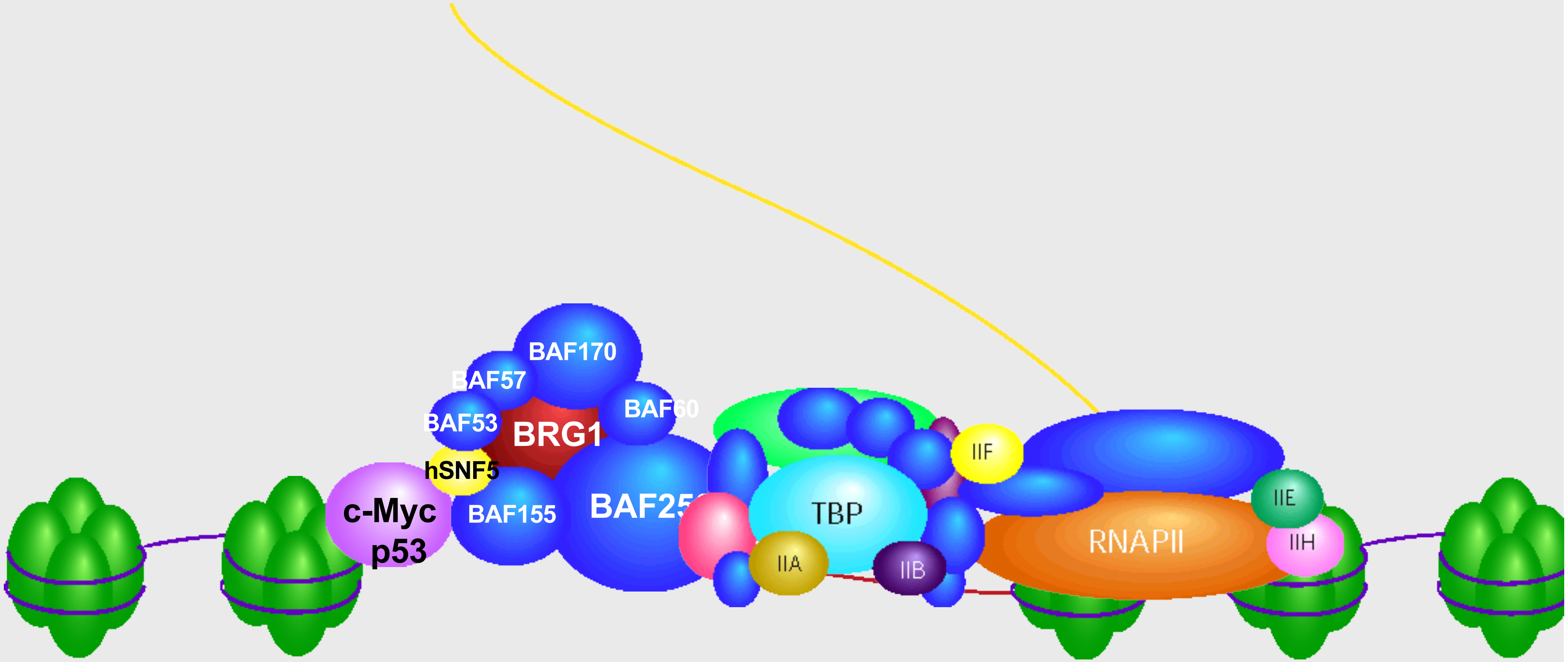
SWI/SNF-Mediated Chromatin Remodeling



SWI/SNF-Mediated Chromatin Remodeling



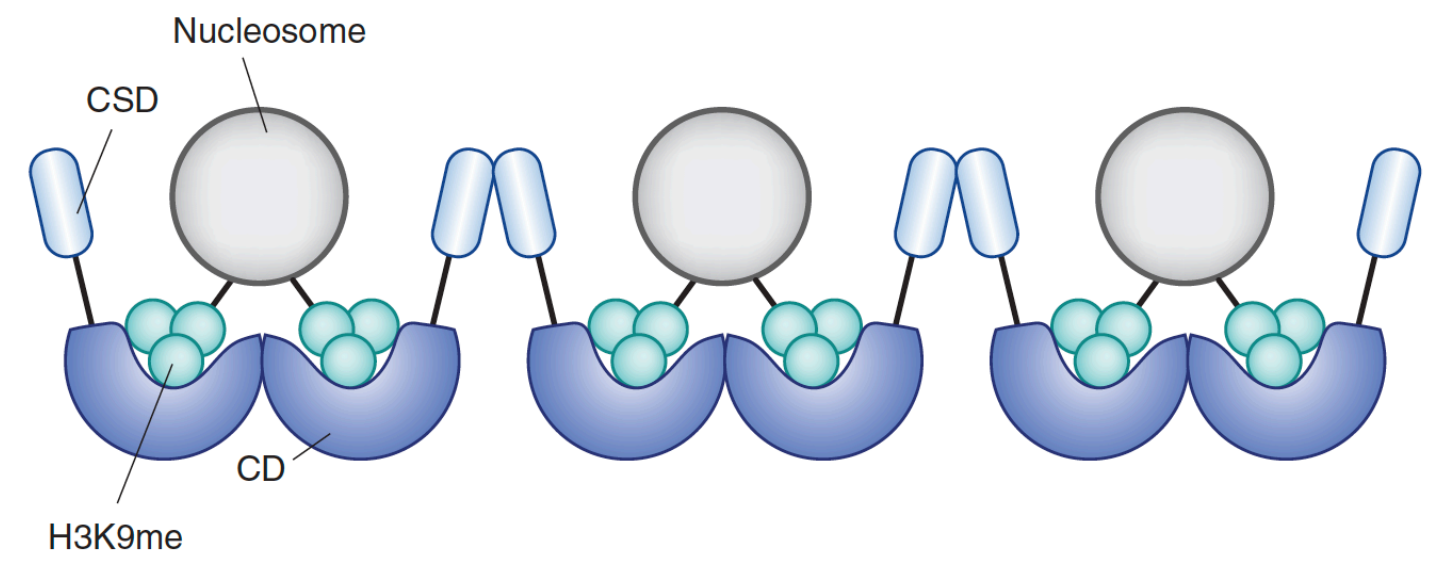
SWI/SNF-Mediated Chromatin Remodeling



Regulation of nucleosome dynamics by histone modifications

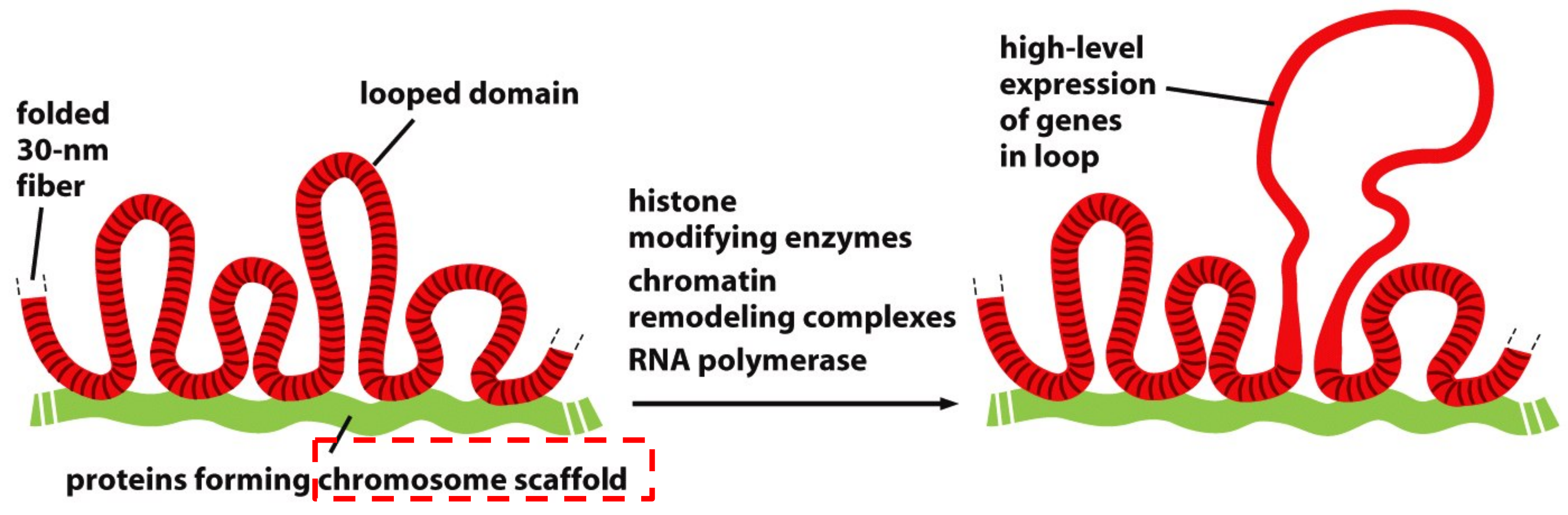
- **Swi6**-mediated **chromatin stabilization**. Swi6 molecules dimerize via their **chromodomains (CD)** to recognize **H3K9-trimethylated histone** tails in a single nucleosome.

- These dimers then contact adjacent dimers via their **chromo-shadow domains (CSD)** to **stabilize nucleosomes** and **promote heterochromatin spreading**

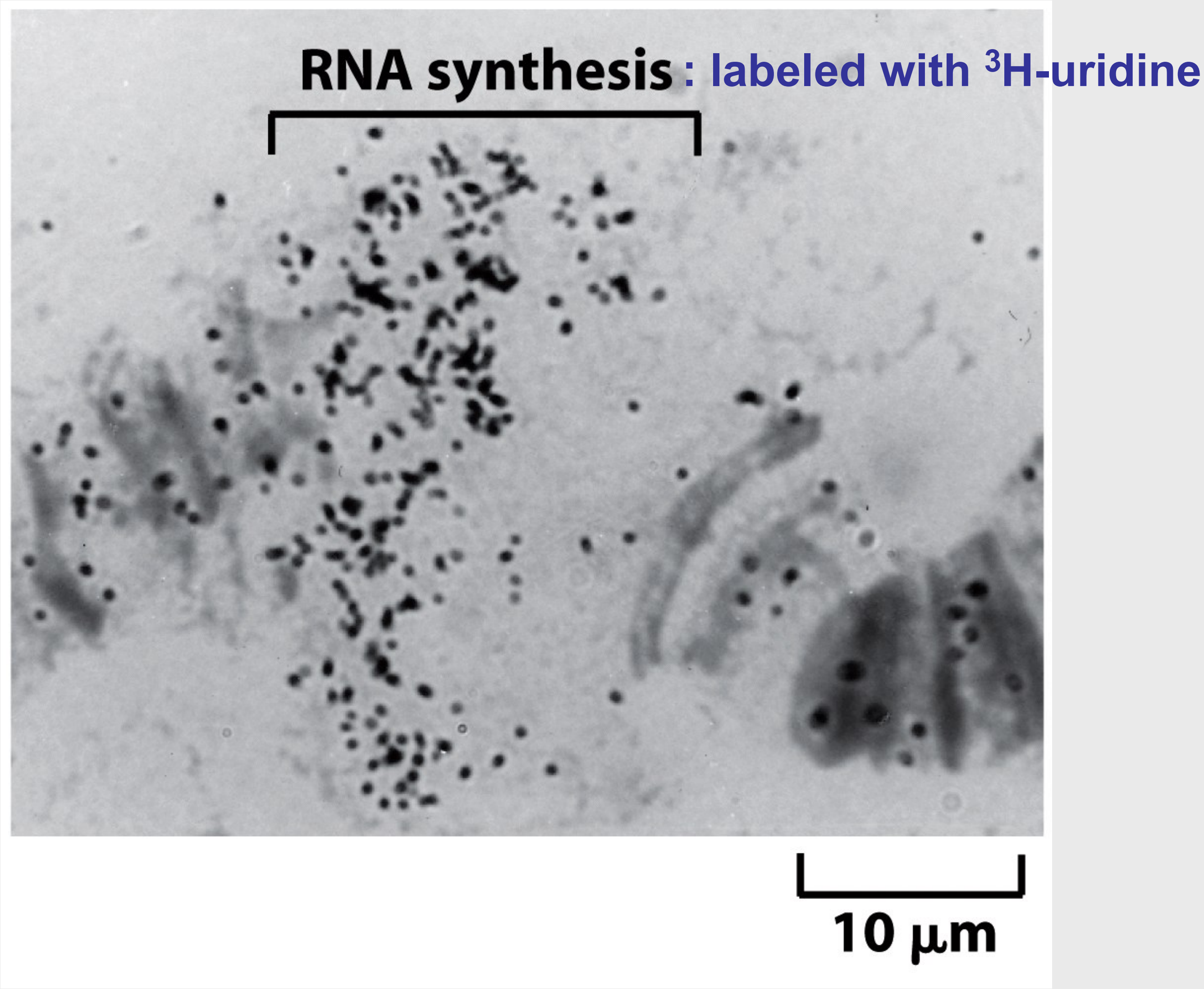


Nature Structural & Molecular Biology, 2013

Loops of Chromatin

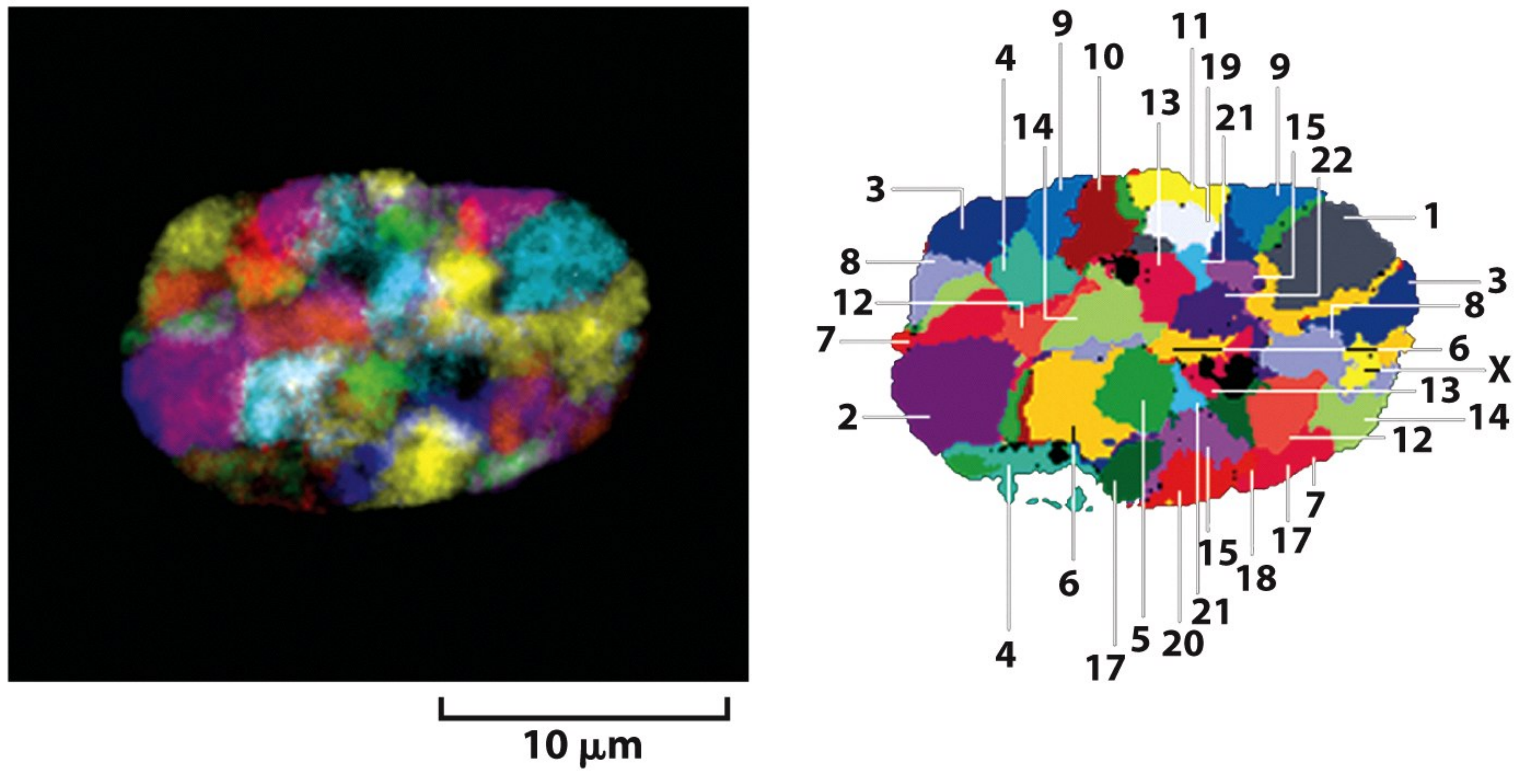


Chromatin loops decondense when the genes within them are expressed

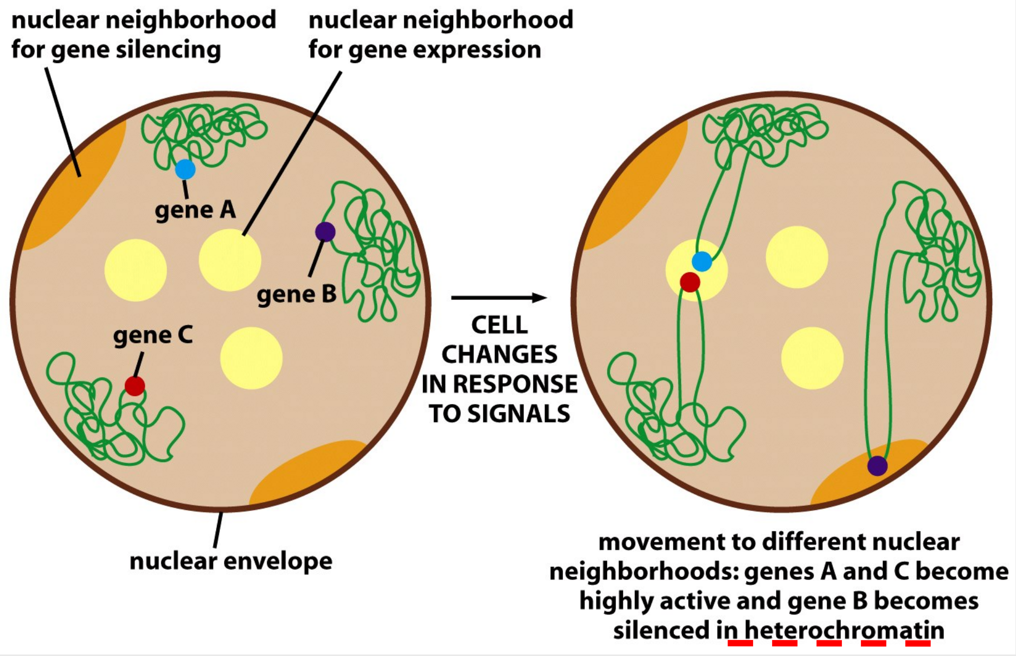


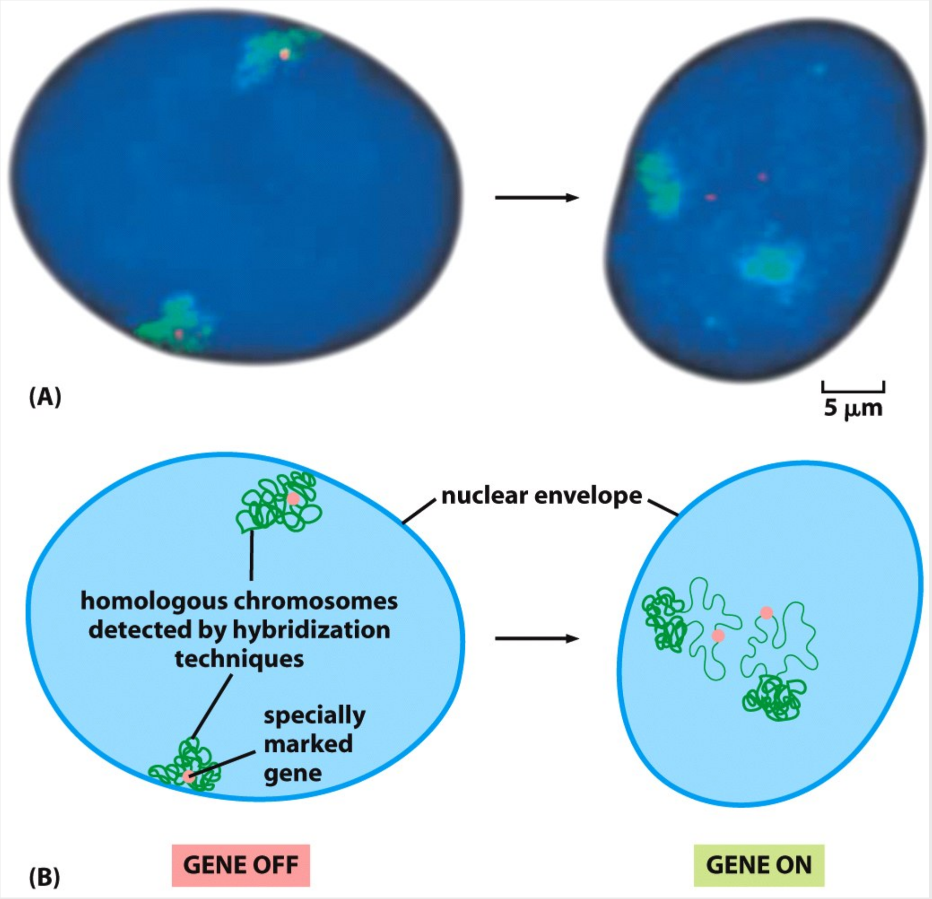
Chromatin can move to specific sites within the nucleus

- **Chromosome territory:**



The position of a gene changes when it becomes highly expressed

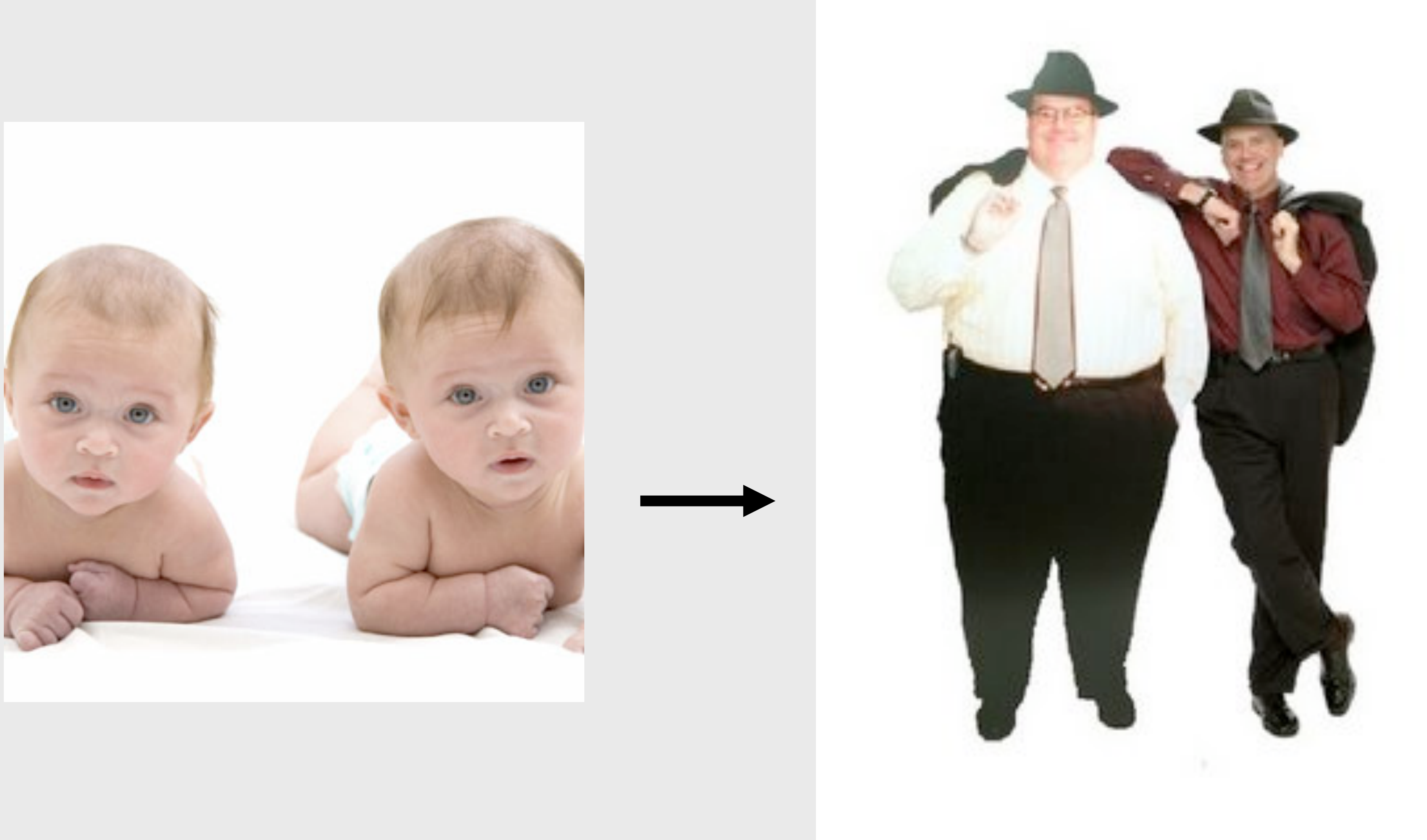




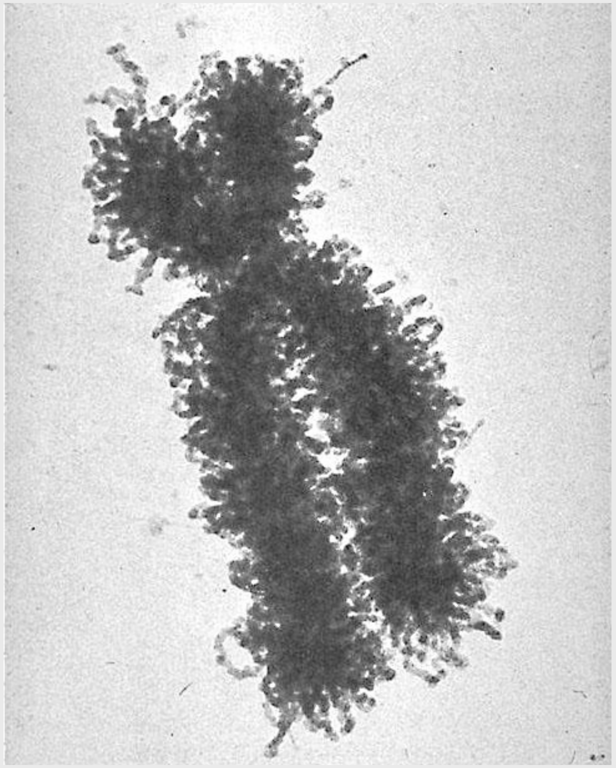
Epigenetics

- The term **epigenetics** refers to changes in appearance (phenotype) or gene expression caused by mechanisms other than changes in the underlying DNA sequence (genotype), hence the name *epi-* (Greek: over; above) *-genetics*

The same genotype with different phenotypes



One genome but many cell types



Chromosome

genetic information

epigenetics

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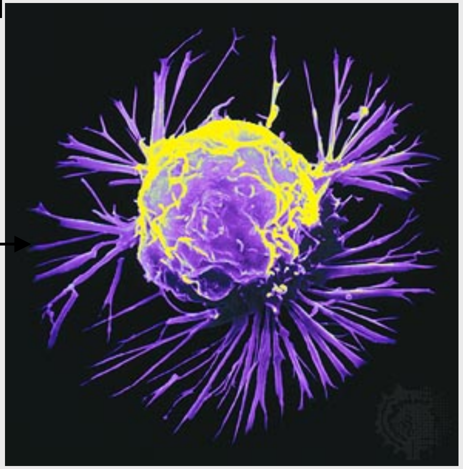
Neuronal cell

genetics/*epi*genetics



Breast cancer cell

>20 Disrupted DNA-methylation and histone-modification genes in cancer

***epi*genetics**

Liver cell

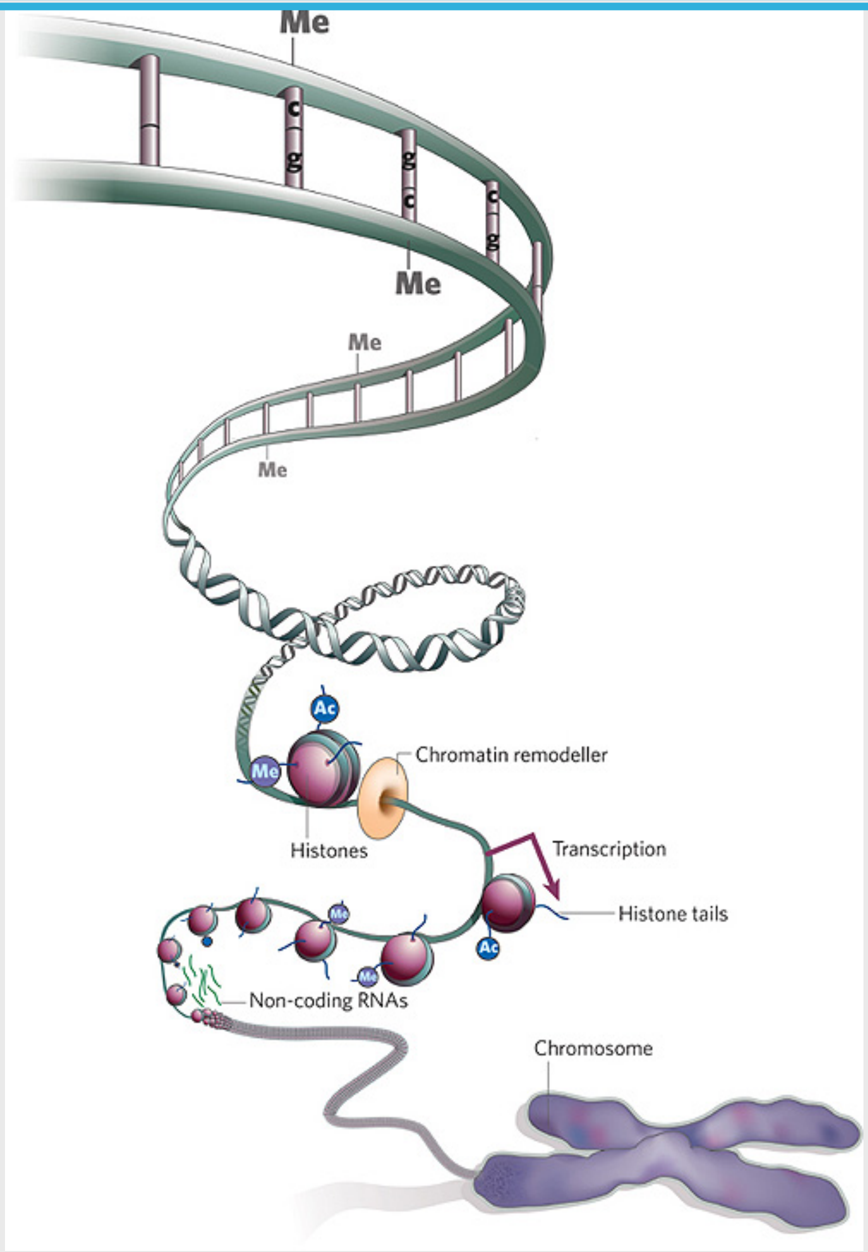
Nature Reviews Genetics, (2007)

Epigenetics: Bookmarks in the book of life

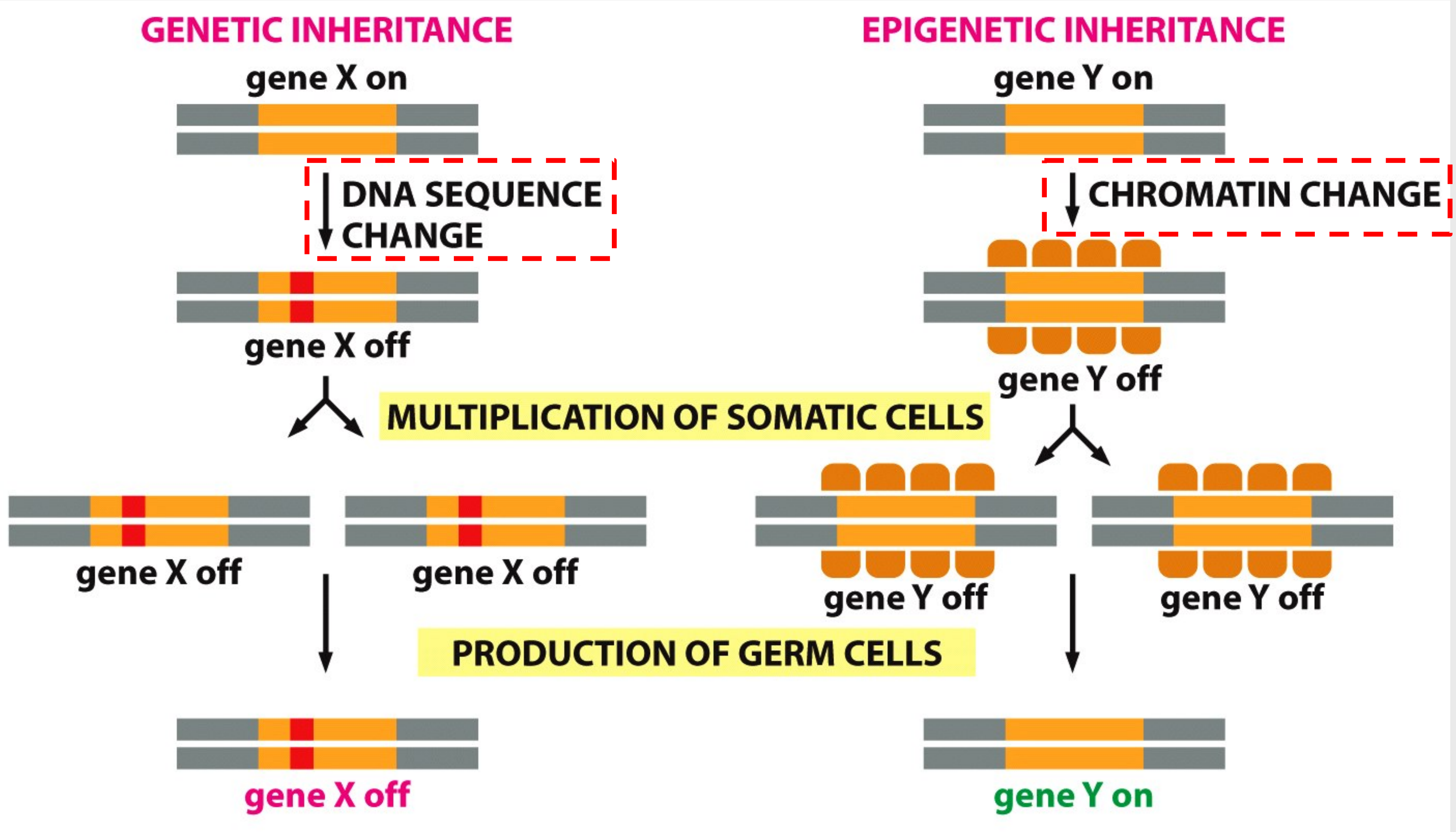
- Specific epigenetic processes include

Gene regulations by small RNAs, **Imprinting**, **X chromosome inactivation**, **Position effect**, **Development/Reprogramming of somatic nucleus**, **Maternal effects**, and **Regulation of histone modifications and heterochromatin.**

Epigenetic Regulations



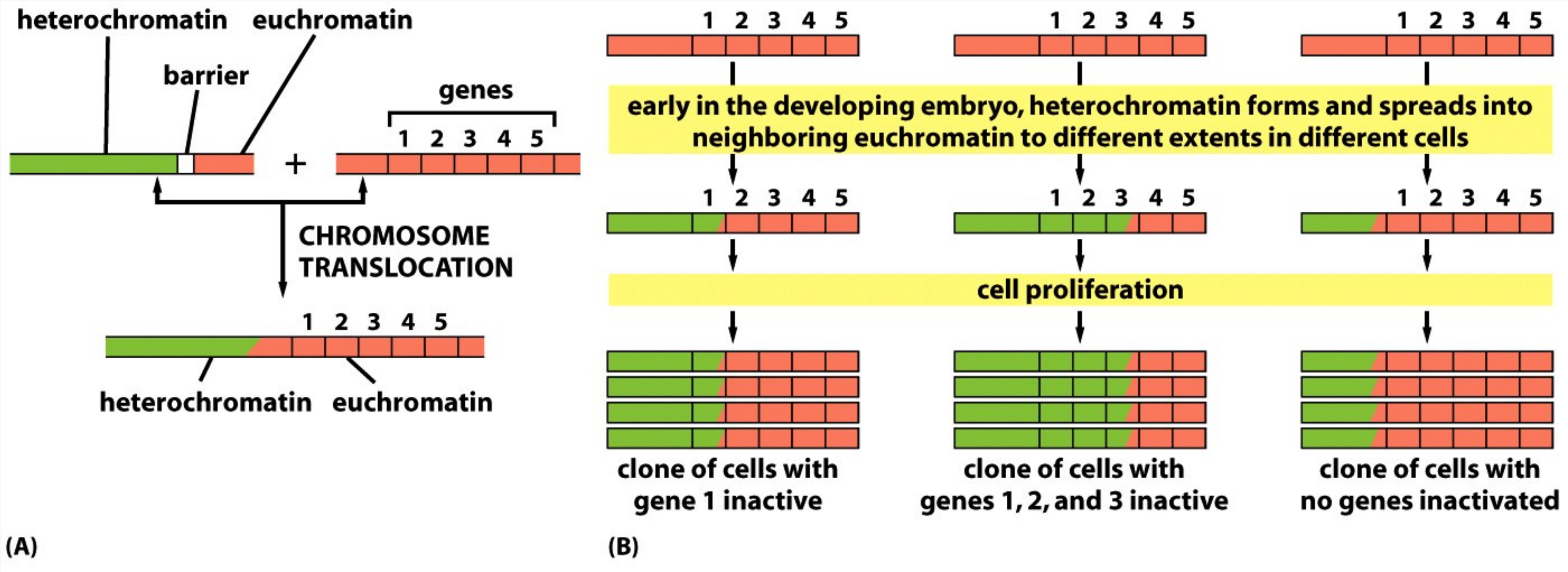
Epigenetic inheritance based on chromatin structures



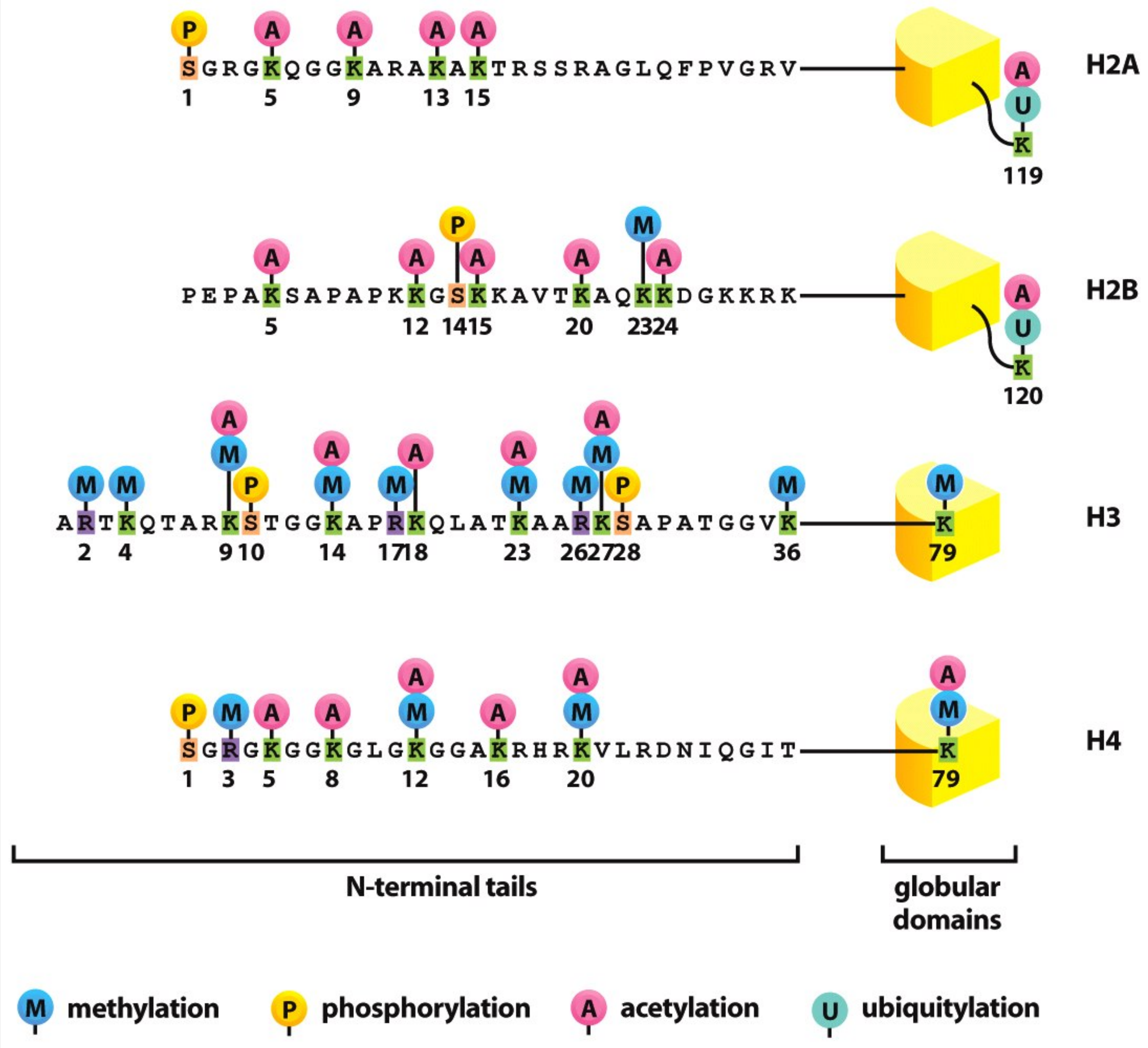
Heterochomatin vs. Euchromatin

- **Heterochomatin:** highly condensed form, genes within are **silenced**
- **Euchromatin:** less condensed form, genes within are **more active**

(Position Effect)

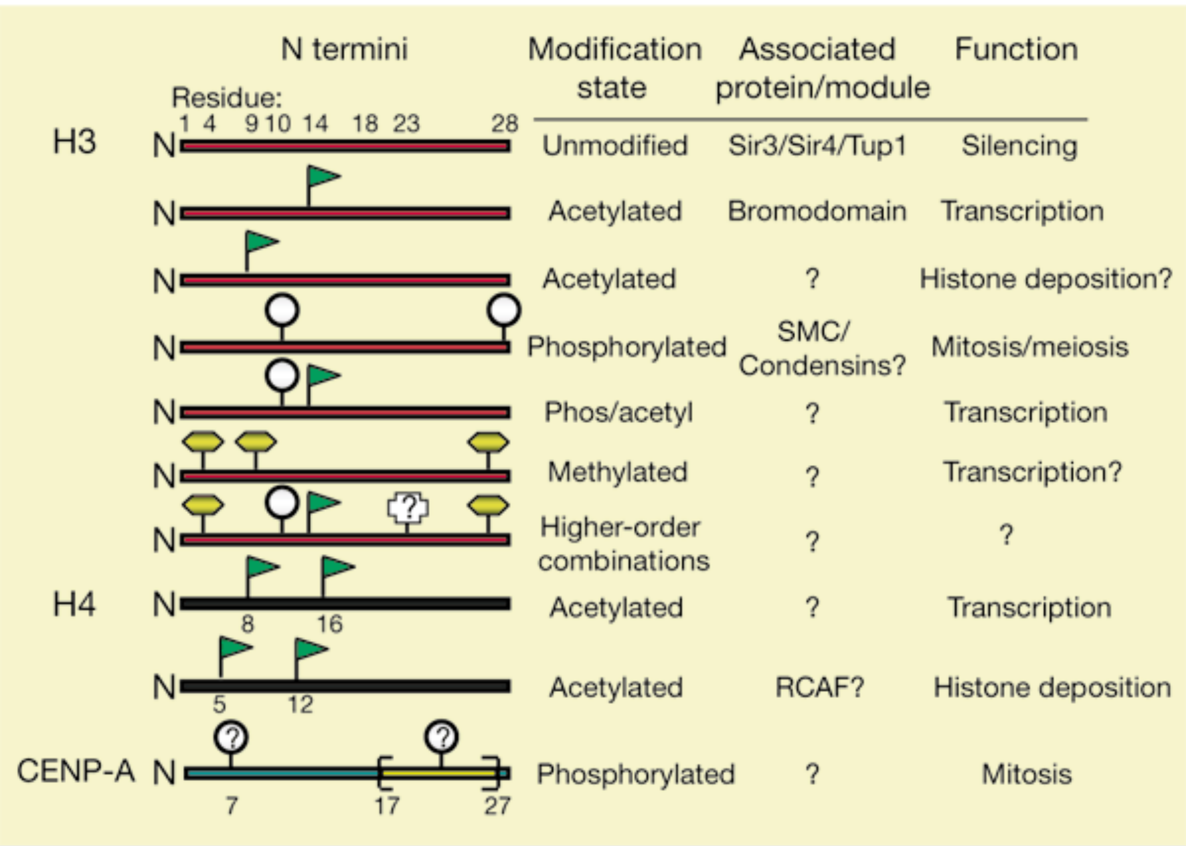


Posttranslational modifications of the histone proteins



Histone Code Theory

- As proposed by David Allis: “that multiple histone modifications, acting in a combinatorial or sequential fashion on one or multiple histone tails, specify unique downstream functions”

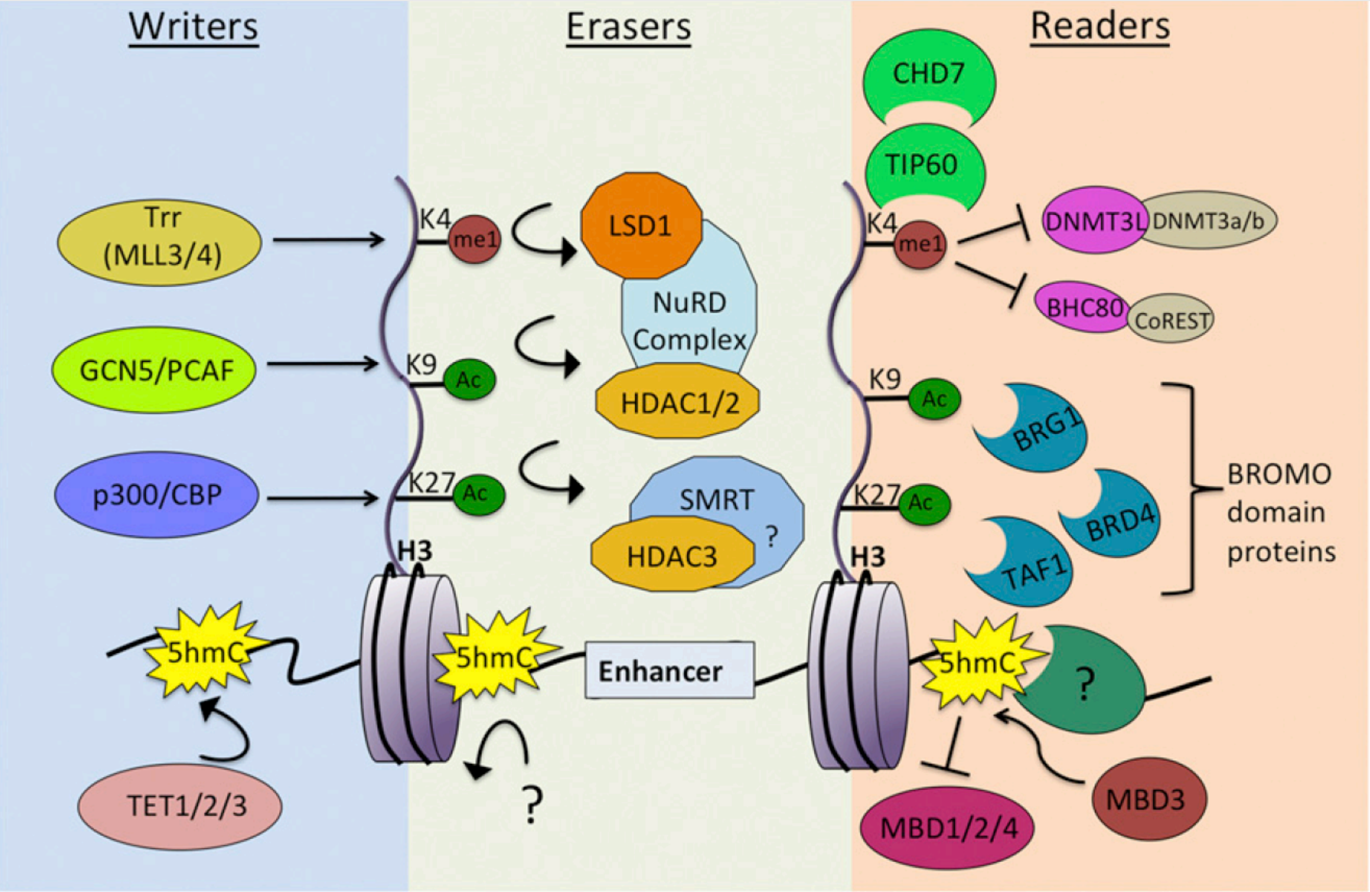


N termini	Modification state	Associated protein/module	Function
H3	Unmodified	Sir3/Sir4/Tup1	Silencing
	Acetylated	Bromodomain	Transcription
	Acetylated	?	Histone deposition?
	Phosphorylated	SMC/ Condensins?	Mitosis/meiosis
	Phos/acetyl	?	Transcription
	Methylated	?	Transcription?
	Higher-order combinations	?	?
H4	Acetylated	?	Transcription
	Acetylated	RCAF?	Histone deposition
CENP-A	Phosphorylated	?	Mitosis

Strahl, B.D. and Allis, C.D., *Nature.* 2000

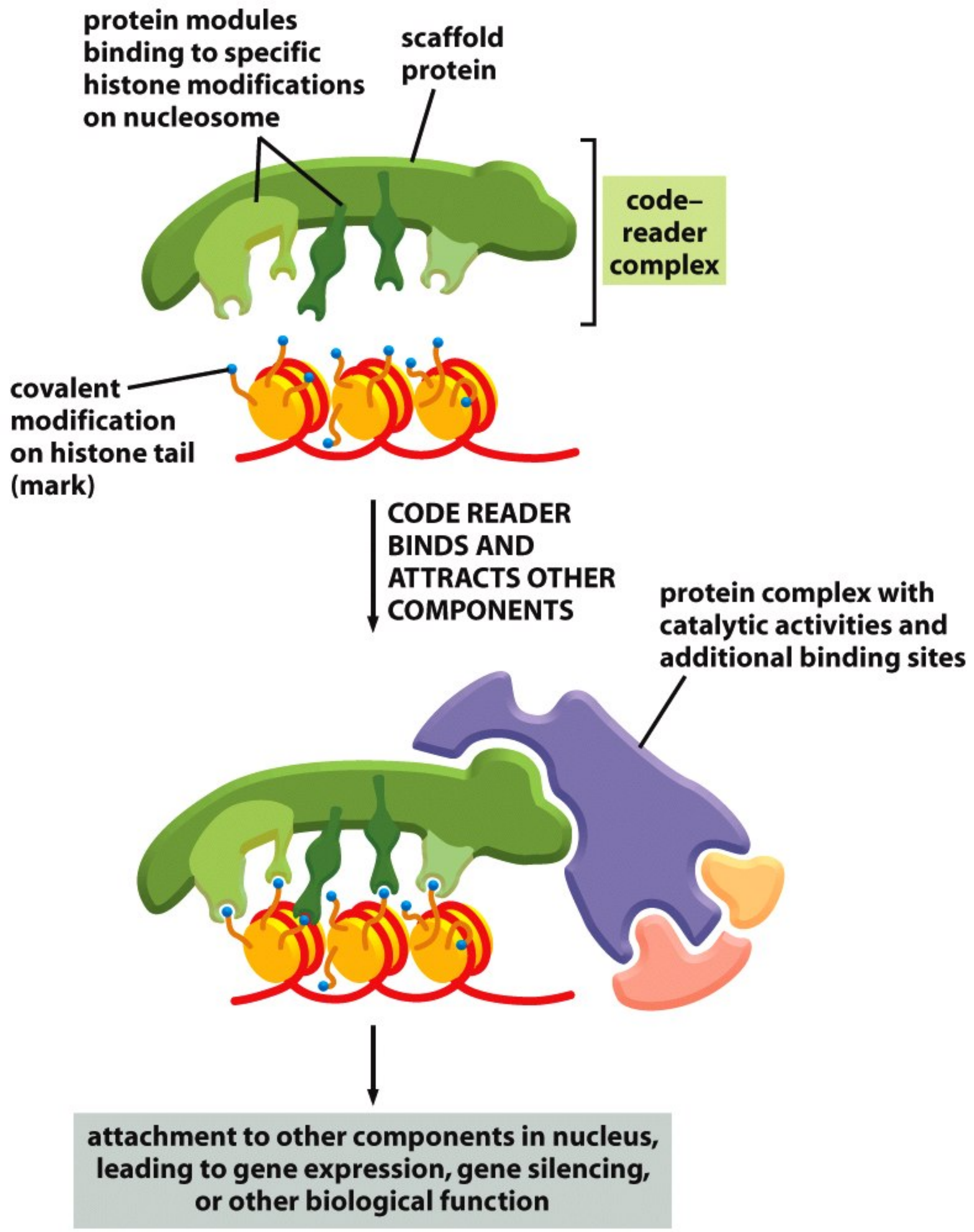
Code-Readers, Writers, and Erasers

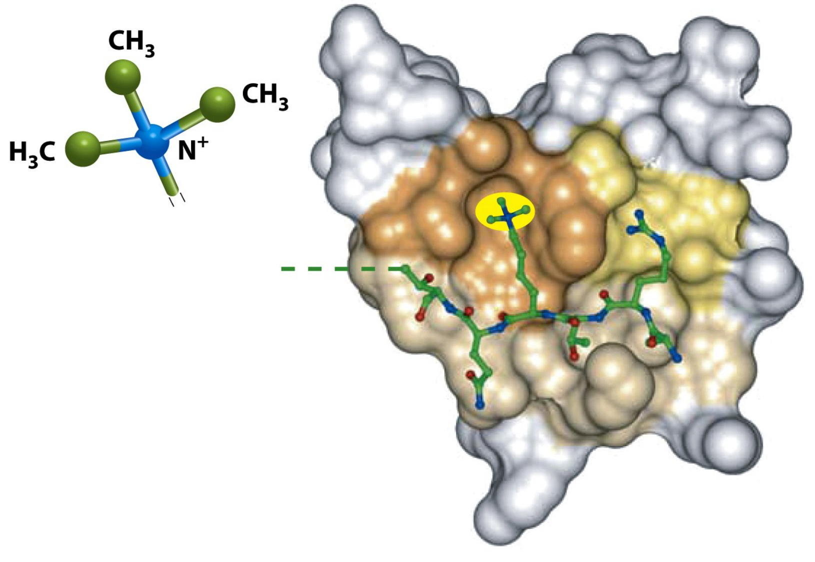
- Proteins capable of adding (**writers**), removing (**erasers**), and recognizing (**readers**) major enhancer-associated chromatin modifications, including H3K4me1, H3K9ac, H3K27ac, and 5hmC, are shown:



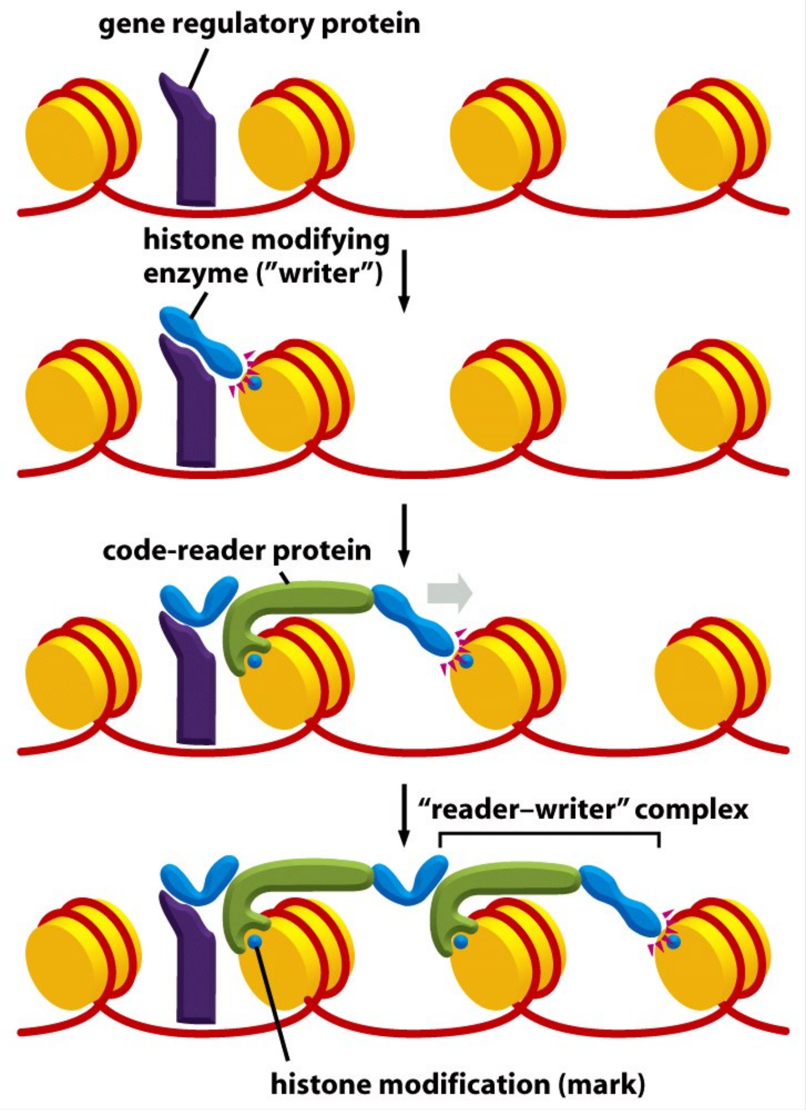
Molecular Cell 2013

Code-reader/writer complex



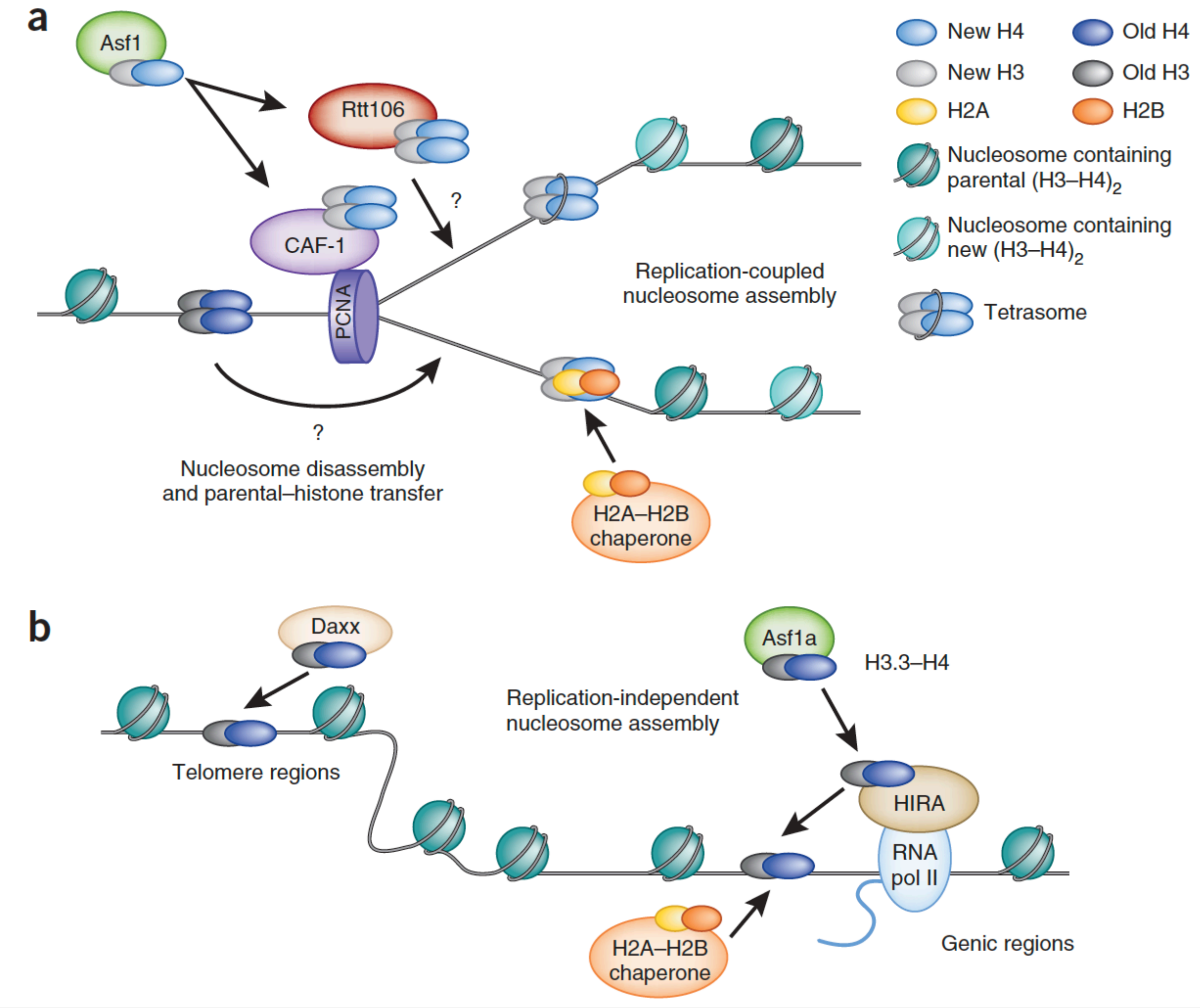


Spreading specific chromatin modifications along a chromosome



Epigenetic Transmission

- **Histone chaperones** are key regulators of replication-coupled and replication-independent nucleosome assembly.



Nature Structural & Molecular Biology, 2013

Histone Chaperones and Nucleosome Assembly

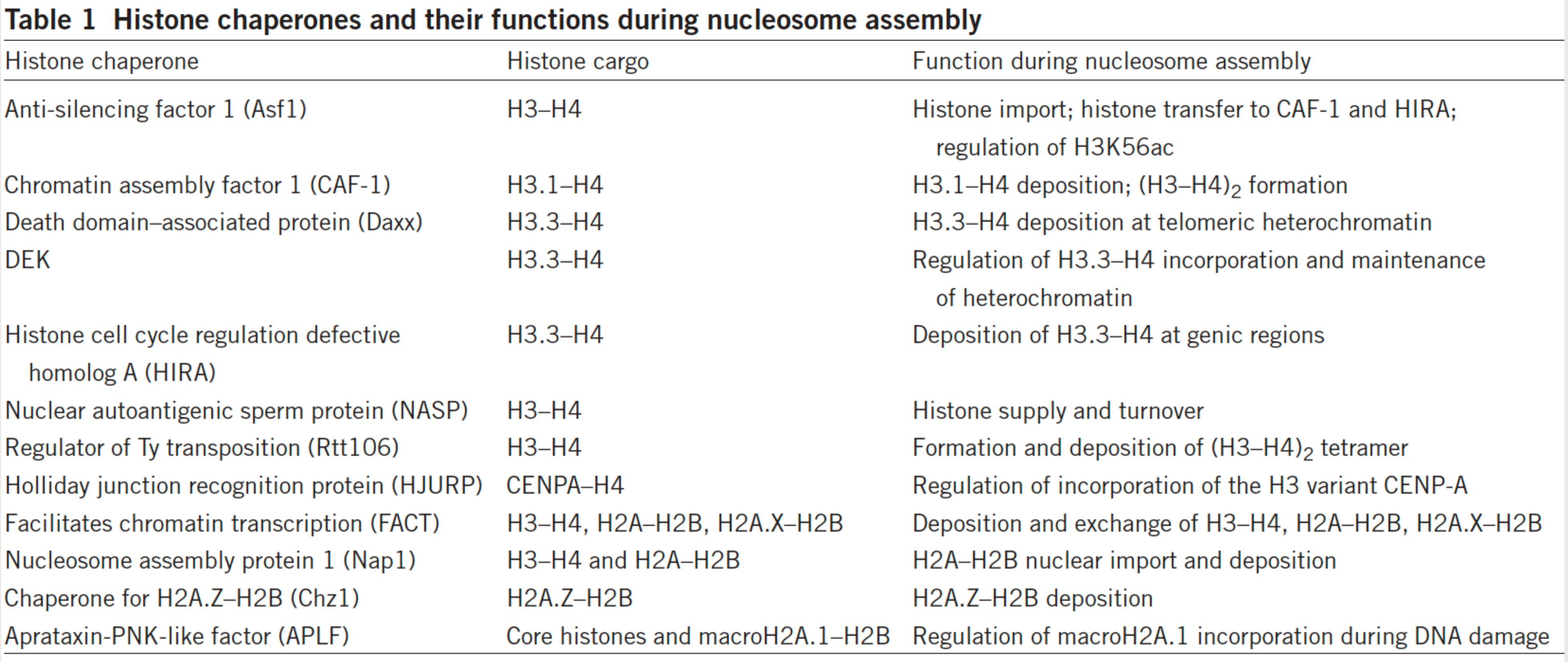
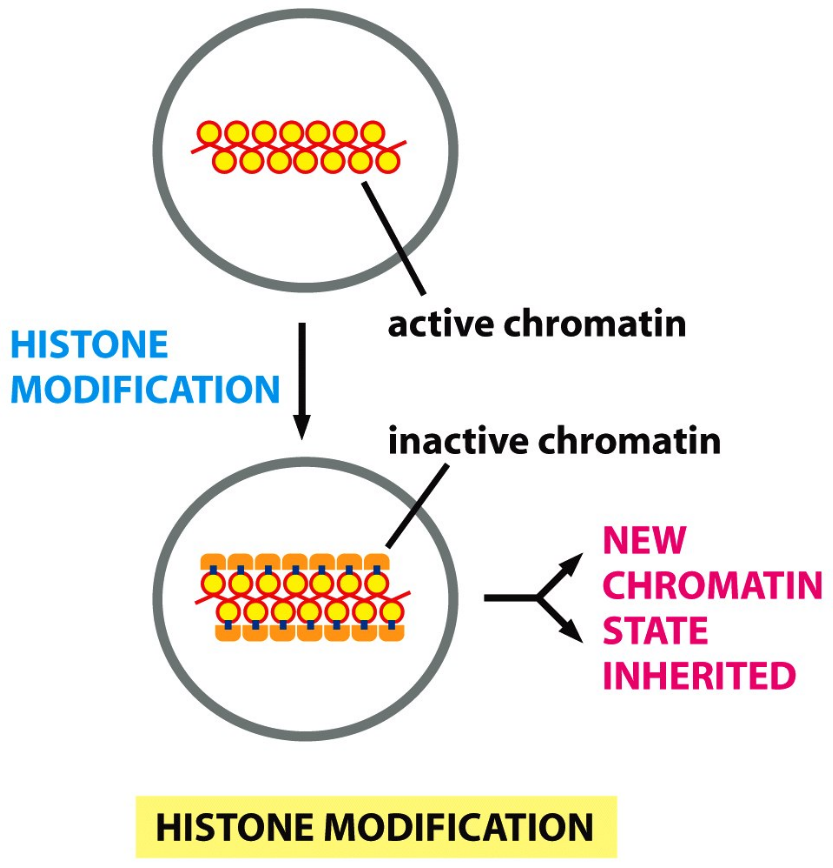


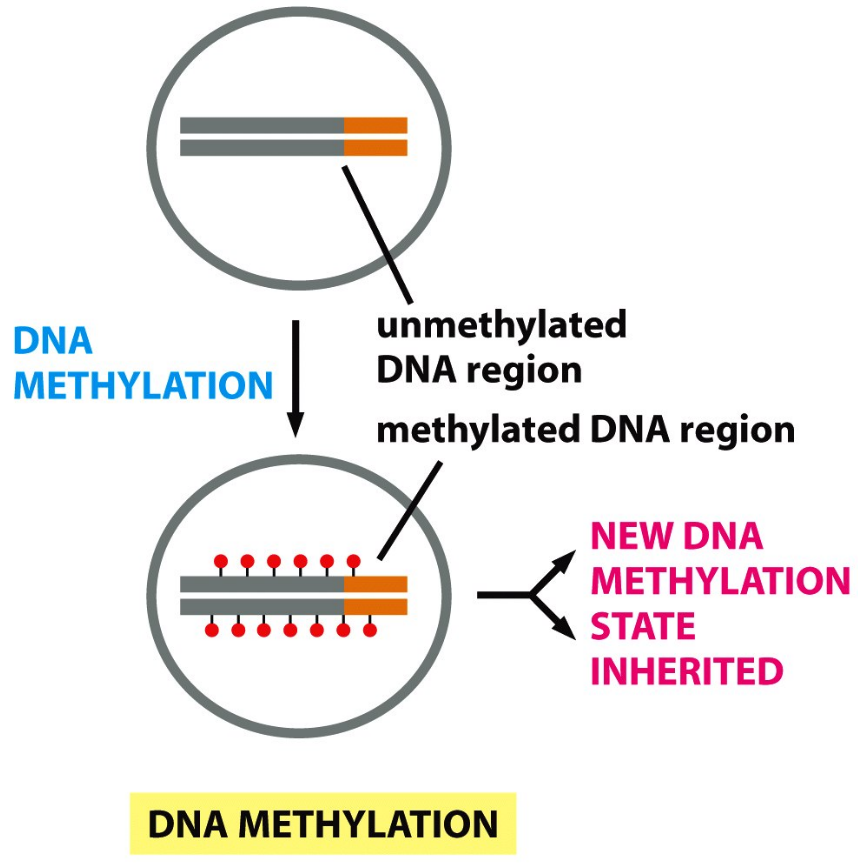
Table 1 Histone chaperones and their functions during nucleosome assembly

Histone chaperone	Histone cargo	Function during nucleosome assembly
Anti-silencing factor 1 (Asf1)	H3–H4	Histone import; histone transfer to CAF-1 and HIRA; regulation of H3K56ac
Chromatin assembly factor 1 (CAF-1)	H3.1–H4	H3.1–H4 deposition; $(H3–H4)_2$ formation
Death domain–associated protein (Daxx)	H3.3–H4	H3.3–H4 deposition at telomeric heterochromatin
DEK	H3.3–H4	Regulation of H3.3–H4 incorporation and maintenance of heterochromatin
Histone cell cycle regulation defective homolog A (HIRA)	H3.3–H4	Deposition of H3.3–H4 at genic regions
Nuclear autoantigenic sperm protein (NASP)	H3–H4	Histone supply and turnover
Regulator of Ty transposition (Rtt106)	H3–H4	Formation and deposition of $(H3–H4)_2$ tetramer
Holliday junction recognition protein (HJURP)	CENPA–H4	Regulation of incorporation of the H3 variant CENP-A
Facilitates chromatin transcription (FACT)	H3–H4, H2A–H2B, H2A.X–H2B	Deposition and exchange of H3–H4, H2A–H2B, H2A.X–H2B
Nucleosome assembly protein 1 (Nap1)	H3–H4 and H2A–H2B	H2A–H2B nuclear import and deposition
Chaperone for H2A.Z–H2B (Chz1)	H2A.Z–H2B	H2A.Z–H2B deposition
Aprataxin-PNK-like factor (APLF)	Core histones and macroH2A.1–H2B	Regulation of macroH2A.1 incorporation during DNA damage

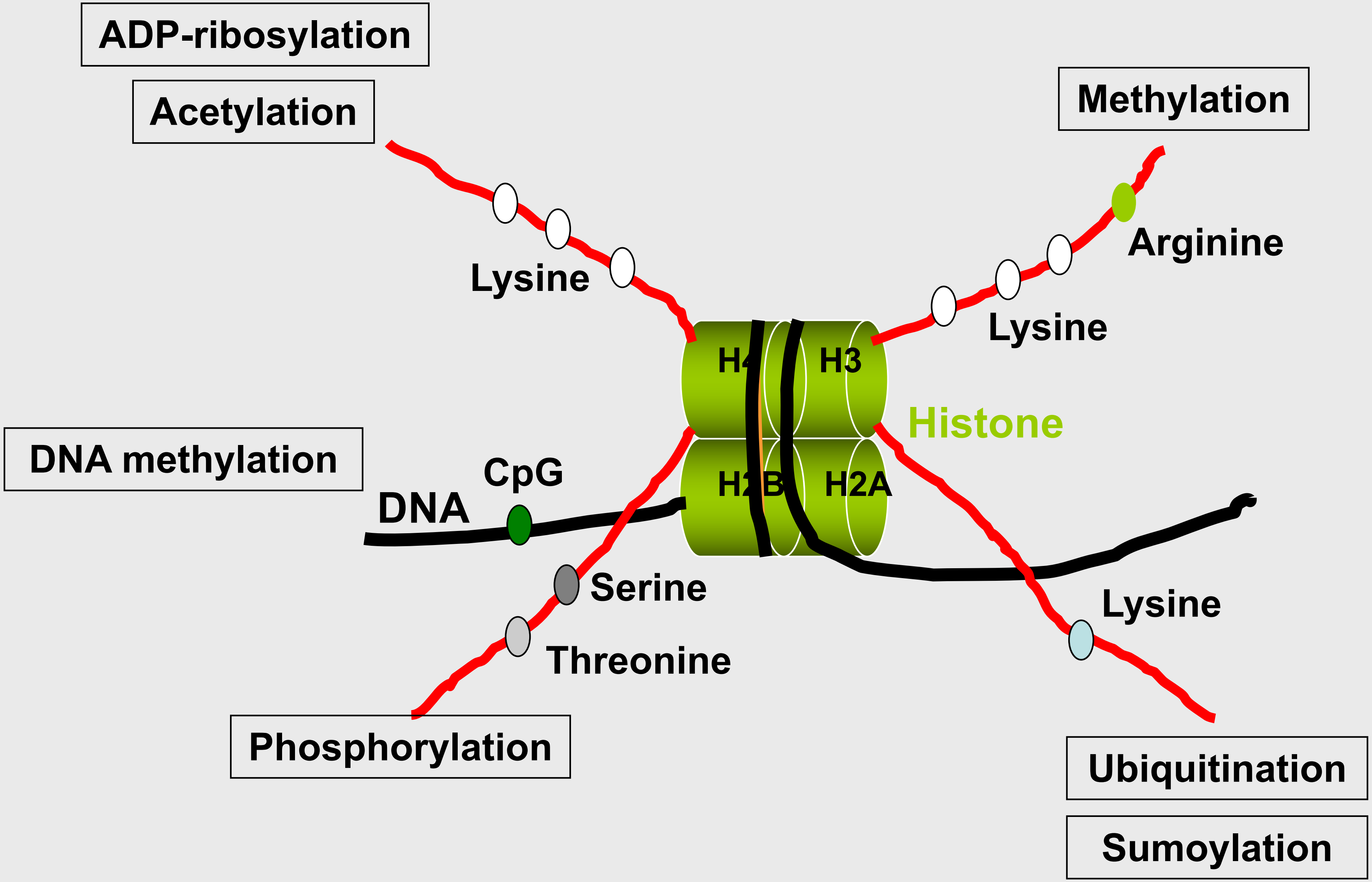
Nature Structural & Molecular Biology, 2013

Two Epigenetic Regulation Controls at Chromatin (DNA + Protein)





Posttranslational histone modifications



ADP-ribosylation

Acetylation

Lysine

Methylation

Arginine

Lysine

Histone

DNA methylation

CpG

DNA

Serine

Threonine

Phosphorylation

Lysine

Ubiquitination

Sumoylation

Acetylation

and

ADP-ribosylation

Histone Acetyltransferases (HATs)

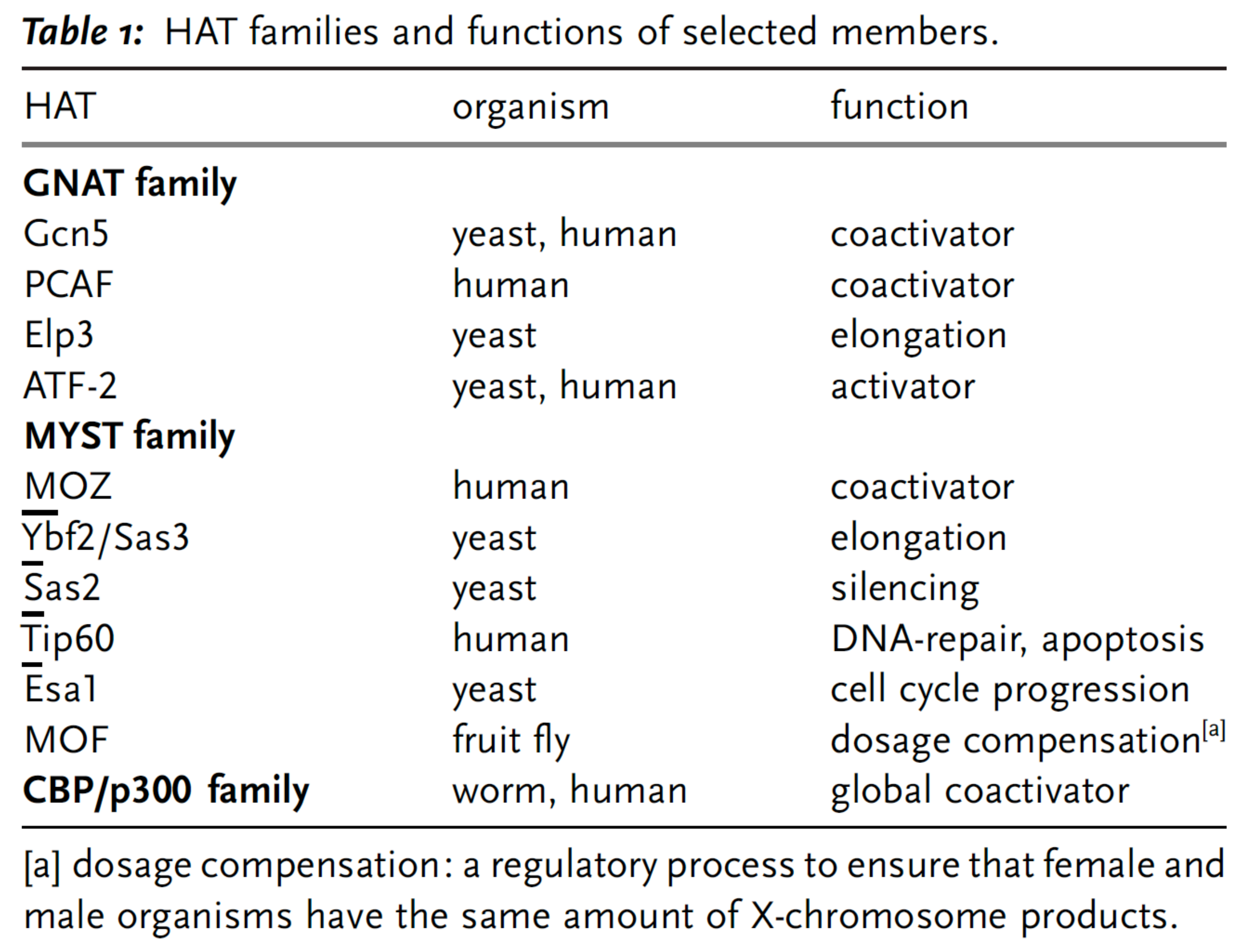
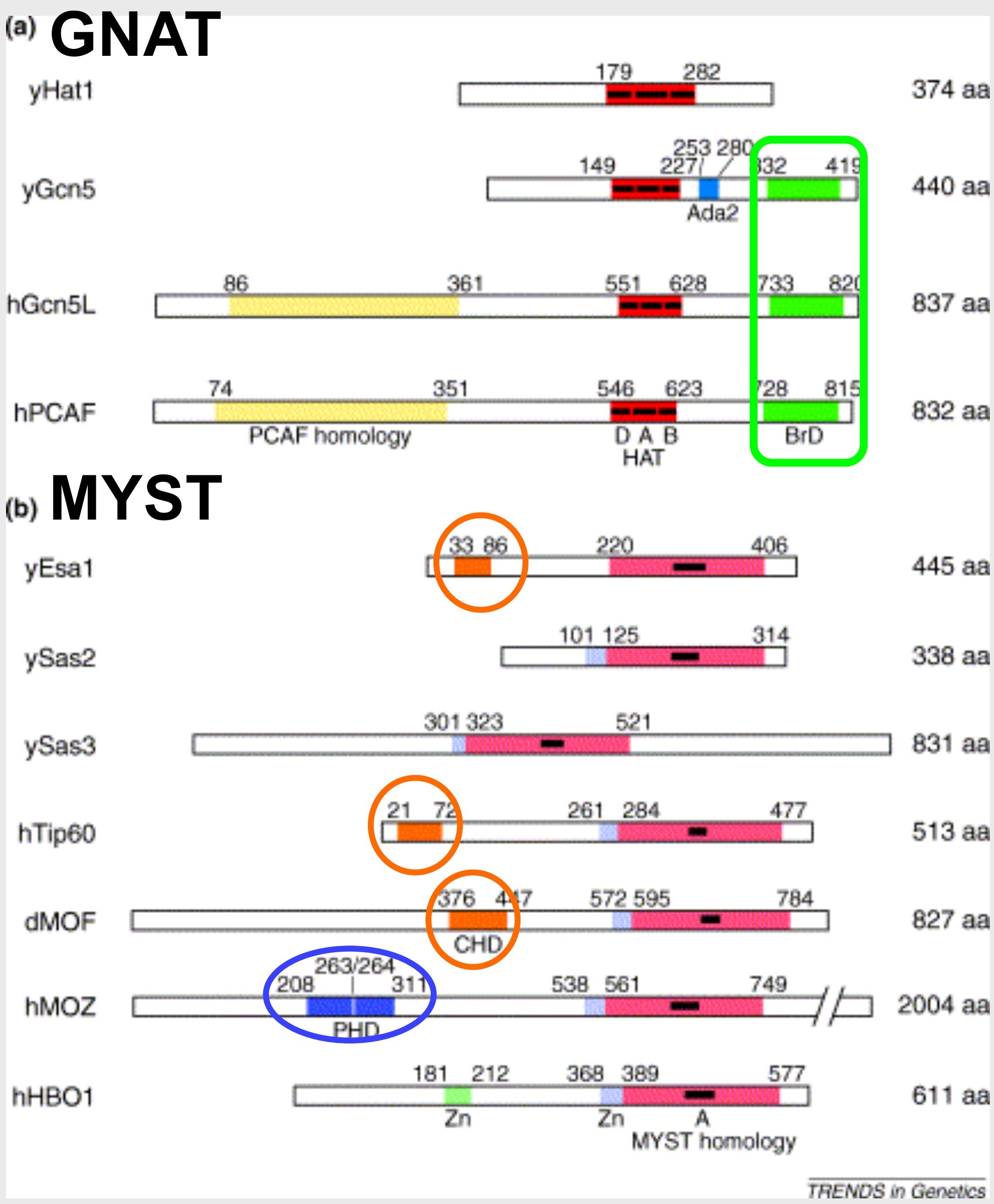


Table 1: HAT families and functions of selected members.

HAT	organism	function
1. **GNAT family**		
Gcn5	yeast, human	coactivator
PCAF	human	coactivator
Elp3	yeast	elongation
ATF-2	yeast, human	activator
2. **MYST family**		
MOZ	human	coactivator
Ybf2/Sas3	yeast	elongation
Sas2	yeast	silencing
Tip60	human	DNA-repair, apoptosis
Esa1	yeast	cell cycle progression
MOF	fruit fly	dosage compensation[a]
3. **CBP/p300 family**	worm, human	global coactivator

[a] dosage compensation: a regulatory process to ensure that female and male organisms have the same amount of X-chromosome products.

Modular domains found in various histone acetyltransferases



1. Green – **Bromodomains**:

   interact with **acetylated-lysine**

2. Orange – **Chromodomains**:

   recognition of **methylated-lysine**

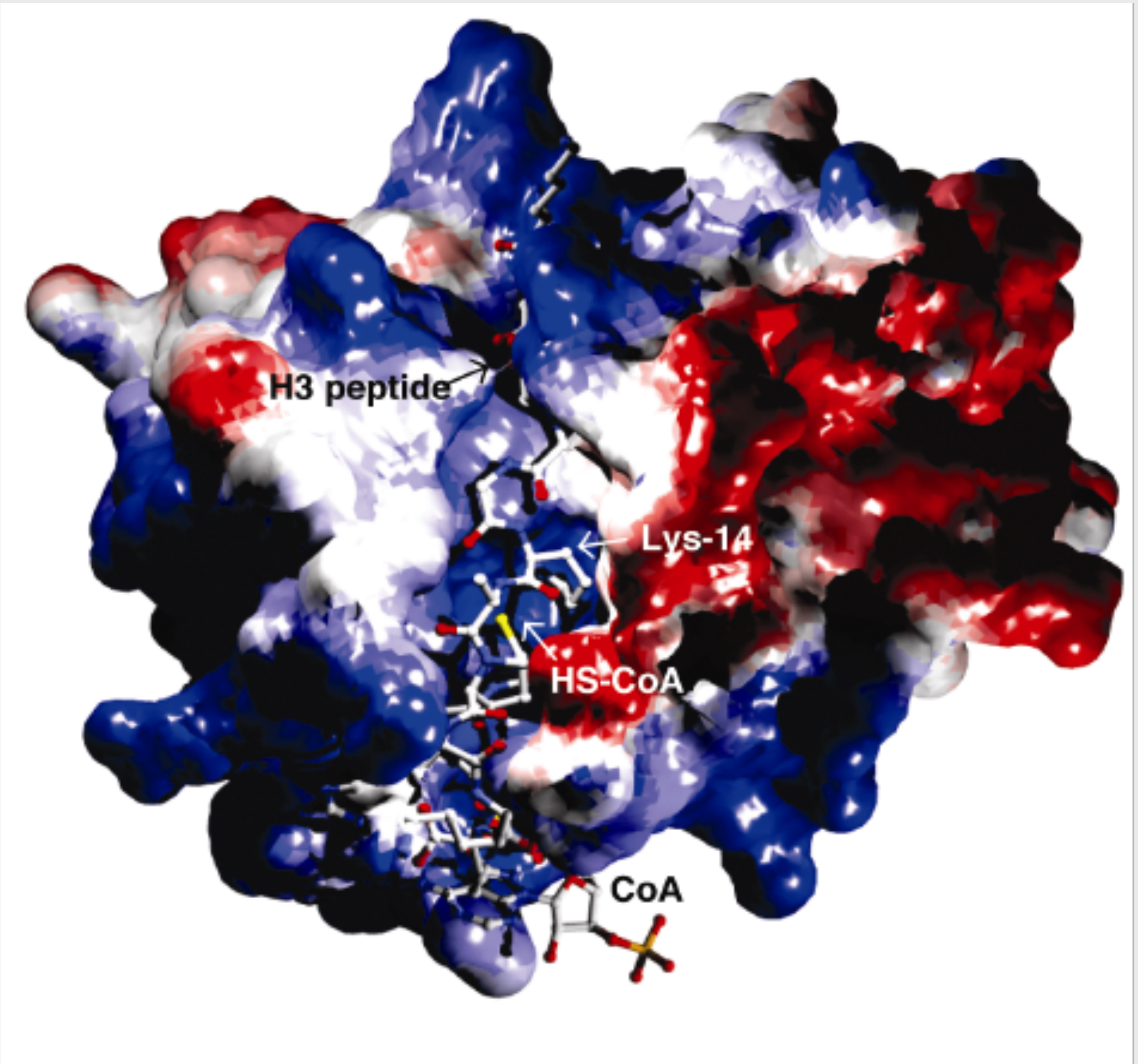
3. **Red** – **GNAT HAT domain**

4. **Pink** – **MYST HAT region**

5. Light Blue & Green – **Zn fingers**

Fr. Carrozza et al. Trends Genet 2003

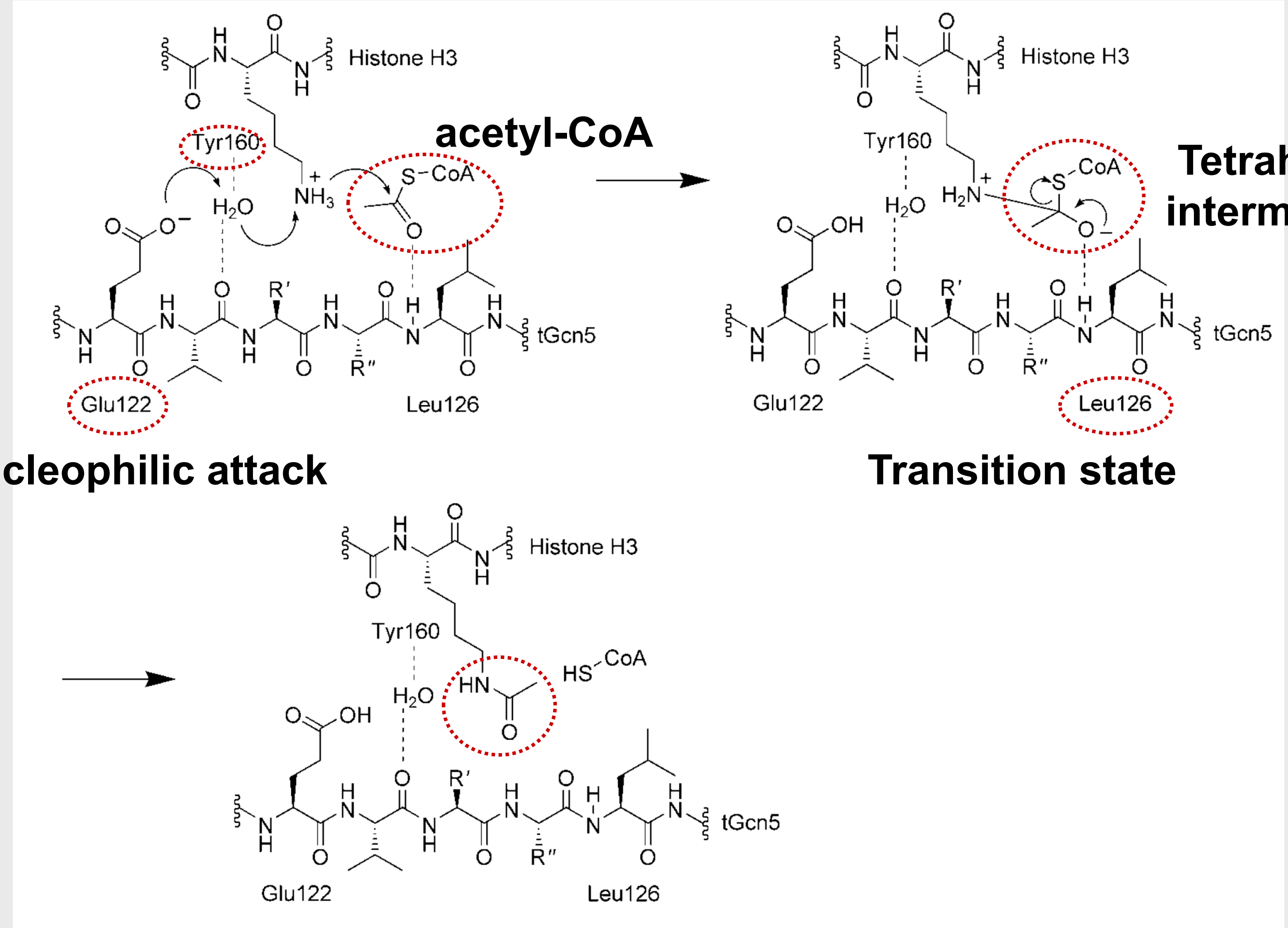
Ligand Bound form of a GCN5-type HAT



tGCN5•CoA•H3 peptide

Rojas et al., 1999

Catalytic mechanism of the HAT, GCN5

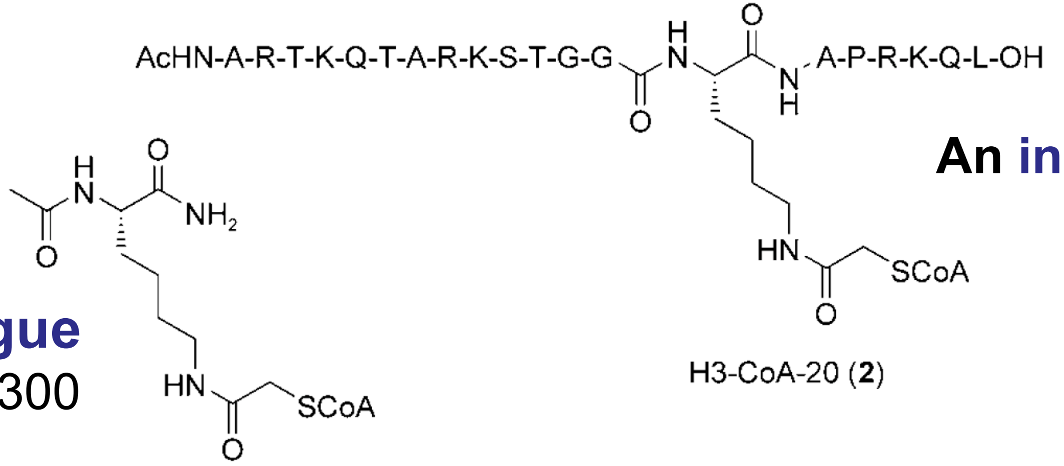

acetyl-CoA

Tetrahedral intermediate

Nucleophilic attack

Transition state

Modulators of the HATs:

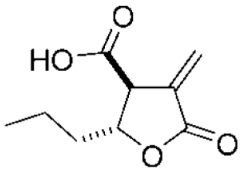


Lys-CoA (**1**)

H3-CoA-20 (**2**)

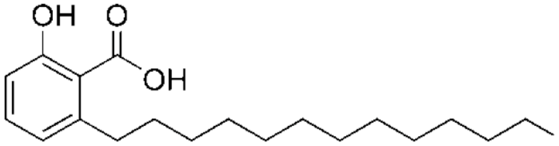
Bisubstrate Analogue
and inhibits the HAT p300

An inhibitor of PCAF



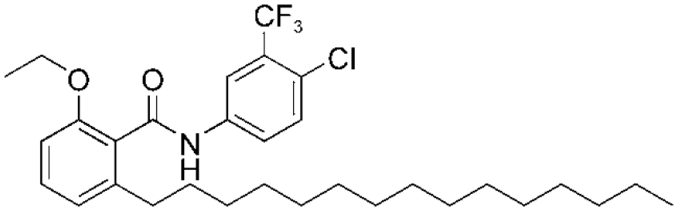
MB-3 (**5**)

cell-permeable inhibitor of the human Gcn5



anacardic acid (**3**)

inhibits both p300 and PCAF

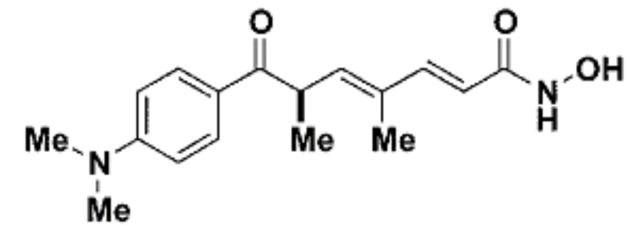


CTPB (**4**) **An activator**

Histone deacetylases families and inhibitors

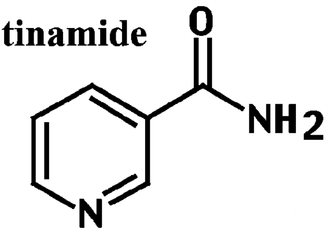
Table I. Classification of Mammalian Histone Deacetylases (HDACs)

Class	Enzyme	Catal. domain	Mechanism of deacetylase activity	Subcellular localization
I (Rpd3-like)	HDAC 1	one	Zn^{2+} dependent	nuclear
	HDAC 2	one	Zn^{2+} dependent	nuclear
	HDAC 3	one	Zn^{2+} dependent	nucleocytoplasmic shuttling
	HDAC 8	one	Zn^{2+} dependent	nuclear
	HDAC 11	one	Zn^{2+} dependent	nuclear
II (Hda1-like)	HDAC 4	one	Zn^{2+} dependent	n. shuttling
	HDAC 5	one	Zn^{2+} dependent	n. shuttling
	HDAC 6 IIb	two	Zn^{2+} dependent	n. shuttling
	HDAC 7	one	Zn^{2+} dependent	n. shuttling
	HDAC 9	one	Zn^{2+} dependent	n. shuttling
	HDAC 10 IIb	one	Zn^{2+} dependent	n. shuttling
III (Sir2-like)	SIRT 1	one	NAD^+ dependent	nucleus
	SIRT 2	one	NAD^+ dependent	cytosol
	SIRT 3	one	NAD^+ dependent	mitochondria
	SIRT 4	one	NAD^+ dependent	NU
	SIRT 5	one	NAD^+ dependent	NU
	SIRT 6	one	NAD^+ dependent	NU
	SIRT 7	one	NAD^+ dependent	NU



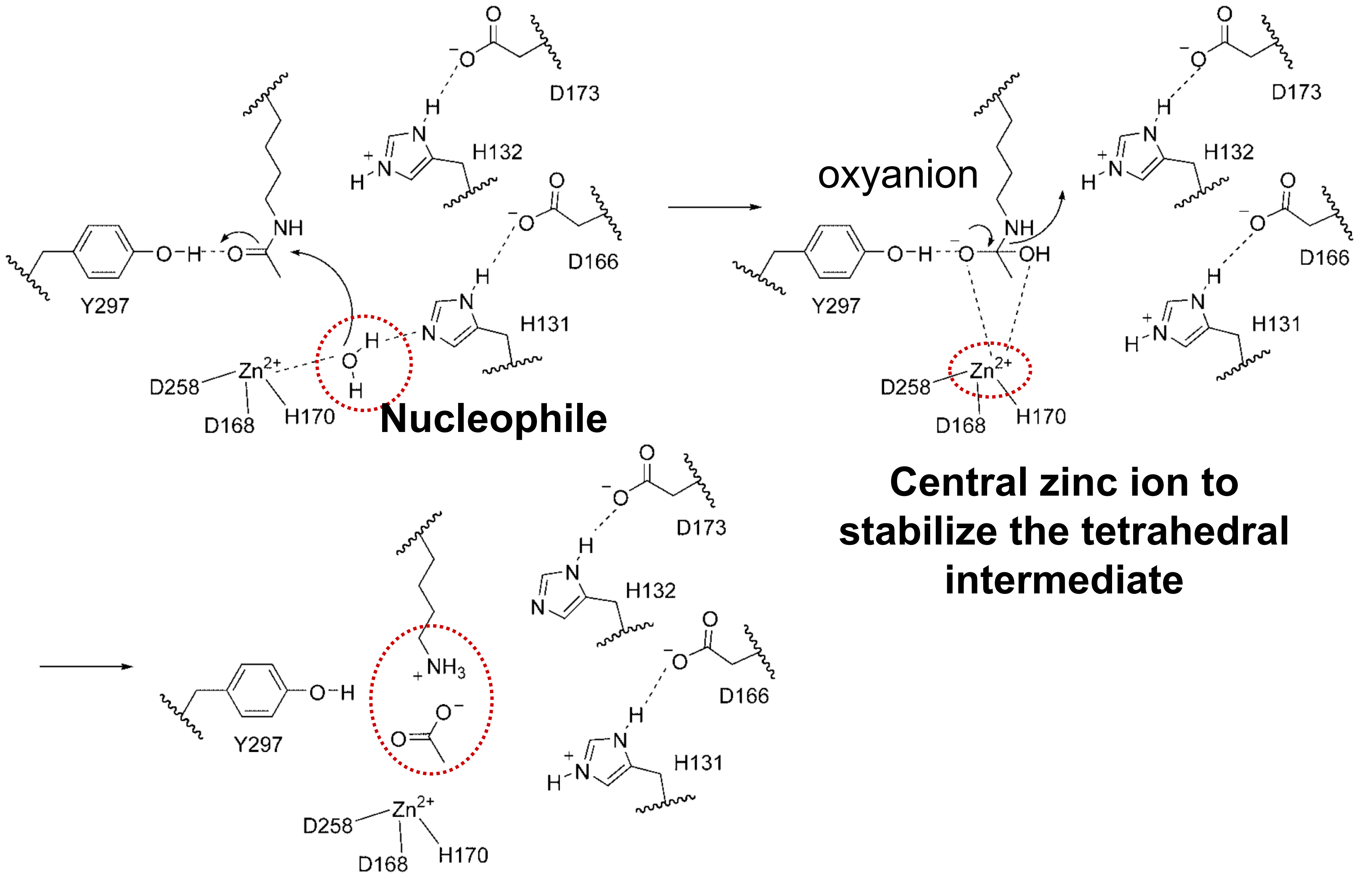
trichostatin A (TSA)

nicotinamide



Mai A. et al., Med Res Rev 2005

Catalytic mechanism of class I and II HDACs



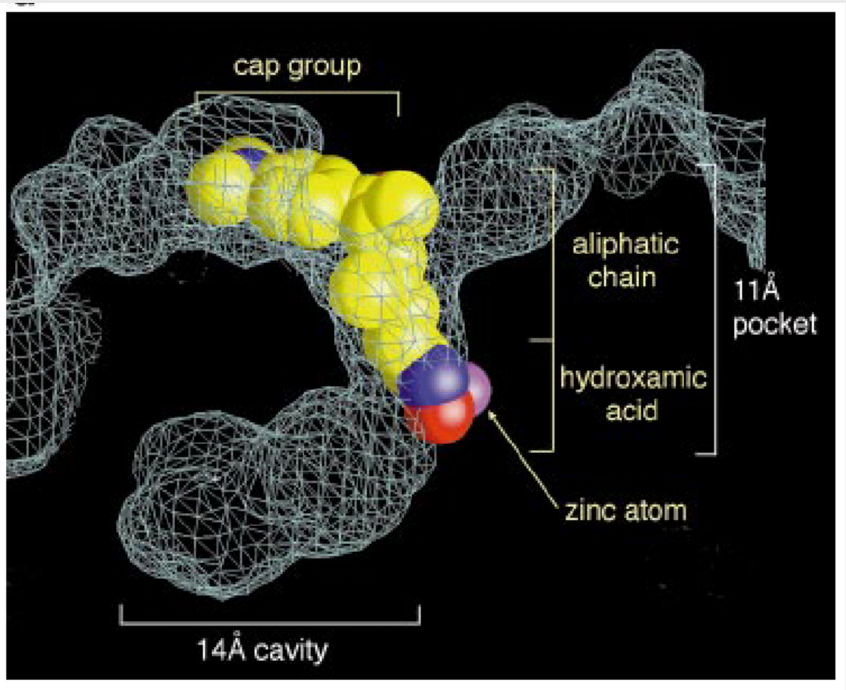
Nucleophile

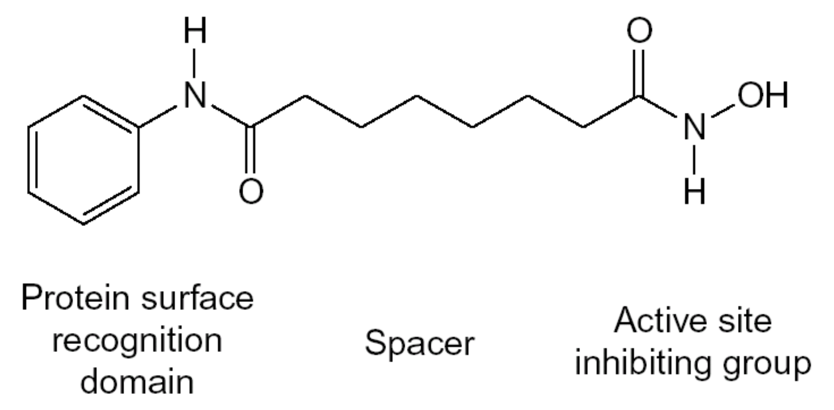
oxyanion

Central zinc ion to stabilize the tetrahedral intermediate

Development of HDAC inhibitors

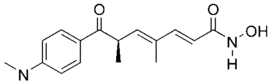
- **Combinatorial approach:
  HDAC paralog-selective inhibitor from a diversity-oriented synthetic process** **(Schreiber SL. *et al*. in PNAS 2003, Chem. Biol. 2003 and JACS 2003)**
- **The crystal structure of the HDAC catalytic core from *Aquifex aeolicus* (hyperthermophilic bacterium) and HDAC inhibitors, suberoylanilide hydroxamic acid (SAHA)**

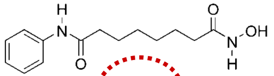


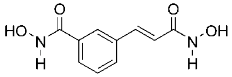


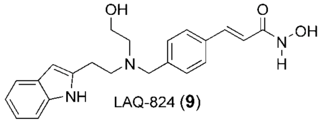
Finnin MS. *et al*., Nature 1999

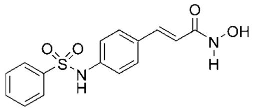
Some HDAC inhibitors

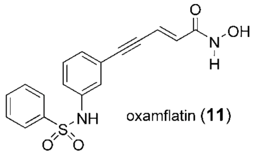

TSA (**6**)

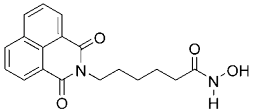

SAHA (**7**)

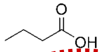

CBHA (**8**)

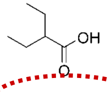

LAQ-824 (**9**)

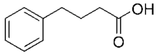

sulfonamide hydroxamic acid (**10**)

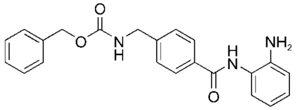

oxamflatin (**11**)

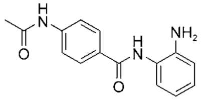

scriptaid (**12**)

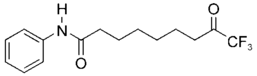

butyric acid (**13**)

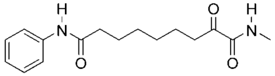

valproic acid (**14**)

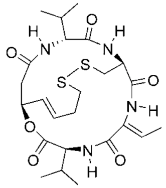

phenyl butyrate (**15**)

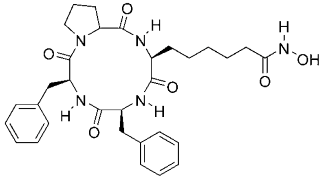

MS-275 (**16**)

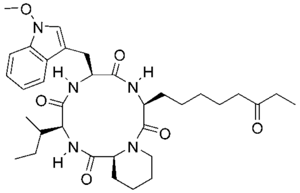

CI-994 (**17**)

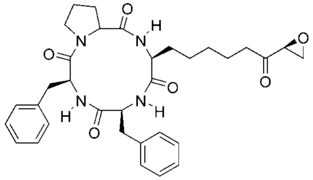

trifluormethyl ketone (**18**)

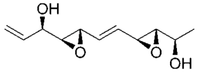

α-ketoamide (**19**)


depsipeptide FK-228 (**20**)


CHAP (**21**)


apicidin (**22**)


trapoxin B (**23**)


depudecin (**24**)

Class III Deacetylases

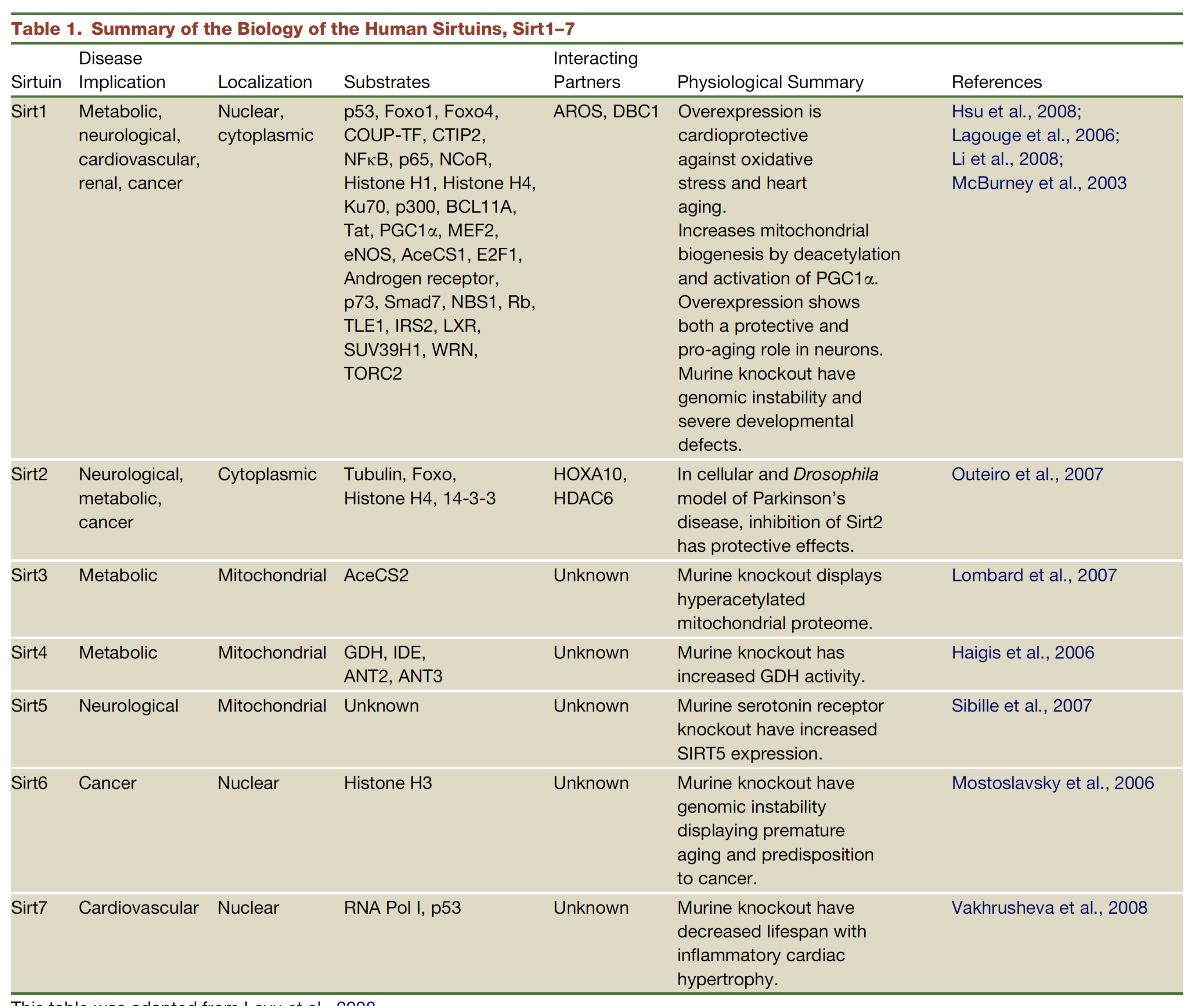


Table 1. Summary of the Biology of the Human Sirtuins, Sirt1–7

Sirtuin	Disease Implication	Localization	Substrates	Interacting Partners	Physiological Summary	References
Sirt1	Metabolic, neurological, cardiovascular, renal, cancer	Nuclear, cytoplasmic	p53, Foxo1, Foxo4, COUP-TF, CTIP2, NFκB, p65, NCoR, Histone H1, Histone H4, Ku70, p300, BCL11A, Tat, PGC1α, MEF2, eNOS, AceCS1, E2F1, Androgen receptor, p73, Smad7, NBS1, Rb, TLE1, IRS2, LXR, SUV39H1, WRN, TORC2	AROS, DBC1	Overexpression is cardioprotective against oxidative stress and heart aging. Increases mitochondrial biogenesis by deacetylation and activation of PGC1α. Overexpression shows both a protective and pro-aging role in neurons. Murine knockout have genomic instability and severe developmental defects.	Hsu et al., 2008; Lagouge et al., 2006; Li et al., 2008; McBurney et al., 2003
Sirt2	Neurological, metabolic, cancer	Cytoplasmic	Tubulin, Foxo, Histone H4, 14-3-3	HOXA10, HDAC6	In cellular and *Drosophila* model of Parkinson's disease, inhibition of Sirt2 has protective effects.	Outeiro et al., 2007
Sirt3	Metabolic	Mitochondrial	AceCS2	Unknown	Murine knockout displays hyperacetylated mitochondrial proteome.	Lombard et al., 2007
Sirt4	Metabolic	Mitochondrial	GDH, IDE, ANT2, ANT3	Unknown	Murine knockout has increased GDH activity.	Haigis et al., 2006
Sirt5	Neurological	Mitochondrial	Unknown	Unknown	Murine serotonin receptor knockout have increased SIRT5 expression.	Sibille et al., 2007
Sirt6	Cancer	Nuclear	Histone H3	Unknown	Murine knockout have genomic instability displaying premature aging and predisposition to cancer.	Mostoslavsky et al., 2006
Sirt7	Cardiovascular	Nuclear	RNA Pol I, p53	Unknown	Murine knockout have decreased lifespan with inflammatory cardiac hypertrophy.	Vakhrusheva et al., 2008

This table was adapted from Lavu et al., 2008

Sirtuin substrates:

Histone and non-histone proteins!

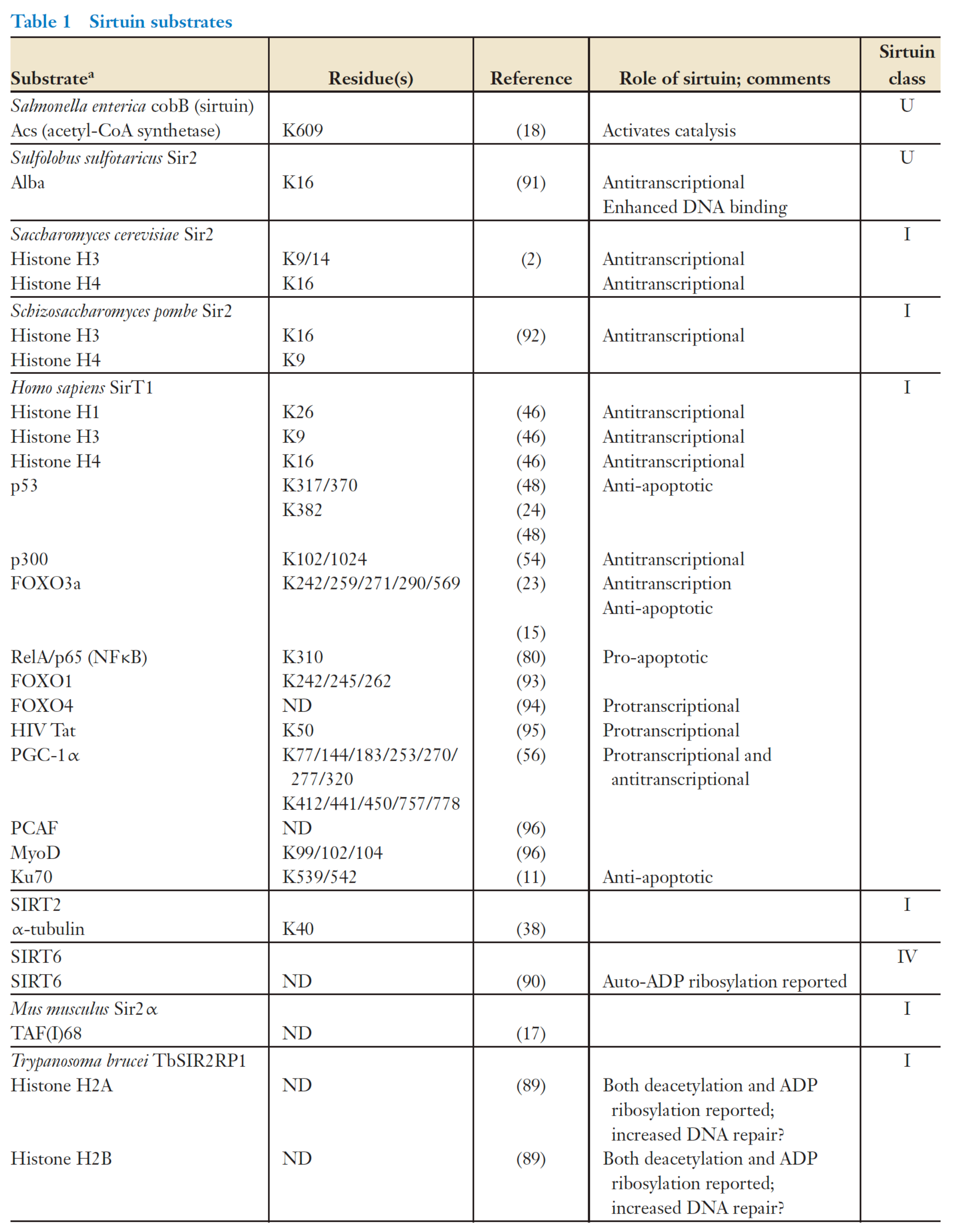
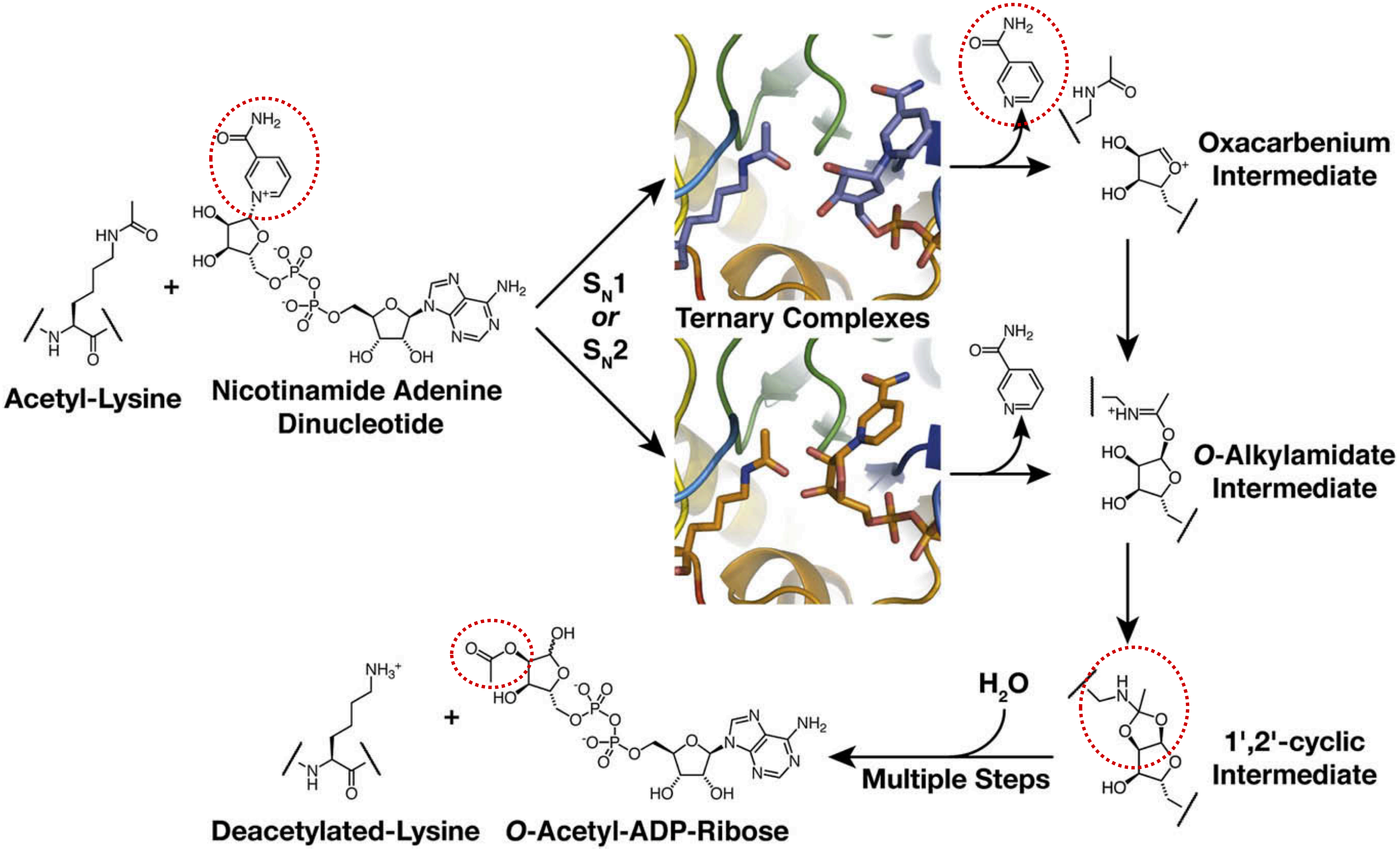


Table 1 Sirtuin substrates

Substrate^a	Residue(s)	Reference	Role of sirtuin; comments	Sirtuin class
Salmonella enterica cobB (sirtuin)				U
Acs (acetyl-CoA synthetase)	K609	(18)	Activates catalysis	
Sulfolobus sulfotaricus Sir2				U
Alba	K16	(91)	Antitranscriptional Enhanced DNA binding	
Saccharomyces cerevisiae Sir2				I
Histone H3	K9/14	(2)	Antitranscriptional	
Histone H4	K16		Antitranscriptional	
Schizosaccharomyces pombe Sir2				I
Histone H3	K16	(92)	Antitranscriptional	
Histone H4	K9			
Homo sapiens SirT1				I
Histone H1	K26	(46)	Antitranscriptional	
Histone H3	K9	(46)	Antitranscriptional	
Histone H4	K16	(46)	Antitranscriptional	
p53	K317/370 K382	(48) (24) (48)	Anti-apoptotic	
p300	K102/1024	(54)	Antitranscriptional	
FOXO3a	K242/259/271/290/569	(23) (15)	Antitranscription Anti-apoptotic	
RelA/p65 (NFκB)	K310	(80)	Pro-apoptotic	
FOXO1	K242/245/262	(93)		
FOXO4	ND	(94)	Protranscriptional	
HIV Tat	K50	(95)	Protranscriptional	
PGC-1α	K77/144/183/253/270/277/320 K412/441/450/757/778	(56)	Protranscriptional and antitranscriptional	
PCAF	ND	(96)		
MyoD	K99/102/104	(96)		
Ku70	K539/542	(11)	Anti-apoptotic	
SIRT2				I
α-tubulin	K40	(38)		
SIRT6				IV
SIRT6	ND	(90)	Auto-ADP ribosylation reported	
Mus musculus Sir2α				I
TAF(I)68	ND	(17)		
Trypanosoma brucei TbSIR2RP1				I
Histone H2A	ND	(89)	Both deacetylation and ADP ribosylation reported; increased DNA repair?	
Histone H2B	ND	(89)	Both deacetylation and ADP ribosylation reported; increased DNA repair?	

The Proposed Mechanism of Sir2 Protein Deacetylases



Acetyl-Lysine

Nicotinamide Adenine Dinucleotide

S_N1 or S_N2

Ternary Complexes

Oxacarbenium Intermediate

O-Alkylamidate Intermediate

1',2'-cyclic Intermediate

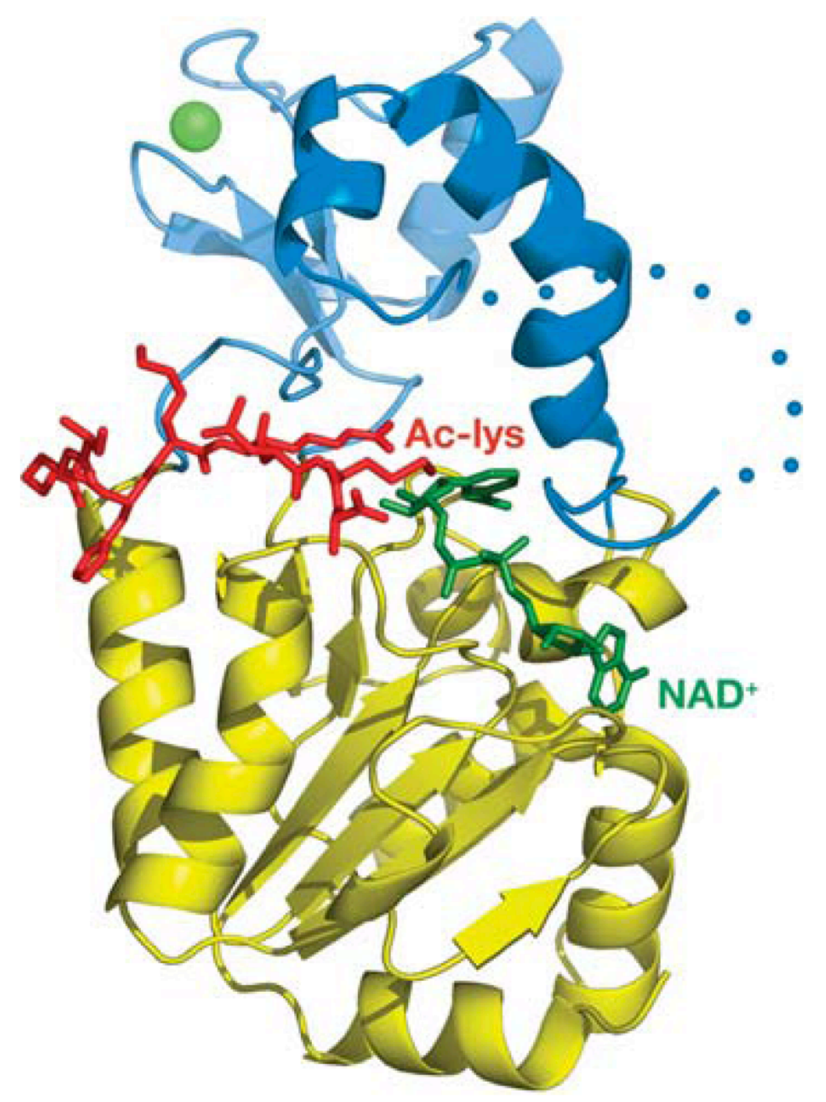
H_2O

Multiple Steps

Deacetylated-Lysine

O-Acetyl-ADP-Ribose

Structure of a Sirtuin bound to Acetylated Peptide and NAD$^+$



Rossmann-fold domain (yellow)

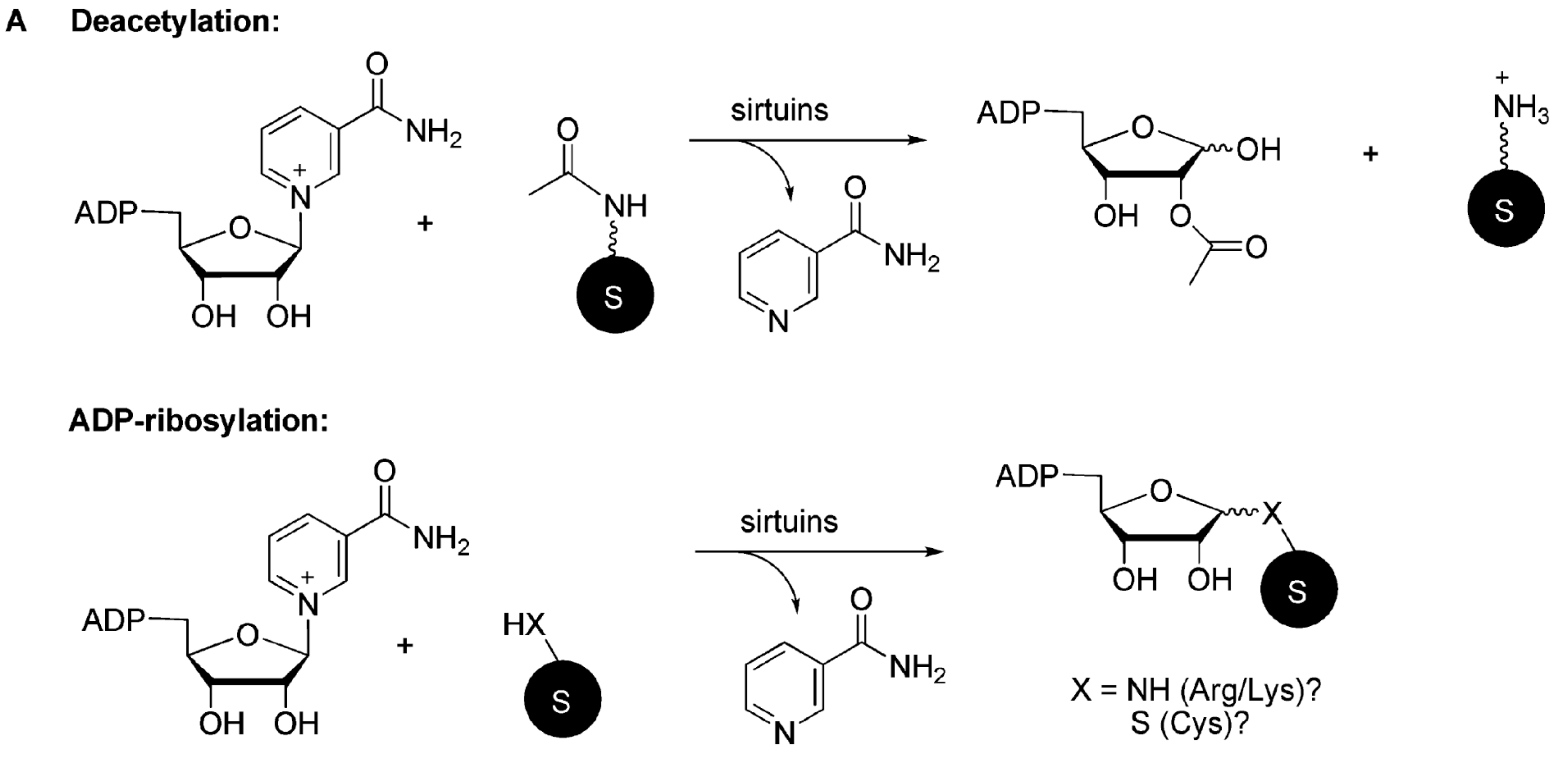
Zinc-binding module (light blue)

Helical module (royal blue)

Acetylated peptide (red)

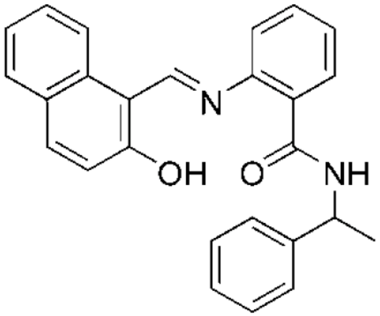
NAD$^+$ (green)

Sirtuin-catalyzed both Deacetylation and ADP-ribosylation reactions

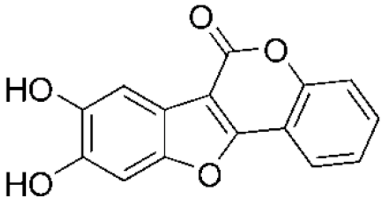


Modulators of sirtuins:

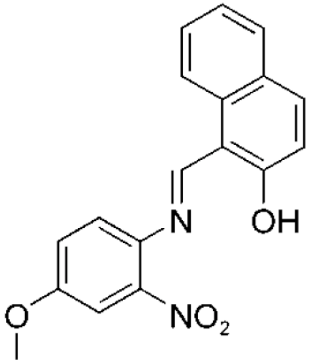
Inhibitors:



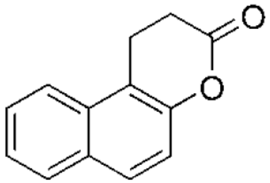
sirtinol (**25**)



A3 (**26**)

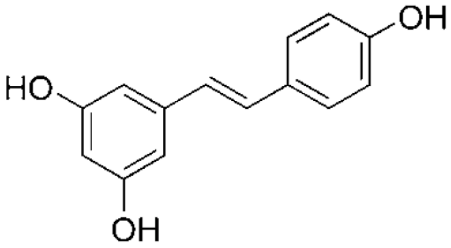


M15 (**27**)

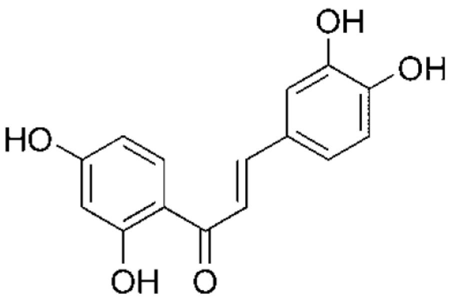


splitomicin (**28**)

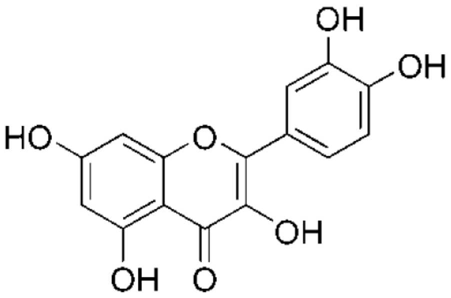
Activators:



resveratrol (**29**)



butein (**30**)



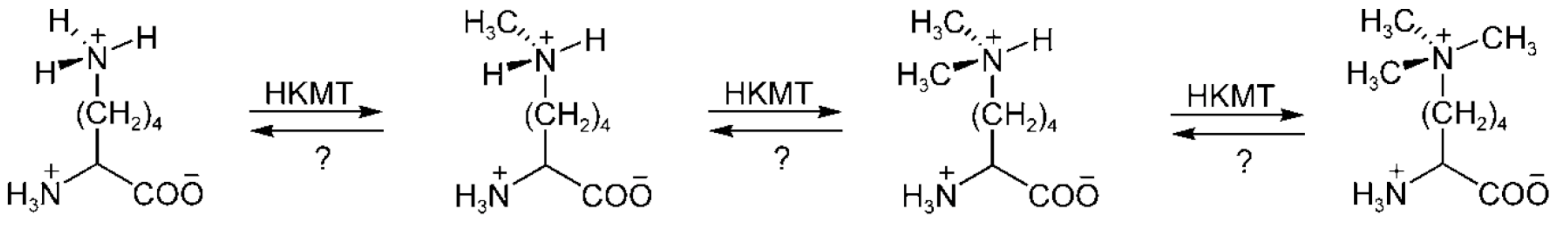
quercetin (**31**)

Methylation

Histone Lysine Methylation

Structure of lysine and its methylated derivatives:

Histone lysine methyltransferase (HKMT)



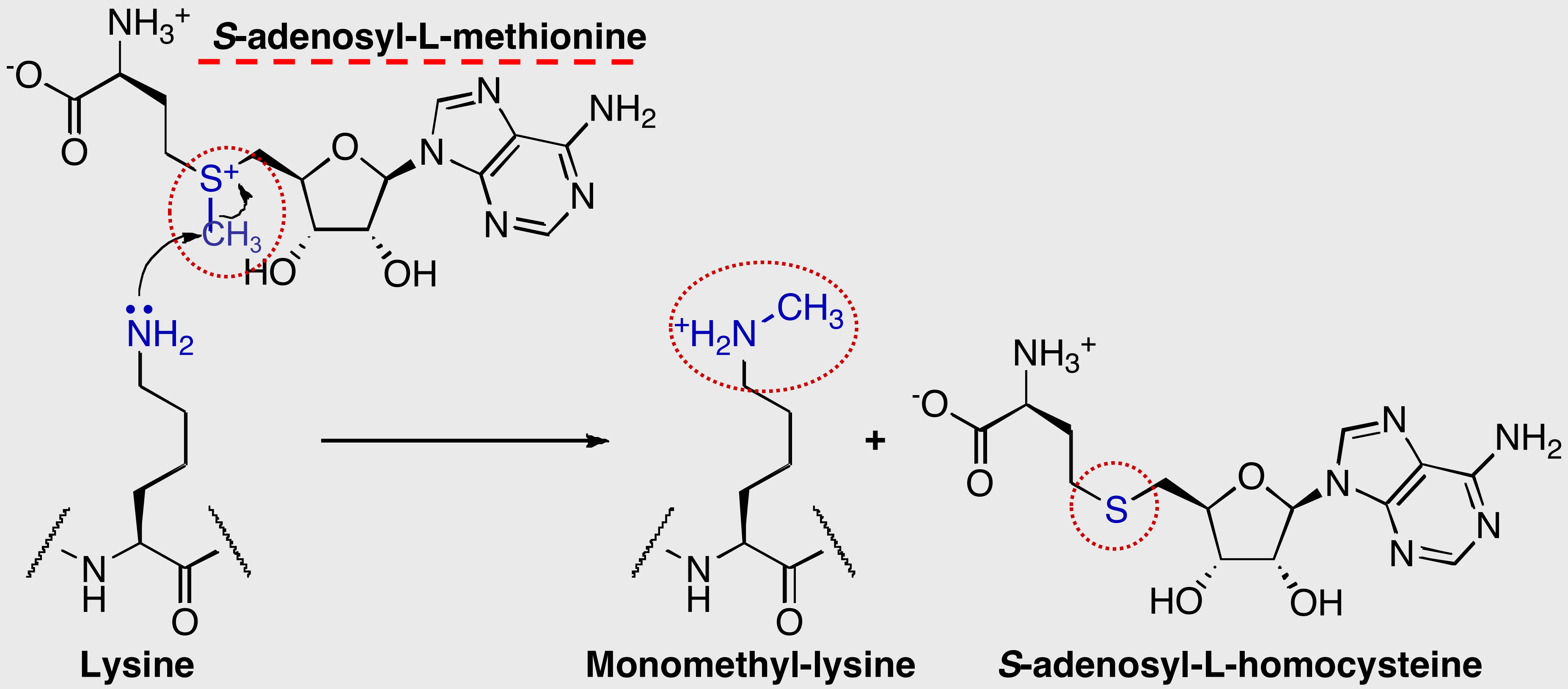
lysine ε-*N*-methyllysine ε-*N*-dimethyllysine ε-*N*-trimethyllysine

mono **di** **tri**

Histone lysine methyl transferases	**Histone substrate specificity**
1. **SET domain-containing**	
SUV39 family	
SUV39H1	H3-K9
2. ***SET1 family***	
SET1	H3-K4, -K79
EZH2	H3-K27
MLL	H3-K4
3. ***SET2 family***	
SET2	H3-K36
NSD1	H3-K36
4. ***RIZ family***	
RIZ1	H3-K9
5. ***SMYD3 family***	
SMYD3	H3-K4
6. **Non-SET domain-containing**	
DOT1	H3-K4, -K79

General chemical mechanism of SAMe-dependent HKMT

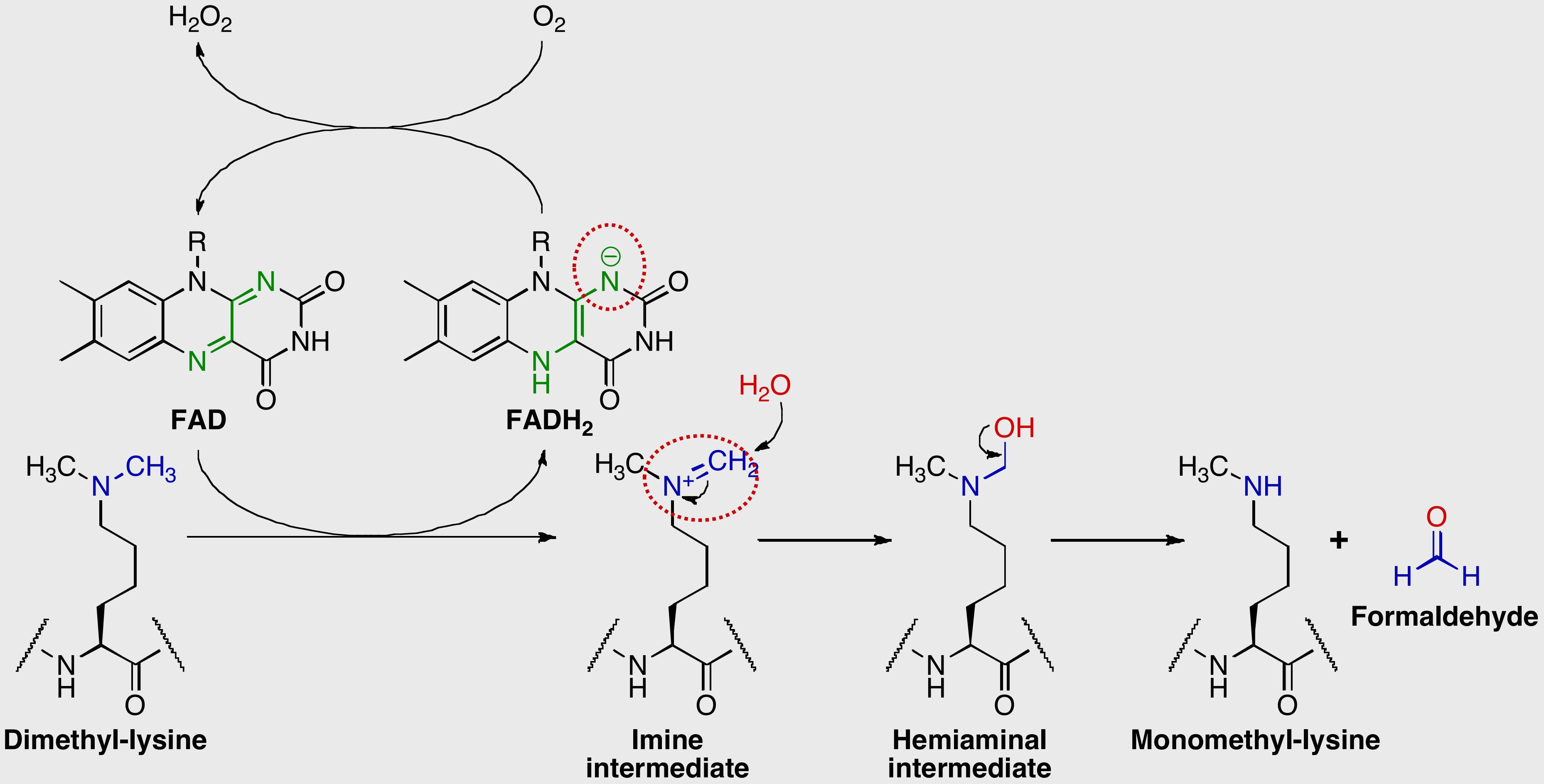
- Both SET domain and Dot1 methyltransferases use a similar mechanism of methyl transfer:



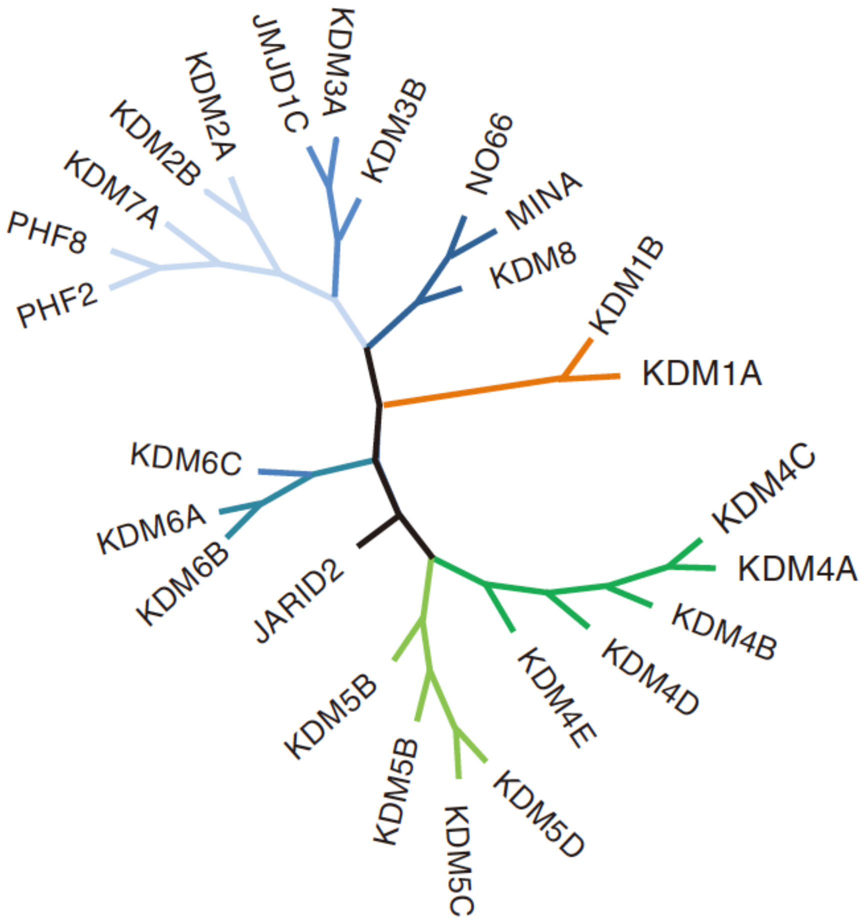
Demethylation of methyl-lysine

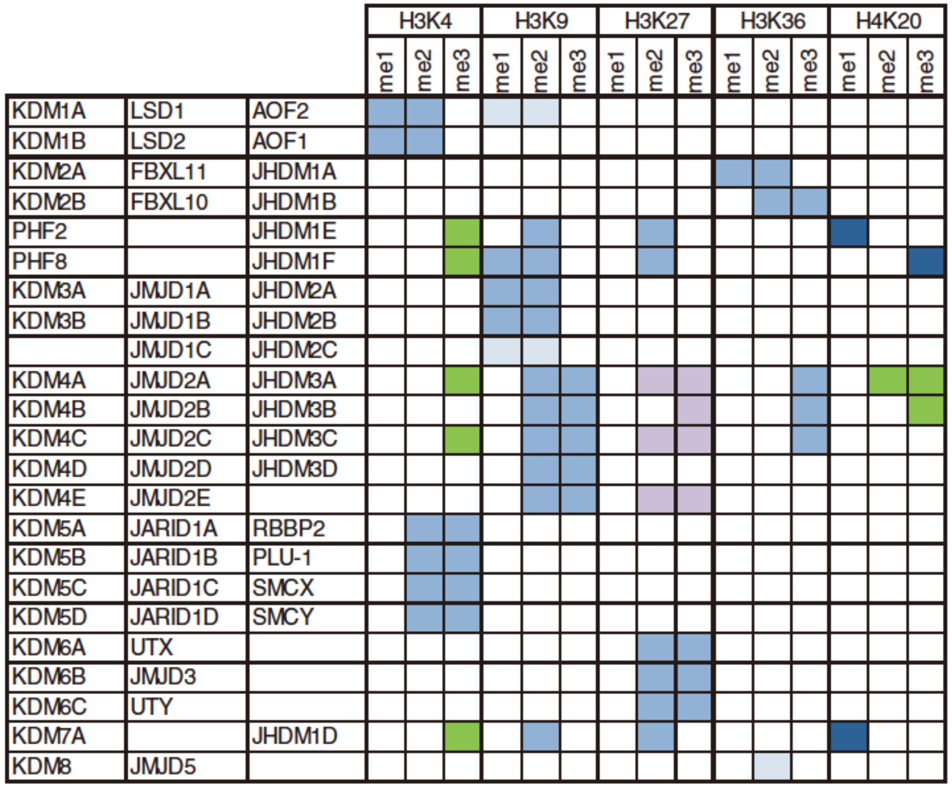
- **Lysine Demethylases:**

Proposed chemical mechanism of LSD/**KDM**: H3-K4, -K9



Histone lysine demethylases





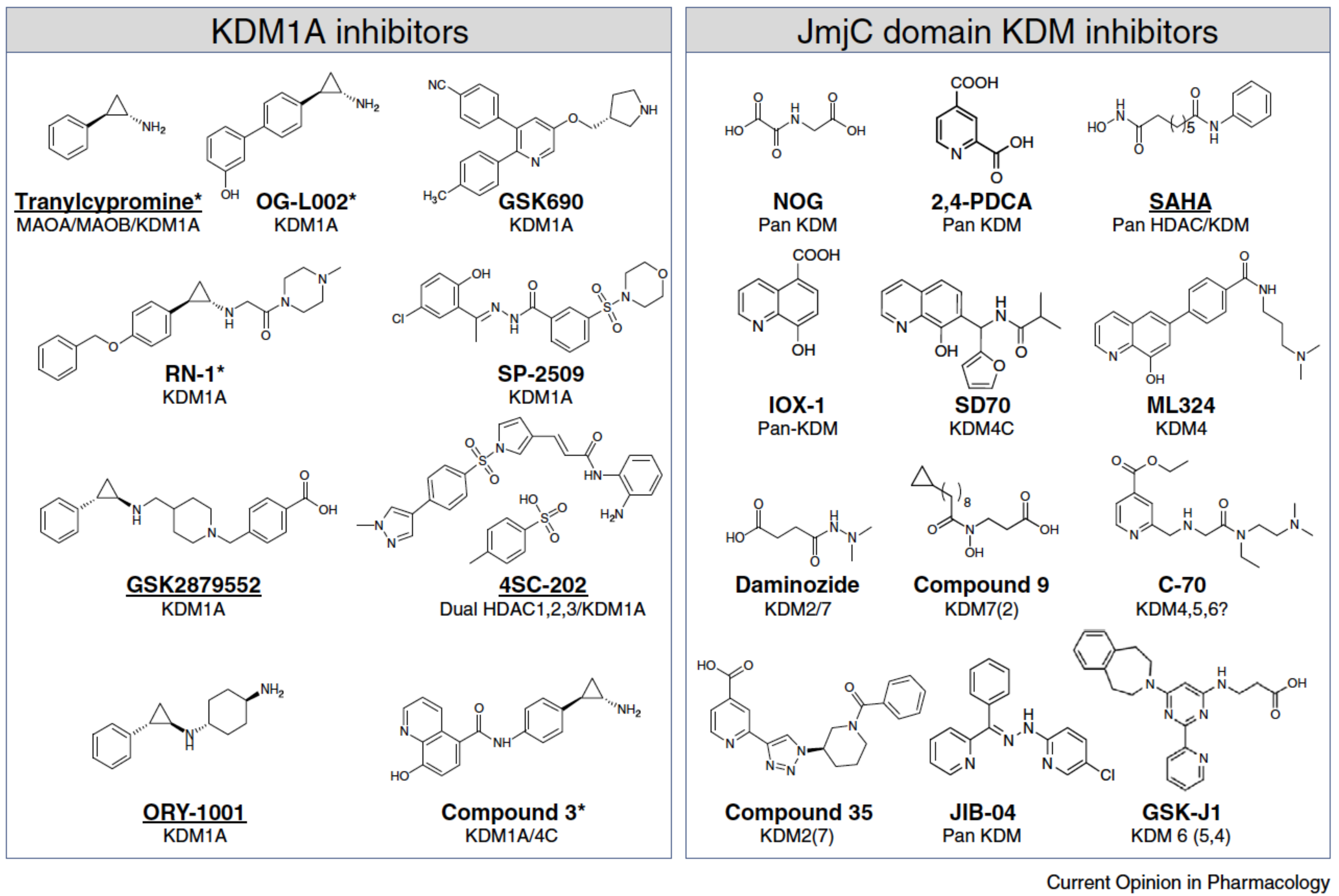
			H3K4			H3K9			H3K27			H3K36			H4K20		
			me1	me2	me3	me1	me2	me3	me1	me2	me3	me1	me2	me3	me1	me2	me3
KDM1A	LSD1	AOF2	🟦	🟦		░	░										
KDM1B	LSD2	AOF1	🟦	🟦													
KDM2A	FBXL11	JHDM1A										🟦	🟦				
KDM2B	FBXL10	JHDM1B											🟦	🟦			
PHF2		JHDM1E			🟩		🟦			🟦					■		
PHF8		JHDM1F			🟩	🟦	🟦			🟦							■
KDM3A	JMJD1A	JHDM2A				🟦	🟦										
KDM3B	JMJD1B	JHDM2B				🟦	🟦										
	JMJD1C	JHDM2C				░	░										
KDM4A	JMJD2A	JHDM3A			🟩		🟦	🟦		🟪	🟪			🟦		🟩	🟩
KDM4B	JMJD2B	JHDM3B					🟦	🟦			🟪			🟦			🟩
KDM4C	JMJD2C	JHDM3C			🟩		🟦	🟦		🟪	🟪			🟦			
KDM4D	JMJD2D	JHDM3D					🟦	🟦									
KDM4E	JMJD2E						🟦	🟦		🟪	🟪						
KDM5A	JARID1A	RBBP2		🟦	🟦												
KDM5B	JARID1B	PLU-1		🟦	🟦												
KDM5C	JARID1C	SMCX		🟦	🟦												
KDM5D	JARID1D	SMCY		🟦	🟦												
KDM6A	UTX									🟦	🟦						
KDM6B	JMJD3									🟦	🟦						
KDM6C	UTY									🟦	🟦						
KDM7A		JHDM1D			🟩		🟦			🟦					■		
KDM8	JMJD5												░				

🟦 effective substrate replicated in vitro using histone tail peptide
░ not replicated in vitro with histone tail peptide, only detected in cells
🟩 binds this residue, may provoke a switch in substrate specificity
■ effective substrate replicated in vitro only when using intact nucleosomes
🟪 weak substrate affinity replicated in vitro using histone tail peptide

Current Opinion in Pharmacology

Maes T., Curr Opin Pharmacol. 2015

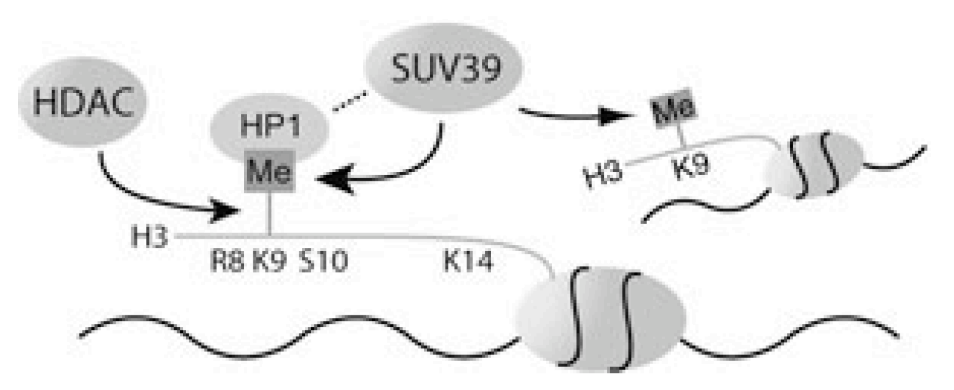
Histone lysine demethylase inhibitors



Current Opinion in Pharmacology

Maes T., Curr Opin Pharmacol. 2015

Gene silencing through histone methylation:

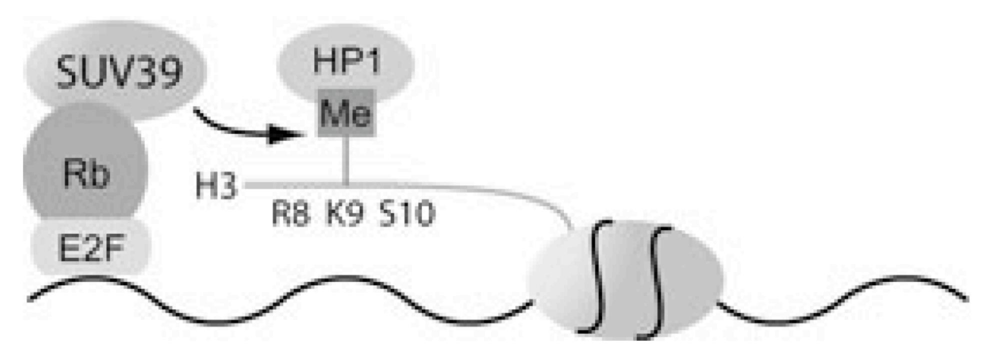


Heterochromatin Protein 1 (HP1)

- **Gene silencing through histone methylation**:

1. **Deacetylation** at H3-K9 by specific HDACs is necessary for subsequent methylation by HKMT activity, *eg.* SUV39.

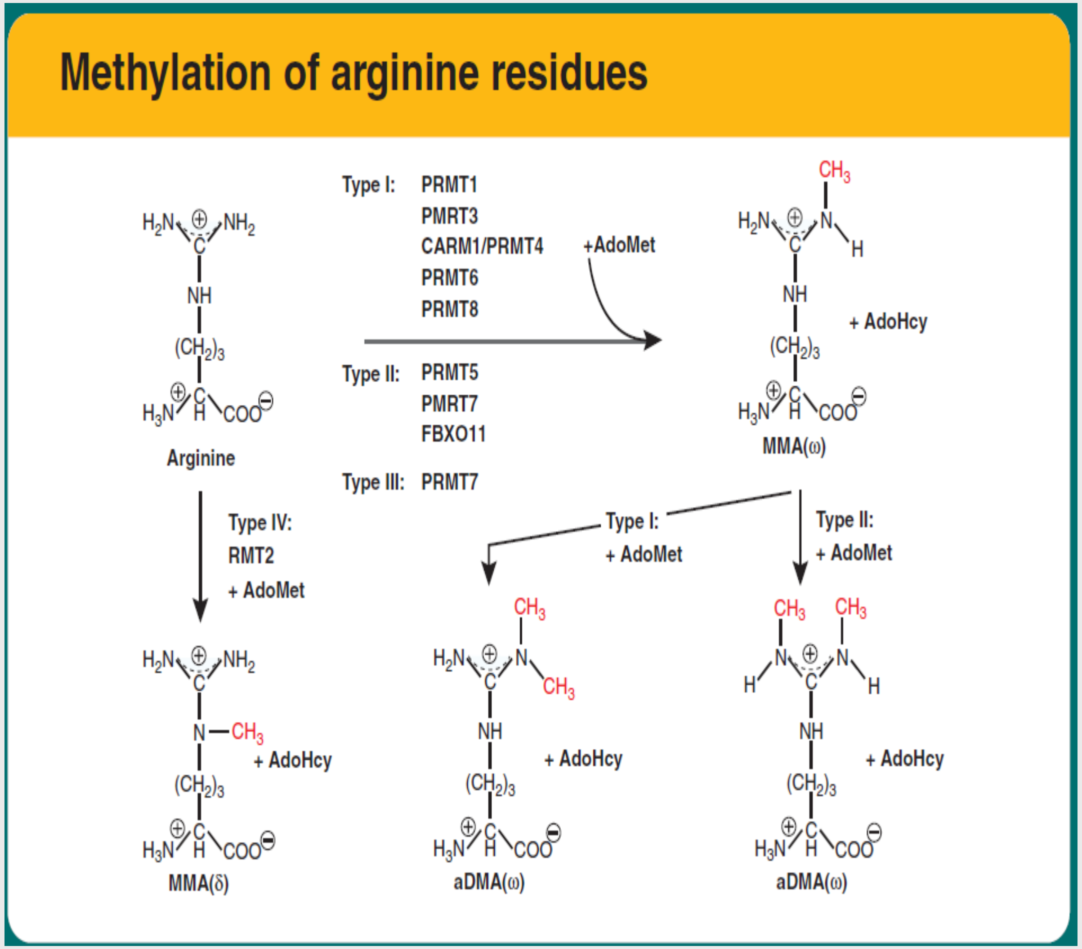
2. **HP1** selectively recognizes methylated H3-K9. The **propagation of heterochromatin** and gene repression is mediated by HP1 recruitment of SUV39 and additional H3-K9 methylation.



- **Histone methylation and transcriptional repression**:
E2F/Rb localize the histone lysine methyltransferase SUV39 at specific promoter sequences, and HP1 initiates transcriptional repression **at euchromatin**.

Histone/Protein Arginine Methyltransferases (H/P-RMTs)

1. Methylation of arginine groups occurs on histone H3 at R2, R17, R26 and on H4 at R3.
2. The **arginine guanidino** function can be either mono- or dimethylated.



J Cell Sci. 2007 Dec 15;120:4243-6.

PRMTs and inhibitor

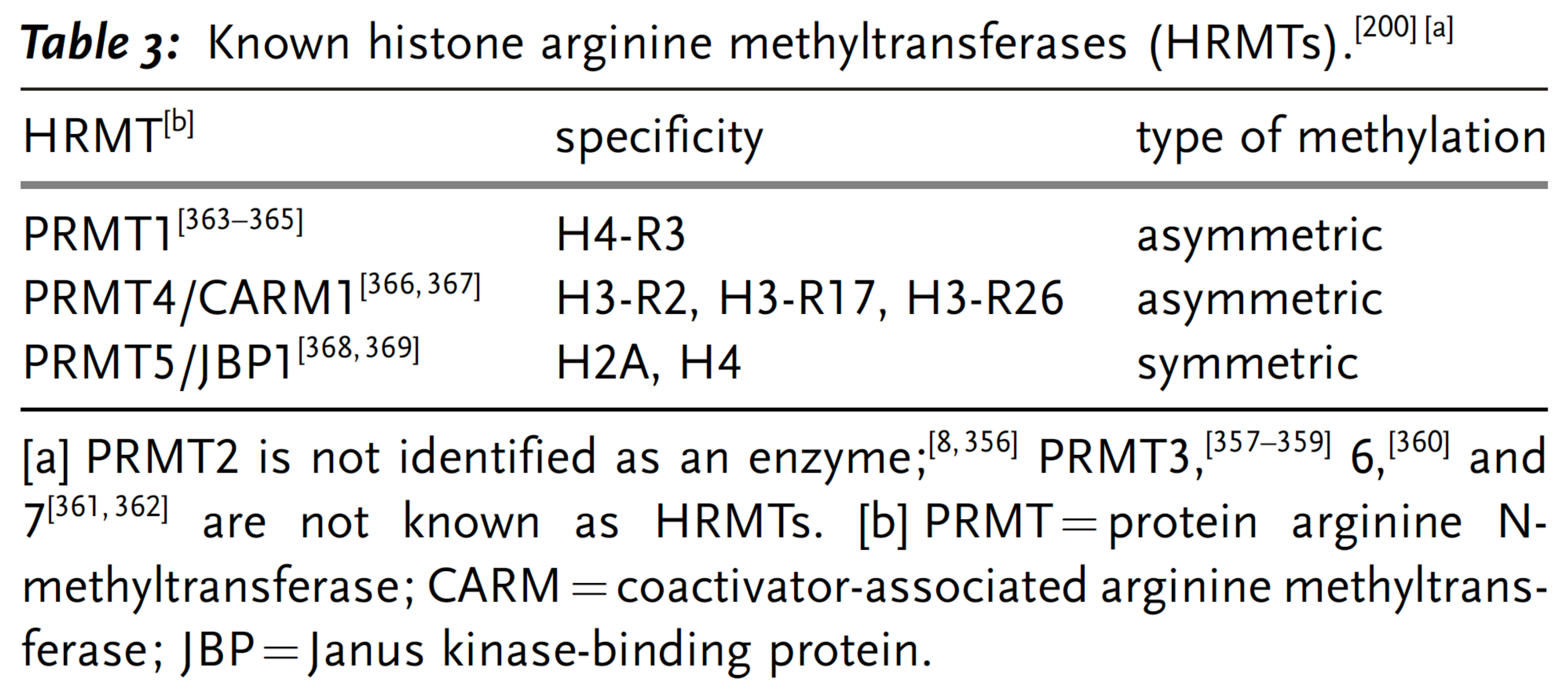
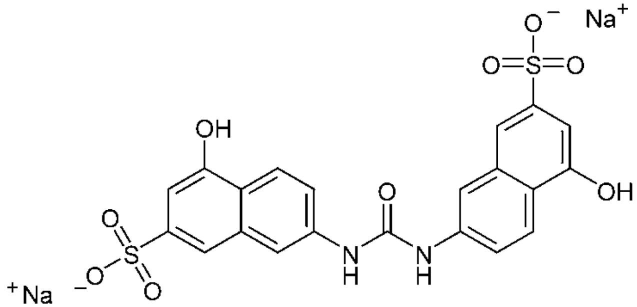


Table 3: Known histone arginine methyltransferases (HRMTs).[200] [a]

HRMT[b]	specificity	type of methylation
PRMT1[363–365]	H4-R3	asymmetric
PRMT4/CARM1[366, 367]	H3-R2, H3-R17, H3-R26	asymmetric
PRMT5/JBP1[368, 369]	H2A, H4	symmetric

[a] PRMT2 is not identified as an enzyme;[8, 356] PRMT3,[357–359] 6,[360] and 7[361, 362] are not known as HRMTs. [b] PRMT = protein arginine N-methyltransferase; CARM = coactivator-associated arginine methyltransferase; JBP = Janus kinase-binding protein.



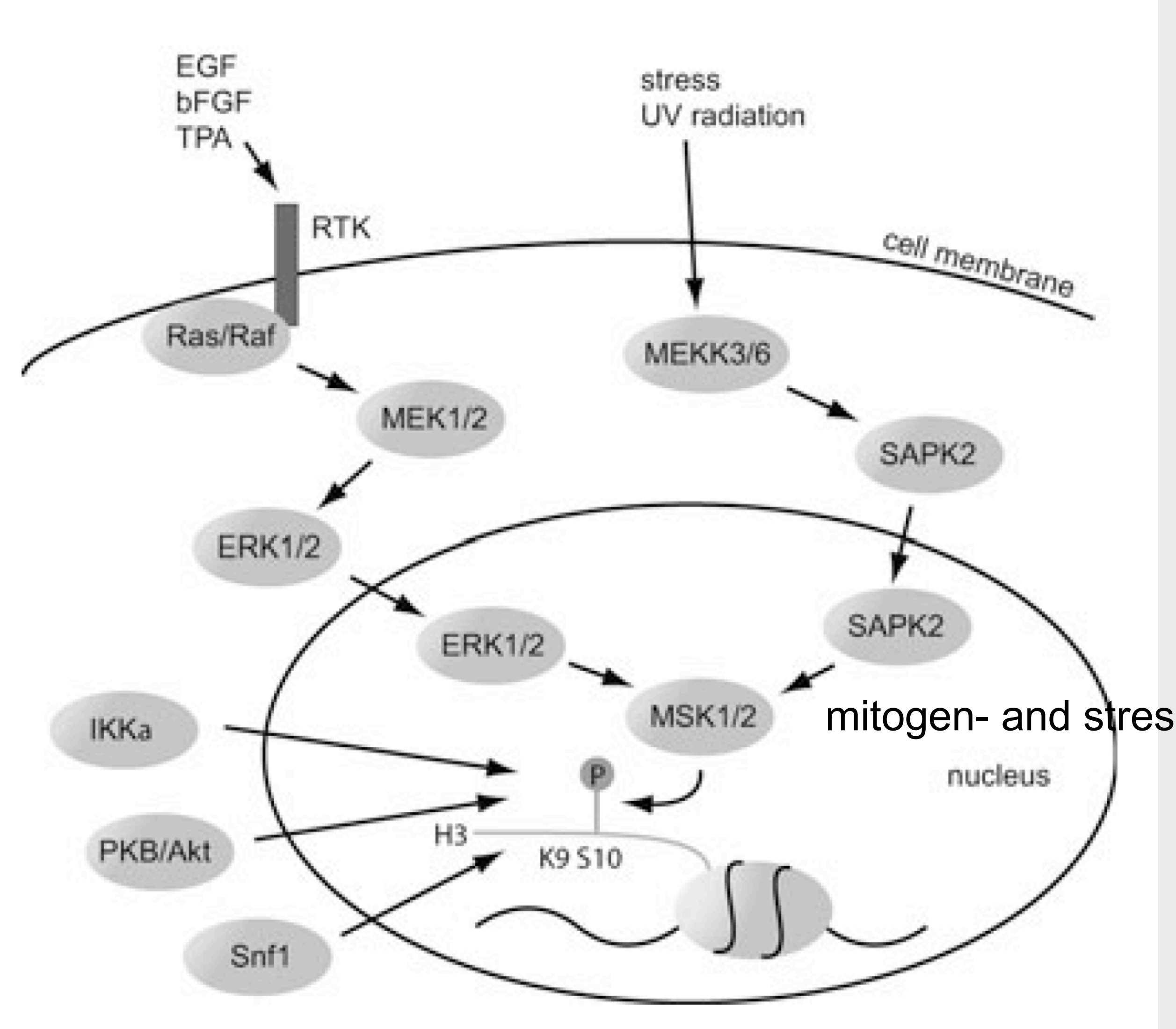
AMI-1 (**39**)

Structure of the specific PRMT inhibitor AMI-1

Phosphorylation

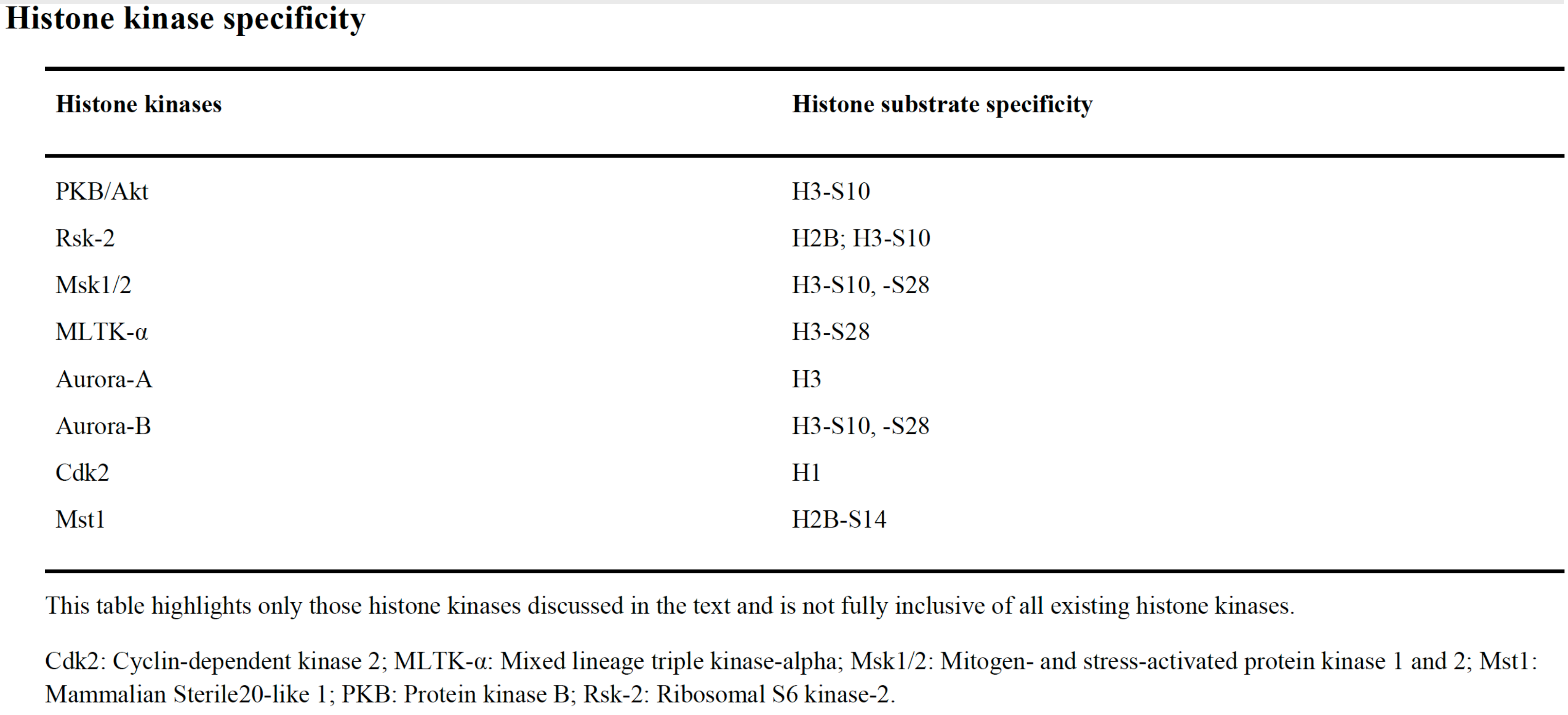
Histone Phosphorylation

Signaling pathways that lead to H3-S10 phosphorylation and gene expression:



mitogen- and stress-activated kinase

Histone Kinases



Histone kinase specificity

Histone kinases	Histone substrate specificity
PKB/Akt	H3-S10
Rsk-2	H2B; H3-S10
Msk1/2	H3-S10, -S28
MLTK-α	H3-S28
Aurora-A	H3
Aurora-B	H3-S10, -S28
Cdk2	H1
Mst1	H2B-S14

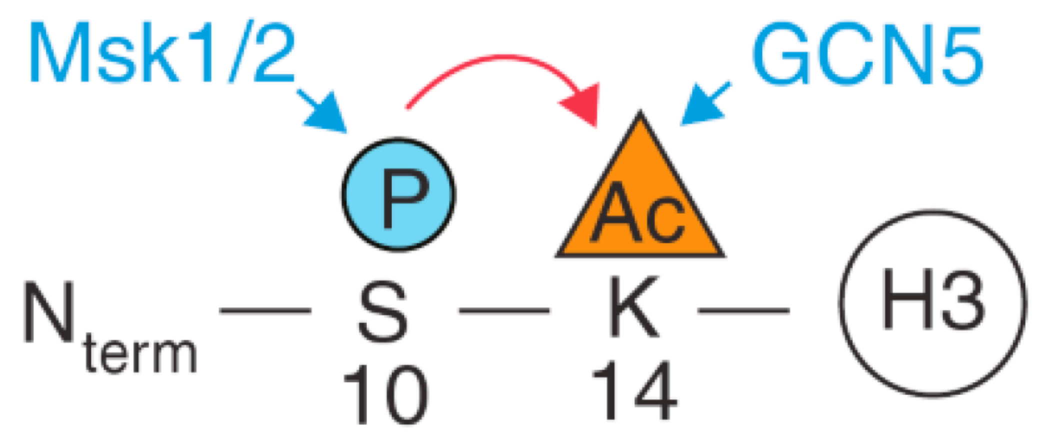
This table highlights only those histone kinases discussed in the text and is not fully inclusive of all existing histone kinases.

Cdk2: Cyclin-dependent kinase 2; MLTK-α: Mixed lineage triple kinase-alpha; Msk1/2: Mitogen- and stress-activated protein kinase 1 and 2; Mst1: Mammalian Sterile20-like 1; PKB: Protein kinase B; Rsk-2: Ribosomal S6 kinase-2.

Cross-talk between phosphorylation and acetylation

Synergistic Model

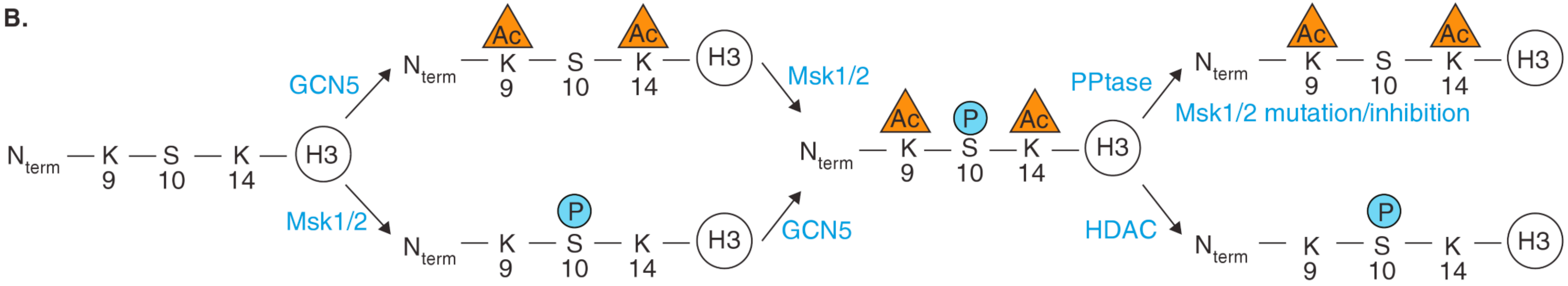
- MSK1/2 phosphorylates H3-S10, which **increases the binding affinity for Gcn5**. Once bound, the HAT activity of Gcn5 results in the acetylation of H3-K14, which activates transcription.



Parallel-Independent Model

- Lysine acetylation occurs equally on both phosphorylated and unmodified Histones.
- Acetylation at H3-K9 and H3-K14 is maintained by an equilibrium established by the activities of HATs and HDACs.

B.



Ubiquitination and Sumoylation

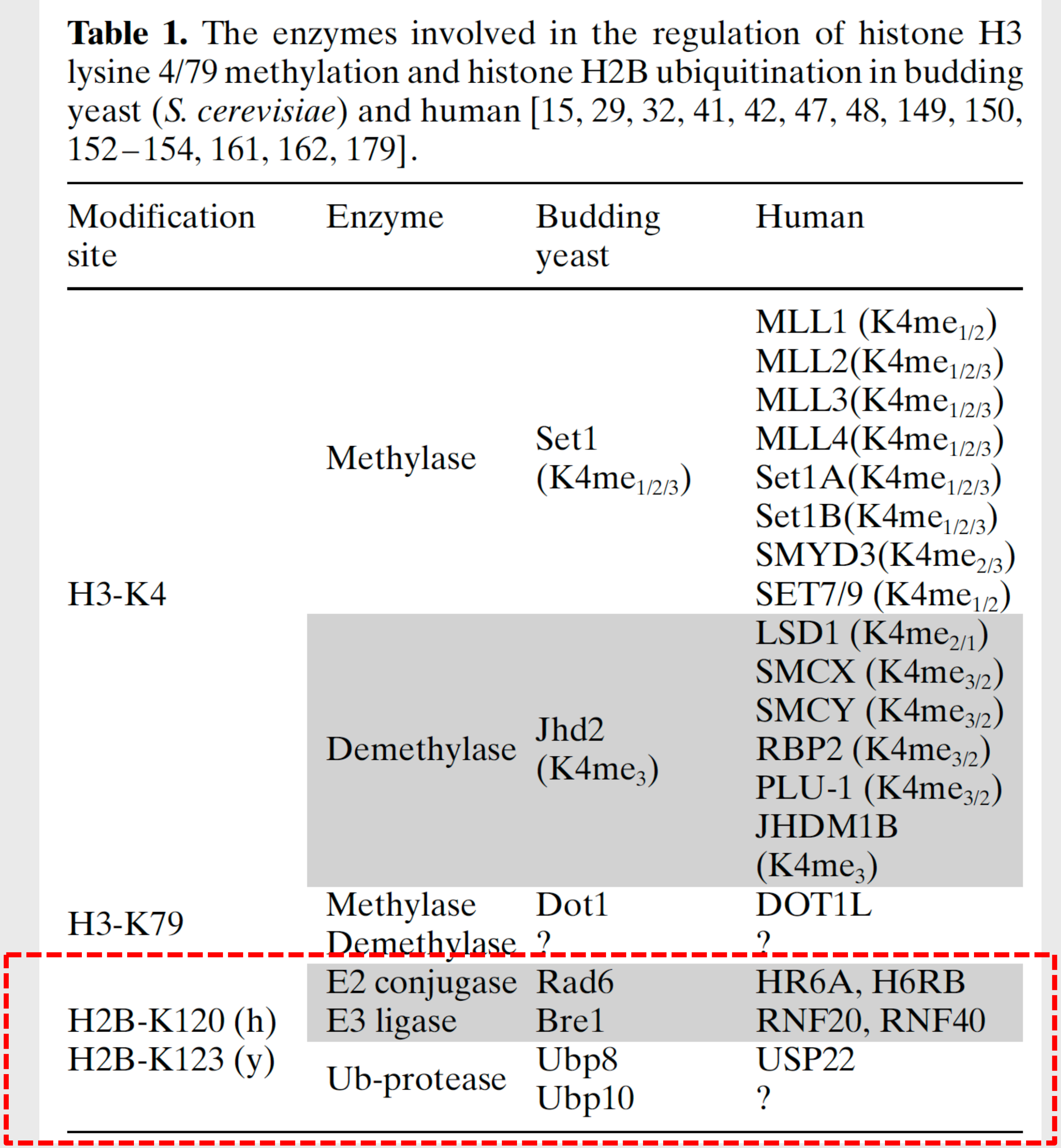
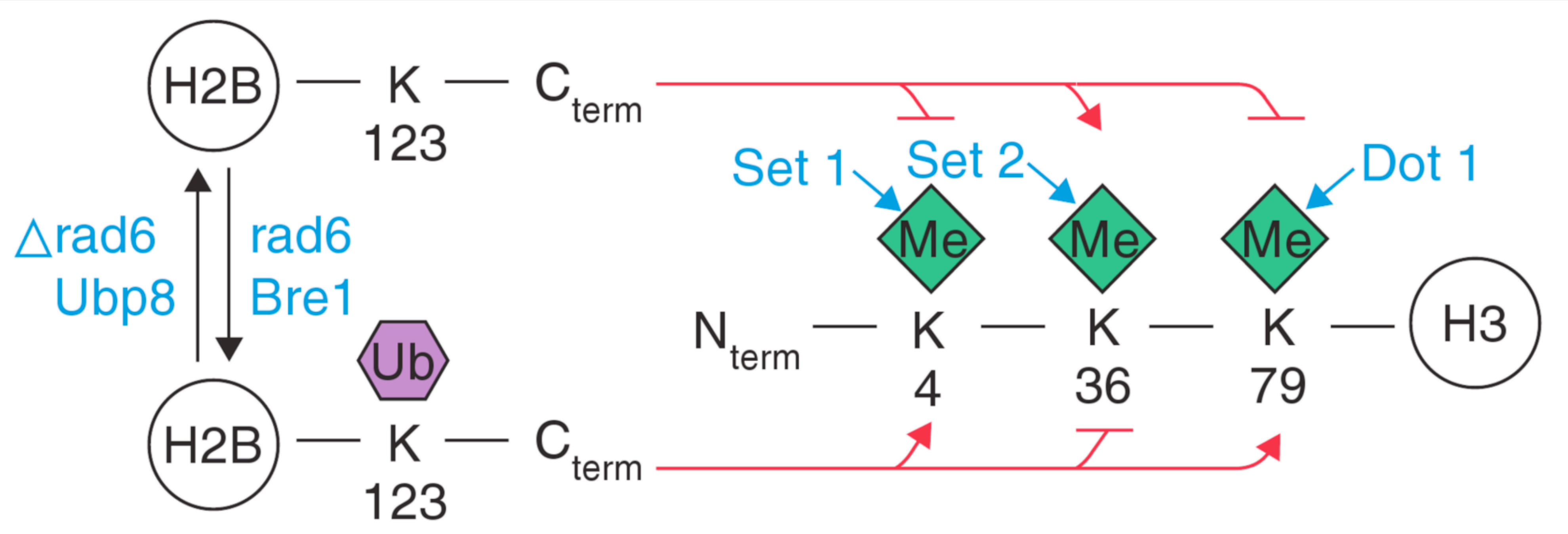


Table 1. The enzymes involved in the regulation of histone H3 lysine 4/79 methylation and histone H2B ubiquitination in budding yeast (*S. cerevisiae*) and human [15, 29, 32, 41, 42, 47, 48, 149, 150, 152–154, 161, 162, 179].

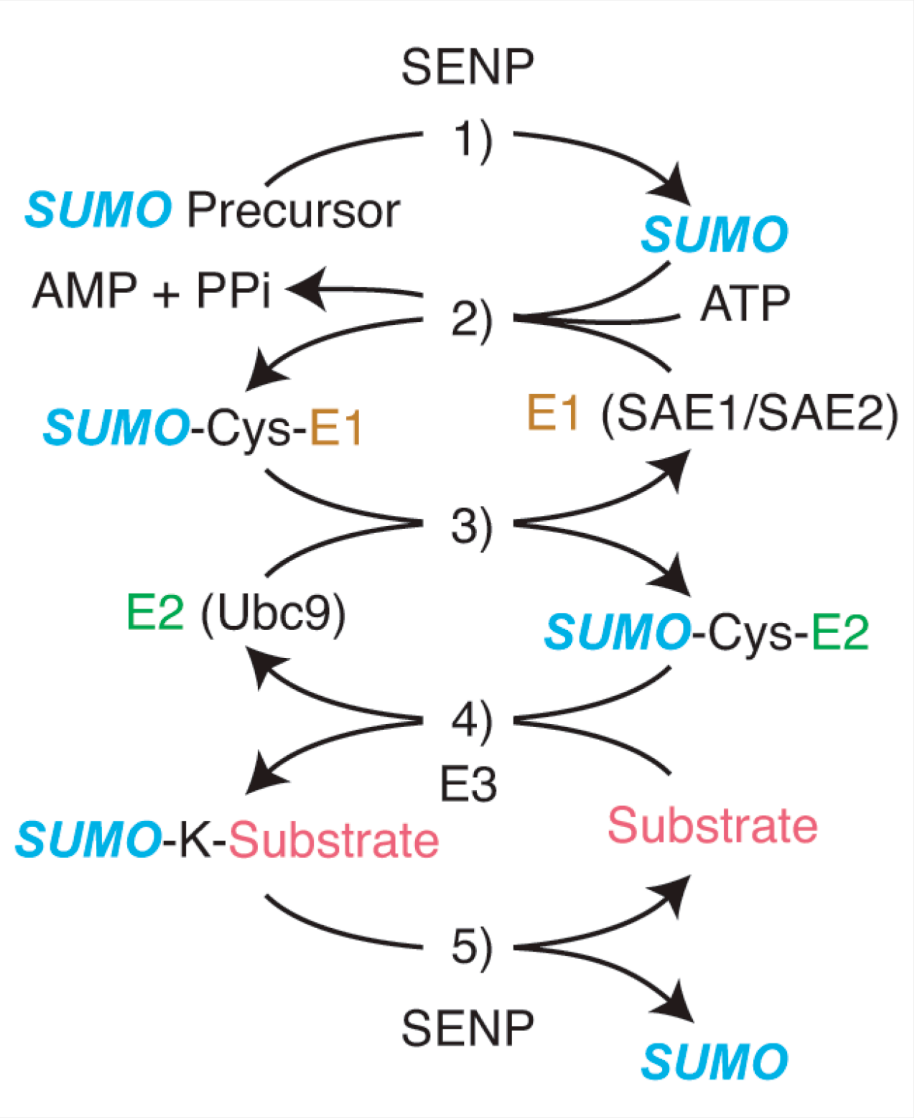
Modification site	Enzyme	Budding yeast	Human
H3-K4	Methylase	Set1 (K4me$_{1/2/3}$)	MLL1 (K4me$_{1/2}$) MLL2(K4me$_{1/2/3}$) MLL3(K4me$_{1/2/3}$) MLL4(K4me$_{1/2/3}$) Set1A(K4me$_{1/2/3}$) Set1B(K4me$_{1/2/3}$) SMYD3(K4me$_{2/3}$) SET7/9 (K4me$_{1/2}$)
	Demethylase	Jhd2 (K4me$_{3}$)	LSD1 (K4me$_{2/1}$) SMCX (K4me$_{3/2}$) SMCY (K4me$_{3/2}$) RBP2 (K4me$_{3/2}$) PLU-1 (K4me$_{3/2}$) JHDM1B (K4me$_{3}$)
H3-K79	Methylase	Dot1	DOT1L
	Demethylase	?	?
H2B-K120 (h) H2B-K123 (y)	E2 conjugase	Rad6	HR6A, H6RB
	E3 ligase	Bre1	RNF20, RNF40
	Ub-protease	Ubp8 Ubp10	USP22 ?

Cross-talk between ubiquitylation and methylation

- **in *S. cerevisiae***
- **Ubp8: Ubiquitin-specific protease**



SUMOylation



1) SUMO-specific proteases (SENP) removes C terminal residues.

2) ATP-dependent activation of SUMO by the SUMO-specific E1.

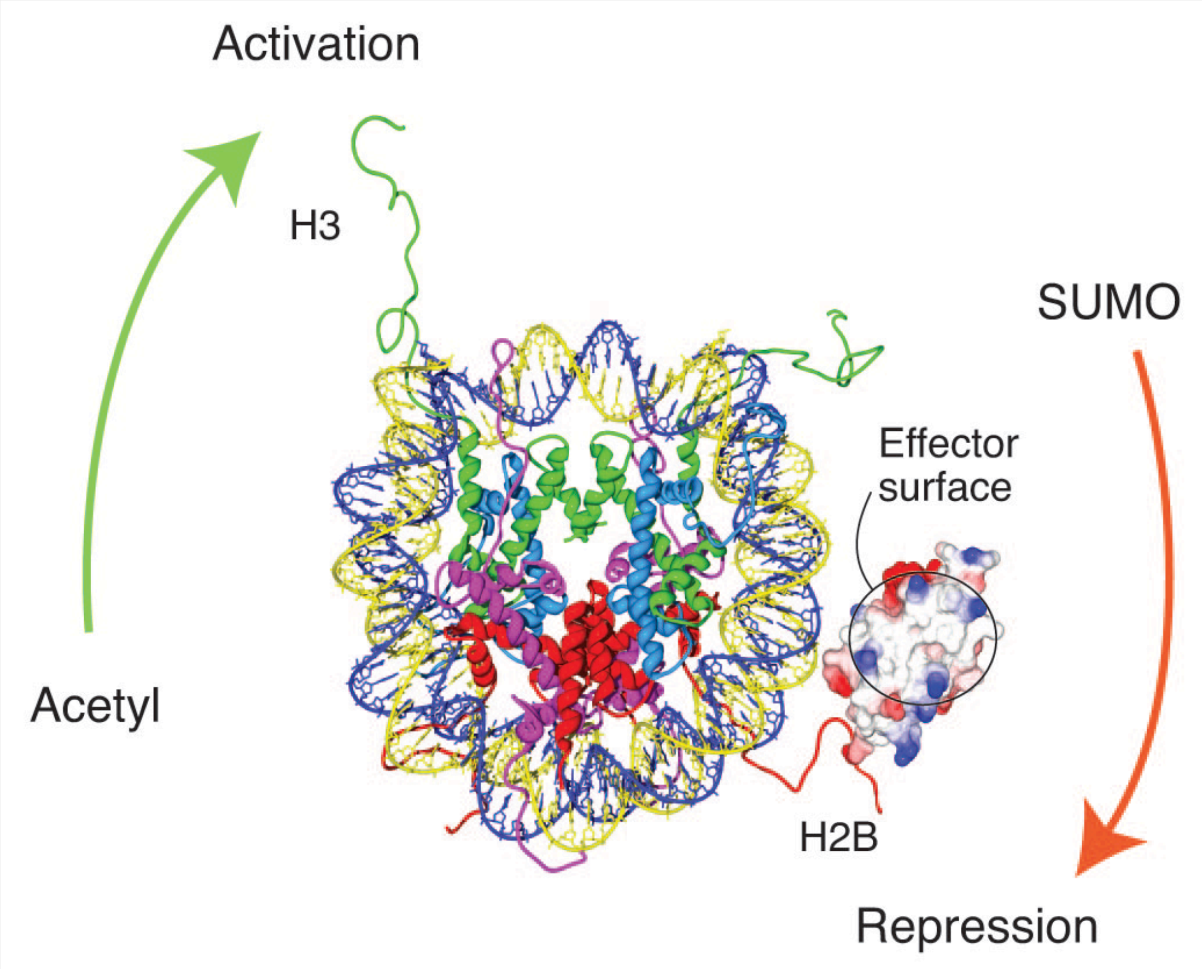
3) The SUMO moiety is transferred to the SUMO E2 ligase UBC9.

4) Ubc9-catalyzed conjugation of SUMO to substrate. This step is enhanced by E3 ligases.

5) SUMO conjugation is reversible through the isopeptidase activity of SUMO-specific proteases.

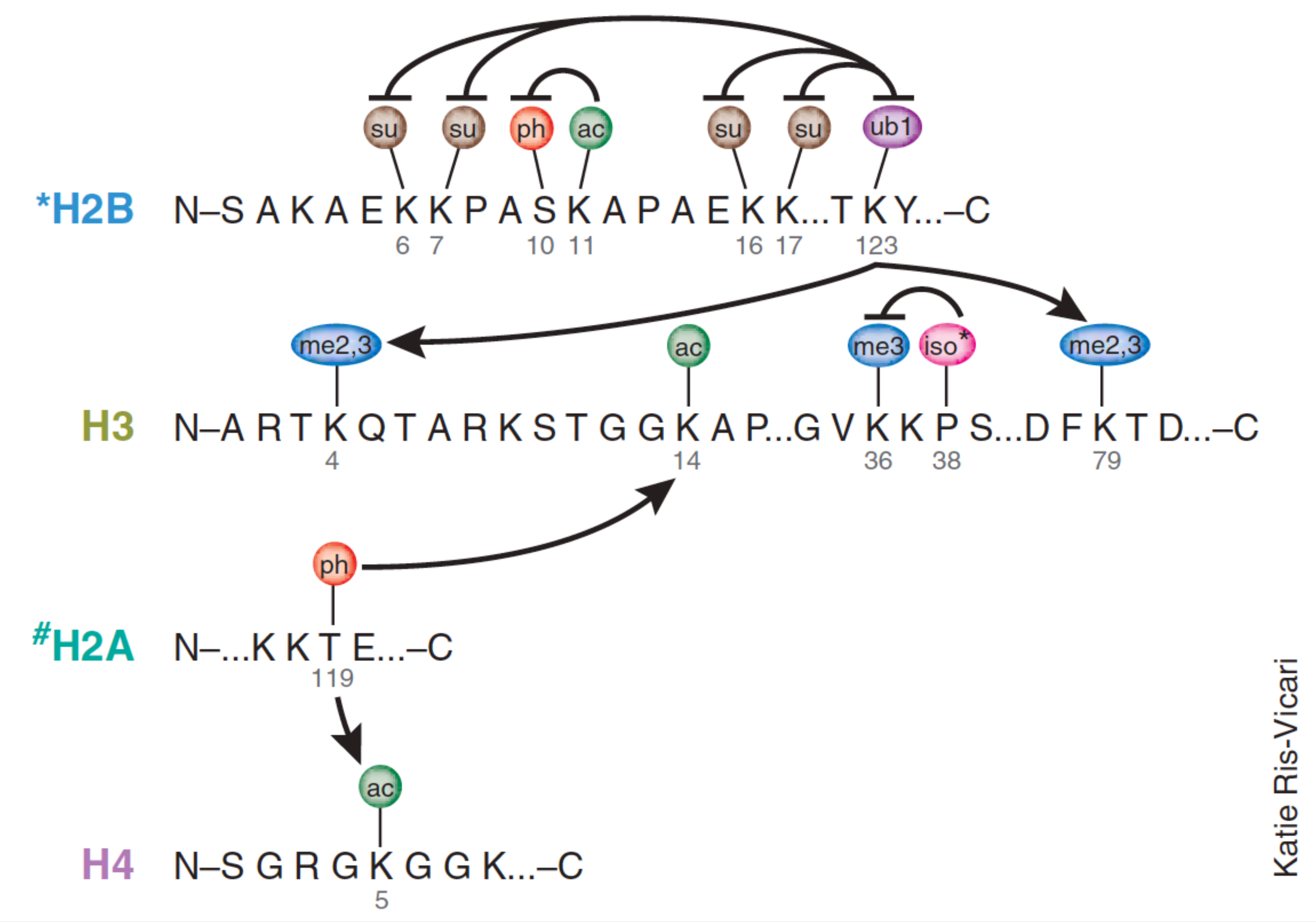
SUMOylation antagonizes Acetylation

- SUMO molecule conjugated to K6 of one of the H2B N terminal tails.
- The basic surface in SUMO essential for its transcriptional repressive function



Cross-regulation of modifications

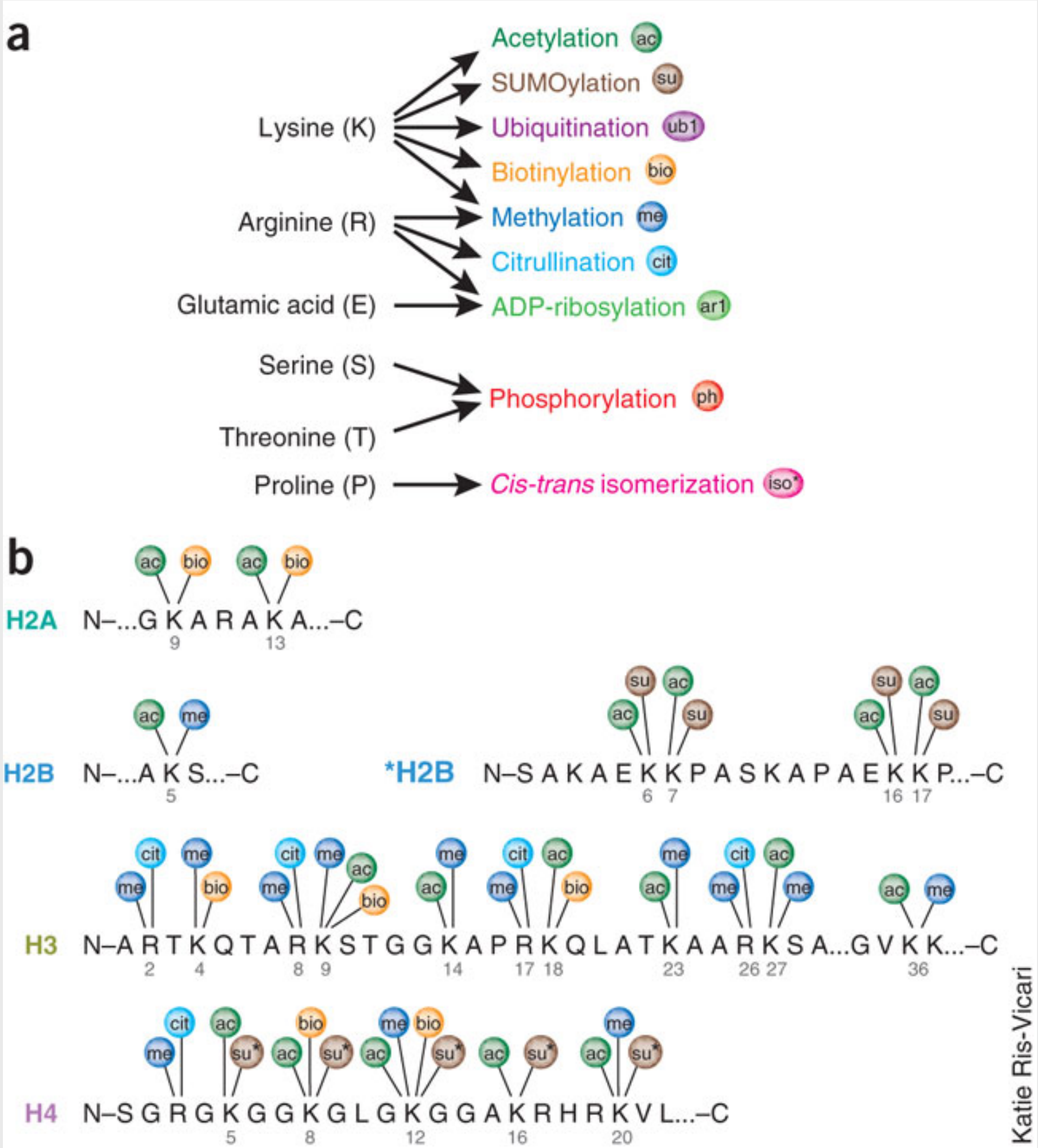
- in *cis* and in *trans:*



Latham JA., Nat Struct Mol Biol. 2007

Other types of histone modifications

- **Citrullination**
- **Glycosylation**
- **Biotination**
- **Proline *cis-trans* isomerization**
- others?

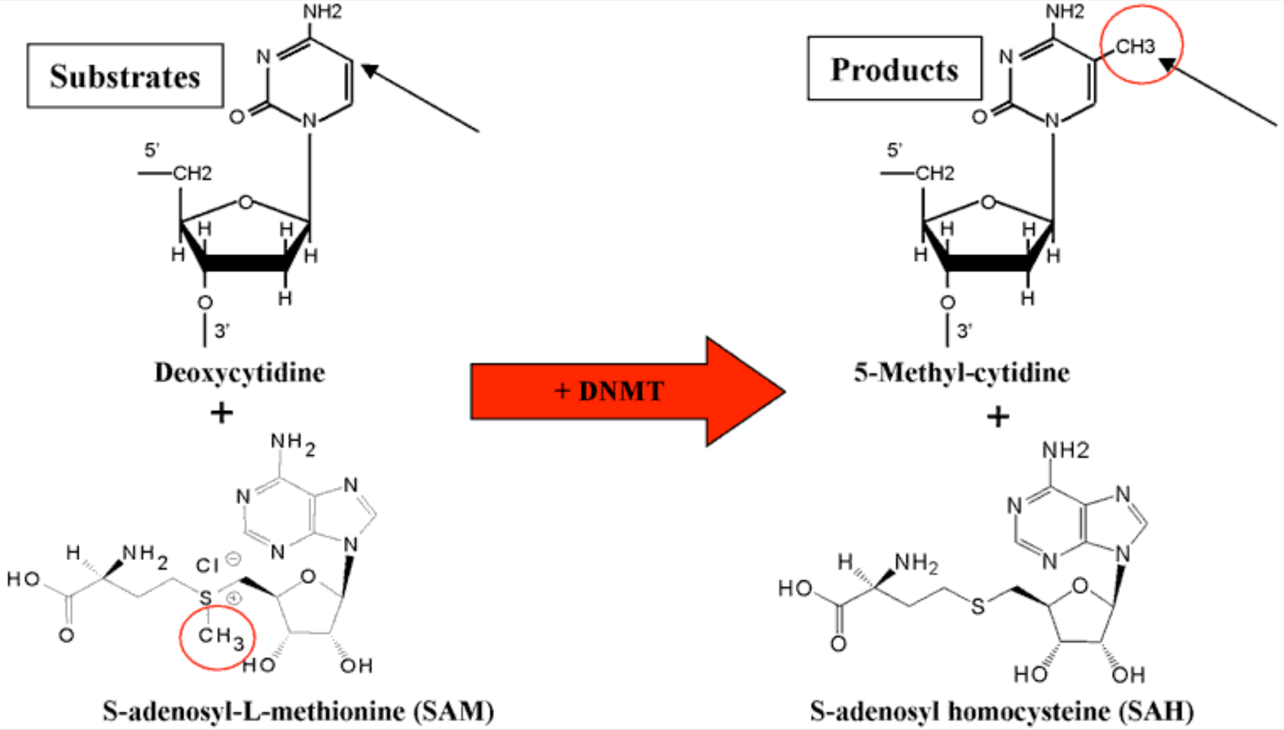


Latham JA., Nat Struct Mol Biol. 2007

DNA methylation

DNA Methylation

- Catalyzed by DNA methylases/methyltransferases use *S*-adenosylmethionine (SAM, SAMe) as methyl group donor.
- Adenine and **cytosine** are methylated more than guanine and thymine.
- Sequence-specific: (1) **CpG island** or (2) 5’GATC3’



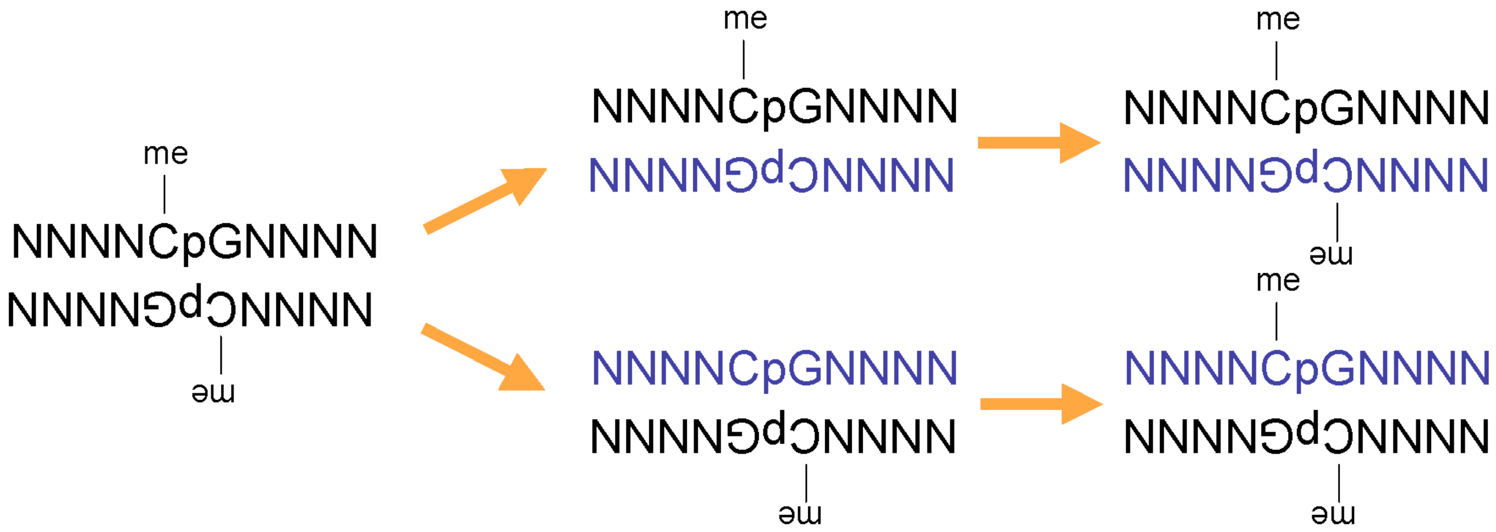
Cellular DNA Methylation Machinery

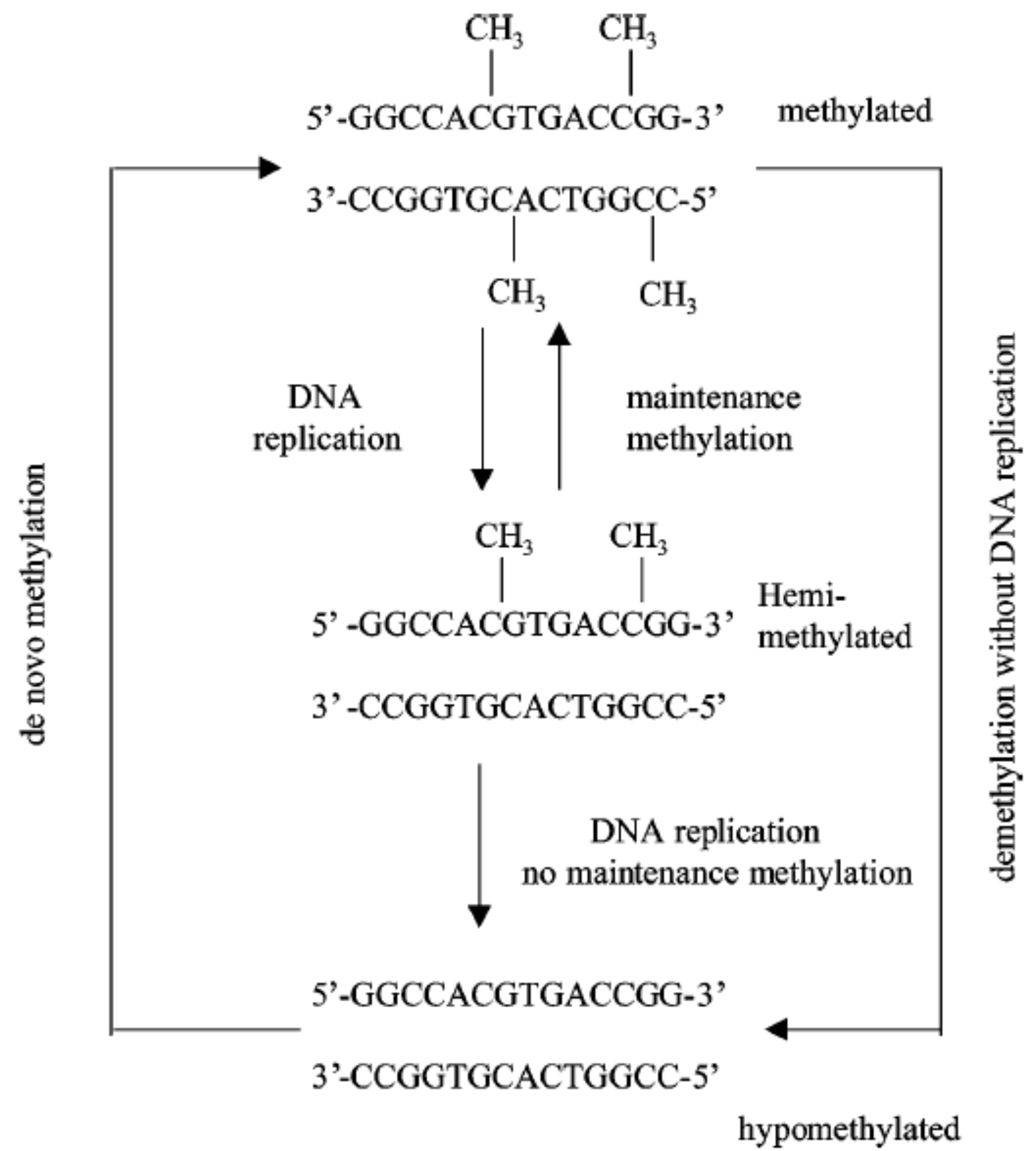
DNMT1 - ‘maintenance methylase’

DNMT3A

DNMT3B

***De novo* methylation**




CH3
CH3
5'-GGCCACGTGACCGG-3'
methylated
3'-CCGGTGCACTGGCC-5'
CH3
CH3
DNA replication
maintenance methylation
CH3
CH3
5' -GGCCACGTGACCGG-3'
Hemi-methylated
3' -CCGGTGCACTGGCC-5'
DNA replication
no maintenance methylation
5'-GGCCACGTGACCGG-3'
3'-CCGGTGCACTGGCC-5'
hypomethylated
de novo methylation
demethylation without DNA replication

DNA methylation patterns are inherited

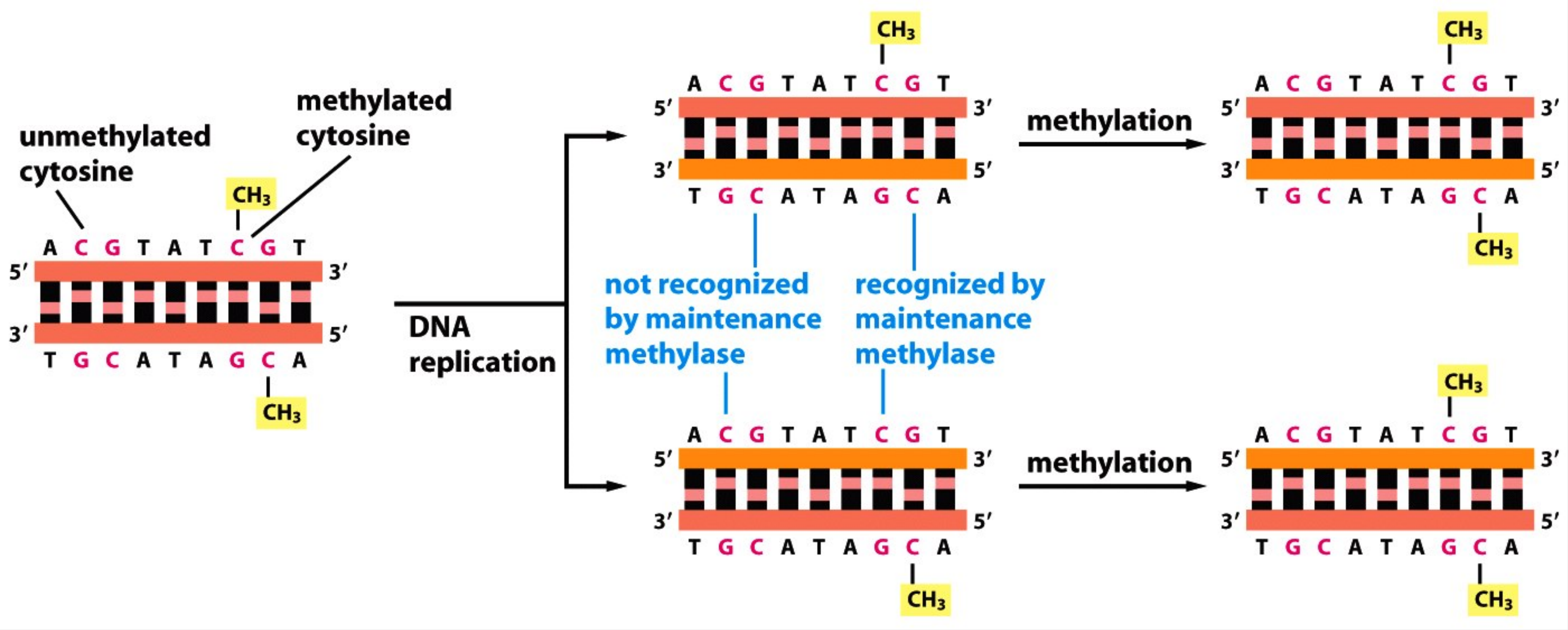
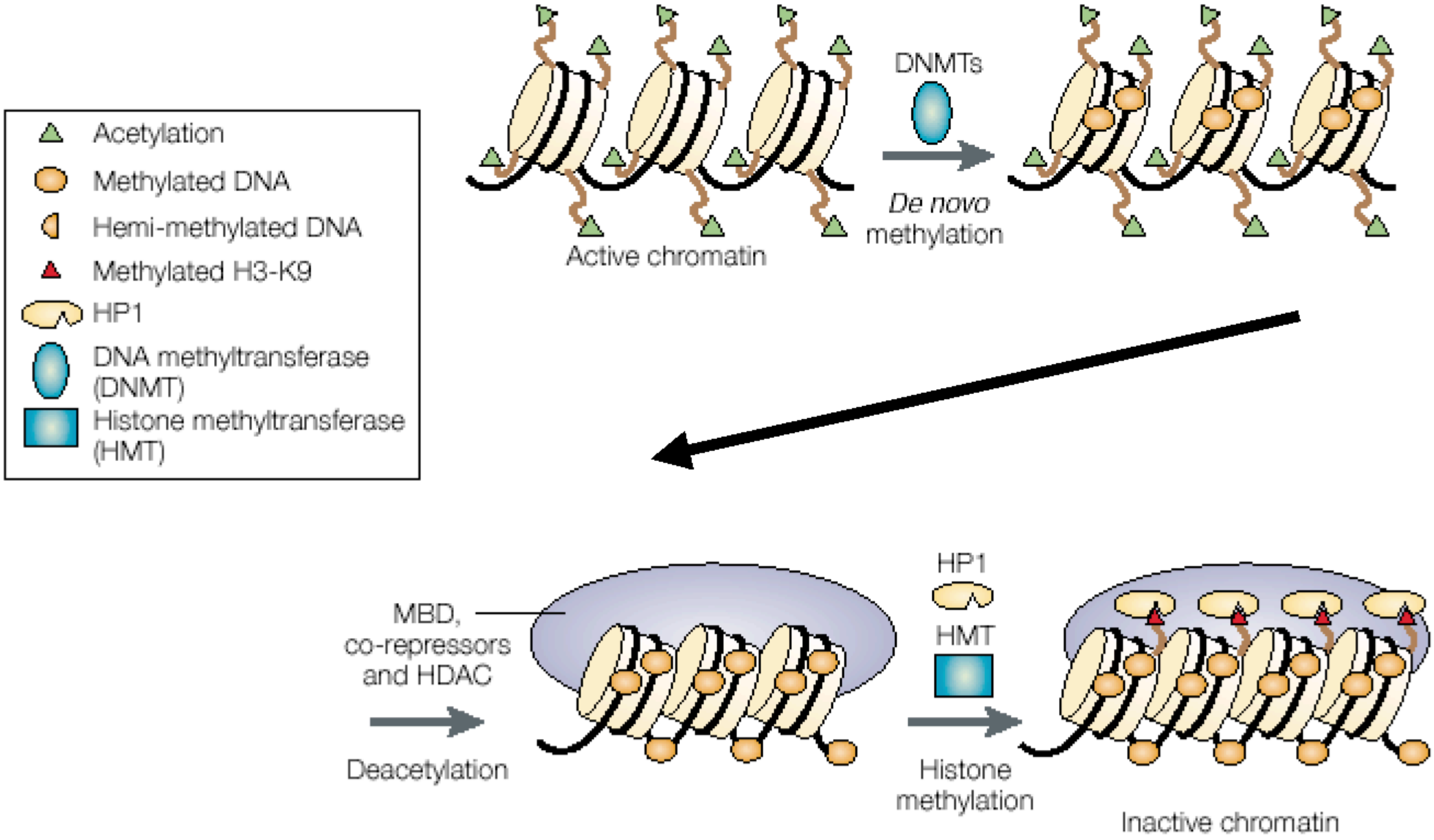


Figure 7-80 *Molecular Biology of the Cell* (© Garland Science 2008)

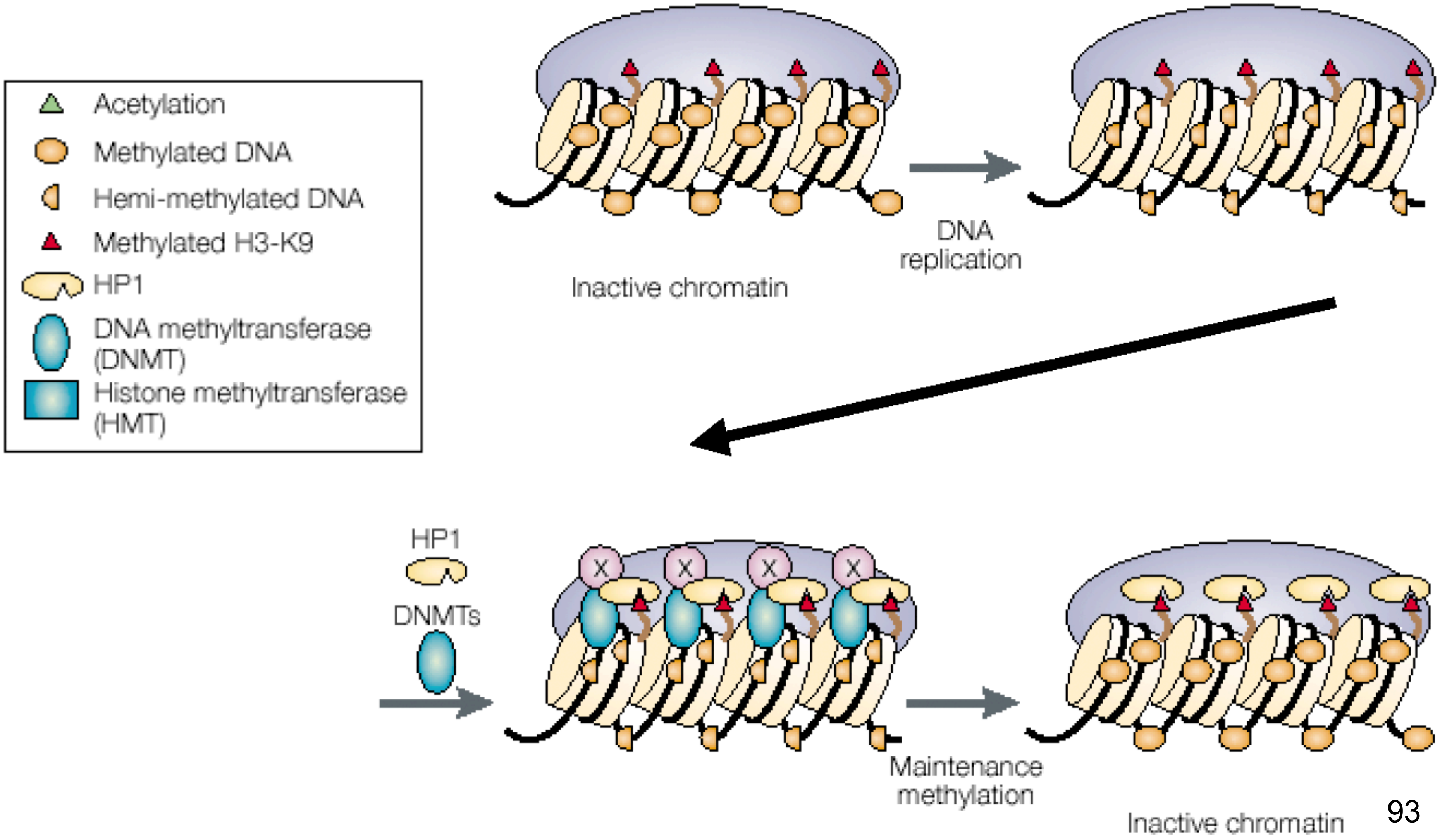
DNA Methylation and Gene Expression

- Cytosine residues in 5’CpG are often post-synthetically methylated
- CpG methylation is involved in long-term silencing of certain gene during development
- The methyl-CpG-binding proteins MeCP1 and MeCP2 interact specifically with methylated DNA and mediate transcriptional repression.

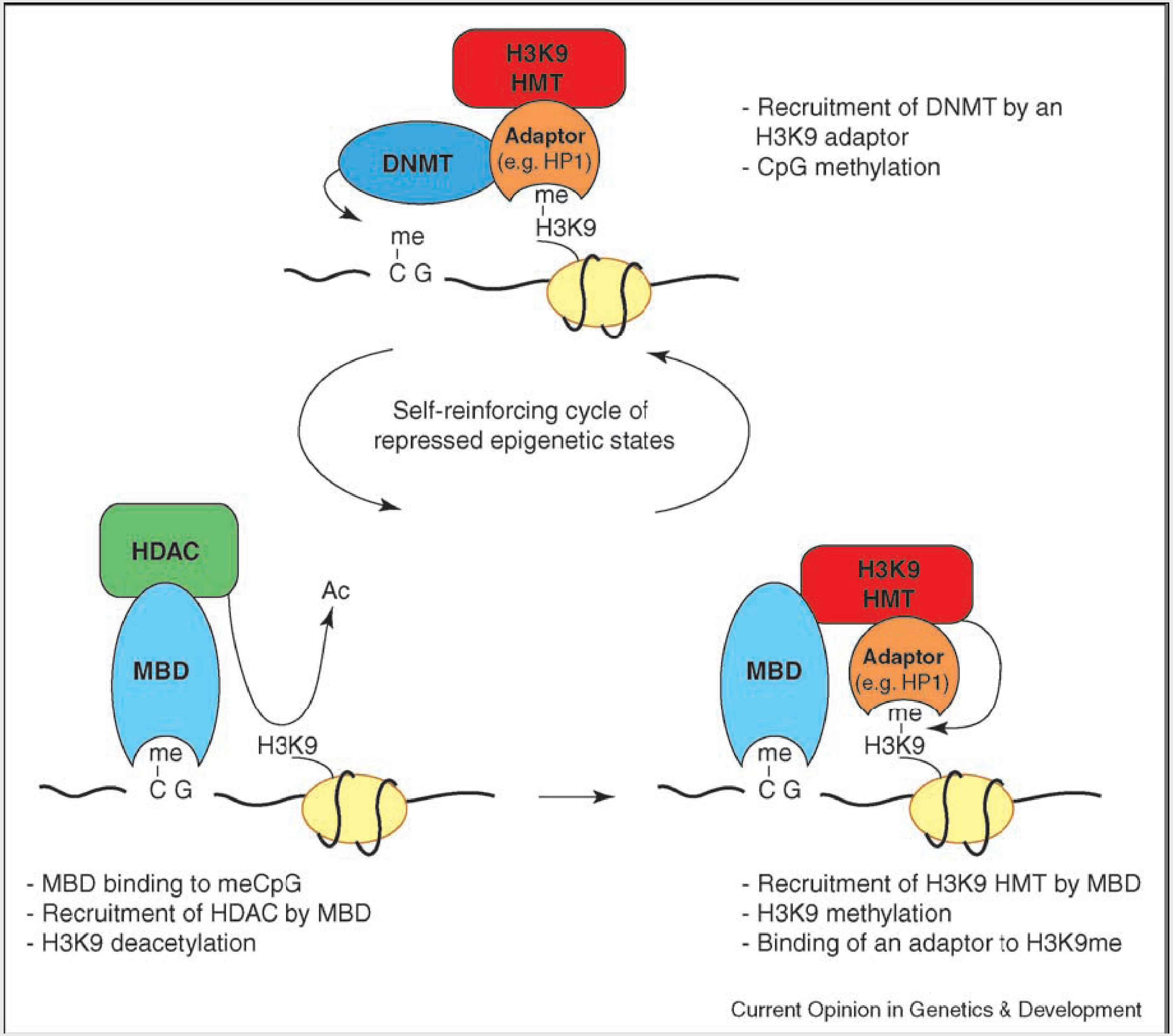
DNA Methylation can Template Histone Methylation



….. and histone Methylation can Template DNA Methylation



Self-Reinforced Model



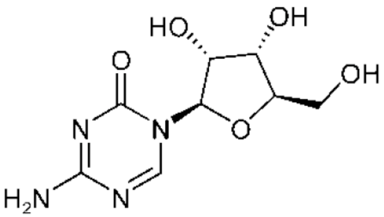
- Recruitment of DNMT by an H3K9 adaptor
- CpG methylation

- MBD binding to meCpG
- Recruitment of HDAC by MBD
- H3K9 deacetylation

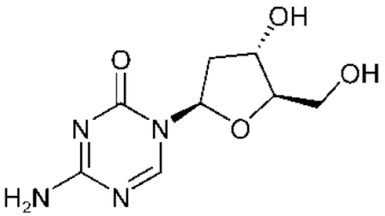
- Recruitment of H3K9 HMT by MBD
- H3K9 methylation
- Binding of an adaptor to H3K9me

Current Opinion in Genetics & Development

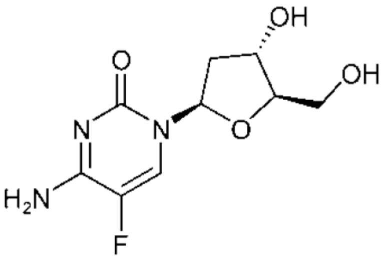
DNA methylation inhibitors



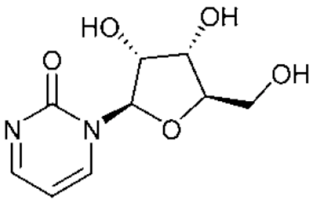
5-azacytidine (**32**)



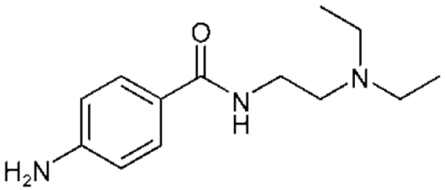
5-aza-2'-deoxycytidine (**33**)



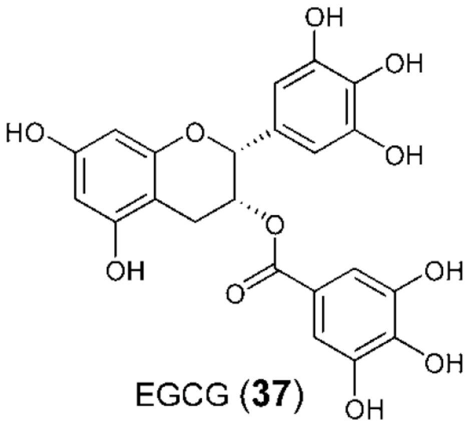
FCDR (**34**)



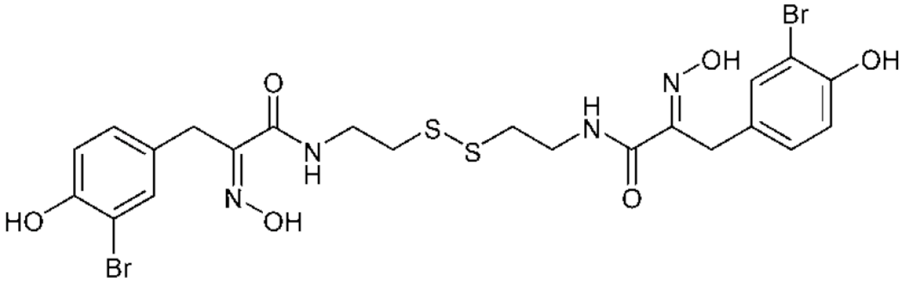
zebularine (**35**)



procainamide (**36**)



EGCG (**37**)



psammaplin A (**38**)

Identifying Methylation Sites

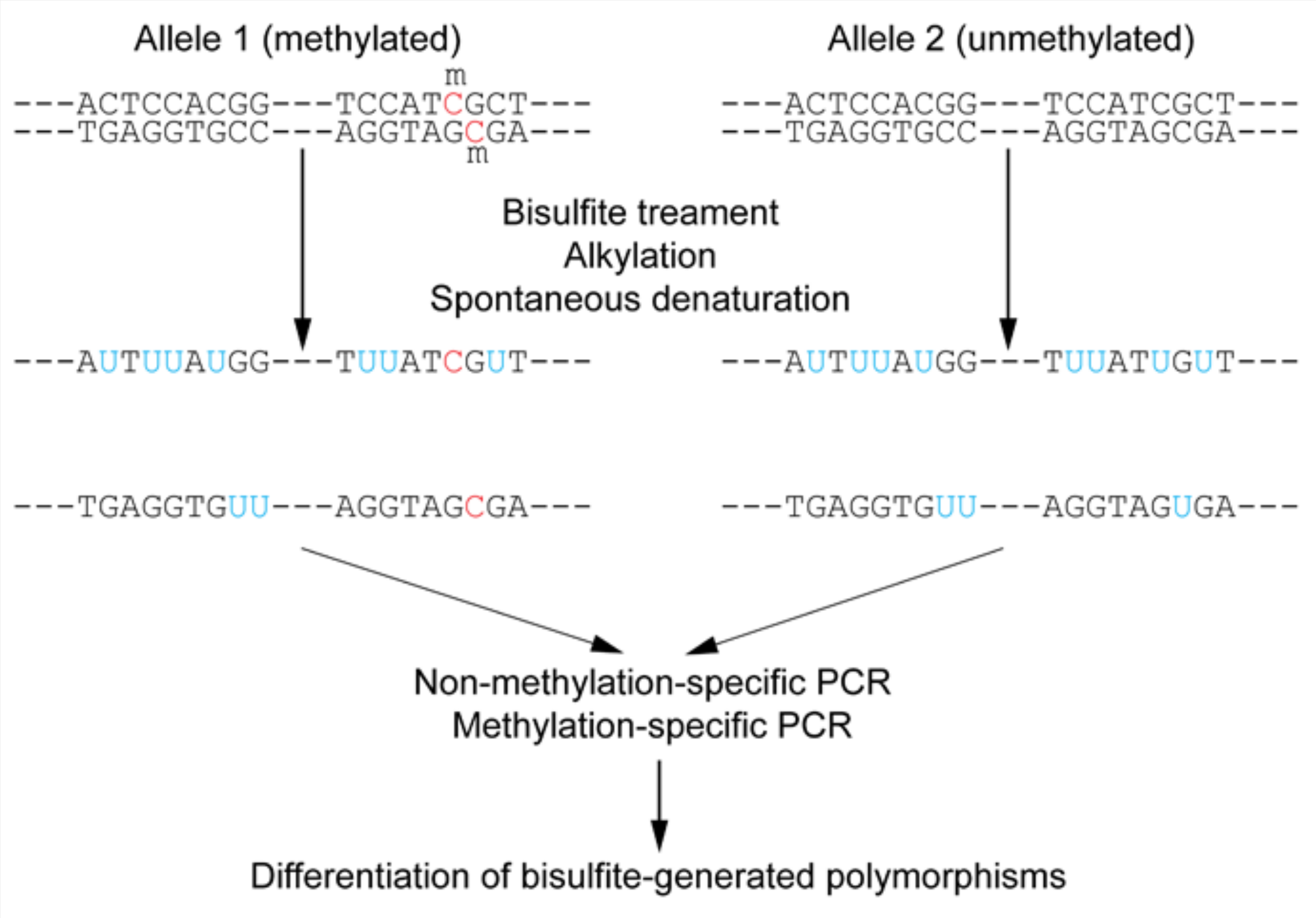
~ **Bisulfite sequencing**:

- Conversion of **non-methylated Cytosine** to **Uracil**
- No change to methylated Cytosine
- Sequenced through computer software identifying locations of methylation
- Two concentrations of methylation (low or high)

~ **ChIP-seq** by the Methyl-binding domain proteins

Bisulfite Sequencing

- **Conversion of unmethylated cystosines to uracil using** **Sodium Bisulfite**



Allele 1 (methylated)

```
             m
---ACTCCACGG---TCCATCGCT---
---TGAGGTGCC---AGGTAGCGA---
                      m
```

Allele 2 (unmethylated)

```
---ACTCCACGG---TCCATCGCT---
---TGAGGTGCC---AGGTAGCGA---
```

Bisulfite treament
Alkylation
Spontaneous denaturation

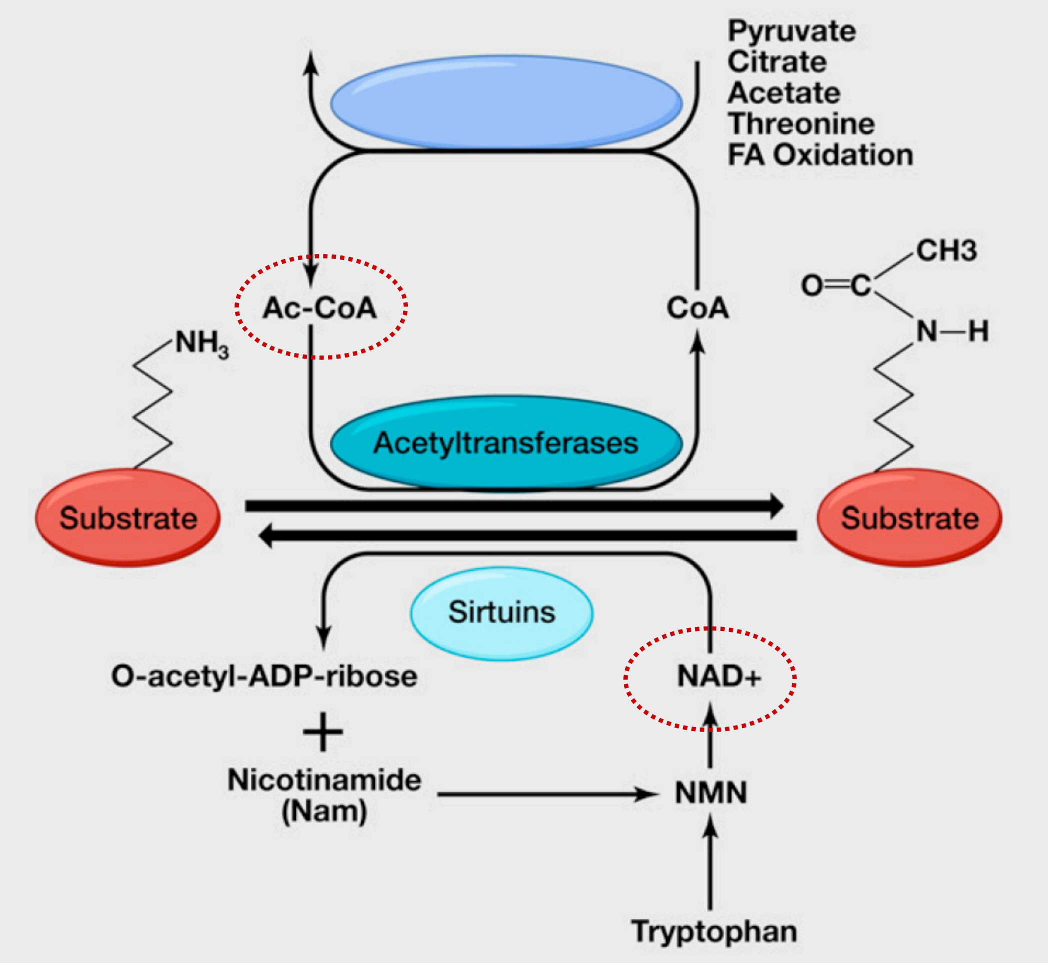
```
---AUTUUAUGG---TUUATCGUT---        ---AUTUUAUGG---TUUATUGUT---

---TGAGGTGUU---AGGTAGCGA---        ---TGAGGTGUU---AGGTAGUGA---
```

Non-methylation-specific PCR
Methylation-specific PCR

Differentiation of bisulfite-generated polymorphisms

Influence of Metabolism on Epigenetics

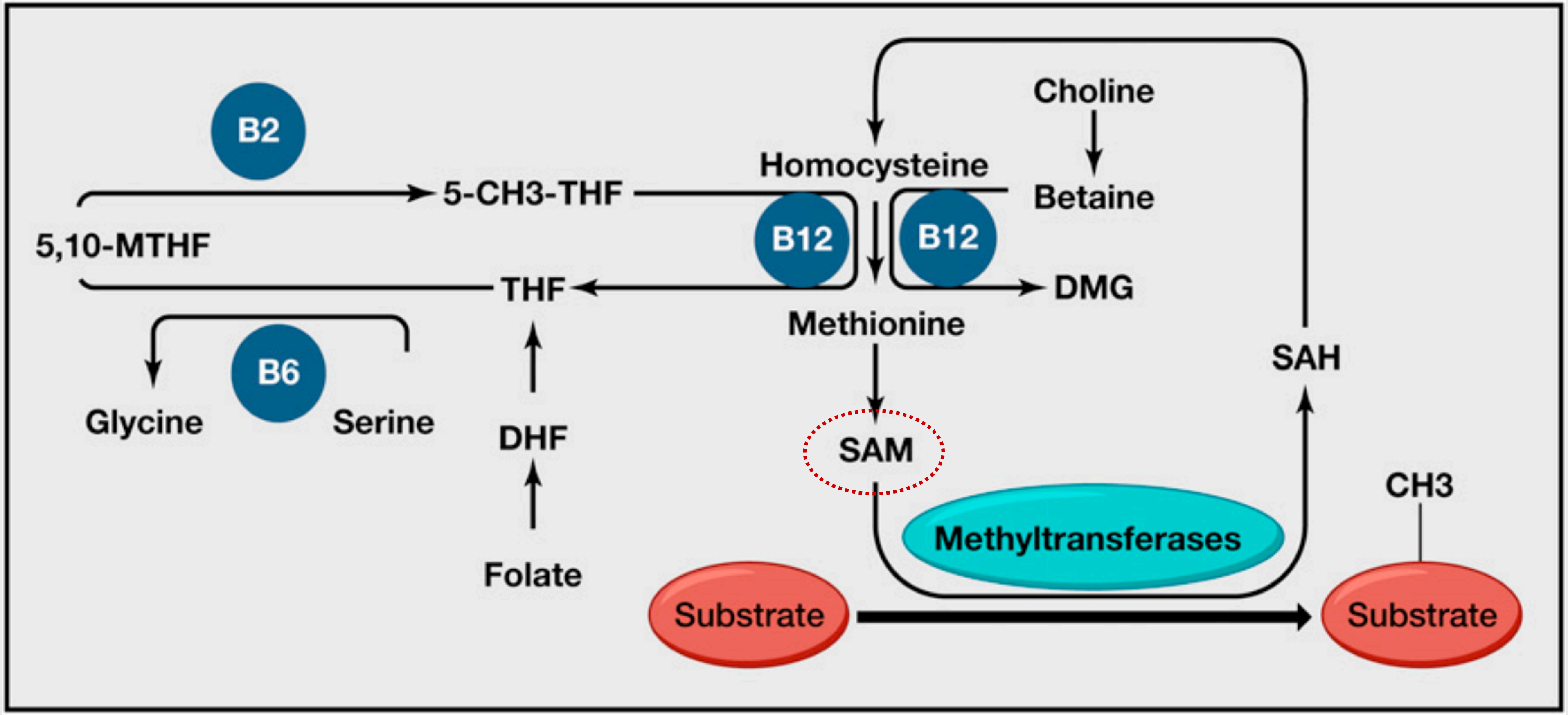
- Metabolism and Acetylation/Deacetylation:



Cell 153, March 28, 2013

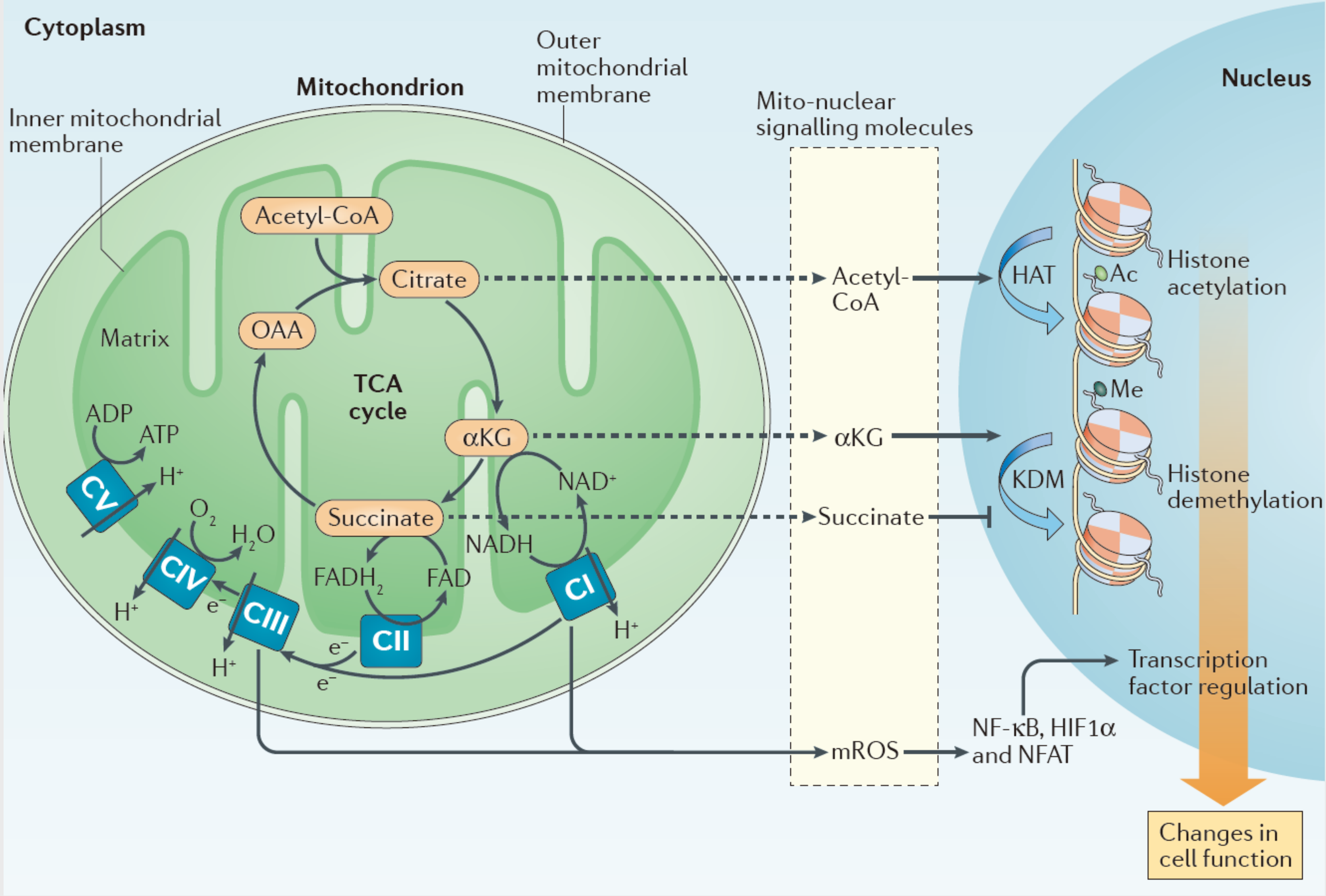
Influence of Metabolism on Epigenetics

- Metabolism and Methylation:



Cell 153, March 28, 2013

Mitochondria as signaling organelles

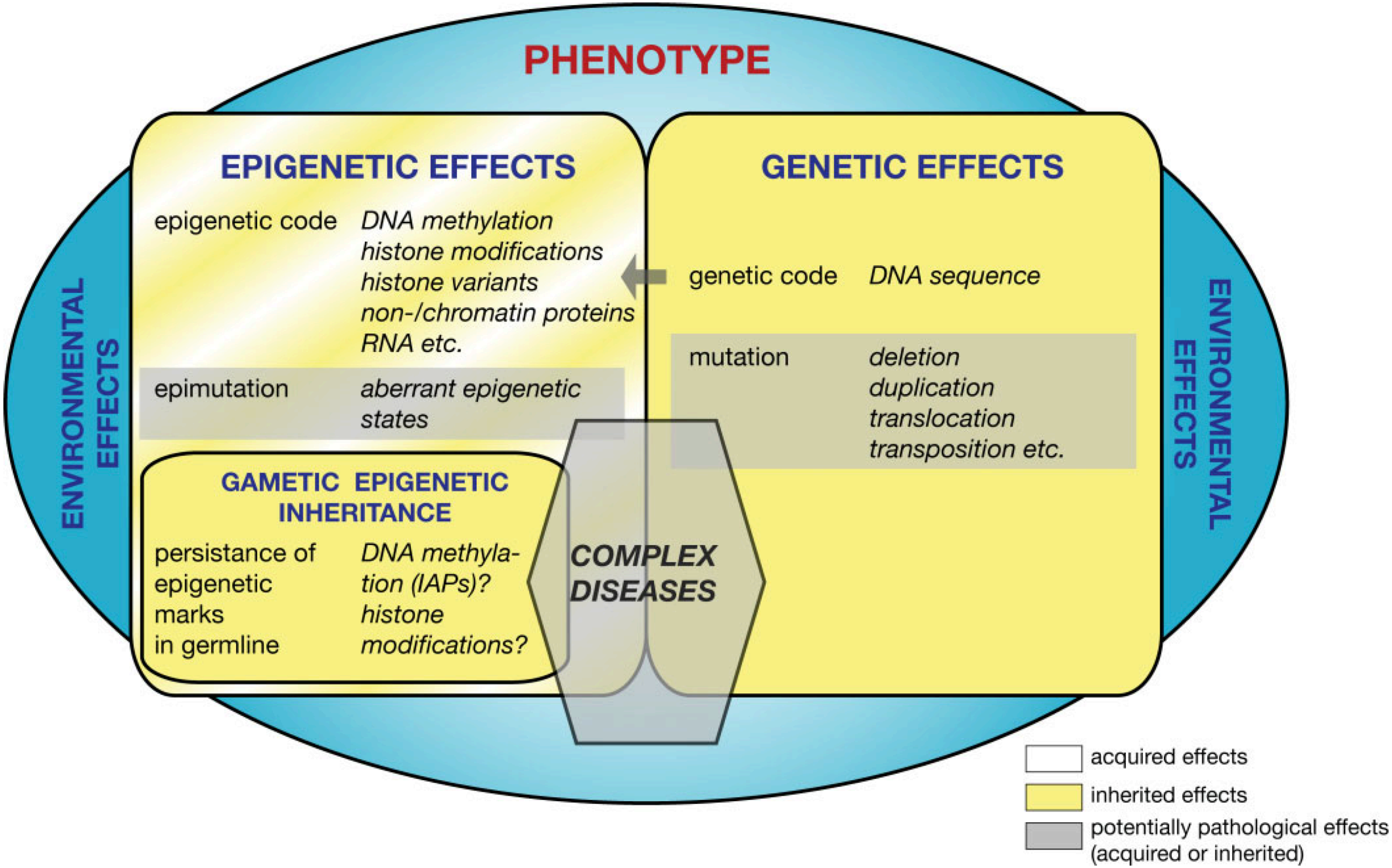


NATURE REVIEWS | **IMMUNOLOGY 2017**

Summary

- Chromatin structure plays a central role many cellular processes.
- Relevant to most aspects of gene expression as well as chromosome stability, DNA replication, recombination and repair.
- Chromatin regulated at local and global levels.
- Misregulation of chromatin and/or DNA methylation states leads to diseases.
- Chromatin is highly dynamic both in terms of structure & chemical composition.

Summary



PHENOTYPE

ENVIRONMENTAL EFFECTS

EPIGENETIC EFFECTS

epigenetic code	*DNA methylation* *histone modifications* *histone variants* *non-/chromatin proteins* *RNA etc.*
epimutation	*aberrant epigenetic states*

GENETIC EFFECTS

genetic code	*DNA sequence*
mutation	*deletion* *duplication* *translocation* *transposition etc.*

GAMETIC EPIGENETIC INHERITANCE

persistance of epigenetic marks in germline	*DNA methylation (IAPs)?* *histone modifications?*

COMPLEX DISEASES

ENVIRONMENTAL EFFECTS

☐ acquired effects

🟨 inherited effects

▨ potentially pathological effects (acquired or inherited)