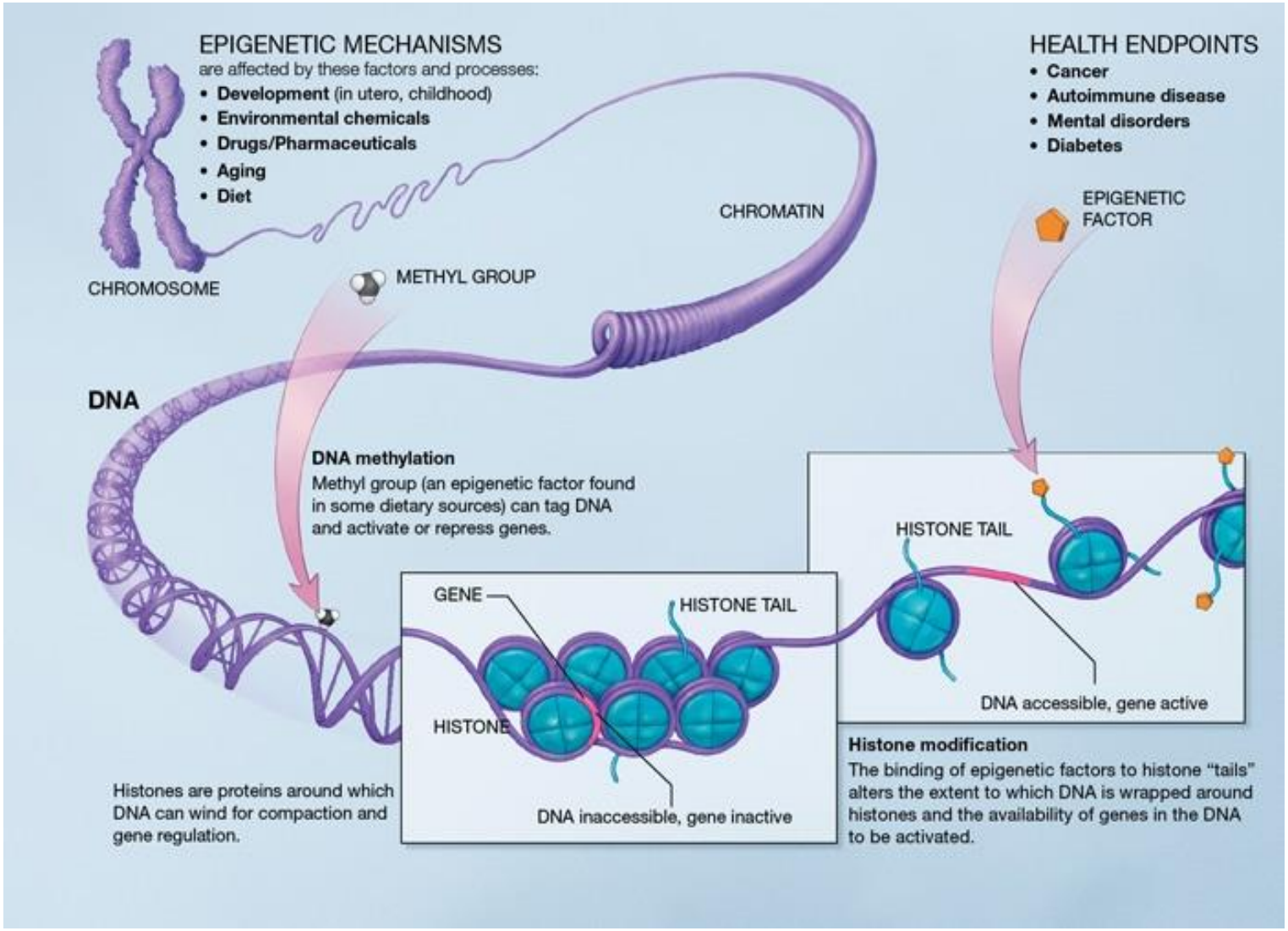
Epigenetic

DNA and Chromatin Structure



Nucleosomes are the basic building block of Chromatin

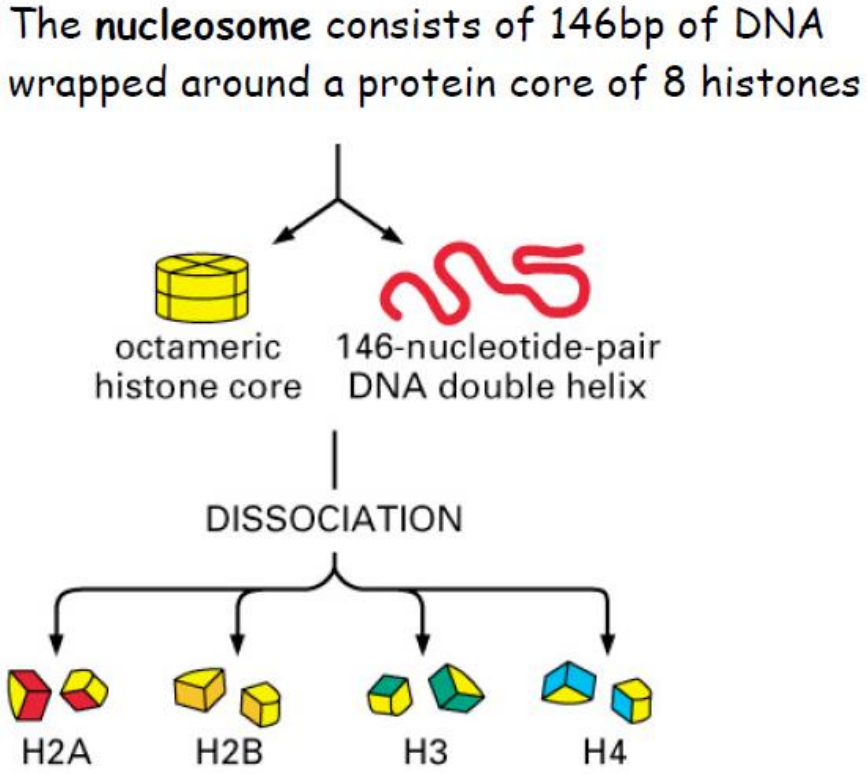
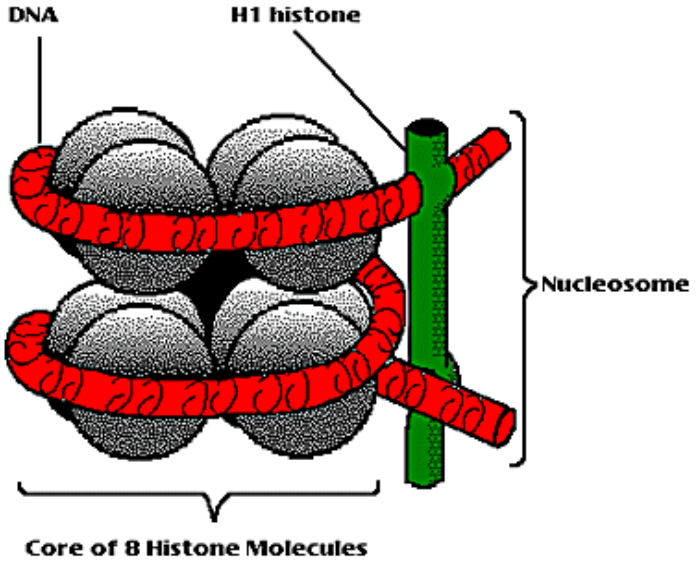


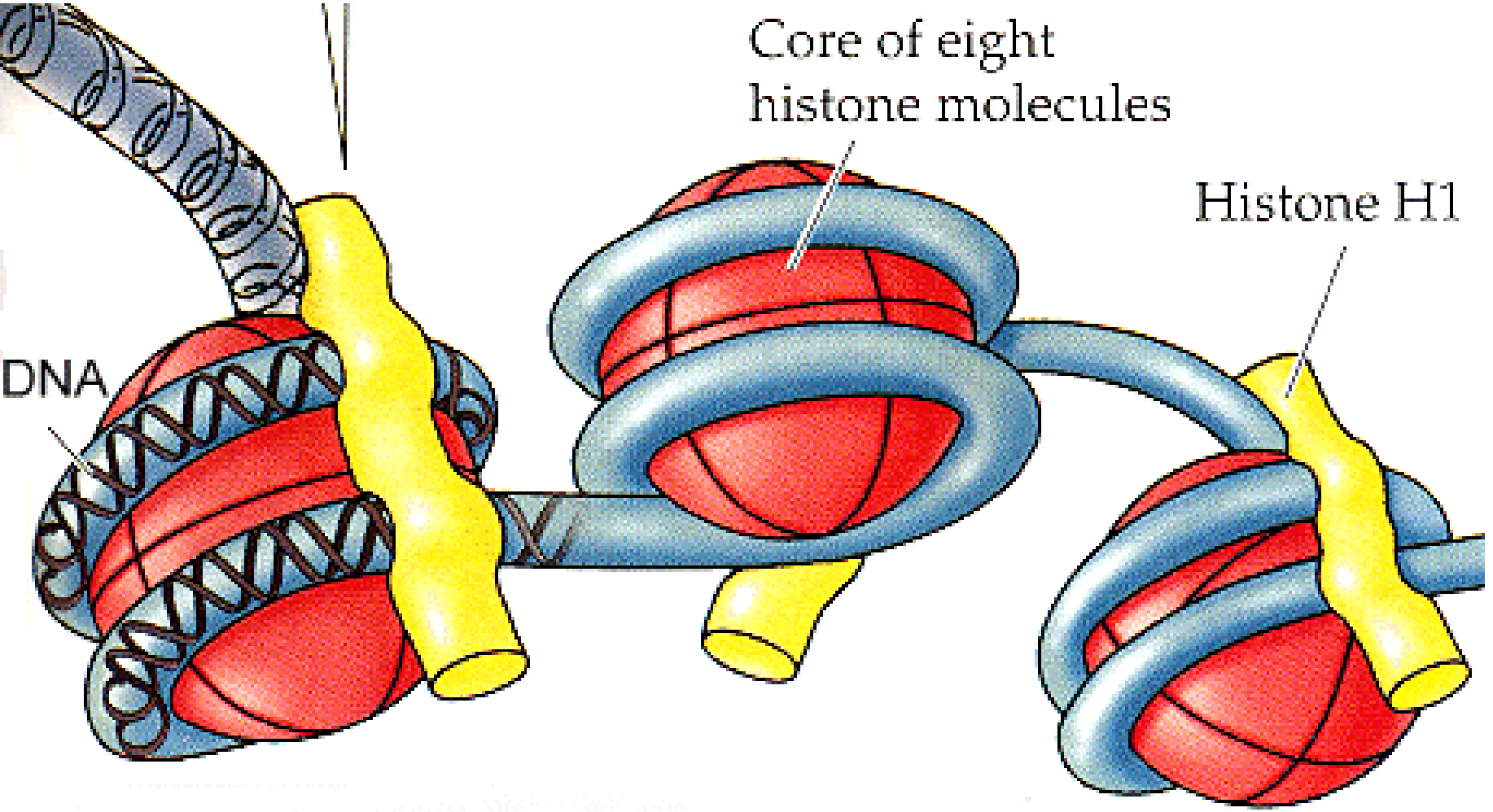
Figure 4–24 part 2 of 2. Molecular Biology of the Cell, 4th Edition.



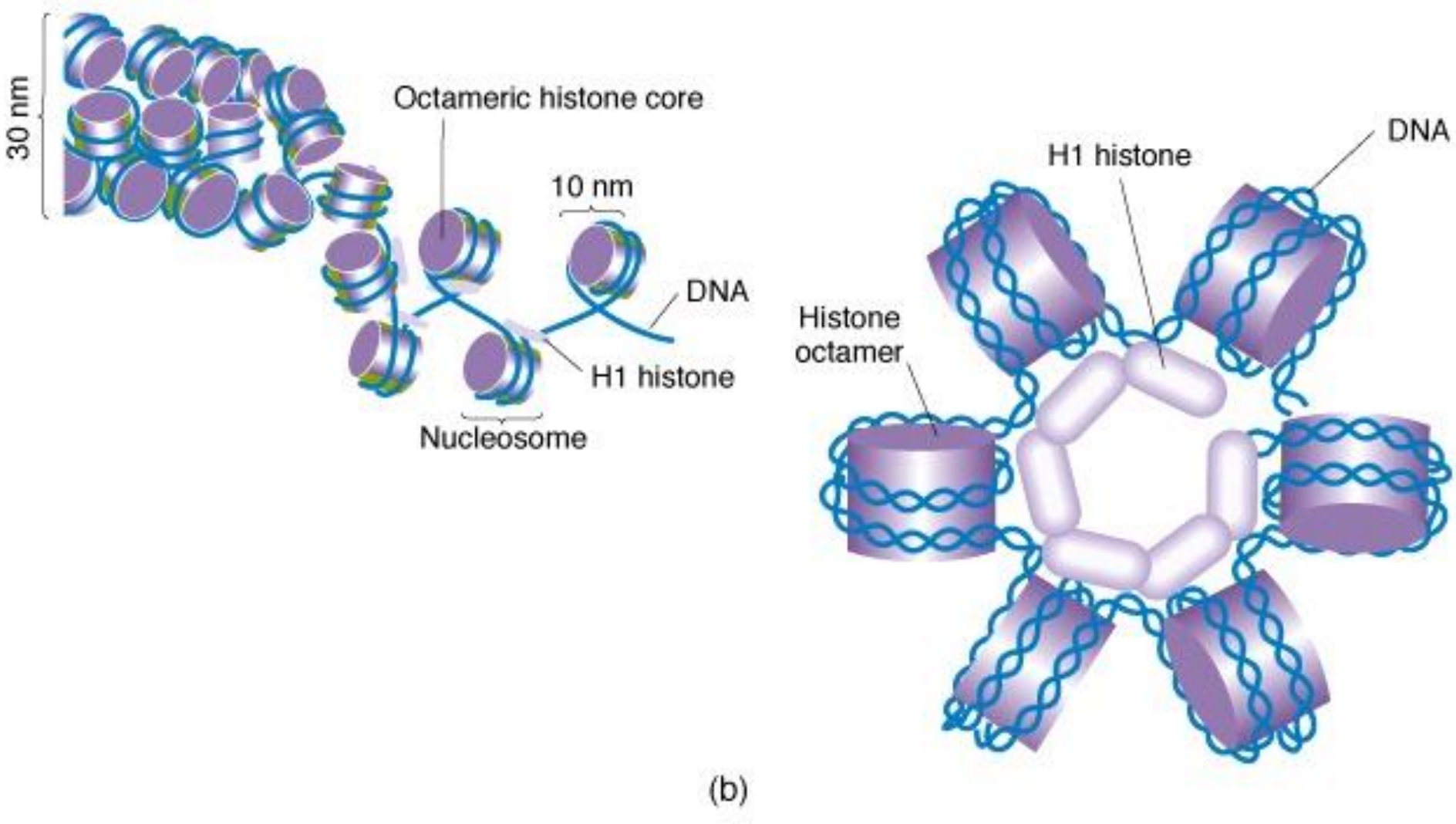
Nucleosome

http://www.accessexcellence.com/AB/GG/nucleosome.gif

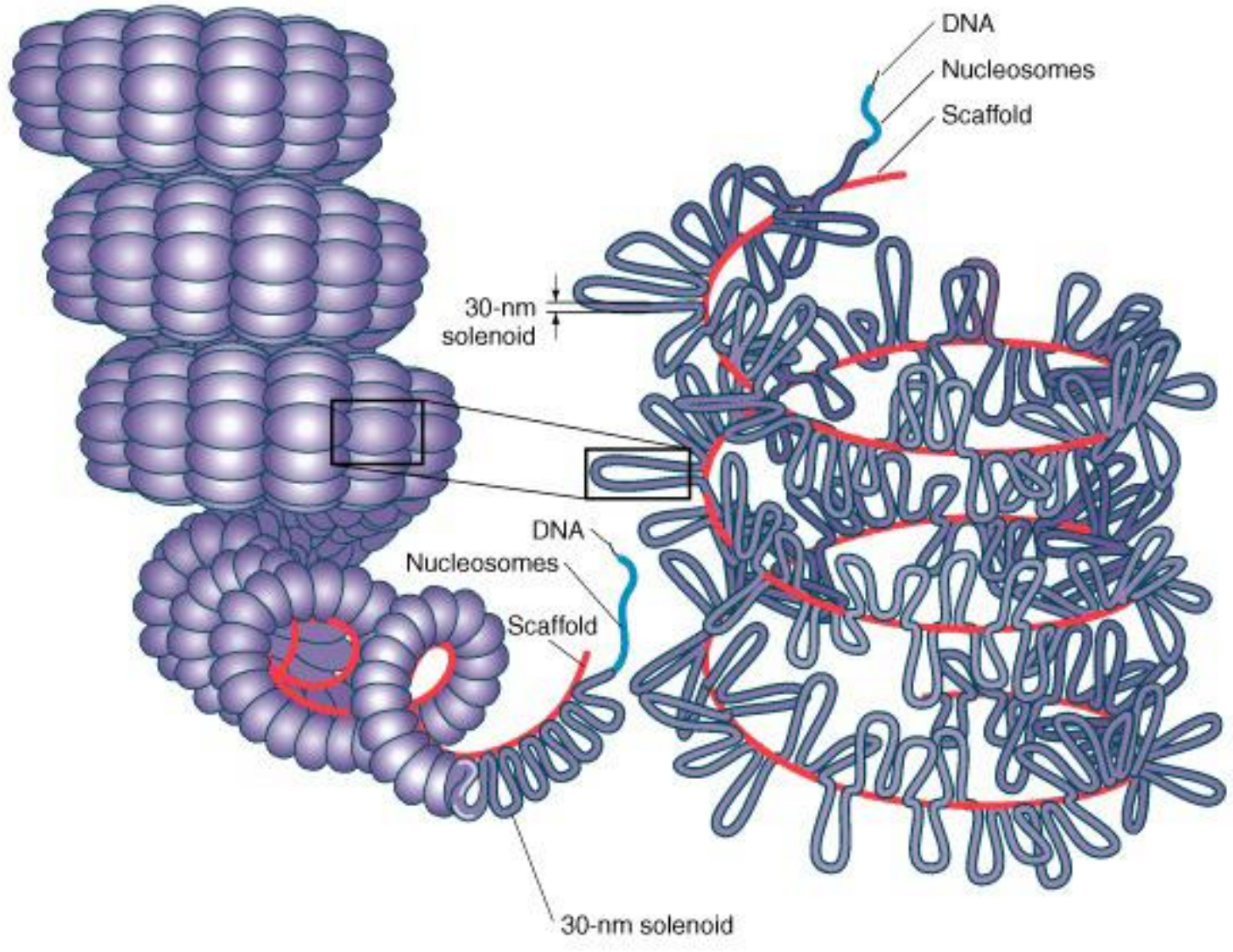
3 nucleosomes shown here



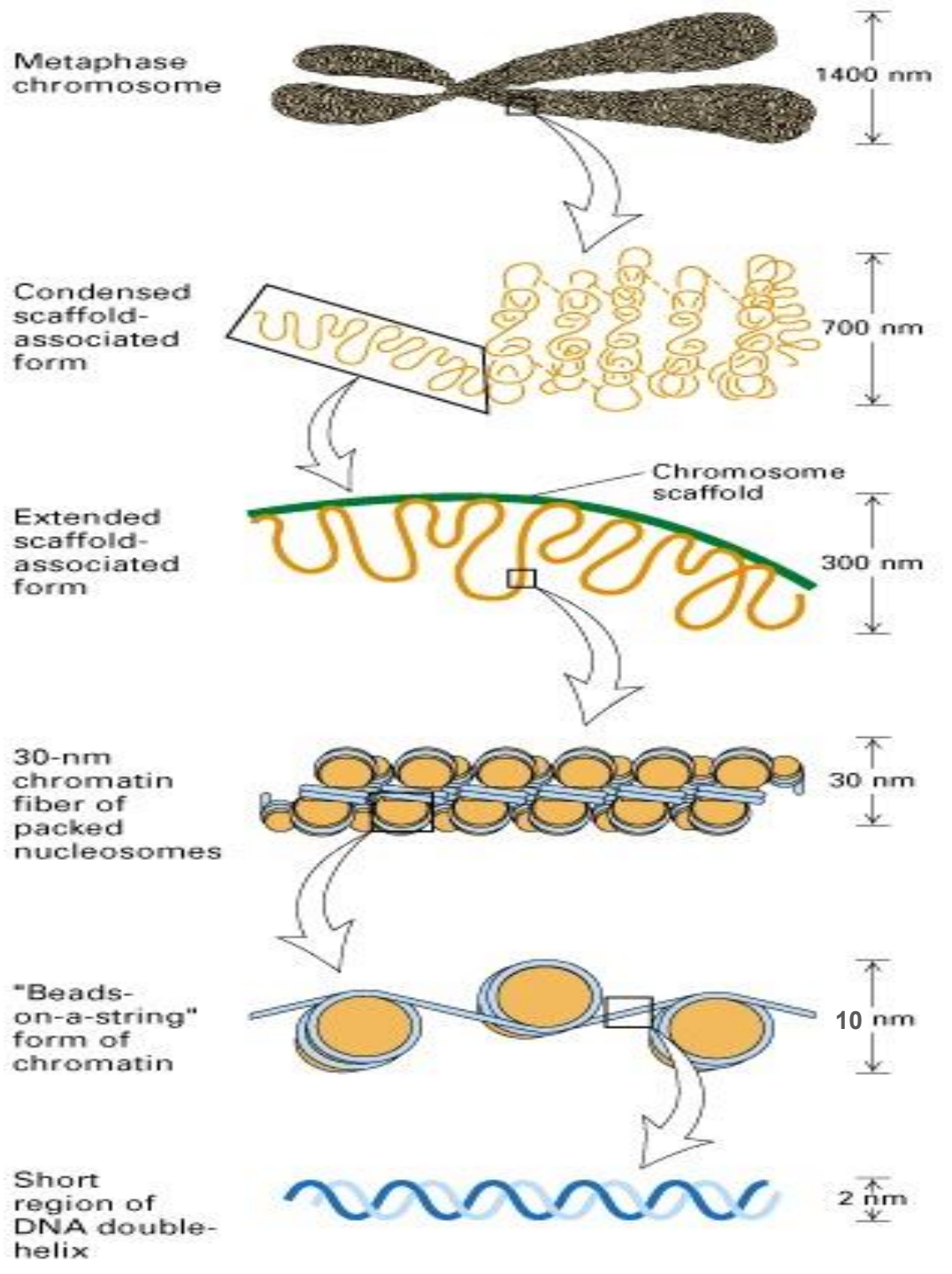
30 nm fiber packing (Solenoids)



Loop Domains



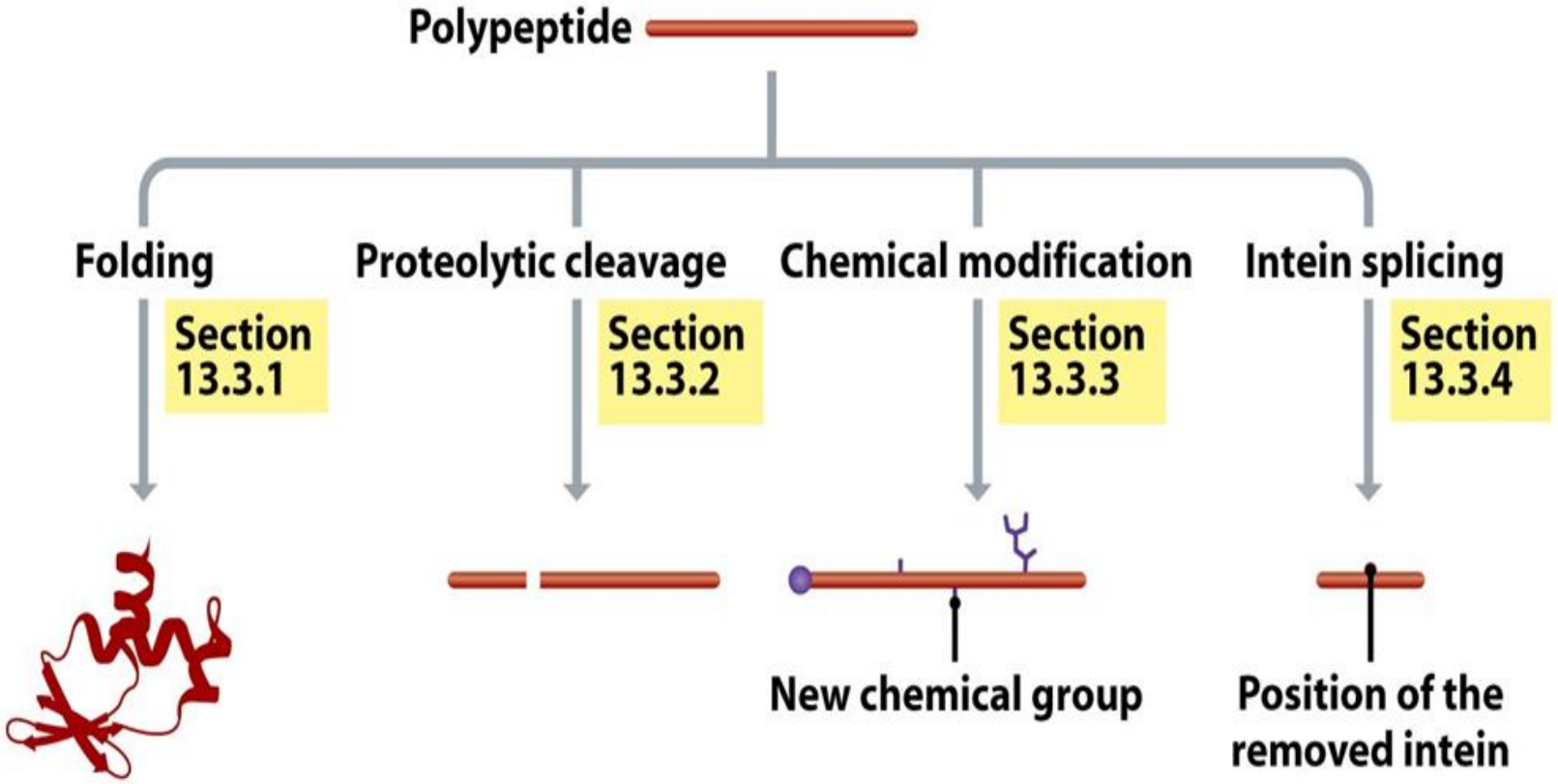
DNA packing overview



There are two types of chromatin found in a nucleus at any given time

Heterochromatin	vs.	Euchromatin
Highly condensed in interphase		Organized in 30nm fiber during interphase
Transcriptionally inactive (contains few genes)		Transcriptionally active
Replicates late in S phase		Replicates early in S phase

Post-translational modifications



Post-translational modifications

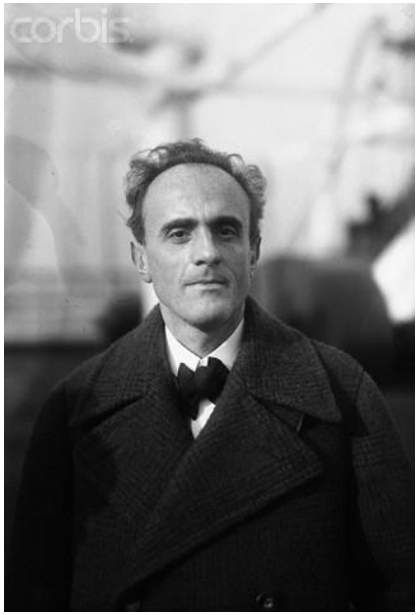
Table 13.6 Examples of posttranslational chemical modifications

Modification	Amino acids that are modified	Examples of proteins
Addition of small chemical groups		
Acetylation	Lysine	Histones
Methylation	Lysine	Histones
Phosphorylation	Serine, threonine, tyrosine	Some proteins involved in signal transduction
Hydroxylation	Proline, lysine	Collagen
N-formylation	N-terminal glycine	Melittin
Addition of sugar side chains		
O-linked glycosylation	Serine, threonine	Many membrane proteins and secreted proteins
N-linked glycosylation	Asparagine	Many membrane proteins and secreted proteins
Addition of lipid side chains		
Acylation	Serine, threonine, cysteine	Many membrane proteins
N-myristoylation	N-terminal glycine	Some protein kinases involved in signal transduction
Addition of biotin		
Biotinylation	Lysine	Various carboxylase enzymes


Me Me Me Ac P Ac Me Ac Ac Me Me P
H3 A R T K Q T A R K S T G G K A P R K Q L A T K A R K S A P
10
20

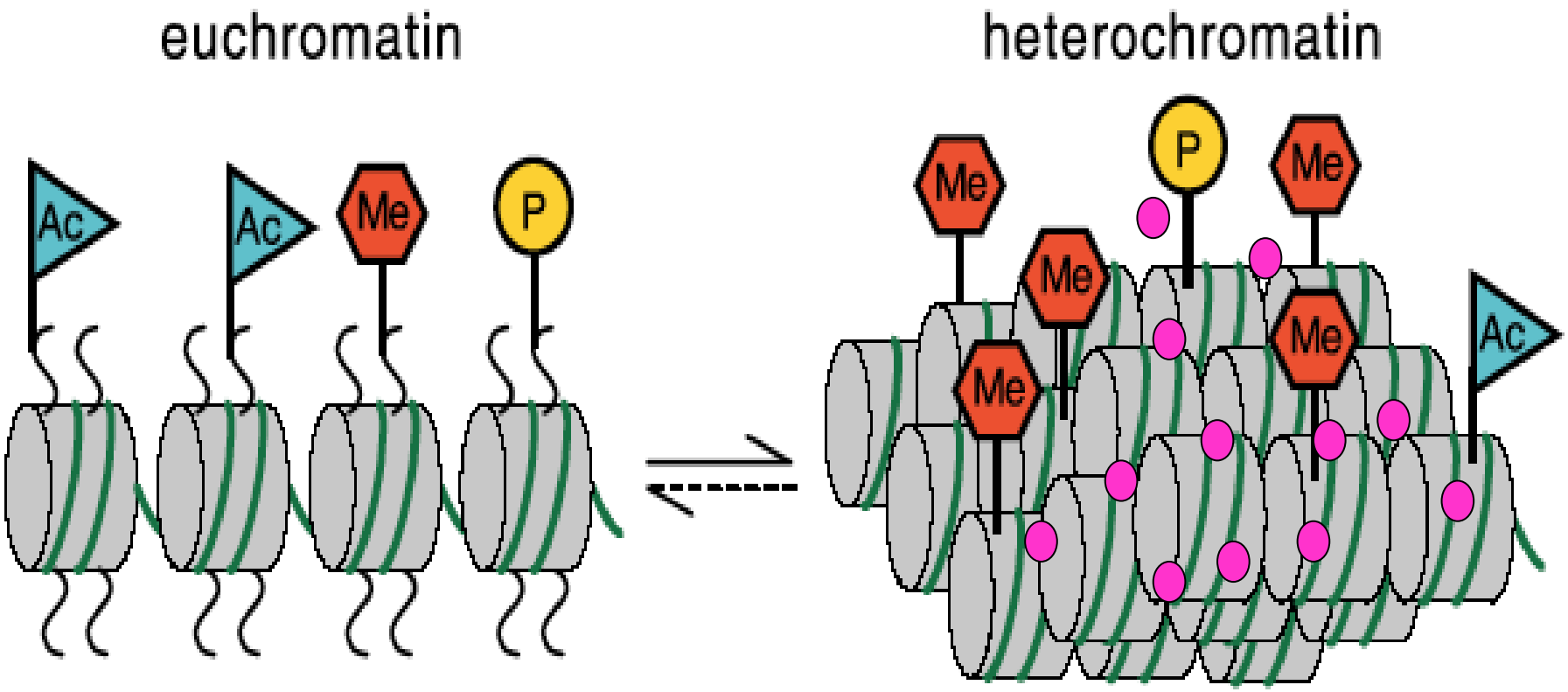
Epigenetic

- History of Epigenetic modifications
  - Discovered by **Paul Kammerer**, a lamarckian evolutionist, in the 1920s.
  - *Epigenetic* was coined by C. H. Waddington in 1942
- Definitions
  - In 2008 → Cold Spring Harbor meeting
    - Stably heritable changes in a DNA without alterations in the DNA sequence",



Characteristics of epigenetic modifications

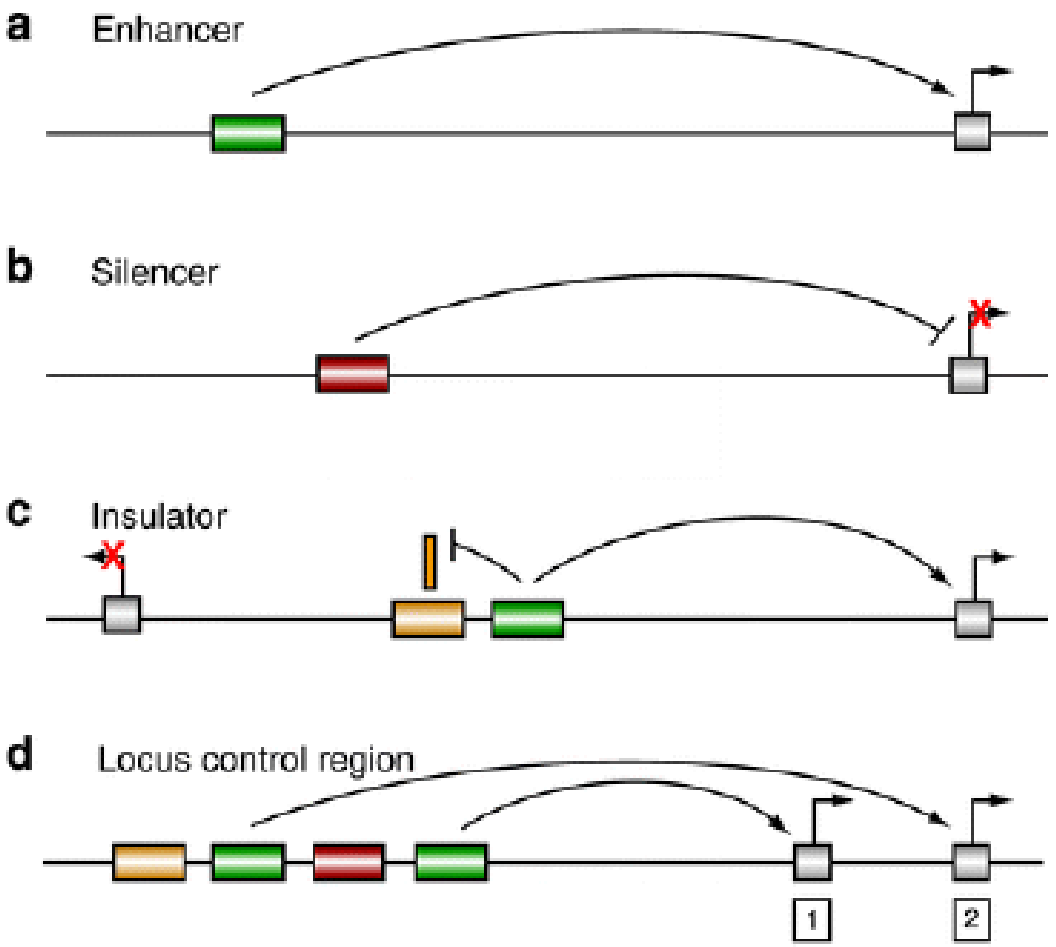
- Epigenetic changes can modify the activation of certain genes, but not the sequence of DNA.
- Epigenetic changes are preserved when cells divide.
- Some epigenetic changes can be transferred to the next generation.



Gene expression

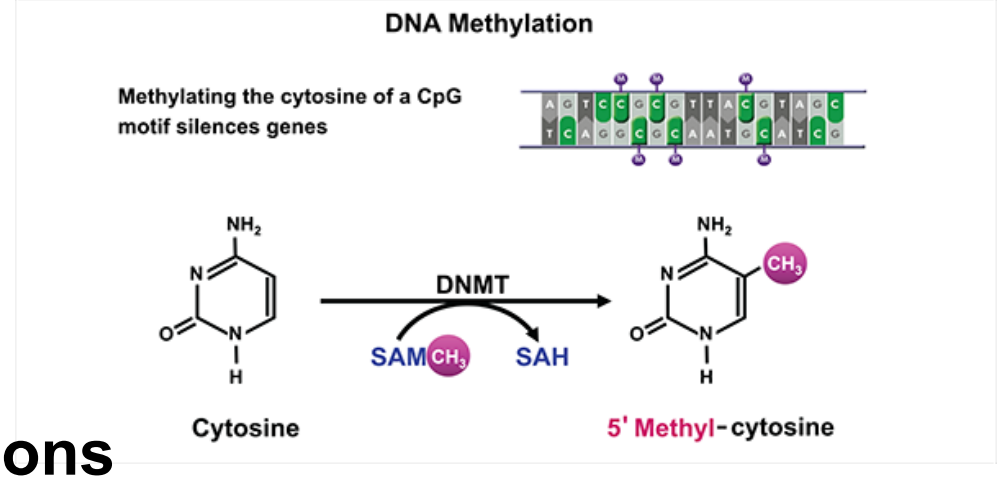
Role of Genomic Cis-Acting Elements

Trans-acting factors bind cis-acting elements

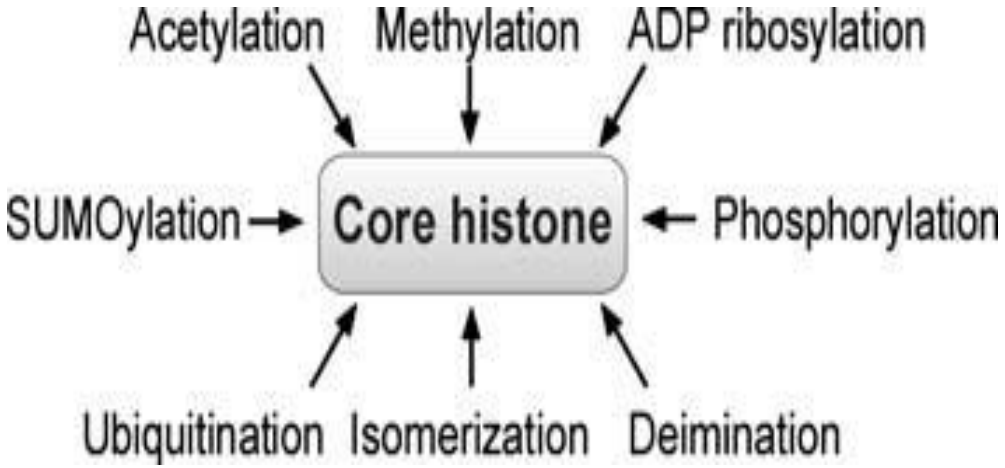


Epigenetic modifications

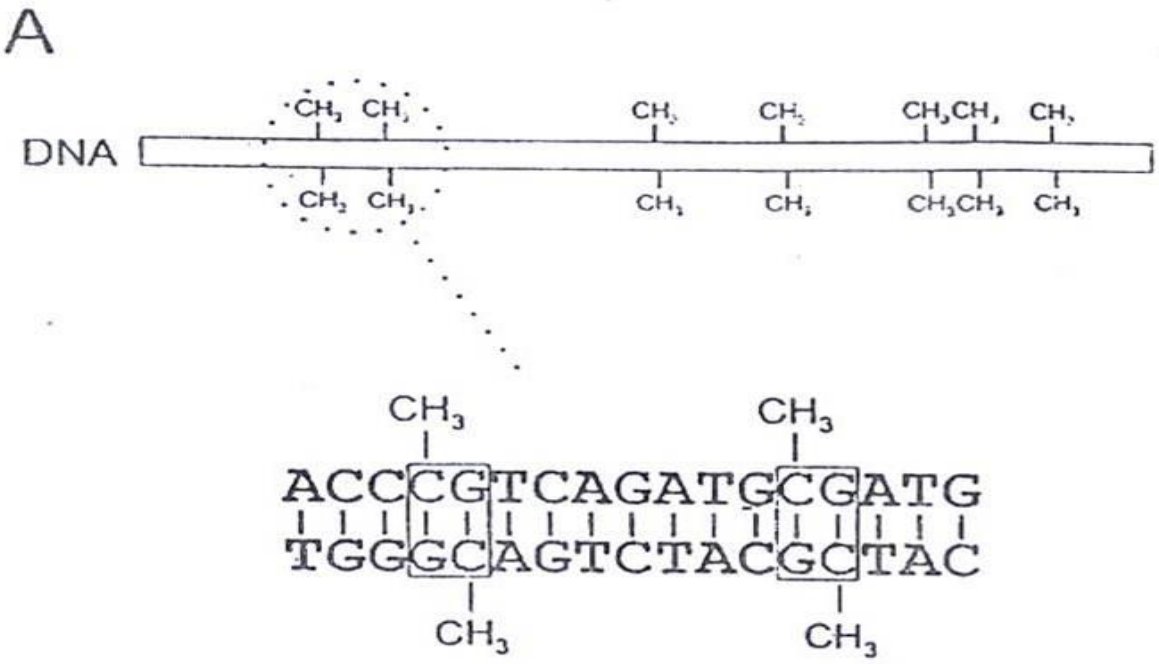
- **DNA modification**

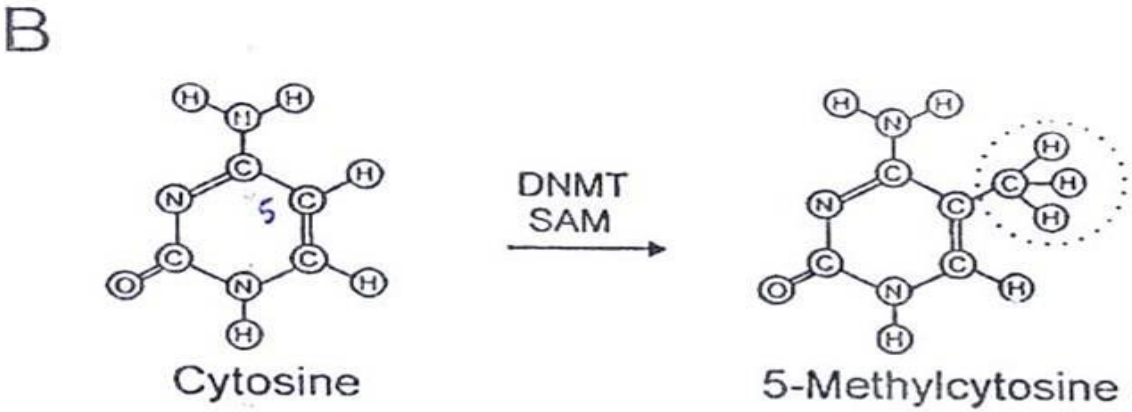


- **Histone modifications**



DNA methylation

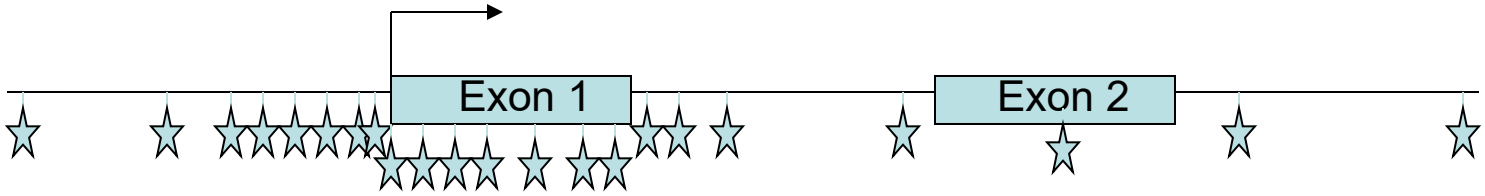


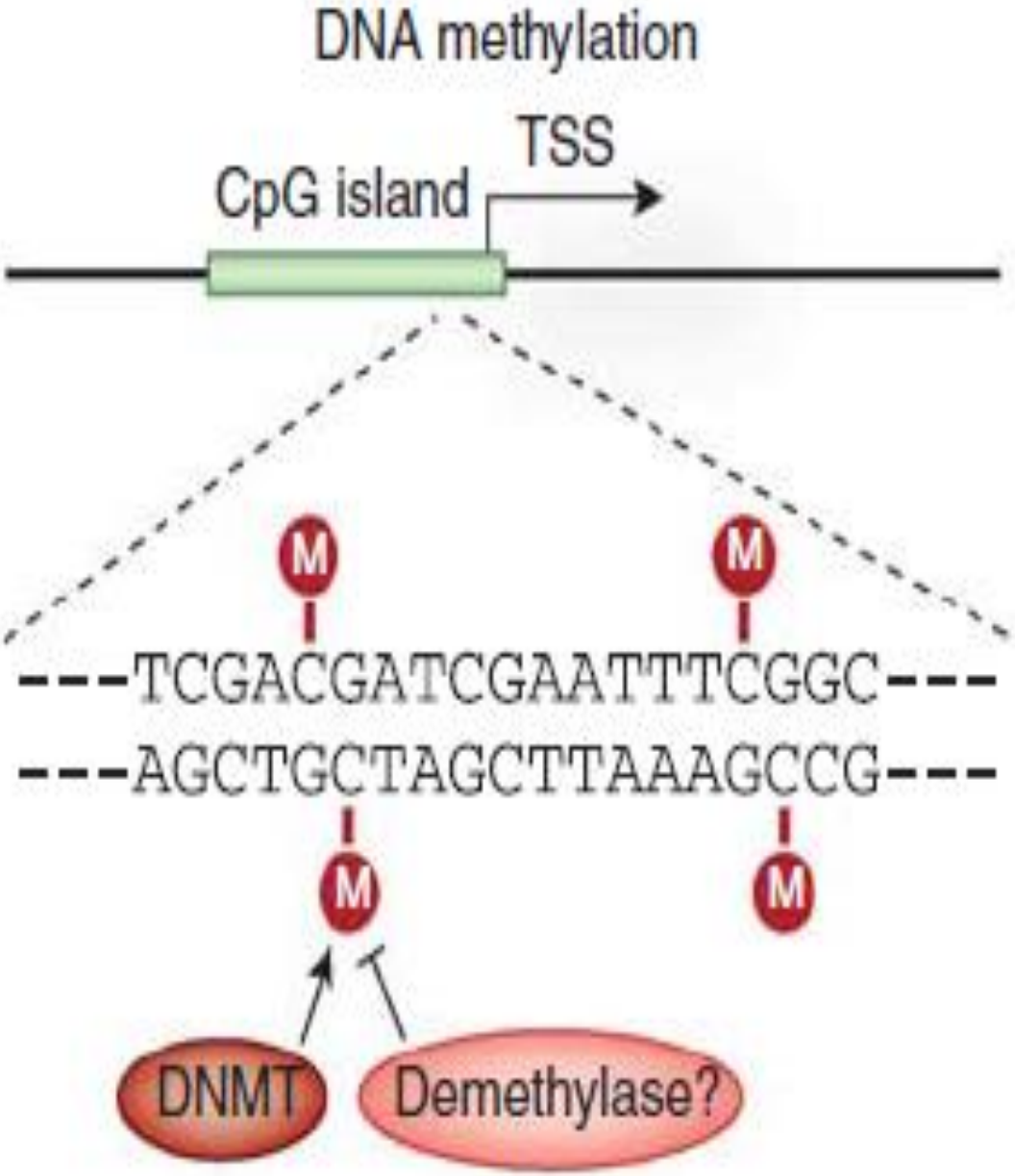


CpG Islands

- Regions of the genome in which the CpG dinucleotide occurs at the EXPECTED frequency

- Usually located in 5` flanking sequence, around the proximal promoter, and/or within the first exon and intron



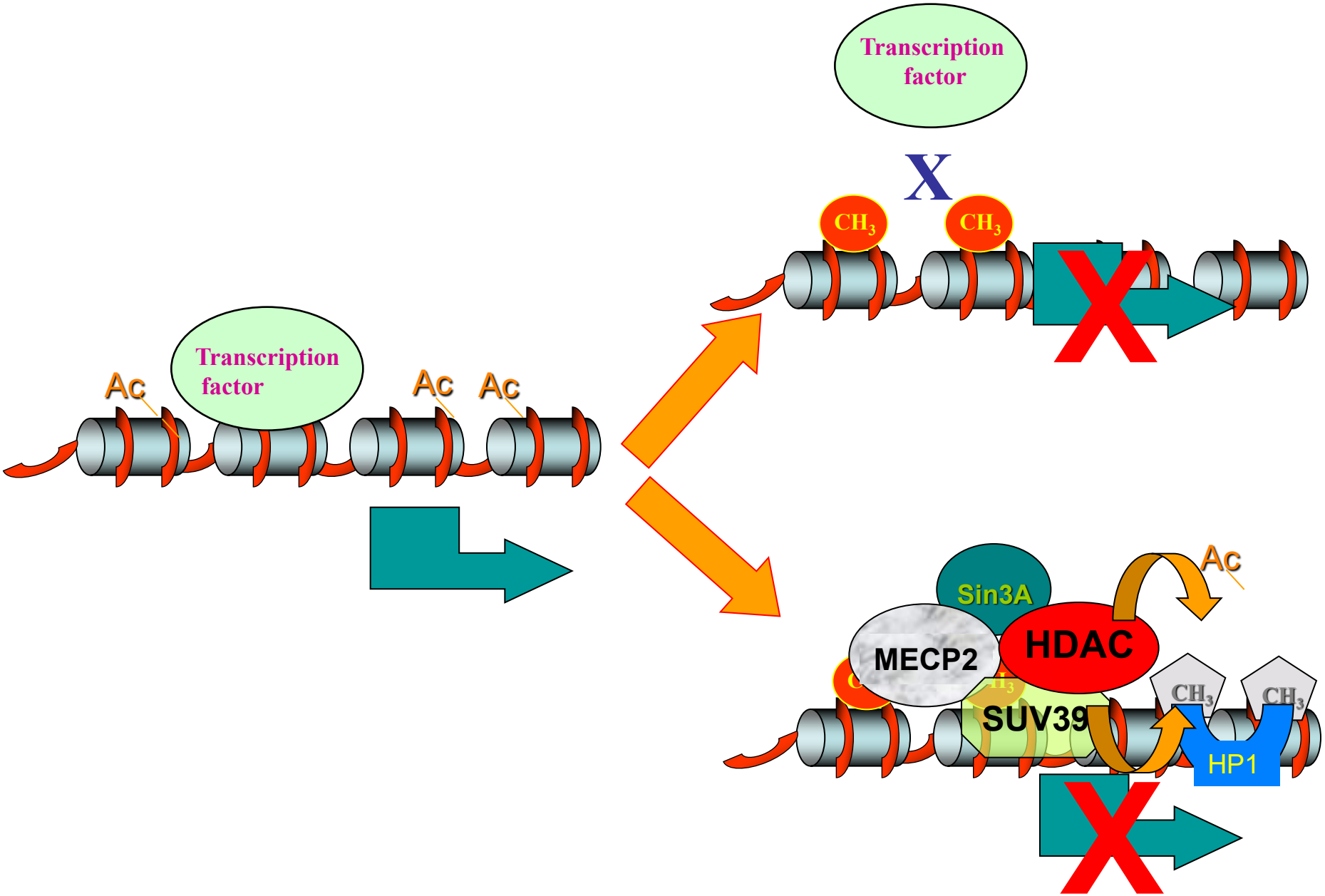


DNA methyltransferases
- DNMT1 (maintenance)
- DNMT3a (de novo)
- DNMT3b (de novo)

DNA demethylase?
- Base excision repair
- GADD45b pathway
- MBD proteins
- TET1 pathway

DNA methylation readers
- MeCP2
- MBD1-4
- Kaiso protein family

DNA methylation silences gene expression by two mechanisms



Histone modifications

Histone octamers assemble from pairs of dimers

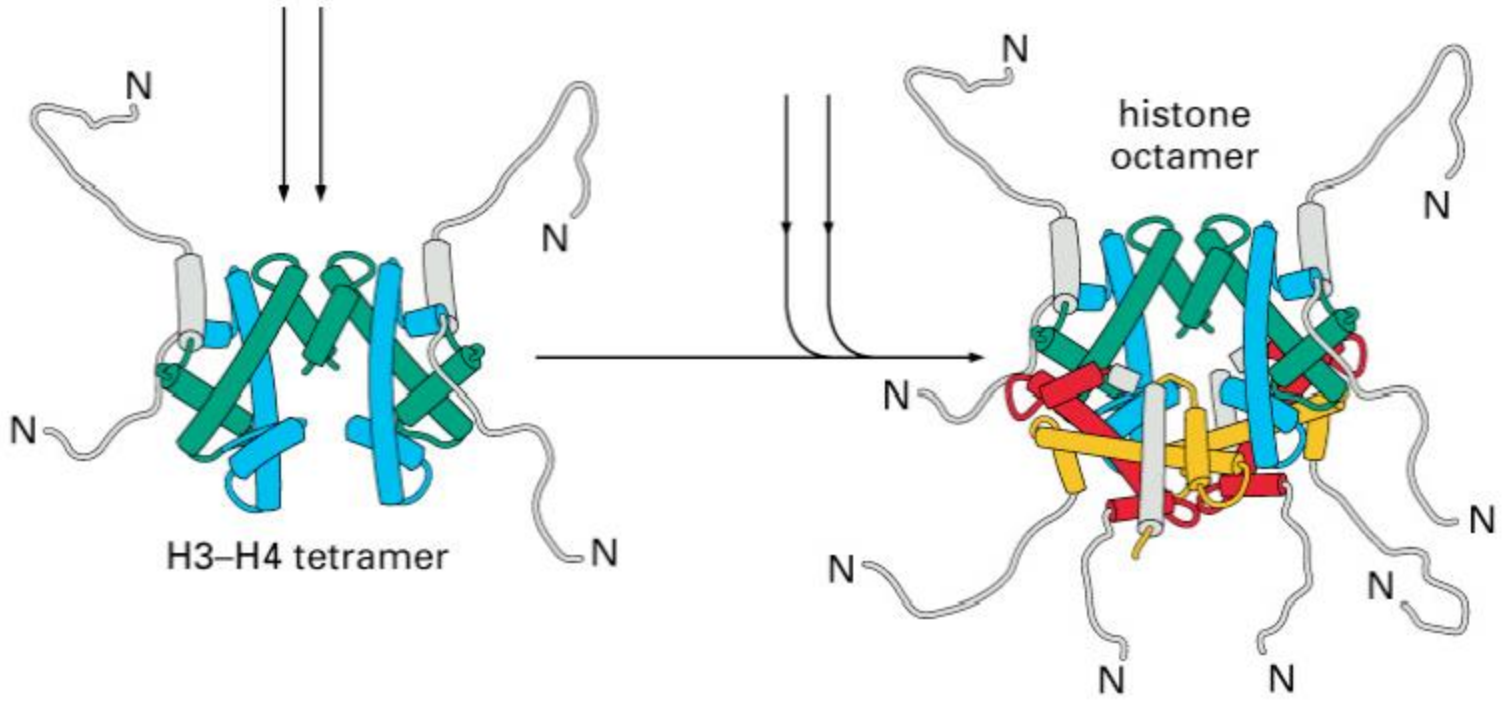
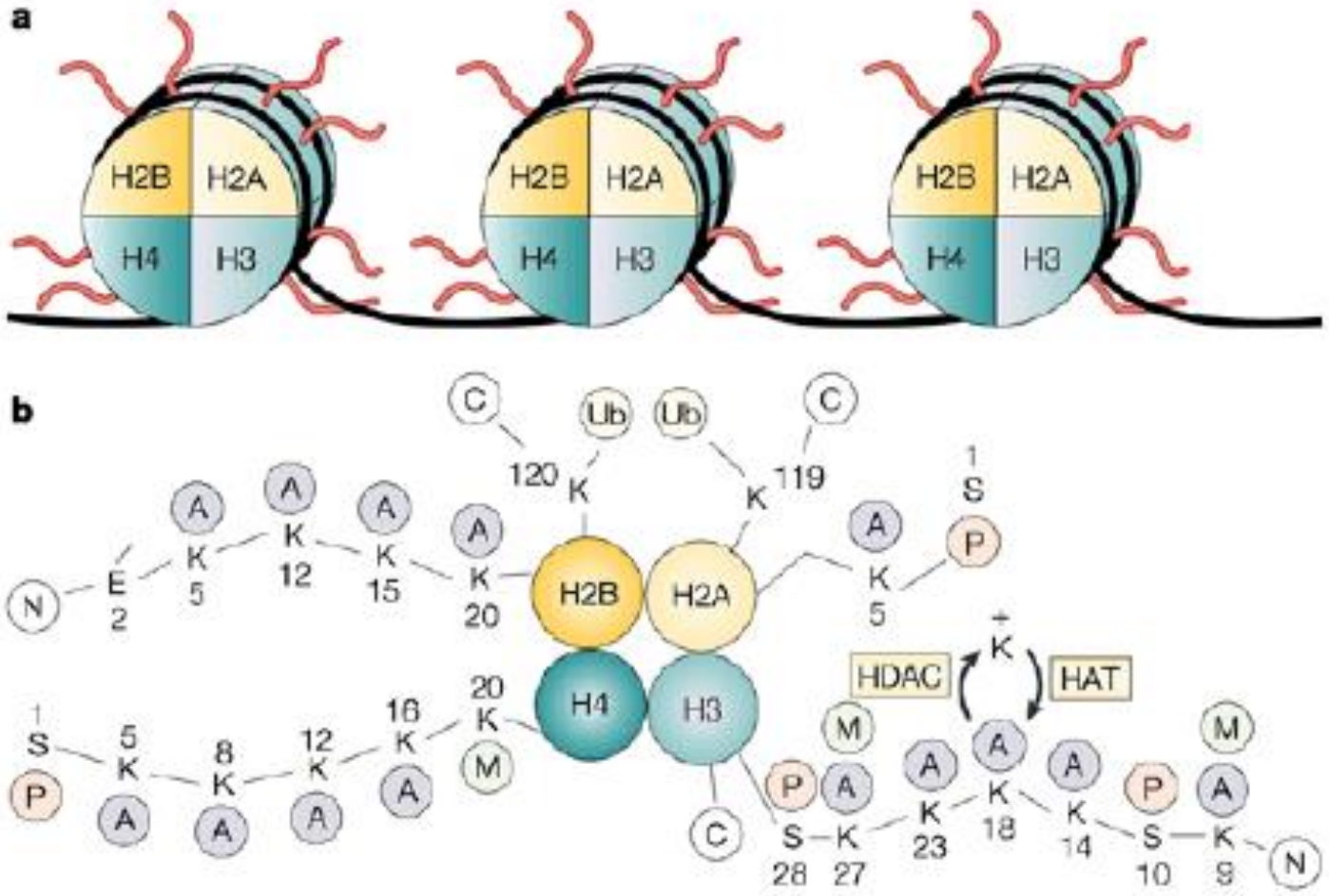
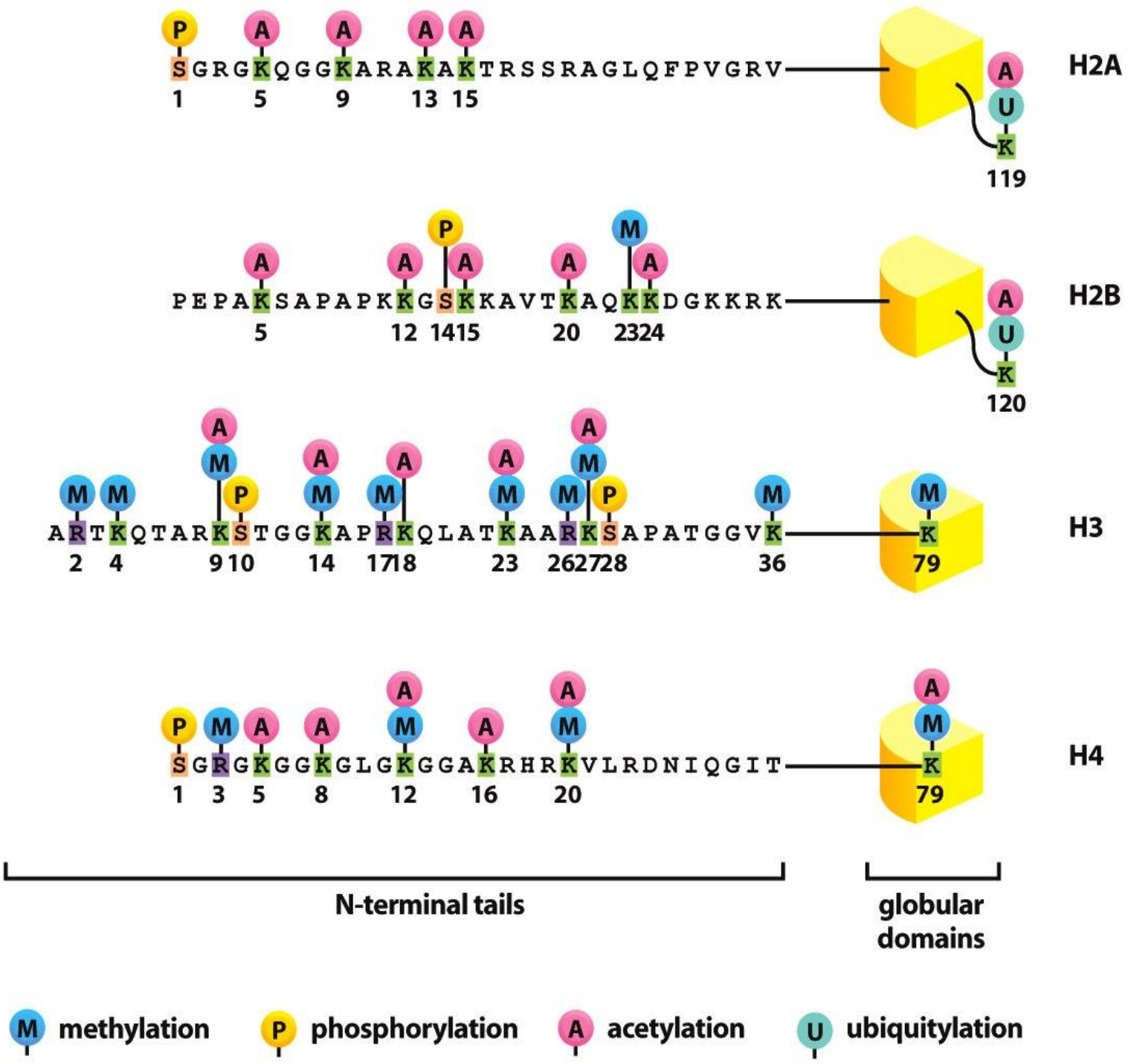


Figure 4–27 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

Detailed View of Chromatin and Transcription




H2A
H2B
H3
H4
N-terminal tails
globular domains
methylation
phosphorylation
acetylation
ubiquitylation

Histone code hypothesis

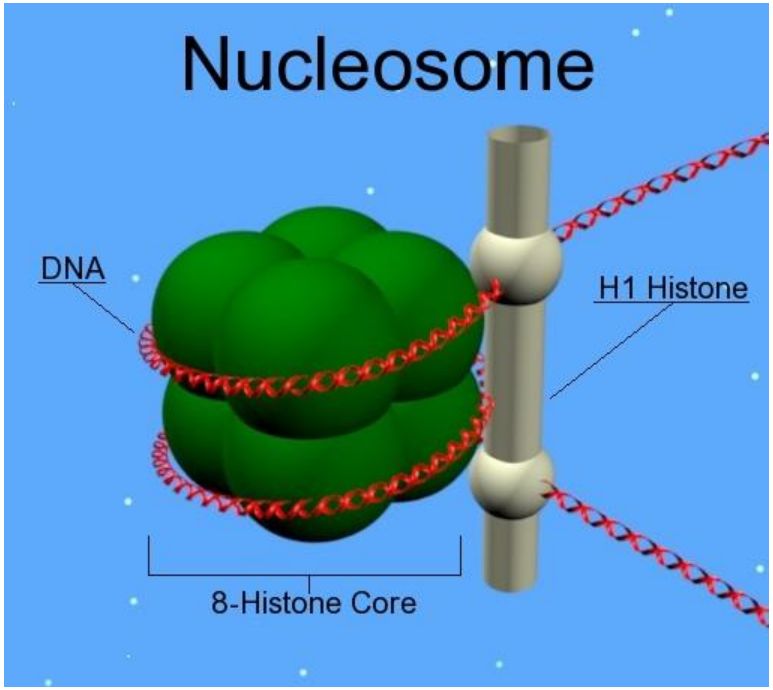
- The **histone code** is a hypothesis that the transcription of genetic information encoded in DNA is in part regulated by chemical modifications to histone proteins.

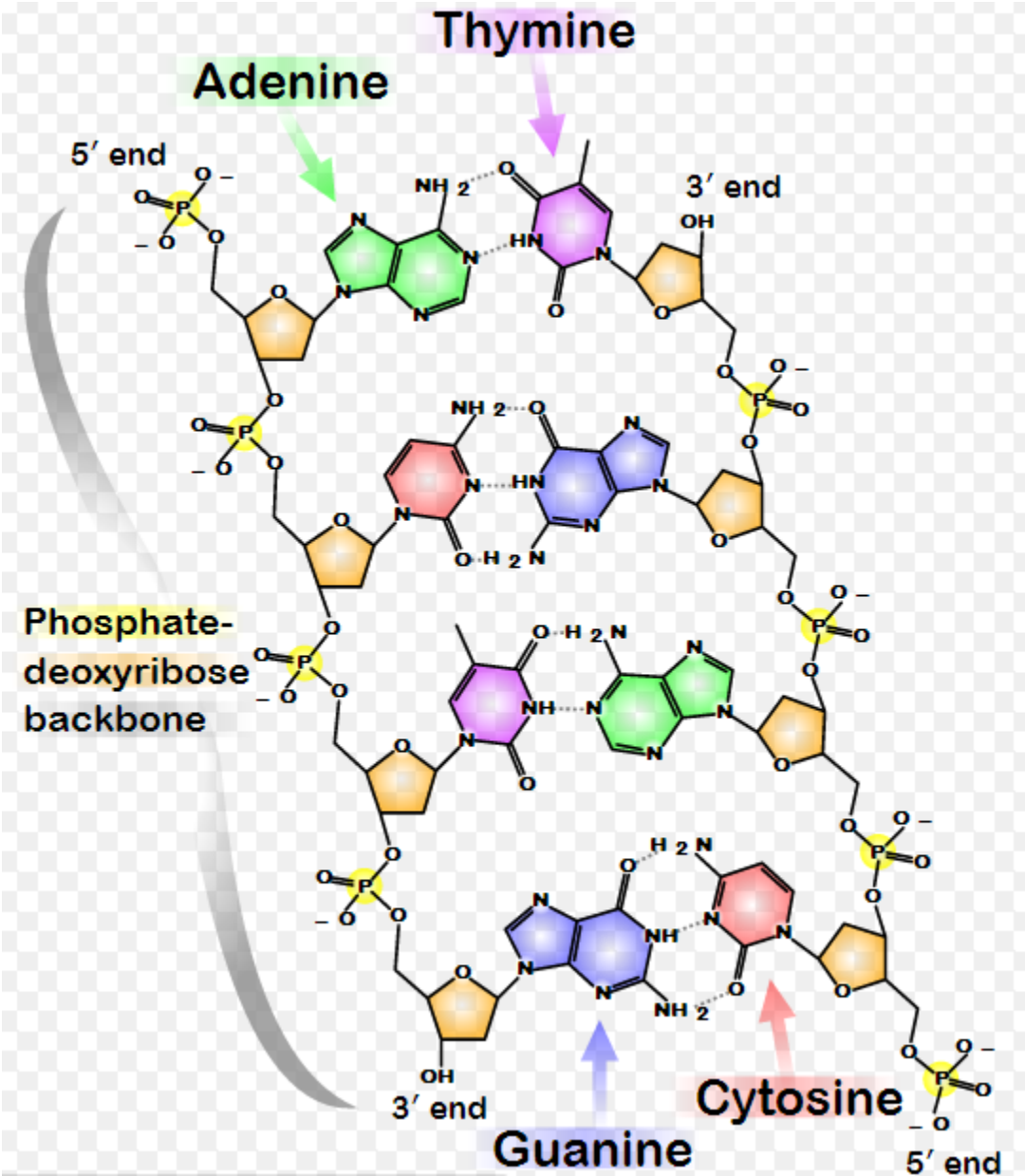
Mechanisms

- **Cis mechanism**→ altering the structure of chromtin

- **Trans mechanism**→ generating a banding platform for effector proteins

Cis mechanism





Modification	Modification Structure (R = chemical functional group)	Charge	Effect
Methylation	R-CH_3	Neutral	Increases packing
Acetylation	R-$COCH_3$	Negative	Decreases packing
Phosphorylation	R-PO_4	Negative	Decreases packing

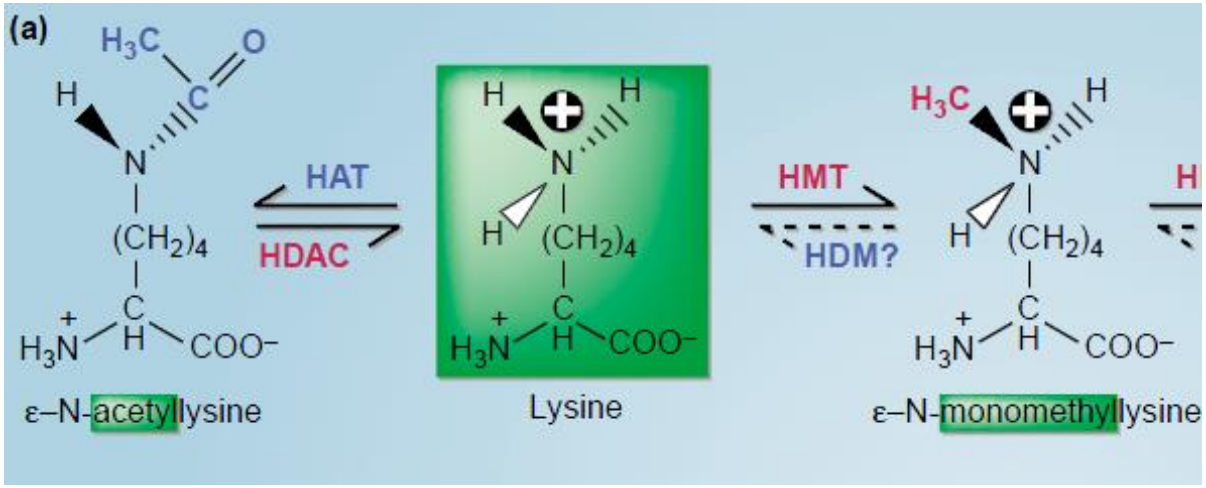


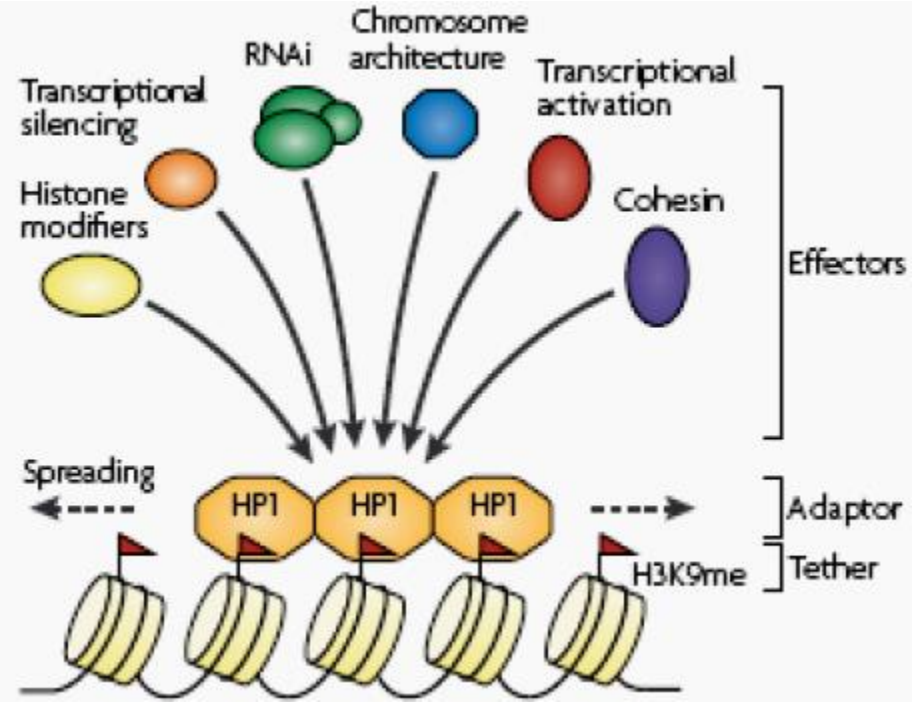
Table 1. Histone Modifications Associated with Transcription

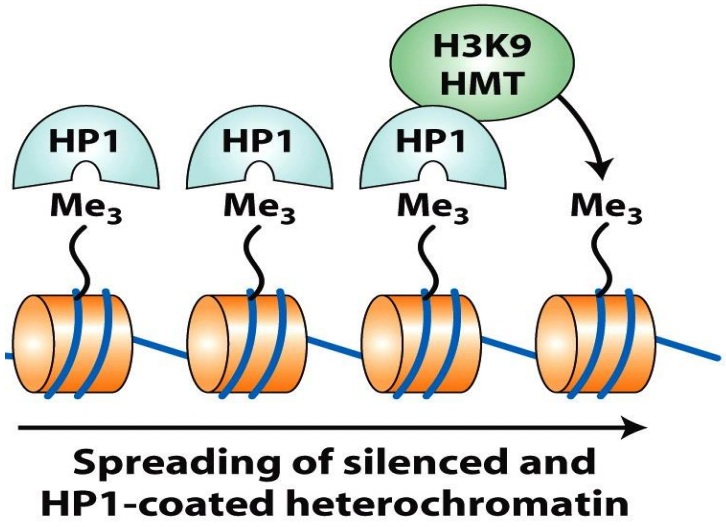
Modifications	Position		Enzymes				Recognition Module(s)[a]	Functions in Transcription
			S. cerevisiae	*S. pombe*	*Drosophila*	Mammals		
Methylation	H3	K4	Set1	Set1	Trx, Ash1	MLL, ALL-1, Set9/7, ALR-1/2, ALR, Set1	PHD, Chromo, WD-40	Activation
		K9	n/a	Clr4	Su(var)3-9, Ash1	Suv39h, G9a, Eu-HMTase I, ESET, SETBD1	Chromo (HP1)	Repression, activation
		K27				E(Z)	Ezh2, G9a	Repression
		K36	Set2			HYPB, Smyd2, NSD1	Chromo(Eaf3), JMJD	Recruiting the Rpd3S to repress internal initiation
		K79	Dot1			Dot1L	Tudor	Activation
	H4	K20		Set9	PR-Set7, Ash1	PR-Set7, SET8	Tudor	Silencing
Arg Methylation	H3	R2				CARM1		Activation
		R17				CARM1		Activation
		R26				CARM1		Activation
	H4	R3				PRMT1	(p300)	Activation
Phosphorylation	H3	S10	Snf1				(Gcn5)	Activation
Ubiquitination	H2B	K120/123	Rad6, Bre1	Rad6		UbcH6, RNF20/40	(COMPASS)	Activation
	H2A	K119				hPRC1L		Repression
Acetylation	H3	K56					(Swi/Snf)	Activation
	H4	K16	Sas2, NuA4		dMOF	hMOF	Bromodomain	Activation
	Htz1	K14	NuA4, SAGA					Activation

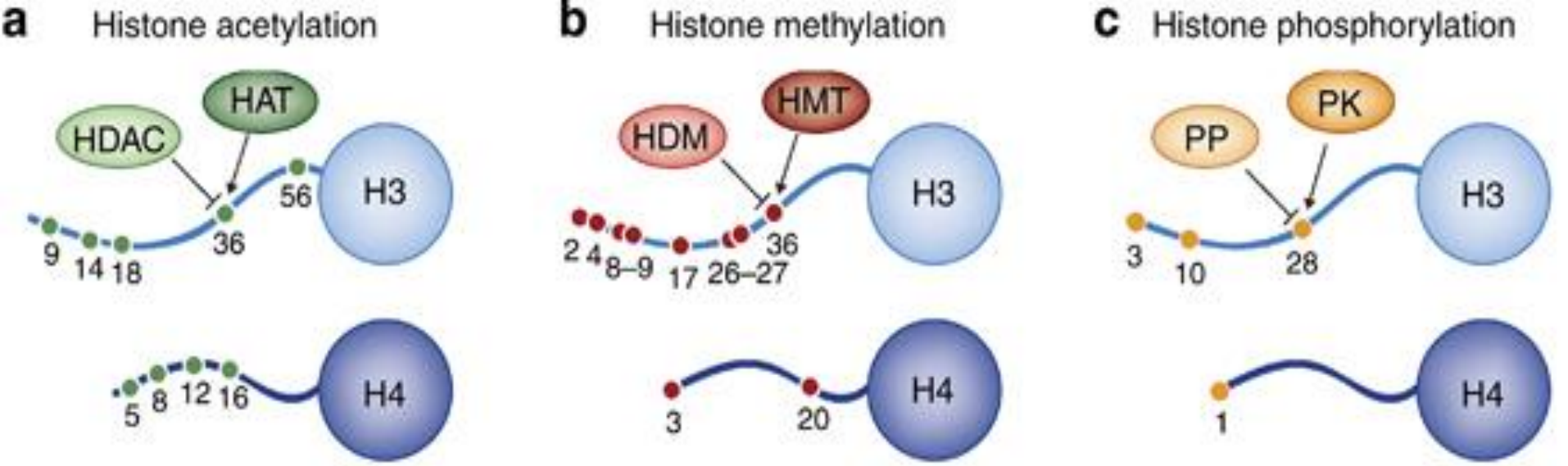
[a] The proteins that are indicated within the parentheses are shown to recognize the corresponding modifications but specific domains have yet to be determined.

Li e. al. (2007)
Cell 128, 707

Trans mechanism







Histone acetyltransferases
- CBP
- p300
- ATF-2
- Tip60

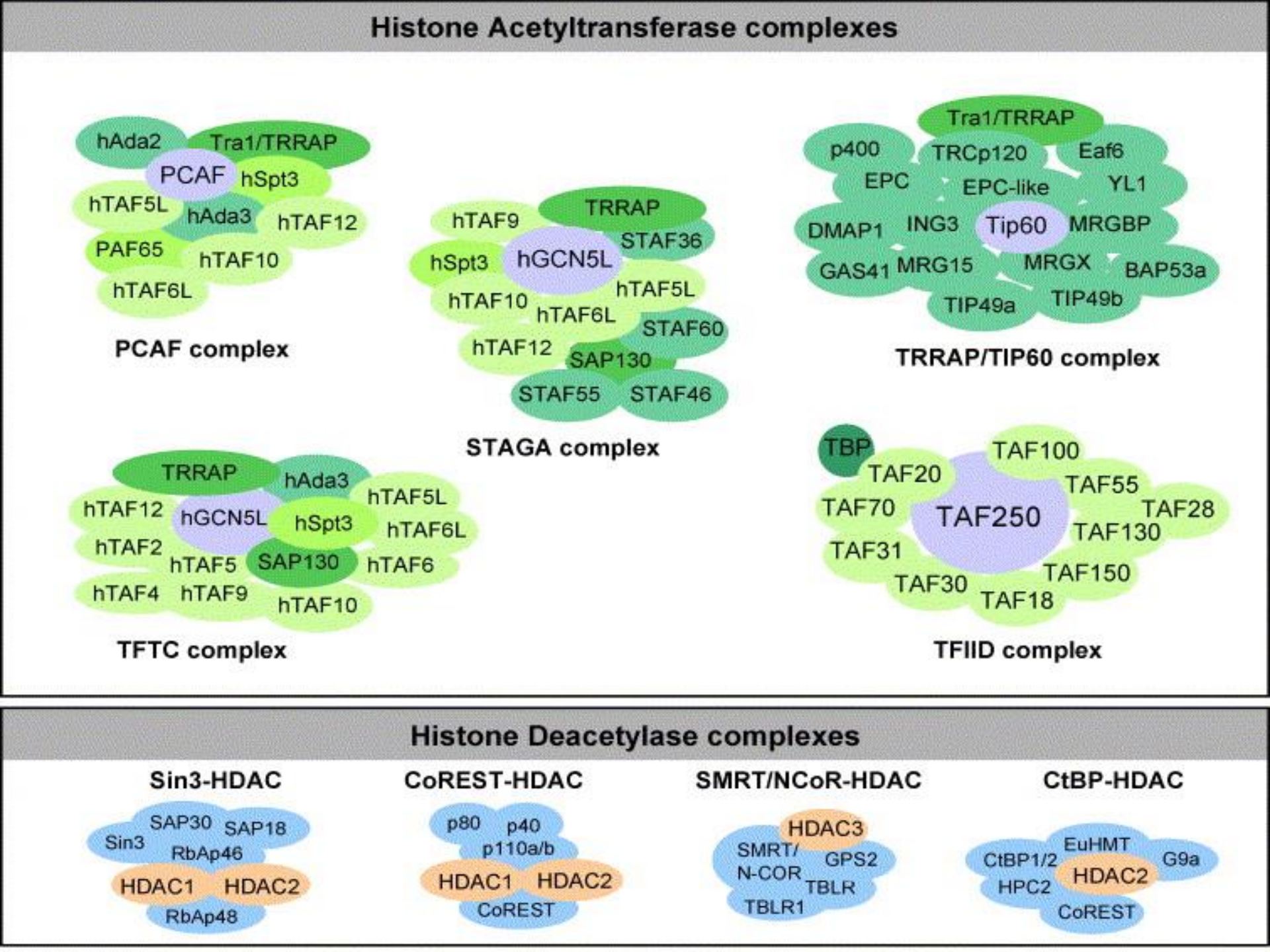
Histone deacetylases
- Class I HDACs
  - HDAC 1, 2, 3, 8
- Class II HDACs
  - HDAC 4, 5, 6, 7a, 9, 10
- Class III HDACs
  - Sirtuins (SIRT1-7)
- Class IV HDACs
  - HDAC 11

Histone methyltransferases
- MLL1
- SetD1a
- Set2
- SetD8
- G9a, GLP
- SUV39H1
- EZH2

Histone demethylases
- MJD2a
- JMJD3
- LSD1
- PHF8

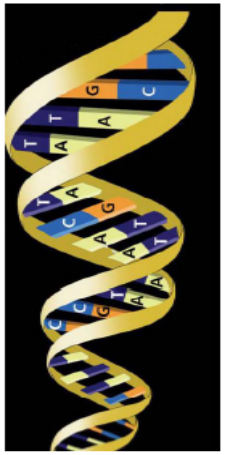
Protein kinases
- MSK1
- MSK2
- RSK
- Aurora B
- CKII
- IKKa

Protein phosphatases
- PP1
- PP2A

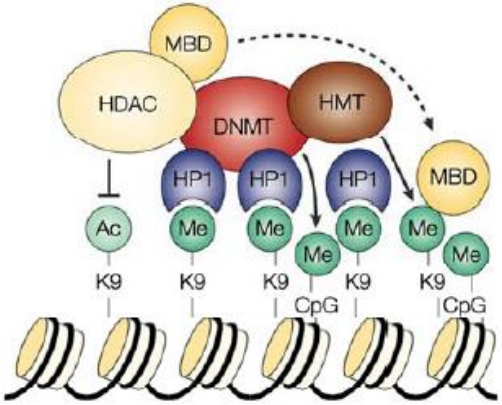

Histone Acetyltransferase complexes
hAda2
Tra1/TRRAP
PCAF
hSpt3
hTAF5L
hAda3
hTAF12
PAF65
hTAF10
hTAF6L
PCAF complex
TRRAP
hTAF9
STAF36
hSpt3
hGCN5L
hTAF5L
hTAF10
hTAF6L
STAF60
hTAF12
SAP130
STAF55
STAF46
STAGA complex
Tra1/TRRAP
p400
TRCp120
Eaf6
EPC
EPC-like
YL1
DMAP1
ING3
Tip60
MRGBP
GAS41
MRG15
MRGX
BAP53a
TIP49a
TIP49b
TRRAP/TIP60 complex
TRRAP
hAda3
hTAF12
hGCN5L
hSpt3
hTAF5L
hTAF6L
hTAF2
hTAF5
SAP130
hTAF6
hTAF4
hTAF9
hTAF10
TFTC complex
TBP
TAF100
TAF20
TAF55
TAF70
TAF250
TAF28
TAF130
TAF31
TAF150
TAF30
TAF18
TFIID complex
Histone Deacetylase complexes
Sin3-HDAC
SAP30
SAP18
Sin3
RbAp46
HDAC1
HDAC2
RbAp48
CoREST-HDAC
p80
p40
p110a/b
HDAC1
HDAC2
CoREST
SMRT/NCoR-HDAC
HDAC3
SMRT/
N-COR
GPS2
TBLR
TBLR1
CtBP-HDAC
EuHMT
CtBP1/2
G9a
HDAC2
HPC2
CoREST

Genetics: the alphabet of life

- **Letters of DNA sequence carry the information**



Epigenetics: the grammar of life



Environmental Factors

- Nutrition, Behavior, Pollution, Sun light, Toxins, Circadian rhythms, Viruses, Bacteria

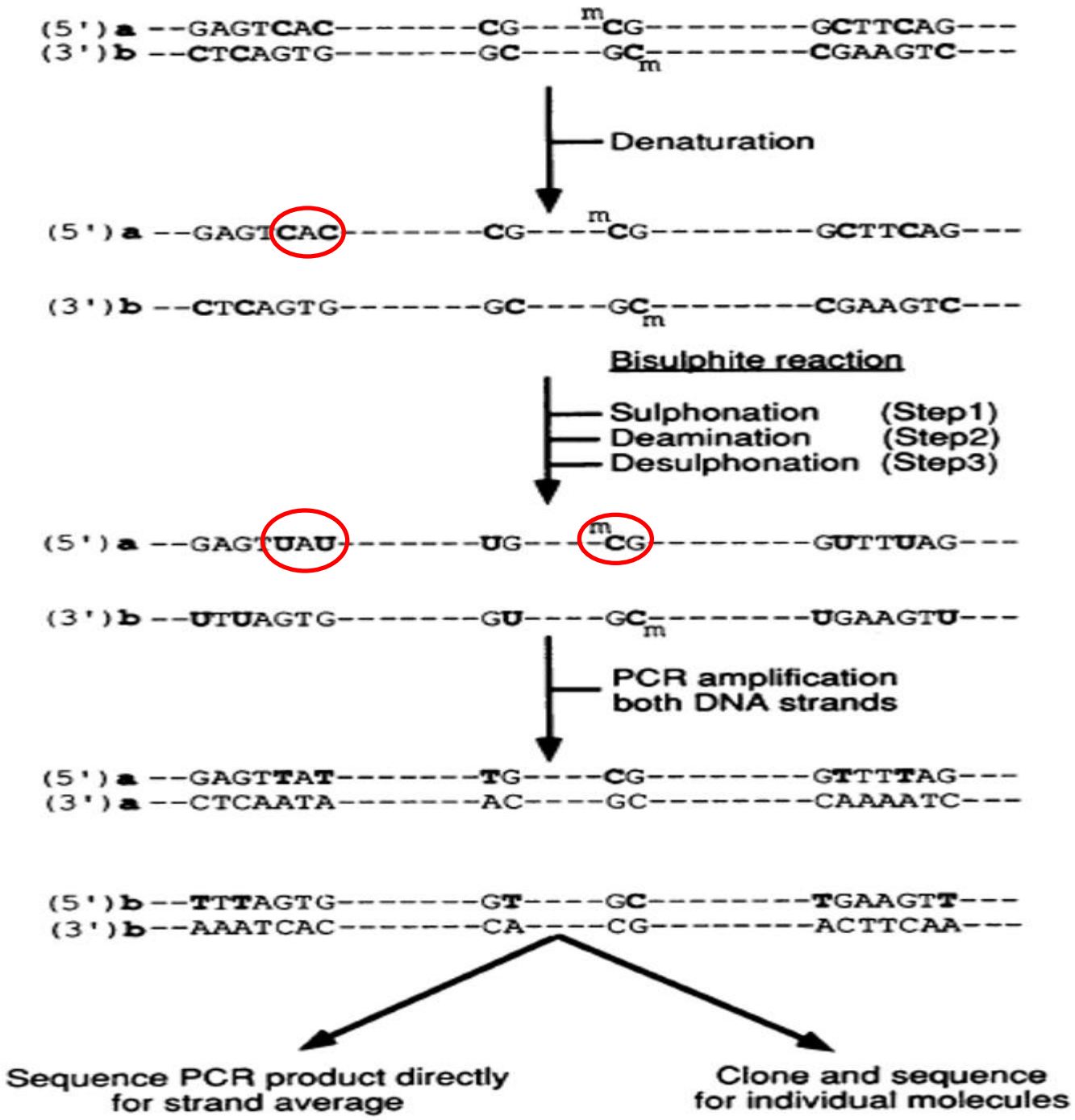
Epigenomics

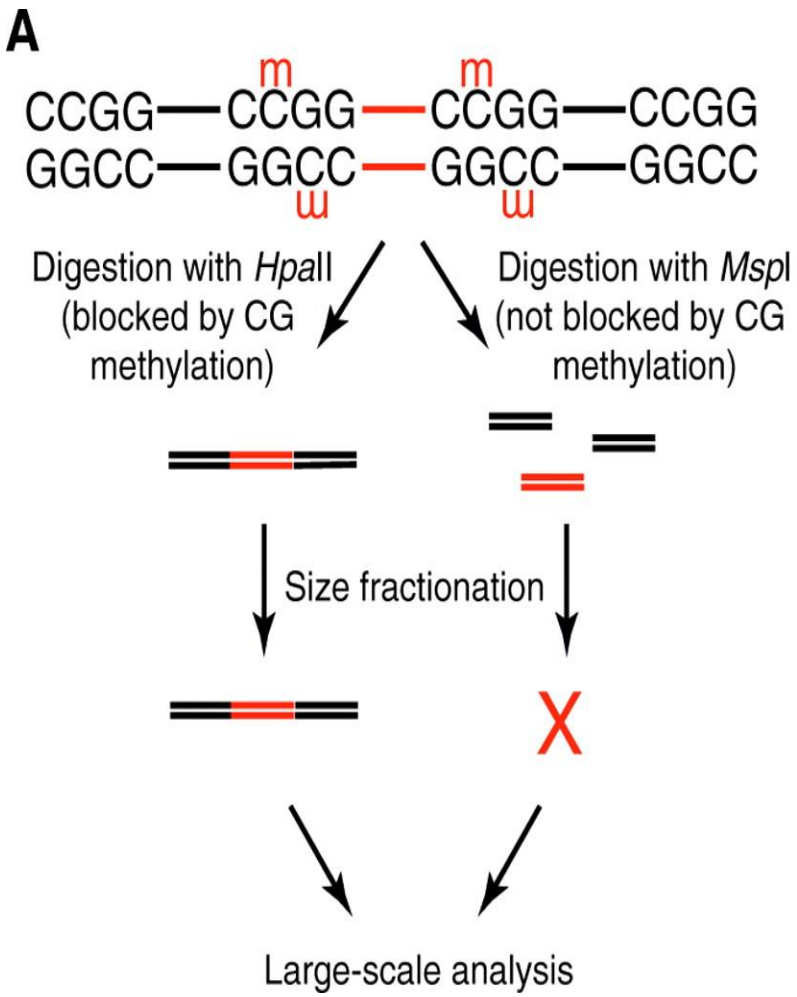
- **Epigenome** → complete set of epigenetic modifications on the genetic material of a cell

- **Epigenomics** is the study of the epigenome

How does one study DNA methylation ?

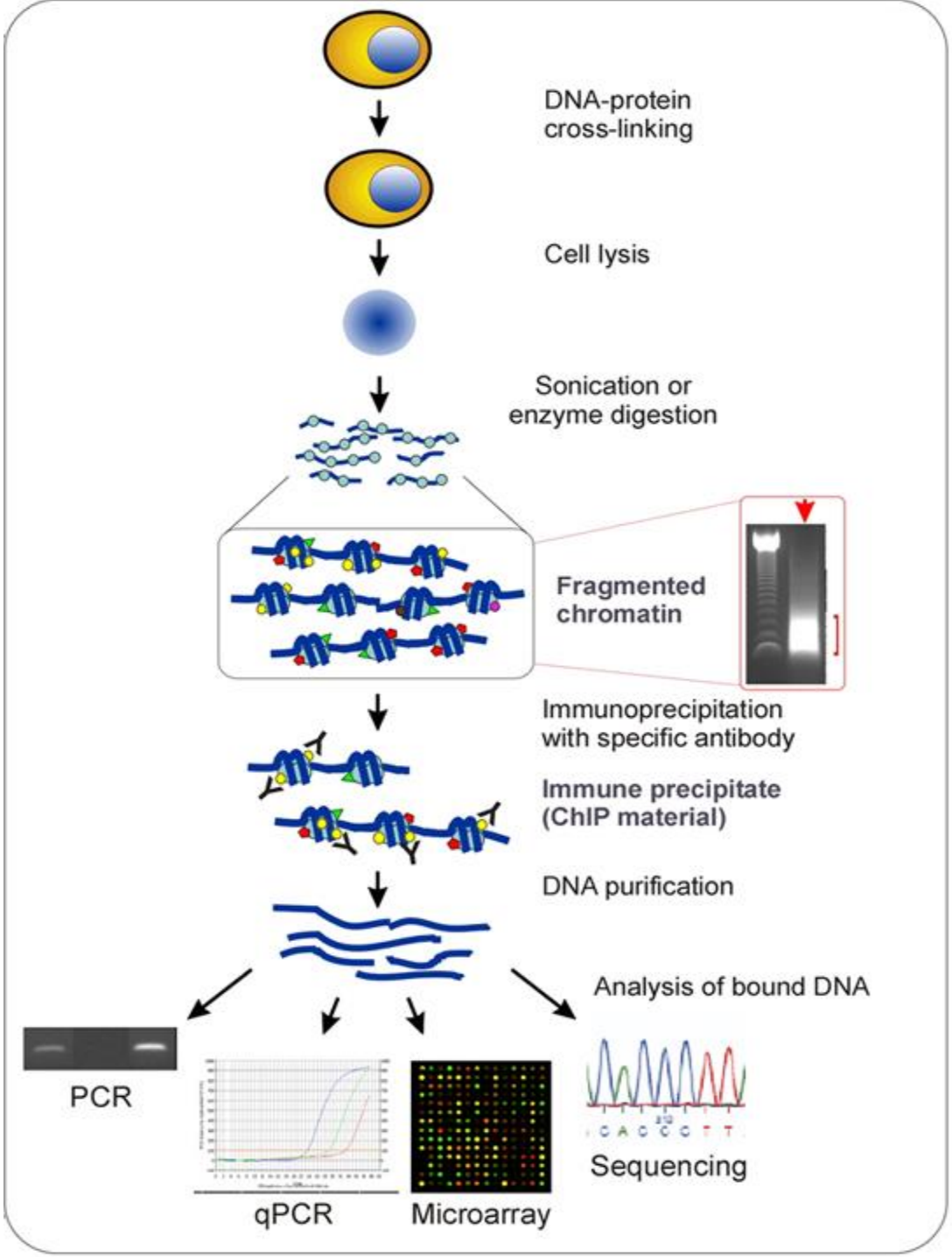
- Bisulphite sequencing
- Methylation-specific restriction endonucleases
  - e.g., HpaII / MspI → $C^{5m}CGG$


(5')a --GAGTCAC--------CG----mCG---------GCTTCAG---
(3')b --CTCAGTG--------GC----GCm---------CGAAGTC---
Denaturation
(5')a --GAGTCAC--------CG----mCG---------GCTTCAG---
(3')b --CTCAGTG--------GC----GCm---------CGAAGTC---
Bisulphite reaction
Sulphonation (Step1)
Deamination (Step2)
Desulphonation (Step3)
(5')a --GAGTTAT--------TG----mCG---------GTTTTAG---
(3')b --TTTAGTG--------GT----GC---------TGAAGTT---
PCR amplification
both DNA strands
(5')a --GAGTTAT--------TG----CG---------GTTTTAG---
(3')a --CTCAATA--------AC----GC---------CAAAATC---
(5')b --TTTAGTG--------GT----GC---------TGAAGTT---
(3')b --AAATCAC--------CA----CG---------ACTTCAA---
Sequence PCR product directly for strand average
Clone and sequence for individual molecules


A
m
m
CCGG—CCGG—CCGG—CCGG
GGCC—GGCC—GGCC—GGCC
w
w
Digestion with HpaII (blocked by CG methylation)
Digestion with MspI (not blocked by CG methylation)
Size fractionation
X
Large-scale analysis

How does one detect histone modifications ?

- Chromatin Immunoprecipitation (ChIP)
  - ChIP-on-chip
  - ChIP-Seq


DNA-protein
cross-linking
Cell lysis
Sonication or
enzyme digestion
Fragmented
chromatin
Immunoprecipitation
with specific antibody
Immune precipitate
(ChIP material)
DNA purification
Analysis of bound DNA
PCR
qPCR
Microarray
Sequencing

Importance of epigenetic modifications studies

- **Embryonic development**
  - Stem cells
  - Differentiation
  - Reprogramming
- **Medicine**
  - Cancer and developmental abnormalities
  - Personalized therapeutic
- **Evolution**