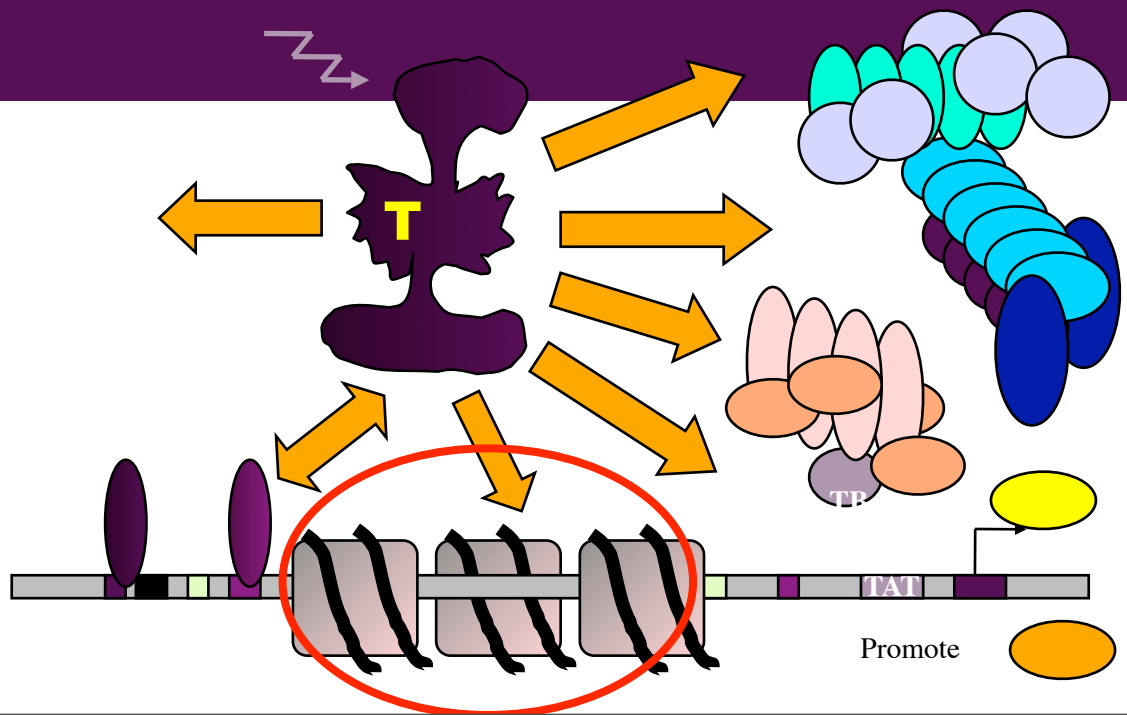
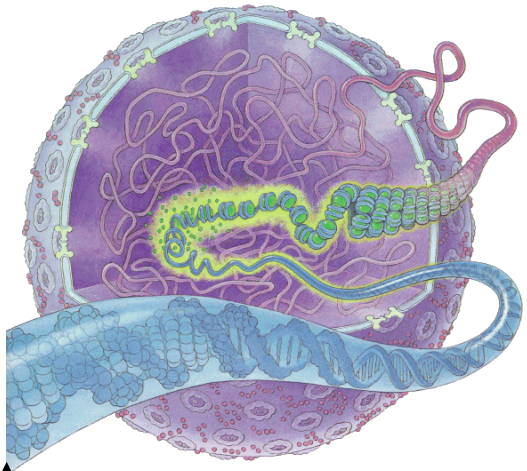
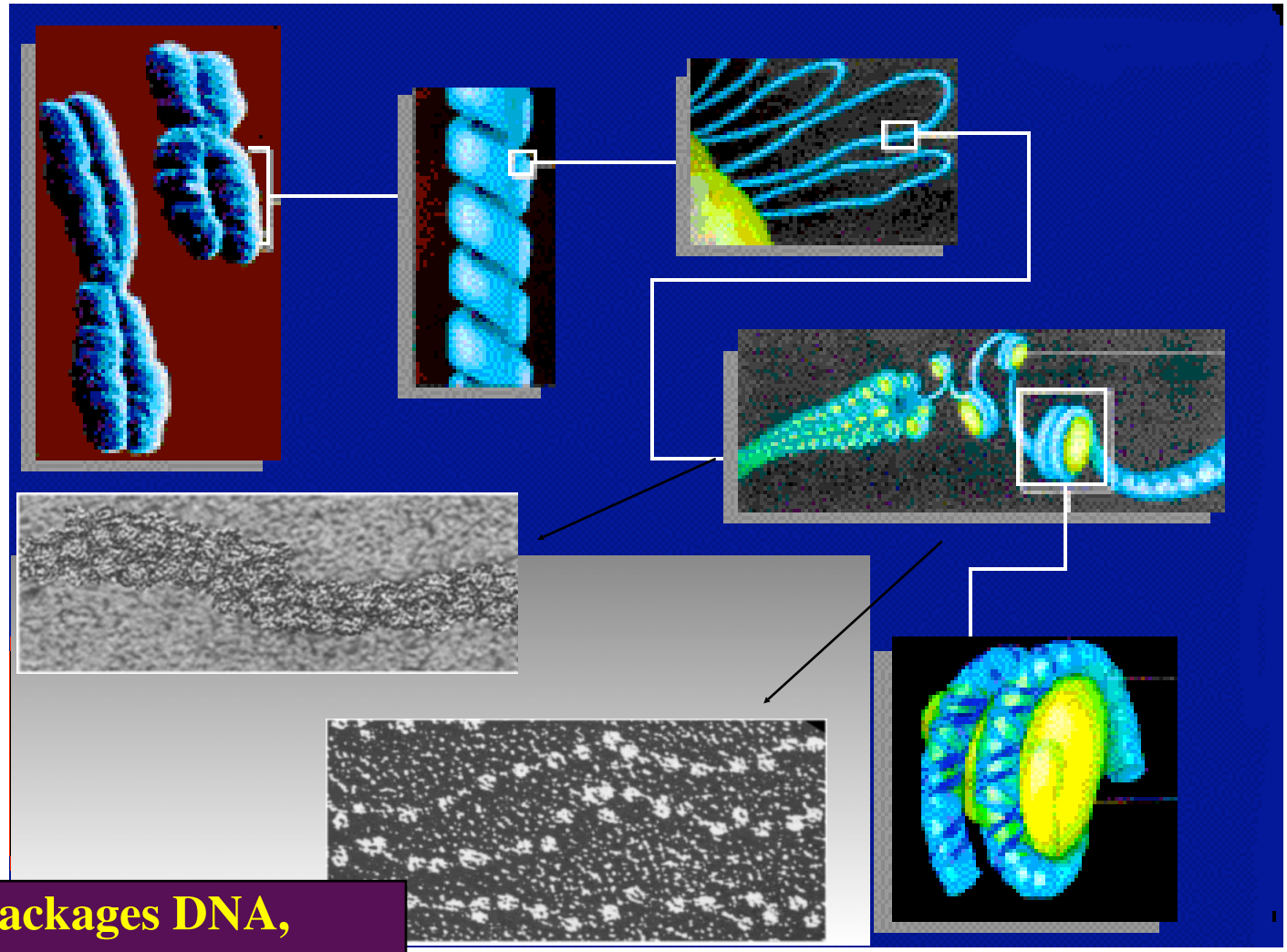
Chromatin
- essential player in trx



Packing of DNA into chromatin



2 m of DNA in a nucleus with a diameter of 5-10 μm

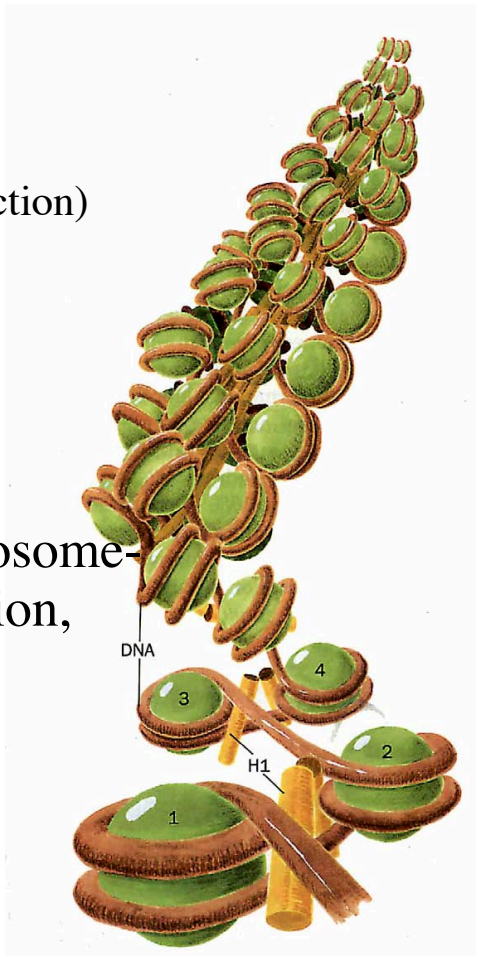


Chromatin not only packages DNA, but also regulates DNA accessibility through modifications in chromatin structure.

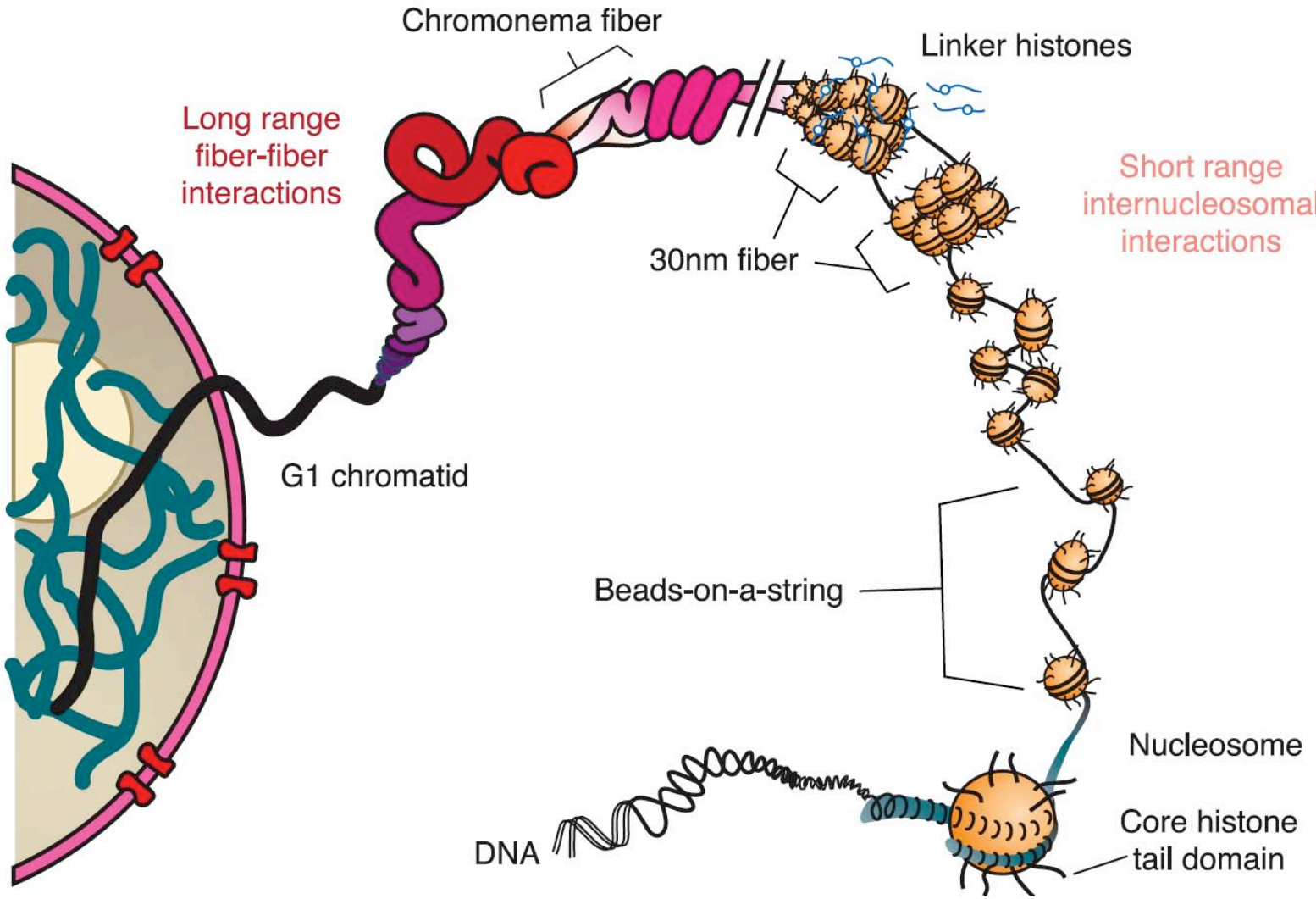
Histones and Nucleosomes

Chromatin-structure - reminder

- **Chromatin - a nucleoprotein complex**
  - Causes condensation and organization of DNA
    - protein:DNA = 2:1 mass ratio
    - histone:DNA = 1:1 mass ratio
- **Several levels of condensation**
    - 10 nm extended fiber with nucleosomes like beads-on-a-string (7x compaction)
    - 30 nm condensed fiber (40-50x compaction)
    - And higher
- **Structural units**
  - strings of nucleosomes compose the primary structural unit.
  - Formation of 30-nm fibers through histone tail–mediated nucleosome-nucleosome interactions provides a secondary level of compaction,
  - tail-mediated association of individual fibers produces tertiary structures (such as chromonema fibers).

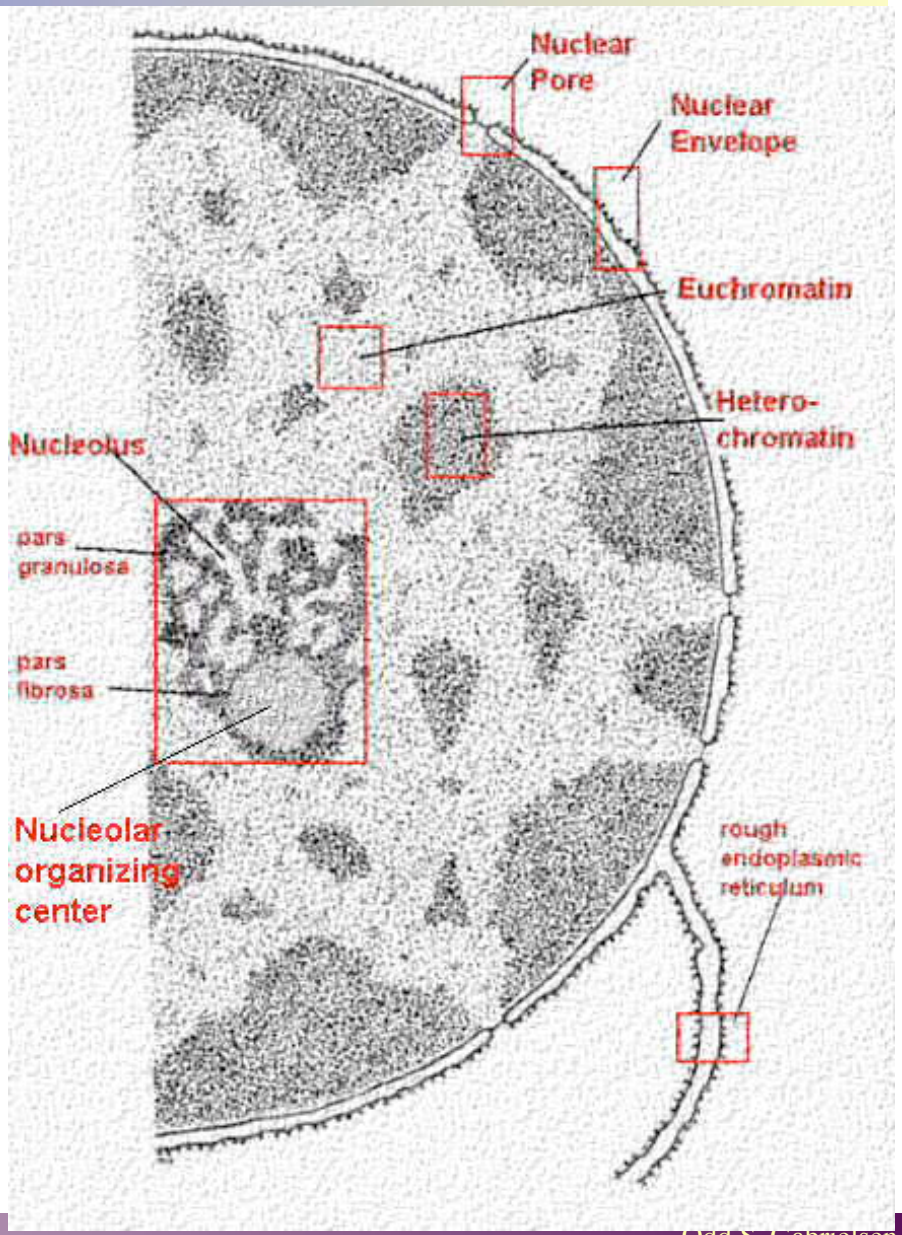


Multiple levels of chromatin folding



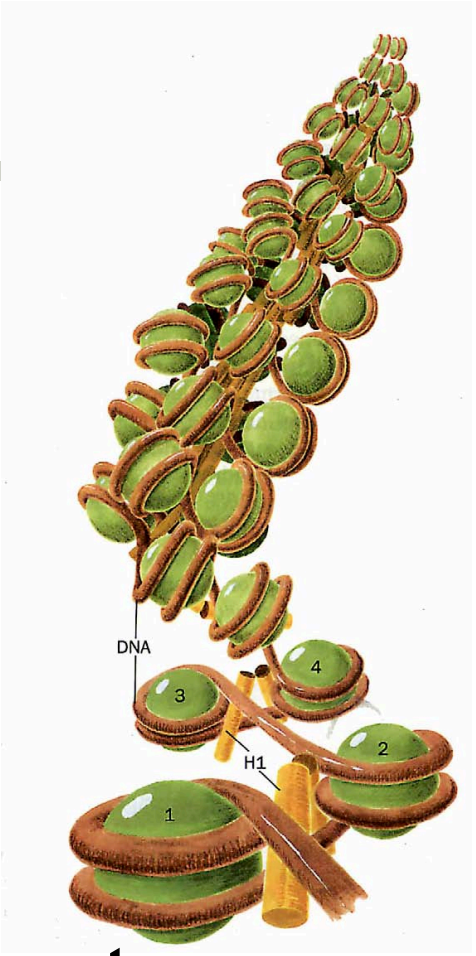
Chromatin compaction influences activity of DNA in transcription

- Heterochromatin - transcriptionally silent
- Euchromatin - transcriptionally active



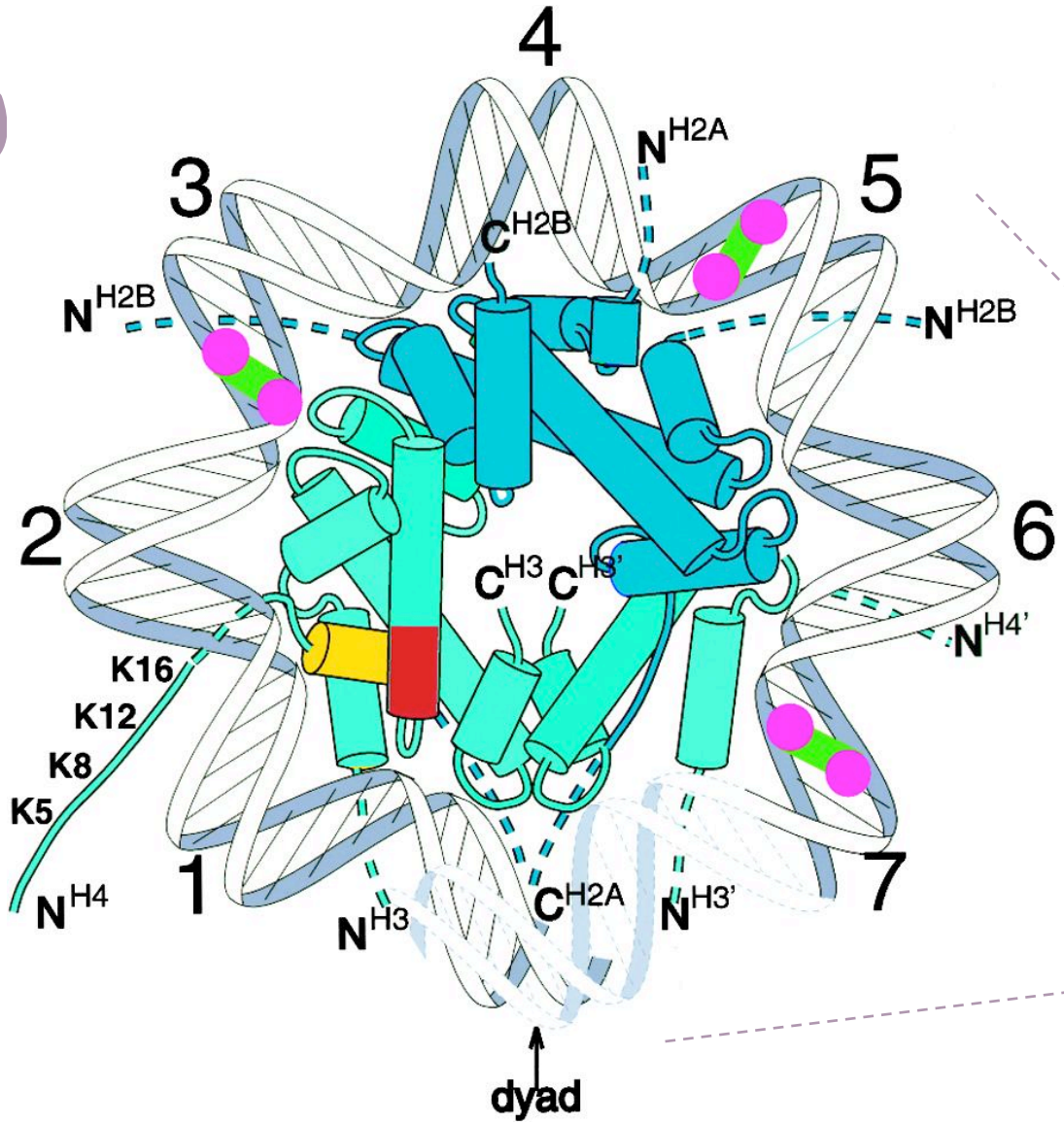
The nucleosome - repeat unit of chromatin

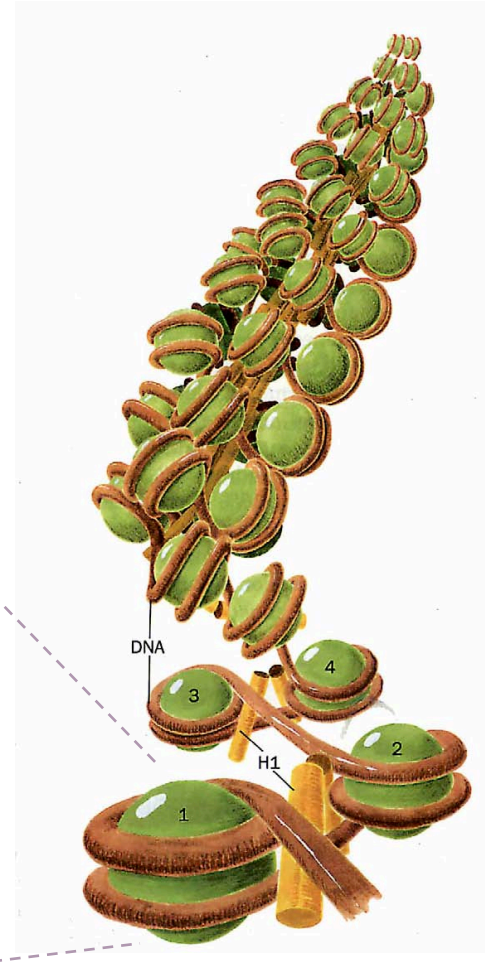
- **Nucleosomes**
  - repeat unit in chromatin
    - Repeat length: 180-210 bp
  - Nuclease digestions
    - Trace of nuclease: **oligo-nucleosomes**
    - More nuclease: **chromatosome** with/ 166bp DNA + octamer + H1
    - Even more nuclease: **core particle** 147 bp DNA + octamer
  - DNA lefthanded superhelix ≈ 2 turns around
    - DNA bended and kinked



- **DNA wrapped 1.65 turns around the histone octamer as a left-handed superhelix**

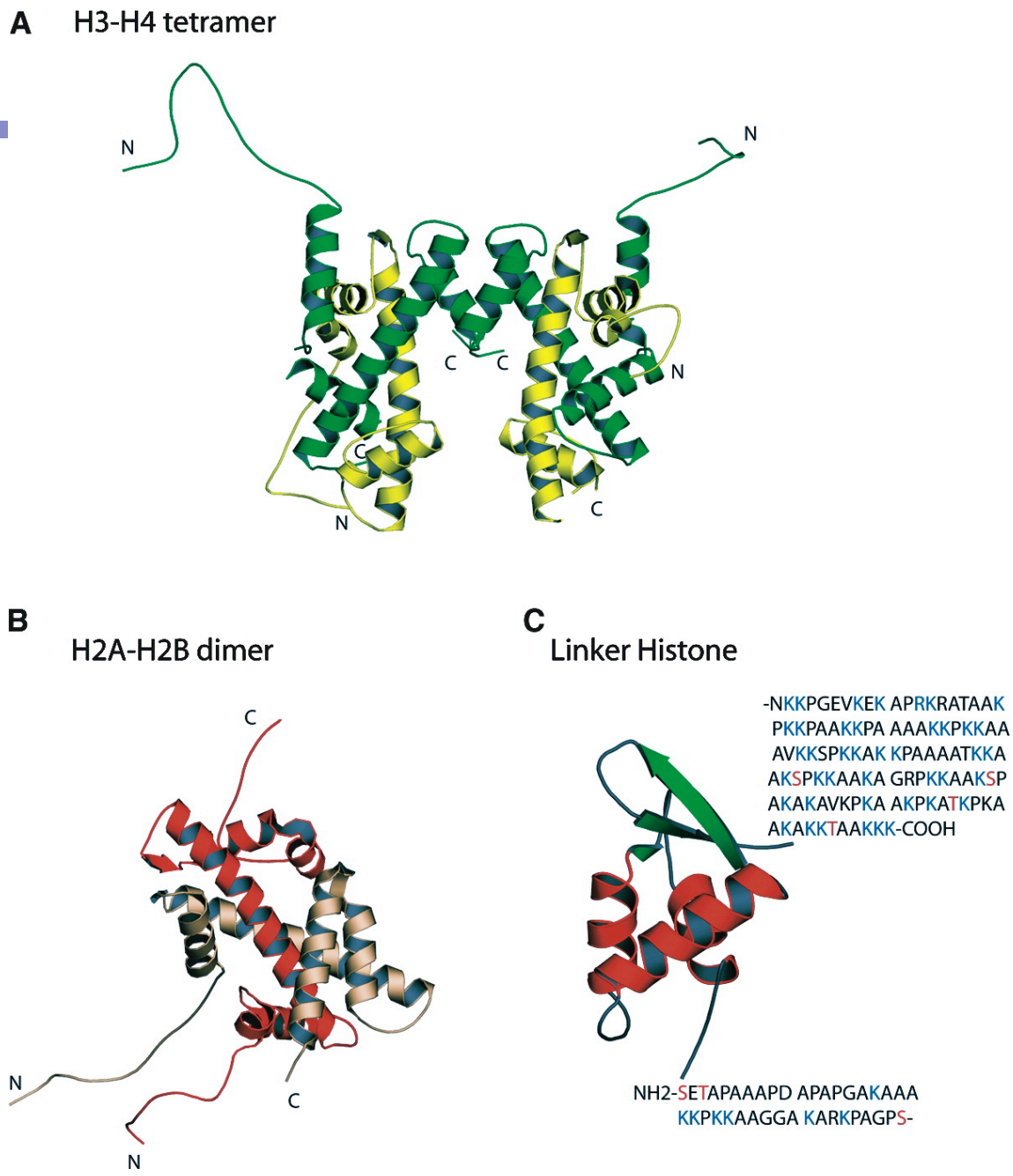
The nucleosome - repeat unit of chromatin





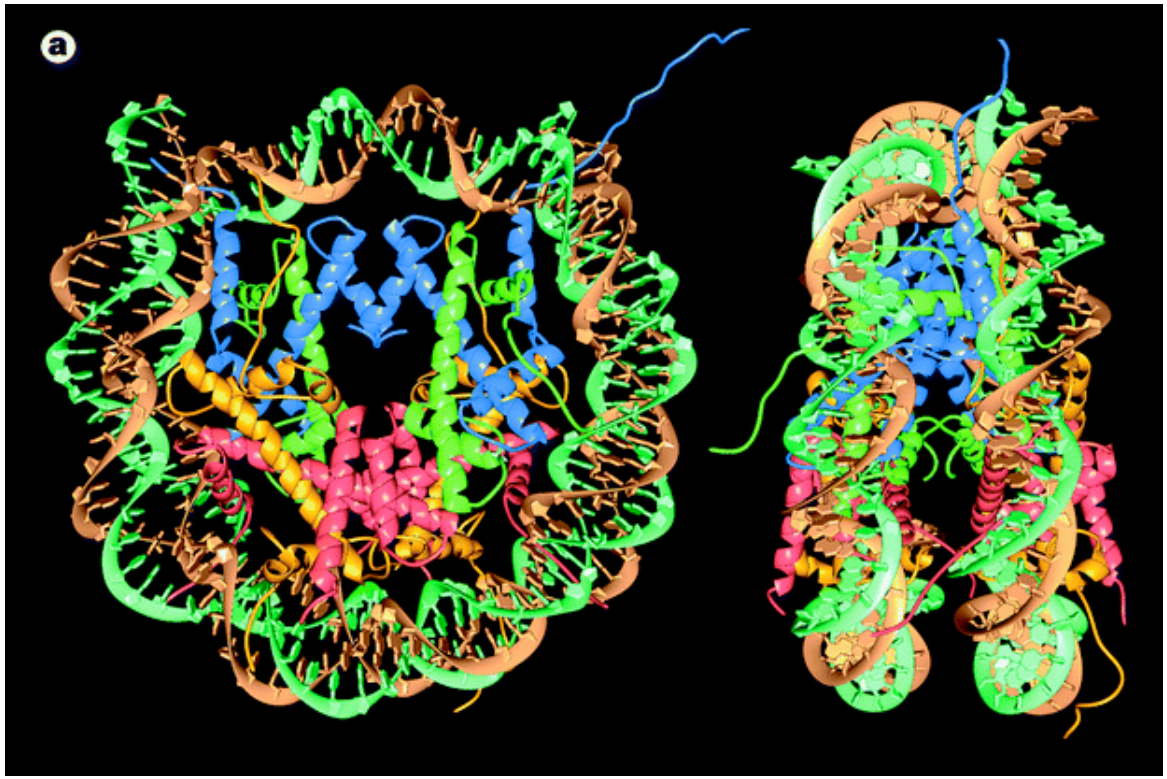
The histones

- **The histone fold**
  - Three-helix core domain
  - Forms a handshake-arrangement
- **Tails**
  - Disordered N-terminal and or C-terminal tails that protrude from the nucleosome (through the minor-groove channels)
  - Ideal located for covalent modifications
- **Octamer = (H2A, H2B, H3 and H4)$_2$**
  - Assembly: H3-H4 tetramer → +2 H2A-H2B dimers



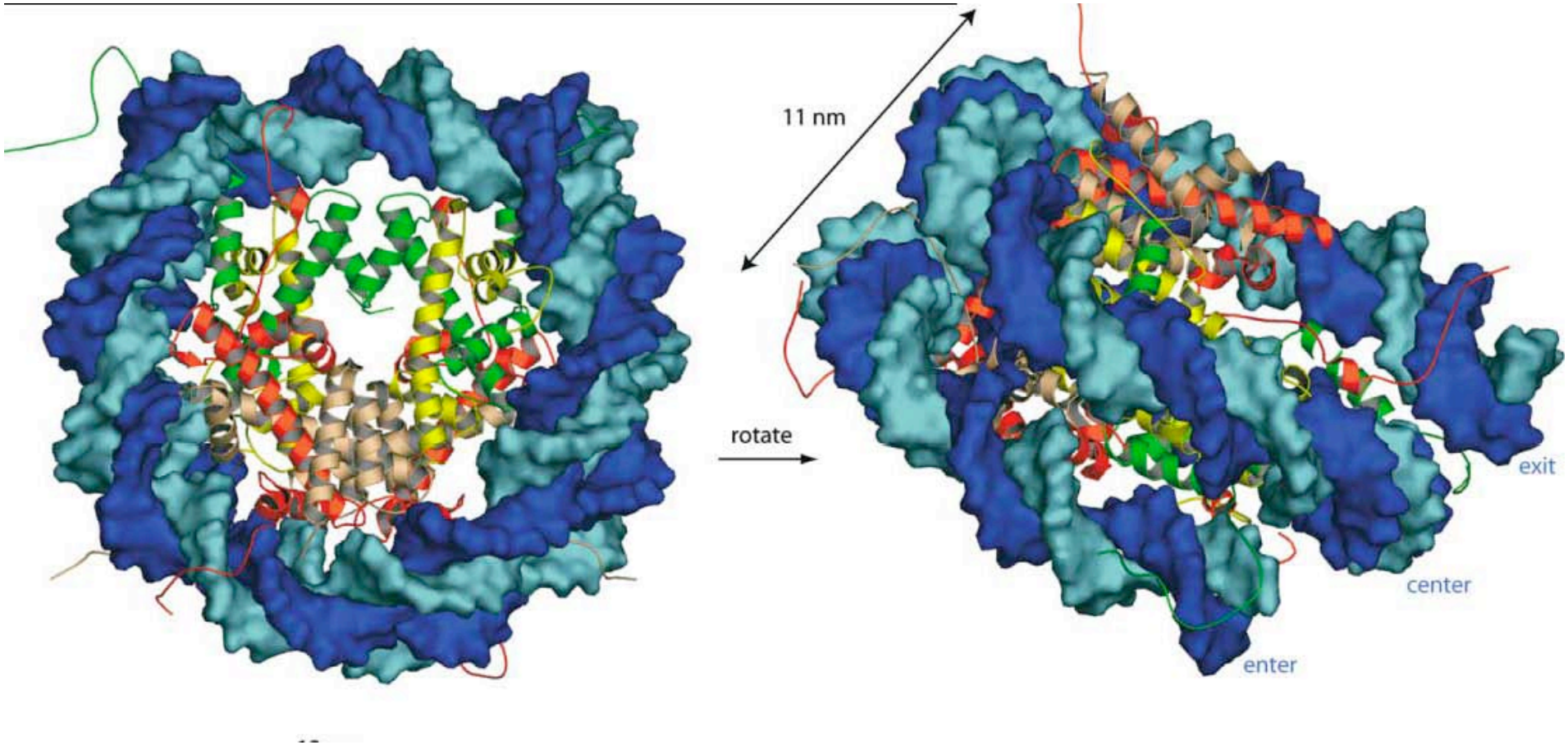
Nucleosome 3D structure

- **Crytals structure of the nucleosome core particle**
  - **low resolution 1984, high resolution 1997**
  - 2.8 Å resolution.
  - 146 bp of DNA wrapped around a histone octamer core
  - Note outside position of histone tails
- **DNA is wrapped around**
  - DNA wrapped 1.65 turns around the histone octamer as a left-handed superhelix
  - 14 contact points between histones and DNA, making the nucleosome one of the most stable protein-DNA complexes under physiological conditions



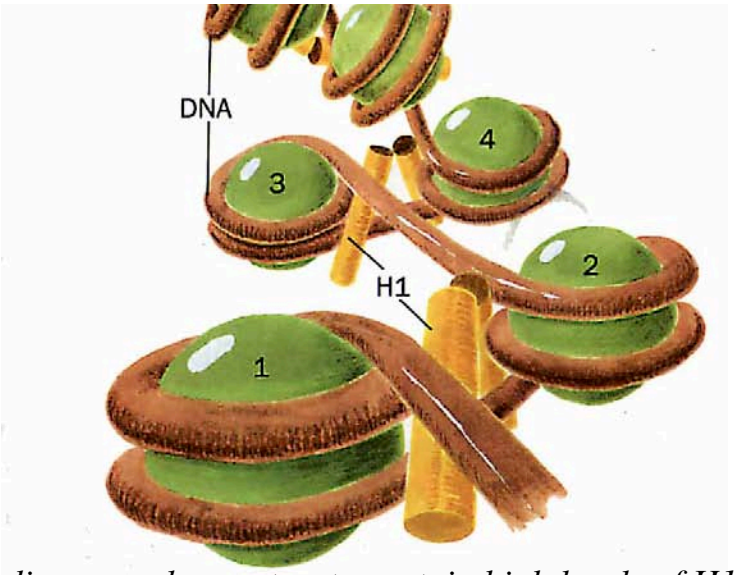
Luger et al., 1997.

Nucleosome 3D structure

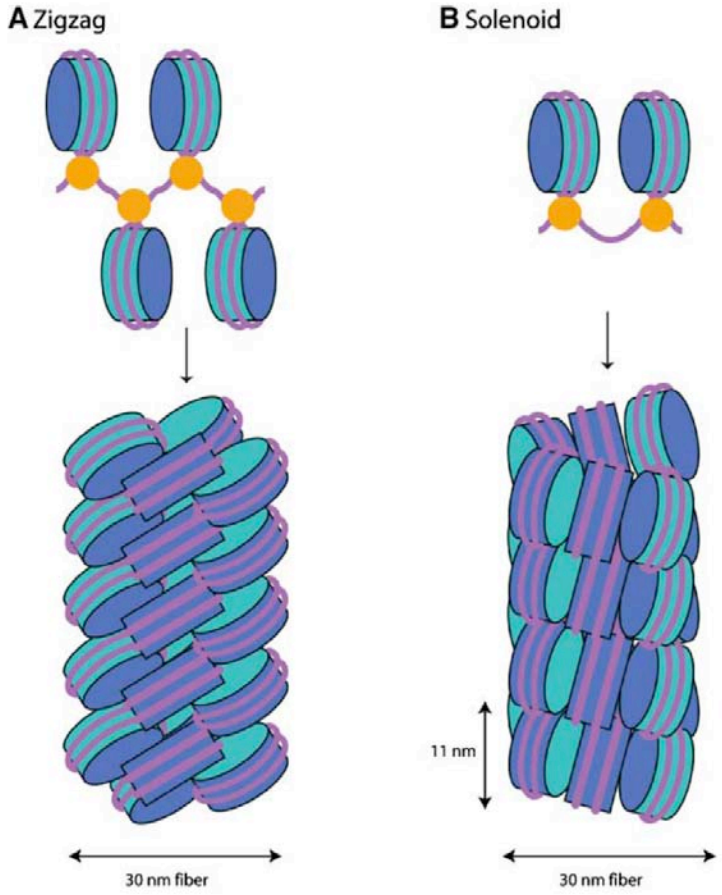


Histone H1 - linker histone

- **H1 linker histone**
  - associated with linker DNA between nucleosomes (about one H1 per nucleosome)
    - Binds DNA at entry/exit
    - stimulates folding 10 nm ➜ 30 nm fiber
    - repressive effect on transcription
  - H1 binds weaker to acetylated nucleosomes



  - *Ordinary nuclear extracts contain high levels of H1*

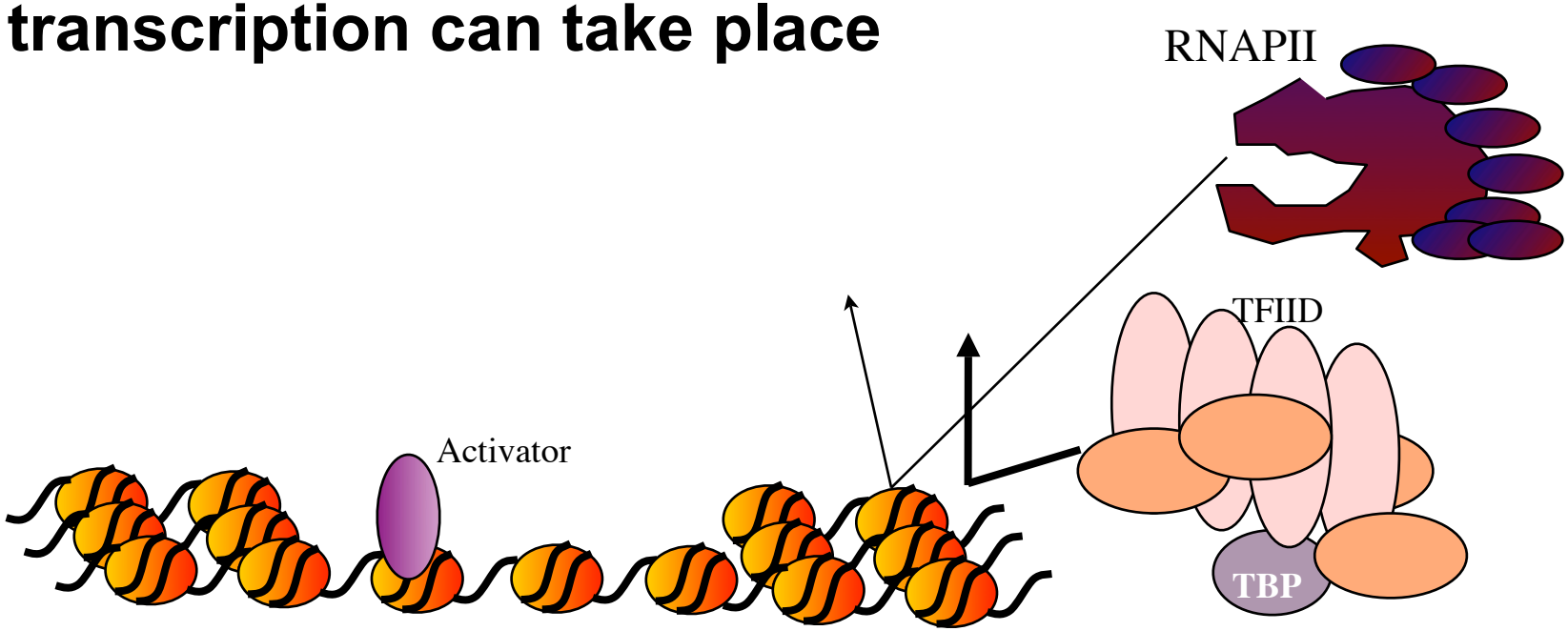


Chromatin - a general repressor of transcription

- unwrapping needed

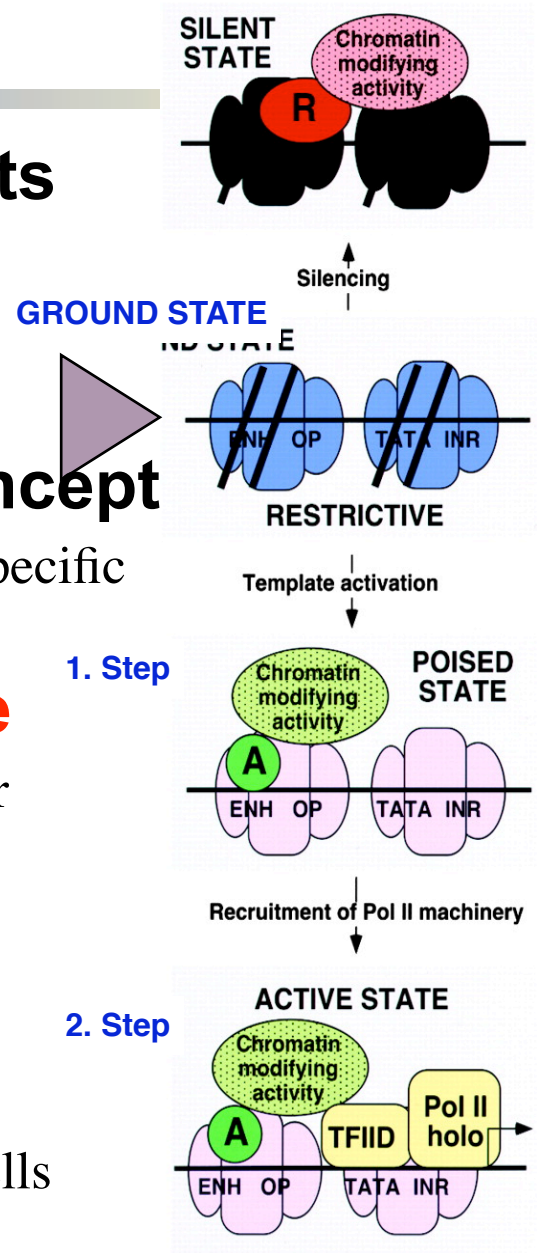
Chromatin restricts transcription

- Chromatin - a general inhibitor of protein access to DNA
- Packed chromatin will block access of basic pol II machinery to core promoters
- Chromatin needs to be unpacked, unwrapped or opened before transcription can take place



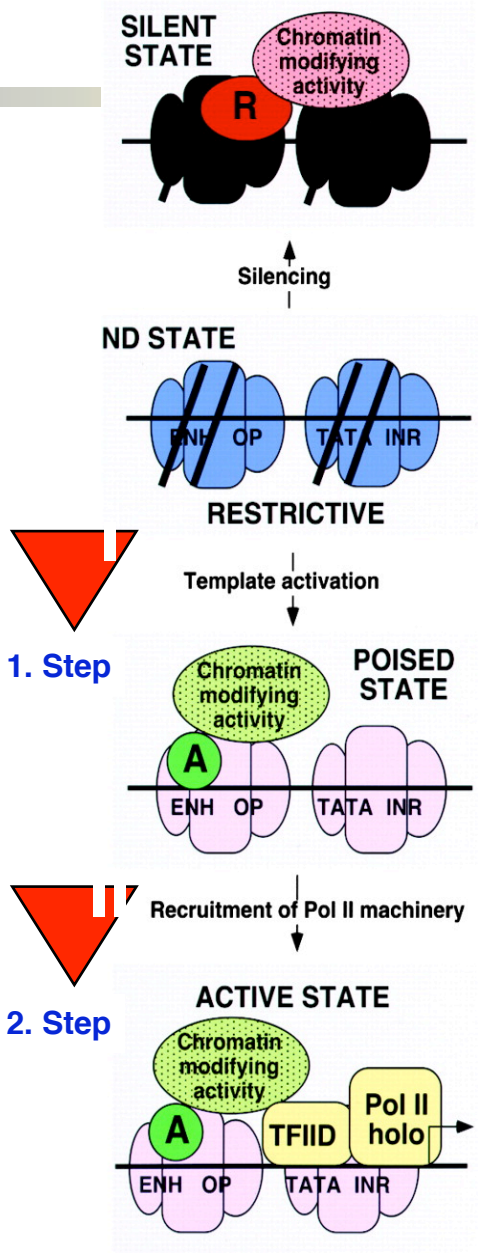
Different logic of gene regulation
- prokaryotes versus eukaryotes

- **Prokaryotic gene regulation - three elements**
  - Promoters recognized by RNA polymerase
  - Operators recognized by repressors
  - Positive control elements recognized by activators
- **"Transcriptional ground state" a useful concept**
  - The inherent activity of promoters in vivo in the absence of specific regulatory sequences (and hence activators and repressors).
- **Prokaryotes: ground state = non-restrictive**
  - No inherent restriction on RNA pol to gain access to promoter
  - Repressors block access
  - Activators required only on some inherently weak promoters
- **Eukaryotes: ground state = restrictive**
  - due to chromatin template
  - A strong core promoter is essentially inactive in eukaryotic cells
  - Essentially all eukaryotic genes require activators



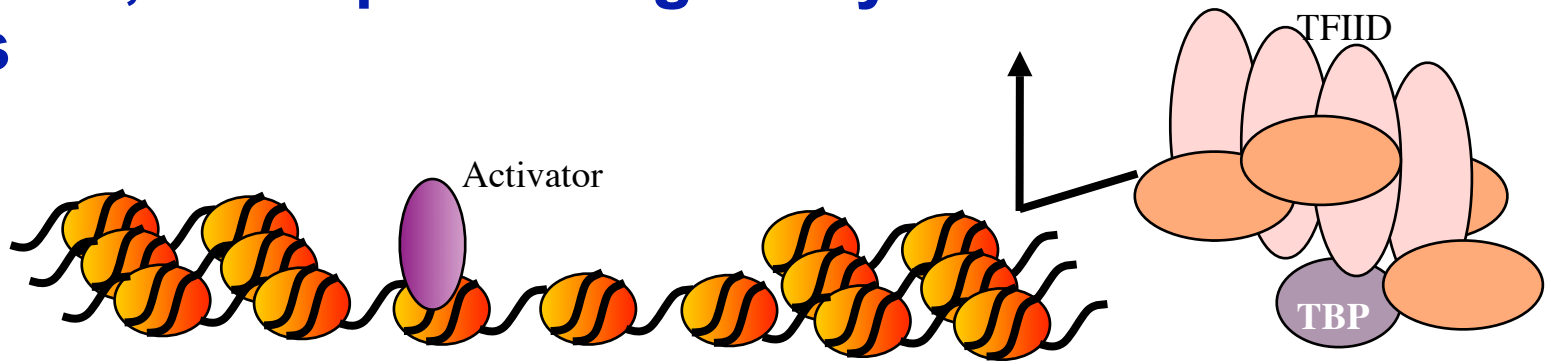
Two steps to activate a gene

- 1. Activation step = **Anti-repression** of inhibitory chromatin structure allowing recruitment of the pol II machinery
- 2. Activation step = **Recruitment** by the activator of components of the pol II machinery



Chromatin maintains the transcriptionally OFF state

- **Chromatin - a general inhibitor of protein access to DNA, but with interesting differences:**
- **TBP unable to bind nucleosomal DNA**
  - Nucleosomes prevent binding of TBP to TATA-elements in vitro
  - TBP does not associate with most core promoters in vivo in the absence of activators
- **Many activators bind their targets in nucleosomal DNA**
- **Chromatin maintains the restrictive ground state by blocking access of basic pol II machinery to core promoters, while permitting many activators to bind their targets**

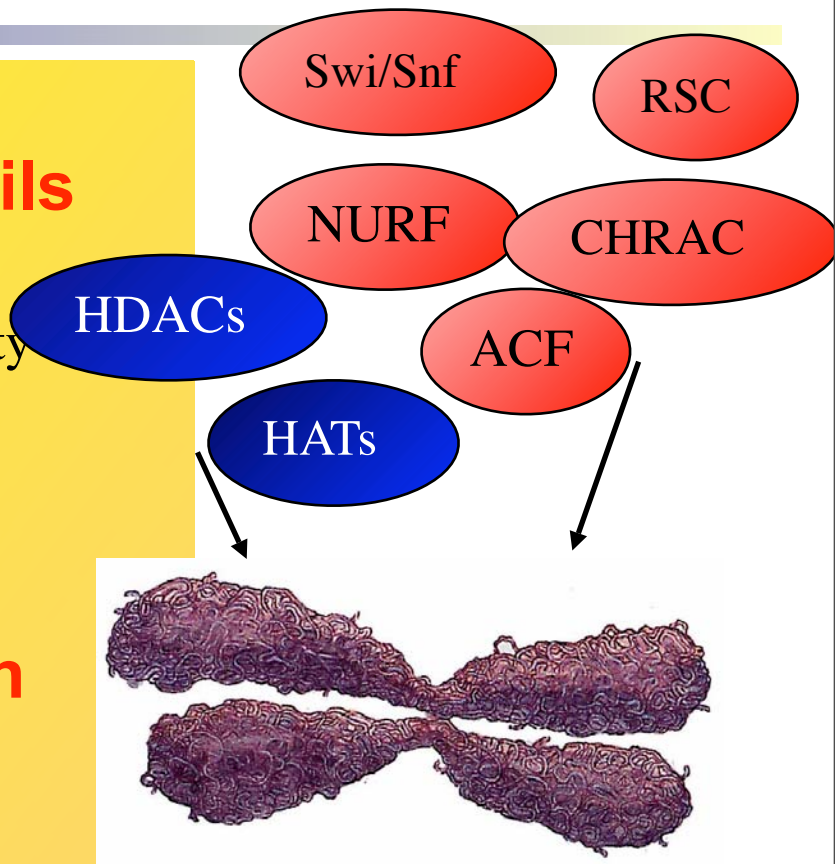


Changing chromatin to allow transcription

Opening and closing the chromatin template

Changing the chromatin-template to help transcription factors

- **1. Opening of chromatin through directed modication of histone tails (acetylation and methylation)**
  - Histone-modification by HAT and HMT activity
  - Basis for the histone code hypothesis
- **2. Opening of chromatin through directed nucleosome mobilization**
  - ATP-dependent process
- **3. Positioning of nucleosomes creates promoters with different requirement for remodeling**



Histone modifications

Histones are among the most conserved proteins known in evolution, but..
…are also among the most variable in post-translational modification.

Nomenclature:
H3K4me3
histone H3, lysine no 4, tri-methylated
H4K16Ac

Table 1. Different Classes of Modifications Identified on Histones

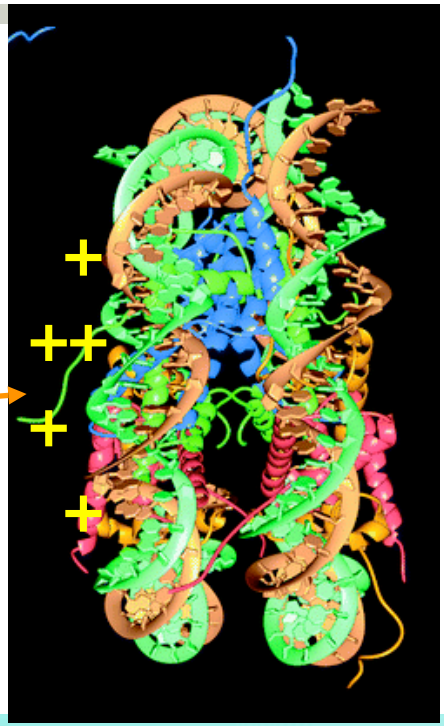
Chromatin Modifications	Residues Modified	Functions Regulated
Acetylation	**K**-ac	Transcription, Repair, Replication, Condensation
Methylation (lysines)	**K**-me1 **K**-me2 **K**-me3	Transcription, Repair
Methylation (arginines)	**R**-me1 **R**-me2a **R**-me2s	Transcription
Phosphorylation	**S**-ph **T**-ph	Transcription, Repair, Condensation
Ubiquitylation	**K**-ub	Transcription, Repair
Sumoylation	**K**-su	Transcription
ADP ribosylation	**E**-ar	Transcription
Deimination	**R > Cit**	Transcription
Proline Isomerization	**P-cis > P-trans**	Transcription

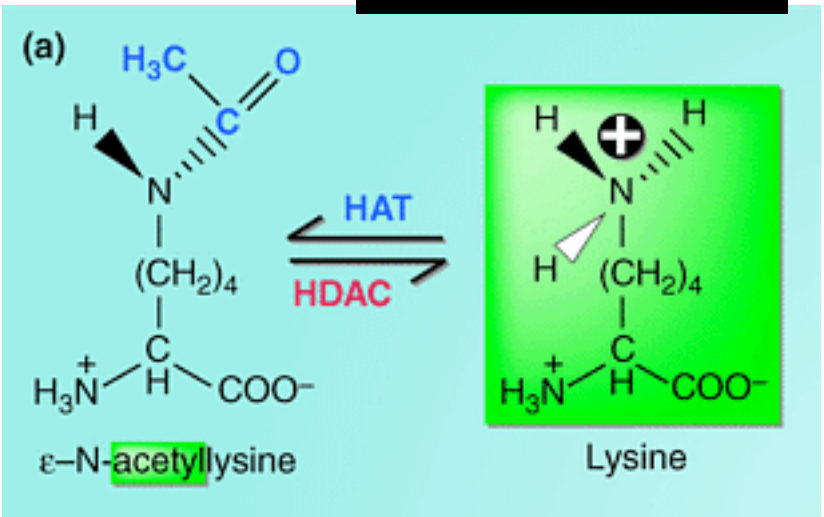
Overview of different classes of modification identified on histones. The functions that have been associated with each modification are shown. Each modification is discussed in detail in the text under the heading of the function it regulates.

Histone acetylation

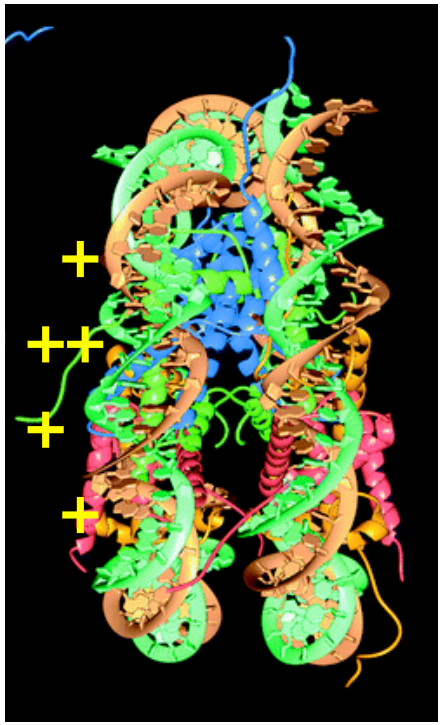
1. Acetylation of core histones

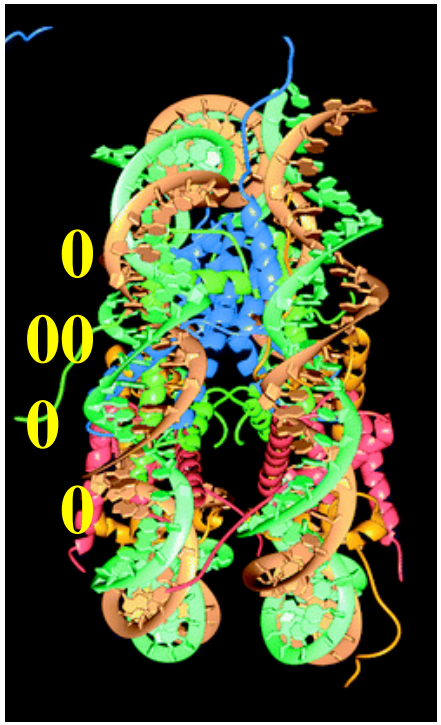
- **N-term tails reversible acetylated in Lys, particularly in H3+H4**
  - The N-terminal tails point outwards and is available for interaction
- **The consequences of acetylation**
  - Acetylation of ε-amino groups in lysines leads to reduced positive charge, believed to weaken the DNA-interaction of the tails.
  - Improves the availability of chromosomal DNA
  - Changes the conformation of nucleosomes and destabilizes internucleosomal contacts
  - May change the interaction with other regulatory proteins

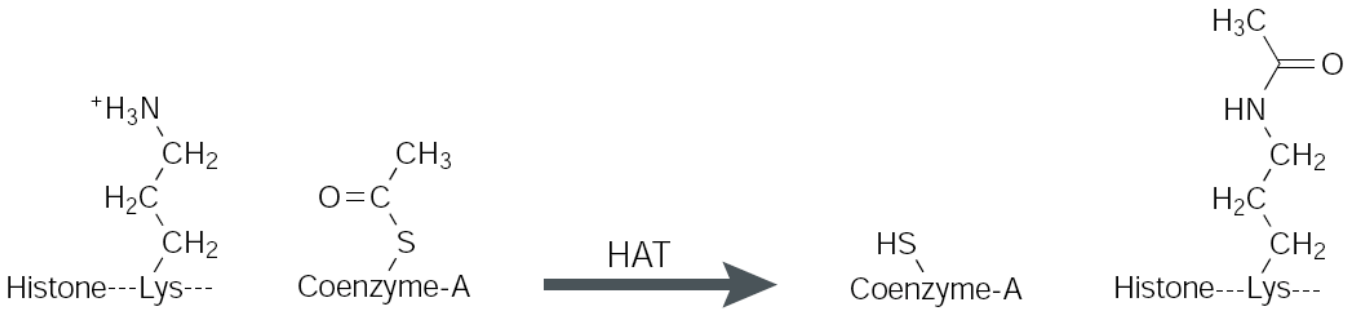




Change of charge



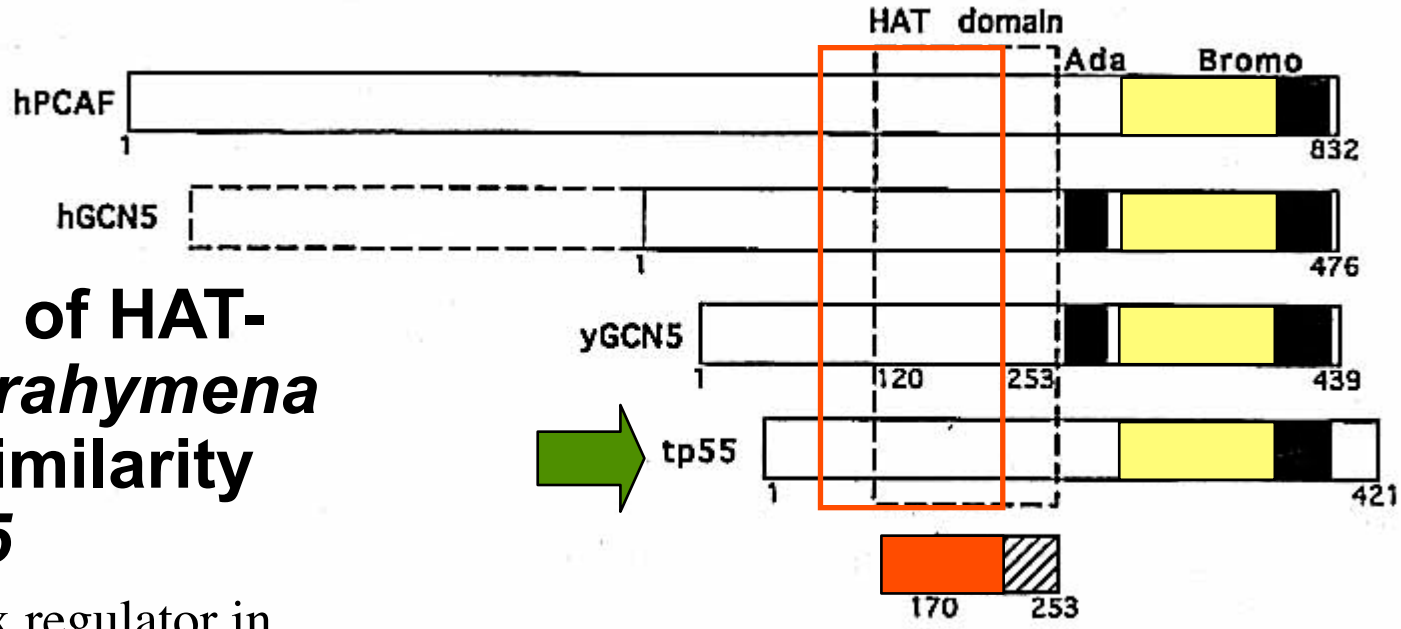




A link histone acetylation - transcription

- **Acetylated histone-tails enriched in transcribed chromatin**
  - Change in level of acetylation using inhibitors of deacetylation (TSA) enhance trx
  - **Chromatin immunoprecipiations (ChIP) show enrichment of acetylated histones in active promoters**
- **Acetylation makes nucleosomal DNA more accessible for TF-binding - a role in "potentiation"**

History - GCN5-related HATs the first link to transcription



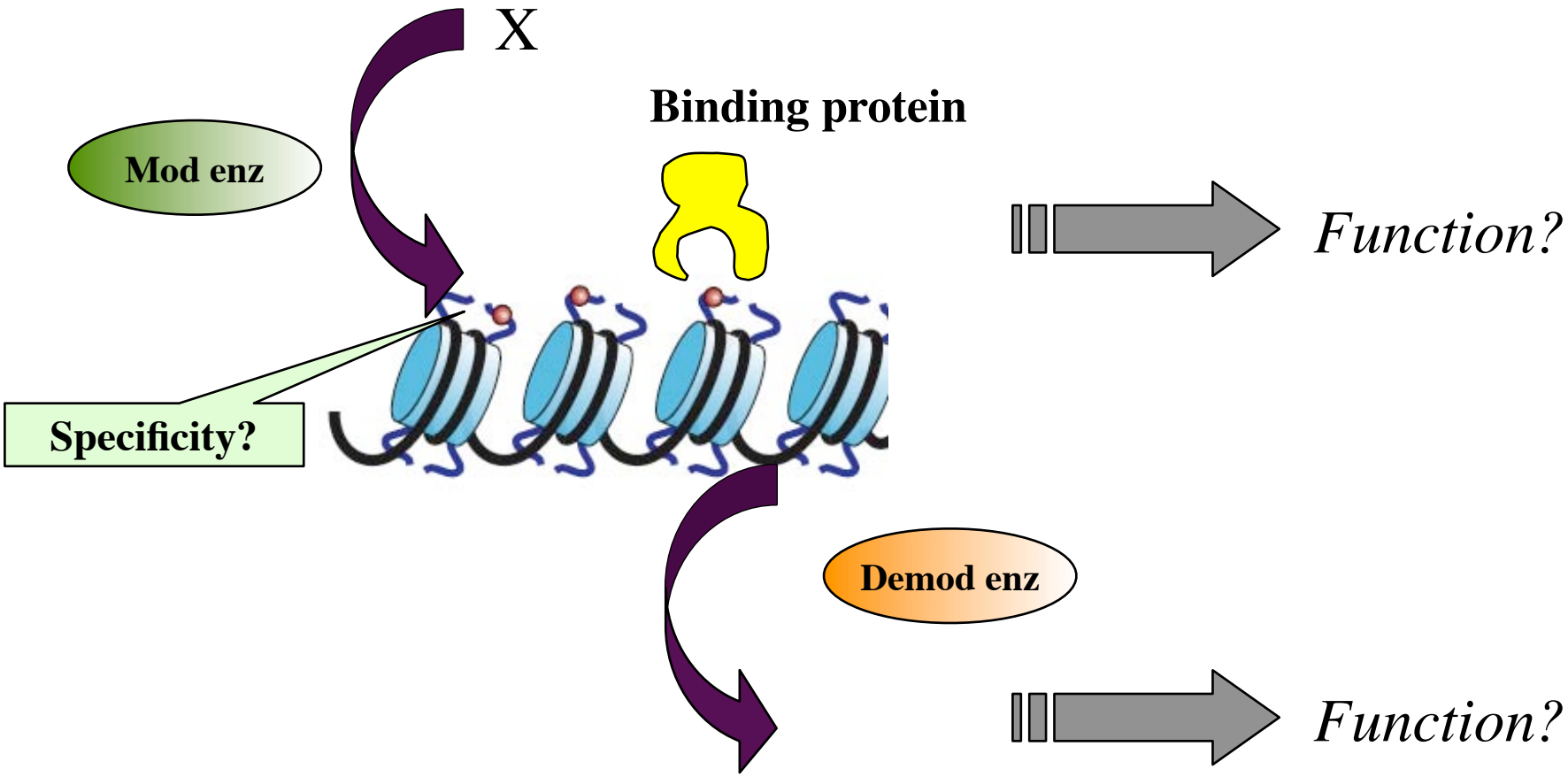
- **The first cloning of HAT-A (p55) from *Tetrahymena* revealed large similarity with yeast *GCN5***
  - Gcn5p = a known trx regulator in yeast
- ***PCAF* and *hGCN5* identified from its homology with *yGCN5***
  - Human GCN5 in short and long form

Trx research - studies of Gcn5p

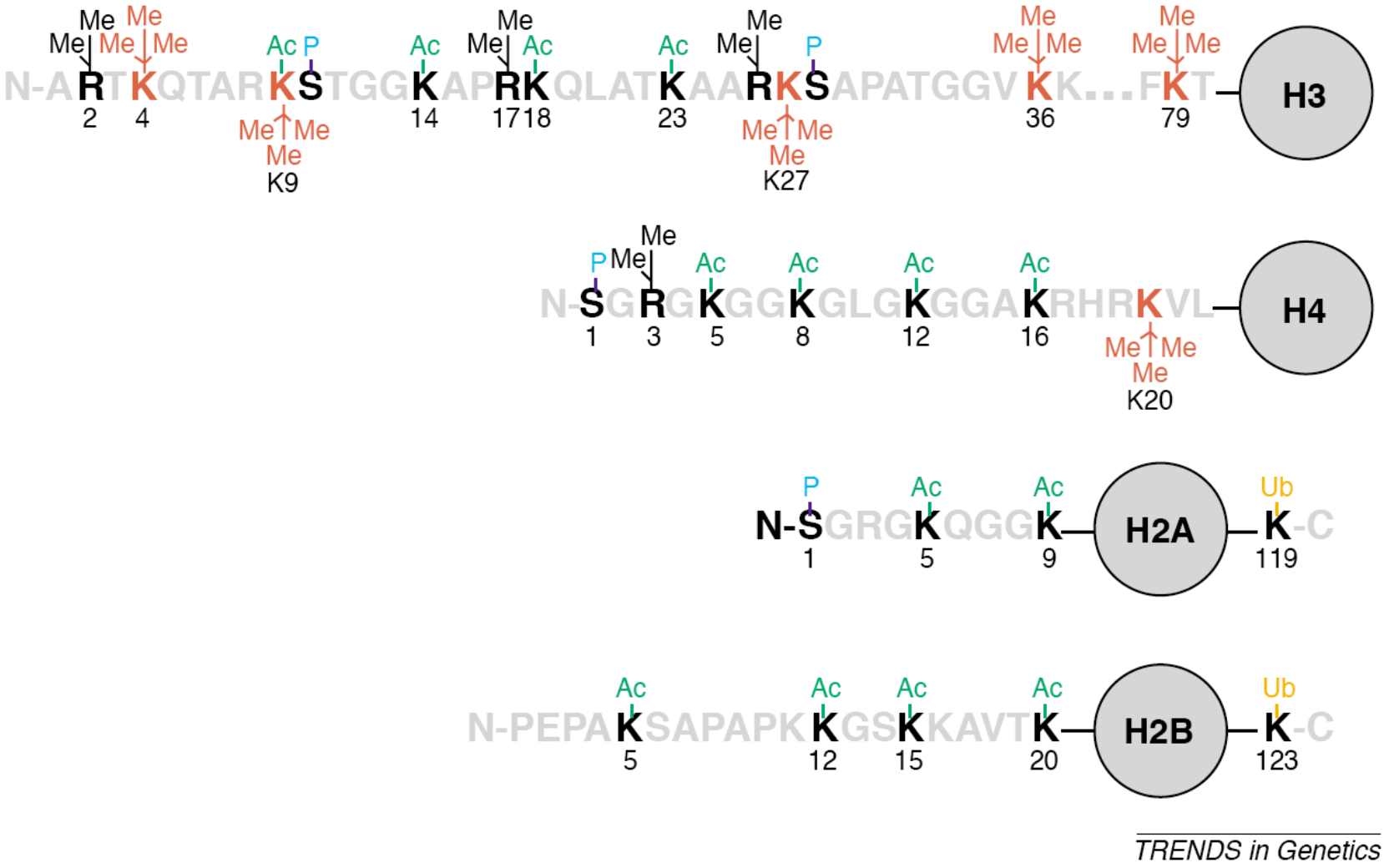
link

Histone research - cloning of HAT

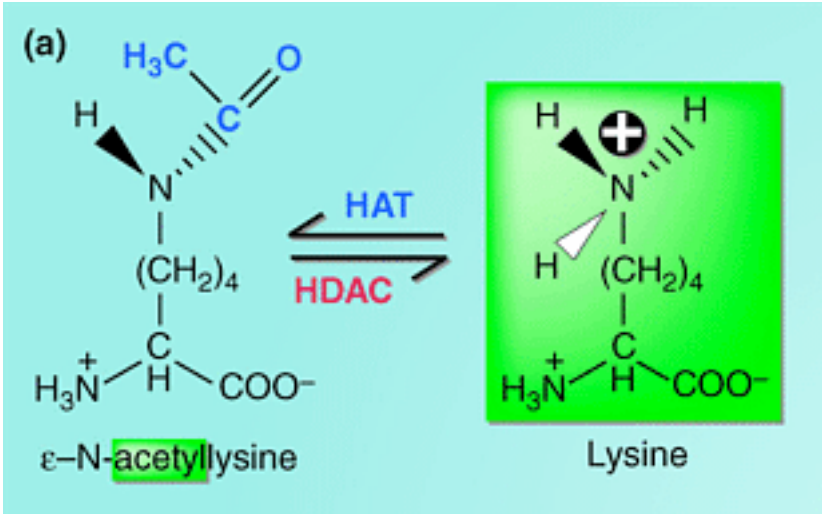
General themes of histone modifications

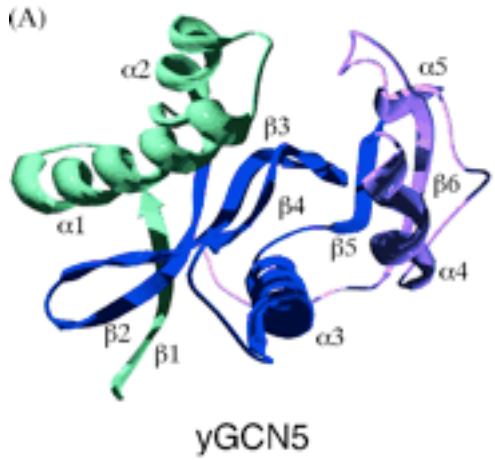


The subtrates: Histone tails - multiple modfications



The enzymes: the HATs

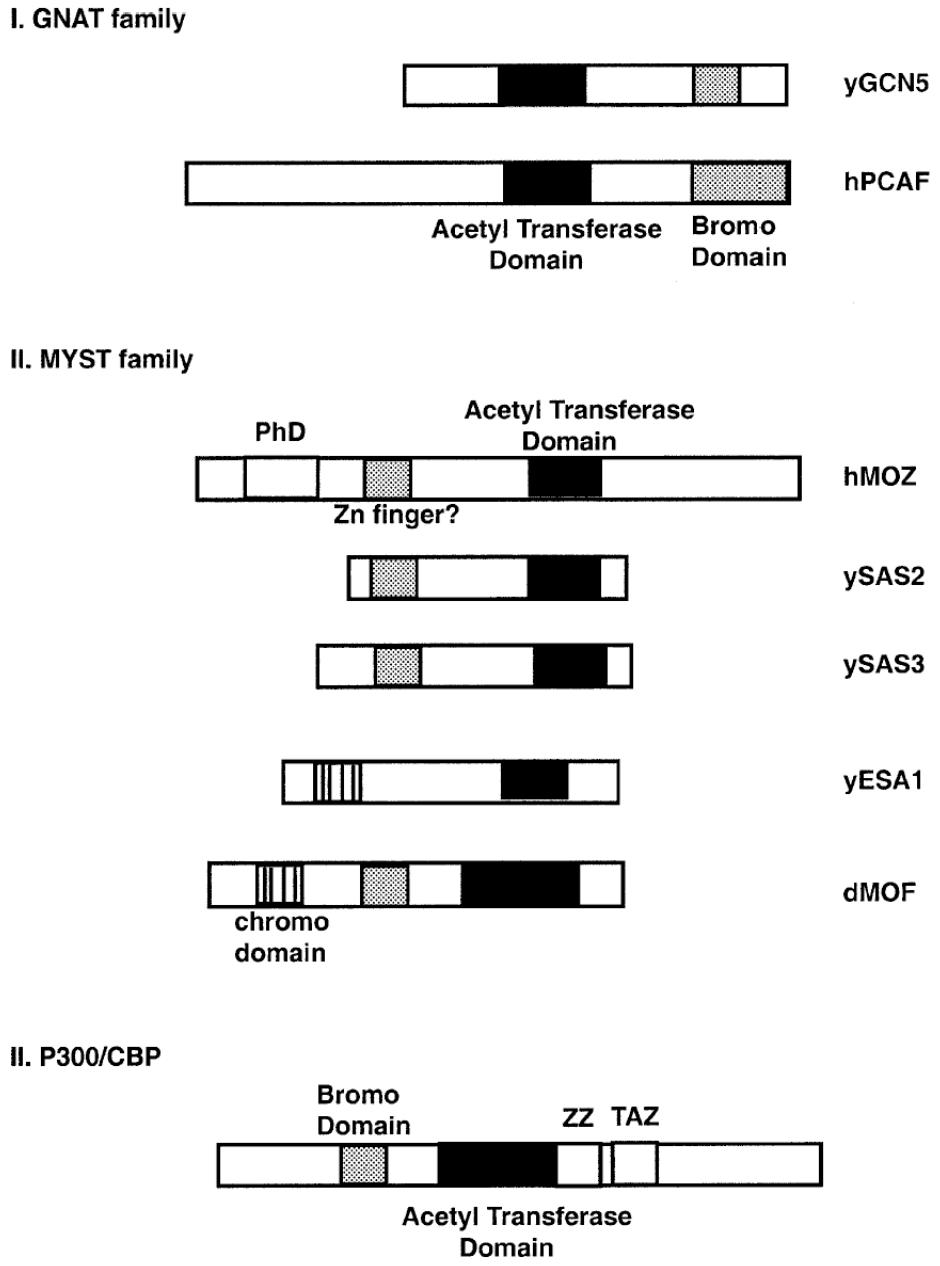




- **Histone acetyltransferases (HATs)**
  - Catalyze the transfer of acetyl groups from acetyl-CoA onto histone tails
  - Two main groups: A-type and B-type HATs
    - A-Type HATs catalyze trx-related acetylations
    - B-Type HATs are cytoplasmic and catalyze acetylations linked to transport of newly synthesized histones from the cytoplasm to the nucleus
- **Histone deacetylases (HDACs)**

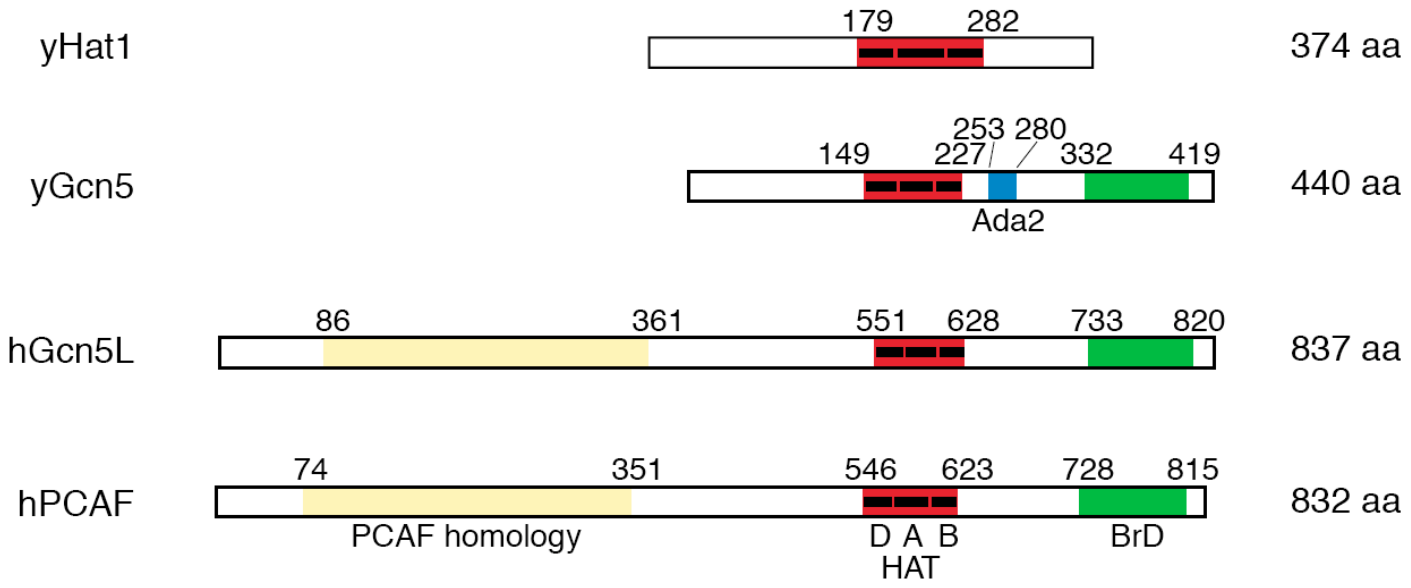
HAT families

- **GNAT family**
  - Gcn5-related N-acetyltransferase
  - Many contains bromodomains
- **MYST**
  - Named for its founding members: MOZ, Ybf2/Sas3, Sas2, Tip60
  - Several contain chromodomains
- **CBP/p300**
- **GTF-HATs**
  - $TAF_{II}250$
- **Nuclear receptor linked HATs**
  - SRC1, ACTR



GNAT-family of HATs

- In larger complexes like
- ySAGA and hSTAGA and hTFTC
  - SAGA (Spt-Ada-Gcn5-Acetyltransferase)
  - 2 MDa complex with 14 subunits incl TAFs
  - Interacts with both TBP and acidic activators
  - Human GCN5 found in a similar complex STAGA
  - TFTC (TBP-free TAF-containing complex)



HAT-enzymes found in larger complexes

Table 1. GNAT-related histone acetyltransferase (HAT) complexes[a–c]

Complex	SAGA	SLIK/SALSA/ SAGA-alt	PCAF	STAGA	TFTC	ADA	HAT-A2	Elongator
Organism	Yeast	Yeast	Human	Human	Human	Yeast	Yeast	Yeast
Substrate	H3/H2B	H3/H2B	H3/H4	H3/H2B	H3/H2B	H3/H2B	H3/H2B	H3/H4
Catalytic	yGcn5	yGcn5	PCAF	hGcn5L	hGcn5L	yGcn5	yGcn5	
HAT subunit	(HAT + BrD)	(HAT + BrD)	(HAT + BrD)	(HAT + BrD)	(HAT + BrD)	(HAT + BrD)	(HAT + BrD)	
	–	–	–	–	–	–	–	Elp3 (HAT)
Accessory subunits	Tra1 (ATM/PI3-K domain)	Tra1 (ATM/PI3-K domain)	PAF400 (ATM/PI3-K domain)	TRRAP (ATM/PI3-K domain)	TRRAP (ATM/PI3-K domain)	–	–	–
	yAda1	yAda1	–	STAF42	–	–	–	–
	yAda2	yAda2	hAda2	–	–	yAda2	yAda2	–
	yAda3	yAda3	hAda3	STAF54	hAda3	yAda3	yAda3	–
	yAda5/Spt20	yAda5/Spt20	–	–	–	–	–	–
	ySpt3	ySpt3	hSpt3	hSpt3	hSpt3	–	–	–
	ySpt7 (full-length)	ySpt7 (C-terminal truncation)	–	STAF65γ	–	–	–	–
	ySpt8	–	–	–	–	–	–	–
	–	–	–	–	hTAF2 ($hTAF_{II}150$)	–	–	–
	–	–	–	–	hTAF4 ($hTAF_{II}130/135$)	–	–	–
	yTAF5 (yTaf90)	YTAF5 (yTaf90)	–	–	hTAF5 ($hTAF_{II}100$)	–	–	–
	–	–	hTAF5L (hPAF65β)	hTAF5L (hPAF65β)	hTAF5L (hPAF65β)	–	–	–
	yTAF6 (yTaf60)	yTAF6 (yTaf60)	–	–	hTAF6 (hTAF80)			
	–	–	hTAF6L (hPAF65α)	hTAF6L (hPAF65α)	hTAF6L (hPAF65α)	–	–	–
	yTAF9 (yTaf17)	yTAF9 (yTaf17)	hTAF9 ($hTAF_{II}32/31$)	hTAF9 ($hTAF_{II}32/31$)	hTAF9 ($hTAF_{II}32/31$)	–	–	–
	yTAF10 (yTaf25)	yTAF10 (yTaf25)	hTAF10 ($hTAF_{II}30$)	hTAF10 ($hTAF_{II}30$)	hTAF10 ($hTAF_{II}30$)	–	–	–
	yTAF12 (yTaf61/68)	yTAF12 (yTaf61/68)	hTAF12 ($hTAF_{II}20/15$)	hTAF12 ($hTAF_{II}20/15$)	hTAF12 ($hTAF_{II}20/15$)	–	–	–
	Sgf29	–	–	–	–	–	–	–
	Sgf73	–	–	–	–	–	–	–
	Ubp8	–	–	–	–	–	–	–
	–	**Rtg2**	–	–	–	–	–	–
	–	–	–	**STAF36**	–	–	–	–
	–	–	–	**STAF46**	–	–	–	–
	–	–	–	**STAF55**	–	–	–	–
	–	–	–	**STAF60**	–	–	–	–
	–	–	–	SAP130	SAP130	–	–	–
	–	–	–	–	–	**Ahc1**	–	–
	–	–	–	–	–	–	–	**Elp1**
	–	–	–	–	–	–	–	**Elp2**
	–	–	–	–	–	–	–	**Elp4**
	–	–	–	–	–	–	–	**Elp5**
	–	–	–	–	–	–	–	**Elp6**

GNAT-related HAT complexes

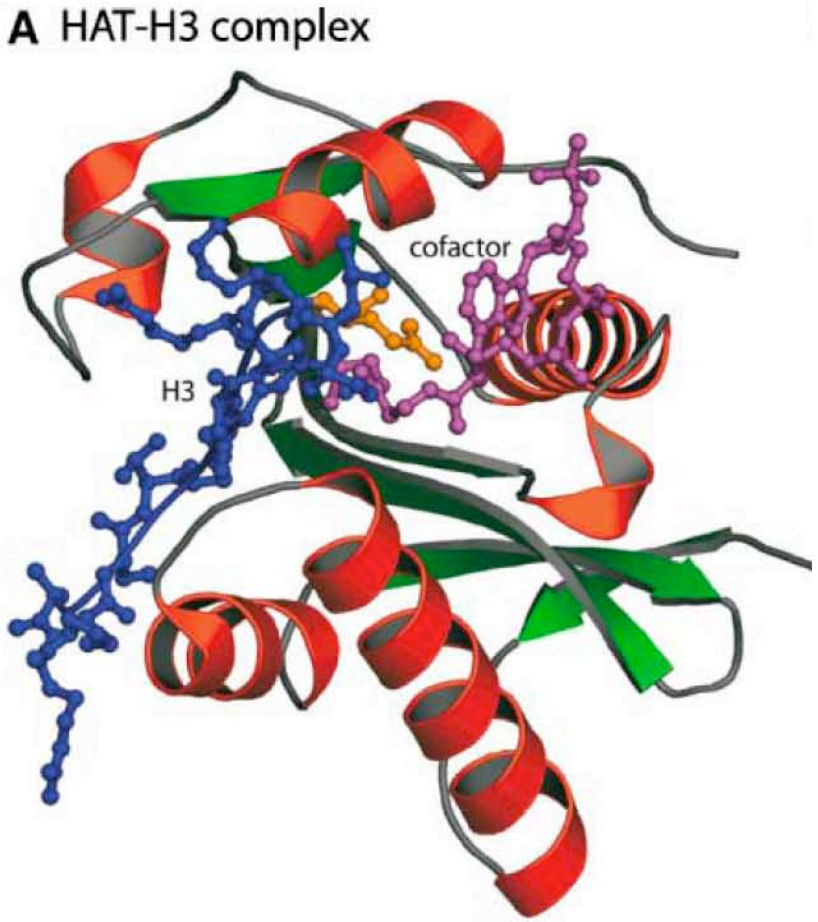
PCAF	STAGA	TFTC
Human H3/H4 PCAF (HAT + BrD)	Human H3/H2B hGcn5L (HAT + BrD)	Human H3/H2B hGcn5L (HAT + BrD)
–	–	–

Activator contact

PAF400 (ATM/PI3-K domain)	TRRAP (ATM/PI3-K domain)	TRRAP (ATM/PI3-K domain)
–	STAF42	–
hAda2	–	–
hAda3	STAF54	hAda3
–	–	–
hSpt3	hSpt3	hSpt3
–	STAF65γ	–
–	–	–
–	–	hTAF2 ($hTAF_{II}150$)
–	–	hTAF4 ($hTAF_{II}130/135$)
–	–	hTAF5 ($hTAF_{II}100$)
hTAF5L (hPAF65β)	hTAF5L (hPAF65β)	hTAF5L (hPAF65β)
–	–	hTAF6 (hTAF80)
hTAF6L (hPAF65α)	hTAF6L (hPAF65α)	hTAF6L (hPAF65α)
hTAF9 ($hTAF_{II}32/31$)	hTAF9 ($hTAF_{II}32/31$)	hTAF9 ($hTAF_{II}32/31$)
hTAF10 ($hTAF_{II}30$)	hTAF10 ($hTAF_{II}30$)	hTAF10 ($hTAF_{II}30$)
hTAF12 ($hTAF_{II}20/15$)	hTAF12 ($hTAF_{II}20/15$)	hTAF12 ($hTAF_{II}20/15$)

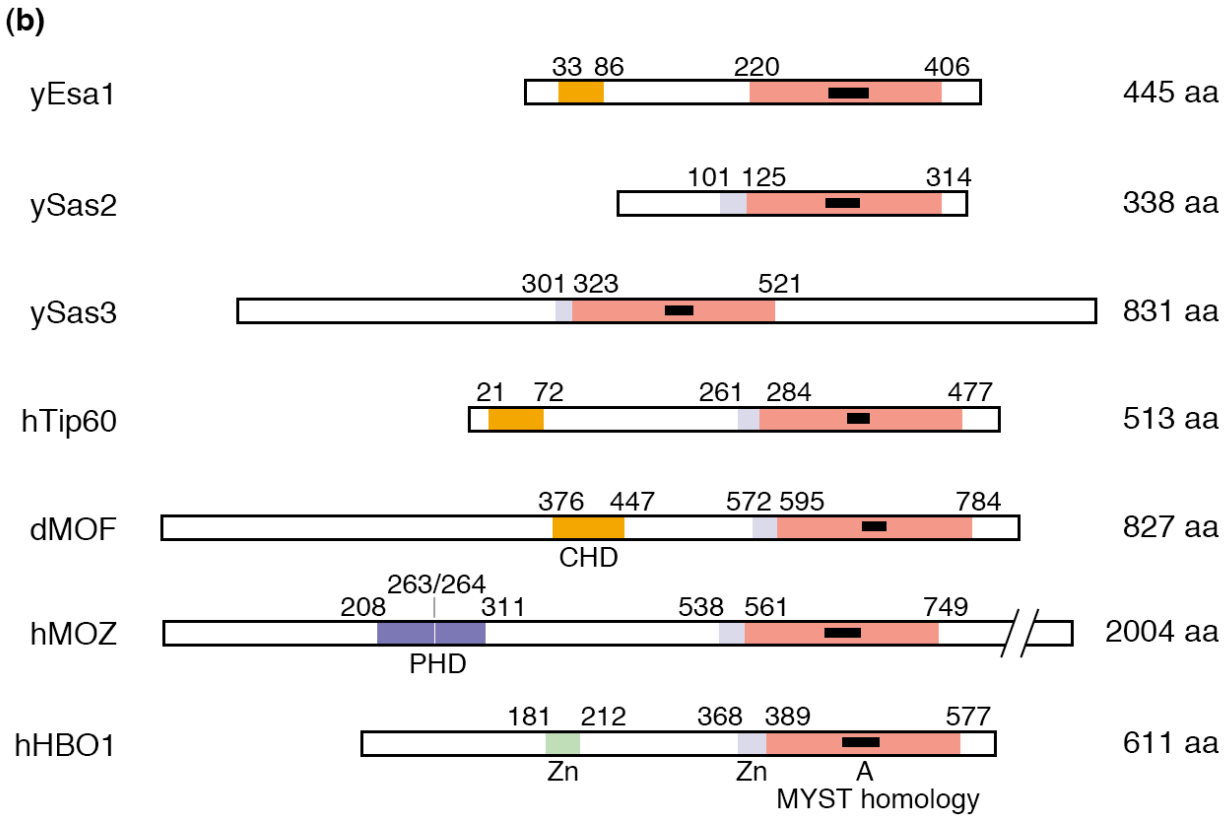
HAT structure

- **The structure of the HAT domain (GNAT-type)**
  - a central conserved core unit important for acetyl-CoA cofactor binding
  - A cleft used for substrate recognition that lies directly over the cofactor pocket
- **Peptide binding pocket**
  - the H3 peptide bound to Gcn5HAT adopts a random coil structure
  - phosphorylation of S10 in the H3 peptide generates additional interactions. Explains how one histone modification can influence the creation of another modification. Phosphorylation at Ser10 can enhance K14 acetylation and together promote transcription



MYST-type HAT complexes

- **Involved in a wide range of cell functions**
  - Trx activation and silencing
- **Five complexes isolated**
  - yNuA4 complex with essential Esa1
  - yNuA3 complex
  - hTip60 complex
  - Some contain chromodomains (recognizing methylated histones)



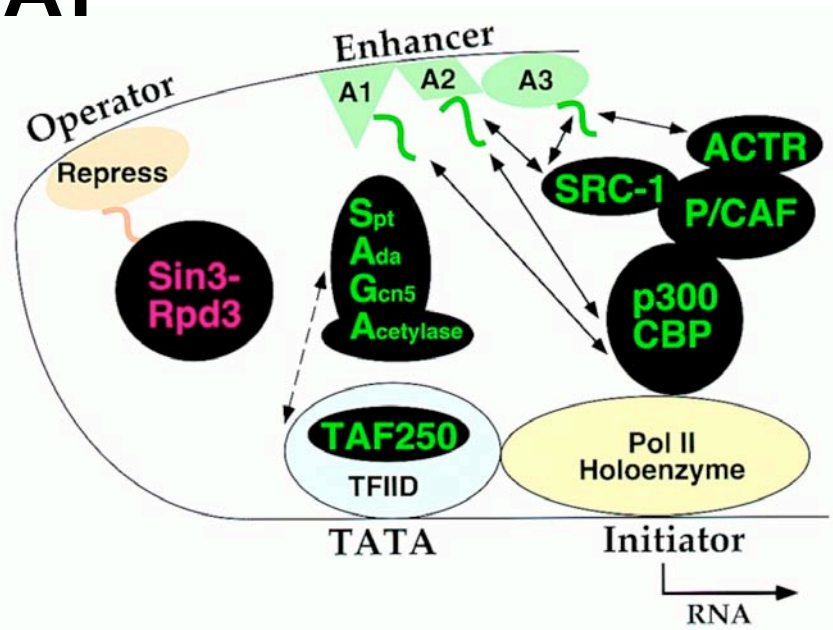
MYST-type HATs

Table 2. MYST-related histone acetyltransferase (HAT) complexes[a,b]

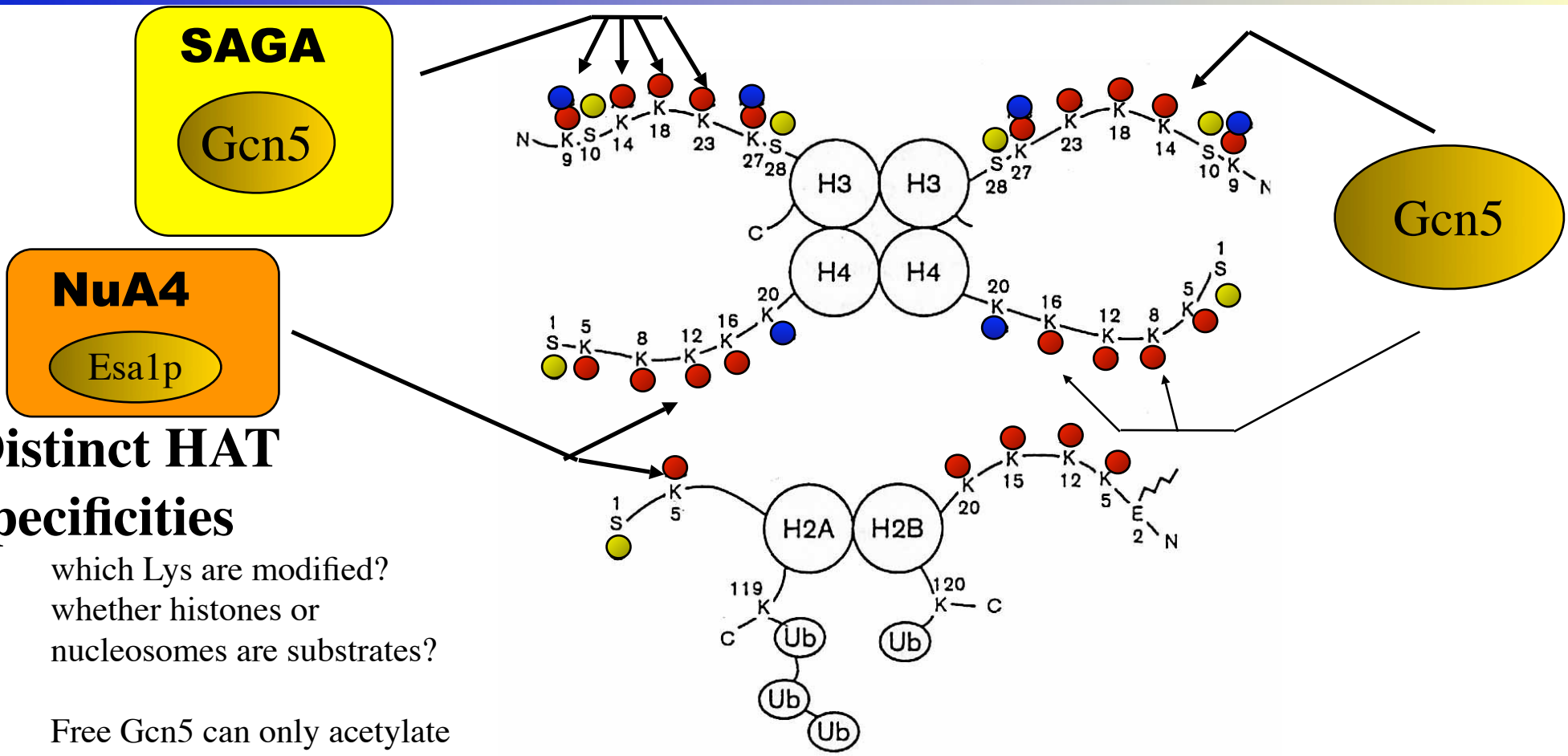
Complex	NuA4	Tip60	MSL	NuA3	SAS (SAS-I)
Organism	Yeast	Human	Fly	Yeast	Yeast
Substrate	H4/H2A	H4/H2A	H4 K16	H3	H4 K16
Catalytic HAT subunit	Esa1 (HAT + CHD)	Tip60 (HAT + CHD)	MOF (HAT + CHD)	–	–
	–	–	–	Sas3 (HAT)	–
	–	–	–	–	Sas2 (HAT)
Accessory subunits	Tra1 (ATM/PI3-K domain)	TRRAP (ATM/PI3-K domain)	–	–	–
	Act1	actin	–	–	–
	Arp4 (actin-related protein)	BAF53 (actin related protein)	–	–	–
	Epl1 (E(Pc) homolog)	EPC[c] (E(Pc) homolog)	–	–	–
	Eaf3 (2 CHDs)	–	MSL3 (2 CHDs)	–	–
	Yng2 (PHD finger; ING1 homolog)	–	–	Yng1 (PHD finger; ING1 homolog)	–
	Eaf1 (SANT domain)		–	–	–
	Eaf2 (SANT domain)	–	–	–	–
	Eaf5	–	–	–	–
	Yaf9 (AF9 homolog)	–	–	Anc1/TAF30 (AF9 homolog)	Sas5 (AF9 homolog)
	Eaf6	–	–	–	–
		p400 (SANT + SWI2/SNF2 domains)			
	–	Tip49 (ruvb-like helicase)	–	–	–
	–	Tip48 (ruvb-like helicase)	–	–	–
	–	–	MLE (ATPase/DEAH helicase)	–	–
	–	–	MSL1	–	–
	–	–	MSL2 (RING finger)	–	–
	–	–	roX RNA	–	–
	–	–	–	–	Sas4

Unexpected large number of HAT-complexes

- **Several coactivators have HAT activity**
  - hTAF250, dTAF230, yTAF130
  - p300/CBP
  - ACTR and SRC-1
  - P/CAF
  - Gcn5
- **Why so many?**
- **Evidence for gene-specific HATs**
  - Mice with p300, CBP, PCAF or GCN5 knocked-out exhibit distinct developmental defects



Histone modification - specificity



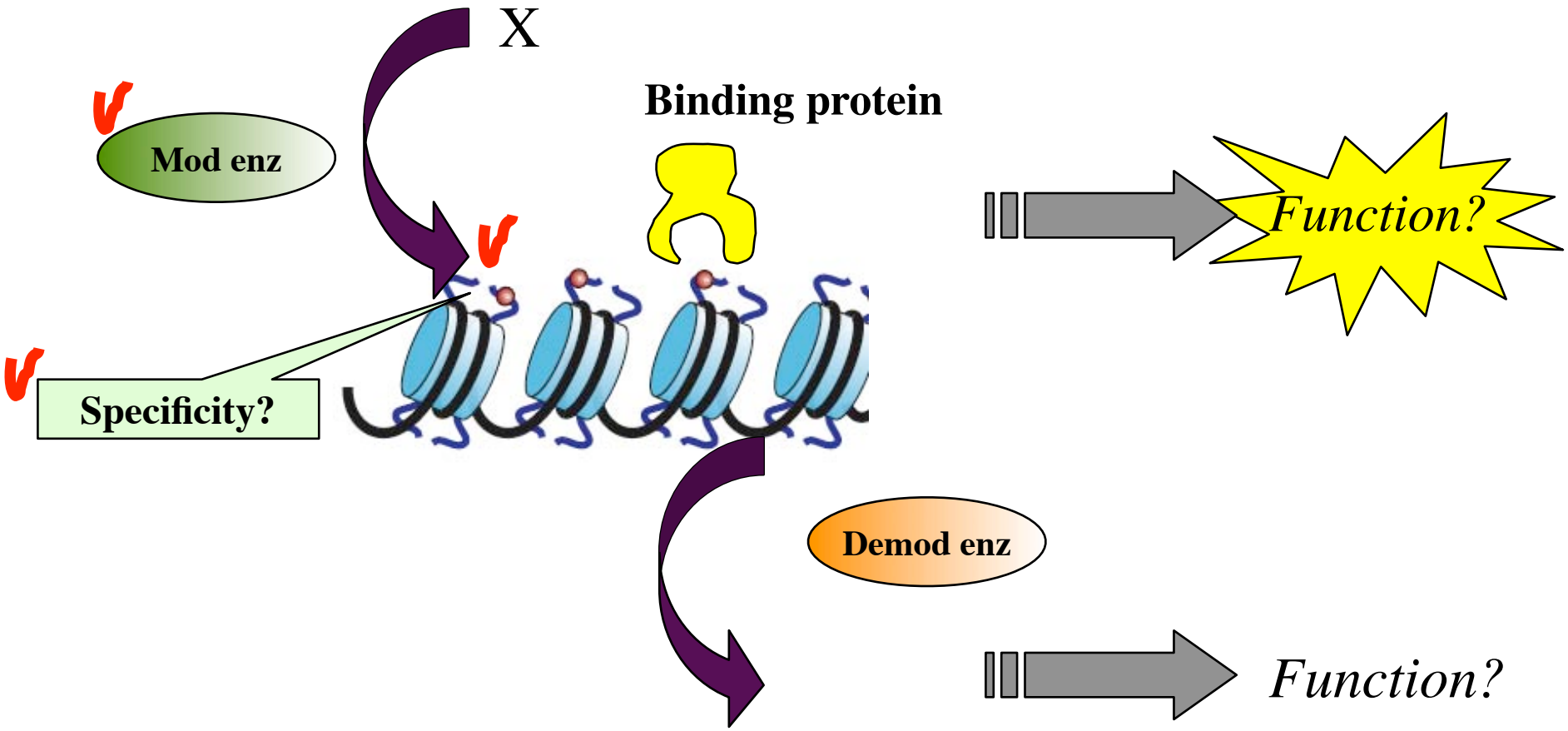
Distinct HAT specificities

which Lys are modified?
whether histones or nucleosomes are substrates?

Free Gcn5 can only acetylate free histones, but as part of the SAGA-complex it modifies also nucleosomes

- Methylation
- Phosphorylation
- Acetylation
- Ubiquitination
- ADP-ribosylation

So far ...

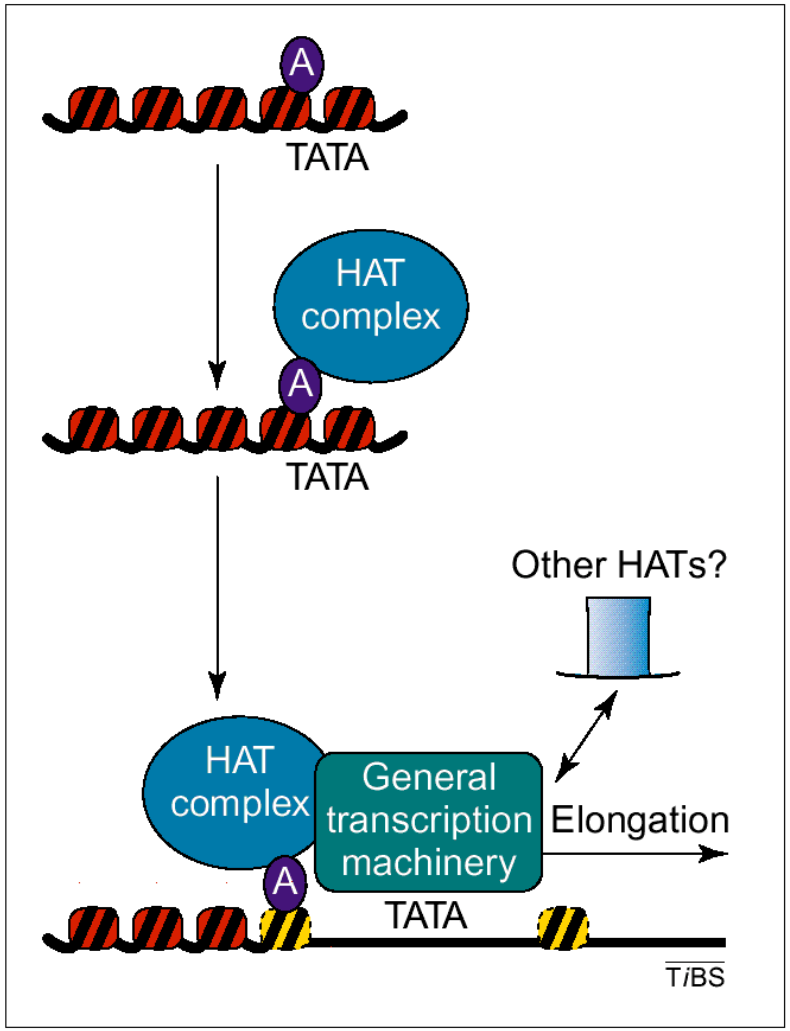

X
Mod enz
Binding protein
Function?
Specificity?
Demod enz
Function?

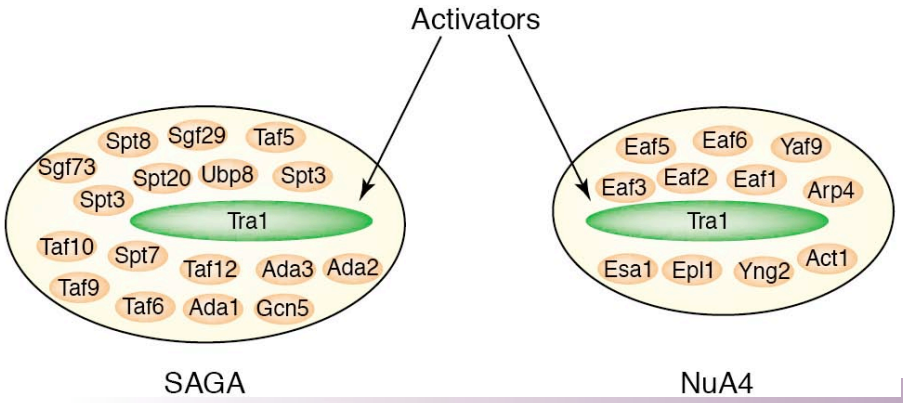
Trx-link: Activation/silencing determined by the equilibrium between HATs and HDACs

- **Acetylation → chromatin activation**
- **Many coactivators = HATs**
  - *Tetrahymena* HAT = homolog of yeast GCN5, a known trx-coactivator
  - The coactivator p300/CBP
  - P/CAF = "p300/CBP associated factor"
  - TAF1: hTAF250, dTAF230, yTAF130
  - Several coactivators for nuclear receptors (SRC-1, ACTR)
  - Point to a coupling between histone- histon-acetylation and gene activation
- **Implies histone acetylation as a directed phenomenon**
- **Present model: HAT-activity becomes recruited to promoters through specfic interactions with activators, leading to local acetylation of nucleosomes around promoters.**

Recruitment - the missing link

- HATs were for a long time not considered interesting for trx because they were assumed to act globally
- Recruitment changed this misconception
- HAT-activity in coactivator-complexes recruited to promoters by TF-interaction

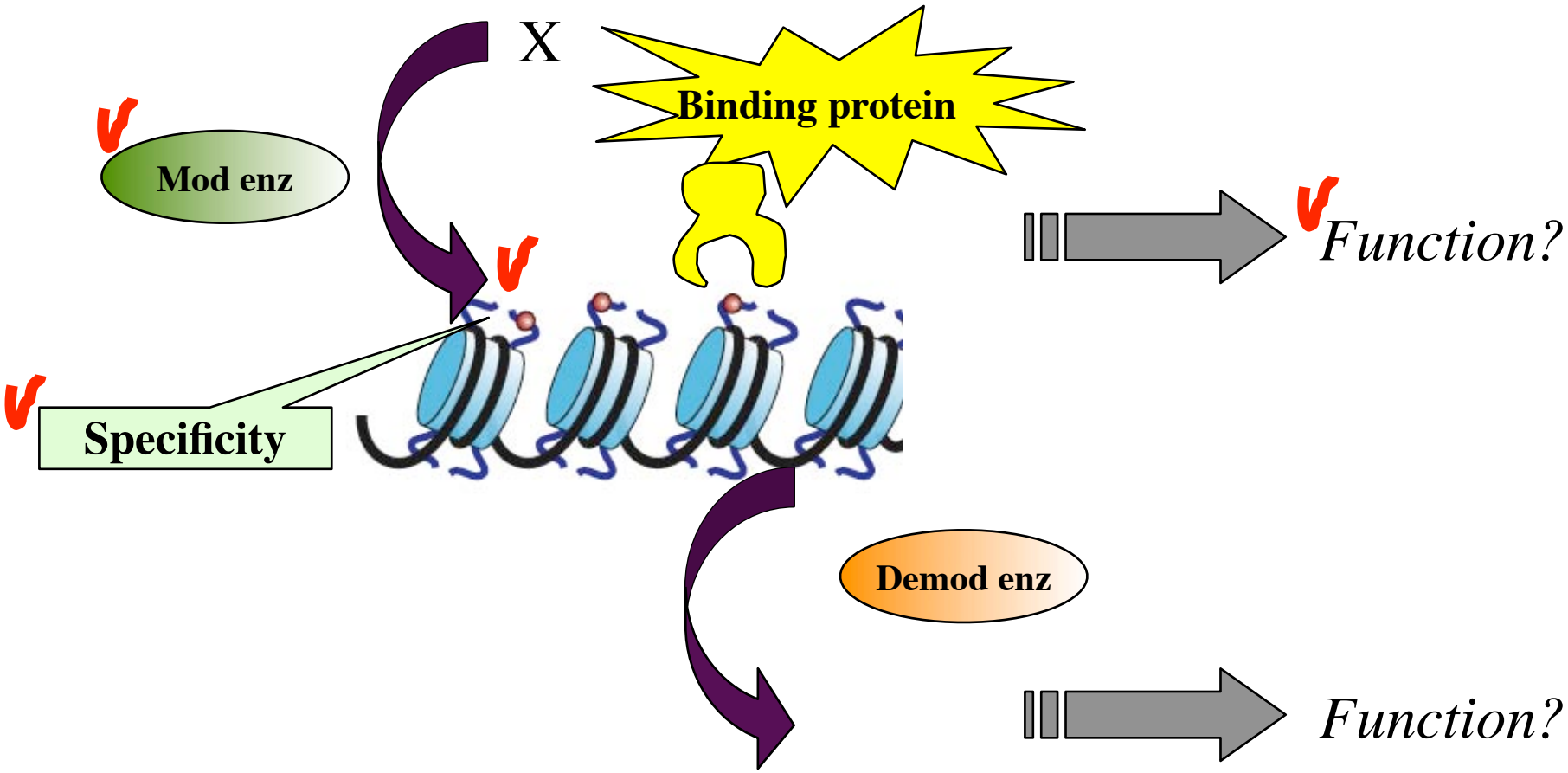




Global versus targeted acetylation/ deacetylation

- **Global acetylation**
  - Bulk acetylation levels surprisingly high
  - On average, 13 of the 30 K residues in a histone octamer are acetylated.
  - this steady-state level of acetylation is maintained by the opposing actions of HAT and HDAC complexes.
- **Targeting**
  - of HAT and HDAC complexes to promoter regions then creates inspecific patterns of hyper- and hypoacetylation in a background of global acetylation that correlate with transcription activation and repression, respectively.
- **Specific pattern or cumulative charge effect?**
  - Distinct patterns of lysine acetylation on histones have been proposed to specify distinct downstream functions.
  - Another view posits that the functions of histone acetylation rely primarily on the number of lysines modified (a cumulative effect). This because of the greater loss of positives charges making acetylated histones easier to displace from DNA.

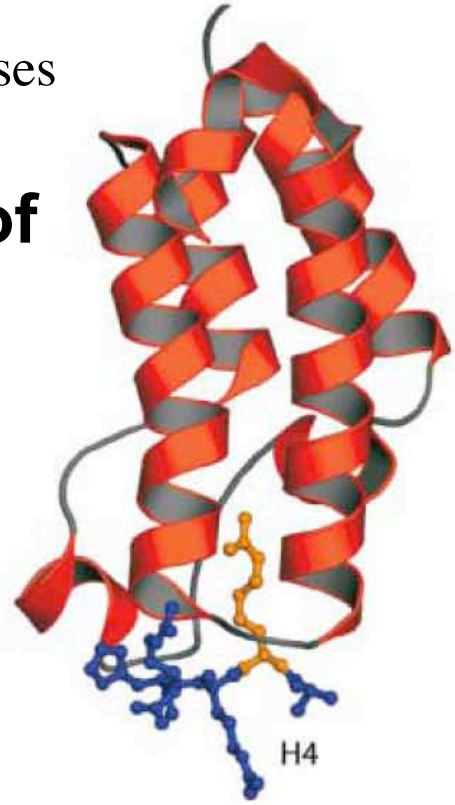
So far ...


X
Binding protein
Mod enz
Function?
Specificity
Demod enz
Function?

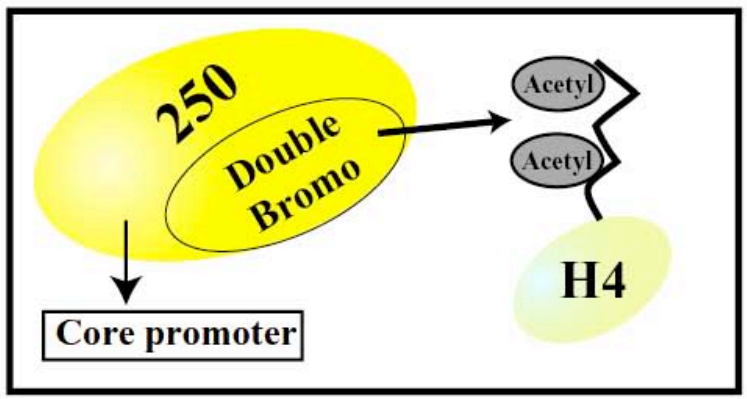
Bromodomains - binding modules for acetylated histones

- **Bromo-domains recognize acetylated lysines in histone tails**
  - 100 aa motif in many chromatin-ass proteins, including HATs
- **Four-helix bundle with a hydrophobic pocket able to interact with acetyl-lys**
  - Single bromo = weak affinity, but affinity dramatically increases with tandem bromodomains such as in $TAF_{II}250$
- **Bromo in HAT logic - ordered recruitment of bromodomain-containing trx complexes**

Bromo-H4 complex



Binding to nucleosome and core promoter



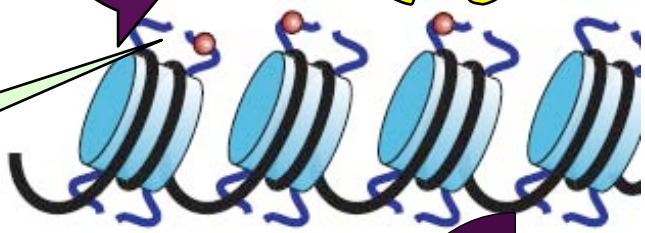
Summary

Several HATs
In large
coactivator complexes

X

Bromo-domain proteins
Binding protein

Mod enz



Specificity

Yes,
complex patterns

Function?

Trx activation

Demod enz

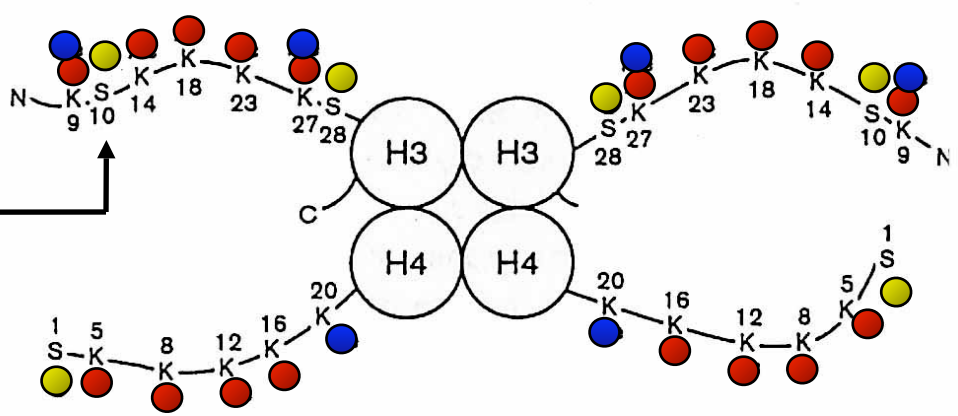
HDACs

Function?

Other histone modifications

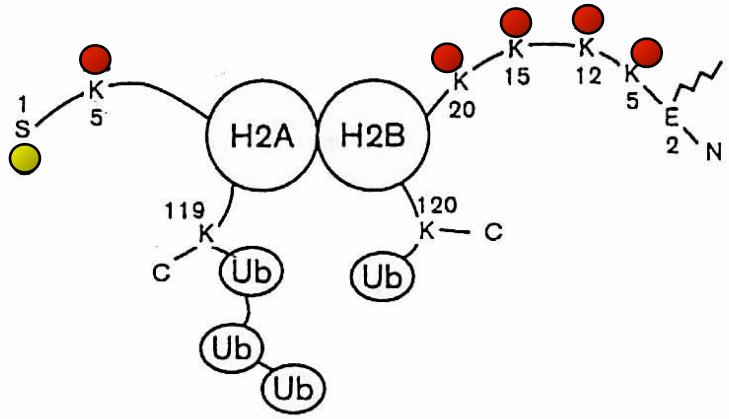
Phosphorylation

H3 Ser10 - mitosis associated
EGF-stimul - Rsk2 mediated
phosphorylation of S10 in H3
Phospho-S10 may enhance
acetylation of surrounding residues



Methylation

!



- Methylation
- Phosphorylation
- Acetylation

Ub Ubiquitination

ADP-ribosylation

Histone methylation

A role in both activation and repression

Histone modifications

Histones are among the most conserved proteins known in evolution, but..
…are also among the most variable in post-translational modification.

Nomenclature:
H3K4me3
histone H3, lysine no 4, tri-methylated
H4K16Ac

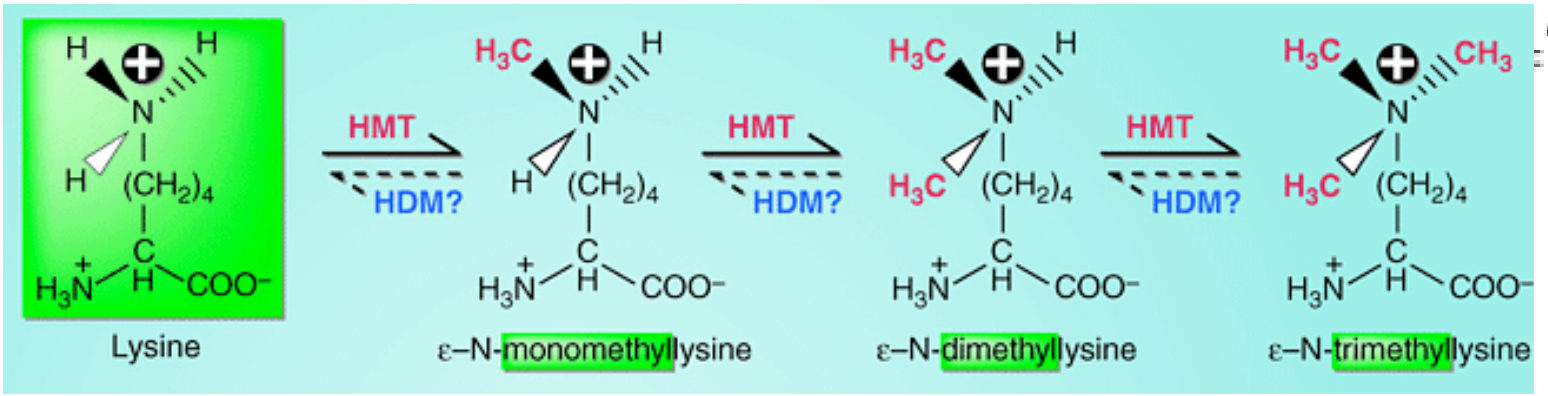
Table 1. Different Classes of Modifications Identified on Histones

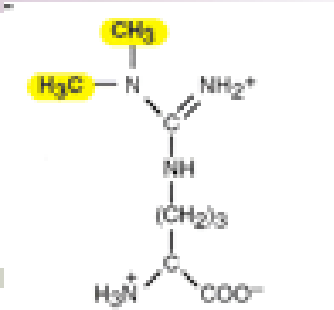
Chromatin Modifications	Residues Modified	Functions Regulated
Acetylation	**K**-ac	Transcription, Repair, Replication, Condensation
Methylation (lysines)	**K**-me1 **K**-me2 **K**-me3	Transcription, Repair
Methylation (arginines)	**R**-me1 **R**-me2a **R**-me2s	Transcription
Phosphorylation	**S**-ph **T**-ph	Transcription, Repair, Condensation
Ubiquitylation	**K**-ub	Transcription, Repair
Sumoylation	**K**-su	Transcription
ADP ribosylation	**E**-ar	Transcription
Deimination	**R > Cit**	Transcription
Proline Isomerization	**P-cis > P-trans**	Transcription

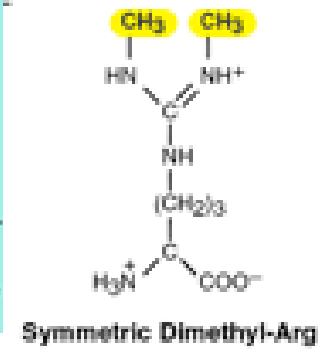
Overview of different classes of modification identified on histones. The functions that have been associated with each modification are shown. Each modification is discussed in detail in the text under the heading of the function it regulates.

Histone methylation

multiple variants on Lys and Arg



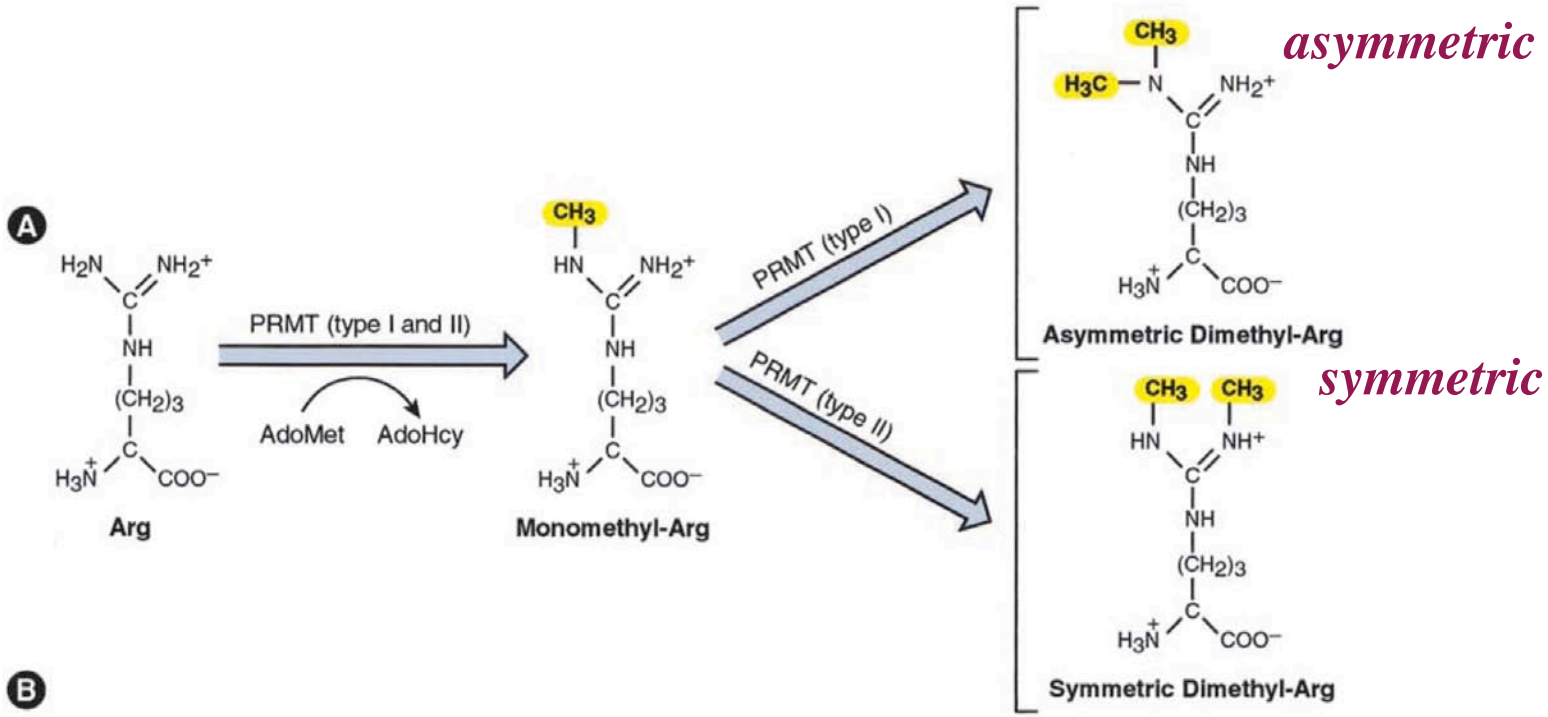




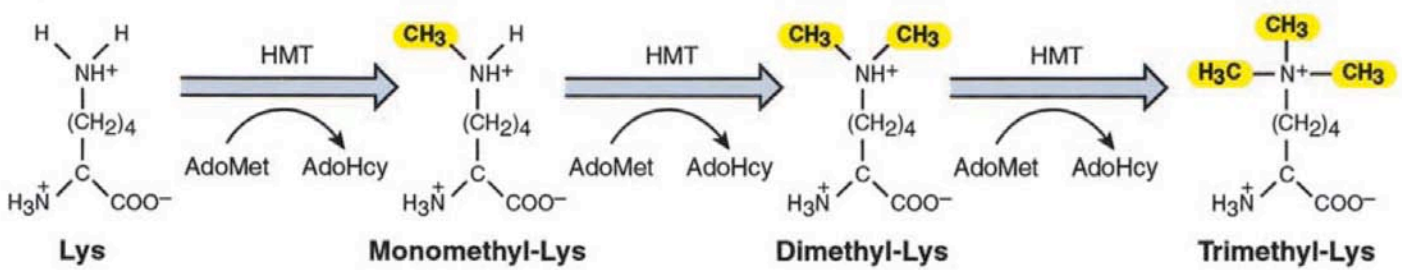
- Both Lys (K) and Arg (R) can be methylated at more than one methyl-group
- Histone demethylases was for a long time thought to not exist, but have just recently been identified
- Methylated residues are present both in eu- and heterochromatin

The substrates: histone tails - arginine and lysine methylation

Arg

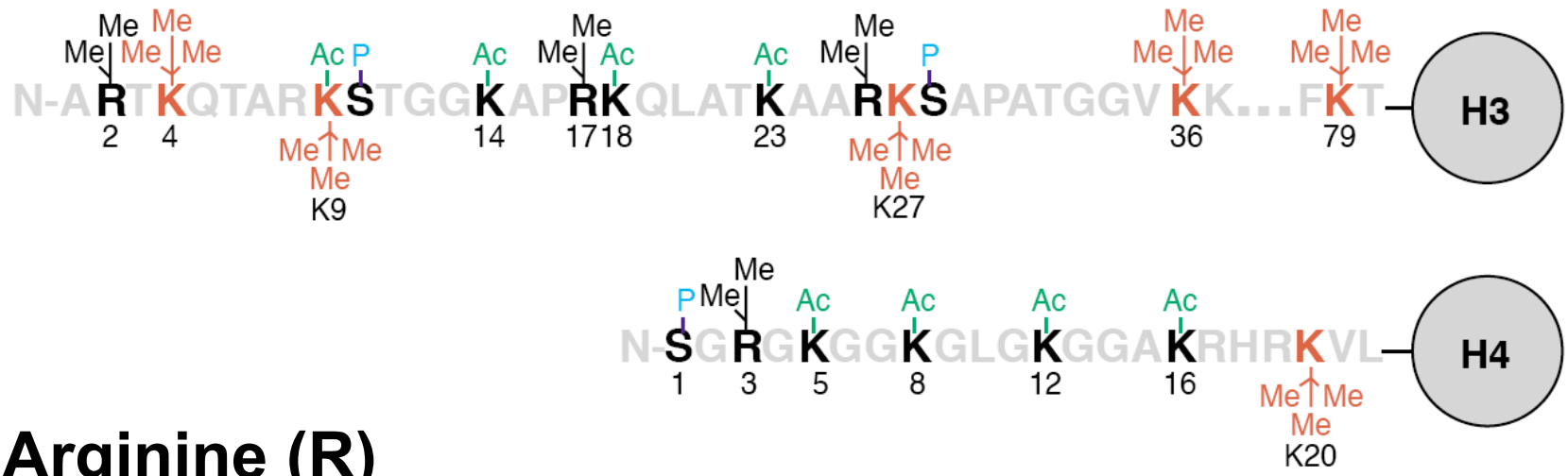


Lys



Increase in the basicity of lys

The subtrates:
Histone tails - multiple methylations



- Black = Arginine (R) methylation
- Red = Lysine (K) methylation
- Above ≈ activation
- Below ≈ repression

The enzymes: Histone lysine methyltransferases (HKMTs)

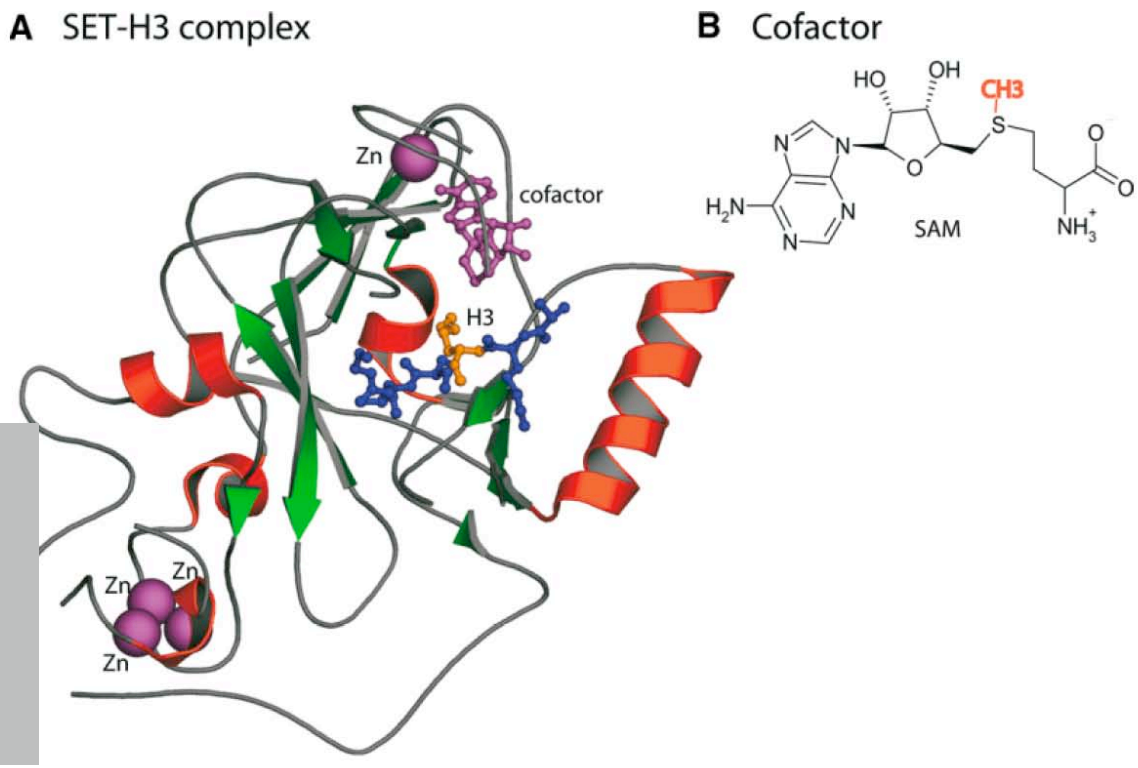
- **The first HKMT, SUV39H1**
  - was identified in 2000.
  - It specifically methylates Lys 9 of histone H3.
  - Later several other HMTs have been identified (see table below)
- **A SET-domain is required for HKMT activity**
- **5 lysines within H3 (K4, K9, K27, K36 and K79) and 1 lysine within H4 (K20) are methylated by specific HKMTs**

Table 1. Histone lysine (K) methyltransferases (HKMTs)[a]

	Enzymes (catalytic subunits)			
Site specificity[b]	***S. cerevisiae***	***S. pombe***	***D. melanogaster***	**Mammals**
H3-K4	Set1 [89–91]	Set1 [92]	Trx, Ash1[c] [37]	Set9[d], ALL-1, MLL, ALR-1 and 2 [93], ALR [93], Set1 [68]
H3-K9	Absent	Clr4	Su(var)3–9 [10], Ash1[c] [37]	Suv39 h, G9a, Eu-HMTase I [11], ESET[e], SETBD1
H3-K27	Absent	?	E(Z)	Ezh2, G9a
H3-K36	Set2 [94]	?	?	NSD1 [67]
H3-K79	Dot1	?	?	Dot1L [60]
H4-K20	Absent	?	PR-Set7,Ash1[c] [37]	PR-Set7

SET-domains

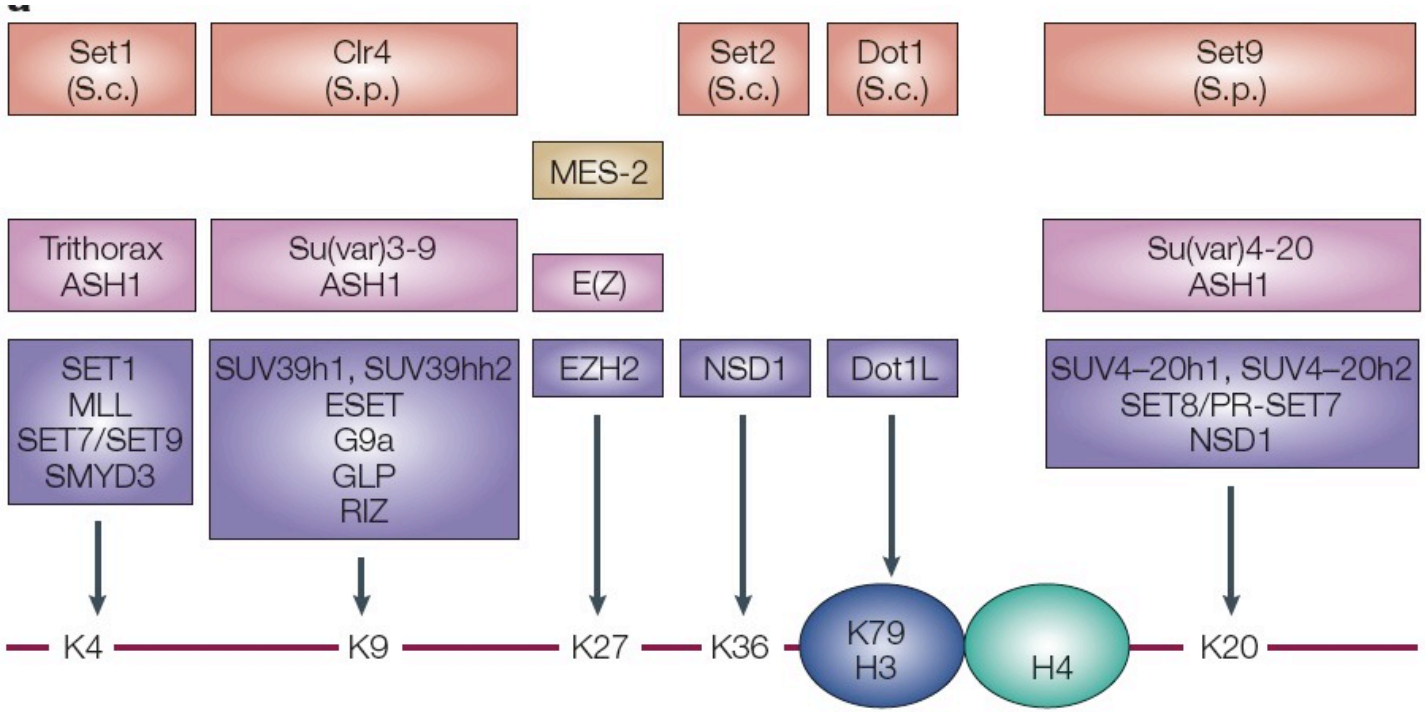
- **Most HKMTs characterized by a conserved SET-domain**
  - Humans: >30 SET-domains
  - Yeast genome: 6 SET-domains
  - >300 found



The structure of DIM5, a homolog of SUV39H1, which methylates lysine 9 (orange) in H3, is shown in complex with H3 tail (blue), cofactor (purple), and four zinc ions (pink).

Many HKMTs identified since

- **yeast,** red
- **worm,** yellow
- **fly,** pink
- **mammalian,**
  - purple



Sum-mary

- 2007

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

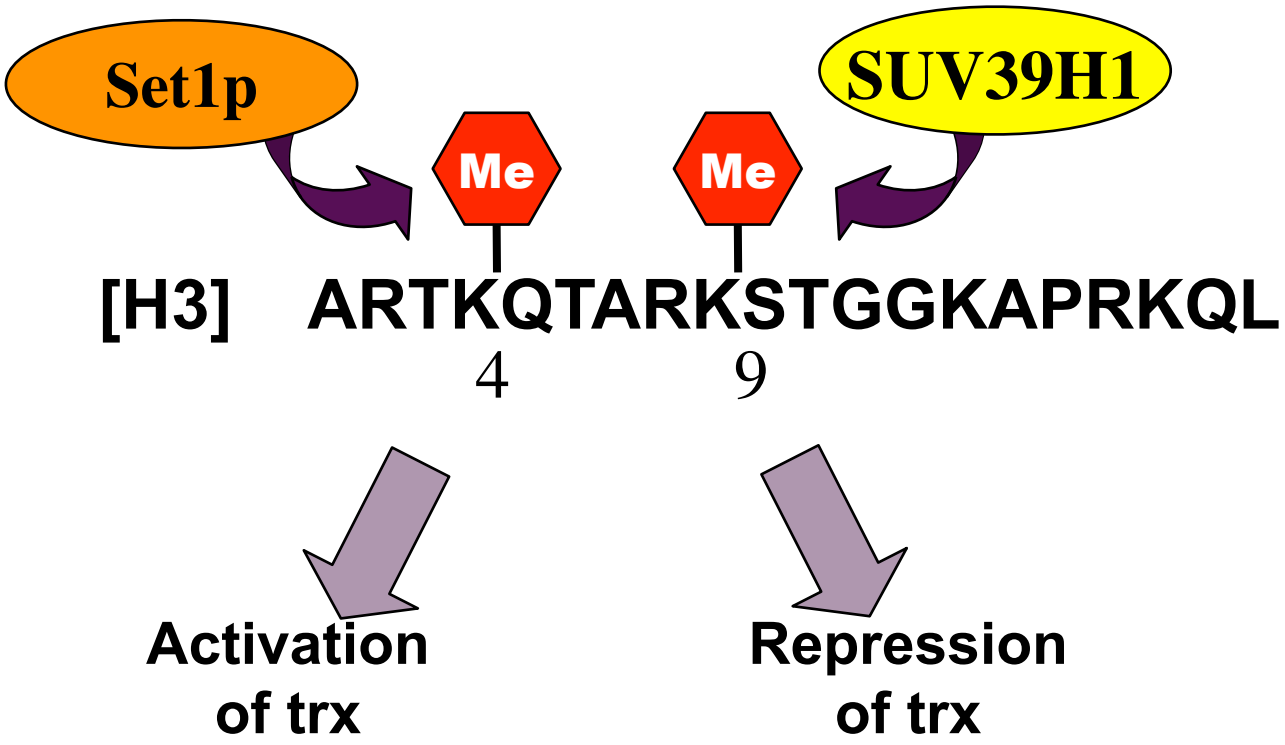
[a] The proteins that are indicated within the parentheses are shown to recognize the corresponding modifications but specific domains have yet to be determined.

Function in long-term epigenetic maintenance?

- Epigenetics = heritable changes in gene expression that do not result from alterations in DNA sequences
- HMTs proposed to be involved in a complex process that controls aspects of short- and long-term transcriptional regulation
- Dogma currently being challenged

Function - Activation or repression? 2 sites - 2 HKMTs - 2 effects

- **Histone methylation was traditionally linked to repression, but turns out to be linked also to activation**
  - H3 K9 methylation correlates with heterochromatin formation.
  - Met-K4 in H3 correlates with trx activation
  - Set1p associated with HAT complex

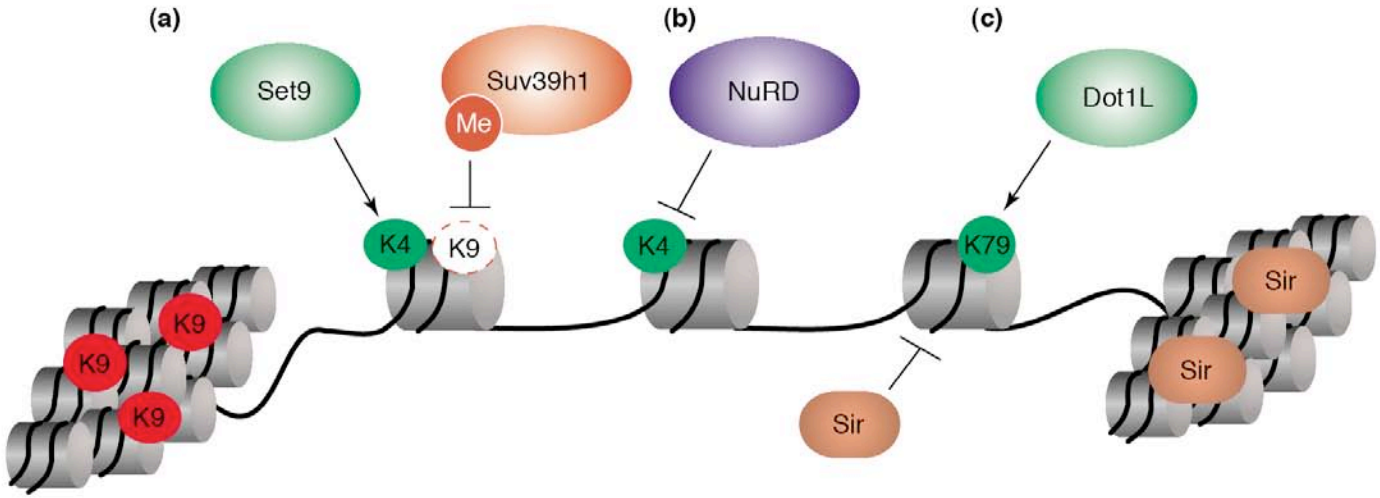


Function of histone methylation

- **Repression (H3-K9, H3-K27, H4-K20) - more next lecture**
  - Numerous reports on silencing effects linked to H3-K9 methylation
  - Heterochromatin formation linked to H3-K9 methylation and HP1 binding
  - Highly condensed centromeric regions related to methyl addition to H3-K9.
  - H3-K9 and H3-K27 methylation might be important for the X chromosome inactivation process.
- **Activation (H3-K4, H3-K36, H3-K79)**
  - H3-K4 methylation is generally associated with trx active chromatin.
  - di-methyl H3-K4 appears to be a global epigenetic mark in euchromatic regions and tri-methylation of H3-K4 correlates with active transcription
  - both H3-K4 and H3-K79 participate in establishing euchromatic regions by preventing the spreading of heterochromatic regions.
- **Role of histone lysine methylation in trx elongation**

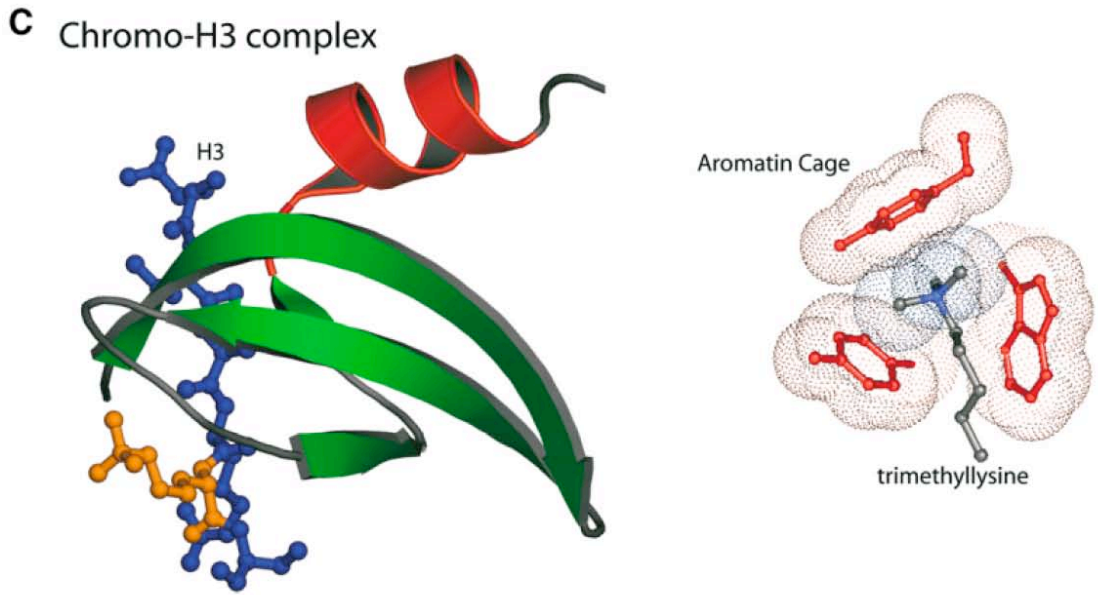
Activating effects of methylation - two levels

- **Chromosome level - preventing spread of heterochromatin**
  - The methylation of H3-K4 specifically impairs methylation at H3-K9, thereby blocking a major pathway of heterochromatin formation
  - binding of the histone deacetylase NuRD repression complex to the H3 N-terminal tail is precluded by methylation at K4, but not K9
- **Gene level**
  - di-methyl H3-K4 appears to be a global epigenetic mark in euchromatic regions and tri-methylation of H3-K4 correlates with active transcription
  - Elongation - more later



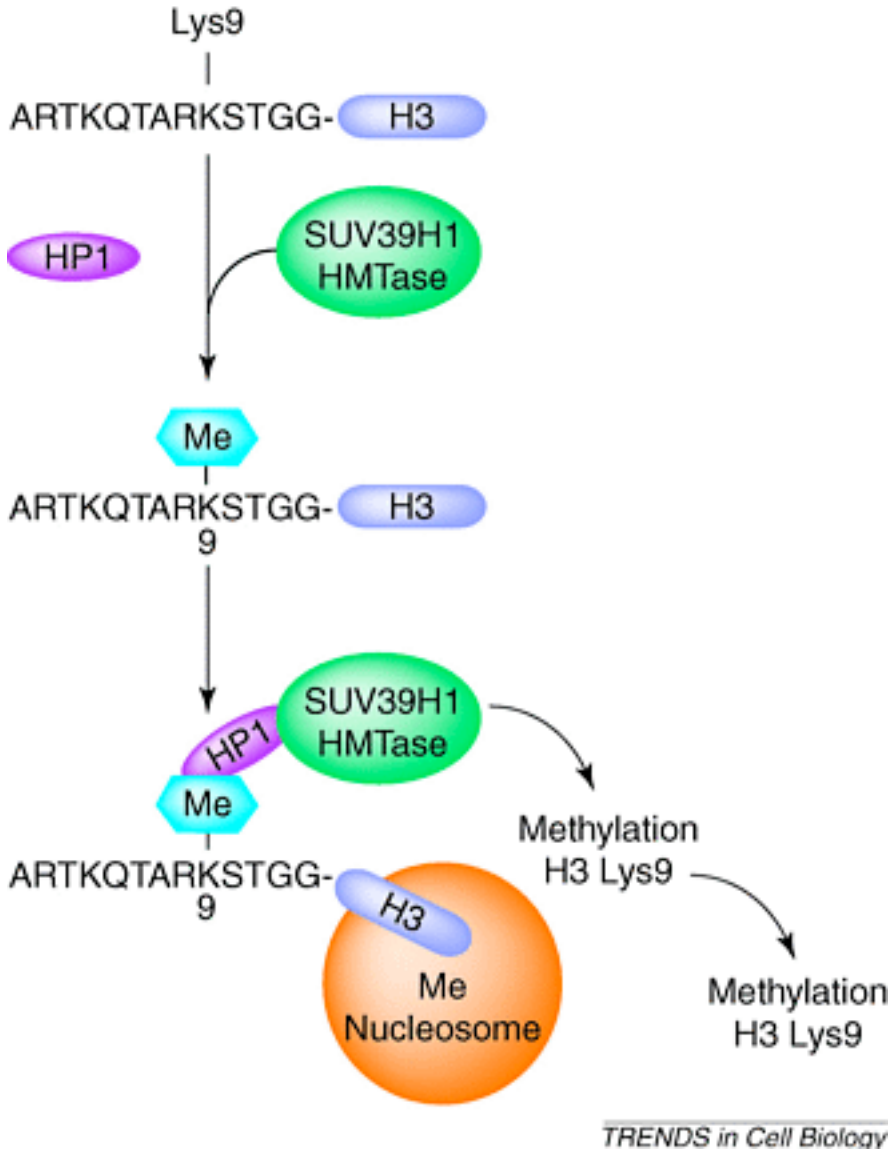
Recognition - the chromodomains

- Methylated lysines are recognized by the conserved chromodomain modules found in several chromatin-associated proteins.
- The chromodomain binds to histone tails bearing methyllysine in a highly specific manner, with the affinity being highest for trimethyllysine and lowest for monomethyllysine.



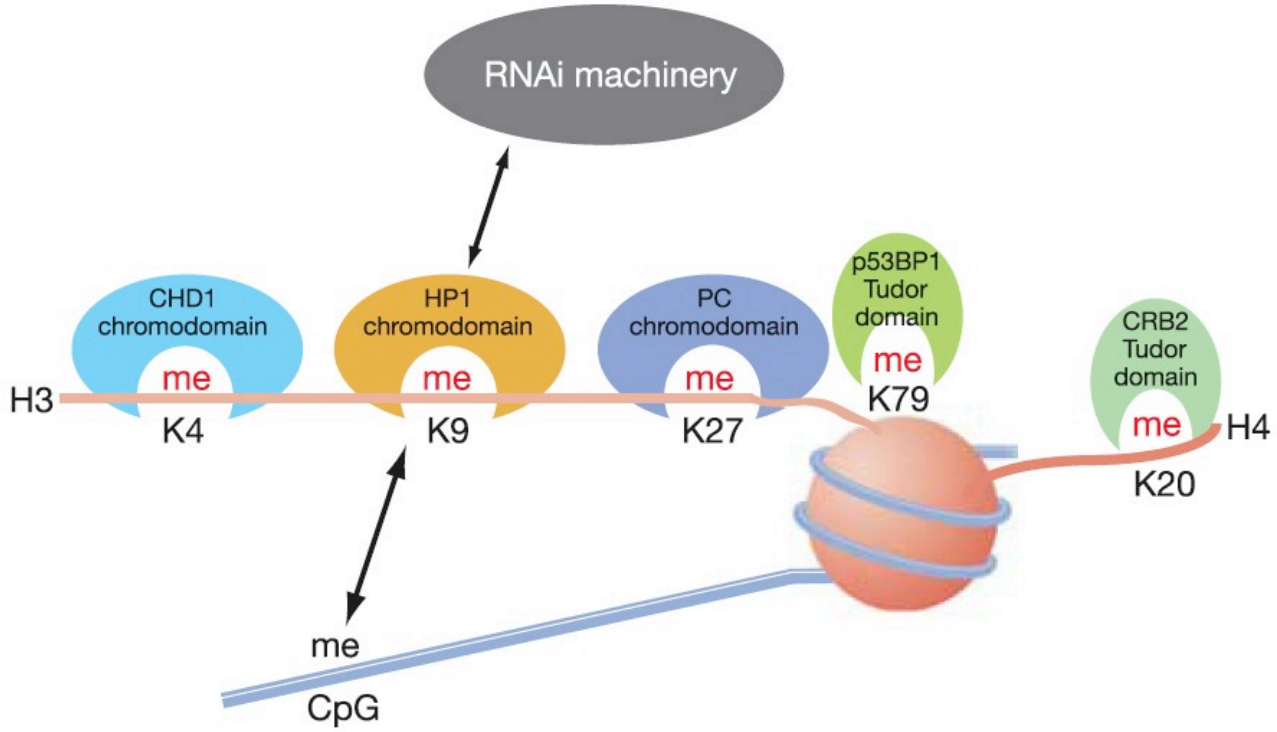
Recognition: Methylated histones recognized by spec chromodomains

- **Chromo-domains as recognition modules**
- **HP1 binds to histones methylated by SUV39H1**
  - H3 K9 methylation correlates with heterochromatin formation.
  - Heterochromatin protein 1 (HP1) is a methyl-lysine binding protein.
  - HP1 recruits SUV39H1 leading to propargation of methylation



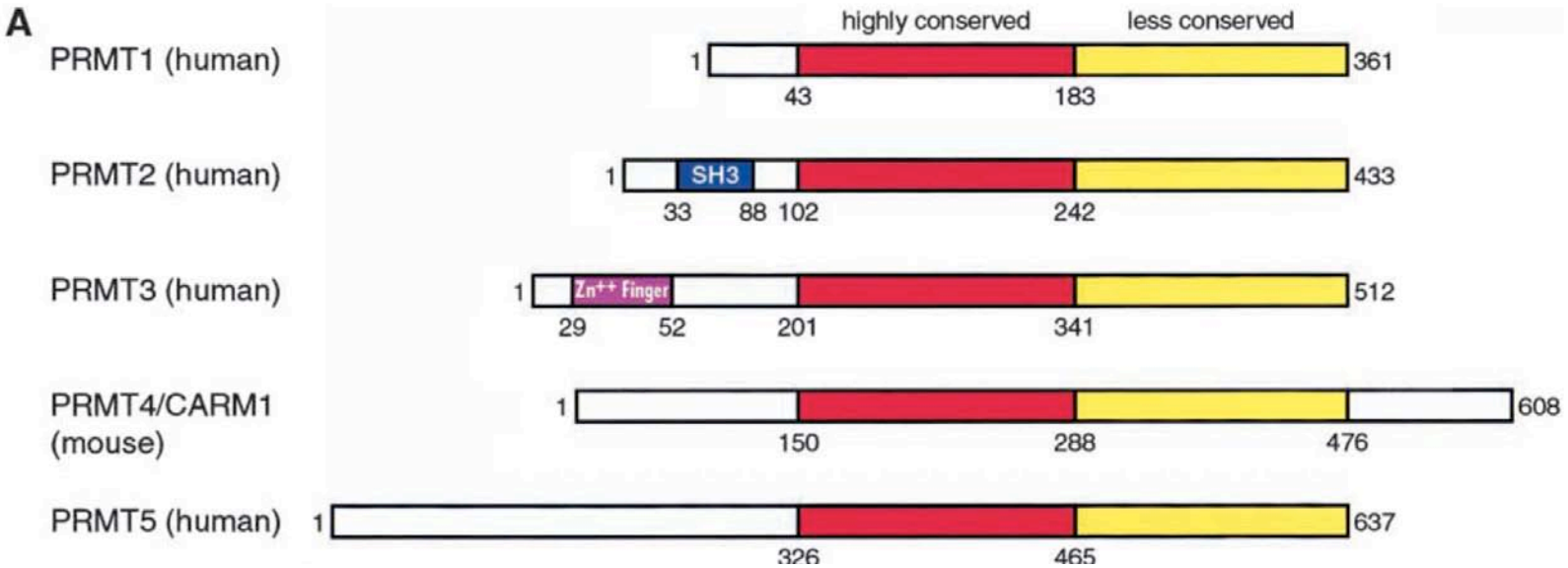
An increasing list of recognition modules

- The repressive proteins heterochromatin protein 1 (HP1) and the Drosophila Polycomb (PC) protein contain a chromodomain that allows them to specifically recognize the repressive methylation marks (H3K9 and H3K27), whereas the chromodomain helicase DNA-binding protein 1 (CHD1) activator protein from yeast uses its chromodomain to bind the activating methylated H3K4.



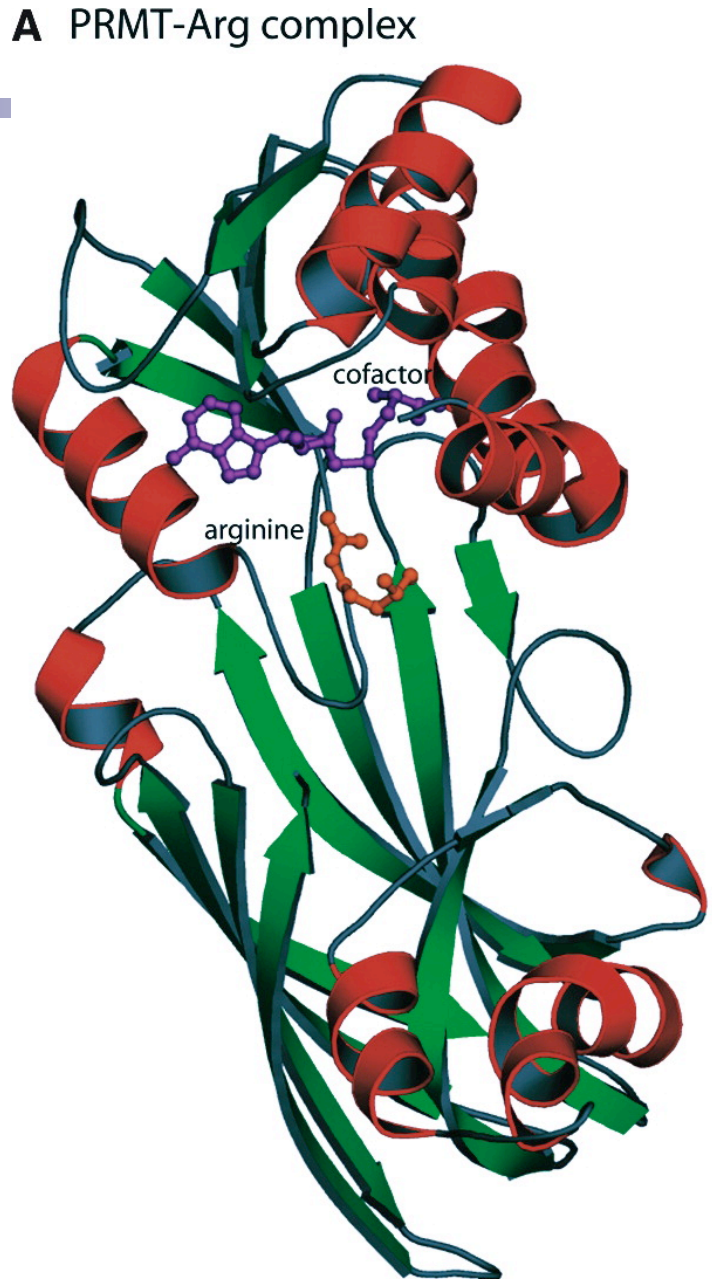
Histone arginine methyltransferases (HRMTs) – PRMTs

- **Several Arg HMTases identified:**
  - Carm1 (Coactivator-associated arginine methyltransferase).
  - PRMT1 (protein arginine methyltransferase).



The PRMT domain

- The catalytic module that methylates specific arginines is known as the PRMT (protein R methyltransferase) domain.
- The PRMT domain transfers the methyl group from SAM to the guanidino group of arginines to produce monomethylarginine or dimethylarginine.
- Some PRMT modules specifically prepare symmetric dimethylarginine and others produce asymmetric dimethylarginine.



Function

- **Methylation of specific arginines in histones H3 and H4 correlate with the active state of transcription.**
  - Ex: Methylation of Arg 3 of histone H4 facilitates H4 acetylation and enhances transcription activation by nuclear hormone receptors.

Arg Methylation	H3	R2	CARM1		Activation
		R17	CARM1		Activation
		R26	CARM1		Activation
	H4	R3	PRMT1	(p300)	Activation

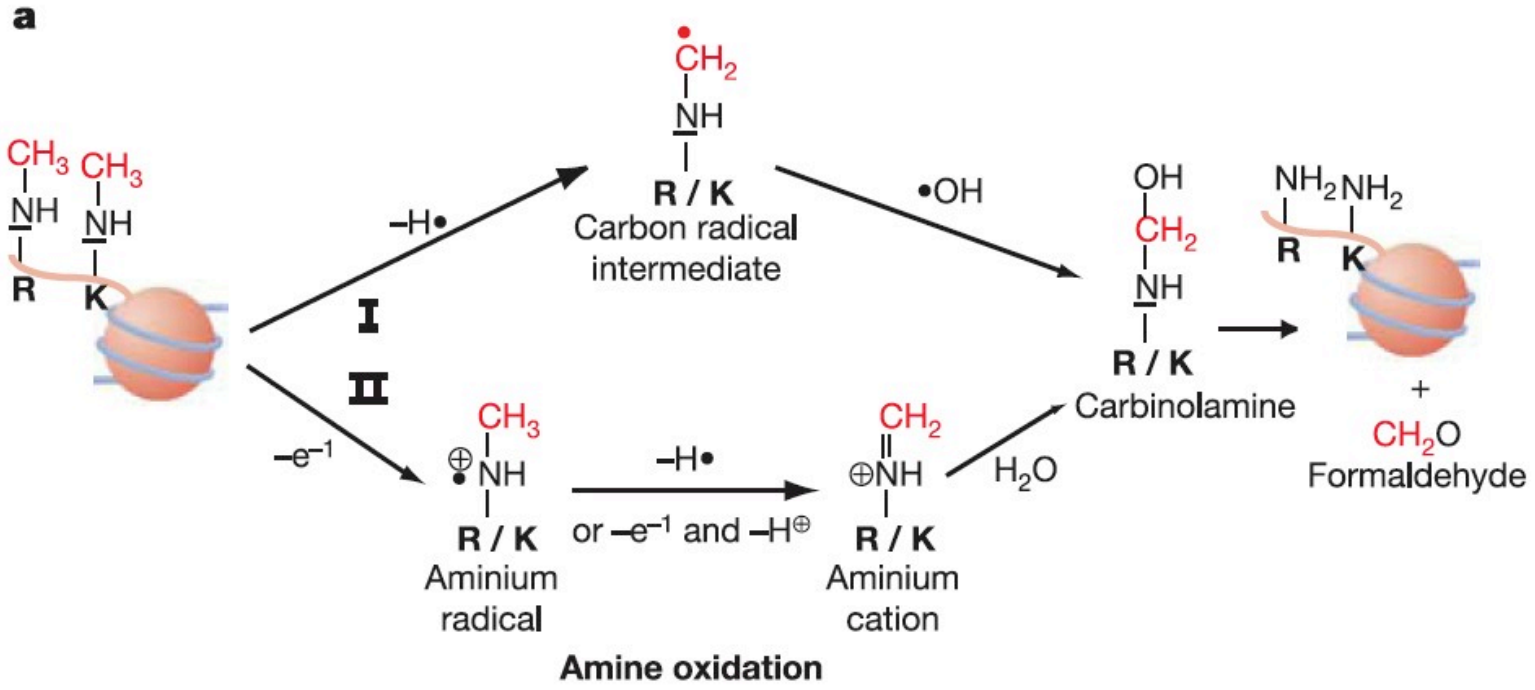
A dogma being challenged

Removing histone methylation?

- histone methylation has for long been believed to be an extremely stable modification,even considered an irreversible epigenetic mark
- histone demethylases was for long not discovered
- Hypothesis - histone methylation = a long-term epigenetic mark
- Still possible to remove?

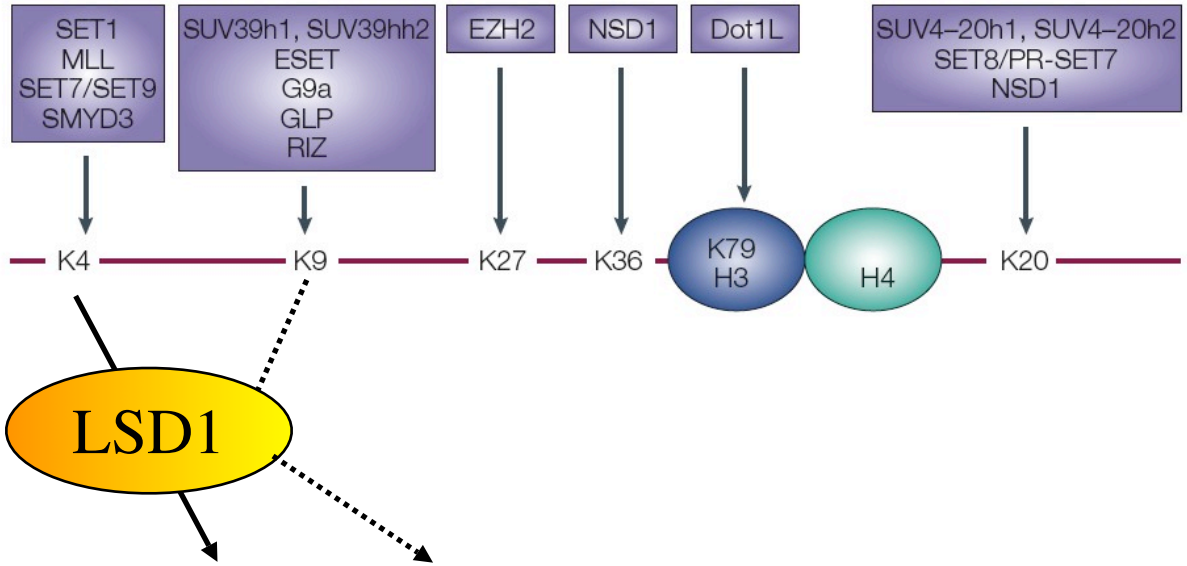
Enzymatic mechanisms

- Amine oxidation
- CH_3 cleaved off after oxidation as formaldehyde



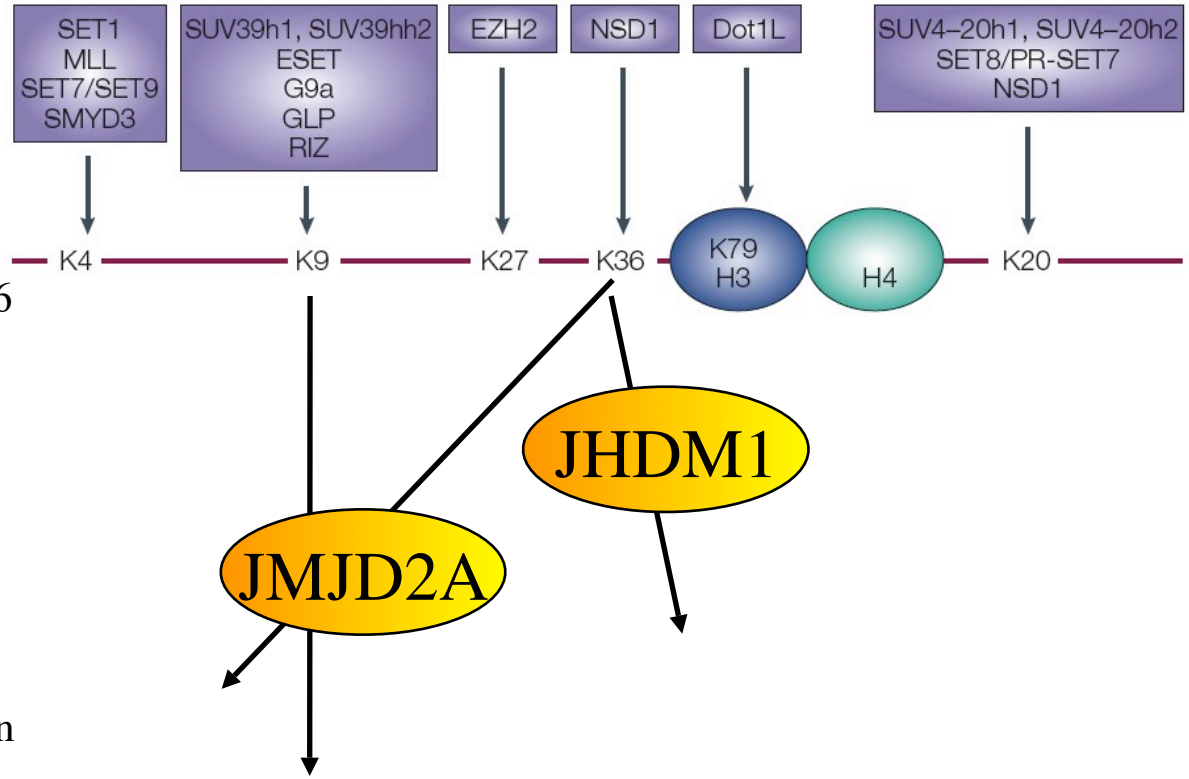
Recently discovered enzymes demethylating Lys: LSD1

- **LSD1**
  - LSD1 (lysine-specific demethylase) is able to demethylate H3K4 using an amine oxidase reaction dependent on FAD (flavin adenine dinucleotide).
  - Demethylation by LSD1 is limited to mono- or di-methylated H3K4: it cannot demethylate tri-methylated H3K4.
  - The androgen receptor alter the specificity of LSD1 from H3K4 to H3K9, and thereby converts the demethylase from a repressor to an activator of transcription.



Recently discovered enzymes demethylating Lys - JmjC-domain enzymes

- **JHDM1**
  - The JmjC domain is a signature motif for a new family of histone demethylases
  - JHDM1 (JmjC domain-containing histone demethylase 1) specifically demethylates histone H3 at lysine 36 (H3-K36).
  - Demethylation generates formaldehyde + succinate and utilizes the same oxidative demethylation mechanism as the AlkB DNA demethylases.
- **JMJD2A**
  - The JmjC domain-containing protein JMJD2A is a lysine trimethyl-specific demethylase able to reverse trimethylated H3-K9/K36 to di- but not mono- or unmethylated products.

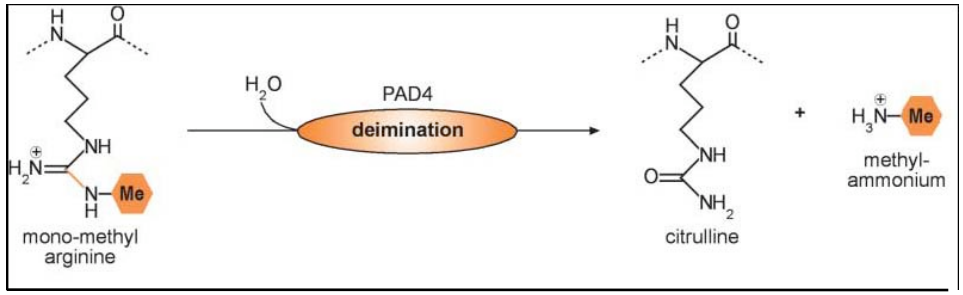


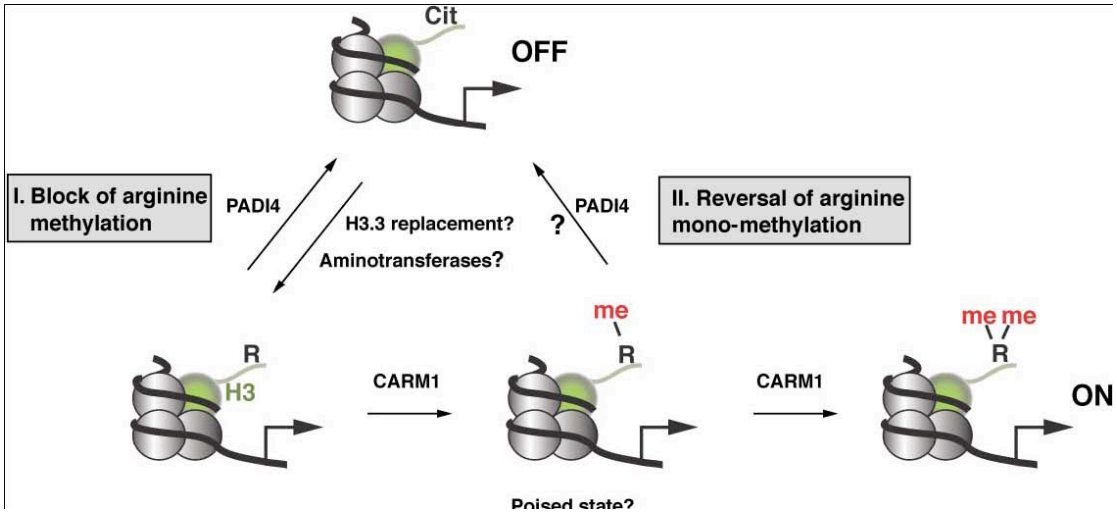
Current list of histone demethylates

Enzymes that Modify Histones	Residues Modified
Lysine Demethylases	
LSD1/BHC110	H3K4
JHDM1a	H3K36
JHDM1b	H3K36
JHDM2a	H3K9
JHDM2b	H3K9
JMJD2A/JHDM3A	H3K9, H3K36
JMJD2B	H3K9
JMJD2C/GASC1	H3K9, H3K36
JMJD2D	H3K9

Recently discovered enzymes demethylating Arg: deimination

- **PADI4**
  - Reversal of arginine methylation in histones can occur through the conversion of the methyl-arginine residue into citrulline.
  - This process is termed deimination, since the methyl group is removed along with the imine group of arginine.
  - The enzyme that mediates this reaction, peptidyl arginine deiminase 4 (PADI4), converts unmodified arginine and mono-methylated (but not di-methylated) arginine to citrulline at specific sites on the tail of H3 and H4.





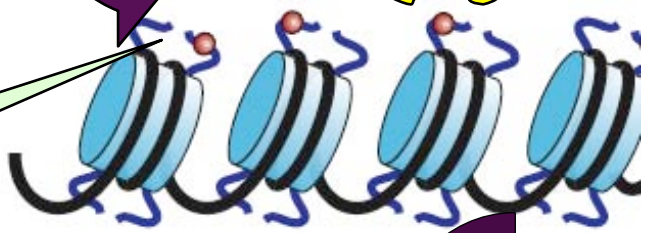
Summary

Several HKMTs
And HRMTs

X

Chromo-domain proteins
Binding protein

Mod enz



Specificity

Yes,
complex patterns

Function?

Trx repression
and activation
Epigenetics

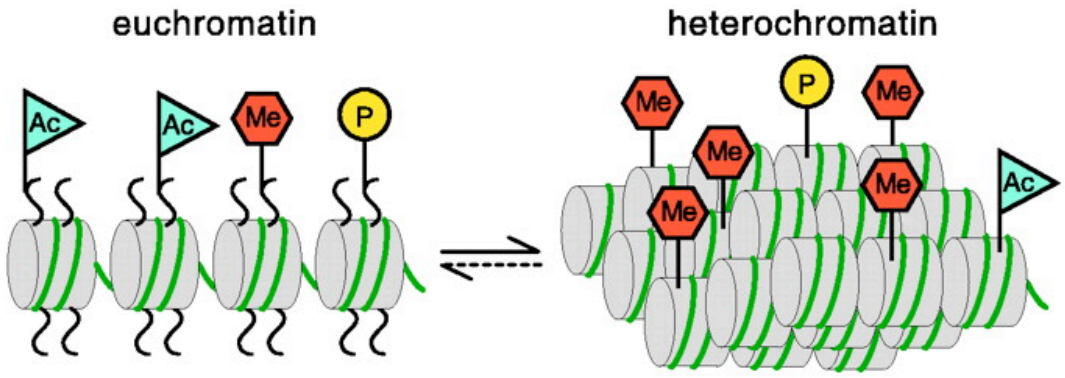
Demod enz

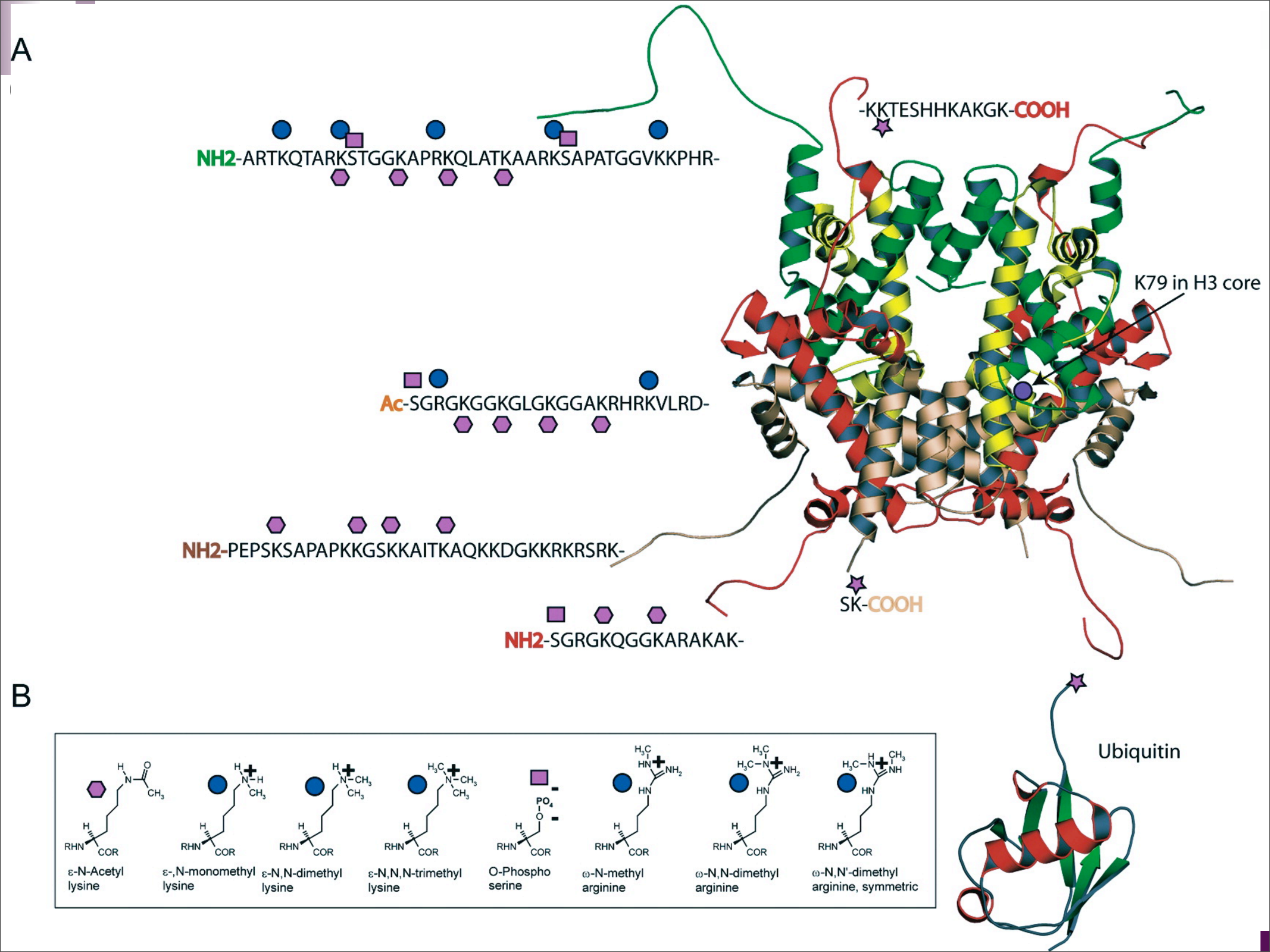
Several new enzymes are
now being discovered

Function?

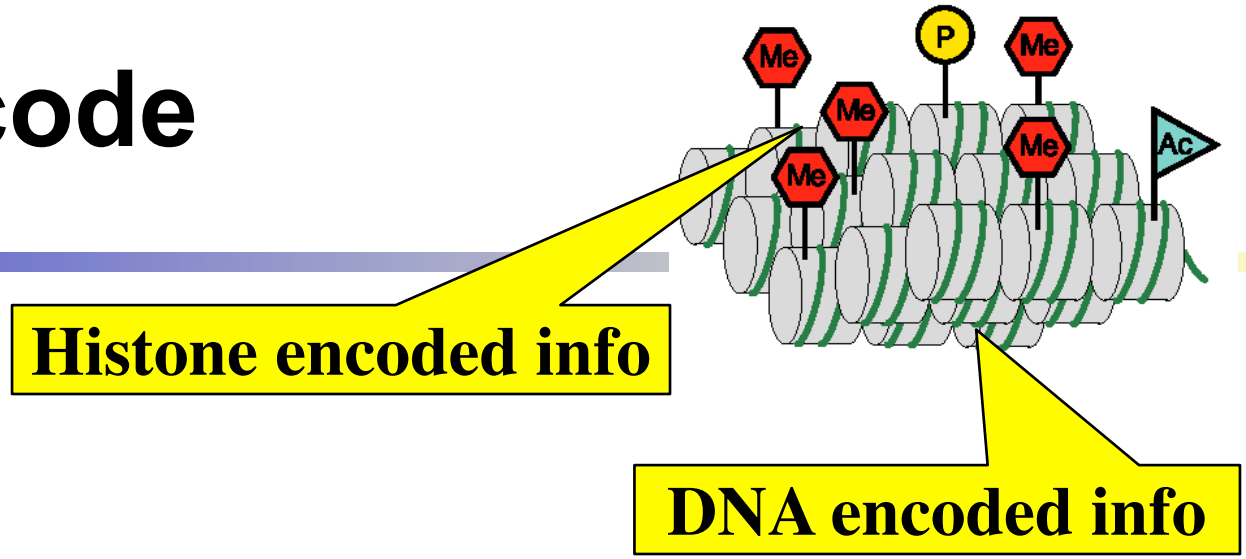
Trx repression
and activation
Epigenetics

The histone code




A
NH2-ARTKQTARKSTGGKAPRKQLATKAARKSAPATGGVKKPHR-
-KKTESHHKAKGK-COOH
K79 in H3 core
Ac-SGRGKGGKGLGKGGAKRHRKVLRD-
NH2-PEPSKSAPAPKKGSKKAITKAQKKDGKKRKRSRK-
SK-COOH
NH2-SGRGKQGGKARAKAK-
B
ε-N-Acetyl lysine
ε-,N-monomethyl lysine
ε-N,N-dimethyl lysine
ε-N,N,N-trimethyl lysine
O-Phospho serine
ω-N-methyl arginine
ω-N,N-dimethyl arginine
ω-N,N'-dimethyl arginine, symmetric
Ubiquitin

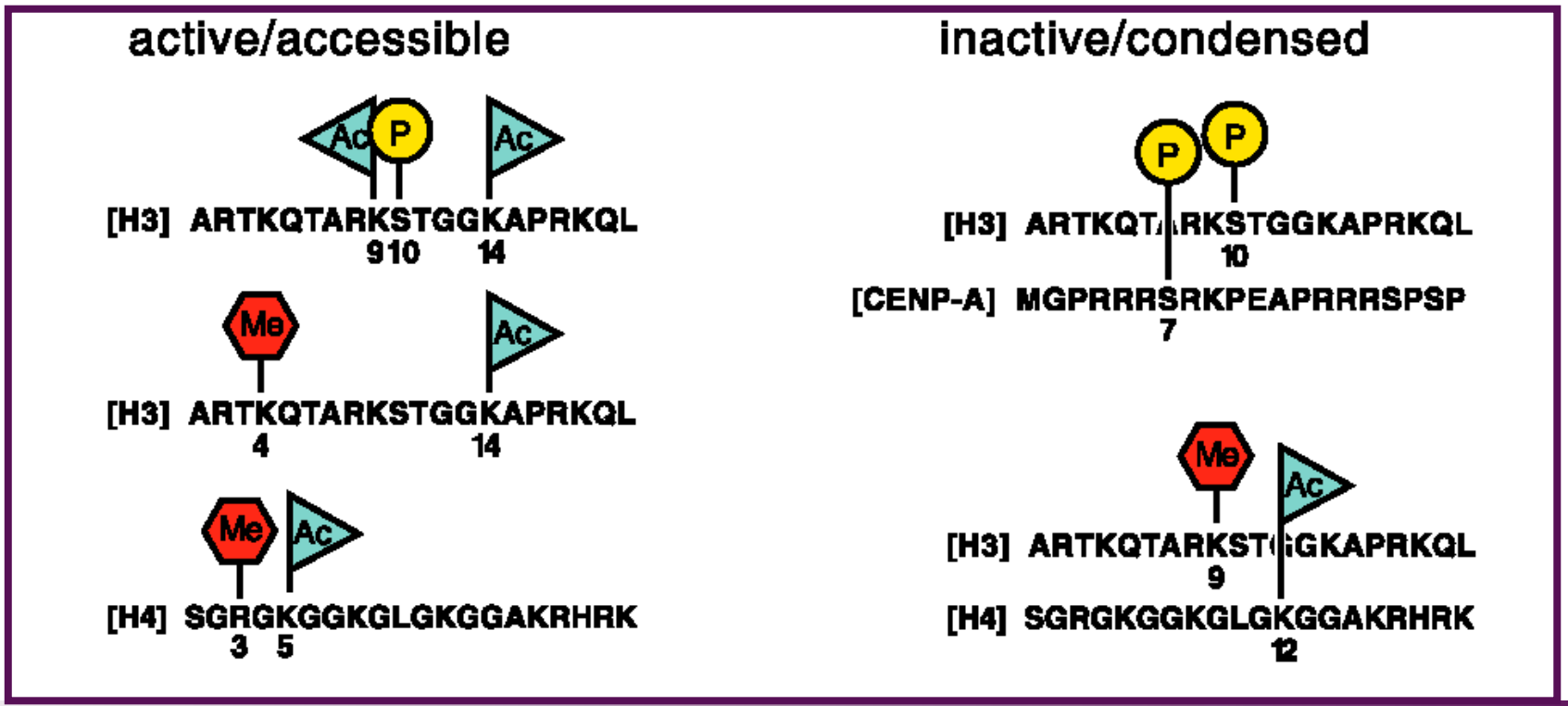
The histone code



- **Modifications of histone tails in specific patterns represent an encoded information extending the information content of the genome beyond the DNA sequence**
- **This hypothesis predicts that**
  - Distinct modifications of the histone tails will induce interaction affinities for chromatin-associated proteins
  - Modifications may be interdependent generate various combinations
  - Local concentrations and combinations of differently modified nucleosomes determine qualities of higher order chromatin

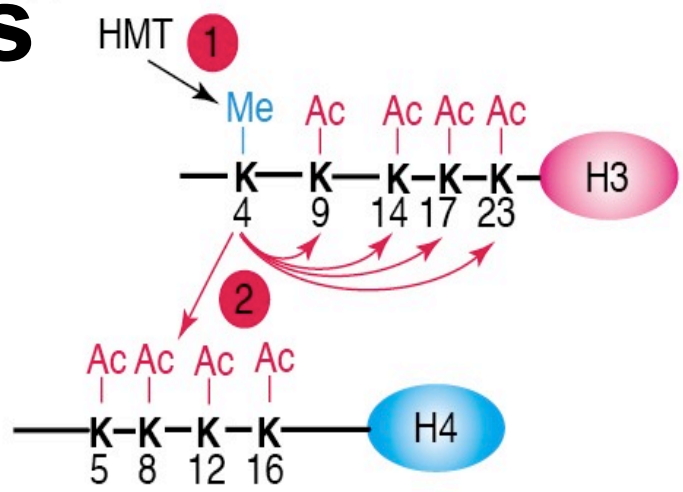
Combinatorial possibilities large - a language?

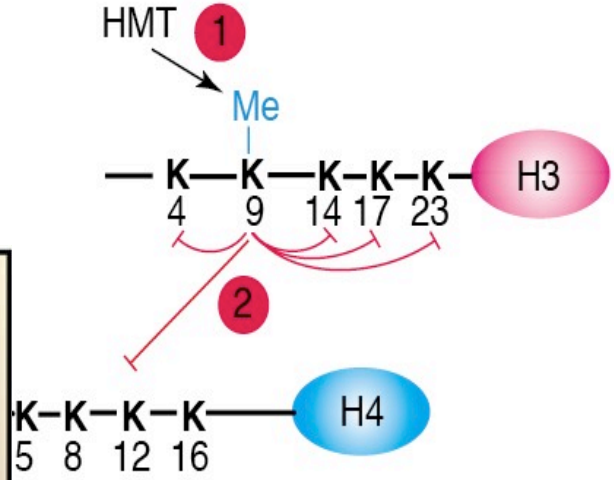
- Acetylated = acitve, deacetylated = inactive
  True but probably too simple
- Many combinations - link to active/inactive chromatin complex
- Number of combinations very high

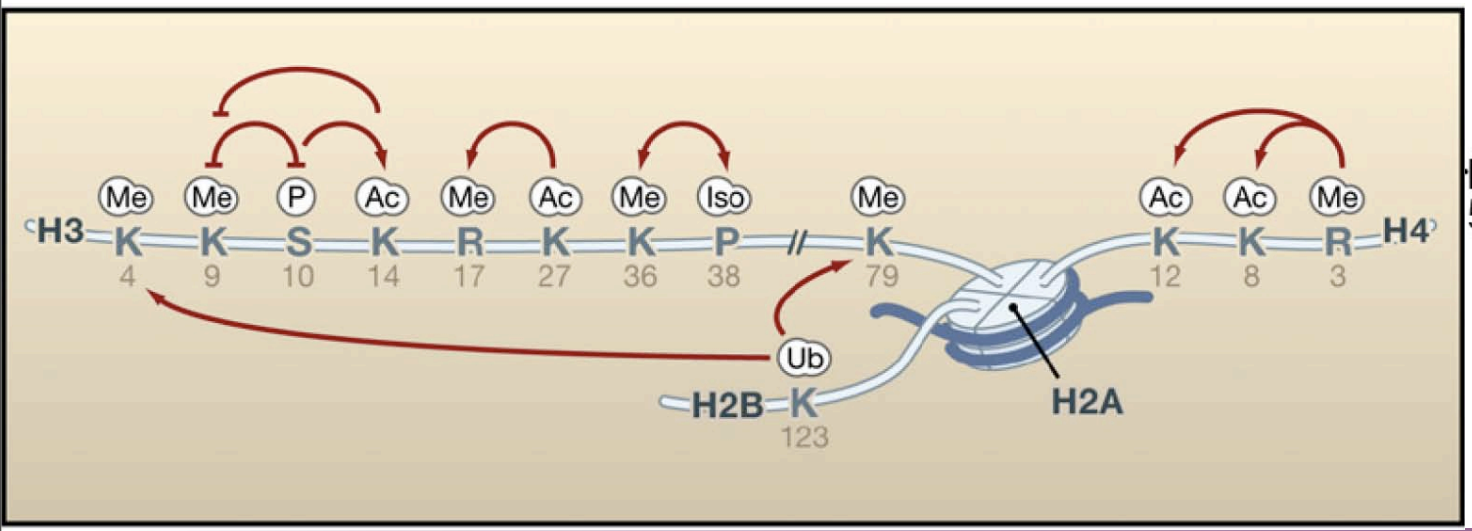


Synergistic or antigonistic modifications of histones

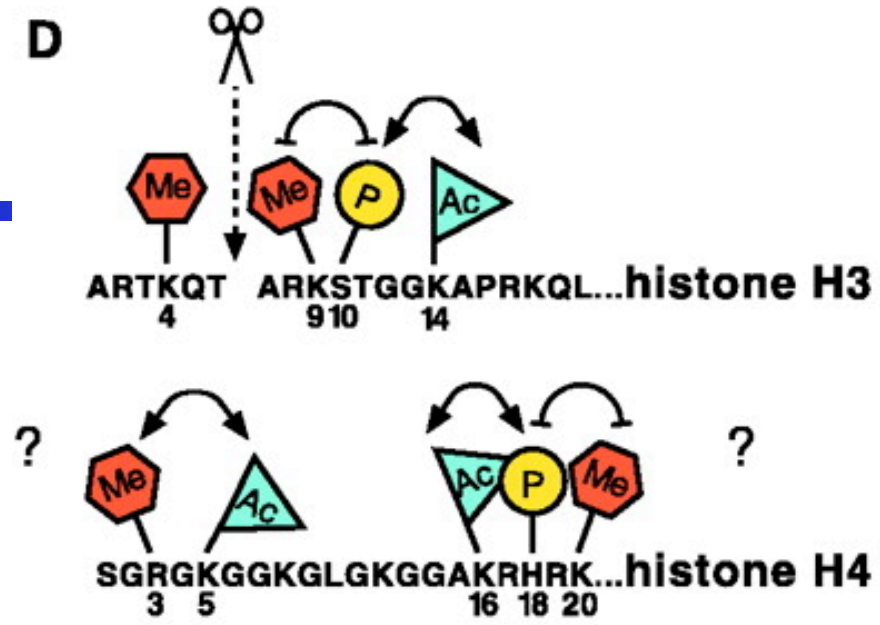
- One modification can enhance or inhibit another modification

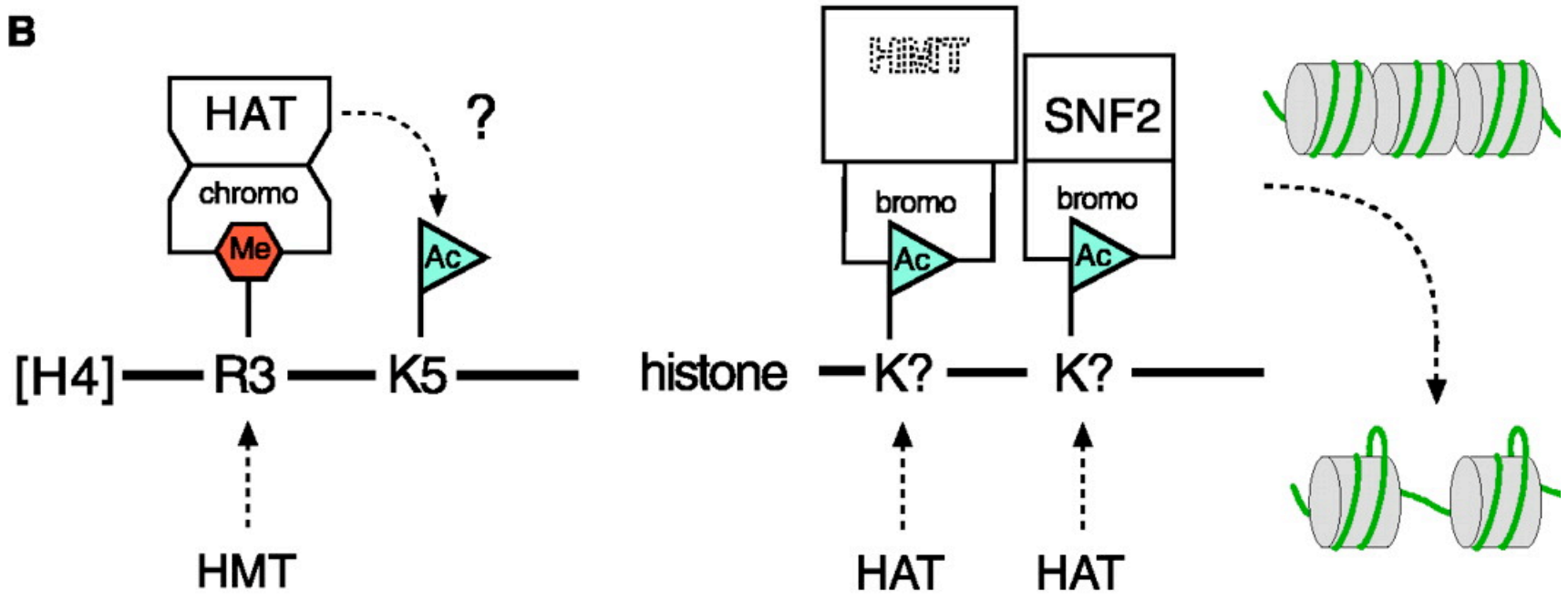






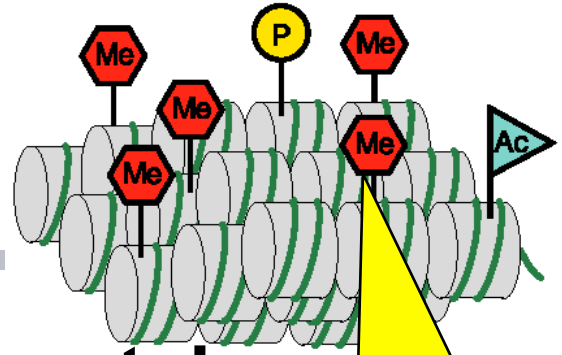
Cross-talk between histone modifications





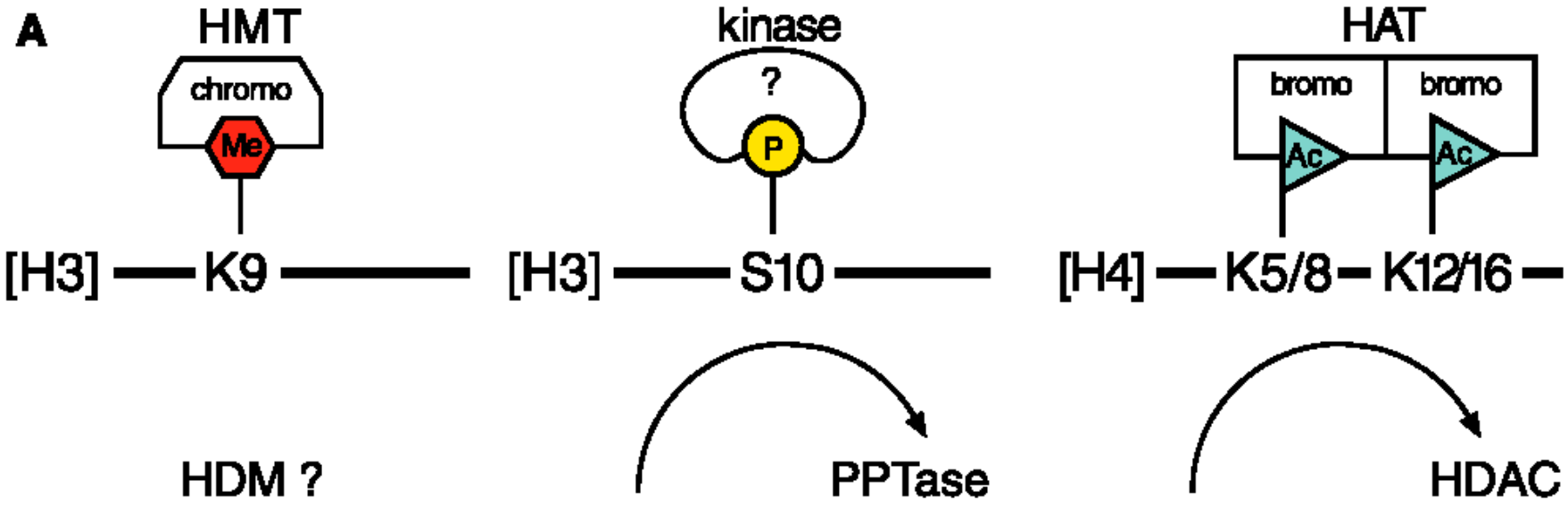
Science 2001, 293, 1074-1079.

Reading / translating the histone code

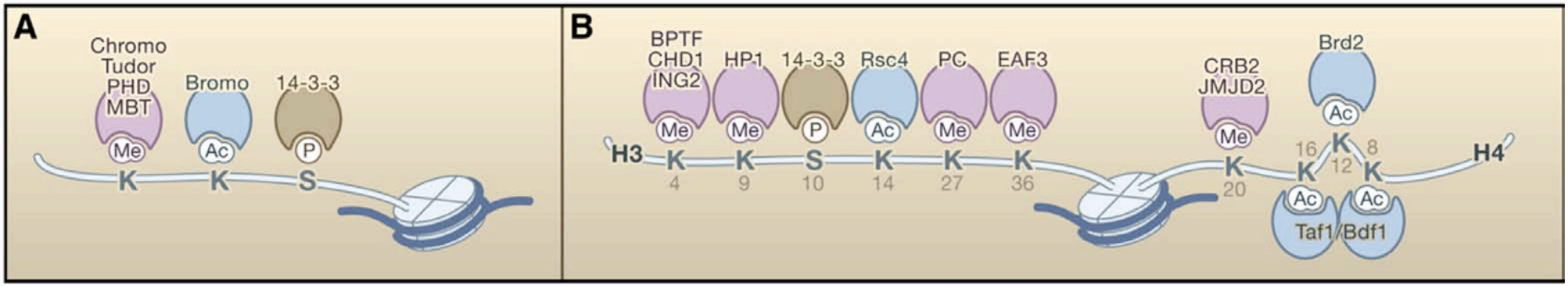


How to read?

- **Modifications = binding sites for protein modules**
  - **Bromodomains** bind acetylated lysines
    - 75 bromo-containing proteins in humans
  - **Chromodomains** bind methylated sites
  - Domains recognizing phosphorylated tails unknown



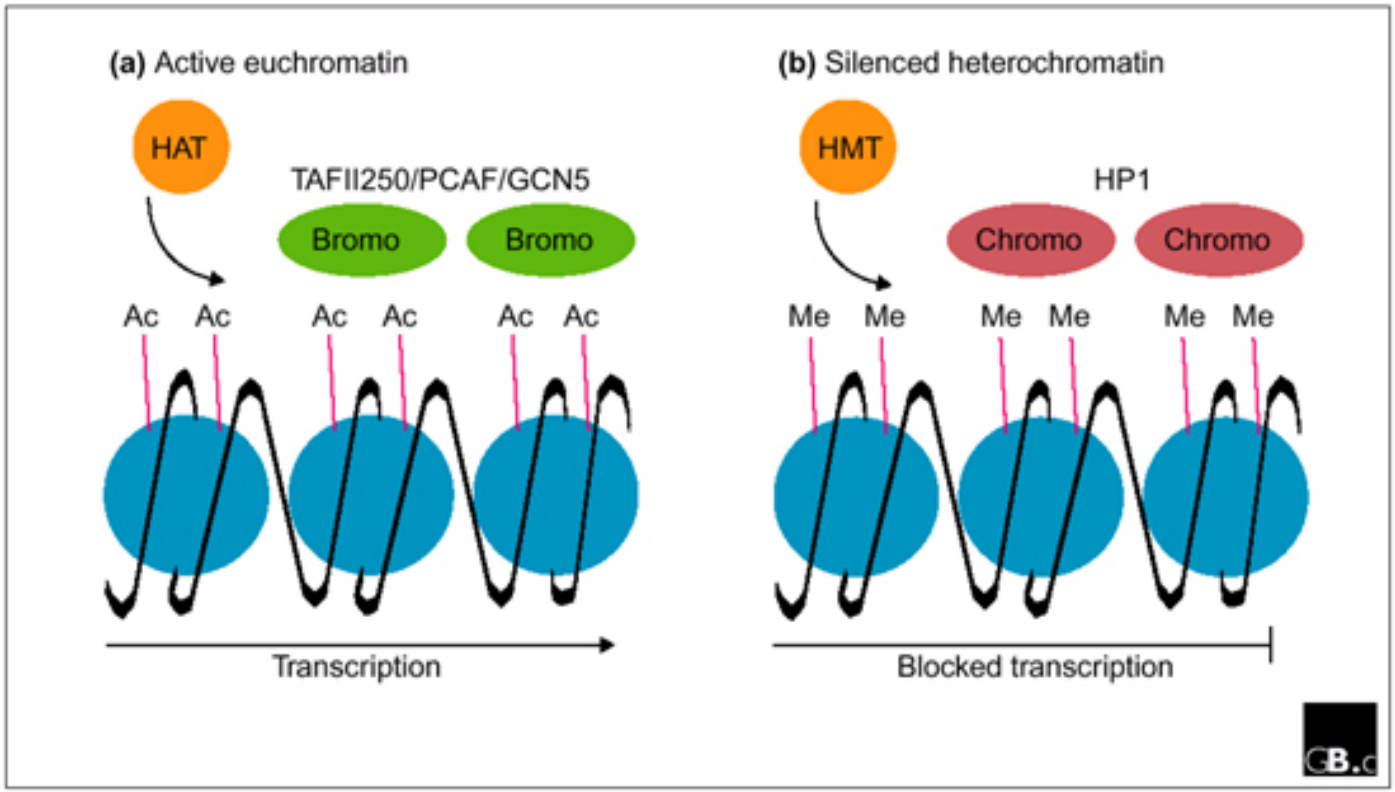
Reading the code



- (A) Domains used for the recognition of methylated lysines, acetylated lysines, or phosphorylated serines.
- (B) Proteins found that associate preferentially with modified versions of histone H3 and histone H4.

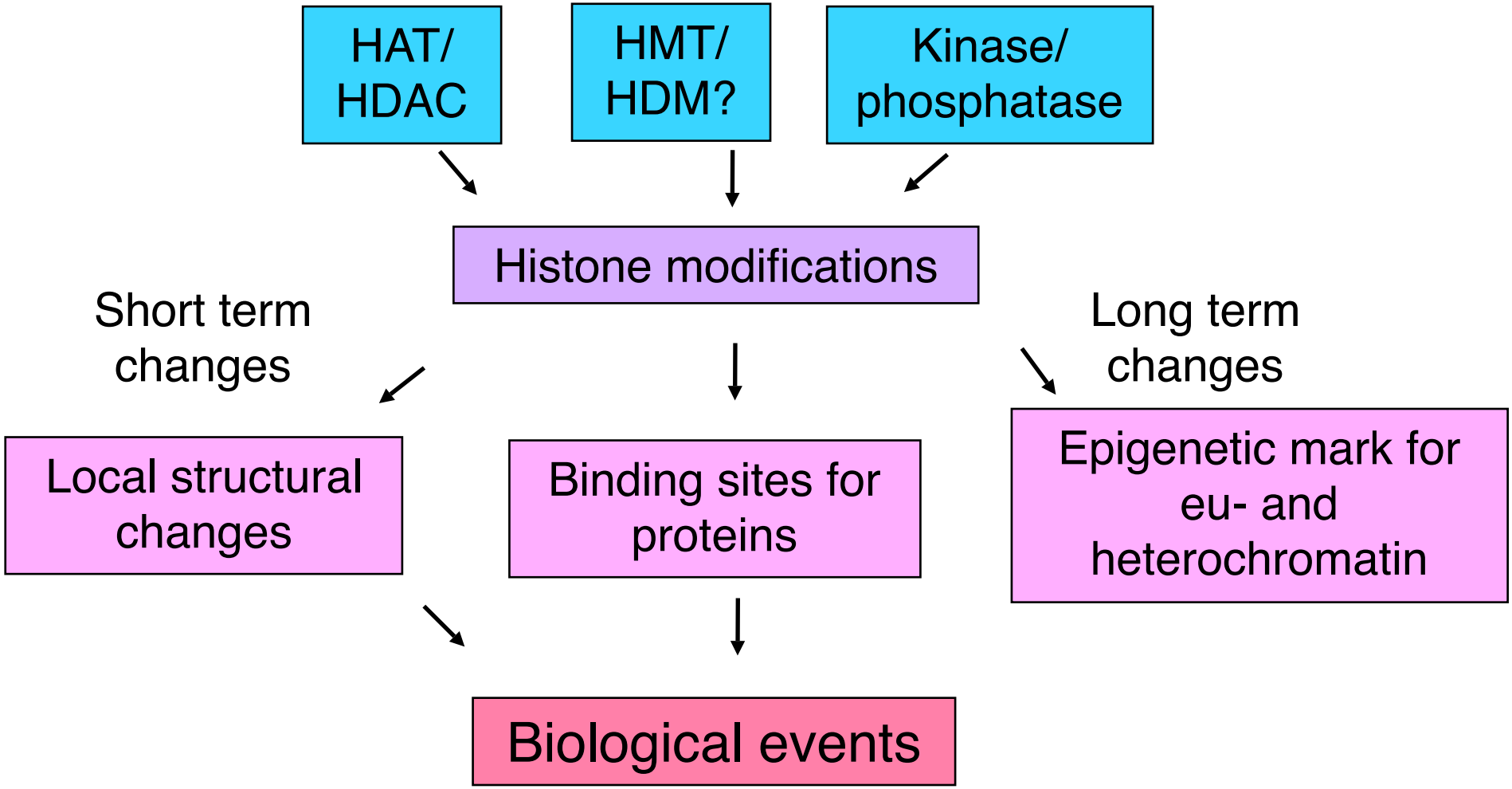
Writing & Reading the histone code

- The authors: HMTs, kinases, HATs, PPTases, HDACs, HDM?
- The readers: Chromo- and bromo domain proteins.



Genome Biology 2001, 2(4): reviews003.1.6

Mendel's gene is more than just a DNA moiety!!

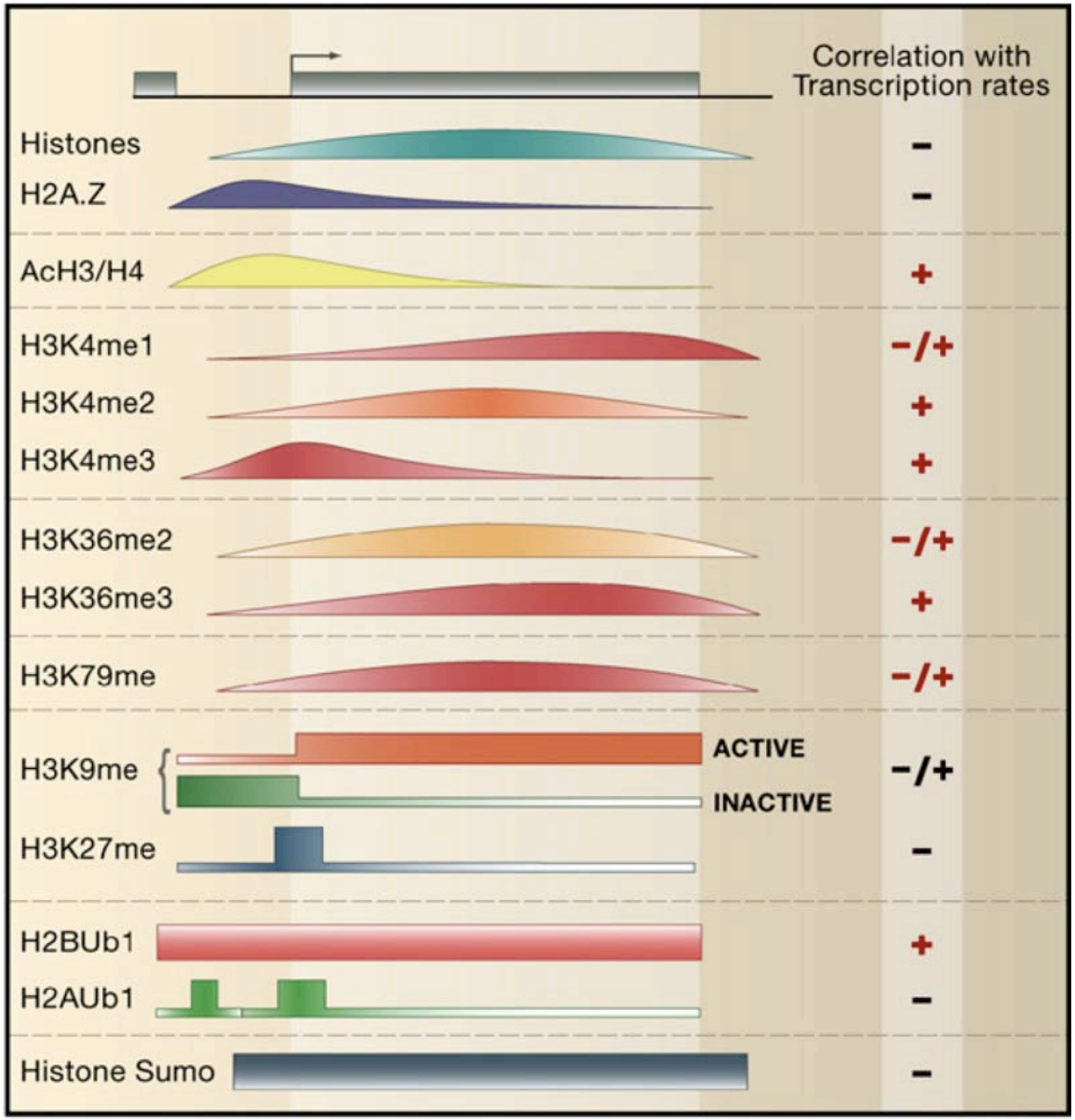


ORF patterns of histone modifications

Distribution of histone modifications along a general ORF (Open reading frame)

H3K4

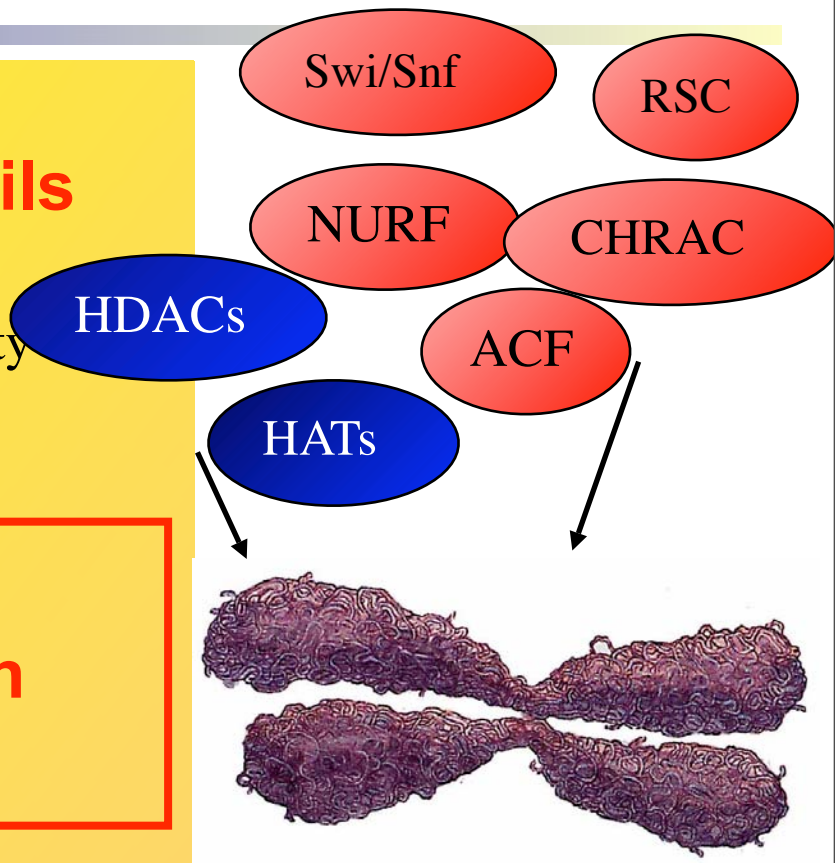
- monomethylation is enriched toward the 3´- end, dimethylation peaks in the middle, whereas trimethylation occurs around the TSS and the 5´-end of the ORF
- The importance of H3K4 methylation might be due to recruitment. Chromatin-remodeling factors(NURF) and histone-modification complexes (hTip60, yNuA3, etc.) contain PHD domains that recognize H3K4 methylation, recruiting complexes to activate/ repress transcription.



ATP-dependent remodelling

Changing the chromatin-template to help transcription factors

- **1. Opening of chromatin through directed modication of histone tails (acetylation and methylation)**
  - Histone-modification by HAT and HMT activity
  - Basis for the histone code hypothesis

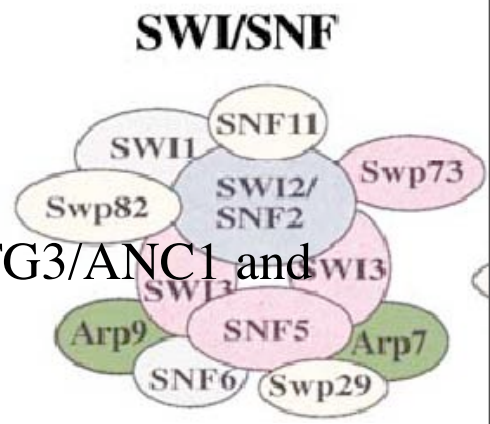


- **2. Opening of chromatin through directed nucleosome mobilization**
  - ATP-dependent process

- **3. Positioning of nucleosomes creates promoters with different requirement for remodeling**

First discovered complex that remodels chromatin: the SWI/SNF complex

- **SWI/SNF complex originally identified genetically as a positive regulator of *HO* and *SUC2* genes**
  - SWI - *HO*-genet inolvert in mating type switching
  - SNF - Sucrose non-fermentor: *SUC2* involvert in sucrose-fermenterng
- **SWI/SNF complex needed for full activity of some TFs**
  - In its absence : GAL4 tenfold reduced
  - basal transcription not affected
- **Exists as a complex of about 2 MDa**
  - Several subunits: SWI1, **SWI2**, SWI3, SNF5,SNF6, SNF11, TFG3/ANC1 and SWP73, +some others
  - Few molecules per cell ≈ 100
- **Chromatin-link: Supressors of defect SWI/SNF complex = chromatin components**
  - several histone mutatants restore activator response
  - Suggested a function in counteracting chromatin-dep repression

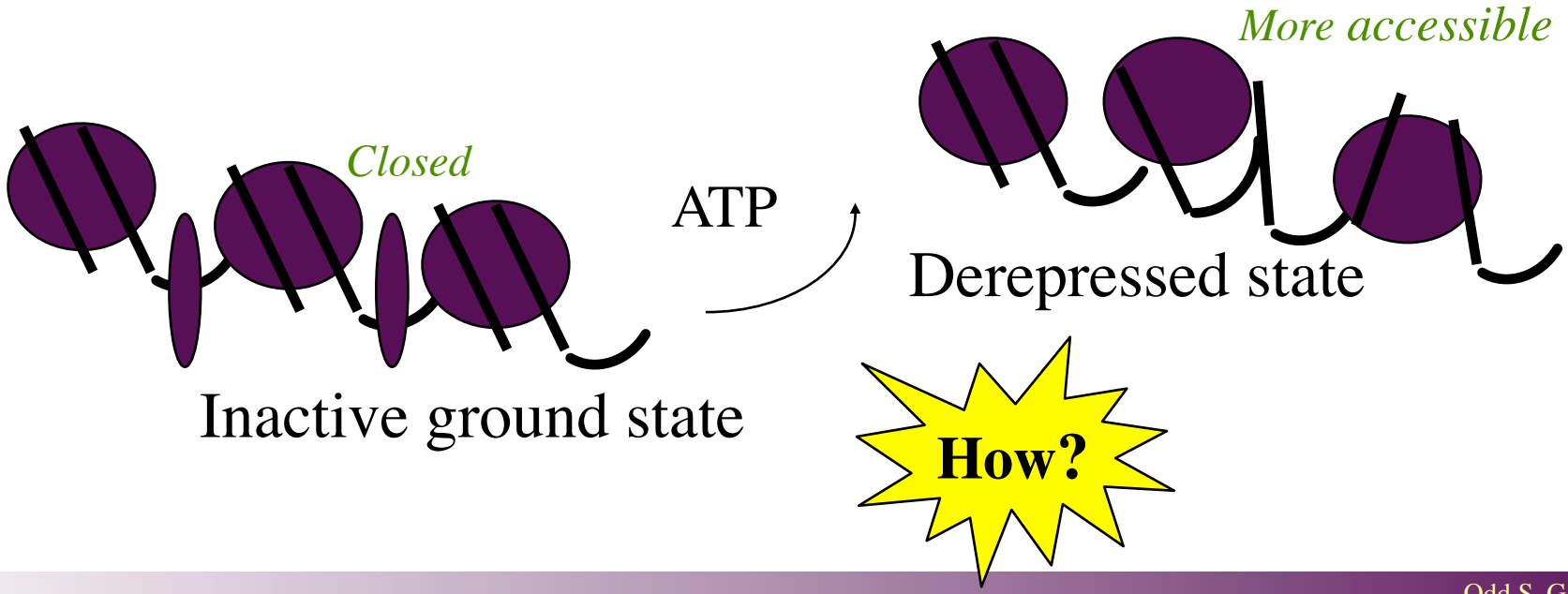


Associates with nucleosomal DNA

- **DNA-interaction**
  - Swi/Snf binds DNA and nucleosomes with high affinity
    - Kd = nM range
  - Binding to DNA is ATP-dependent and targets minor groove
  - After binding to DNA starts an ATP-dependent remodelling of nucleosomes
    - 1000 ATP molecules consumed pr min
  - The Nucleosome is not removed during this process

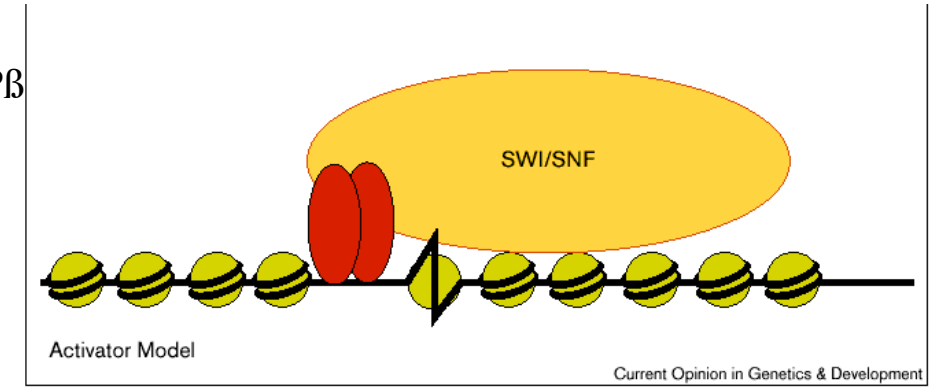
SWI2 - the motor in the machinery - running on ATP

- **SWI2-subunit harbors a DNA-stimulated ATPase activity**
  - mutated ATPase → defect SWI-SNF function
- **Function: Use ATP hydrolysis to increase the accessibility of nucleosomal DNA**



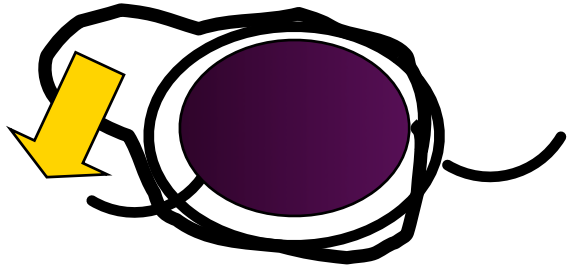
Recruitment

- **Swi/Snf acts probably only on a subset of genes**
  - Genome-analysis in yeast shows that <5% of all gener require Swi/Snf
  - Limited number of molecules pr cell (100-500 Swi/Snf pr celle)
  - Swi/Snf must be recruited to those promotere requiring Swi/Snf function
- **Recruitment model**
  - Gene-specific activators recruit Swi/Snf-complex directly
  - Several activators shown to have direct interaction with the Swi/Snf-complex
    - GCN4, SWI5, GAL4-VP16, GR, C/EBPß

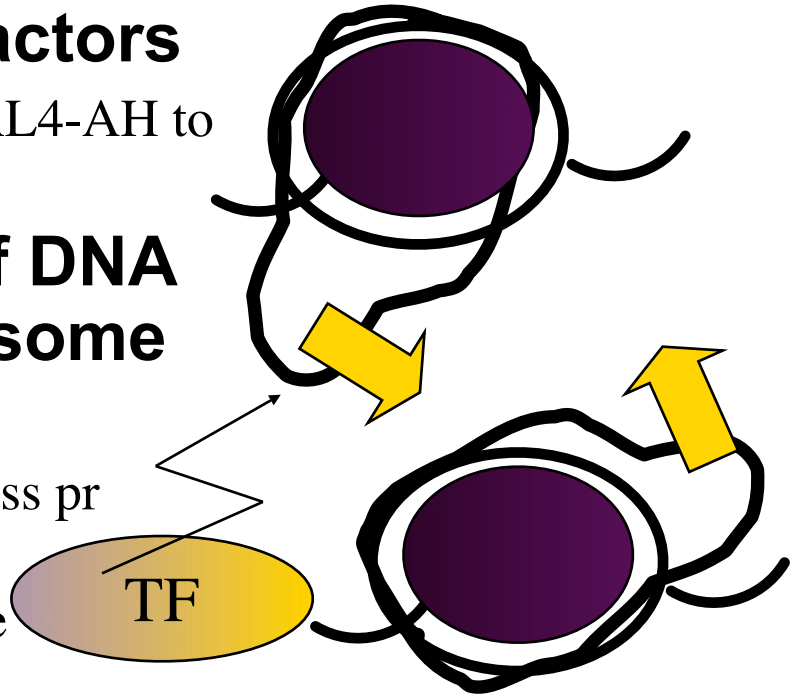


Mechanims of nucleosome disruption *unwrapping / unpeeling*

- **Unclear exactly what ”disruption” means**
  - Structurally changed nucleosomes or removal of the octamer?
  - Or some sort of altered DNA conformation

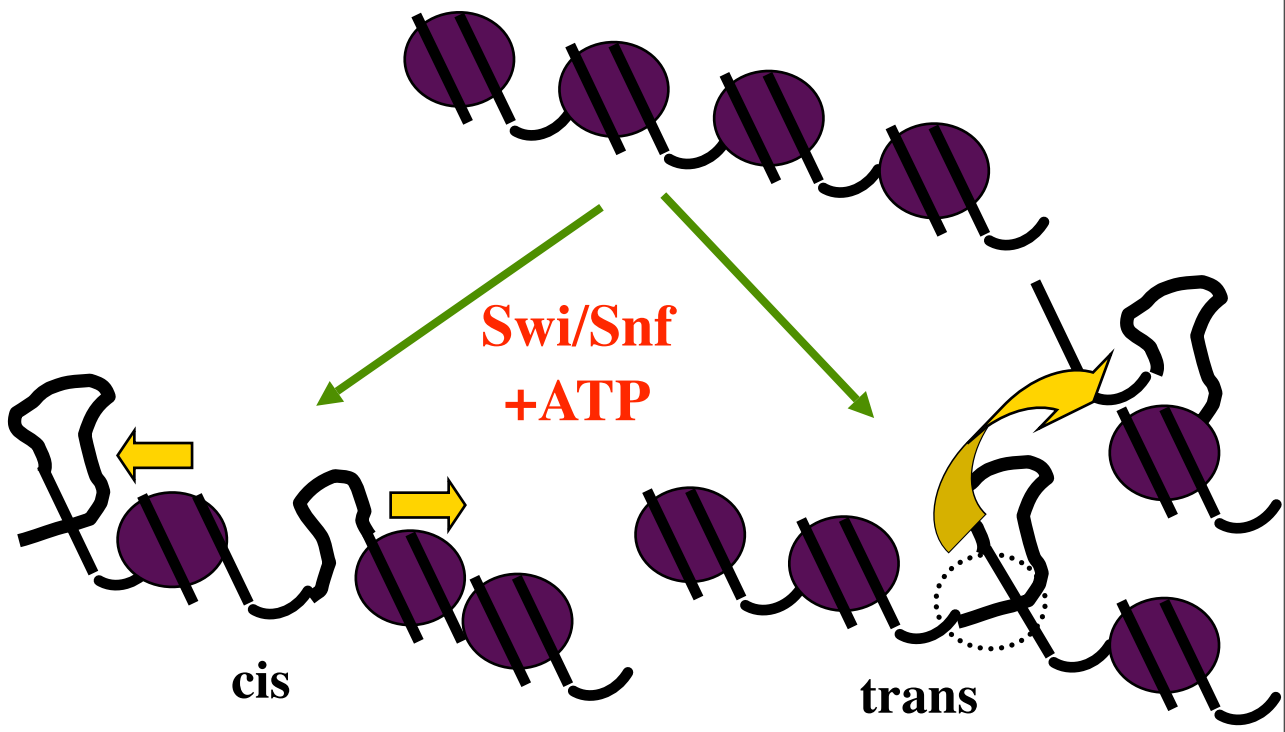


- **Remodeled chromatin is more accessible for TFs and other factors**
  - Purified complex stimulates binding of GAL4-AH to nucleosmal DNA 10-30-fold
- **Model: partial ”unwrapping” of DNA from the surface of the nucleosome provides DNA access**
  - Reduced DNA-mass relative to protein-mass pr nucleosome
  - Reduced DNA-supercoiling pr nucleosome

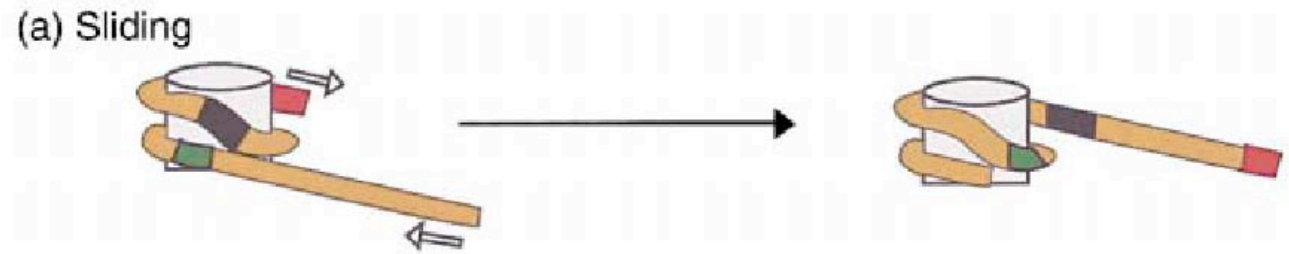


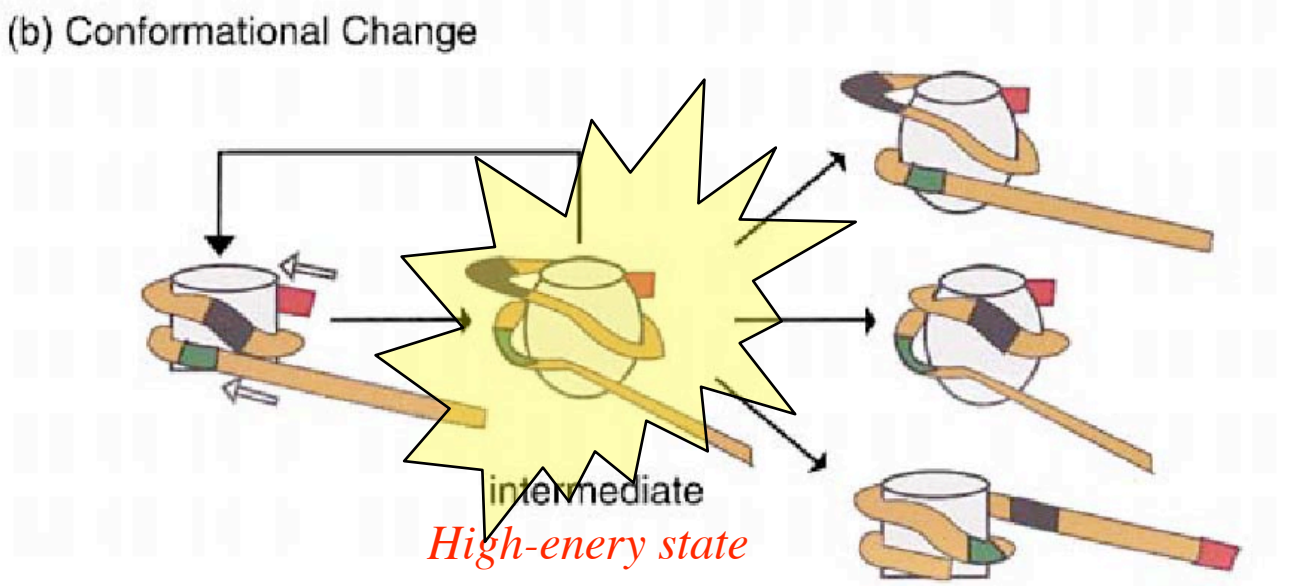
Remodeled chromatin
also through ”sliding” or ”transfer”

- **Removal of nucleosomes can happen by local displacement along the same strand**
  - in *cis* (sliding)
  - by ”spooling” or ”twisting”
- **Or by transfer**
  - i *trans* (transfer)



Two main mechanisms

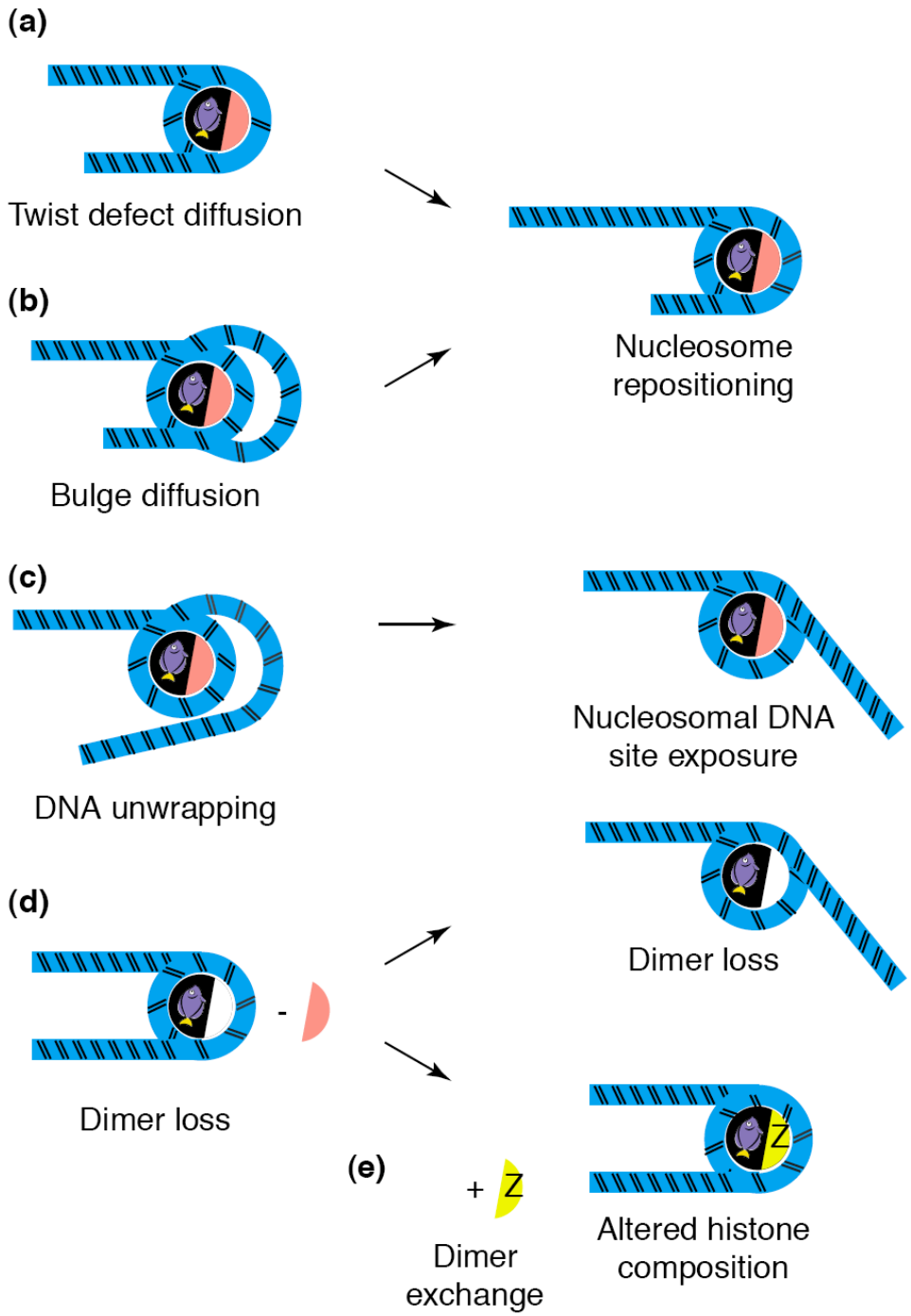




High-enery state generated by ATP?

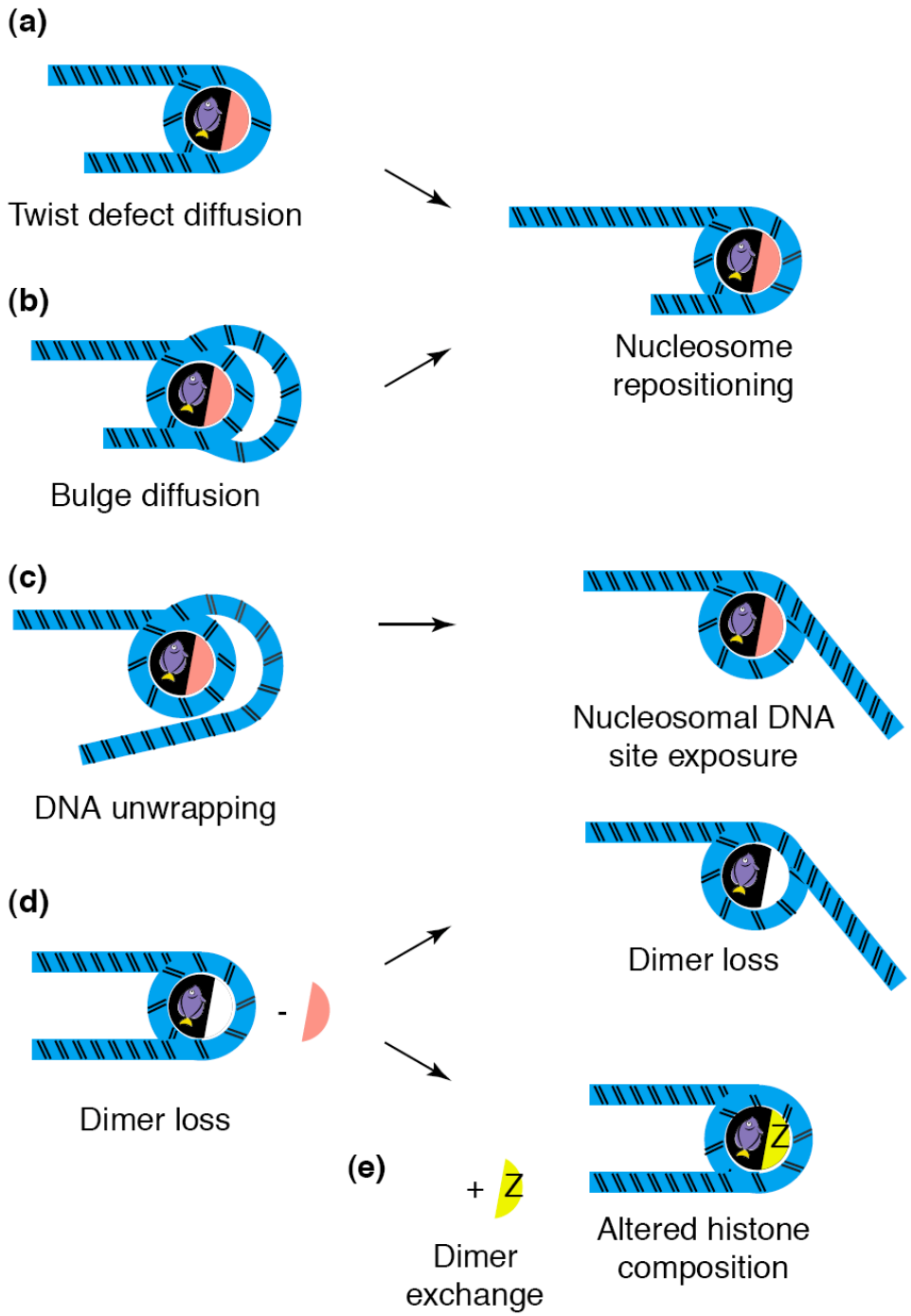
More mechanisms

- ATPase motors that drive these enzymes seem to function as ATP-dependent DNA translocases with limited processivity
- Can also be regarded as ‘nucleosome isomerases’



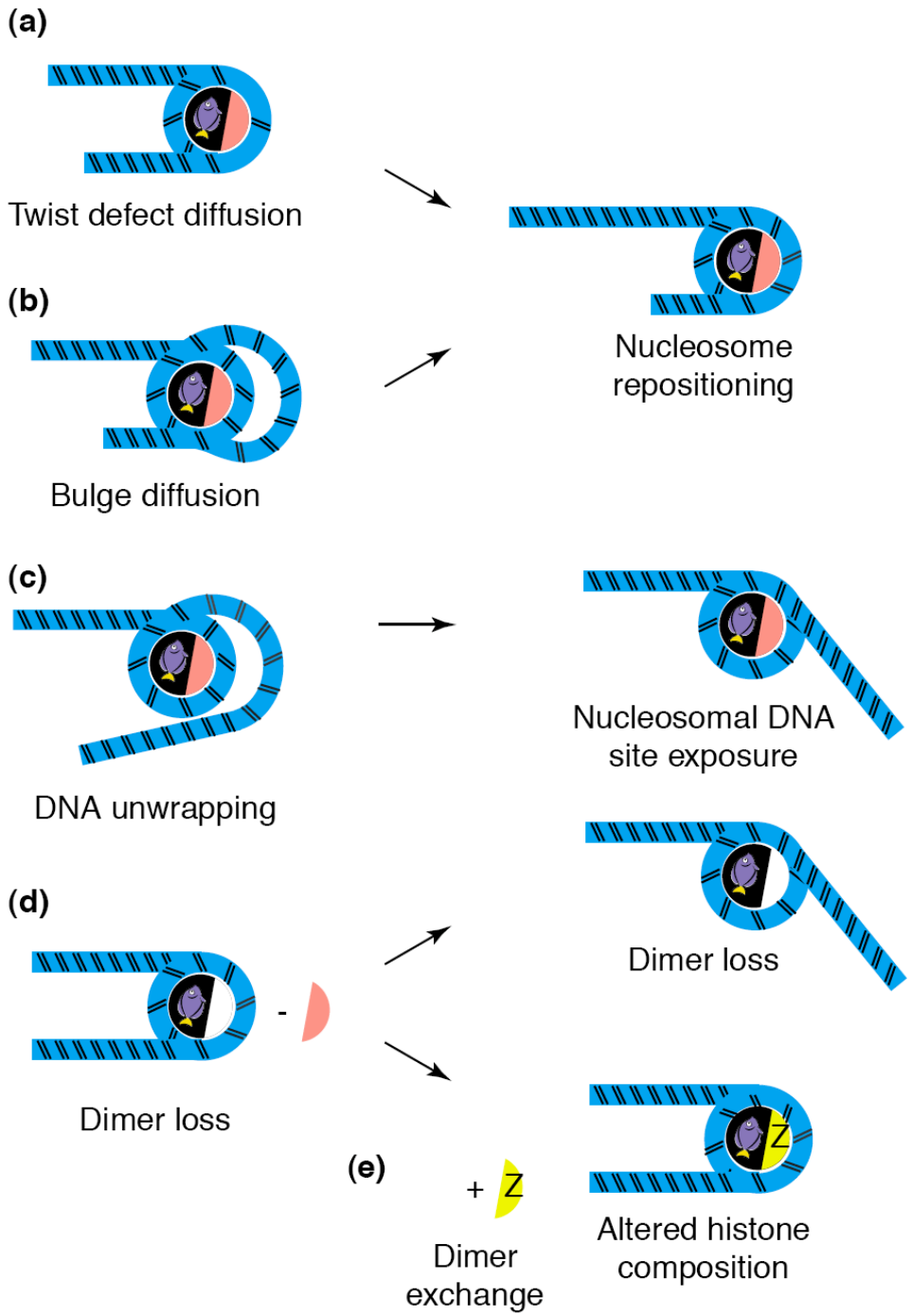
Mechanisms

- **1. Twist defect diffusion**
  - In this model, small local alterations from mean DNA twist ('defects') propagate around the nucleosome
- **2. Bulge diffusion**
  - In the bulge diffusion model, unpeeling of DNA from the histone octamer at the entry/exit of the nucleosome and subsequent rebinding of more distal sequences to the same histone contact points would establish nucleosomal particles harbouring excess DNA.
  - Subsequent migration of the bulge around the nucleosomal superhelix would result in the nucleosome apparently stepping - 40-60 bp



Mechanisms

- Histone dimers can be removed or exchanged between nucleosomes during the course of remodelling reactions
- Furthermore, remodelling may cause alteration of chromatin composition

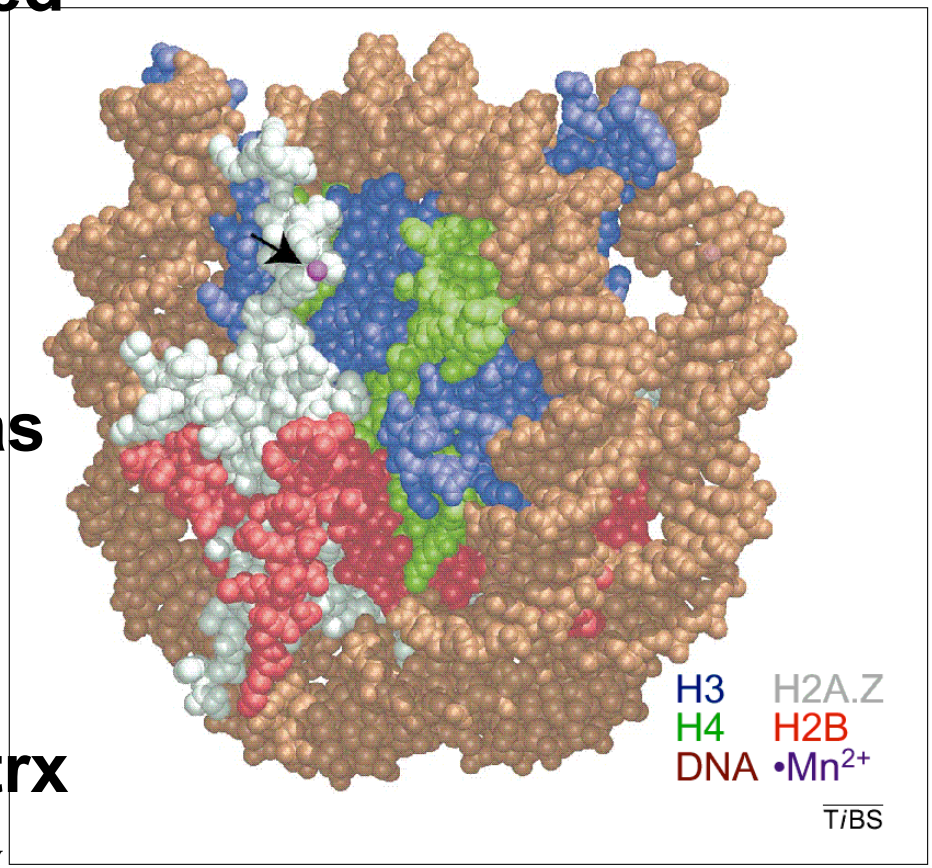


Histone eviction and variant incorporation

- **Eviction - displacement of histones**
  - Histone dimers of H2A and H2B can be rather easily exchanged in and out of nucleosome
  - Entire histone octamers, including H3 and H4, can also be displaced (evicted) or exchanged under certain circumstances
  - TF binding, chromatin-remodeling complexes and actively transcribing Pol II can all mediate histone displacement.
- **Variant histone incorporation**
  - Many variant forms of histones exist, distinguished from canonical core histones mainly by being expressed outside of S phase and incorporated into chromatin in a DNA replication-independent manner.

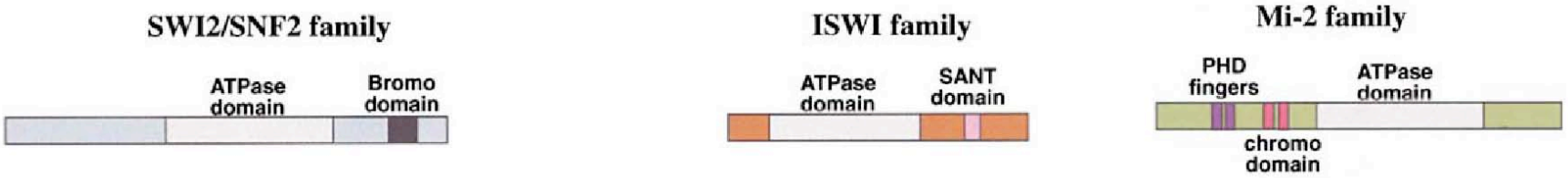
Example: Histone variant H2A.Z

- **Only 60% conserved relative to H2A**
- **Associated with transcribed regions**
  - Facilitates TBP binding
  - Is evicted upon activation
- **Essential**
- **The H2A.Z nucleosome has distinct features**
  - Altered contacts
  - Metal binding site
- **H3.3 also assocated with trx**
  - Different from canonical H3 in only four amino acids

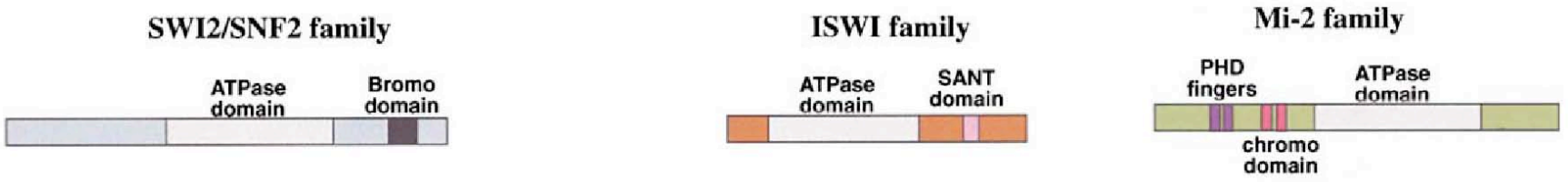


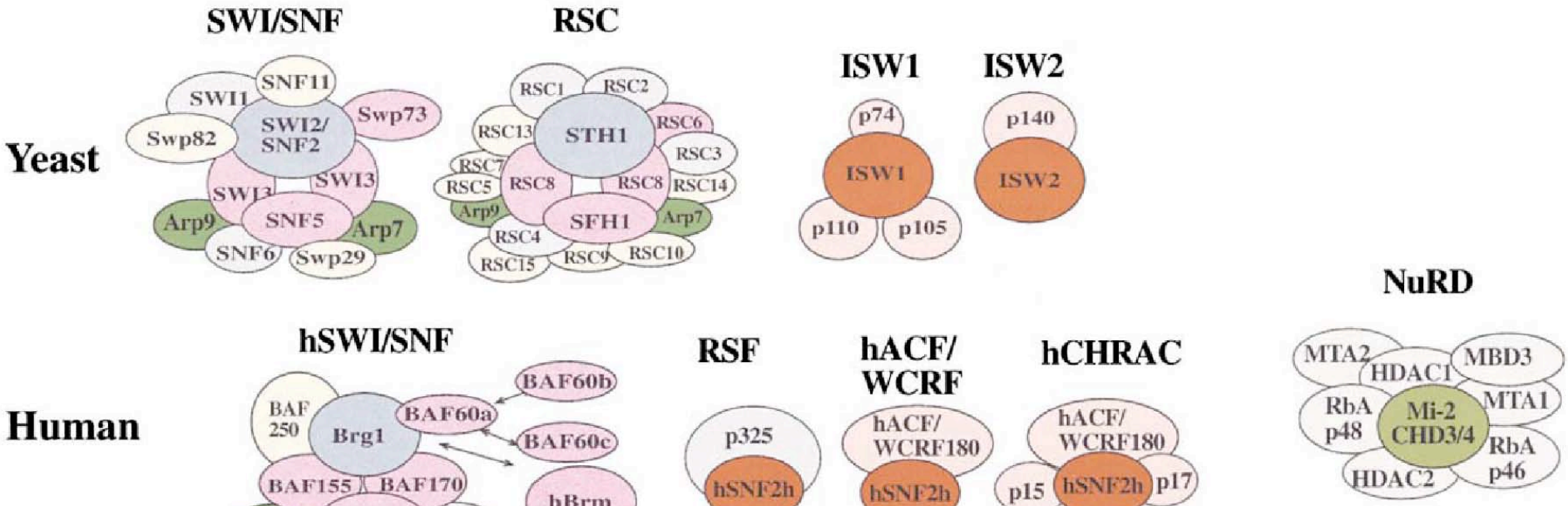
Three families of ATP-driven remodeling factors

- **Swi/Snf-familien**
  - Yeast: Original Swi/Snf + more abundant RSC
  - *Drosophila* dSWI/SNF with brahma (brm)
  - Humant: hSWI/SNF
    - Med BRG1 and hBRM
- **Mi-2/CHD-familien**
  - Mi-2 complex
  - NuRD
- **ISWI-familien**
  - yeast: **ISW1** and **ISW2**
  - *Drosophila* **NURF**(Nucleosome remodelling factor) and **ACF** and **CHRAC**
  - Human **RSF and hACF** and **hCHRAC**

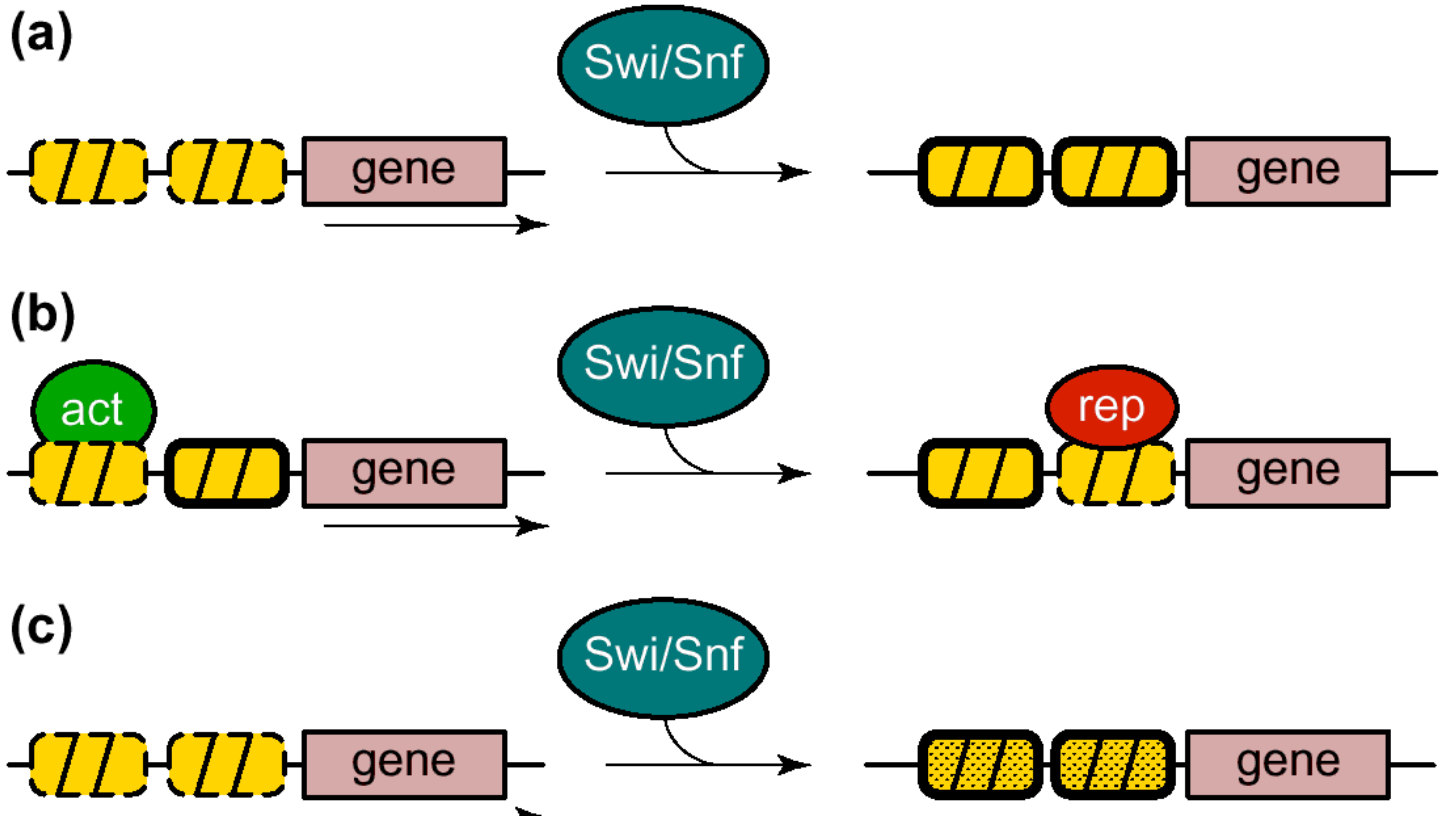


Three families of ATP-driven remodeling factors





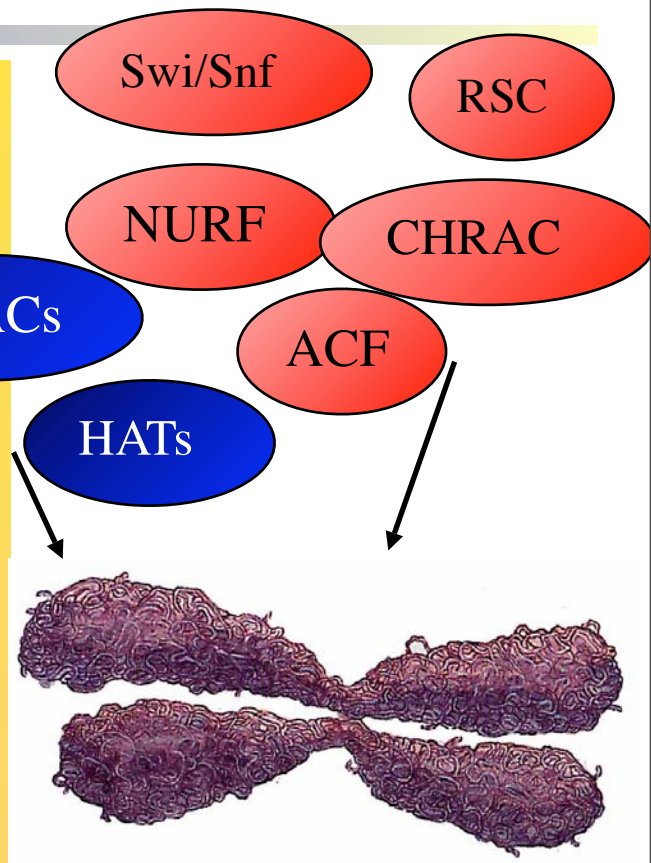
Also repression by Swi/Snf



Histone modification and chromatin remodelling together

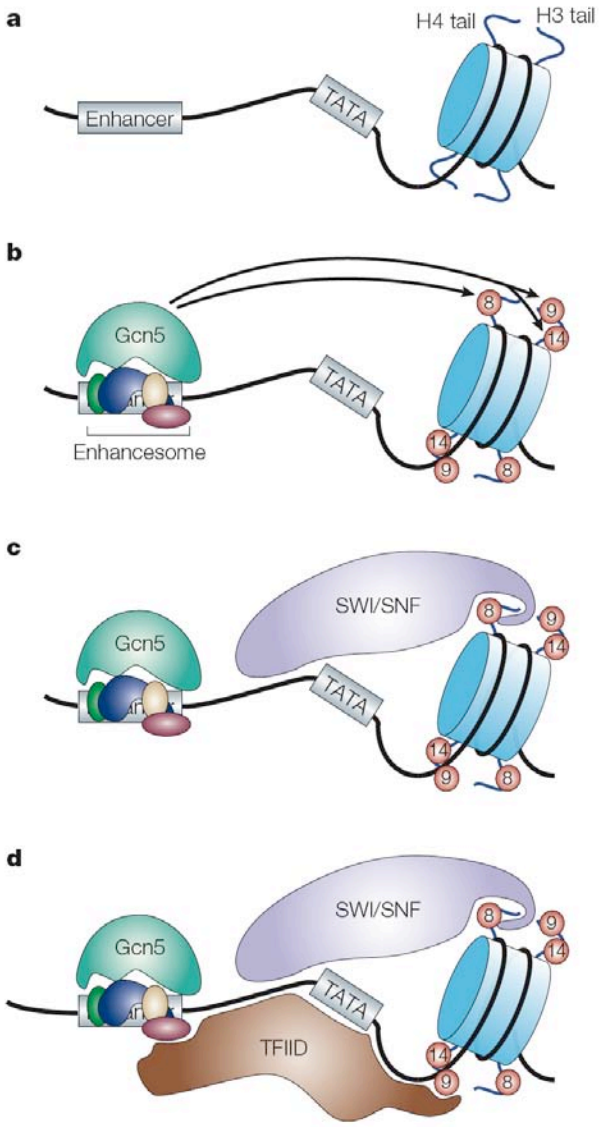
Changing the chromatin-template to help transcription factors

- **1. Opening of chromatin through directed modication of histone tails (acetylation and methylation)**
  - Histone-modification by HAT and HMT activity
  - Basis for the histone code hypothesis
- **2. Opening of chromatin through directed nucleosome mobilization**
  - ATP-dependent process
- **3. Positioning of nucleosomes creates promoters with different requirement for remodeling**



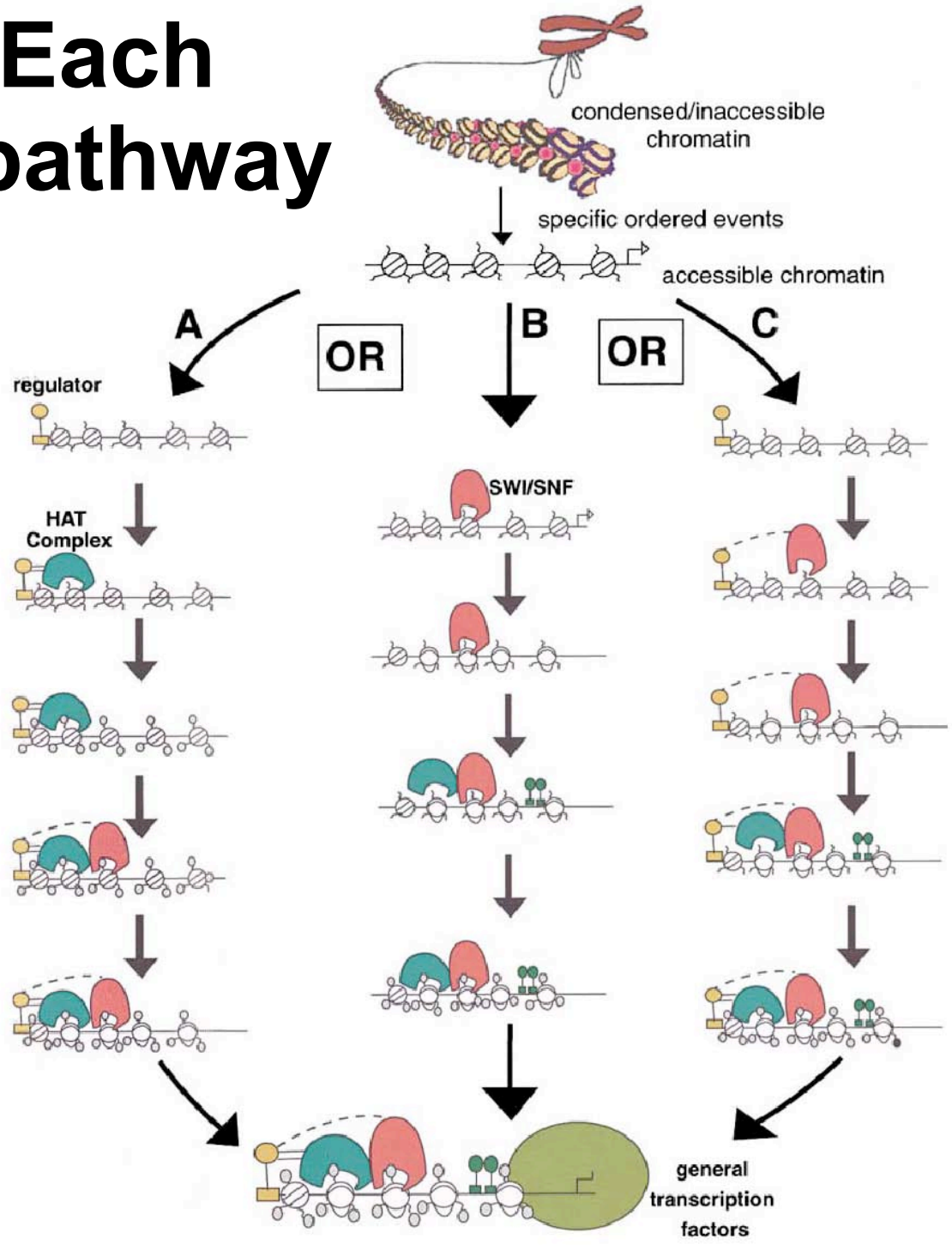
Recruitment followed by further associations and modifications

- Once targeted, SAGA acetylates histone H3 in the vicinity of the promoter.
- Targeted acetylation by SAGA stabilizes its binding and that of a targeted SWI/SNF chromatin-remodeling complex.
- This requires the bromodomains of Gcn5 and Swi2, respectively, which bind to acetyl-lysine.
- Due to bromodomains in other proteins, acetylation might stabilize interactions of several complexes



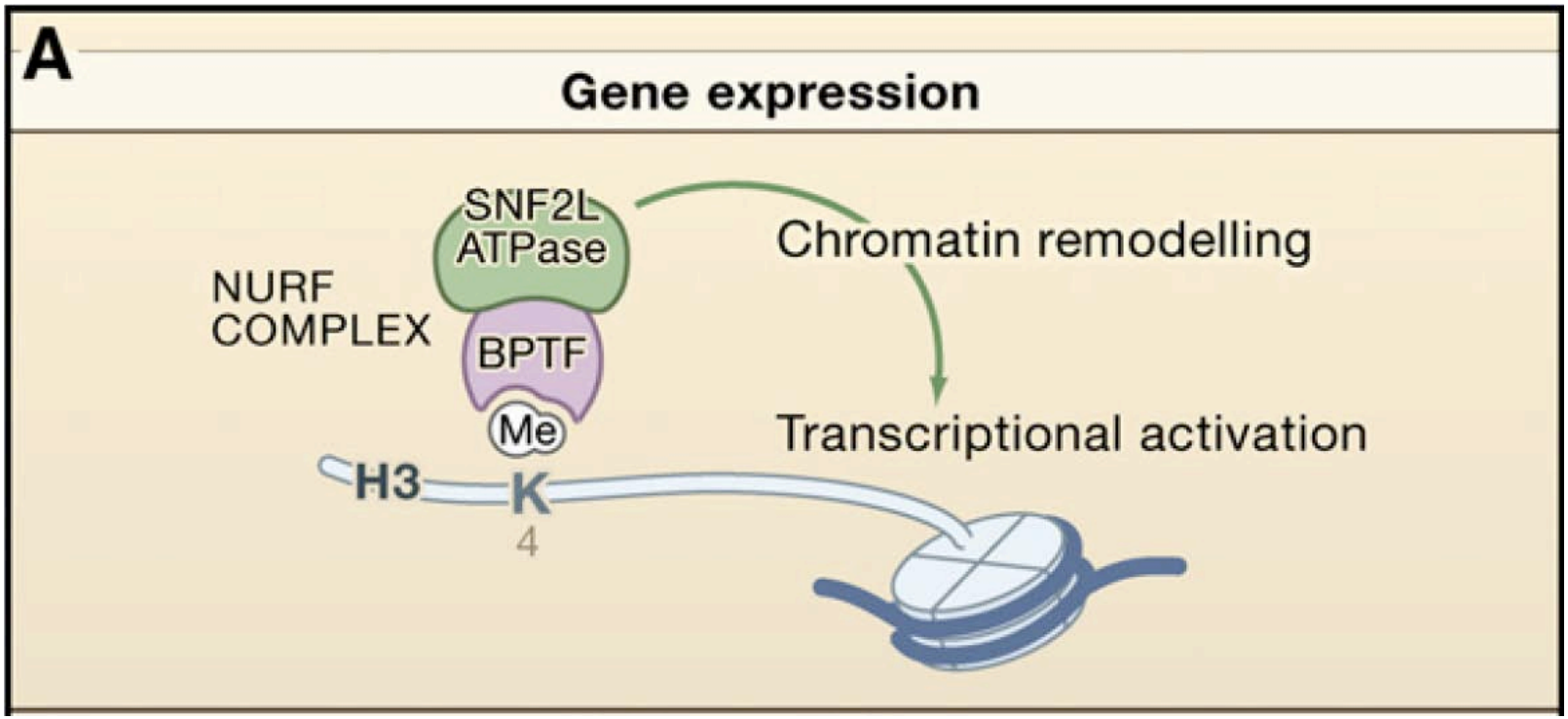
Order of action: Each gene a specific pathway

- It appears that there is no obligate order for function of ATP-dependent remodelers and covalent modifiers that is general for all promoters.
- Rather, it seems that each individual promoter will work using a set order of action by these complexes that differs from promoter to promoter.



Linking Histone modifications and chromatin remodelling

- **Recruitment of remodelling complexes may be controlled by histone modifications**
  - Recruitment of the NURF complex, which contains a component BRTF recognizing H3K4me and a component-remodeling chromatin.



Kouzarides, T. (2007) Chromatin modifications and their function. Cell, 128, 693-705.

Nucleosome positioning

Changing the chromatin-template to help transcription factors

- **1. Opening of chromatin through directed modication of histone tails (acetylation and methylation)**
  - Histone-modification by HAT and HMT activity
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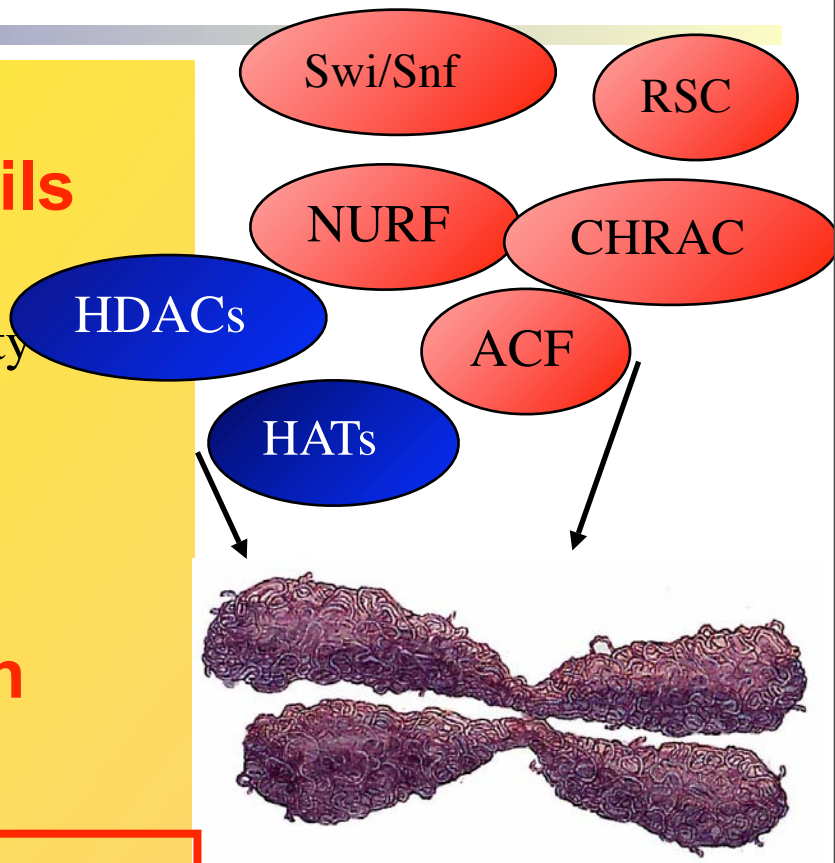
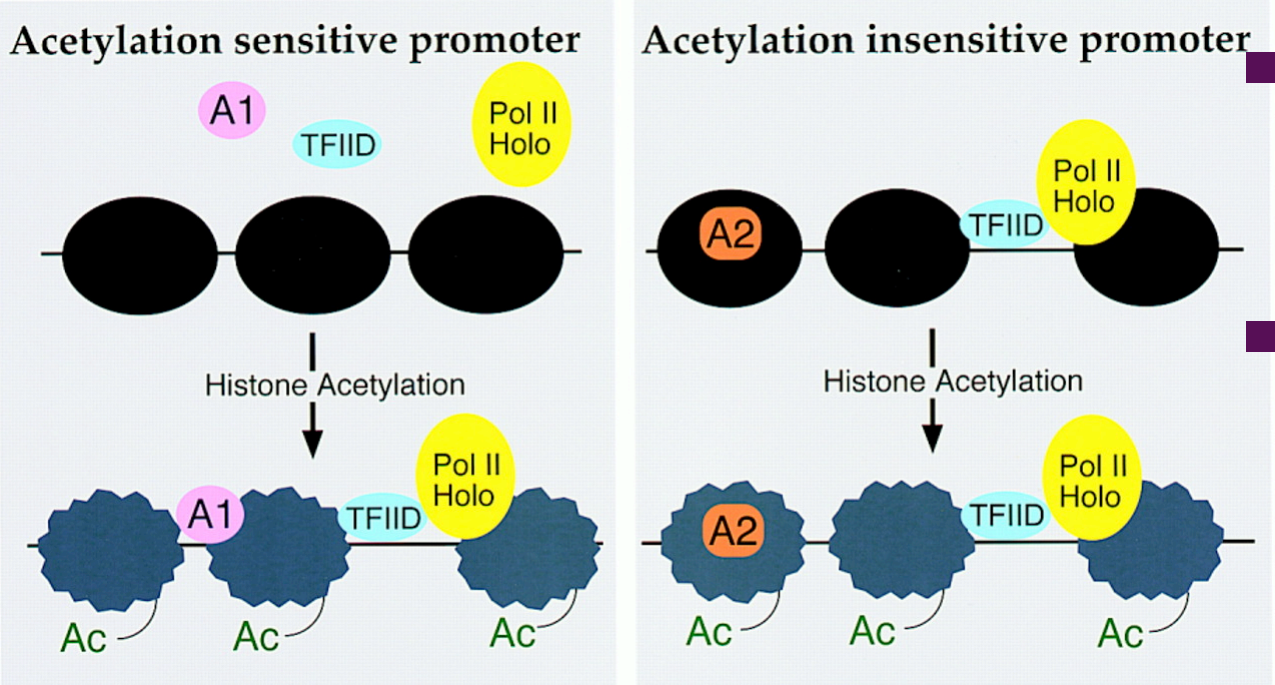


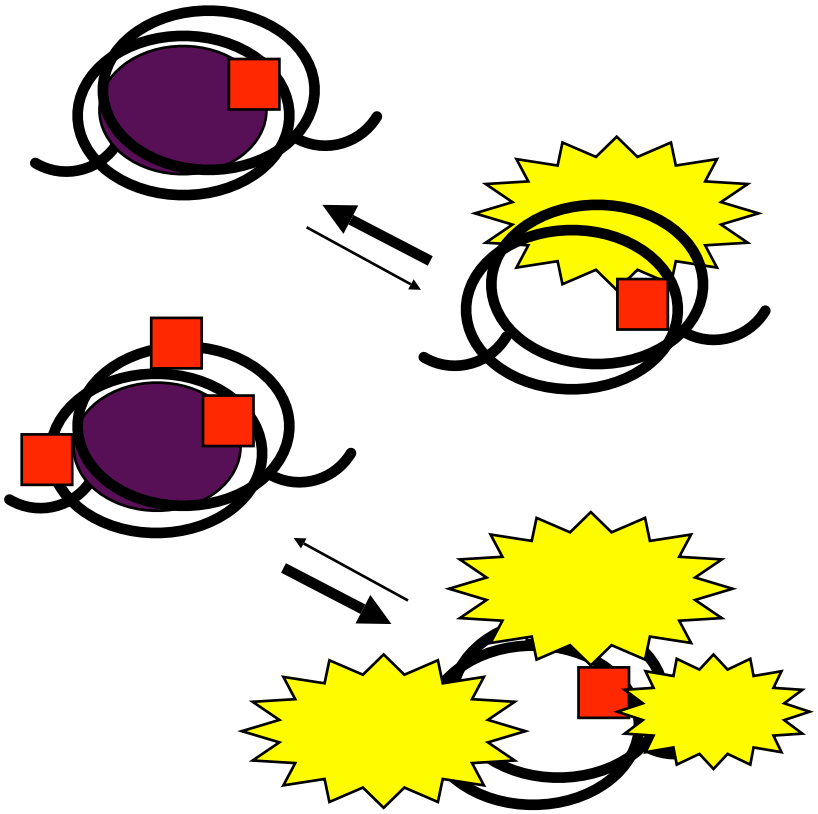
3. Different promoters differ in their dependence on remodeling



- Promoters vary w.r.t. dependence on remodeling
- AT-rich regions can be relative nuclesome-free and hence ”poised” for activation
- Nucleosome position may be decisive

Access: some TFs can invade chromatin alone

- **Some cis-elements available for TFs without disruption of the nucleosome**
  - Others hidden. These elements require nucleosome displacement before TF binding
  - Multiple TFs bound in a cooper-ative fashion necessary for invasion
- **The concept that nucleosomal DNA is somewhat accessible to TFs has been reinforced recently**
- **TF-binding sites mapped either to nucleosome-free regions or within a nucleosome.**
- **Nucleosome density at promoter regions is typically lower than that in the coding region**



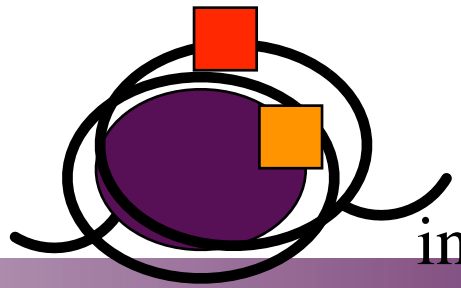
Eukaryotic cells tend to position sequence-specific TF-binding sites within accessible regions

Nucleosome positioning - translational

- **Def: Translational positioning**
  - = position along the DNA-strand
- **Nucleosomes in vivo: - having a specific translational positioning?**
- **position determined partially by DNA-sequence, partially by bound TFs**
  - DNA seq varies with respect to flexibility, bendability → bending around octamer occurs in preferred positions
    - thermodynamic pref for AT-rich in the minor groove pointing inwards and GC-rich on the outside
    - TFs may act as fixed blocks determining positioning of neighbouring nucleosomes

Rotational positioning of nucleosomes

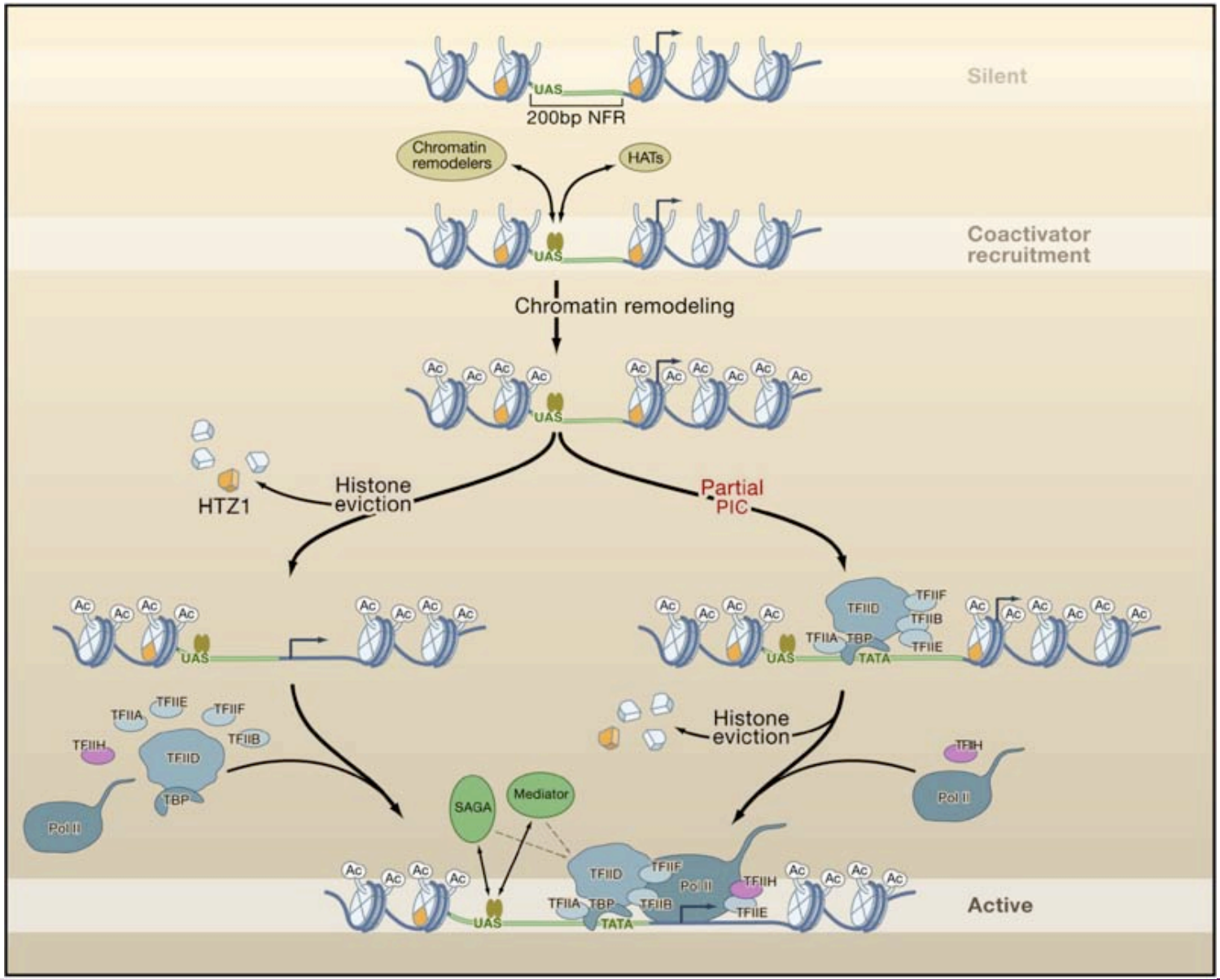
- **Def: orientation of DNA relative to octamer surface ("in-out")**
- **Determine availability of sites**
  - A role in presentation of cis-elements
  - 5 bp sliding of a response element will change rot.pos. from "in" to "out"
- **A classical example**
  - The MMTV LTR example:
    - +hormone → GR goes to nucleus → binds rotationally exposed GRE → recruits SWI-SNF complex → H1 dissociates → NF1 + OCT site become exposed for binding → TFIID recruited → activation



in/out

A combined model example

- **Open regions** (NFR nucleosome free regions)
- **Remodel-ling**
- **Histone modifi-cations**
- **Histone eviction**



Examples of questions for the exam

- **Histone code**
  - *The "Histone code hypothesis" has become a key to understand how the transcriptional apparatus interacts with chromatin. Describe briefly key elements in this hypothesis, including how code patterns are generated and how they are read (decoded).*
- **Methylation**
  - *The packing of genes into chromatin reduces their accessibility for the transcriptional apparatus. Hence, processes have evolved that carefully control the accessibility of genes for transcription. One set of mechanisms involves post-translational modification of histone tails. Explain some key elements related to the methylation of histone tails: what type of modifications occur, how these modifications relates to transcriptional activity, which proteins are involved in generating these modifications (and demodifications) and how these modifications are recognized by other proteins.*
  -